# Assessment for Production of Algal Bio-Chemical Compounds using Wastewater and its Application for value-added Products

## THESIS

SUBMITTED TO
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LUCKNOW

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*Dedicated*

*to*

*My Parents*

*&*

*Respected Teachers*

# CANDIDATE'S DECLARATION

I, **Rifat Azam**, solemnly declare that the research work embodied in this thesis entitled **"Assessment for Production of Algal Bio-Chemical Compounds using Wastewater and its Application for value-added Products"** carried out by me under the guidance and supervision of **Professor Naveen Kumar Arora, Head, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow (U.P.), India** and Co-supervision of **Dr. Richa Kothari, Associate Professor, Department of Environmental Sciences, Central University of Jammu, Samba (J&K), India,** is an original work and is also approved by Departmental Research Committee (DRC).

I further declare that to the best of my knowledge, this thesis does not contain part of any work submitted for the award of any degree either in this University or any other University around the globe. It is further undertaken that the thesis is essentially free from all kinds of plagiarism.

**Date:** 16 February, 2022
**Place: Lucknow**

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# CERTIFICATE

This is to certify that the thesis titled **"Assessment for Production of Algal Bio-Chemical Compounds using Wastewater and its Application for value-added Products"** submitted by **Ms. Rifat Azam** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations -1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

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**Place: Lucknow**

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# ABBREVIATIONS

| | |
|---|---|
| SHWW | Slaughterhouse wastewater |
| OSCCW | Open sewage contaminated channel wastewater |
| OPS | Open pond system |
| PBR | Photobioreactor |
| CPBR | Closed photobioreactor |
| FP-PBR | Flat panel photobioreactor |
| VC-PBR | Vertical column photobioreactor |
| $CO_2$ | Carbon dioxide |
| TAG | Triacylglyrides |
| VAP | Value-added products |
| TDS | Total dissolved solid |
| BOD | Biological oxygen demand |
| COD | Chemical oxygen demand |
| TES | Total dissolved solids |
| CGF | Chlorella growth factor |
| NCIM | National Collection of Industrial Microorganism |
| MLD | Million litre per day |
| V/V | volume by volume |
| L/D | Light/Dark |
| OD | Optical chemistry |
| TPBR | Tubular photobioreactor |
| VC-PBR | Vertical column photobioreactor |
| HE | Harvesting efficiency |
| ATP | Adenosine triphosphate |
| GS-PBR | Gas based photobioreactor |
| EC-PBR | Electrical coil based photobioreactor |
| TES-PBR | Thermal Energy storage based photobioreactor |
| ACM | Annual cost method |

# Chapter 1
# Introduction

## 1.1. Introduction

Planet earth is facing challenges to address the demands of clear and clean water, due to several reasons such as (i) drought, (ii) rise in population, and (iii) deforestation. Water is an important natural resource that covers about 71% area of the earth's surface, of which 97% is in the ocean and 3% is available as freshwater, which can be found in polar ice caps, glaciers, atmosphere and soil. Only 0.5% is available for drinking purposes from this 3% is freshwater. The poor condition of water bodies is not only an indicator of environmental pollution, but also poses a risk for ecosystem hygiene. So, the quality of water bodies is very important for both environmental and economical scales. Due to the increase in population and industrialization, there is a consumption of a large amount of freshwater thus generating a huge amount of wastewater. The wastewater getting discharged directly into the environment creates a risk for ecosystem and human health.

Wastewater can be classified based on its origin like industrial, agricultural, and municipal wastewater. Industrial wastewater is a type of wastewater coming out of different industries, and agricultural wastewater is a type generated from different agricultural processes and dairy farming. The municipal and domestic wastewater together produces the largest amount of wastewater. The sources of the above types of wastewaterincludesolid contents like faeces, food, and detergent residue as well as sand particles (Garcia-Martineza *et al*., 2019; Manasa and Mehta, 2020). In addition, domestic and municipal wastewater contains large quantities of pharmaceutical products, pathogens, untreated domestic waste, garbage, biodegradable organic compounds, odor and heavy metals which further leads to an increase in chemical oxygen demand (COD), biological oxygen demand (BOD), and the organic load of the wastewater (Wang *et al.,* 2017).

Wastewater may contain various types of contaminants and heavy metals *i.e.,* molybdenum, zinc, arsenic, magnesium, lead, copper, and mercury *etc.*

The United Nations reported that 80% of water is discharged as wastewater without any proper treatment (UNESCO, 2017; Li *et al*., 2019). About ~12.6 million people died due to air pollution, water, soil, and chemical exposures (Yadav *et al*., 2019). Consequently, every type of pollutant contaminating water requires treatment before its discharge into the environment. In addition, the availability of a high amount of BOD, COD, nitrate, and phosphate causes eutrophication in water bodies (Ibrahim *et al*., 2019). The amount of hydrocarbons, pesticides, phenolics, polychlorinated biphenyls, polyaromatic, and antibiotics also pose a harsh risk, especially to the aquatic ecosystem.

Different methods of treatment are employed to remove pollutants from wastewater like physical, chemical, and biological methods (Saleh *et al.*, 2020). The physical method consists of membrane filtration, UV radiation, and adsorption, which are mostly used for wastewater pretreatment (Udaiyappan *et al.*, 2017). The conventional methods consist of sedimentation, anaerobic digestion, and denitrification that allow the reduction in the number of nutrients from wastewater (Raouf *et al.*, 2019). However, these methods are not economically viable due to low nutrient removal efficiency and generate a high quantity of sludge.

The existing methods for the treatment of wastewater are neither entirely sustainable nor could resolve the issue of scarcity of water (Ibrahim *et al*., 2019). Therefore, biological-based wastewater treatment may act as a promising tool for its revival with valuable resources, from natural water and wastewater too. This strategy may thus help in strengthening the green decisive factor of "best from waste". In turn, biological wastewater treatment may prove to be good to fulfill the biological remediation process for reducing the consumption of natural resources towards protecting the environment. Wise attention is warranted for the use of advanced cutting-edge techniques in the treatment of wastewater with an aim of resource recovery and low cost too. Therefore, in the upcoming sections of this Chapter, basic information on water resources, their utilization, sources of water pollution, types of pollutants present in water are discussed. Impacts of these in terms of the water crisis and the major industrial sources responsible for large wastewater discharges are also discussed. Various wastewater treatments are shown in Figure 1.1.

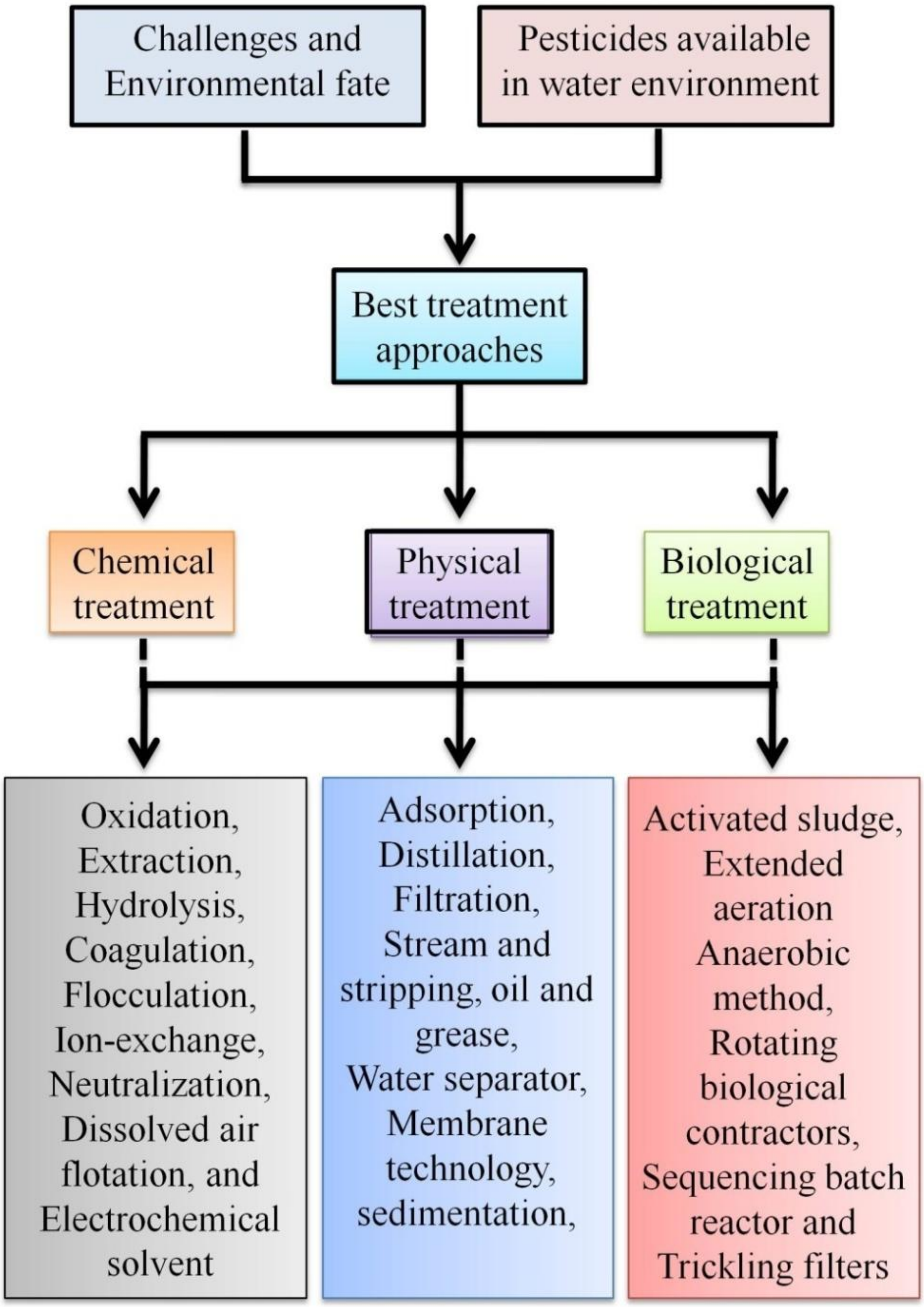



**Figure 1.1. Different methods of chemical, physical and biological treatments of wastewater** (Singh *et al.*, 2019)

Water requirement is high nowadays due to the demand for agricultural processes, urbanization, and industrialization. Major water resources include the following:

**1.2. Groundwater**

Groundwater is the fresh water that comes from rain or melting ice and snow that soaks into the soil and is stored in small pores between the rocks and soil particles. Groundwater has a vital role in the hydrological cycle, as it is also important for plant growth, soil formation, and for human activities like drinking, washing, cooking, and other related daily needs. It is obvious that it is a very tough task to observe the groundwater flow, its mapping, modeling, monitoring, and subcellular flow and needs expert handling. It is also important to know its sustainability and the reasons for its contamination. Noteworthy, existing and forthcoming threats to the groundwater quality and quantity require consideration especially from policy and developmental viewpoints, climate change, extraction, fossil fuel exploration and natural pollutants like arsenic and radium, the underground storage tanks, industrial pollutants, and agrarian use and its pollution (Edwin *et al*., 2017).

**1.3. Surface water**

Surface water comes from rivers, lakes as well from freshwater wetland. It is restored by precipitation, evaporation, ocean and sub-surface leakage. There are many factors like lakes storage capacity, wetlands, artificial water reservoirs, precipitation, and evaporation time, which are responsible for the quantity of the surface water and their loss. For the estimation of the availability of water in India, several organizations and individuals are working in these sectors (Gangwar, 2013). The major cause of water loss is through plant transpiration and evaporation. The Ganga-Brahmaputra-Barak-Meghna are considered as an important source for the use of water resources at national level. In India, the Ganga basin is second in terms of flow rate and it is the largest storage for the utilization of water (Saroha, 2017). Water flow in a river depends on the size of its water catchment area.

### 1.4. Sources of water pollution

The water pollutants come from point and non-point sources. The point sources are the direct point of origin of pollutants like pipe connected from a factory, oil spill out of a tank, wastewater coming from industries directly, and other waste discharges, whereas non-point sources of pollution are those that comes from different unidentified links and deliver the contaminants into the ground and surface water that create pollution in the environment. In point sources, pollutants come from large areas like agricultural field that are fully covered with fertilizers or pesticides. It runs off and reaches streams, rivers as well as in soil that contaminates the ground and surface water. Whereas, non-point sources are a big problem all over the world. The non-point source of pollutants comes from rainfall and snowfall. Its occurrence is not regular, it comes anytime anywhere, so it is called irregular or accidental occurrence of pollutants. Some examples of non-point sources are the wastes runoff from unnoticed/unidentified sources like agricultural fields, city waste *etc.* Sources of water pollution are depicted in Figure 1.2. The water pollution sources consist of natural sources, sewage wastewater, agricultural wastes, oil pollution, radioactive substances, river, and marine dumping *etc*. in general way which are discussed in detail as follows:

#### 1.4.1. Natural sources

The natural sources of pollutants come from near by vegetation, rainwater, dust, storms, rocks, volcanoes, and natural runoff. Therefore, rainwater is considered as a naturally occurring source of pollution in a water body that takes pollutants from the air and dissolves it.

### 1.4.2. Domestic sewage

Domestic waste comes from household or residential areas, it consists of 99% water and the remaining 1% is solids. It comprises of organic and inorganic components. The organic components are proteins, fats, carbohydrates, inorganic constitute salts, grit and metals. The main problems connected with sewage waste that spread the diseases, which is responsible for the bad smell. In addition, the organic pollutant leads to the depletion of oxygen and kills the fishes too. It contains a huge amount of nutrients, which occur in the form of nitrate and phosphate, which causes the problem of eutrophication in water bodies. There are various methods for the treatment of sewage or disposal that may be employed like the land filling treatment options, incineration *etc.*

### 1.4.3. Agricultural waste

Agricultural wastes come from agricultural activities like manure, oil, fertilizer, pesticides, and herbicides, which is also global level contributor of pollution. Various heavy metals like copper, chromium, zinc, mercury *etc.* that comes from chemical fertilizers, used for the growth of vegetables and fruits by farmers also responsible for pollutionin aquatic systems (UNESCO, 2017). Nitrate and phosphate from the agricultural run-off are the major chemical contaminants in freshwater sources. According to the available literature, 38% of water bodies in the European Union are contaminated due to agricultural pollution (US EPA, 2017). Similarly, the USA and China also share surface-water pollution due to the agricultural sector only. Three main agricultural production systems categories in the literature are crops, livestock and aquaculture. The major pollutants discharged from these in water bodies are nutrients, pesticides, salts, sediments, organic matter, pathogens, metals and emerging pollutants like drugs residues, feed additives, *etc.* During crop production and livestock production/farming, excess nitrogen and phosphates move/leach into water courses and leads to diffused water pollution. Phosphate solubility is also not easy as other nutrients gets adsorbed on the soil layer and enters into water aquifers (FAO/WHO, 2016). Higher salinity is noticed with advanced practices of irrigation and it directly affects the bio-diversity of microorganisms, algae and plant varieties. Sedimentary movements are always carriers for physical and chemical pollutants and cause damage to the water bodies. Excreta of livestock consist of many zoonotic microorganisms and multicellular parasites which areharmful for the health and hygiene of the several ecosystems. Hence, agricultural pollution occurs in a wide area and its sources are not easy to recognize, so it becomes hard to control the pollution through discharges from agricultural water (Pathak *et al.*, 2015). Tables 1.2 indicate the impact of agricultural pollutants on surface and groundwater as well as human health is enlisted as per available literature.

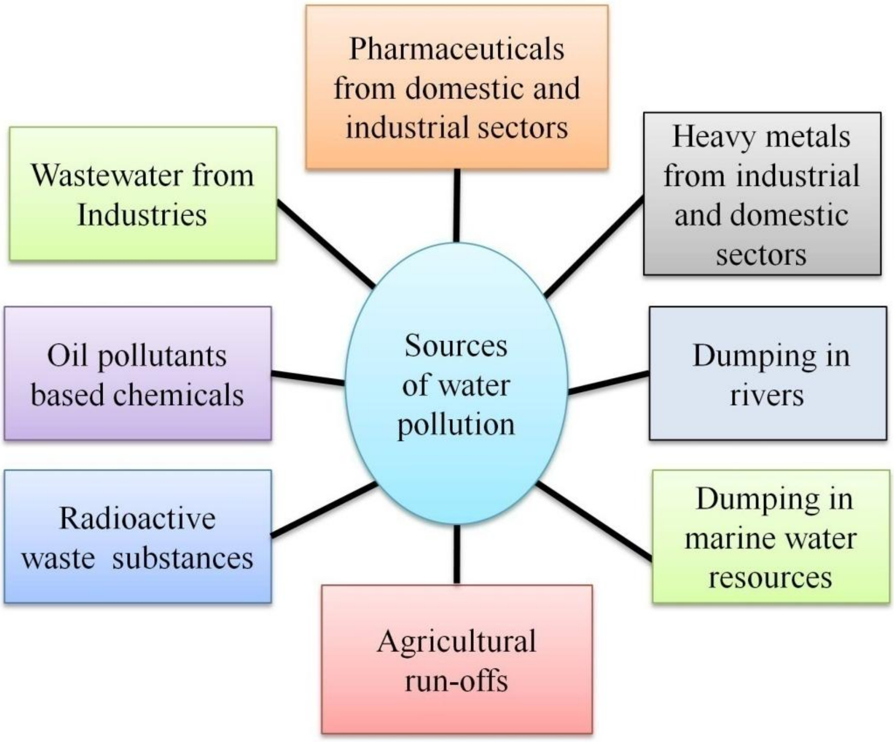



**Figure 1.2. Different sources of water pollution in water bodies**

### 1.5. Types of pollutants present in water

Water pollution is defined as the presence of unwanted substances in water, thus it degrades the quality of water and makes it toxic for human health and the surrounding environment. Water is polluted by both activities either natural or anthropogenic. Water is contaminated due to the occurrence of pollutants, which are further classified into three categories: biological, physical and chemical pollutants. The biological pollutants consist of bacteria, viruses, worms, and protozoans. The chemical pollutants comprise of nutrients, organic waste and heavy metals (Manyatshe *et al.,* 2017; Artabe *et al.,* 2020) whereas, the physical pollutants change the physical properties of water like color, temperature, and suspended solids (Staff, 2017). Some radioactive materials are emitted from anthropogenic activities like nuclear power plants, nuclear weapons and radioactive appliances. Common radionuclide present in the drinking water includes uranium and thorium. The presence of naturally occurring radium and uranium causes dangerous effects on the body of human beings. They cause cancerous effects on bones and toxic effects on the kidney (Owolabi, 2017; 2018). The suspended solids are those pollutants that are released from industrial, sewage, and municipal wastewater. Therefore, there are three types of suspended solids: sand material, organic content and tiny particles present in wastewater. The pathogens are microorganism that can cause disease; it includes bacteria, viruses, and parasites (Dwivedi, 2017). Various waterborne diseases come from different sources that contaminate the water body and cause diseases. Some specific bacterial and pathogen organisms that cause serious problems for human beings as noticed in drinking water sources are summarized in Table 1.1.

Industries manufacture very useful products, but at the same time, generate a large amount of waste products in the form of solid, liquid, and gas. The main problem in industrial society is the consumption of water. Due to the reduction of consumption of water, many approaches are adopted like wastewater recycling. Both industrialists and government have a quick action to solve this problem (Ilyas *et al.*, 2019) at the global level. The familiar origins of contaminants are point sources and non-familiar origins are non-point sources. So, it is very difficult to manage the non-point sources due to multiple routes,color, nitrite, fluoride, heavy metals and chemical byproducts are the common contaminants in water bodies from industries. These types of industrial waste materials are always create the problem for all the spheres (atmosphere, lithosphere, and hydrosphere) of the ecosystem. So, to reduce the impacts of contaminants on these spheres directly or indirectly, research activities should be promoted with sustainability. Although all the spheric components are important but in the present research work, we focused our objectives on hydrosphere *i.e.* water resources. Different factors, sources, impacts on surrounding, natural and anthropogenic contaminants all are taken in a loop for study in this research work.

### 1.6. Water crisis

Due to the high demand for fresh water in the agriculture sector and industrial sector water consumption and unequal division of drinking water sources are the main factors for the water crisis.

**Table 1.1. Infectious organisms reported in drinking water sources**

| **Organism** | **Disease** | **Remarks** | **References** |
|---|---|---|---|
| **Bacteria** | | | |
| *Escherichia coli*<br>*Legionella pneumophila*<br>*Yersinia enterocolitica*<br>*Vibrio chlorella* | Gastroenteritis<br>Legionellosis<br>Yersiniosis<br>Cholera | Diarrhoea<br>Severe respiratory illness<br>Diarrhoea<br>Dehydration | Dennehy, 2019 |
| **Viruses** | | | |
| *Adenovirus (31 types)*<br>*Enteroviruses (67 types)*<br>*Norwalk agent*<br>*Reovirus*<br>*Rotavirus*<br>*Hepatitis A* | Respiratory disease<br>Meningitis<br>Gastroenteritis<br>Gastroenteritis<br>Gastroenteritis<br>Infectious hepatitis | -<br>-<br>Vomiting problem<br>-<br>-<br>Fever, Jaundice | Greening and Cannon, 2016 |
| **Protozoa** | | | |
| *Cryptosporidium*<br>*Balantidium coli*<br>*Cryptosporidium*<br>*Entamoeba histolytica*<br>*Giardia lambia* | Cryptosporidiosis<br>Balantidiasis<br>Cryptosporidiosis<br>Amoebic dysentery<br>Giardiasis | Diarrhoea<br>Dysentery<br>Diarrhoea<br>Long time diarrhea<br>with bleeding problem | Hemphill *et al.*, 2019 |
| **Helminthes** | | | |
| *Trichuris Trichuria*<br>*Taenia solium*<br>*Hymenolepis nana*<br>*Ascaris lumbricoid*<br>*Dracunculus medinensis*<br>*Fasciola hepatica* | Trichuriasis<br>Taeniasis<br>Hymenoleopsis<br>Ascariasis<br>Dracunculiasis<br>Fasciolosis | Whipworm<br>Pork tapeworm<br>Dwarf tapeworm<br>Roundworm<br>Guinea worm<br>Sheep liver fluke | Ghaffar, 2015 |

**Table 1.2. Agricultural pollutants and their impacts(FAO, 2016; Sasakova *et al.*, 2018; Lwimbo *et al.*, 2019)**

| **Agricultural processes and associated pollutants** | **Impact on surface water** | **Impact on groundwater** | **Impact on human health** |
|---|---|---|---|
| Fertilizers | Flow of nutrients cause eutrophication | Leakage  of nitrate into groundwater causes pollution | Cancer problem, kidney effect, Liver and lungs infection |
| Pesticides | Pesticides runoff due to heavy rainfall that contaminates the surface water | Leakage of pesticides into groundwater that contaminates the groundwater | Abnormalities in reproduction, Hormonal disorder, Cancer |
| Manure/organic matters | Surface water contaminated through heavy metals, pathogens available in manure | Nitrogen contaminated the groundwater | Salmonellosis, Listeriosis |
| Feedlots and aquaculture | Eutrophication help to increase the sediment in the water that causes the loss of the aquatic life | Feedlots might leak nitrogen and other metal into groundwater causing pollution in groundwater | Drugs and antibiotics resistance |
| Ploughing | Some chemicals absorbed from sediments particles cause contamination | - | - |
| Irrigation | Run-off pesticides cause surface water contamination, pathogen contamination | Leaking of nutrients as well as salt cause groundwater contamination | Decrease important nutrients from the body, Decrease immunity |

Although there is a huge difference in approaches for the estimation of present water demand at the national and international level (Addams *et al*., 2009). Also, there is an extensive fear for the water resources getting deteriorated nowadays based on its need and utilization (Kumar *et al*., 2012). Therefore, the discharge of industrial wastewater towards the river and water bodies without any treatment may cause pollution in water bodies. Therefore, new processes are required that looks for the need/ recovery of water resources and their distribution at national as well as international level as per their climatic conditions, industrial sectors and their requirements for water, assessment strategies for water quality need specific attention.

### 1.6.1. Climatic conditions

Climate change has begun to affect worldwide resources, through precipitation patterns, warming, and extreme climate conditions. So, there is an urgent requirement to review the water management processes, particularly for those areas, where the demographic changes and climatic conditions are going at a very faster rate to regulate a sustainable and safe supply of water. In addition, the advanced technology solutions and practices can improve the water utilization efficiency users should be the main target group for the management of water to reduce the loss of water. Increasing the non-conventional alternatives is also a good strategy towards the protection of water resources (Morote *et al.,* 2019). Water quality degradation should be of global concern, due to impacts on biodiversity, human health and ecosystem sustainability. The possible impacts on water resources quality by rising demands and climate change arising from the spreading pattern of contaminations should be considered and evaluated reduce the water scarcity (Tzanakakis *et al.,* 2020; Agha kouchak *et al.,* 2020).

### 1.6.2. Industrial processes

Untreated water that is generated from industrial processes causes pollution when discharged with/without treatment. There are several types of toxic as well as chemical pollutants present in industrial wastewater, which are classified into three classes like organic matter, heavy metals, and fouling substances. The pollutants released from industries into waterways are very dangerous for the aquatic environment. While as, there are many standards and guidelines implemented for water resources, but unfortunately, not evaluated or implemented in the proper way (Bharagava *et al.*, 2018). There are many health issues due to this industrial wastewater discharged after processing, and it pollutes the water. The major pollutants released from industries are heavy metals, microorganisms, non-heavy metals, and organic compounds. Diarrhea, vector borne diseases, blindness, cutaneous, paralysis, and liver are caused through contaminated water (Yadav *et al.,* 2017). About 22% of the world's water is used as an industrial process as reported by (Burek *et al*., 2016).

Table1.3 presents the state-wise sewage generation capacity which is generated from urban centers in India. Due to the pollution in drinking water, the sewage is generated at an estimated value of 72,368 MLD and utilization is 20,235 MLD only and the remaining quantity is let out as untreated sewage (CPCB, 2020). Table 1.4 Indicates water consumption from different industries in India based on available literature.

### 1.6.3. Water quality and health

According to Madhav *et al.,* (2018) the surface and groundwater are being contagious due to biological, chemical, inorganic, and organic toxic materials. The occurrence or existence of disease-causing microorganisms in the water bodies is considered a vital parameter of water quality.

**Table 1.3. State-wise sewage generation capacity of urban centers-India (CPCB, 2020)**

| States/Union territory | Sewage generation (MLD) | States/Union territory | Sewage generation (MLD) |
|---|---|---|---|
| Andaman and Nicobar Islands | 23 | Nagaland | 135 |
| West Bengal | 5457 | Rajasthan | 3185 |
| Assam | 809 | Sikkim | 52 |
| Andhra Pradesh | 2882 | Tamil Nadu | 6421 |
| Arunachal Pradesh | 62 | Telangana | 2660 |
| Dadra and Nagar Haveli | 67 | Uttarakhand | 627 |
| Himachal Pradesh | 116 | Mizoram | 103 |
| Gujarat | 5013 | Uttar Pradesh | 8263 |
| Goa | 176 | Jharkhand | 1510 |
| Haryana | 1816 | Punjab | 1889 |
| Jammu and Kashmir | 665 | Pondicherry | 161 |
| Lakshadweep | 13 | NCT OF Delhi | 3330 |
| Madhya Pradesh | 3646 | Maharashtra | 9107 |
| Bihar | 2276 | Orissa | 1288 |
| Karnataka | 4458 | Manipur | 162 |
| Kerala | 4256 | Meghalaya | 118 |
| Chattisgarh | 1203 | Tripura | 237 |
| Chandigarh | 188 | | |
| **Total** | **-** | **-** | **72368** |

**Note: Sewage generation is predicted based on water supplies and generation of sewage**

**Table 1.4. Annual water consumption from different industries in India (CEA, 2016; Joseph *et al.*, 2019)**

| **Different industries** | **Annual consumption (Million cubic meters)** | **Total water consumed from industries in (%)** | **Water demand perunit production (Billion cubic meters)** |
|---|---|---|---|
| Thermal power plant | 35, 157.4 | 87.87 | 0.06 |
| Steel industry | 516.6 | 1.29 | 0.52 |
| Fertilizers | 73.5 | 0.18 | 0.07 |
| Pulp and paper industries | 905.8 | 2.26 | 0.91 |
| Textiles | 829.8 | 2.07 | 0.83 |
| Sugar | 194.9 | 0.49 | 0.19 |
| Engineering | 2019.9 | 5.05 | 2.02 |
| Others | 314.2 | 0.78 | 0.31 |
| **Total** | **40, 012.0** | **100.0** | **40.01** |

All over the world, contaminated and dirty water is the main reason for the 4 million diarrhea cases and 1.5 million deaths among children of upto 5 years of age. According to the literature, in India, about one-third of rural groundwater is biologically infected with arsenic and fluoride and moreover, human activities also affect its quality UNICEF (2013). Therefore, the proper remediation techniques are required to check the water quality and that was introduced by the Ministry of Water Resources and encouraging current initiative has been for the creation of new protocols for drinking water monitoring (MDWS, 2013). There are some common factors, which affect the quality of water, are enlisted depicted below in Figure 1.3.

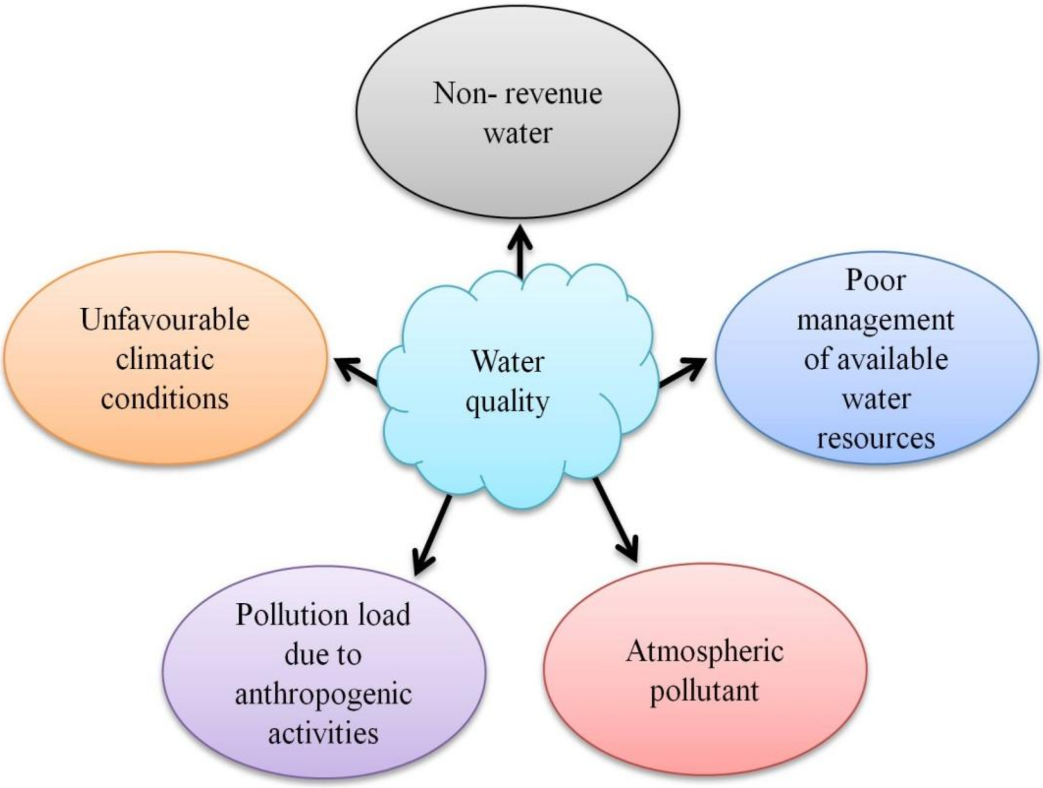



**Figure 1. 3. Factors affecting the water quality**

**1. 7. Treatment options for wastewater**

There are different types of wastewaters according to the processing and treatment steps opted for discharging the water at small to large scale agricultural wastewater, animal wastewater, industrial wastewater, municipal wastewater, confectionary wastewater, and textile wastewater, which are discussed in the upcoming sections of this Chapter.

### 1.7.1. Agriculture based wastewater

The availability of untreated pesticides in soil and water causes harmful effects on human health, like blindness, blurred problem, dizziness, and nervous problem. There are many chronic diseases, which are interlinked with pesticides, that include birth defects, respiratory disorders, cardiovascular problems, hormone imbalance, diabetes, asthma, pulmonary diseases, which are explored by many research studies (Zhang *et al.,* 2018). The removal of pesticides depends upon the types of pesticide material and efficiency for their treatment process. There are several treatment options that are used for the removal of pesticides from water. So, the techniques involved physical, chemical, and biological methods for treatment. Every treatment methods have its advantages, in terms of reliability, environmental impact, operability, and their efficiency. The chemical-based wastewater treatment consists of an advanced oxidation process that includes ozonation coagulation and fenton treatment. Adsorption is also a good process that is used for the purification of water. It shows very effective results for the purification of environmentally and publically stressful contaminants. In biological treatment process, there are two types of treatment processes *i.e.*aerobic and anaerobic treatment plant. Therefore, treatment options are activated sludge, bio-augmentation activated sludge, anaerobic and aerobic treatment, and membrane bioreactor (Saleh *et al.,* 2020).

### 1.7.2. Dairy wastewater

The dairy wastewater is mainly produced from milk products, and the detergent used to clean the animals. Amount of wastewater depends on the production of the milk, milk products produced, and the usage of pure water. In some studies, it has been calculated approximately 0.5-15L of the waste per liter of processed milk products are produced (Rad *et al*., 2014). Although the wastewater produced from the dairy waste is in small amount but, it may pose serious jeopardy to the flora and fauna of the area if not appropriately treated before its safe disposal. Thus, its treatment and safe disposal are very needful to protect the environment. Moreover, the available conventional methods of wastewater treatment are often loaded beyond design flows, causing its inefficient operation leading to excess financial burden thus affecting the company's bottom line (Karadag *et al* 2015). Presently, the method and techniques used for the treatment of dairy wastewater include sequence batch reactor, the up-flow anoxic/aerobic sludge, membrane bioreactor, anaerobic treatment, and electrochemical treatment (Goli *et al.*, 2019). It has been noticed that after the treatment of dairy wastewater the BOD, COD and suspended solids were reduced by these methods. The dairy wastewater and its products with treatment processes for the removal of pollutantsare delineated in Table 1.5 and 1.6., as follows:

### 1.7.3. Slaughterhouse wastewater

Slaughterhouse wastewater is that wastewater that comes after the waste released sacrifices of animal's either sheep, goat, chicken, or buffaloes after processing from that source. Though it requires considerable treatment for it sustainable and protected release to the environment because of having a high content of organic and nutrient pollutants present. For the same reason, physical, biological, and chemical treatment is used for the degradation of slaughterhouse wastewater (Bustillo-Lecompte and Mehrvar, 2017). Algal-based wastewater treatment is the best method for the treatment of meat processing wastewater. It cleans the water bodies and therefore protects from eutrophication (Jais *et al*., 2017). In developing countries, slaughterhouses are among the key sources of water pollution and GHG emission. Pathogens released from slaughterhouses may also infect humans exposed to the water bodies and make the water unfit for swimming, drinking, and irrigation purposes (Um *et al*., 2016). Major Asian countries experience severe bloody diarrhea, gastrointestinal diseases, liver malfunctions, and in some cases even death due to the incidence of viruses, helminthes eggs, protozoa, and bacteria in slaughterhouse wastewater Pavlidis *et al*., (2020). As well as in swine slaughterhouse wastewater is proliferating, along with the human population.

As a result, swine wastewater treatment is necessary for the security of the surrounding environment. For the removal of organic matter in swine slaughterhouse wastewater, the stabilization ponds and constructed wetlands anaerobic digester method provides a money-making solution for wastewater treatment with a high quantity of organic waste, resulting in the production of energy (Gonzalez-Tineo *et al.,* 2020). *Chlorella sorokiniana., Scenedesmus bicellularis., Scenedesmus dimorphus., Scenedesmus obliquus., Tetraselmis sp., Tetraselmissuecica., Phormidium sp., Chlamydomonas sp., Desmodesmus sp.,* are the algal species found in literature to treat the wastewater from swine slaughterhouses (Behl *et al.,* 2020; Wang *et al*., 2020; Zheng *et al.,* 2020). (Yaakob *et al.,* 2018) investigated the fact that wastewater coming out of the chicken slaughterhouse have many impacts on the environment and the receiving water bodies. They confirmed that direct discharge of chicken slaughterhouse wastewater can have a negative impact on the environment. Therefore, a proper treatment system is required for the slaughterhouse wastewater to release it in the water bodies such that it will have zero or least impact on the environment.

### 1.7.4. Aquaculture wastewater

Aquaculture is considered as the fastest rising industry in the world as a feed. It needs a high quantity of freshwater for the culturing of fish. It releases a high amount of wastewater into the aquatic environment. The main source of nutrient and pollutant load comes from the left behind feed, the fertilization of ponds fishes, and the residues of aquatic animals (Garcia-Martineza *et al*., 2019).

**Table 1.5. Dairy wastewater and its composition with various dairy products**

| Milk processing | pH | COD(g/l) | $BOD_5$(g/l) | Total nitrogen (g/l) | Total phosphorous (g/l) | References |
|---|---|---|---|---|---|---|
| Milk reception | 7.18 | 2.54 | 0.8 | - | - | Janczukowicz *et al.*, 2008 |
| Mixed dairy | 4-11 | 0.5-10.4 | 0.24-5.9 | 0.01-0.66 | 0-0.6 | Kolev Slavov, 2017 |
| Dairy sewage | 9.1±6.7 | 2.04 | 1.08-2.81 | 0.02 | 0.03 | Tawfik *et al.*, 2008 |
| Fluid milk | 5-9.5 | 0.95-2.4 | 0.5-1.3 | - | - | Demirel *et al.*, 2005 |
| Yoghurt | 4.53 | - | 6.5 | - | - | Un *et al.*, 2013 |
| Butter | 12.08 | 8.93 | 0.22-2.65 | - | - | Janczukowicz *et al.*, 2008 |
| Ice-cream | 5.1-6.96 | 5.2 | 2.45 | - | 0.014 | Karadag *et al.*, 2015 |
| Cheese | 3.38-9.5 | 1-63.3 | 0.59-5 | 0.018-0.83 | 0.005-0.28 | Vialkova *et al.*, 2019 |
| Cottage cheese | 7.83 | 17.65 | 2.6 | - | - | Janczukowicz *et al.*, 2008 |
| Cheese whey | 3.92-6.5 | 50-102.1 | 27-60 | 0.2-1.76 | 0.12-0.53 | Carvalho *et al.*, 2013 |
| Hard cheese whey | 5.8 | 73.45 | 9.48 | - | - | Janczukowicz *et al.*, 2008 |
| Soft cheese whey | 5.35 | 58.55 | 26.77 | - | - | Raghunath *et al.*, 2016 |
| Soft cheese whey | 4.5 | 79 | - | 2 | - | Janczukowicz *et al.*, 2008 |
| Effluent from | 5-9 | 1.07-2.18 | 0.59-1.21 | - | - | Geilman*et al.*, 1992 |
| Washing wastewater | 10.37 | 14.64 | 3.47 | - | - | Janczukowicz *et al.*, 2008 |

**Table 1.6. Different treatment processes and their removal efficiencies**

| **Treatment process** | **Characterization** | **Removal efficiency (%)** | **References** |
|---|---|---|---|
| Coagulation | Ferrous chloride as a coagulant | COD: 88%, 76%, 45%, 28% and 82% | Shivsharan *et al.*, 2013 |
| Adsorption | Low-cost adsorbents: activated carbon, dust, fly ash | TSS: activated carbon had a good removal efficiency | Budhiary and Sumantri, 2021 |
| Membrane process | Reverse osmosis process | TOC: 99.8%<br>Lactose: 99.5% | Andrade *et al.*, 2014 |
| Membrane process | Ultrafiltration + reverse osmosis | Dairy industry wastewater can be reused and recycled | Melchiors *et al.*, 2016 |
| Electro-coagulation | The soluble aluminum anode used | Nitrogen: 81%<br>Phosphate 89% | Markou *et al.*, 2017 |
| Electro- flocculation | Iron electrodes | Organic matter: 97.4% | Yonar and Sivrioglu, 2017 |
| Combined electrodes system | Iron and aluminium electrodes | Only 20-minute electrolysis was sufficient for the treatment of COD | Bazrafshan *et al.*, 2013 |
| Electro- coagulation | Direct-current-aluminium plates were utilized as sacrificial electrodes | COD: 87% | Beneois *et al.*, 2016 |

Therefore, due to the high pollution load, the aquaculture requires proper treatment before discharging it into the environment, because it gives a negative impact on aquaculture industries. The wastewater contains various types of pollutants, and high organic contents. Wang and his co-workers observed that the cultivation of algae with a variety of wastewaters offers an instantaneous reduction in the biomass production cost and helps equally in the bioremediation of water. Additionally, algae are known as a good source of value-added products due to their valuable bio-chemical profile present in (Gonzalez-Delgado *et al.,* 2017). They build up various pharmaceutical and neutraceuticals materials present in their cell bodies which may be used as a cosmetic, food, and pharmaceutical industry. One of the main advantages of using these microorganisms are their ability to grow in unsuitable conditions for other crops, needing merely some nutrients and sunlight. Algal biomass is rich in proteins that compete favorably, in terms of quantity and quality, with conventional food proteins such as soybeans, eggs, and fish (Ejike *et al*., 2017). The discharge limits of different wastewaters are described in Table 1.7 as per national and international standards.

### 1.7.5. Municipal wastewater

Municipal wastewater usually comes from a mixture of wastes originating from kitchens, toilets, and bathrooms at a central wastewater treatment plant (Tang *et al*., 2020). The nutrients present in municipal water that is discharged into the freshwater bodies lead to eutrophication which affects the aquatic environments (Huang *et al*., 2020). The two main nutrients present in municipal wastewater are phosphorous and nitrogen, their reuse may reduce not only eutrophication but may also act as sustainable fertilizer sources too if recovered (Kehrein *et al*., 2020; Collins *et al*., 2018). The water quality degrades when the untreated wastewater is discharged into the receiving water body (lakes, streams, and rivers) which are creating problems for the consumption of clean water by human beings. Presently, there is an exponential growth rate in the urban population leading to the high amount of wastewater, so the reuse of water is a matter of concern for sustainable development. Due to the severe freshwater scarcity, it would be a revolution if the process of reuse and recycling of the water will be in progress. In some cases, it has been noticed that the untreated wastewater is released directly into the ground and the natural drainage system that causes eutrophication and pollution in the downstream areas.

The wastewater treatment has lately shifted from pollution control to resource exploitation in terms of technological viability, economically, sustainable development, and societal needs. So, there is a burning requirement of phycoremediation practice to treat wastewater sustainably, which can be further exploited for fuel and related value-added products. However, the requirement of hard work is needed to decrease the elevated rate of biofuels. Many researchers have been taken a suitable algal species for the research purpose is an integrated approach (Aziz *et al*., 2020; Shokravi *et al*., 2020).

In conventional wastewater treatment system, a huge volume of freshwater is an essential requirement to carry out a little amount of human excreta from the laboratory to the wastewater treatment plant. Moreover, nutrients present in the toilet are diluted by groundwater intrusion, industrial wastewater, and rainwater (Jasim, 2020). Thus the present challenge is to sort out the way to utilize various new cutting-edge technologies to get rid of the wastes from the municipal wastewater and recover the organic/inorganic compounds sustainably. For that reason, the utilization of alternate resources is necessary to produce new fertilizers (Jastrzębska *et al*., 2019). Application of source separation sanitation systems in black water treatment plans show a promising alternative for proficient nutrient revival from human urine and faeces.

**Table 1.7. Discharge limits of different wastewater based on Indian/World standards**

| **Parameters** | **Slaughterhouse wastewater (world bank)** | **Dairy wastewater (CPCB)** | **Textile and Dye wastewater (CPCB)** | **Municipal wastewater (CPHEEO)** |
|---|---|---|---|---|
| pH | 6-9 | 6.5-8.5 | 6-8.5 | - |
| TDS | - | | - | - |
| TSS | 50 | 150 | 100 | - |
| COD | 125 | - | - | - |
| BOD | 30 | - | 100 | 10 |
| Nitrate | 10 | 10 | - | 10 |
| Phosphate | 6-9 | 5.0 | - | - |
| Sulphide | - | 2.0 | 1000 | - |
| Fluoride | - | 2.0 | - | - |
| Oil and Grease | - | 10 | - | - |

**All the parameters were in mg/l except pH**

On the other hand, the technical solutions for the removal of nutrients from black water is still a matter of concern that include evaporation tanks, anaerobic digestion, compost filters, and membrane reactors, with restrictions like insufficient elimination of fecal indicators, little to no nutrient elimination and filter clogging (Moges *et al*., 2020). Algae characterizes a sustainable and chemical-free alternative for wastewater treatment due to their ability to uptake heavy metals, nutrients, reduce BOD, pathogens, and its ability to put up with high load wastewater. Algae also signify an environmental friendly substitute to the energy concentrated physical and chemical removal method for phosphate. In addition, wastewater remediation using algae is a low-cost nutrient source for algae cultivation, harvesting the algae can prove to be a resource for the production of various valuable by-products *i.e.,* fertilizers, biofuels, and lipids.

### 1.8. Biological treatment options

Wastewater treatment is a global issue that can't be managed by a single technology, due to the high level of variables, contaminations, and regional level of the conventional treatment process, which is focused on the removal of suspended solids, and biological oxygen demand was reduced through activated sludge process. This biodegradation process engages the breakdown of organic and inorganic compounds from wastewater which is very important to prevent eutrophication in rivers and lakes. The capacity of degradation by these conventional technologies is very limited, due to heavy metals, the high load of nutrients, and xenobiotic conditions. Algae have the metabolic capability for the performance of phototrophic, mixotrophic metabolism. They signify the different wastewater sources for biological treatment. The algal-based wastewater treatment has focused mostly on conventional algae and cyanobacteria that include *Chlorella sp., Scenedesmus sp., Arthrospira, and*

*Nannochloropsis* due to their high efficiency for the extraction of high levels of lipid and starch (Ansari *et al.,* 2019). So, conventional and advanced treatment methods are used to treat different industrial wastewater for the removal of pollutants.

### 1.8.1. Conventional treatment options

The conventional method is defined as a combination of physical, chemical, and biological methods, which are used for the removal of contamination from wastewater. The conventional method is the best option for the elimination of heavy metals, chemical precipitation, carbon adsorption, ion exchange, and membrane evaporation process. The conventional wastewater techniques are trickling filter, activated sludge, rotating biological contractor techniques. In addition, rotating biological contractors and trickling filters are temperature sensitive. Trickling filters need more cost for construction as compared to activated sludge. Activated sludge contains a high amount of microorganisms like fungi, bacteria, and protozoa and it exists as unfastened. The rotating biological contractor is known as the biological treatment method for organic wastewater. Hence, the aerobic biological contractor is mostly used in industrial and domestic wastewater treatment whereas, the membrane bioreactor techniques are used for the treatment of suspended growth and biological wastewater-treatment system (Rajasulochana and Preethy, 2016; Aslam *et al.,* 2017).

### 1.8.2. Advanced treatment options

The complete removal of pollutants and other toxic substances after the secondary treatment is called as advanced treatment method. In addition, several processes are used in the advanced treatment process to satisfy the target which includes suspended solids, biological oxygen demand, dissolved solids, plant nutrients and toxic substances. The application of advanced and innovative techniques requires improvement in the water quality for further use through the recycling and reusing process. Therefore, due to all these problems related to contamination in water bodies, an advanced treatment process is the best alternative for the complete removal of contamination from wastewater. Presently, conventional-based treatment technologies face several problems in the context of chemical consumptions, requirement of high energy, and space requirements. Moreover, proficient removal of limits for handling more water instead of fixed design capacity as well as lacking skill power is the main issues in this technology and causes further problems in conventional treatment either technologically or operational. So, researchers are trying to develop new techniques for wastewater treatment to solve the overall problems. Advanced treatment techniques require an advanced oxidation process, low-cost adsorption materials, bioflocculant, nanomaterial, membrane technology *etc.*, (Gedda *et al.*, 2021; Sadegh *et al.*, 2021). The utilization of advanced treatment technology with a combination of conventional techniques may result in effective treatment that helps in the reuse and recycling of treated wastewater. All the conventional and advanced treatment processes are described with detailed pros and cons in Chapter-2.

### 1.9. Wastewater and Biofuels: Integrated approach

It is indicated that biofuels is a renewable source of energy as compared to the geopolitical instability and poisonous effect of fossil fuel energy. Biofuels can be classified into two categories of primary and secondary biofuels. The primary biofuels are obtained from the plant material and animal dry waste, and the secondary biofuels are classified as those generation biofuels that are released from animal and plant material directly or indirectly (Rodionova *et al.*, 2017). Ethanol is the first generation biofuels that is derived from food rich in starch and the $2^{nd}$ generation biofuels is bioethanol which is obtained from oil-rich plant seeds such as jatropha and $3^{rd}$ generation biofuels is obtained from algae, cyanobacteria, and other microbes. Based on current knowledge algae is considered the best feedstock for the production of biofuels (Kothari *et al.,* 2019; Ahmad *et al.,* 2018; Singh *et al.,* 2019). There are several species of algae that have the potential to produce biofuels from algae as an optional source of energy. At present time ethanol, biodiesel, fatty acids, triglycerides, and carbohydrates are the main sources of biofuels. Algal biomass has the ability for the production of highest amount of biomass in terms of protein, lipid and carbohydrates can be utilized as an alternative source of bioenergy (Ahmad *et al.,* 2018; Pathak *et al.,* 2019). The harvested algal biomass can be utilized for the synthesis of various biofuels products like biodiesel, bioethanol, biohydrogen, and biogas (Poudyal *et al*., 2016). Figure 1.4 indicating the algal biomass based treatment of wastewater for the cultivation and production of nutrients and its biomass utilized for biofuels production.

According to the discussion in the above sections of this Chapter, we can conclude that wastewater is the drawback of the “Industrial Revolution” but now is the time for sustainable technologies to maximize the water resources and recovery of resource materials from the waste/wastewater. Among the various techniques cited by researchers, utilization of wastewater for energy recovery is gaining attention nowadays. There are various experimental technologies discussed and investigated by the researchers (Kang *et al.,* 2019). The use of algal biomass for the treatment of wastewater offers an enormous potential for bioenergy options (Kothari *et al.,* 2019; Al-Jabri *et al.,* 2021; Bhatia *et al.,* 2021). Henceforth, this integrated approach is taken as a major objective for this research study, and relevant researches are cited with detailed mechanism, influencing factors, and applications in Chapter- 2.

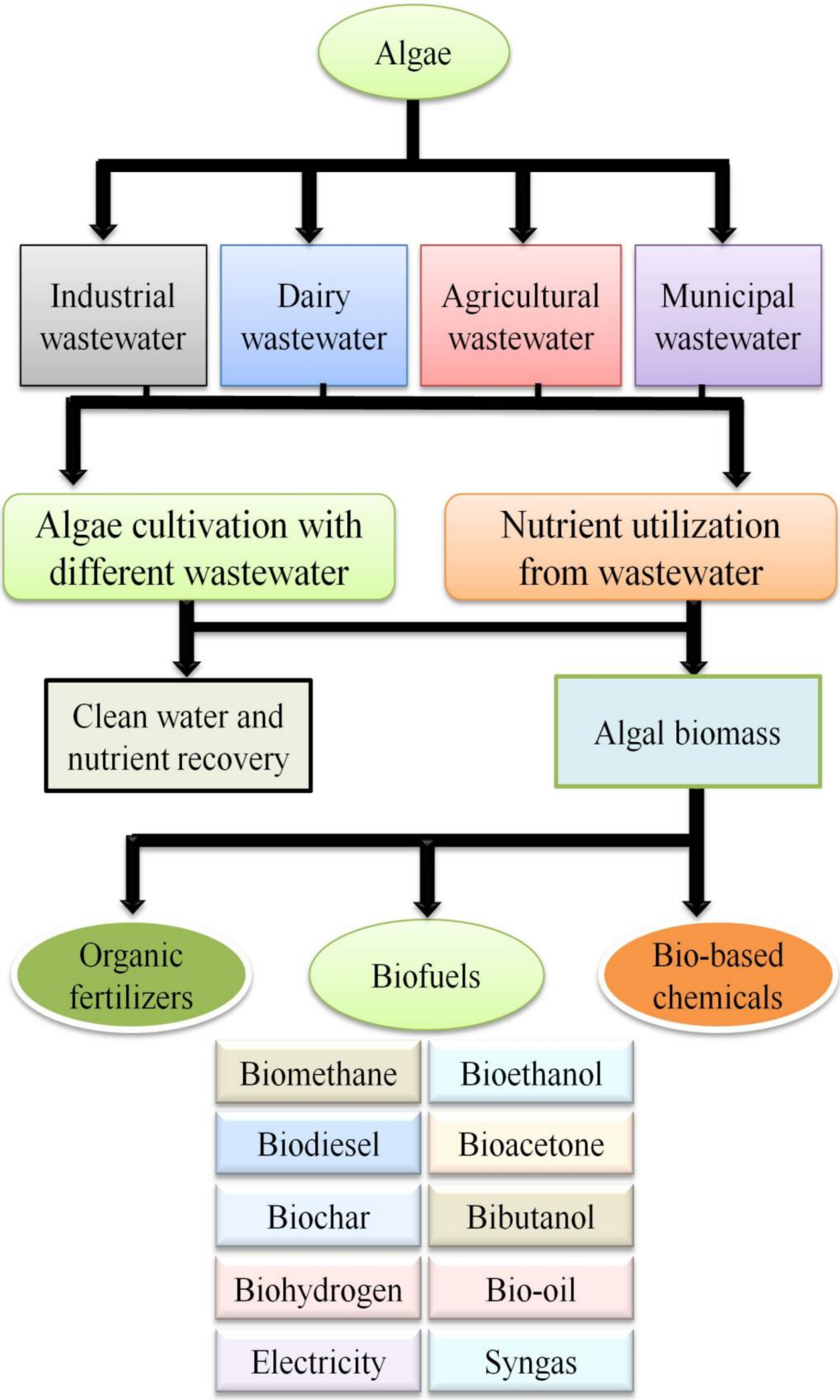



**Figure 1. 4. Wastewater: Integrated approach for biorefinery**

### 1.10. Applications of algal biomass

Due to their chemical composition, algae can be used as nutritional supplements, protein, dyes polyunsaturated fatty acids, and antioxidants (Matos *et al.*, 2017; El-Shenody *et al.*, 2019). There are several algal applications and health-related benefits given in the Figure 1.5. It helps for the production of energy and nutrient recycling, and biomass extensively used as feed ingredients. There are several strains *(Dunaliella salina.*, *Nanochloropsis sp.*, *Haematococcus pluvialis*, *Dunaliella sp.*, *Spirulina sp.*, and *Spirulina platensis)* of algal extract, which are used as a bio-based alternative feedstock for the production of various cosmetics products like anti-aging cream, UV-protectants and antioxidants. It helps to prevent hair follicles as well as loss from hair fall (Johnson *et al.*, 2018).

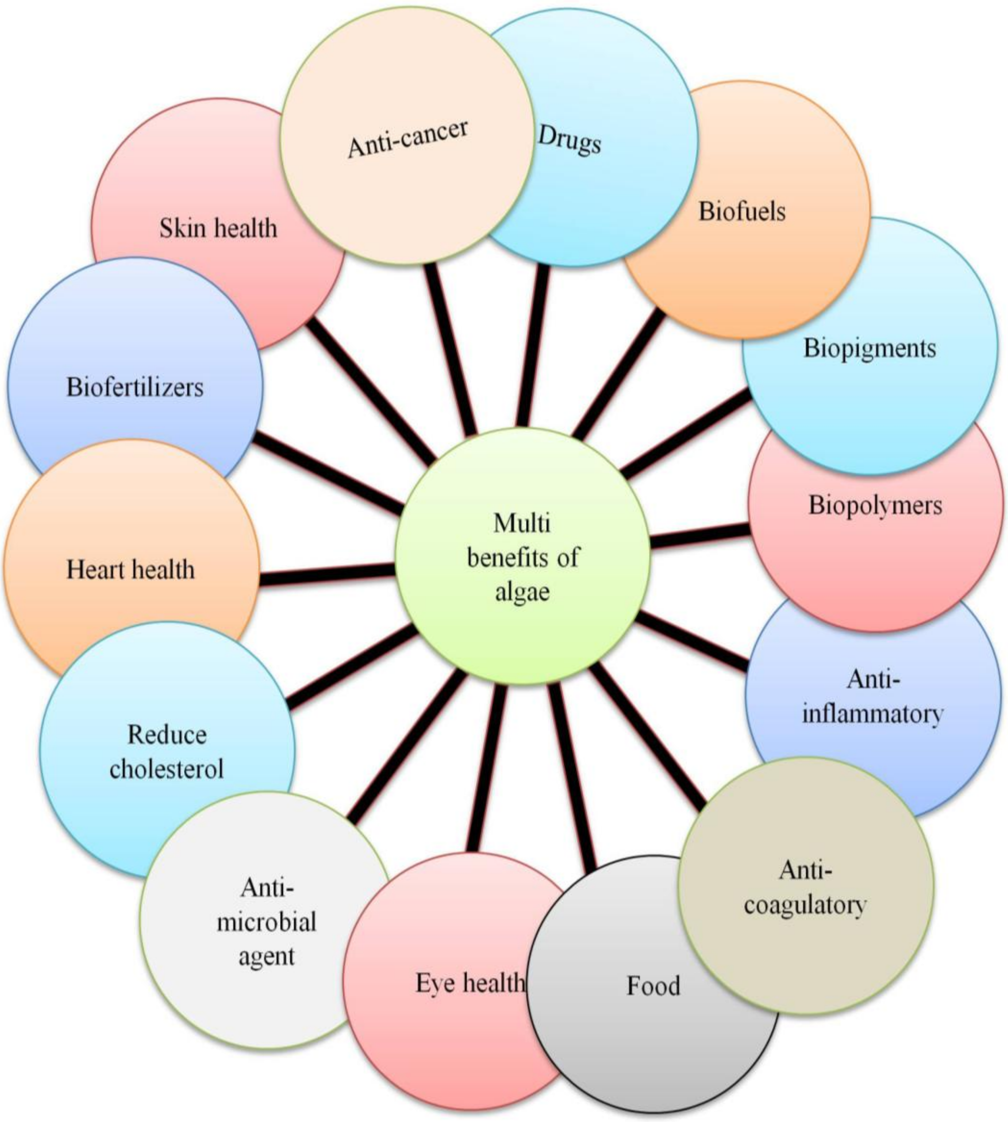



**Figure 1.5. Benefits of algal biomass for various purposes**

Algae are considered for the production of more oil for the development of biodiesel as compared to other terrestrial plants for *e.g.,* jatropha, rapeseed, and soya. In spite of all this, they are used for commercial applications. They can convert any type of wastewater into a low environmental impact on wastewater, and their effluent is used as a biofertilizer for the improvement of the fertility of the soil. As per the literature survey, a lot of exposure is emphasized based on biodiesel production from algae, but there is limited utilization of algae for the exploration of neutraceuticals and cosmetics industries (Bhalamurugan *et al.,* 2018; Piwowar and Harasym, 2020). For better results based on valuable products, biomass is required in high amounts, and future research should take into consideration this aspect. So, the selection of algal strains and bioprocessing are the best technologies for the successful utilization of algal biomass for various applications of further development of these valuable products. Therefore, this Chapter is designed with the objective to provide basics on types of water resources, sources of water contamination and pollution, types of pollutants, and their impacts. Further, some major wastewater contributors are also discussed in this Chapter. Different treatment options (conventional and advanced) are also discussed in this Chapter. Alternative strategies with an integrated approach for wastewater treatment are also part of this Chapter, very particularly regarding the use of algal biomass for wastewater treatment and their wide range of applications. Although, detailed discussion with the available literature on the integrated way of wastewater treatment and resource recovery with value-added end-products is delineated in Chapter- 2 of this research study with objectives designed to complete this research work.

Therefore, keeping in mind all the challenges, the following objectives have been designed to prove the concepts by experimental validations.

## 1.11 Objectives of the Study:

1. Selection, collection and Physico-chemical analysis of different wastewater samples.
2. Collection of algal biomass (wild/known strain) and its bio-chemical analysis and growth with selected water samples.
3. To assess the quantitative analysis of chlorophyll and protein content in the selected algal strains with the prescribed selections as given in objectives 1 and 2.
4. To study the co-relational analysis between bio-compounds with selected water samples using selected algal strains (as per objective 2).
5. To investigate the applications of harvested biomass (wild/known strain) for various neutraceuticals values at the commercial scale.
6. To assess the harvesting of known algal biomass with a designed and fabricated bioreactor to scale-up the biomass at a large scale with special reference to temperature and light variation only.
7. To study the techno-economic assessment of selected approach for the integration of low- cost wastewater treatment with commercial applications.

# Chapter 2
# Review of Literature

## 2.1. Wastewater: recent trends of treatment

Chemical and biological contamination of water has become a major problem in the last several years affecting the society significantly. The industries discharging wastewater contains undesirable contaminants which are very toxic. Based on this, society and researchers need more efforts for the protection of water resources. The poor condition of water quality is due to the increase in population and anthropogenic activities at a global scale. Anthropogenic activities include agricultural processes and commercial activities. It comprises of high level of contaminants available such as nitrogen which is in the form of nitrite, nitrite, ammonia, protein, drugs, and pathogens. Furthermore, other contaminants available are toxic metals (cadmium, chromium, arsenic, copper, zinc, mercury, nickel, and lead) from different sources in wastewater. The physical, chemical and biological treatment process is best for the minimization of nutrient and pollutant load from wastewater (Jasim, 2020). Conventional treatment plants consist of primary and secondary processes to remove contamination from wastewater. Primary treatment processes include neutralization, equalization, separation of oil and grease. It involves physical separation, screening, grit removal, and then sedimentation instead of secondary treatment (Wang *et al.*, 2021). Secondary treatment is the main step for the removal of pollutants which is remained after primary treatment. Mostly, the biological process is taken for a treatment process called as activated sludge process. The activated sludge process is a highly concentrated process for the utilization of micro-organisms like protozoa, fungi and bacteria for the removal of contaminants from wastewater (Rajasulochana and Pretty, 2016). Therefore, primary and secondary treatment processes in wastewater decrease the organic load from wastewater like biochemical oxygen demand. Despite of all these techniques, the contamination is still present in wastewater so, requires some advanced treatment options. The replacement of conventional technology by algal-based techniques is a part of research nowadays as a result the process needs less energy consumption as compared to the conventional treatment system. The nutrient recovery after secondary treatment from wastewaters has been planned as an alternative for tertiary treatment, thus permitting to decrease the nitrogen and phosphorus concentration in the form of the conventional treatment process with wastewater (Kehrein *et al.,* 2020). Therefore, the next section has specified the wastewater treated with algal biomass. Thus algal-based wastewater treatment process is a sustainable, low cost and eco-friendly treatment technology as per recent available literature conventional and advanced treatment options are discussed in detail with pros and cons in upcoming sections of this Chapter. Table 2.1 delineating the different processes of wastewater treatment and their advantages and disadvantages.

### 2.1.1. Conventional treatment options

The primary reason for the treatment of wastewater is to protect human health and drinking water crisis management, and also to restrict the spreading of dangerous diseases (Drahansky *et al.,* 2016). Some general terms are used to increase the treatment level are preliminary, primary, secondary and tertiary/advanced wastewater treatment. Conventional methods are used for the removal of dissolved heavy metals are coagulation, ion exchange, evaporations and membrane processes (Wang *et al.,* 2021). The selection of any particular treatment technique depends upon factors like types of waste, concentration, heterogeneity of effluent, cleanup based on requirement and their economic factor. So, the cost-effective options technology is high in demand in the current scenario. Therefore, the biological materials either living or non-living microorganisms to remove the toxic metals from industrial wastewater has gained benefits, due to their good performance and low-cost involvement. Various biochemical-based flocculent are being utilized for the harvesting of algal biomass efficiently, but for a while these chemicals deform and denature the algal biomass structurally and morphologically. In addition, there are a variety of treatments either onsite or offsite treatment process. It consists of trickling filters, activated sludge, constructed wetlands, and membrane bioreactor. Whereas, the biological treatment process is best because it takes diverse wastewater constituents to give energy for the metabolism of microbes and also the building block for the synthesis of cells (Samer, 2015).

The primary and secondary processes are involved in the treatment of large particles and organic matters respectively. After this treatment, many unwanted matters will remain in the treated water, and the tertiary treatment is applied for the removal of pollutants from the wastewater. These treatment processes are generally considered as physical, chemical and biological processes. The primary treatment constitutes two steps viz. preliminary as well as sedimentation process. The preliminary process mainly contains screening, skimming tank and grit chamber, which are used for the removal of large particles, fats and oil from wastewater. Its main function is to protect the clogging valves of the wastewater treatment plant (Guyer, 2018). In the case of grit chamber, it is used for the removal of sand, broken glasses and metal fragments. It is necessary to remove these kinds of materials from polluted water bodies for the protection of equipments.

In addition, several types of grit chambers are applied for removal systems, which are as vortex, horizontal, and aerated grit chambers are used in wastewater treatment plant. Whereas, in skimming tanks, the removal of floating matter, wax, oil, soap and grease. The floating matter is badly affecting the performance of the activated sludge process. Therefore, the primary settling tanks are used for the removal of suspended particles and organic matter (Patziger *et al.,* 2016). In secondary treatment, the biodegradable materials as well as organic compounds are despoiled through the process of microorganisms. The activated sludge which has a high amount of microorganisms like bacteria, protozoa and fungi which are available as a free clumped mass of very small particles that are reserved as suspension are used for the removal of organic matter from wastewater. It is nominated as a biological treatment method that utilizes an adjourned growth of organisms that remove suspended solids and biological oxygen demand. In recent times, biosorption has been considered as a cost-effective and capable alternative for the removal of heavy metals from wastewater. Many studies have been reported for the utilization of algal biomass and the removal of metal from wastewater. Numerous microorganisms were engaged to remove nutrients and toxic chemicals (He and Chen, 2014). In trickling filter technology, wastewater treatment is associated with an unnumbered filter, where the influent is directly applied at the top of filter media. The trickling filter is an attached growth process that involves a percolating filter with a rotational distribution arm that distributes the wastewater above the packing bed of plastic or other coarse materials (Samdani and Choudhary, 2017). The rotating biological contractor (RBC) consists of a chain of narrowly circular spaced plastic disks that are associated with a rotational hydraulic shaft (Habibi and Vahabzadeh, 2013). About 40% of the bottoms of all plates are steeped in discharged wastewater and the film which cultivates the moved disk in and out of the wastewater. Rotating biological contractors consist of a unique and superior alternative to degrade organic and nitrogenous compounds. Whereas Sharma and Philip, (2016) reported 70% of nitrogen removal from coke wastewater by the application of rotating biological contractor. Figure 2. 1 illustrates the mechanism of activated sludge process, membrane bioreactors, trickling filters and rotating biological contractors.

### 2.1.2. Advanced treatment options

The tertiary treatments are called advanced treatment methods. This type of treatment removes a significant amount of nitrogen, phosphorous, heavy metals, biodegradable organic matter, pathogenic bacteria, and virus. Whereas, there are several advanced treatment technologies, which are utilized for the removal of contamination, including membrane separation, electrodialysis and disinfection (Serra *et al.*, 2014). The advantages and disadvantages of conventional wastewater and advanced treatment options are given in Table 2.2.

#### 2.1.2.1. Disinfection method

The disinfection process is a tertiary treatment method that is mostly used for the treatment of municipal water. The process is used for destroying the pathogenic microorganisms which include viruses and bacteria, and some chemicals used like chlorine is called disinfection. The chlorination, bleaching powder, chlorine gas, and chorus acid are used for the disinfection of municipal wastewater (Roy *et al.*, 2016).

#### 2.1.2.2. Reverse osmosis

Reverse osmosis is a method that is used for the removal of inorganic minerals (magnesium, calcium, potassium and fluoride) and organic compounds like pesticides. In the reverse osmosis process, the wastewater is passed via a semi-permeable membrane through osmotic pressure. It consists of small pores where water can simply flow. So, the reverse osmosis process takes part in the purification of wastewater. Cellulose, acetate and polyamide are the important membranes that are mostly used (Pervov *et al.*, 2018).

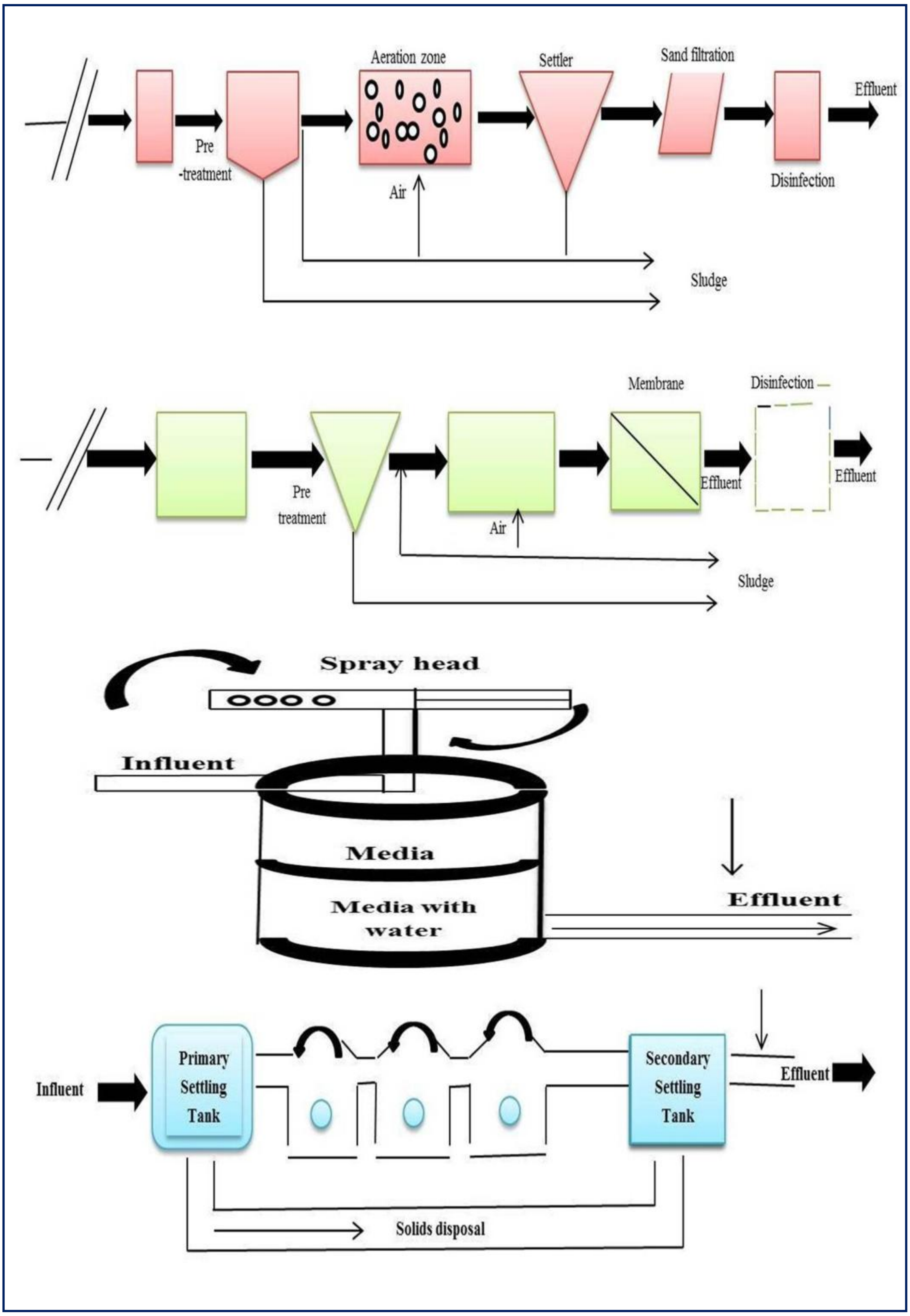



**Figure 2.1. Different aerobic processes of wastewater treatment:**
**(a) Activated sludge process, (b) Membrane bioreactors,**
**(c) Trickling filters, (d) Rotating biological contractor**

**Table 2.1. Various wastewater treatment process and their advantages and disadvantages**

| Processes | Advantages | Disadvantages | References |
|---|---|---|---|
| Coagulation/ Flocculation | Low cost, Characteristics of good sludge settling and dewatering, Reduce the COD and bacterial inactivation capacity | Requirement of non-reusable chemicals, Physicochemical analysis of the wastewater, Sludge generation volume increased, Low arsenic removal | Crini and Lichtfouse, 2019 |
| Membrane filtration | Requirement of small space, Very simple, Efficient and rapid, Don't require any chemical,It contains all types of dyes salts and minerals | For small as well as medium industries, Money utilization is too high, Requirement of high energy | Giwa and Ogunribido, 2012 |
| Biological methods | High removal of BOD, Biodegradation of organic contaminants is simple, Economically attractive and well accepted by the public, White-rot fungi produce a huge diversity of extracellular enzymes that have high biodegradability capability | It requires maintenance and management of microorganisms, Slow process, Dull decolorization, Low biodegradability of certain molecules (dyes) | Santal and Singh, 2013 |
| Ion exchange | A broad range of commercial products which is available from numerous manufacturers, Simple equipment, establishment and testing procedure is good, Easy control and maintained | It needs a large volume and column, Fast saturation and clogging of reactors, Precipitation of metals and blocking of the reactor | Miller *et al.*, 2009 |
| Photochemical | No sludge generation | Byproducts Formation | Bartolomeu *et al.*, 2018 |

| Processes | Advantages | Disadvantages | References |
|---|---|---|---|
| Chemical precipitation | Technologically very simple and integrated physicochemical process, Economically beneficial and efficient, Efficient for metals and fluoride removal, Reduce COD | Chemical consumption, Unproductive for the removal of heavy metal ions at low concentration, Sludge production high treatment, Management, Cost problem | Crini and Lichtfouse, 2019 |
| Adsorption | This equipment is technologically simple, and adaptable for various treatment options, Huge amount of contaminants can be targeted, Target a wide variety of contaminants extremely efficient process | Its performance based on material types, Method is nonselective and destructive, Comparatively high investment | Zdarta *et al.*, 2019 |

#### 2.1.2.3. Electrodialysis

Electrodialysis is the best process for the removal of cations and anions which are present in water. Due to the applied potential, the ions present in water migrate through ion exchange. These membranes are found between the cathode and anode electrodes. During the process, cations will migrate towards cathode whereas anions will migrate towards anode upon applying an external potential. In this process, cations allow the cation-selective membranes and anion allow the anion-selective membranes and as a result the water obtained gets purified. It is cost-effective and favorable process for product recovery, due to the limited requirement of chemicals. In the industrial sector electrodialysis is mostly used for the desalination of brackish water for the production of drinking water. Therefore, many applications of electrodialysis are focused on biochemistry, pharmaceuticals and food processing (Sajjad *et al.*, 2020; Zhao *et al.*, 2018).

## 2.2. Algal biomass and wastewater: Integrated approach

Various researchers cited in the previous section provide a basic view on conventional and advanced wastewater treatment options. Keeping in view, all the above-discussed advantages and disadvantages of advanced options, phycoremediation *i.e.*, use of algal strains are best for wastewater treatment/remediation. Hence, as per our objective designed for this particular research, phycoremediation is taken as a major treatment option for wastewater treatment and harvested biomass is utilized for various value added-products So, to study the different dimensions involved in their integrated approach for algae and wastewater, upcoming section is framed with practical challenges, their application for future as per the literature available by researchers of this specialized area. Algae have the potential to grow in different wastewaters and convert sunlight and atmospheric carbon dioxide into biomass.

**Table 2.2. Advantages and disadvantages of conventional and advanced wastewater treatment options**

(Gedda *et al.,* 2021; Rathour Dhatwalia, 2016; Morin-Crini and Crini, 2017; Crini and Lichtfouse, 2019)

| Technology | Advantages | Disadvantages |
|---|---|---|
| Coagulation | Low principal cost, Less-effort method, Good sludge settling and dewatering character | Generates a large amount of sludge, Physico-chemical monitoring of the effluent (pH) |
| Trickling filters | Potential for removing high quantity of BOD | Strong odor generation, High cost |
| Rotating biological contractors | Generate low amount of sludge, Operational cost affordable, Airing through environment | Requirement of huge area to run this system, System protection in cold climatic conditions is difficult |
| Activated sludge process | Airing through environment, Available in domestic to industrial scale, Viable for the removal of high quantity of nitrogen, phosphorous, COD and BOD, Operating system is very simple, Outstanding effluent quality | Sludge concentration can be monitored |
| Disinfection | Low cost, Toxic toward pathogens, The ability for the removal of ammonia, Iron and manganese | Difficult for handling due to their dangerous nature |
| Reverse osmosis | Present in domestic toward industrial purposes, Low amount of energy is enough, Easily maintained, Easily replaced membrane | Before purifying the water pretreatment is very important |
| Electrodialysis | Membrane regenerate easily, Water pretreatment is not necessary | Only ions can be removed, The membrane required for selection based on the quality of water |
| Up-flow anaerobic sludge blanket reactor | Energy demand, Requirement of land, Economical, Efficiency for the removal of organic and BOD is excellent, Wastewater can be utilized for farming | Need a large amount of granular seed sludge |
| Phytoremediation, Biological | Low cost, Less energy-intensive, High efficiency with | Climatic conditions act as a controlling |

| **Technology** | **Advantages** | **Disadvantages** |
|---|---|---|
| activated sludge, Microbiological treatment, Enzymatic decomposition | pollutant load removal, Zero-waste concept, The microorganism process for biodegradation of organic contamination is simple, Attractive economically, Publically acceptable | factor,<br>Required for creating a favorable environment, Slow process, Poor decolorization, Mixed culture composition changes during the decomposition process |
| Chemical precipitation | Technologically simple equipment, Efficient for fluoride and metal removal, Not metal selective, COD extensively reduced | Physicochemical monitoring of the effluent, Sludge produced in high amount, Ineffective removal of metal in low concentration |
| Flotation | Efficient for the removal of small particles, Metal selective, Retention time low, Utilized as an efficient tertiary treatment in pulp and paper industry | Lost energy, Operational cost and maintenance no negligible, Requirement of chemical |
| Chemical oxidation | Simple process, Generation of ozone onsite, Good removal of color and odor | Requirement of chemical, Production, Transport and management of oxidant, No effect on salinity, Sludge generation |
| Ion exchange, Chelating resins, Selective resins | Large amount of commercial products available from several manufacturers, Simple equipment | Initial cost of selective resins, Beads easily fouled by particulate and organic matter, Strong oxidants |
| Incineration, Catalytic oxidation, Thermal oxidation | Simple process, Highly efficient, Sludges | Initial investment cost, High running costs Storage and transport of the effluents |
| Advanced oxidation process | Production of insitu reactive radicals | Laboratory scale, Economically non-viable for small and medium industries |

Their cells also have the capability to convert and energy storage instead of using it for their growth and development. So, therefore, algal biomass can be explored as new system for biofuels and other end products that are considered as potential candidates for fossil fuels due to sustainability, renewability, and short life cycle for algal growth (Arun *et al.*, 2021). Algal biorefinery development could be efficient for decreasing the demand for fossil fuel and greenhouse gas emissions, which mitigate the problems associated with global warming and climatic changes. It is considered as an important feedstock for the production of biofuels because algae can be cultivated throughout the year with high biomass productivity (Bhatia *et al.,* 2021; Goswami *et al.*, 2021). In addition, they are considered as potential candidates for the recovery of resources from different types of nutrient-rich wastewater. These are produced from different industrial sectors that include dairy, pharmaceutical, swine, aquaculture, food, textile industries as well as from municipalities. Whereas, wastewater constitutes numerous toxic chemicals and pathogens, which affect the ecosystem.

Furthermore, in irrigation untreated wastewater causes several issues including unwanted vegetative growth, causing several plant-related diseases, which reduce the quantity and quality of the crops. Unprocessed wastewaters also cause chemical and biological contamination in ground water, which contains another negative penalty. Conventionally, wastewater generated from various sectors is treated by using chemical (flocculation, neutralization, oxidation and disinfection) processes as well as physical (grit chamber, floatation, and screening) processes (Libuti *et al.,* 2018). But all these treatments are very costly, and require a secondary process for the treatment of unwanted pollutants from polluted water. According to Ren *et al.,* (2019) these types of technologies represent a green clean and sustainable treatment, which recovered about 95% of nutrients from wastewater. When algae is cultivated with wastewater, it produces biomass, containing biochemical profiles, which are carbohydrates, lipid, protein and some valuable compounds that are beneficial for the production of biofuels and other value-added products. After that, the treated water can be applied for agricultural purposes as well as the irrigational sector. So, this type of treatment technology is considered as an integrated approach for the utilization of wastewater with algae for the production of biofuels, biodiesel, biogas, recycling of agricultural processes (Garcia *et al.,* 2019; Li *et al.,* 2021).

Presently, many researchers used algae for processing dairy wastewater to produce biomass and it's used as value-added products. It can treat wastewater by the reduction of nutrient and pollutant load from wastewater (Braz *et al.,* 2020; Adesra *et al.,* 2021). Algal cultures may thus help in contributing significantly to the management of the wastewater ecosystem by providing an economical and environmental friendly wastewater treatment system. The use of dairy effluent in the culture of algae is very beneficial to minimize the utilization of freshwater, it also reduces the price of nutrients adding up, removing the left over nitrogen, phosphorus and help to produce algal biomass as bioresources with high value biofuels. However, many existing studies proved the potential use of algae for dairy wastewater treatment (Kothari *et al*., 2013; Nagarajan *et al*., 2020) for the removal of nutrient and pollutant load and its application is used for various purposes. Therefore, the products obtained from dairy wastewater with algal biomass after-treatment of the harvested algal biomass can be utilized as supplements for various animal products like fish farming and animal feeding. In swine slaughterhouse wastewater, contain a high amount of ammonia that creates pollution and causes toxic impact on the aquatic environment. So, it is very essential to treat the swine wastewater before its release into the surrounding area as well as into the water body. It needs treatment in reducing the nutrient and pollutant load from swine wastewater. According to Cheng *et al.,* (2019) as certained that *Scenedesmus sp.* is best for the remediation of swine slaughterhouse wastewater. Slaughterhouse wastewater is also a carrier of zoonotic diseases which are further transferable to humans (Prabhakar *et al*., 2017). Furthermore, the macronutrients like phosphorus and nitrogen may cause eutrophication (Baker *et al*., 2021). So, the release of these nutrients also triggers unnecessary algal growth. Finally, slaughterhouse wastewater contains compounds, like unionized ammonia and chromium that are directly toxic to marine life (Al-Gheethi *et al.,* 2017). Wastewater is a good source for the cultivation of algae which have different advantages including low-cost organic and inorganic medium for growth, maintaining large-scale cultivation, potential to provide trace elements. Currently, various algal strains (*Chlorella vulgaris., Chlorella pyrenoidosa., Chlamydomonas reinhardii., Spirulina sp., Tetraselmis sp., Picochlorumsp., Chlorellasorokiniana., Scenedesmus obliquus., Nannochloropsisoculata., Tetraselmissuecica., Chlamydomonas sp., Phormidium sp., Desmodesmus sp.)* have been explored for wastewater treatment that includes dairy, municipal, agricultural, brewery, domestic, textile and slaughterhouse wastewater. Kothari *et al.,* (2012) reported that using of *Chlorella pyrenoidosa* treated with dairy wastewater for harvesting of algal biomass is a sustainable approach for biofuels production. Therefore, due to the best result reported by several researchers, the algal based wastewater is an integrated approach for the remediation of different industrial wastewaters.

### 2.3. Influencing parameters

Algal growth is influenced by a diversity of cultural parameters, which are light intensity, salinity, pH, nutrients availability, temperature, carbon dioxide, concentration of dissolved oxygen and other factors like selection, mixing, screening of algae affect the biomass productivity and their growth. Therefore, these cultural parameters should be optimized for the improvement of biomass productivity. As a result, the culture condition depends from species to species, all of these growth factors are necessary to be specific for fruitful algal cultivation. In a stimulated cultivated system, several environmental factors such as light, carbon, and nutrient concentration is an important part for the growth and development of algae (Daliry *et al.,* 2017). Generally, the overall process of algal cultivation is divided into two upstream and downstream processes. For the enhancement of best algal growth, several parameters are required for optimization to achieve the desirable biochemical compounds, whereas in downstream cases, the algal biomass involves, harvesting, dewatering, flocculation, lipid and biodiesel extraction as well as bioethanol production. Therefore, there is a strong relationship between algal growth and environmental factors.

**2.3.1. Process parameters**

The parameters which affect the algal biochemical compounds are as: temperature and light intensity, media composition with nutrient concentrations. For the process of photosynthesis, algae require only nutrients and light for the production of lipids, carbohydrates and proteins. These metabolic products are connected to the environment and nutritional value which includes light, temperature and carbon dioxide, and also available nutrients and the presence of other organisms. Hydrogen, carbon, and oxygen are required as non-mineral nutrients for algal growth. Micronutrients and macronutrients are the two non-mineral nutrients that manipulate the biochemical composition of algae. Micronutrients containing iron and manganese are required in little amount while other elements such as zinc, cobalt, boron, molybdenum and copper are essential trace elements. Macronutrients include phosphorus, nitrogen, sulfur, magnesium and potassium. pH, temperature and heavy metals are the important factors that affect algal growth and metabolism. Instead of all these but the temperature is also a very important factor that affects the algal growth rate cell size and biochemical composition and nutrient requirements. On increasing, the temperature the lipid content and protein synthesis decreased the growth rate of algae. Light intensity also affects the composition of algal cell which exhibits the reduction of protein content and increases the level of lipid. It affects the growth rate of algae through its impact on, photosynthesis (Igiri *et al.,* 2018; Santos *et al.*, 2020). Low light intensity is beneficial for protein concentration while lipid is effective as compared to protein. Some components of light which affect the regular process like chlorophyll synthesis, photo damage repair, and cell division. Another factor is pH which is required for the cultivation of algae which impacts algal metabolism. Nitrate and phosphate are the two important factors for the growth of algal cells and metabolism. Nitrate form proteins and nucleic acid and phosphate are the key component of phospholipids (Metsoviti *et al.*, 2020).

**2.3.1.1. pH**

The pH has huge importance in the culture of algae, because besides distressing the algae themselves, it determines the mineral and carbon dioxide solubility in the medium. Various factors affect the composition, buffering capacity, the quantity of dissolved carbon dioxide, temperature and metabolic movement of the cell influence the culture medium of pH. The pH level of culture differs based upon species, that affect the algal growth, but the pH of algae differs from 6 to 8 (Qiu *et al.,* 2017; Rai and Gupta, 2017). In the case of *Scenedesmus almerienesis*, the optimal pH ranges from 7.5 to 8.5 and the biomass productivity is strongly reduced, when the pH value is above 9.0. It is a significant parameter for the growth of algae, it examines the solubility of existing nutrients (potassium, nitrate and phosphate) it gives an amazing impact on algal metabolism. For the cultivation of *Spirulina platensis* the optimum pH was found best 7.0-8.0 and the best growth was observed with 8.0 (Fagiri *et al.,* 2013). Tripathi *et al.,* (2015) reported that the pH of *Scenedesmus sp.* range from 7 to 10 but results indicate the optimal pH of this species was 8.0.

In case of *C. vulgaris* it facilitates the treatment of acidic water during the metabolic process of propagation, as the metabolites increase the pH of the water body. Therefore, the most favorable range of pH for *C. vulgaris* was 5–9. Therefore, the investigating effects of physical factors on pH becomes particularly significant in this method. For the exploration of single and multivariate interaction, effect on pH will be explored in the orthogonal analysis and response surface methodology (RSM) respectively. The value of solution pH is measured to be an essential assessment factor for the interactions of water body and the growth of algae (Safi *et al.,* 2014). In biochemical profile the content of lipid, protein and carbohydrates changed at different temperature. The temperature between 20℃ -35℃, the carbohydrates and protein content of *S. obliquus* increase first and then decrease, and the maximum carbohydrates and protein content was found with 25℃. When temperature is above 25℃, the carbohydrates and protein content are reduced. Due to the increase in temperature the carbohydrate and protein content also decreased. In the exponential phase of cultivation, the lipid content increased and maximum lipid content was observed with 35℃ at 24% of the content. Similar finding was observed with *Nannochloropsis oculata* with doubled content of lipid and growth (Zhang *et al.,* 2019). Hence the optimization of pH is necessary to obtain the maximum algal growth.

**2.3.1.2. Light**

The presence of light is also an important factor which helps in the growth and productivity of photosynthetic microorganisms. Light is considered as the main indicator for the photosynthetic process for the growth of microorganisms, if it is maximized it gives good results. Due to mutual shading, the irradiance inside algae cultures is not homogeneous but a function of light intensity, culture depth, and biomass concentration. Gonclaves *et al.,* (2016) evaluated the light effect on the growth of algae and also nutrient uptake. Based on their report the optimal condition of algae was dependent not only light, but on the wavelength and the photoperiod to which the cell was exposed. As algae require light intensity for its growth and metabolic activity, basically the culture is strongly influenced by this parameters either quality or quantity basis. Although due to the feasible light sources, the biochemical profile of algae give a value-added products based on their valuable culture, it all depends upon light. Therefore, the cultivation of algae requires light sources either natural or artificial. The artificial sources of light for the cultivation of algae are light-emitting diodes (LED), fluorescent lamps and halogen lamps, and incandes-cent bulbs. All these light sources differ each other in energy consumption, wavelength distribution, spectrum and cost. Because of all these characteristics, the fluorescent light and light emitting diode are the best source of light for the production of algal biomass. Takache *et al.,* (2015) reported that the cycles are responsible for the photosynthetic conversion and productivity of biomass. The light variation and wavelength have an important impact on algal growth. The maximum biomass productivity was reported 35.10 mg/l/d at a light intensity of 150 µmol/m$^2$. There are three light conditions, which affect algal growths are light limitation, light inhibition and light saturations. Therefore, due to the increase of light intensity in *S.*

*obliquus* that exhibits the protein content decreased. In *Phaeodactylum tricornutum*, similar results (18 μE/m) were reported by (Zhang *et al.,* 2019). Daliry *et al.,* (2017) reported the maximum growth rate and lipid production with *Chlorella vulgaris* at light intensities of 5000–7000 lx.

**2.3.1.3. Temperature**

Temperature is the most important environmental factor for the optimization of algal growth, nutrient requirement, cell size and biochemical profile. The algal cultures absorb heat by radiation from the light sources, because due to increase in temperature of the culture. For the growth of algae the optimal temperature ranges from 20- 35 °C even though some mesophilic species can bear upto 40 °C. When the temperature is below these limits the yield of algal strain gets reduced. In addition due to seasonal changes, the variations during day and night time have a significant effect on algal cultivation. Usually, high temperatures between 25-35 °C maintains the algal growth. Based on investigation, both the high and low temperature can help in the lipid productivity. Several studies have been reported that the temperature have a positive impact on algal growth, biomass and lipid yield at an optimal pH temperature of 28.63 °C and 6.51 respectively (Deb *et al.,* 2017) whereas, for the prevention of overheating of algal culture, there are several studies which have been done by various researchers by using dark colored sheet for shades, spray water on bioreactor surface. Therefore, the temperature is also a noteworthy factor, In case of *Chlorella vulgaris* (Singh *et al.,* 2015) demonstrated that the optimum temperature for the growth of this strain is 25°C whereas, some strains like *Ulva sp., Chlorella, Nanochloropsis, spirogyra, botryococcus and Chlamydomonas,* few brown, red algae as well as blue -green algae can grow between this temperature 20℃-30℃. San *et al.,* (2015) investigated the utilization of *Nannochloropsis gaditana* for the monitoring of pH, temperature and dissolved oxygen by using data logging system and control software. An optimized temperature is required for the biomass productivity (0.19l/day) was obtained at a dilution rate of (0.3l/day). Hence, due to the increase in optimal temperature it enhances algal growth. Algal culture heated by strong solar radiation is a big problem especially in humid climatic conditions, when there is no evaporation process. The temperature differs from species to species in different algal strains (Mehta *et al.,* 2018). Some changes occurred with changes in temperature shift is the modification in the level of unsaturated fatty acids in lipid membranes. The *Botryococcus braunii,* green algae that secretes extracellular lipids, which were observed with three different temperatures (18, 25 and 32 °C). Some researchers reported that temperature did not affect the lipid content as well as a similar finding was reported with *Nannochloropsis oculata* and *Chlorella vulgaris* both of them have an optimum temperature of 25°C.

**2.3.1.4. Nitrogen**

Nitrogen is an important source of macronutrient for the growth of algae which plays an important role in the synthesis of protein, lipid and carbohydrate. Nitrogen is the fundamental factor which is responsible for algal structure and function of protein. It is known as $2^{nd}$ rich abundant element present in algal biomass which contains about 1 to 14% concentration of dry mass. It is also responsible for the formation of protein, vitamins, nucleic acid, and photosynthetic pigments (Sajjadi *et al.,* 2018). Algal cell provides nitrogen in the form of inorganic nitrate and it becomes a biochemically active compound which is sequentially recycled in between the algal cell and fulfill the physiological demand. Recently various researchers investigated that when the nitrogen are in stress condition the algal growth and lipid production rate-regulated under stress condition. (Zarrinmehr *et al.,* 2020) support the nitrogen-limited condition for lipid production, but on the opposite side, it decreases the algal biomass productivity. Hence, in the above discussion it plays a vital role for the enhancement of biomass and biochemical profile of algae and their productivity. Algae can incorporate nitrogen in the form of nitrite, nitrite, ammonia and urea as well as it can be utilized for the culture of algae, biomass and lipid production. Thus, it can be concluded that nitrogen concentration favors the production of highest biomass (Zienkiewicz *et al.,* 2020). For the production of lipid in algal cell, nitrogen is a very effective technique for the increasing of lipid production. Many researchers reported in the literature, that nitrogen is sufficient for the production of lipid and the enhancement of biomass productivity. Due to the lowest and highest concentration of nitrogen inducing the stress condition in algal cell of *Chlorella zofingiensis,* for the production of biomass. Results indicate that the *Isochrysis galbana* decreases, when the nitrogen sources are below 288 mg/l (Zarrinmehr *et al.,* 2020).

**2.3.1.5. Phosphorous**

Phosphorus is another necessary nutrient for algal growth and many cellular metabolic activities that includes nucleic acid synthesis, energy transfer, and deoxyribonucleic acid. The range of phosphorous is between 0.05- 3.3% as per dry biomass. Phosphorous is present in various forms in wastewater and natural environments such as metaphosphate, orthophosphate, pyrophosphate and polyphosphate (De alva *et al.,* 2018). However, several studies have been reported the ratio of nitrate and phosphate analysed the effect on algal growth. In *Chlorella vulgaris* the nitrate and phosphate ratio was 8:1 which is suitable for algal growth. The decrease of phosphorous may cause accumulation in some microalgal species, but the effect is lower that caused by the deficiency of nitrogen. In case it is available or not, it may affect the growth and productivity of algal species. Feng *et al.,* (2012) reported that the *Chlorella zofingensis* showed that phosphorous is a good suitable source for the generation of lipid. There are various algal species (*Eustigmatophytes sp., Chlorella zofingiensis, Scenedesmus obtusus, Scenedesmus sp.,* and *Chlorella vulgaris*) which are cultured for the utilization of production of biodiesel, biomass productivity and lipid extraction (Yang *et al.,* 2018). The optimum concentration of phosphorous in algae is in range between 0.001-0.179 g/l. It is an efficient environmental pressure for the induction of lipid accumulation. The limited concentration of phosphorous continuously supports the growth, lipid accumulation especially under stressed conditions (Patel *et al.,* 2017; Yang *et al.,* 2018). Algae have the potential for the absorbance of inorganic phosphate from wastewater within the range 70-90% in comparison to the optimal level which is needed for growth. So, therefore it can be concluded that phosphorous is a necessary macronutrient for the production of lipid. Although, the phosphate uptake from algal can reach saturated, due to limits of light, decrease the level of oxygen and carbon dioxide in the culture medium (Solovchenko *et al.,* 2016).

#### 2.3.1.6. Carbon

Carbon is an essential nutrient which is important for algal growth, photosynthesis, and reproduction. About 50% concentration of carbon is present in algal biomass. It is considered as an important source of nutrients, based on structural component and their biochemical compounds like fatty acid, carbohydrate, protein, carotenoids, and pigments. Carbon sources occurs in the form of carbondioxide which plays an important role in the growth and development of algae (Lin and Wu, 2015). Therefore, nutrient consist of macro as well as micro, are an essential factor for the growth development and their biomass. Various ranges of algal species are preferred for glucose as a source of carbon that can be easily assimilated for the production of biochemical compounds. Various studies have been reported that 30g/l and 20 g/l of glucose are used as a carbon source for the productivity of lipid in *Auxenochlorella protothecoides* and *Chlorella vulgaris* (De Moraes *et al.,* 2017). Hence it can be concluded that all the processing parameters play a vital role in the growth of algae and their biomass productivity from laboratory to commercial scale. But its culture completely depends upon the media, bioreactor type and their geographical location. Therefore, harvesting techniques are much influenced by the end of algal biomass.

**2.3.2. Working parameters**

Algae are an important source which is considered as the promising feedstocks for the production of biofuels due to many benefits because they can be grown as non-arable land and wastewater. In addition, the algal-based biofuels are considered as a good candidate for the replacement of fossil fuel. In general, there are two types of microalgal cultivation system: open pond and closed pond. For the feasibility of this process, the algal-based biofuels production should be cost-effective. On the other hand, the algal growth and biomass productivity depends upon environmental factors such as carbondioxide, temperature, pH, salinity and light, all these factors directly affect the growth of algae (Chowdury *et al.,* 2020).

**2.3.2.1. Bioreactor**

Bioreactor is transparent vessels which are known as open and closed reactor and are made up of transparent water proof materials, and it has the capability to provide favourable conditions for growing the microorganisms.  Bioreactor is brought out for biochemical process which is linked with microbes like fungus, plant cell and algae for the production of biological products are bio-oil, biodiesel, biogas, bioethanol and biohydrogen and some value-added based bio-chemical compounds are pigment, carbohydrate, lipid as well as fatty acids and some other uses food, fodder and fertilizer as per reported by Kothari *et al.,* (2017). Algae need a suitable climatic condition for growth and development. Perfect bioreactor accessories, are Sparger, Agitator and Jacket. The sparger device is used for the management of culture medium in the bioreactor as well as the agitator is used for the mixing of gas, heat transfer and maintain the uniformity inside the vessel. The jacket helps to maintain the constant temperature, when the algal biomass is cultured in the bioreactor.

**2.3.2.2. Bioreactor types**

The process of microalgal cultivation consists of two types of products that is open and closed pond system. In open pond system, it is cultivated in outdoor for the consumption of maximum sunlight (Kumar *et al.,* 2015) as well as closed photobioreactor it is associated with an artificial environment. Scientifically, the term photobioreactor is used for the growth of algal biomass and its development. There are several types of bioreactors that are used for algal biomass cultivation, but for the designing and fabrication of bioreactor for suitable algal growth production is a very difficult task (Ahmad *et al.,* 2017). Algae are considered as a multifaceted organism used in several applications for commercial level, which help for the production of biofuels and also value-added products (Kothari *et al.,* 2017). Algae have the potential for fast-growing, due to their photosynthetic capability, it builds large quantity of bio-products inside the cells that make it a valuable end products by utilizing the industrial wastewater. In addition, algae cultivation don’t need any type of fertile soil, freshwater in large amount, pesticides herbicides comparative to other crops (Khan *et al.,* 2018). The cultivated algae help in the reduction of atmospheric carbondioxide through the process of photosynthesis. Several treatment technologies have been evolved for the production of algae. In general, the culturing of algae can be classified into two categories which are as open pond system and the photobioreactor (Posadas *et al.,* 2017). Hence, for the best results in case of algal growth system, bioreactor plays a vital role for the development of biomass density.

**2.3.2.2.1. Open pond system**

For the cultivation of algae in open pond system either for natural or artificial production of bioethanol has been widely investigated worldwide. The most important open pond system contains tanks, circular pond, shallow pond and raceway ponds. In open pond system, they are economically valuable and favourable, but the main issue is due to land cost, availability of water, low productivity and proper climatic condition. It is mostly used for the commercial purposes of cultivation (Chia *et al.,* 2018). There are various algal species that have some extreme level of environmental conditions, due to their high concentration of nutrients, alkalinity and salinity. Inspite of all these limited characteristics the *Dunaliella, Spirulina* and *Chlorella* are the best examples, which are cultivated in open pond bioreactor system. *Spirulina* is a filamentous cyanobacterium that can be easily grown in alkaline, saline brackish lakes and freshwater. It convert nutrients into cellular matter and releases oxygen with the help of photosynthesis, it requires nitrogen, carbon and phosphorous for growth (Acien *et al.,* 2017). Due to its high content of proteins (60-70 g/100g) it is widely considered as a good source of minerals and vitamins. In industries *Spirullina* is produced from open pond. Whereas, in *Chlorella* it produce intra and extracellular compounds that is proteins, carbohydrates and lipids (Uebel *et al.,*2019). They have many benefits compared to higher plants that contain high biomass, efficiency for the direct utilization of nutrients as wastewater. There are some health benefits of ingestion of *Chlorella* it prevents peptic ulcer, atherosclerosis. Similarly, *Dunaliella* are cultured for antioxidants activity, proteins, glycerol and lipids estimation. It is used as a food supplement (Costa *et al.,* 2019). In order to obtain superior light condition, some modified raceway ponds were developed with temporary light, but this modification was not success, as it was unable for industrial production (Ahmad *et al.*, 2017). In previous times, there are various types of open ponds system that are applied for open, shallow and circular pond systems, which are used for the cultivation of algal biomass.

**2.3. 2.2.2. Raceway pond system**

Raceway photobioreactors are the most important and it is widely used for algal culture systems in industrial sector. It is mostly used at a commercial scale, and it is operated at the depth of 15-30 cm. There are various algal strains like *Chlorella Spirulina.*, *Spirulina platensis.*, *Botryococcus braunii.*, *Scenedesmus sp.*, *Haematococcus Dunaliella.*, widely used with this type system (Kusmayadi *et al.*, 2020). It is connected with baffle, paddlewheel and channels whereas the paddlewheel are used for the maintenance of culture medium, suspension of algal cells, and avoid sedimentation. It is economically feasible and favourable for the sustainable development of commercial products of algae. There are several studies that investigate the design of open raceway pond system, for the reduction of energy cost and increase the growing efficiency of algae. In raceway pond the global productivity obtained were very high in specific growth rate. Van den hende *et al.*, (2014) utilized the aquaculture wastewater in raceway pond system, they analysed that the raceway pond system is best for the removed of total nitrate (31%) and total phosphate (64%) chemical oxygen demand (28%) and biochemical oxygen demand (53%), suggesting to reduce the threat of bacteria from algal growth could be reduced at low level. Same observation was reported by Liu *et al.*, (2017) interaction between algae, bacteria or fungi in other wastewater sources. The uses of aquaculture based wastewater treatment have low cost, but this system is more influenced with external environment.

**2.3. 2.2.3. Unstirred pond system**

Generally, the natural water systems are devoid of a stirred unit that leads to poor mixing, but lesser cost for commercial scale culture. The lagoons, lakes, and ponds are the common photobioreactor. It supplies a simple, economic and suitable approach for the operational and monitoring process of the culture. The natural pond system is usually a lesser amount of half meter in deep for the penetration of light in water and it is absorbed by the algal cells. It provides a reasonable price and is clearly produced for commercial purposes (Ihsan, 2020). These are very simple ponds in terms of construction without any special condition. However, due to the lack of stirrer, it causes less mixing that becomes a limiting factor for the cultivation of biomass at pilot scale. Many studies revealed that plastic transparent cover is a good solution to protect the unstirred pond systems from contamination (Ihsan, 2020; El-Baz, 2018). Some researchers reported that use of plastic film is a better alternative to cover the water surface for the maintenance of temperature *e.g., Dunaliella salina.*, can be cultured in these kinds of open systems for commercial purposes. For the culturing of algae, this method is very convenient and feasible to monitor the culture process (Han *et al.,* 2017; El-Baz, 2018). Therefore, this type of pond has very limited applications, like poor growth, competitive growth with contaminants from protozoa, bacteria and viruses, in spite of all these problems that occurred at the time of cultivation, it is very difficult to handle. For the production of β-carotene in Australia, unstirred ponds are used for the cultivation of *Dunaliella salina.* The applications of pesticides/herbicides in some way to control biological contaminations in such types of unstirred ponds for algal cultivation (Mendes *et al.,* 2013).

**2.3. 2.2.4. Circular pond system**

The circular pond is mostly used in Asia for culturing the *Chlorella sp.* The idea of utilizing the rounded pond which contains long rotating arm was encouraged by the circular reactor treated with wastewater hence the circular pond is almost similar to wastewater treatment pond. This kind of pond is constantly 20-30 cm in deep and 40-50 m in diameter (Molazadeh *et al.,* 2019; Ting *et al.,* 2017). In the centre of pond, the long rotating arm is set, which work like a clock that performs like a paddlewheel which is famous in the structure of that raceway pond. It is clearly indicated that the mixing of media culture and algal cells are more capable as compared to the unstirred pond, but as the algae are uncovered to the surroundings, the contamination is unavoidable. In these types of photo-bioreactor, the flow rate of gas is the main parameter that needs to be regulated, as it affects the growth of algae. Therefore, the circular system is more convenient compared to the unstirred system, but the cultivated algae is viable for the contamination of the surrounding environment (Han *et al.,* 2017; El-Baz, 2018).

**2.3.2.3. Closed photobioreactors**

To resolve the troubles which are generated from an open system and for the achievement of the better yield of algae biomass, the closed vessel has been developed, which does not allow direct mass transfer among the culture media and the atmosphere that contains a large amount of energy consumed, which seems uneconomical (Yen *et al.,* 2019). Therefore, closed photobioreactors are generally considered to be very difficult for handling in pilot scale. The closed photobioreactor system has great value for the production of value-added products that includes cosmetics, bio-pharmaceuticals, human health foods and biofuels which are obtained from algae that become more efficient for the development of suitable and sustainable closed photobioreactor have high potential. The current common closed photobioreactors generally included are vertical tube, flat panel, stirred tank, horizontal tube and their modified configurations (El-Baz, 2018; Show *et al.,* 2017).

**2.3.2.3.1. Tubular photobioreactor**

The tubular photobioreactor is the most commonly used closed algae cultivation systems. Some current studies reported that these types of reactor are mostly used to treat the wastewater. These systems are comparable to those used in the pure cultivation of algae. It clearly shows that these types of wastewater are more diverse and the concentration of pollutants in wastewater is higher than the others (Płaczek *et al.,* 2017). The wastewater consists of municipal, industry, livestock and poultry breeding wastewater. Therefore, the organic load of wastewater is very high. In addition, the tubular reactors are more efficient for operation mode, such as semi-batch, turbid state and continuous (Yen *et al.,* 2019; Ting *et al.,* 2017). This kind of bioreactor has the potential to realize large-scale production because it is easy to handle. There are various studies that are used in tubular bioreactor for the treatment of different wastewater at large scale. For large scale tubular photobioreactor, is used with the working volume of 8-380. Di-Termini *et al.,* (2011) reported that the horizontal tubular photobioreactor is best for the treatment of municipal wastewater treatment plant under controlled conditions. The airlift and bubble columns are considered with vertical tubular types of bioreactor. It is connected at the bottom of photobioreactor in the form of air sparger (Yen *et al.,* 2019). The shape of bubble column is cylindrical with a diameter of 20 cm. It is basically considered as low fabrication cost, efficient amount of gaseous mixing. The results indicated that about 100% of pollutants were removed from wastewater with controlled conditions, which was extensively higher than that in outdoor bioreactors without any light, temperature as well as other conditions (Chang *et al.,* 2018). *Chlorella vulgaris* cultivated in tubular photobioreactors is the best source for the production of biodiesel (Frumento *et al.,* 2013).

**2.3.2.3.2. Flat panel photobioreactor**

Flat panel photobioreactor is a common type of photobioreactor, which is rectangular in shape, and its especially used for pure cultivation of algae. It can be exposed to indoor artificial light sources or outdoor sources uncovered to sunlight. It is made up of transparent or semi-transparent materials which are as plexiglass, polycarbonates, and plastic bags. It contains a short path light that can be easily penetrated in the cultural liquid. The air bubbles are mainly used for mixing, which are generated from the air sparger. The pump is mostly used to supplement air bubbles through the air sparger which circulate the algal cell suspension (Ihsan, 2020; Elrayies, 2018). The main advantages of flat panels include high surface area to volume ratio, convenient to clean, not-too-serious accumulation of dissolved oxygen. The unit is flexible and it is suited for scale-up. In the meantime, the main limitations of the flat panel is expensive to control the temperature, biofouling near the internal surface, hydrodynamic stress which is generated from aeration (Ting *et al.,* 2017; Elrayies, 2018). In *Chlorella sorokiniana* the biomass achieved in flat panel bioreactor was 0.20 g/l/d was reported by (Wolf *et al.,* 2016) as well as the maximum biomass produced from *Chlorella zofingiensis*. On the other side, in flat panel bioreactor *Chlorella* produced low, but it requires baffle insertion, to improve the productivity between 0.6-1.1 g/l/d. The depth of culture in flat-panel bioreactor was 7 cm, while the light condition was 2.5 and 3 cm, respectively (Zeng *et al.,* 2013).

**2.3.2.3.3. Vertical column photobioreactor**

The vertical column photobioreactor consists of two types of vertical tube photobioreactor, airlift and bubble column. Both of them consist of an attached air sparer which is at the bottom of the reactor, converting the spared gas into tiny bubbles to ensure algal cells suspension and to improve the mass transfer, that captures carbondioxide and release oxygen. Under this process, the bubbles compel the algal cells for the transportation of the external illuminated surface to the axial dark zone which is called as "light flash effect" meant for the improvement of photosynthetic activity for mixing of algae. The bubble column reactor has many advantages which contain low cost, simple configuration, high surface area, and satisfactory mass transfer (Ting *et al.,* 2017). The airlift reactor helps in the improvement of bubble column but it has two interconnecting zones inside one of them is the riser and the other is called down comer. There are several studies on the airlift reactor that claimed the advantages as follows: High mass transfer efficiency, well mixing with low shear stress, low energy consumption, good for immobilization of algae on moving particles. The photosynthetic activity and average biomass productivity in the vertical column of the bioreactor were obtained in 0.20 g/l/d in *Chlorella (*De Morais *et al.,* 2007).

**2.4. Harvesting approaches**

The biomass harvesting process is the removal of biomass from the culture medium. It consists of physical, chemical and biological methods. According to Mata *et al.,* (2010) the method involved for the recovery of algal biomass from the main culture, that contributes 20-30% of total biomass cost. These are some techniques that are used for the harvesting of biomass such as, flocculation, sedimentation, centrifugation, flotation and electrophoresis. The harvesting method depends upon the properties of algal cell size their density and the preferable condition of the final products. Algal-based biofuels production methods include the isolation, selection of algal strain, biomass growth, screening, cultivation techniques, lipid production, harvesting of biomass and transesterification. The process of isolation and strain selection is the best step for the utilization of algae as sustainable biomass feedstock for the production of biofuels due to the maximum growth and lipid extraction. Several researchers (Kothari *et al.,* 2017; Mofasser *et al.,* 2017) have examined the isolation of algae having a potential for the production of different beneficial products. Therefore, for the optimal production process, it requires the native microalgal strains which are capable for adopting the local climatic conditions (Sero *et al.,* 2021). Therefore, the main objective of this study is maximum lipid extraction from algal biomass and its utilization for value-added products. Several research centers either national or international provide cultured biomass that contains high lipid productivity, such as NCIM (Pune, India), The ANAAC (Australia), UTEX (USA), CCAP (UK), SAG (Germany), NIBS (Japan). The upstream techniques involve three steps: screening, maximum biomass and lipid production, selection of efficient cultivation system (Park *et al.,* 2019). In upstream and downstream, it requires big efforts for the improvement of biomass production. It improves the microalgal strains platform by genetic manipulation for the development of new organisms for higher lipid extraction.

To establish the new strategy that makes it suitable for the best performance in the downstream stage for the promotion of up-gradation of lipid productivity (Peng *et al.,* 2019). The screening step cover three main steps like physiological growth, production of metabolites, sensitivity toward predators and pathogens. In terms of growth, it comprises of high cell density, specific growth rate, and environmental acceptable variances such as temperature, pH, carbondioxide, salinity and light as s well as photosynthetic efficiency. The screening methods like media culturing, light intensity and time taken for sampling are also considered. The selection of algal strain for the production of biofuels is depended upon the climatic and parametric conditions. Isolation method like pour, streak and spread plate is used for purifying the strains whereas, regular cleaning is must in the upstream process, if wastewater is utilized for the production (Sydney *et al.,* 2021).

**2.4.1. Flocculation**

Flocculation is a process that increases cell recovery through sedimentation, and the flocculent is added to the system. The main aim of this process is to increase the algae particle size and it is considered as the first step of harvesting. The load that occurs on the surface of algae can be changed through flocculants. The common flocculants are aluminum chloride, aluminum sulphate and ferric chloride. Therefore, the flocculants are poisonous in high concentrations. Flocculants should be low-priced, harmless and valuable at low concentrations. Chitosan is a cationic polymer, nontoxic flocculant agent which is used to treat the wastewater in the food industry (De Godos *et al.,* 2017; Branyikova *et al.,* 2018). However, the use of algal biomass greatly depends on algal biomass harvesting concentration and their technology. Flocculation indicates a comparatively low-cost and proficient approach for algal harvesting and biomass at pilot scale (Xu *et al.,* 2018; Pandey *et al.,* 2019). However, in traditional most of the chemical flocculants covalently bind to the algal surfaces, contaminating the final product, which significantly limits their application. Xu *et al.,* (2021) reported that utilization of kinetics of flocculation of *Chlorella vulgaris* with chitosan and walnut protein extract at pH (7) the kinetics flocculation reached up to 40 after the incubation of 1 hour. But chitosan showed upto 80% of flocculation with *Chlorella vulgaris* upto

20 minutes. The total protein content in walnut protein extracted was observed with *Chlorella vulgaris* was 89.6 ± 2.3%. Another researcher utilized the dehulled walnut the total protein content was obtained (16.5± 0.3%) in their study (kong *et al.,* 2019; Li *et al.,* 2020). Pandey *et al.,* (2019) used waste egg shell as a bioflocculant to investigate the changes in their efficiency for the harvesting of *Chlorella pyrenoidosa* which influence the pH on zeta potential was also studied. Hence, bio-flocculant-based harvesting is a good method for the dewatering of algal biomass from an aqueous solution and algal cell surface are environment-friendly and cost-effective approach.

**2.4.2. Centrifugation**

It is a process that involves the application of centripetal acceleration for the separation of algae from culture medium and it may be the fastest cell recovery method on the basis of density gradient. The centrifuge disks are easily sterilized and clean which can be used to any kind of algae. Due to high gravitational force, the cell is exposed, which changes the cell structure and recovers the fragile of algal biomass which requires low centrifugation (Nazari *et al.,* 2020). Harvesting by centrifugation method requires high efficiency, but this method is not possible for continuing the culture reported by (Najjar and Abu-Shamleh, 2020).

**2.4.3. Filtration**

Filtration is a method by which the filter is used for the particles which are in suspended form and retained as a physical separation process. In solid and liquid separation processes the filtration process is safe and is suitable for large-scale algae.

**2.4.4. Flotation**

Flotation is a process where the gas or air bubbles are directed to solid particles and convert them into liquid form it is called as separation process. It is an efficient process for the removal of cell than sedimentation. It can easily confine the particle that is below 500 mm in diameter (Chen *et al.,* 2011). Based on the bubble size in this process, the application is divided into dispersed flotation and dissolved flotation.

### 2.4.5. Electrophoresis

Electrophoresis is a method that is used for the separation of algae with no requirement of chemical. There are many benefits for using this technique, including environmental compatibility, efficiency, versatility, safety, energy, and selectivity, but some factors such as high cost makes that this method to be rarely used on a large scale (Uduman *et al.,* 2010). The main option for selecting and harvesting of algal biomass is depended upon the type of bio-product preferred. Therefore, for the requirement of high value-added products like food, drugs and aquaculture there is a requirement of continuous process to obtain high biomass (Mata *et al.,* 2010).

## 2.5. Resource recovery

Large amount of algal cultivation contributes the sustainable development for the production of biomass at large scale for the utilization of value-added products. On the basis of observation, many algal species are cultivated that have high potential, but lack of information to run for commercial purposes (Peteiro, 2018; Arun *et al.,* 2020). Large amount of algal biomass is needed to complete the feedstocks for the sustainable approaches for bioethanol. It can be cultured with different methods and conditions. It needs light as a source of energy for converting the absorbed water and carbon dioxide into biomass with the help of photosynthesis. Algae require nitrogen and phosphorous as the main nutrients, and other requirements for the growth are macro and micronutrients. The macronutrients are calcium, magnesium and potassium, whereas micronutrients are boron, cobalt, molybdenum, manganese and zinc (Zarrinmehr *et al.,* 2020). In addition, for algae cultivation with wastewater is a good source of nutrients for algal growth. Therefore, various applications of wastewater that are released from agriculture and food industries can encourage the algae cultivation. During the growth period, the algae passes via different phases like lag phases, exponential phase, stationary and death phase (Khan *et al.,* 2018). Different algal species may vary on the basis of their requirement for the growth medium. Therefore, the basic need is same for all species, it contains essential nutrients, iron, phosphorous, nitrogen, organic and inorganic carbon sources. *Chlorella* and *Spirulina* have the efficiency to remove pollutants from rubber effluent. It has the efficiency for biosorbent and present in both freshwater and marine water (Seon *et al.,* 2019). The *Chlorella vulgaris* and *Scenedesmus* were grown in textile wastewater, the nitrogen was reduced by both of them with a high reduction rate in dissolved solids and they achieved 33% of phosphate removed by *Chlorella vulgaris* with 100% concentration of textile wastewater. The removal efficiency of algae also depends on the type of wastewater and species type for the remediation. About 60% and 87% of nitrate and phosphate were removed by *Chlorella pyrenoidosa* (Kothari *et al*., 2012) whereas 90% of nitrate and 70% of phosphate were removed from dairy wastewater by using of *Chlamydomonas polypyrenoideum* (Kothari *et al*., 2013). The most important parameters in algal culturing depend on the type of bioreactor used. If algae can be cultured in large scale in open ponds, that is comparatively inexpensive, but they become easily contaminated. For commercial purposes, algae can be grown in different wastewater to obtain double benefits for cleaning of water body and production of biomass. In addition, seawater is also good options for the cultivation of algae. Using sea water on behalf of freshwater reduces the cost of production. Algae culture depends upon the factors like availability of nutrients are (nitrogen, phosphorous, potassium) temperature, salinity, pH, inorganic carbon, oxygen, light intensity and also carbon dioxide (Morales *et al.,* 2018). Other factors that requires for the successful condition of the culture of shaking, mixing and their dilution rate.

### 2.5.1. Energy options

The most important applications of algal biomass are used as algal-based biofuels on behalf of petrochemical fuels. It has the capability to replace the fossil fuels. It clear that biofuels are a renewable source of energy as compared to the poisonous effects of fossil fuel energy. Algal based biofuels are clean and renewable fuel which can be used without any harm to the environment. Algal biomass is highly accepted for the production of biofuels, due to their high lipid content and sugar in algal biomass. It has the capability to produce biofuels through different routes its biomass can be transferred through the process of transesterification. It gives different benefits for cultivation of algae it doesn't require arable land, high photosynthetic properties compared to terrestrial plants, not extra nutrient required (Bhatia *et al.,* 2021; Goswami *et al.,* 2021). Therefore, algal biomass needs drying for efficient extraction of lipid from it, where drying the wet algal biomass could be very energy-intensive. Whereas, anaerobic digestion of whole wet biomass could be cost and energy-effective. The potential biogas yield from algal biomass was reported to be as high as 200–600 ml/g of organic content. Pyrolysis is another technique that is utilized for dried algal biomass for oil production (Adamczyk and Sajdak, 2018). Biofuels can be classified into two categories: primary and secondary biofuels (Shuba *et al.,* 2018 Beacham *et al.,* 2017). The primary biofuels are obtained from the plant material and animal dry wastes, as well as the secondary biofuels, are classified as three generations that are released from animal and plant material directly or indirectly (Rodionova *et al.,* 2017). The biofuels are obtained from algae due to their different potential that includes alcohols, which are taken through the fermentation process of algal biomass and double approach of fermentation and hydrolysis, transesterification and gasification. In present time the ethanol, biodiesel, fatty acids, triglycerides and carbohydrates are the main sources of biofuels. The harvested algal biomass can be utilized for the synthesized of various biofuels products like biodiesel, bioethanol, biohydrogen and biogas (Ratnapuram *et al.*, 2018).

**2.5.1.1. Biogas**

Biogas is a colorless combustible gas that is produced by the biological breakdown of organic matter; occurring in the absence of oxygen. It comes from "biogenic materials" and it is generated from aerobic digestion of biodegradable materials like cow dung, biomass, green waste and agricultural residue. It is comprised of a mixture of different gases, mainly methane, carbon dioxide and other gases including hydrogen. It is considered as an ecofriendly strategy for the production of energy from biomass, and its residues are used for soil conditioning. In *Scenedesmus sp.,* the amino acid was investigated for biogas production results indicate that the residual biomass provides a better biomass yield as compare to raw material (Ramos *et al.,* 2014). Biogas consists of 50-75% methane, 25-50% carbondioxide, minor quantity of other gases are nitrogen and phosphorous. It plays an important role in future energy scenario, and it can be applied as a renewable and sustainable source of electricity (Bose *et al.,* 2020; Siciliano *et al.*, 2018). Mainly moist plants (sorghum, sugarcane, vegetable and sugar root), as well as some non-food crops marine crops (microalgae and macroalgae) and some waste manure, are most appropriate for the production of biogas (Sole-Bundo, 2019; Karthikeyan *et al.,* 2018). Algal biomass consists of various molecules, which can transform into biogas through the anaerobic digestion process. In spite of all these efforts, it can be utilized for heat, electricity generation as well as transport. Additionally, anaerobic digestion can be used for recycling of nutrients from wastewater and after that treated water could be recycled for the cultivation of algae (Wong *et al.,* 2018; Reddy *et al.,* 2020).

#### 2.5.1.2. Bioelectricity

Bioelectricity is a process by which electricity is generated with the help of an anaerobic digestion process, using organic substrates by microbes. There are various parameters involved in microbial fuel cell technology toward power generation include power density, columbic efficiencies and sometimes chemical oxygen demand removal rate which evaluates the effectiveness of the device. Microbial application in the bioremediation at the same time results in the generation of electricity makes microbial fuel cell technology a highly advantageous proposition which is used in various sectors of municipal, industrial and agricultural waste management. Kim *et al.,* (2015) successfully achieved bioelectricity production from domestic wastewater. Hence as per the best of our knowledge, microbial fuel cell attached with wastewater treatment is a possible solution for the achievement of bioeconomic way for the production of bioenergy. Several researchers (Kim *et al.,* 2017; Ali *et al.,* 2019) have been investigated that wastewater treated with microbial fuel cell for the removal and recovery of contaminants includes: heavy metals, ammonia and chemical oxygen demand by utilizing the biological degraded organic matter for the production of electricity (Munoz cupa *et al.,* 2020). Chen *et al.,* (2020) observed that in their study, use of anaerobic moving-bed biofilm and microbial fuel cell for the generation of bioelectricity treated with pulp/paper wastewater, indicate that the chemical oxygen demand removal efficiency was 65.6% and microbial fuel cell (51.3%) was observed respectively.

#### 2.5.1.3. Biohydrogen

Biohydrogen is a good source of fuel having outstanding benefits as compared to other gaseous or liquid fuel. Lignocelluloses biomass is a richly available raw material that can be utilized as an economical and renewable substrate for the production of biohydrogen. Therefore, algae produces biohydrogen naturally through photolysis, and its biomass can be utilized as a feedstock for fermentative biohydrogen. The biomass obtained from algae contains a high quantity of carbohydrates, protein and lipids therefore, it is recognized as a potential feedstock for the fermentation of biohydrogen production (Nagarajan *et al.,* 2020). Renewable energy sources decrease the greenhouse effect and provide an alternative source for increasing the globally demand of energy (Nagarajan *et al.,* 2020). Nevertheless, due to some technical barriers like developing low-energy process to harvest algal cells, difficulties in continuous production of biomass at large scale, the presence of invasive species in large-scale ponds, low light penetration in dense algal cultures, and the lack of cost-effective bioenergy transporter extraction process, are required to overcome before using algae as an economically feasible biofuel feedstock (Mehariya *et al.,* 2020; Liu *et al.,* 2020). An experimental investigation was done , that suggested tannery wastewater, municipal and slaughterhouse wastewater have the potential for cultivating the three strains (*Chlorella variabilis., Scenedesmus sp., and Chlorella sorokiniana*) for the reduction of pollutant load from wastewater safely (Maizatul *et al.,* 2017). The gasification process is used with different types of waste materials which include municipal solid waste, sewage sludge, agricultural and forest biomass, food waste, animal manure has been considered a popular technique for hydrogen production. Prasertcharoensuk *et al.,* (2019) reported that parameters have high impact on hydrogen production through the process of lignocellulosic biomass waste gasification. Zhang *et al.,* (2020) reported about 28.9% of hydrogen content obtained from food waste through the process of anaerobic digestion and gasification.

**2.5.1.4. Biodiesel**

Biodiesel is a renewable fuel that can be manufactured via vegetables oils, animal fats and recycled biomass materials. It is also known as "green diesel". There are three main types of biofuels are bioethanol, biodiesel and bio-jet fuel. Bioethanol is used in engine that burn gasoline. Biodiesel consists of long chains and contains alkali esters the process is known as transesterification. Bioethanol is an alcohol made up of fermentation, mostly from carbohydrates produced in plants as sugar or starch. The essential edible oils, palm, sunflower, soyabean, mustard and peanuts have been used for fuel from hundred years. Presently, 350 oil producing crops are considered globally for the production of biodiesel. The emitted greenhouse gases that are produced from fossil fuels are the prime object behind taking the decision for biodiesel as an alternative fuel. It can be utilized as an outstanding alternative fuel for the diesel engine, mainly low carbon content makes it possible alternative for heating oil. By taking into consideration, biodiesel can cycle carbon into the atmosphere instead of releasing stored carbon. The parametric effect for the synthesis of biodiesel by using different feedstock on the basis of temperature and time by using transesterification process is given in Table 2.4. The feedstock selection is the main factor to produce biodiesel. These are the feedstock which are used for the synthesis of biodiesel are jatropha (35-60%), karanja (27-39%), rubber seed (40-50%), algae (30-70%), canola (40-45%), linseed (40-44), soybean (15-20%), sunflower (25-35%), peanut (45-55%), palm (30-60%), and coconut (63-65%) as per the values indicate the oil content present in various feedstock (Lin *et al.*, 2017). Due to high demand for biodiesel, various studies have been identified for low cost oil sources like vegetables oil, fish waste from fishes activities (Rajak and Verma, 2018). Barik *et al.*, (2018) reported that food waste which is released from kitchen was utilized for the production of biodiesel, by dried the food waste and methanol is used as a solvent, about 33% of biodiesel was produced. The physico-chemical properties of biodiesel are discussed in detail on the basis of their kinematic viscosity, density and flash point in Table 2.3.

### 2.5.2. Bioactive Compounds

The freshwater based algal biochemical compounds are proteins, lipids, carbohydrates, chitin, nucleic acids and nucleotides. The marine water biochemical compound are carotenoids, vitamin B12, glycoprotein amino acid, fatty acid, carrageen and asymmetric carotenoids, these are different compound as compared to fresh water algal biomass (Pereira *et al.*, 2020). The bioactive compounds derived from algae have antioxidant, anti-inflammatory and antimicrobial activities. The nucleic acid and phospholipids are the most abundant compounds in fresh water based algal biomass whereas amino acid is the most important compound in marine water based algal strains. Bioactive compounds are studied with valuable effects on human health (Hao *et al.*, 2018; Neumann, *et al*., 2019). Some sp. of algae are *Botryococcus braunii., Arthrospira., Chlorella vulgaris., Haematococcus pluvialis., Dunaliella salina* and *Nostoc* have been identified as antioxidant, anti-inflammatory, anticancer and antifungal activity (Hao *et al*., 2018). In biotechnological field, algae is known as highly efficient for bio-active compounds production, which are used in medicines, cosmetics and pharmaceutical industry (Prabakaran *et al*., 2020; Hao *et al.*, 2019). Algae are considered as useful resources due to their growing rate for bioprocessing to attain environmental-friendly products. The growth efficiency of algae shows high potential for the production of bio-products and biofuels that increases the concentration of valuable bio-chemical compounds (Velazquez-Lucio *et al.,* 2018). Algae are useful for bio-remediation of agro-industrial wastewater and the assessment techniques for the assessment of environmental toxicants that is pesticides, heavy metals and pharmaceuticals (Nie *et al.,* 2020). It can be cultivated not only for a food sources but also for feedstock and high lipid content.

**Table 2.3. Physico-chemical characteristics of biodiesel**

| **Properties** | **Kinematic viscosity40 (◦C)** | **Density (g/cm$^3$)** | **Flash point (◦C)** | **References** |
|---|---|---|---|---|
| Jatropha | 4.1-4.3 | 0.835-0.884 | 92-181 | Amid *et al.*, 2020 |
| Castor | 3.5-5.6 | 0.853-0.870 | 161-236 | Roy *et al.*, 2020 |
| Neem | 2.93–4.90 | 0.840–0.896 | 133–257 | Shrivastava *et al.*, 2019 |
| Algae | 2.41-4.3 | 0.841-0.937 | 114-243.5 | Wei *et al.*, 2020 |
| Camalin sativa | 4.04-4.94 | 0.8820.893 | 65-142 | Yilmaz and Atmanli, 2017 |
| Corn | 4-4.7 | 0.871-0.883 | 73-111 | Rangabashiam *et al.*, 2020 |
| Pure diesel | 2.3-5.5 | 0.814-0.844 | 76 (Min) | Jagtap *et al.*, 2020 |
| Animal fat | 4.3-5.7 | 0.875-0.880 | 83–176 | Simsek and Uslu, 2020 |
| Moringa oleifera | 4.03/3.63 | 866.1/834.3 (kg/m$^3$) | 189.0/71.5 | Teoh *et al.*, 2019 |
| Argemone Mexicana | 4.38/2.8 | 870/830 (kg/m$^3$) | 193/65.5 | Singh *et al.*, 2020 |
| Karanja | 4.42/2.78 | 881/831 (kg/m$^3$) | - | Agarwal *et al.*, 2015 |
| Calophylluminophyllum | 4.9762/3.4926 | 871.8/834.7 (kg/m$^3$) | 92.6/68.5 | Monirul *et al.*, 2016 |
| Pongamia | 10.29/2.3 | 912/824 (kg/m$^3$) | 175/53 | Nantha Gopal *et al.*, 2015 |
| Fish oil | 4.74/3.05 | 885/850 (kg/m$^3$) | 114/56 | Gnanasekaran *et al.*, 2016 |
| Rice bran oil | 4.98/3.58 | 887/843 (kg/m$^3$) | - | Dhamodaran *et al.*, 2017 |
| Rapeseed oil | 4.8/2.6 | 874/850 (kg/m$^3$) | >140/68 | Raman *et al.*, 2019 |
| Chicken fat | 5.3/3 | 889.7/829 (kg/m$^3$) | 169/63 | Turkcan, 2020 |

**Table 2.4. Parametric effect on biodiesel synthesis**

| **Feedstock** | **Time (Minutes)** | **Temperature (◦C)** | **Types of transesterification** | **Yield (%)** | **References** |
|---|---|---|---|---|---|
| Jatropha oil | 480 | 60-80 | Homogenous base | 96.8 | Rathore *et al.*, 2015 |
| Soyabean oil | 60 | 60 | Heterogenous transesterification | 90 | Lu *et al.*, 2015 |
| Sunflower oil | - | 23-60 | Homogenous base | 99 | Likozar and Levec, 2014 |
| Silybum Marianum seed oil | 75 | 60 | Carbon acid esterification and homogeneous base transesterification | 96.9 | Reyero *et al.*, 2015 |
| Waste lard | 20 | 50 | Ultrasoundassisted transesterification | 96.8 | Adewale *et al.*, 2016 |
| Canola oil | - | 45 | Homogenous base | 95 | Fadhil *et al.*, 2016 |

#### 2.5.2.1. Pigments

Pigments derived from algae have neuroprotective properties in pharmaceutical products that are used for the prevention of neurogenerative diseases (Chew *et al.,* 2017). They are classified into three categories: carotenoids, chlorophyll and phycobiliproteins which are responsible for green, orange/yellow and blue/red colors, respectively (Levasseur *et al.,* 2020). From numerous years, these compounds have been shown positive health prevention measures such as vitamin precursors, antioxidant properties, immune activators and anti-inflammatory agents (Hamed, 2016). Kalra *et al*., (2020) observed that the plants are the major source of carotenoids. Algae are used for the development of algal cell factories and sustainable production of valuable supplement as nutritional purposes (Levasseur *et al.,* 2020; Sathasivam *et al.,* 2019). It can be used as a source of pharmaceutical industry for increasing market value, antiviral and antifungal infections (Rosales-Mendoza *et al.,* 2020). Some compounds like lutein, palmitoleic acid and oleic acid have been used as antimicrobial and antioxidant properties. It has the potential for the prevention of diseases (Santosh *et al*., 2019; Kiran *et al*., 2021). Pigments are widely used in different industries like cosmetics, aquaculture, neutraceuticals and pharmaceutical industry. The main pigments that are obtained from algae are phycoerythrin, chlorophyll a, b, c, phycocyanin, β-carotene, astaxanthin, fucoxanthin lutein and phycobilliprotein. Some algae which are exploited from centuries for food as well as health prevention measure. Therefore, they are mostly used for food supplements, pharmaceutical and natural colors are the main sources of bioactive molecules (Garcia *et al.,* 2017).

#### 2.5.2.2. Carbohydrate

Carbohydrates are synthesized in the form of dropping sugars like sucrose, lactose, glucose, fructose and polysaccharides. For the production of bioethanol, algal cell are composed of several complex carbohydrates that is cellulose, glycogen, starch and agarose (Khan *et al.,* 2018). Carbohydrates are monosaccharides, oligosaccharides and polysaccharides both of which are comprised of metabolic and functional structure. For the production of carbohydrates, the algal strains (*Botryococcus braunii*., *Haematococcus pluvialis., Nannochloropsis gaditana* and *Scenedesmus obliquus*) was investigated for high amount of carbohydrates content (0.76%, 0.74%, 15.34 and 13.69) respectively. Infact, algal polysaccharides are gaining more attention in cosmetic industry as hygroscopic agents and it is used as antioxidants for current application, which includes body lotions as well as creams. Similarly, another study was analyzed by using swine, aquaculture, textile and food wastewater with different strains *Phormidium sp., Tetraselmis suecica., Chlamydomonas sp.,* and *Scenedesmus sp.,* for carbohydrates contents (16%, 10.62%, 19.5% and 22.1%) was obtained respectively (Behl *et al.,* 2020; Andreotti *et al.,* 2020).

#### 2.5.2.3. Protein

Algae have potential due to their rich protein content availability and its dependent upon the environmental factor and strain used. These molecules consist of several functions which are used for nutritional, pharmaceuticals and cosmetics products (Khanra *et al.,* 2018; Siqueira *et al.,* 2018). Some species *Chlorella, Arthrospira,* have high protein content and considered as an alternative source of protein for human being (Khanra *et al.,* 2018). It produces top quality proteins and well-balanced diet as well as amino acid, according to WHO guidelines are legally known as nutritional food which avoid physical condition from disorders and abnormalities (Bernaerts *et al.,* 2019). Therefore, *Spirulina* has been cultivated as a protein supplements for human food which contain tablets, pastes and pills. The most important thing in case of *Spirulina* about 4 gram of protein is found that is why it is known as "superfood" by World health organization (WHO) by (Grahal *et al.,* 2018). The *Chlorella* and *Spirullina* protein are much better than the protein obtained from other sources like chicken, milk, beef (Grahl *et al.*, 2018).

Inspite of all these valuable beneficial nutritional values, more strains of algae are needed to be explored for study. These two strains are considered as best, due to their potential for future commercialized processes for human nutrition.The *Tetradesmus obliquus., Asterarcys quadricellulare.,* are the algal strains that contain maximum content of protein the values obtained are 34.29±0.0 and 35.78±0.77 respectively. Finding shows that protein content exceeds the limit of lipid and carbohydrates contents in both strains of algae. Due to the best results, the carbohydrates and protein content are applied as a feed for livestock, and it is utilized for commercial application and it can be explored for biofertilizers (Do *et al.,* 2019). Table 2.6 describe the bioactive compounds obtained from algae in detail.

#### 2.5.2.4. Lipids

Algae are considered as a key way for the production of biodiesel. There are several processes available for converting algal biomass into biofuels. It contains biochemical conversion, chemical reaction, thermochemical conversion and direct combustion. Transesterification is a chemical reaction process that is commonly employed for converting lipids into biodiesel (Bhalamurugan *et al.,* 2018). Hence, algae are considered to be the only option for producing biodiesel in a sustainable way as they have the ability to grow even on wastewaters and adapt themselves for changing environmental conditions. Lipid plays an important role in algal growth and their metabolic activity (Kottuparambil *et al.,* 2019). The lipid content in algae ranges from (2 to 77%) it depends on the species and their environment according to Safi *et al*., (2014). The biochemical profile of algae is given in Table 2.5 with different strains of algae. There are several strains of algae, which are investigated with wastewater for the production of biodiesel are *Chlorella vulgaris., Scenedesmus dimorphus., Scenedesmus sp.,* which are analyzed by different researchers in their study (Mondal *et al.,* 2017; Bhalamurugan *et al.,* 2018; Molino *et al.,* 2018). A study investigated by Sharma *et al.,* (2020) by using different concentrations (25%, 50%, 75% and 100%) of wastewater and BG-11 media used for algal cultivation. As per the observation, the maximum lipid content (25.1%) was obtained with 75% concentration. (Sharma *et al.,* (2020) observed that the algal consortia grown in untreated carpet industry wastewater indicate the low value of protein (53.8%) carbohydrate (15.7%) and lipid (5.3%) as compared to other studies.

**Table 2.5. Biochemical composition of different algae**

| **Algal strains** | **Protein (%)** | **Carbohydrate (%)** | **Lipid (%)** | **References** |
|---|---|---|---|---|
| *Chlorella sp* | 64.2 | 10.5 | 14.8 | Ge *et al.*, 2018 |
| *Scenedesmus* | - | 22.1 | 20.18 | Ji *et al.*, 2015 |
| *Micratinium reisseri* | - | - | 20 | Renuka *et al.*, 2013 |
| *Chlamydomonas sp* | 51.9 | 19.5 | 11 | Behl *et al.*, 2020 |
| *Desdosesmus sp* | 39.6 | 24.4 | 23.5 | Do *et al.*, 2019 |
| *Nanochloropsis oculata* | 36 | 7.3 | 49.8 | Parsy *et al.*, 2020 |
| *Tetraselmis suecica* | 50.2 | 10.62 | 25.06 | Andreotti *et al.*, 2020 |
| *Porphyridium cruentum* | 28-35 | 17-22 | 0.5-0.8 | Cecal *et al.*, 2012 |
| *Phaeodactylum tricornutum* | 34.8 | 16.8 | 16.1 | Tibbetts *et al.*, 2015 |
| *Dunaliella primolecta* | 12 | - | - | Slocombe *et al.*, 2013 |
| *Dunaliella sp*<br>*Scenedesmus sp*<br>*Nanochloropsis sp* | 34.17<br>31<br>30 | 14.57<br>28<br>10 | 14.36<br>15<br>22 | Kent *et al.*, 2015 |
| *Chaetoceros mulleri* | 59 | 10 | 31 | Velasco *et al.*, 2016 |
| *S. obliquus* | - | - | 35 | Breuer *et al.*, 2012 |
| *C. zofingiensis* | - | - | 24.5 | Zhu *et al.*, 2014 |
| *Ankittrodesmus falcatus* | - | - | 59.6 | Singh and Singh, 2015 |
| *Phaeodactylum tricornutum* | - | - | 3.26 | Yodsuwan *et al.*, 2017 |

| **Algal strains** | **Protein (%)** | **Carbohydrate (%)** | **Lipid (%)** | **References** |
|---|---|---|---|---|
| *Entomoneis paludosa* | - | - | 2.62 | Cointet *et al.*, 2019 |
| *Rhdomonas sp* | - | - | 30.3 | Latsos *et al.*, 2020 |
| *Chlorella vulgaris* | 6.49 | 50.6 | 23.2 | Praveen and Loh, 2016 |
| *Nanochloropsis oceanic* | - | - | 58 | Zienkiewicz *et al.*, 2020 |
| *Picocystis salinarium* | - | - | 33.87 | Delgado *et al.*, 2021 |
| *Anaebaena cylindrical* | 43- 56 | 25-30 | 4-7 | Biller *et al.*, 2014 |
| *Botryococcus brunni* | 40 | 2 | 33 | |
| *Chlorella pyrenoidosa* | 57 | 26 | 2 | |
| *Chlorella vulgaris* | 41-58 | 12-17 | 10-22 | |
| *Scenedesmus quadricauda* | 47 | - | 1.9 | Priyadarshani and Rath, 2012 |
| *Spirogyra sp.* | 6-20 | 33-64 | 11-21 | |
| *Spirulina maxima* | 60-71 | 13-16 | 6-7 | |
| *Spirulina platensis* | 42-63 | 8-14 | 4-11 | |
| *C. Sorokiniana* | 39.3 | 29.4 | 23.3 | Deng *et al.*, 2020 |
| *N. Oceanica* | 18.1 | 20.7 | 48.3 | Li *et al.*, 2020 |
| *Synechocystis* | - | 27.1 | 28 | Ashok kumar *et al.*, 2019 |

**Table 2.6. Different applications of bioactive compounds obtained from algae**

| Algae Species | Different Compounds | Uses | References |
|---|---|---|---|
| *Chlorella sp* | Astaxanthin | Powders, Tablets, Nectar, Noodles and Powder extracts | Hu *et al*., 2016 |
| *Spirullina* | Astaxanthin | Tablets, Powders, Beverages extracts, Chips, Pasta and liquid extracts | Sowjanya and Manjula, 2016 |
| *Pyropiayezoensis* | Carotenoids, vitamin B12, PGP glycoprotein | Food applications, Anti-inflammatory and Antoxidative activity | Choi *et al*., 2015 |
| *Chlamydomonas reinhardtii* | - | Human nutrition, Biofuels | Adarme-Vega *et al*., 2012 |
| *Emilianiahuxleyi* | Calcium carbonate, Dimethyl sulfide | Human nutrition, Weather influence | Endo *et al*., 2016 |
| *Haematococcus pluvialis* | Carotenoids, Astaxanthin | Neutraceuticals, Pharmaceuticals | Gonzalez-Delgado *et al*., 2017 |
| *Phaeodactylum tricornutum* | Lipids | Human nutrition, Biofuels | Karas *et al*., 2015 |
| *Nannochloropsis gaditana* | Lipids | Human nutrition, Biofuels | Iwai *et al*., 2015 |
| *Odontellaaurita* | Fatty acids | Pharmaceuticals, Cosmetics, baby food | Xia *et al*., 2014 |
| *Porphyridium cruentum* | Polysaccharides | Pharmaceuticals, Cosmetics | Santosh *et al*., 2019 |
| *Ostreococcus lucimarinus* | Asymmetric carotenoids | Antioxidant molecules, human nutrition | Botebol *et al*., 2015 |
| *Nanochloropsis sp* | Eicosapentaenoic acid (EPA) | Cardiovascular benefits | Asgharpour *et al*., 2015 |
| *Porphyridium purpureum* | Arachiodonic acid | Improve the normal growth rate | Su *et al*., 2016 |

| **Algae Species** | **Different Compounds** | **Uses** | **References** |
|---|---|---|---|
| *Nannochloropsis* | Eicosapentaenoic acid (EPA) | Cardiovascular benefits | Asgharpour *et al.*, 2015 |
| *Phaeodactylum* | Eicosapentaenoic acid (EPA) | Mental support | Cui *et al.*, 2021 |
| *Porphyridium* | Eicosapentaenoic acid (EPA) | Anti-inflammatory | Kavitha *et al.*, 2016 |
| *Nanochloropsis oculata* | Peptides | Anticancer | Samarakoo *et al.*, 2013 |
| *Arthrospira platensis* | Phycocyanin | Antioxidants | Wollina *et al.*, 2018 |
| *Isochrysis sp* | Phenolics | Food applications | Widowati *et al.*, 2017 |
| *Dunaliella salina* | Lutein | Antioxidants | Fu *et al.*, 2014 |
| *Phaeodactylum tricornotum* | Eicosapentaenoic acid (EPA) | Cardiovascular benefits | Peng *et al.*, 2014 |
| *Porphyridium purperum* | Arachidonic acid | Improve normal growth | Su *et al.*, 2016 |
| *Dunaliella salina* | β-carotene | Antioxidant, anti-allergic and inflamatory | |
| *Haematococcs pluvialis*<br>*C.zofingenisis* | Astaxanthin | Anti- inflammatory, Antioxidants | Ciccone *et al.*, 2013 |
| *Scenesdesmus sp* | Lutein | Anti- inflammatory, Antioxidants | Saha *et al.*, 2020 |
| *Muriellopsis sp* | Lutein | Antioxidants | Molino *et al.*, 2020 |
| *C. sorokiniana* | Lutein | Anti- inflammatory | Xie *et al.*, 2019 |
| *Chlorella sp*<br>*Crypthecodinium cohnii*<br>*Haematococcu spluvialis* | Lutein, β-carotene<br>Docosahexaneoic acid,<br>Astaxanthin, lutein, Glycerol, | Neutraceuticals | Santhosh *et al.*, 2016 |

| Algae Species | Different Compounds | Uses | References |
|---|---|---|---|
| *Nanochloropsis gaditana*<br>*Scenedesmus almeriensis*<br>*Chlamydomonas reindardtii* | Eicosapentaneoic acid, glycerol | | |
| *Chlamydomonas nivalis*<br>*Chlorella zofingiensis* | Canthaxanthin | Antioxidants, Anti- inflamatory | Sathasivam *et al.*, 2019 |
| *Tetraselmis suecica*<br>*Chlorella sorokiniana* | Lutein | Diabetic, Retinopathy | Sansone *et al.*, 2017 |
| *Ankistrodesmus brunii* | Lutein | Antioxidant and Anticancer activity | Liu *et al.*, 2017 |
| *Poryphyridium sp* | Polysaccharides | Anti-inflammatory, Antiviral | Bule *et al.*, 2018 |
| *P. cruentum* | - | Immune function | Koyande *et al.*, 2019 |
| *Chlorella stigmatophora*<br>*Phaeodactylum tricornutum* | Polysaccharides | Antioxidant, Reduce free radicals | Chen *et al.*, 2018 |
| *Odontellaaurita*<br>*Salpingoeca marina* | Fucoxanthin and Zeaxanthin | Anticancer activity, Prevention<br>of osteoporosis, Cholestrol, gastric, ulcer | Kiran *et al.*, 2021<br>Liu *et al.*, 2017 |
| *Chlorella sp* | Glycoprotein | Used as dietary items, Tablets and capsules | Caporgno and Mathys, 2018 |
| *Phaeophyta* | Polysaccharides | Antifungal, Anticancer | Lekshmi and Kurpa, 2019 |
| *Rhodophyta* | Polysaccharides | Anti-inflammatory | Morokutti-kurz *et al.*, 2017 |
| *Chlorophyta* | Polysaccharides | Antiprotozoa, Reduce coronary heart disease | Gaikwada *et al.*, 2020 |

| Algae Species | Different Compounds | Uses | References |
|---|---|---|---|
| *Rhodophyta* | Polysaccharides | Antihelminthic, Anti-inflammatory | Olasehinde *et al.*, 2017 |
| *Chaetoceros calcitrans* | Polysaccharides | Decrease occurance of chronic disease like obesity, arthritis and cardiovascular diseases | Amin *et al.*, 2018 |
| *Isochrysis galbana* | Polysaccharides | Diabetes, Improve brain potential | Katiyar *et al.*, 2021 |
| *Nanochloprosis sp* | Eicosapentaenoic acid | Cardiovascular disease, Improve the function of brain | Vitor *et al.*, 2021 |
| *Nanochloropsis sp* | Eicosapentaenoic acid | Valuable for coronal heart patient | Levassuer *et al.*, 2020 |
| *Pyramimonas sp* | Docosahexaeonic acid | Anti- inflammatory, Utilized as a food for pregnant women | Kiran *et al.*, 2021 |
| *P cruentum* | Vitamins | Reproduction | Koyande *et al.*, 2019 |
| *Porphyridium sp* | Arachidonic acid | Vasoconstricter | Paliwal *et al.*, 2017 |
| *Crypthecodium sp* | Docosahexaeonic acid | Cardiovascular disease control | Long *et al.*, 2018 |
| *Dunaliella sp* | Glycoprotein | As a supplements like tablets | Gallaso *et al.*, 2019 |
| *Naviculaatomus* | Arachiodonic acid | Platelets aggregation | De Moraise*t al.*, 2015 |
| *Tetraselmis* | Vitamins | Cholestrol control, Utilized as anticancer | Delasoie *et al.*, 2018 |
| *Spirulina* | Glycoprotein | Utilization for Tablets and powder | Marque- Escobar *et al.*, 2018 |

### 2.6. Applications of algal based compounds

Algae are used for commercial, biotechnological and industrial applications. For commercial applications, algae are the sources of protein, carbohydrates, enzyme and biofuels production. Besides many vitamin and minerals like vitamin B1, B2, B6, A, C, B, potassium, niacin, iron, calcium and magnesium richly occurs in algae. There are some important biotechnologically applicable algae such as *Cyanobacteria*., *Haematococcus pluvialis.*, *Chlorella vulgaris*., *Dunaliella salina* and *Spirulina maxima* which are widely used for commercialized and nutritional purposes for humans and animal feed (Sathasivam *et al*., 2019; Garcia *et al*., 2017). For industrial applications, algae are used as a biofertilizer in aquaculture, and pharmaceutical industry. The sp. which are used for aquaculture are *Tetraselmis.*, *Chlorella.*, *Isochrysis.*, *Phaeodactylum.*, *Chaetoceros*., *Pavlova*., *Skeletonema.*, *Thalassiosira* and *Nannochloropsis* (Bikker *et al*., 2016).

There are several high value products which include pigments, polyunsaturated fatty acids, phycobiliproteins which are used for neutraceuticals and pharmaceutical purposes which are obtained from algae (Yakoob *et al*., 2014). They are rich sources of natural bioactive compounds antifungal, antiviral, antibacterial, antioxidant, anti-inflammatory and hypercholesterolemia properties (Suganya *et al*., 2016). Phycocyanobilins and phycoerythrobilins are used as antioxidant in the decorative cosmetic industry (Hamed, 2016).

Henceforth, algal-based wastewater treatment represents cost-effective and efficient treatment techniques, by which the algal biomass is the only renewable resource which has a high uptake capability of pollutant removal. After an extensive survey of the literature and on the basis of bio-based value-added products and their influencing parameters as well as the residual biomass, which are applied for future applications, provides a new view for bio-economy. Therefore, algal biomass has high potential to solve all these problems that are associated with wastewater to fuel conversion.

# Chapter 3
# Materials & Methods

### 3.1. Introduction

This Chapter is explaining about the experimental plan with methodology procured in this research study. The analytical techniques used in present study are explained. Algal species and wastewater selection (different concentrations) with different physical parameters are also outlined. Experimental procedures adopted with phycoremediation approach for wastewater treatment are also discussed. Harvesting of known algal biomass with highest growth rate in coupling with wastewater at lab scale is also assessed with designed bioreactor to scale-up the biomass at large scale as per objective designed. The co-relation analysis between bio-chemical compounds (chlorophyll, carbohydrate, protein, carotenoids and lipid) with selected water samples using selected algal strains also investigated. Investigated approaches selected to complete the objectives of this study as per the findings with concluding remarks in provided in Chapter-4, 5, 6 and 7.

### 3.2. Reagents, chemicals and glasswares

To avoid the chances of contaminations, all the reagents were prepared by using deionised/distilled water for experimental study. All glasswares and plastic containers were used after washing and rinse with Milli-Q water in experiments. All chemicals applied for the research work were of analytical grade. The chemical used for this experimental study is given in Annexure-1.

### 3.3. Characteristic features of algal species used in this study

*Chlorella pyrenoidosa* is a single-celled freshwater alga (as illustrated in Figure 3.1 (a)) with higher chlorophyll concentration used in this research study.

*Chlorella vulgaris* is green algae (as illustrated in Figure 3.1 (b)) in the genus *Chlorella,* Division Chlorophyta. In this study, two strains are used (*C. pyrenoidosa* and *C. vulgaris*) for the completion of this research work. Therefore, these two strains are selected to complete the objectives designed for this research study based on their availability and adaptability to different wastewaters as cited in literature. Only *C. pyrenoidosa* has been cultivated with all concentrationsof wastewater and best concentration observed with algal growth further investigated with *C.vulgaris* with all the selected water samples. Classification of *C. pyrenoidosa* and *C.vulgaris* is mentioned in Table 3.1.

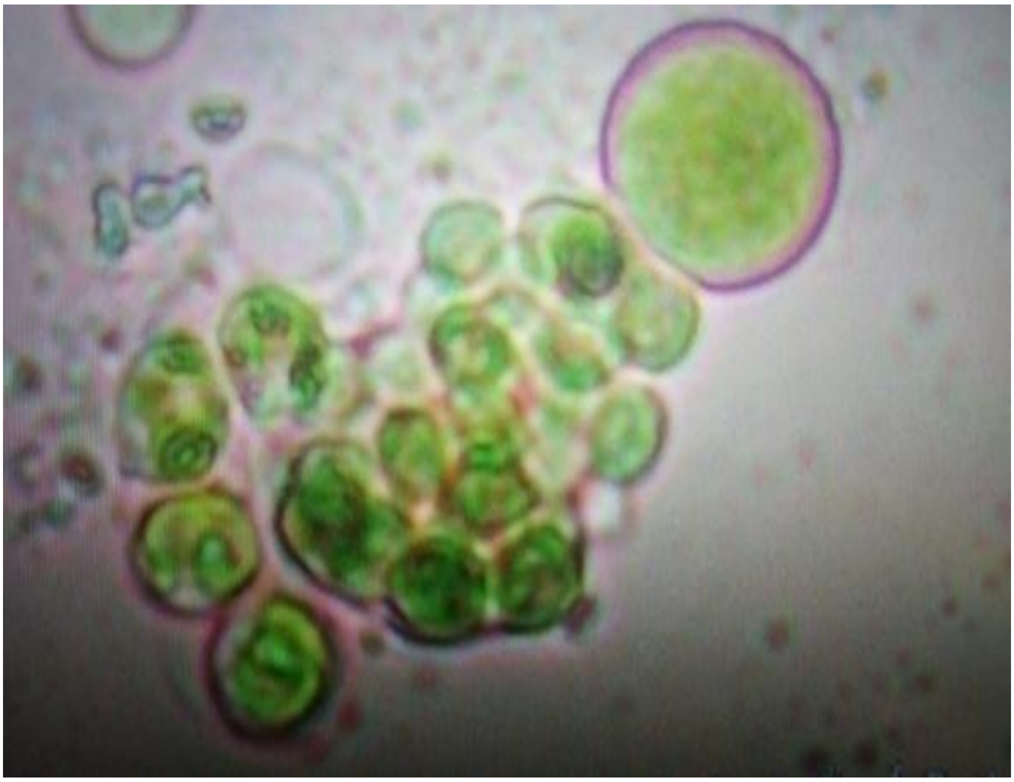


**Figure 3.1 (a). Microscopic image of *C. pyrenoidosa***

**Table 3.1 (a). Scientific classification of *C. pyrenoidosa***

| | |
|---|---|
| **Kingdom** | Plantae |
| **Division** | Chlorophyta |
| **Class** | Trebouxiophyceae |
| **Order** | Chlorellales |
| **Family** | Chlorellaceae |
| **Genus** | *Chlorella* |
| **Species** | *Pyrenoidosa* |

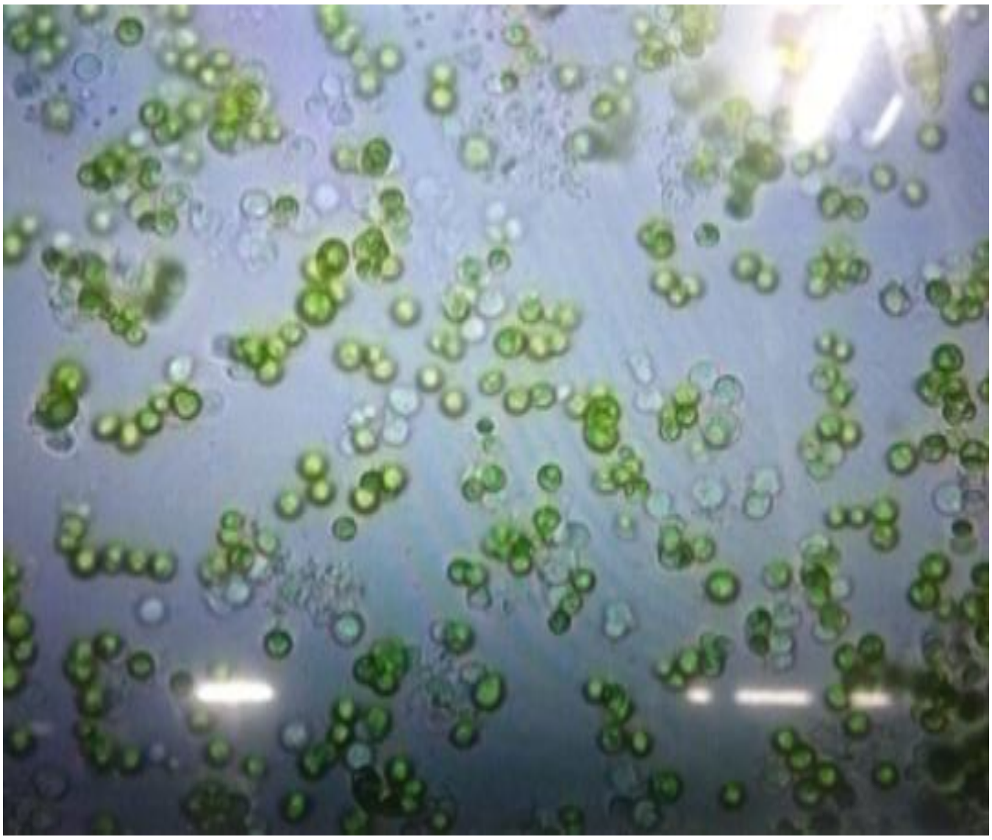


**Figure 3.1 (b). Microscopic image of *C. vulgaris***

**Table 3.1 (b). Scientific classification of *C. vulgaris***

| | |
|---|---|
| **Kingdom** | Plantae |
| **Division** | Chlorophyta |
| **Class** | Trebouxiophyceae |
| **Order** | Chlorellales |
| **Family** | Chlorellaceae |
| **Genus** | *Chlorella* |
| **Species** | *vulgaris* |

### 3.3.1. Nutrient media and culture conditions

The sample of *C. pyrenoidosa* was acquired from National Collection of Industrial Microorganism (NCIM 2738), Pune, India, whereas *C. vulgaris* was obtained from culture laboratoryof Department of Environmental Science, in university campus. Both cultures retained in BG-11's growth medium as summarizes in Table 3.2. Algal cultures were manually agitated to provide homogenous nutrient distribution as well as to avoid algal threads stickiness on reactors wall and sedimentation. Nutrient medium and flasks in which algal cultivation took place were sterilized by autoclaving at 15psi and 121°C temperature for 20 minutes. The algal culture medium was inoculated with algal cell density equivalent to 0.1 at 665 nm and incubated at 25±2°C in 12h light (10 $Wm^{-2}$)/dark cycle. Algal cultivation was carried out in ambient condition as well as artificially controlled conditions. Algal biomass growth was measured by taking optical density of 680 nm by using UV–Vis spectrophotometer (Model no. Systronics 2203). All experimental flasks were inoculated with an initial optical density of 0.05 algal cell densities taken from mother culture of both strains.

### 3.3.2. Biochemical composition of *C. pyrenoidosa* and *C. vulgaris*

Biochemical compounds of algal biomass play a crucial role to produce multiple end products. Algal biomass based green economy is best alternative to support the best bioenergy/alternative options on behalf of fossil-fuel based energy options. A wide range of industries are being targeted for the use of algal biomass: (i) food industry (bio-emulsifier, edible coating *etc.*) (ii) Cosmetic industry (antioxidants, antibacterial cream, other skin enhancement lotion *etc.*) (iii) pharmaceuticals (Formulation of vaccines, healing agent, immune modulatory agents, inflammatory agents *etc.*) (Kothari *et al.,* 2017).

**Table 3.2. Media composition of BG-11**

| **Stock solution of Nutrients** | **Quantity** |
|---|---|
| *$NaNO_3$ | 15.0 g |
| **$K_2HPO_4$ | 2.0 g |
| **$MgSO_4.7H_2O$ | 3.75 g |
| **$CaCl_2.2H_2O$ | 1.80 g |
| **Citric acid | 0.30 g |
| **Ammonium ferric citrate green | 0.30 g |
| **$EDTANa_2$ | 0.05 g |
| **$Na_2CO_3$ | 1.00 g |
| Trace metal solution: | |
| *$H_3BO_3$ | 2.86 g |
| *$MnCl_2.4H_20$ | 1.81 g |
| *$ZnSO_4.7H_2O$ | 0.22 g |
| *$Na_2MoO_4.2H_2O$ | 0.39 g |
| *$CuSO_4.5H_2O$ 0.08g | 0.08 g |
| *Co $(NO_3)_2$. $6H_2O$ | 0.05 g |

***per litre; **per 500ml**

The characteristic features regarding bio-chemical compounds of *C. pyrenoidosa* and *C. vulgaris* were observed by using methods *i.e.* Lowry method, phenol sulphuric acid, Bligh and Dyerwith BG-11 media. Therefore, it is possible to produce value-added products by using both *Chlorella* sp. as it has significant amount of bio-chemical compounds. In this experimental plan, *C. pyrenoidosa* was experimentally investigated with different concentrations of wastewater for biomass growth, whereas growth of *C. vulgaris* was assessed with only best optimized resulted concentration of wastewater to check its potentiality on comparative basis.

**3.4. Wastewater: selection and characterization**

In order to establish green and sustainable approaches for algal cultivation system, wastewaters have been selected as a source of nutrients for algal growth and development. After extensive literature survey, three types of wastewater, Slaughter house wastewater (SHWW), Open-sewage contaminated channel wastewater (OSCCW), and Poultry excreta Leachates wastewater (PELW) were selected as a source of nutrient for algal cultivation. The initial physico-chemical parameters was assessed to study the color, odor, temperature, TDS (total dissolved solid), pH, BOD (biochemical oxygen demand), COD (chemical oxygen demand) was analyzed with the help of standard method prescribed by American Public Health Association (2012). Four different concentration (25%, 50%, 75% and 100%), was taken for setup the process and BG-11 media was taken as a control, as per our objective in study of SHWW, OSCCW and PELW. Figure 3.4 demonstrate the various steps involved in wastewater treatment processes.

**3.4.1. Sampling sites**

The selected wastewater samples were collected in sterilized 2 litres of glass bottles (20 litres) and subsequently stored at 4ºC for further analysis. All the wastewater

(SHWW, OSCCW and PELW) samples were collected in the month of (January, May and September) in a year. Figure 3.2 clearly illustrating the generalized process scheme of SHWW, OSCCW and PELW. Optimizations of wastewaters concentration have been done to examine maximum algal growth. Experimental plan consists of physico-chemical assessment of selected wastewater. Pollution removal efficiency of alga was analyzed in this section.

**3.4.1.1. Slaughterhouse wastewater**

Slaughterhouse wastewater was collected from Kasai Bara near local market of Aminabad Lucknow (Uttar-Pradesh), India (26$^{o}$50’N 80$^{o}$55’E) which have the capacity to generate gallons of wastewater per day. All the samples were collected with sterilized glass bottles and stored at 4 $^{o}$C for the analysis of wastewater. At that time the biological waste released from slaughterhouses after the scarifying process mostly in rural areas, the wastewater dump into the local canals or nearby areas that pollute the environment which affects human health and hygiene. It requires major treatment due to the high organic and nutrient pollutant present, its needs safe discharge into the environment. Therefore, the treatment process and final discharge of slaughterhouse waste (solid/liquid) require proper treatment to save the environment and also human health. Although slaughterhouse wastes materials can be degraded by chemical, physical, and biological processes. Thus, algal-based wastewater treatment is a good alternative for the treatment and disinfectant of slaughterhouse wastewater among other conventional methods cited by researchers for wastewater treatment. This type of wastewater consists of a large amount of BOD, COD, nitrate, and phosphate. The nitrogen and phosphorous released into the environment without any treatment create pollution in river bodies, lakes, and sea that disturb the ecosystem, and various hazardous impacts due to low dissolved oxygen, algal bloom, fish kill, undesirable pH, and cyanotoxin production.

### 3.4.1.2. Open sewage contaminated channel wastewater

The OSCCW (Open sewage contaminated channel wastewater), was collected from *Naalahs* (broad channels of flow of wastewater) of Uthrethia near Babasaheb Bhimrao Ambedkar University campus, Lucknow (Uttar-Pradesh) India (26$^{o}$76'N 80$^{o}$93'E) that flows with large amount of wastewater per day, collected from different unidentified *Naalis* of nearby residential as well as commercial communities drainage systems. Although quality and flow of wastewater changes on per day as well as seasonal variations as per observations but random sample was collected from the area of *Naalahs* where the maximum discharges from various *Naalis* (narrow channels/drainages of wastewater). Samples collected from these broad open channels are referred as open sewage contaminated channel wastewater in this study. Wastewater from these "*Naalis*" of different communities mixed together in the "*Naalahs*". So, proper check on these non-point sources is lacking by governmental agencies particular to Indian systems. These OSCCW poses a serious threat for freshwater bodies, when mixed or linked to rivers or lakes from cities. Hence, to minimize the contamination of freshwater resources reuse and recycling of wastewater is the only solution. There is an immediate need of phycoremediation process that can sustainably reduce pollution load of wastewater and can be exploited for fuel and renewable chemicals.

### 3.4.1.3. Poultry Excreta Leachate Wastewater (PELW)

Poultry wastewater was taken from Southcity, near Babasaheb Bhimrao Ambedkar University, Lucknow (Uttar-Pradesh) India (26$^{o}$76'N 80$^{o}$93'E) for this study for physio-chemical characterization and algal growth only. Due to the large amount of production/consumption of eggs, chicken and meat standing high level generation of pollution load all over the world. This production level creates large volume of waste materials from poultry farms. Waste materials contain fecal excreta, bedding materials urine, and feathers. Inspite of this, waste management methods are very risky for land as well as water bodies. It contains nitrogen and phosphorus compounds such as uric acid, ammonical nitrogen, carbonaceous compounds and minerals. Whereas, due to the high amount of discharges of pollutant from poultry farming to the open environment is very effective for flora and fauna as well as human being. Therefore, requirement of management of poultry waste is very important part, to prevent the environment from contamination. Thus, utilization of available nutrients as a resource for algal cultivation and remediation of nutrients and pollution load has gained worldwide attention. This waste was not further evaluated for other experimental investigations due to unavailability of poultry waste during COVID-19 lockdown. Therefore, only two wastewaters were taken to investigate for further to complete the designed objectives.

### 3.4.2. Physico-chemical characterization

Physico-chemical parameters of selected wastewater were analyzed by following the standard analytical procedures prescribed by APHA (2012). All the physicochemical parameters (TDS, TS BOD, COD, nitrate and phosphate) was investigated and clearly described in detailed in Annexure 1 of this study. The protocol for the investigation of experimental work is divided into three phases (Phase I, Phase II and Phase III) and it is given in detailed in annexure section 1.

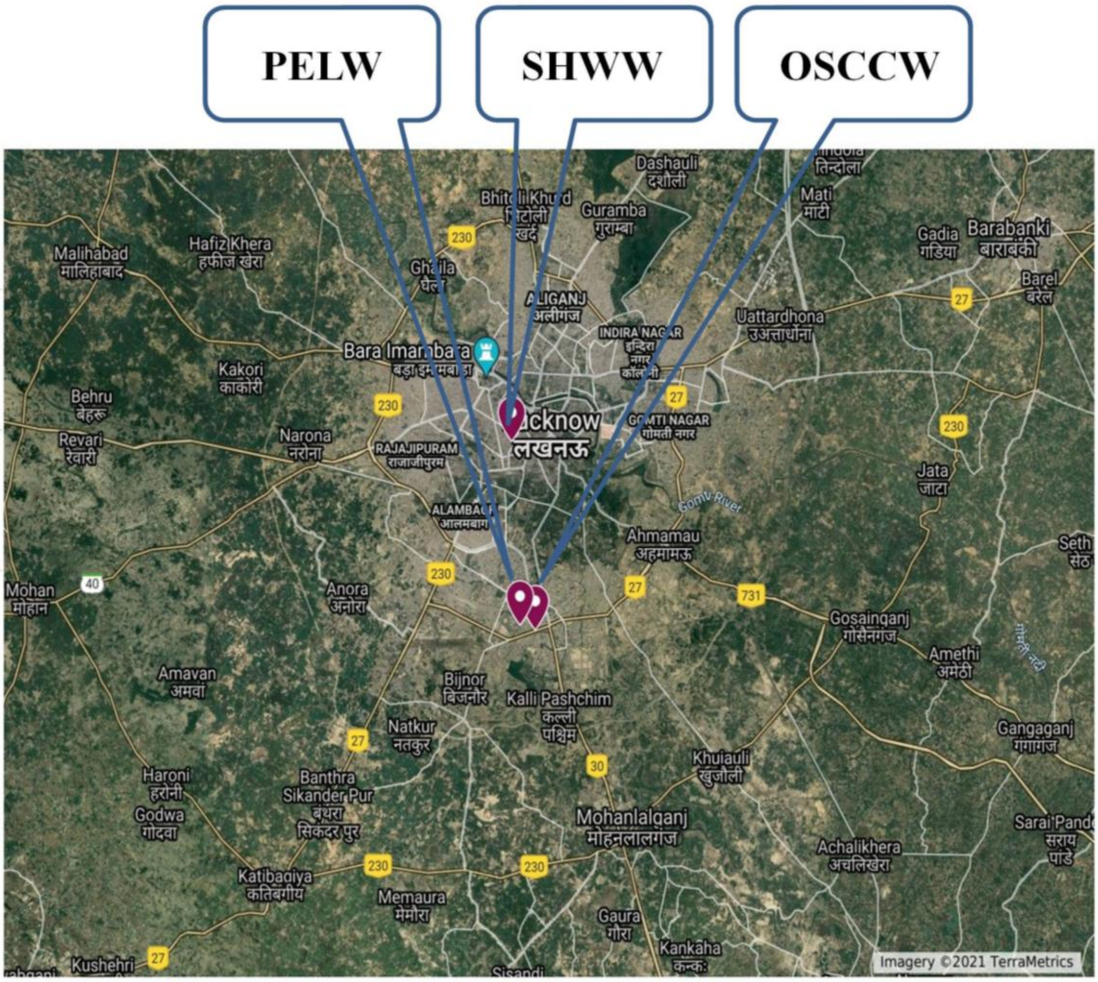



**Figure 3.2. Sampling sites of wastewater for this study:**

**(a) SHWW (b) OSCCW (c) PELW**

### 3.4.3. Concentrations of selected wastewater samples

Different concentrations of selected wastewaters were prepared to observe the optimized concentrations of wastewater that supports maximum algal growth. Its applicability as nutrient medium for cultivation of both *Chlorella* sp. have also been studied. Therefore, different concentrations (25%, 50%, 75% and 100%V/V) of wastewater were selected for optimization process using *Chlorella* sp. recommended nutrient medium (BG-11) and tap water were taken as a control for this experimental study. Best optimized concentrations obtained from experiments were further taken as growth medium for *C. vulgaris* with SHWW and OSCCW.

### 3.5. Algal bio-chemical compounds

The biochemical characterization of selected algal biomass for carbohydrate, protein, lipid, carotenoids and chlorophyll were estimated by standard methods. Carbohydrate was estimated by Anthrone method (Dreywood, 1946) at an absorbance of 620 nm. Lowry method is used for the estimation of protein at wavelength of 660 nm (Lowry *et al.,* 1951). The chlorophyll is estimated by methanol method for the analysis of chlorophyll content in the cells (Mackinney, 1941) with different wavelength at an absorbance of 750 nm, 666 nm, 475 nm and 653 nm by spectrophotometer (all the methods used in biochemical analysis of algal biomass are discussed in Annexure-1). Biomass concentration was determined by using UV-Vis spectrophotometer (Model no. Systronics 2203) of the culture absorbance at the wavelength of 680 nm. The lipid was estimated by using the standard method of Bligh and Dyer (1959) to determine the lipid content.These parameters were analyzed on every 5$^{th}$ day of experiment. Chapter 6 is designed with findings of bimolecules from both strains of *Chlorella* with selected wastewaters.

### 3.6. Experimental set-up

As per the objectives of study, decided for this research work, experimental plans were formulated and divided into three phases (Phase-I, II, and III) to make it more fruitful with significant findings as per methodology adopted. To enhance the algal biomass production with low-cost harvesting techniques simultaneously treatment of SHWW and OSCCW parametric characterization is taken as general objective for this study as Phase-I in Chapter-4 and 5, whereas, Phase-II covers the efficiency and co-relational study in between bio-compounds as per availability of growth nutrients in wastewater for value-added end products (neutraceuticals) studied and discussed in Chapter-6 in particular. Phase-III of this study covering the techno-economic assessments of selected approach with effect of bioreactor on growth conditions elaborative in Chapter-7. Concluding remarks with future recommendations on overall research study is provided in Chapter-8. The overall process of algal cultivation for biofuels generation of this experimental study has been illustrated in Figure 3.4. Table 3.3 showing the Chapterization of objectives as per phases.

### 3.7. Statistical analysis

All the experiment was carried out in triplicate and demonstrated in mean ± standard deviation which were calculated by using MS Office Excel (2007). The growth kinetics was measured by Graph Pad prisms software v6 (student version) for the calculation of growth rate by applying the exponential equation. The correlation analysis between the physico-chemical and biochemical parameters of algal strains was analysed with the help of Metabo Analyst, Statistical Analysis software (student version). Two different sigmoidal functions (logistic and modified Gompertz models) were applied for algal growth and biomass prediction and nutrient load reduction by algae was evaluated by using first order model. The modeling work was done using Origin Pro (2021a) software package.

**Table 3.3. Experimental plan used for present research work**

| **Phase-I (Chapter-4 and 5)**<br>**Chapter-4: Phycoremediation of slaughterhouse wastewater and their impact on algal bio-chemical compounds using both *Chlorella* species with correlation study.**<br>**Chapter-5: Phycoremediation of open-sewage contaminated channel wastewater and their impact on algal bio-chemical compounds using both *Chlorella* species with correlation study.** | **Phase-II (Chapter-6)**<br>**Algal derived biocompounds using wastewater: Theorectical Assessment for value-added products and their applications.** | **Phase-III (Chapter 7)**<br>**Techno-economic assessment:comparative study.** |
|---|---|---|
| • **Phycoremediation of SHWW using algae *Chlorella pyrenoidosa* and *Chlorella vulgaris*:**<br>• Optimization of different wastewater concentrations to obtain maximum algal growth, parameters selected are:<br>  • Pollutant load<br>  • BOD<br>  • COD<br>  • Nutrient Load<br>  • Nitrate | • Carbohydrate<br>• Protein<br>• Lipid<br>• Chlorophyll a, b<br>• Carotenoids<br>• Pigment | Photobioreactor with optimized concentration of wastewater for algal biomass and bio-chemical compounds with best algal strain. |

| | | |
|---|---|---|
| • Phosphate<br>• Impact of physico-chemical parameters on algal growth, Co-relational study between biomass productivity and biochemical compounds composition (protein, carbohydrate, chlorophyll, carotenoids) via heat maps.<br>• Kinetic models. | | |
| • **Phycoremediation of OSCCW using algae *Chlorella pyrenoidosa and Chlorella vulgaris.***<br>• Optimization of different wastewater concentrations to obtain maximum algal growth.<br>• Impact of physico-chemical parameters on algal growth, Co-relational study between biomass productivity and biochemical compounds composition (protein, carbohydrate, chlorophyll, carotenoids) via heat maps.<br>• Kinetic models. | | |

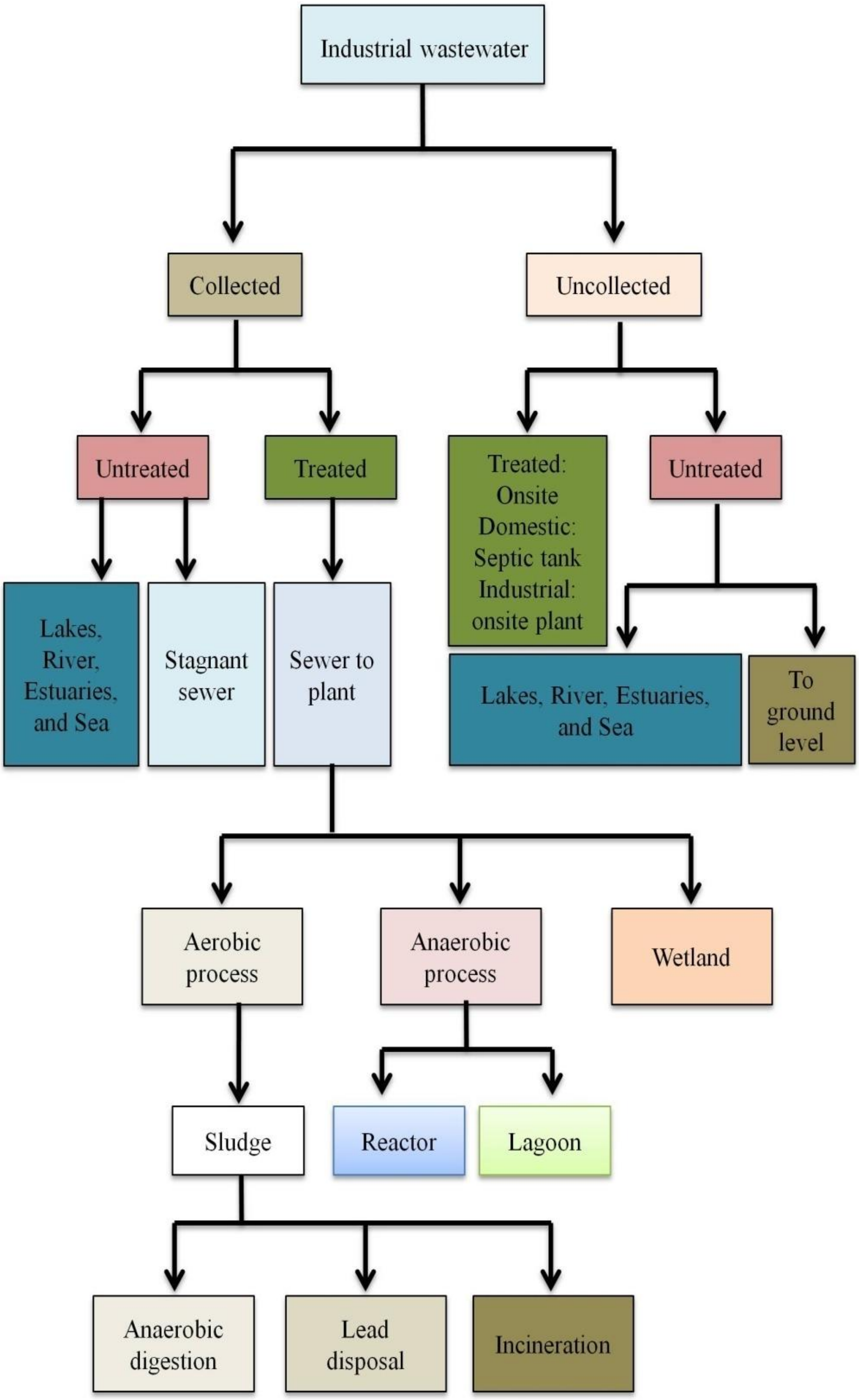



**Figure 3.3. Flow chart depicting various steps involved in wastewater treatment processes**

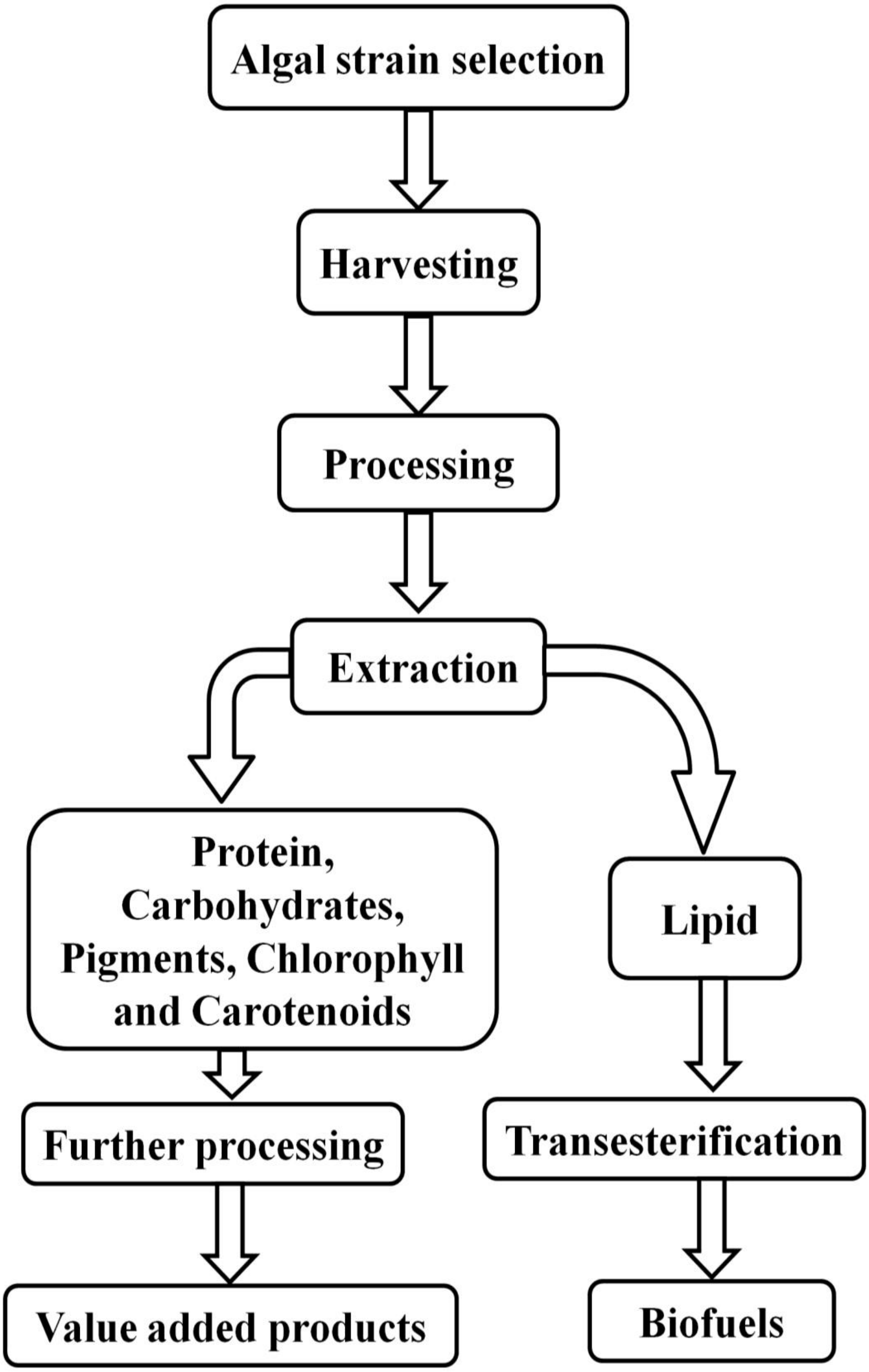



**Figure 3.4. Process applied for algal cultivation to bio-chemical profile**

### 3.8. Phase-I: Phycoremediation of slaughterhouse wastewater and their impact on algal biochemical compounds using both *Chlorella* species with correlation study

The prime objective of experiment is to culture the algal biomass under various parameters to obtained maximum biomass. The present experimental work is focused on phycoremediation approach for treatment of slaughterhouse wastewater parameters to obtained desired/ enhanced algal biomass production. The main focus of this experimental study is to impart the direct affects on algal biomass productivity and their biochemical compounds (protein, chlorophyll, carbohydrates, carotenoids and lipid). Application of growth kinetics *i.e.* specific growth rate and biomass productivity is also a significant part of this experiment set-up to understand the cross-interaction between parameters and algal growth rate. The relationship between different physio-chemical parameters and their effect on bio-chemical compound has also been studied by the application of statistical analysis *etc.* Metabo Analyst, statistical Analysis software (student version) correlation co-efficient analysis, clearly describes the significant positive and negative correlation between parameters and biochemical compounds. SHWW samples initially characterized with parameters as.

#### 3.8.1. pH

pH is a significant parameter of algal biomass growth as it investigates the solubility of essential available nutrients with noteworthy impact on algal metabolism. In order to evaluate the best suited pH value for optimum growth of *Chlorella* sp *i.e. C. pyrenoidosa* and *C. vulgaris*, pH in culture media were investigated experimentally for algal biomass growth. The pH was analyzed by a digital pH meter Model No-009 (1) A.

### 3.8.2. Nutrients

Nutrients (nitrate, phosphate, carbon and other micronutrients) play an important role for better algal growth, biomass and their development. As per the requirement of our experimental set-up, nitrogen, phosphorus, carbon (in form of salt and gas) has been taken as major source of nutrients for algal growth. So, nutrient removal is an important process for the treatment of slaughterhouse wastewater. There are different treatment process for the removal of nutrient are advanced treatment process, aerobic and anaerobic stabilization pond as well as batch reactor.

#### 3.8.2.1. Nitrate

Nitrate is the main limiting factor for the growth of algal biomass and it plays an important role for the synthesis of carbohydrates, protein and lipid. Generally, the concentration of nitrogen influences the algal growth and biochemical profile. Nitrate is mostly used for the culturing of algae as compared to ammonia. For the estimation of nitrate Catolde *et al.,* (1975) method was applied by using UV-Vis spectrophotometer (Systronics 2203) by taking absorbance at the wavelength of 410 nm. The detailed protocol is given in Annexure-1.

#### 3.8.2.2. Phosphate

Phosphorous is the important fundamental source of macronutrients that play a good role for the formation of protein/nucleic acid, DNA, RNA and ATP respectively. The nutrient content is the main factors which influence algae cultivation, temperature, light intensity, salinity, pH, mixing and also carbon dioxide. It is essential component, which promote the algal growth, lipid productivity and fatty acid contents that regulate the metabolic activity. The phosphate was estimated by stannous chloride method by using UV-Vis spectrophotometer (Systronics 2203) by taking absorbance at the wavelength of 680 nm and the term was given by (Sletten and Bach, 1961). All the detailed protocol regarding phosphate estimation is given in Annexure-1.

### 3.8.3. Kinetics of algal growth

The growth of algae can be studied under different growth phases which is lag exponential, declining, stationary and death phases. In lag phase of algae occurs, at the time of inoculums transferred from one medium to another for growth conditions. Mostly, in exponential phase the algal growth is considered for the calculation of growth, and the duration is depend upon the size of inoculums and the capacity of culture conditions for the support of algal growth. For the measurement of algal growth kinetics, the specific growth rate was observed by using Eq. (2) as follow:

$$\mu = In(P_o - P_t)/(t_1 - t_0) \qquad (2)$$

Where, μ is the specific growth rate (mg $L^{-1}$ $day^{-1}$) of *C. pyrenoidosa*. The graph Pad Prism Software v6 (student version) used to calculate the growth rate by applying the exponential Eq. (3)

$$P_t = P_O exp(r \times t) is \qquad (3)$$

Eqs. (2 and 3), $P_o$ and $P_t$ are the size of the population at initial and final at time t and r is the growth rate of population (Van *et al*., 2015). The declining, stationary and death phases can be predicted by observing the growth curve of algae. The diminishing of algal biomass generally occurs when require a specific factor for cell division that inhibit the reproduction. Stationary phase of algae is characterized with zero net growth rates and no longer remained for active metabolism and gradually enters in to the death phase. In present study kinetic parameters was obtained from growth curve of algae on varying culture conditions.

### 3.8.4. Biochemical analysis

The biochemical characterization of selected algal biomass for carbohydrate, protein, and chlorophyll were estimated by (APHA, 2012) standard methods. The bio-chemical compounds consist of (carbohydrate, protein, lipid, carotenoids and chlorophyll) is already discussed in section 3.5 of this Chapter. The following equation was used for the estimation of biomass, lipid, carbohydrates, protein and lipid productivity of SHWW these are as:

$$\text{Biomass productivity (mg/l/d)} = \text{BY(mg/l)/CT (days)} \quad (3.1)$$

Where, BY is biomass yield and CT is cultivation time

Lipid productivity (mg/l)/d

$$= \text{BP (mg/l/d)/lipid content (\%)/100} \quad (3.2)$$

Where, BP is biomass productivity in mg/l/d, lipid content is in percentage per day biomass weight

Protein productivity (mg/l/d)

$$= \text{BP (mg/l/d)* PC (\%)/100} \quad (3.3)$$

Where, BP is biomass productivity (mg/l/d), PC, is protein content in percentage as per dry weight of biomass (%)

Carbohydrates productivity (mg/l/d)

$$= \text{BP (mg/l/d)* CC (\%)} \quad (3.4)$$

Where, BP is biomass productivity is in (mg/l/d)* CC is carbohydrate content is in percentage per dry biomass weight (%).

**3.8.5. Correlation analysis**

Pearson correlation coefficient is statistical tools which are called as Pearson product-moment. It is used to measure correlation between two variables to understand the positive or negative correlation between two different variables of physic-chemical parameters of SHWW and algal biochemical profile (chlorophyll, protein and carbohydrate). Pearson correlation co-efficient (a statistical analysis) has been applied with the obtained data. The present study is focused on the correlation between two variables of different concentrations (25%, 50%, 75%, and 100%) of SHWW parameters. The high numerical value of the correlation between two variables shows the long association between two variables.

### 3.8.6. Kinetic Models

Generally, algae growth follows an "S-shape" curve which is characteristically non-linear. Non-linear growth can be easily modeled using the sigmoid functions. In this study, the growth of *C. vulgaris* and *C. pyrenoidosa* cultivated in SHWW was simulated using two different sigmoid functions viz., logistic and modified Gompertz models. The forms of the models are given below:

$$y = \frac{P}{1+(\exp)^{-\mu_m(\lambda - t)}}$$

$$y = P_{\exp}\left\{-\exp\left[\frac{\mu_m}{P}(\lambda - t) + 1\right]\right\}$$

Where, y represents the predicted algal biomass in terms of OD, P is the maximum biomass production potential, $\mu_m$ is the maximum specific growth rate, λ is the lag phase (days) while t is the time of phycoremediation experiments, respectively.

Moreover, the time-course changes in the nutrient/pollutant load reduction by algae were evaluated using the first-order reaction model. The form of the first-order model is given below:

$$\log\frac{[C_i]}{[C_t]} = -kt$$

In this, $C_i$ and $C_t$ are the initial and final concentrations of slaughterhouse wastewater pollutant at time t while $k$ represented the rate constant of pollutant removal (unit/day). The modeling work was done using Orignin Pro (2021a) software package.

### 3.9. Phase-I: Phycoremediation of open-sewage contaminated channel wastewater and their impact on algal bio-chemical compounds using both *Chlorella* species with correlation study

The main objective of this experiment is for culturing of algal biomass under different parameters for the production of maximum biomass, lipid production, nutrient and pollutant removal efficiency by using different concentration (25%, 50%, 75% and

100%) with *C. pyrenoidosa*. As per the finding after lab scale the 50% concentration was found best with algal growth and biomass productivity. The best result of 50% concentration was further investigated with *C. vulgaris* to check the potentiality of both strains. Furthermore, 50% concentrations of OSCCW with two species of *Chlorella* (*C. vulgaris* and *C. pyrenoidosa*) were selected in a comparative way at lab-scale. In OSCCW, comparative study was investigated with bio-chemical compounds (carbohydrates, chlorophyll, pigments, protein and lipid) of algae and physicochemical parameters of both strains. The correlation study was performed for the biochemical profile of two *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) with parameters physico-chemical of OSCCW 50%concentration which indicates the significant variation among them.

### 3.9.1. pH

pH is an essential parameter for the boosting of algal biomass growth as it analyze the solubility of important available nutrients with noteworthy impact on metabolism of algae. The biomass concentration is completely depends upon the pH of the culture medium, because it influence the efficiency of nutrient absorption and their photosynthesis, whereas, low pH causes enzymatic inhibition in photosynthetic process. The pH was analyzed by using a digital pH meter Model No-009 (1) A.

### 3.9.2. Nutrients

Nutrients (nitrate, phosphate, carbon, and other micronutrients) play a noteworthy role for algal biomass growth and development. On the basis, of the requirement for our experimental set-up, nitrogen, phosphorus, carbon has been taken as major source of nutrients for the growth of algae.

#### 3.9.2.1. Nitrate

Nitrate is the key factor for the growth of algal biomass, which is considered as first nutrient. The algae consume the nutrient that results show the reduction of pollution over time of course. The nitrate was estimated by Catolde *et al.,* (1975) method by using UV-Vis spectrophotometer (Systronics 2203) by taking absorbance at the wavelength of 410 nm. The detailed protocol is given in Annexure-1.

#### 3.9.2.2. Phosphate

Phosphorous is important parameters which contain macronutrients and play a significant role for the formation of protein/nucleic acid and DNA/RNA respectively. Algae have the potential to remove the phosphate from wastewater. The phosphate was estimated by stannous chloride method and the term was given by (Sletten and Bach, 1961) by using UV-Vis spectrophotometer (Systronics 2203) by taking absorbance at the wavelength of 680 nm. All the detailed protocol regarding phosphate estimation is given in Annexure-1.

### 3.9.3. Kinetics of algal growth

Algal growth can be studied under various growth phases such as lag phase, exponential phase, declining phase, stationary phase and death phase. In present study kinetic parameters was obtained from growth curve of alga on varying culture conditions. Growth kinetic is also studied and used for Phase-II of experimental plan.

Generally, algae growth follows an “S-shape” curve which is characteristically non-linear. Non-linear growth can be easily modeled using the sigmoid functions. In this study, the growth of *C. vulgaris* and *C. pyrenoidosa* cultivated in OSCCW was simulated using two different sigmoid functions viz., logistic and modified Gompertz models. The forms of the models are given below:

$$y = \frac{P}{1 + (\exp)^{-\mu_m\,(\lambda - t)}}$$

$$y = P_{exp}\ \left\{-\exp\left[\frac{\mu_m}{P}(\lambda - t) + 1\right]\right\}$$

Where y represents the predicted algal biomass in terms of OD, P is the maximum biomass production potential, $\mu_m$ is the maximum specific growth rate, $\lambda$ is the lag phase (days) and t is the time of phycoremediation experiments, respectively.

Moreover, the time-course changes in the nutrient/pollutant load reduction by algae were evaluated using the first-order reaction model. The form of the first-order model is given below:

$$\log \frac{[C_i]}{[C_t]} = -kt$$

In this, $C_i$ and $C_t$ are the initial and final concentrations of OSCCW pollutant at time t, while *k* represented the rate constant of pollutant removal (unit/day). The modeling work was done using Origin Pro (2021a) software package**.**

**3.9.4. Biochemical analysis**

The following equation was used for the estimation of biomass, lipid, carbohydrates, protein and lipid productivity of OSCCW. The productivity of carbohydrate, protein, and lipid were analyzed by equations 2., and 4 respectively that were suggested by Ansari *et al.*, (2017). The chlorophyll a, chlorophyll b, and carotenoid were measured at the stage of stationary phase, when the nitrogen concentration was in low point. The collected cells of both *Chlorella* sp. were washed 3 times by using distilled water, than centrifuge all the samples for 5 minutes at 13,000 rpm. For the extraction of chlorophyll and carotenoid contents, the supernatant was observed with different wavelength (470 nm, 666 nm, 653 nm and 750 nm) of spectrophotometer. Data are means of triplicate determinations ± standard deviations. The equation used for the estimation of chlorophyll a, chlorophyll b and total carotenoid, which is given below (Wellburn, 1994).

$$\text{Carbohydrate productivity} = \text{BP (mg/l/d)} * \text{CC (\%)}/100 \quad (2)$$

$$\text{Protein productivity} = \text{BP (mg/l/d)} * \text{PC (\%)}/100 \quad (3)$$

$$\text{Lipid productivity} = \text{BP (mg/l/d)} * \text{LC (\%)}/100 \quad (4)$$

Where, BP is the biomass productivity and CC, PC and LC represent carbohydrate, protein and lipid content respectively.

Chlorophyll a ($C_a$) = $15.65A_{666}$ - $7.34A_{653}$

Chlorophyll b ($C_b$) =$27.05A_{653}$ -$11.2A_{666}$,

Carotenoid =($1000A_{470}$ -$2.86C_a$ - $129.2C_b$)/221

**3.9.5. Correlation analysis**

The correlation analysis was investigated between two variables to investigate the positive and negative interaction/correlation. In present work, the correlation study was performed between two variables, for the biochemical profile of the two *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) with parameters physico-chemical of OSCCW 50% concentration which indicates the significant variation among them.

**3.9.6. Kinetic Models**

Generally, algae growth follows an "S-shape" curve which is characteristically non-linear. Non-linear growth can be easily modeled using the sigmoid functions. In this study (Chapter-5), the growth of *C. pyrenoidosa* and *C. vulgaris* cultivated with OSCCW was simulated using two different sigmoid functions viz., logistic and modified Gompertz models. The forms of the models are given below:

$$y = \frac{P}{1 + (\exp)^{-\mu_m (\lambda - t)}}$$

$$y = P_{\exp} \left\{ -\exp\left[ \frac{\mu_m}{P} (\lambda - t) + 1 \right] \right\}$$

Where y represents the predicted algal biomass in terms of OD, P is the maximum biomass production potential, $\mu_m$ is the maximum specific growth rate, $\lambda$ is the lag phase (days) while t is the time of phycoremediation experiments, respectively.

Moreover, the time-course changes in the nutrient/pollutant load reduction by algae were evaluated using the first-order reaction model. The form of the first-order model is given below:

$$\log\frac{[C_i]}{[C_t]} = -kt$$

In this, $C_i$ and $C_t$ are the initial and final concentrations of OSCCW pollutant at time t while *k* represented the rate constant of pollutant removal (unit/day). The modeling work was done using Orignin Pro (2021a) software package.

**3.10. Phase-II: Algal derived Bio-chemical compounds using wastewater: Theoretical Assessment for value-added products and their applications**

The main objective of this work is culturing of algal biomass under various parameters for the production of maximum biomass. In this work the bio-chemical compounds are derived from algae by treating with different wastewater (SHWW, OSCCW) for the estimation of valuable results, which is based on value-added products. Algae play an important role in daily diet such as rich in proteins, carbohydrates and lipids and are considered as an essential food for human beings and animal feed all over the world. It have the ability for the achievement of value-added products like methanol, ethanol, oil, biogas, diesel, biohydrogen, long chain hydorocarbon, phycobiliprotein , phycocyanin, phycoerythrin, vitamins (*e.g.* A, B1, B2, B6, B12, C, E, nictitate, folic acid, biotin and pantothenic acid), protein, antihelmintic, polysaccharides, ecosapentaenoic acid, arachidonic acid, docosahexaenoic acid, omega-3 fatty acid (Kothari *et al.*, 2017). However, regardless of these challenges is associated completely harvesting of algal biomass. Whereas, harvesting of algal biomass is an important part of this experimental research for value-added products.

### 3.10.1. Carbohydrate

Carbohydrates occurred in the form of dropping sugars like sucrose, lactose, glucose, fructose and polysaccharides. For the production of bioethanol, algal cell are composed of several complex carbohydrates that is cellulose, glycogen, starch and agarose. Carbohydrates are monosaccharides, oligosaccharides and polysaccharides have both comprised of metabolic and functional structure. Infact, algal polysaccharides are gaining more attention in cosmetic industry as hygroscopic agents and it is used as an antioxidants. It is natural source of highly valuable fascinating and biologically active compounds.

### 3.10.2. Protein

Algae have a potential due for their rich protein content availability and its all depend upon the environmental factor and strain used. These molecules consist of several functions which are used for nutritional, pharmaceuticals and cosmetics products. In developing countries particularly some species *Chlorella, Dunaliella salina,* have high protein due to their high protein content of algae, mainly in *Arthrospira, Chlorella* and *Dunaliella salina* have been considered as an alternative source of protein for human being.

### 3.10.3. Chlorophyll

Chlorophyll is considered an omnipresent green pigment which is naturally occurred. It has potential because it is used as a dyeing agent in food and medicinal purposes. It is extensively used as a constituent in health and hygiene products such as air fresheners, antiperspirants, lozenges, and against foul smells.

### 3.10.4. Carotenoids

Carotenoids are also a pigment that is lavishly found in algae. These highly rich molecules ranging from yellow to red are mostly known for their antioxidant property and their dyeing power. It is frequently utilized as food, feed, cosmetics and pharmaceutical products. Algae have the potential for the production of an extensive range of pigments: β-carotene, lycopene as well as violaxanthin, astaxanthin, zeaxanthin, anteraxanthin, neoxanthin and lutein. Some algal species are used for the production of value-added products which are as: *Dunaliella salina, Scenedesmus obliquus, Nanochloropsis oceanic, Nanochloropsis oculata* and *Scenedesmus quadricauda*.

### 3.11. Phase-III: Techno-economic assessment: comparative study

Techno-economic study was carried on the basis of industrial discharges for the production of algal biomass. The cultivation of algae plays an important role for the development of urban and rural areas on the basis of their socio-economic status. It increases the chances of labor and the settlement of small scale industries as a purpose of business in different sectors. The techno-economic assessment systems is totally depends upon its research development, commercial aspects and environmental elements. Therefore, algae have a potential for the production of bioenergy based valuable product for different purposes.

#### 3.11.1. Bioreactor

Algal cultivation with bioreactor is a significant process for the enhancement of biomass in pilot scale under controlled conditions with different factors like pH, temperature, light, photoperiod and nutrients. There are several factors which effect the growth of algae are: nutrient, carbon sources and temperature. The techno-economic assessment system depends on its research, development, commercial aspects and environmental elements. Keeping in view of all the objectives, algal biomass-based biofuels production with bioreactors analysis and its assessment are evaluated theoretically and experimentally are provided in Chapter-7. Therefore, the process, which is done through a lab- scale, is unable to compile the requirements in a pilot scale.

### 3.11.2. Algal derived bio-chemical compounds

In this experimental study algae play an important role for the production of bio-chemical compounds, which is very beneficial for health. The bio-chemical compounds consist of (protein, chlorophyll, carotenoids, carbohydrates and lipid) are a good source for the production of algal biomass for various applications. In this study, only 50% concentration of SHWW and OSCCW were investigated with *C. pyrenoidosa* and *C.vulgaris.* There are various bio-chemical compounds which are derived from algae are pigments, polysaccharide, lipids, antioxidants, growth promoting compounds and other compounds.

### 3.11.3. Impacts of bioreactor design and fabrication on growth and bioactive compounds

During real-time conditions optimization of light and temperature is a technical challenge. So, here in this advanced stage laboratory to reactor scale wastewater treatment (SHWW and OSCCW), we focused on ambient temperature (ranges 27-32 °C) and natural day-night light cycle (12:12 h) to carry out the experiment study with the selected algal strains. There are many developed method, which are used for high productivity, biomass quality and its density, to prevent the contamination of algal culture.

The treatment processes of wastewater with bioreactor have many advantages due to cost saving, reuse and recover of resource, water reuse and nutrient rich fertilizers. So, utilization of bioreactor for wastewater treatment for the production of large scale cultivation of algae for biomass production is a valuable approach. Therefore, this Chapter summarized the basic requirements for the completion of experimental objective from Phase-1, II, and III, whereas experimental findings are given in detailed in Chapter-4, 5, 6 and 7.

# Chapter 4

# Phycoremediation of slaughterhouse wastewater and their impact on algal biochemical compounds using both Chlorella species with correlation study

## 4.1. Introduction

The slaughterhouse wastewater (SHWW) is produced from different slaughterhouses is one most focused issues by researchers as it contains diluted blood, protein, fat, and suspended solids, resulting in a high organic and nutrient concentration in this wastewater, as well as partially solubilized residues, resulting in a highly contaminating effect in bodies if released untreated. Moreover, the slaughterhouse industry is one of the largest ones, which has economic importance for country under the category of the livestock sector. India is the fifth largest exporter of meat at the international level among the various meat-producing countries (Kumar *et al.,* 2018). For the processing of meat, the slaughterhouse produces a high amount of discharges after the cleaning and washing process. So far, no reports are presented on the use of discharge water assessed neither at the national or international level. It may be due to the non-point source at the commercial level means very small to very large, based on the number of animals sacrificed for meat on daily basis. Based on the Indian context, very little data are presented on the use of water foot printing in slaughterhouses, but authorized information regarding published records is not available. Although, physio-chemical characterization of SHWW was reported by various researchers in peer-reviewed articles (Aleksic *et al.,* 2020; Musa *et al.,* 2021). Kitrungloadjanaporn *et al., (*2017) and Kothari *et al., (*2017) also reported that the high concentrations of nutrients present in the SHWW make a favorable condition for the growth of algae and biofuel production. Slaughter waste just like any other waste can be detrimental to humans and the environment if accurate safety measures are not in use. Slaughterhouse waste is not discharged in the proper way that causes public health risks and nuisance in most nearby areas like soil and water that pollute the environment as well as infect the flies and other disease vectors. However, without any treatment wastewater discharges to the environment that largely contributes to the eutrophication. In this Chapter, algae showed high potential of treating different types of wastewater that consist of domestic, dairy, textile and municipal wastewater and alternatively produce high biomass by utilizing their organic matter as a nutrient for growth (Kothari *et al.,* 2013; Maizatul *et al.*, 2017). On the other hand, if the same concept is developed with controlled /known algal strains to treat the SHWW, it could provide new insight into the era of both water and energy crises as different types of phycoremediation approaches can treat different types of wastewater more efficiently in comparison to other conventional methods like chemical coagulation, electro-coagulation, bed sequencing batch reactor and anaerobic treatments. Nevertheless, all these methods are costly and need centralized systems for the treatment of wastewater in one place, and this could be the major challenge with these existing technologies. For hygienic reasons at the time of slaughtering need to use a large amount of water in the washing process produces a large amount of wastewater discharge. The major environmental problem associated with this SHWW consists of a large number of suspended solids and liquid waste as well as odor generated at the processing time that is very difficult to accept. In Nigeria, many slaughterhouses dispose their wastewater directly into streams and rivers without any treatment and the slaughtered meat is washed with the same water. Discharge into groundwater is a major concern, especially due to the unmanageable nature of contaminants (Muhirwa *et al.,* 2010). The processes of adsorption and trapping by fine sandy materials, clays and organic matter can remove pathogenic organisms and some dissolved organic matter during the passage of polluted water through the soil, thus reducing the microbial loads.

Hence, the present study explores to assess the impact of variation in the concentration of SHWW for the growth of *C. pyrenoidosa* and *C. vulgaris* for high biomass production. Pollutant removal through algal strain with an integrated approach of biomass production showed the benefits for biomass growth using wastewater as a resource media (Salama *et al.,* 2017; Ahmad *et al.,* 2019). Different compositions of wastewater (textile, dairy, pond, municipal and poultry) are treated with algal biomass by absorption of nutrient-rich organic pollutants and their transformation into biomass which can be used to produce biodiesel by transesterification process as reported by the researchers with different algal sp. such as *C. sorokiniana., Chlamydomonas sp., C. vulgaris* and *C. pyrenoidosa.* In SHWW, blood consist of the high pollution load followed by fats in the wastewater body and is considered as one of the major pollutants, which has the highest COD. For adequate management, it is important to know the quantity being generated daily, weekly and yearly, their characteristics as well asexisting management facilities. It is also important to understand the methods of handling and disposing of waste with proper knowledge of the basic characteristics of the waste. Keeping in view, *C. pyrenoidosa* and *C. vulgaris*was taken in this experimental study for the assessment of algal growth and bio-chemical compounds using SHWW with an aim for treatment and one of the important value-added products *i.e.* bio-oil. The novelty of the work is based on the growth of both strains of *C. pyrenoidosa* and *C. vulgaris*that was done by using different kinetic models by nutrient availability in SHWW via phycoremediation.

Therefore, the objective of this Chapter is focused on a novel approach of bioremediation via various algal strains. Presently, the algal biomass production at the commercial level uses the existing freshwater resources which are not sustainable due to their demand for drinking and other purposes and is available at a high cost. It is also an alarming condition for the environmentally sustainable part and contributes as one of the factors in capital production cost. Thus, the utilization of wastewater as an alternative source for freshwater and to reduce the cost in a dual way *i.e.* as a medium for biomass and providing low-cost source nutrients from those instead of high-cost chemicals, decrease the production cost and break the limitations with its wide applications.

### 4.2. Materials and methods

To study the objectives of this Chapter, two *Chlorella sp.* (*C. pyrenoidosa* and *C. vulgaris*) has been used for this experiment. It is further divided into physio-chemical characterization of SHWW, to know the real factual composition of selected SHWW. The kinetics models have been applied for the analysis of algal growth by using the logistic model that has shown better fitness to predict various decisive parameters as compared to the modified Gompertz model. Algal growth and optimization on different concentrations of wastewater to check the nutrient media, culture condition and biochemical profile of algae. Utilization of *Chlorella sp.* for removal of nutrient load and pollutant load in SHWW. In addition, the first-order model was helpful to determine the rate constant of the pollutant removal process. Correlation analysis investigation has been applied in between pollution load and biochemical profile of selected algae for possible outcomes in terms of value-added products.

#### 4.2.1. Reagents, chemical and glassware

Details of experimental plan for this study for reagents, chemicals and glassswares were given in section 3.2 of Chapter-3.

#### 4.2.2. Physical and chemical parameters of SHWW

The physico-chemical parameters of SHWW were assessed for this experimental study by using the standard scientific protocol (APHA, 2012)are shown in Table 4.1 with discharge limits prescribed by WHO, EU, US, Canadian and Australian standards.

**Table 4.1. Worldwide standards limits for the discharges of SHWW (Bustillo-Lecompte and Mehrvar, 2017)**

| S. No | Parameters | Present study *(C. pyrenoidosa)* | Present study *(C. vulgaris)* | World Bank Standards | EU Standards | US Standards | Canadian Standards | Australian Standards | Indian Standards |
|---|---|---|---|---|---|---|---|---|---|
| 1 | pH | 10.16±0.32 | 10.7±0.089 | 6-9 | n.a. | 6-9 | 6-9 | 5-9 | 5-9 |
| 2 | Color | Dark red | Dark red | - | - | - | - | - | - |
| 3 | Odor | Offensive smell | Offensive smell | - | - | - | - | - | - |
| 4 | TDS | 774.66±70.39 | 675.54±1.86 | - | - | - | - | - | - |
| 5 | COD | 3429.33±61.04 | 3243.38±1.61 | 125 | 125 | n/a | n/a | 3×BOD | 250 |
| 6 | BOD | 1798.33±14.01 | 1625.58±1.35 | 30 | 25 | 26 | 5-30 | 10-15 | 100 |
| 7 | Nitrate | 331.66±7.50 | 298.75±0.77 | 10 | 10 | 8 | 1 | 0.1-15 | 10-50 |
| 8 | Phosphate | 34.13±1.32 | 26.44±0.86 | 6-9 | n.a. | 6-9 | 6-9 | 5-9 | 5-9 |

### 4.2.3. Characteristics features of algal species

For this study, two *Chlorella sp.* (*C. pyrenoidosa and C. vulgaris*) have been selected to complete the objectives of this research work. Details are provided in previous Chapter (Chapter-3).

### 4.2.4. Nutrient media and culture conditions

The nutrient removal investigation was carried out in 2 liter conical flask with a working volume of 1 litre of different concentrations (25%, 50%, 75% and 100%) by using SHWW. In both *Chlorella sp.* the experiment was carried out in 500 ml of conical flasks, which consists of different concentrations of SHWW. The quantity of biomass was analyzed from $0^{th}$ to $15^{th}$ day of inoculation for the reduction of pollutant load in SHWW. Samples were centrifuged at 5000 rpm for 10 minutes at 4 ˚C and subsequently, 0.45 mm filter paper was used to filtrate. Algal biomass growth was measured by optical density by using a spectrophotometer (Model no. Systronics 2203) at the wavelength of 680 nm. The filtrate was used to analyze BOD, COD, nitrate and phosphate. The nutrient load was measured by using Equation (1) after the removal efficiency of pollutant load (APHA, 2012).

$$\text{Removal efficiency (\%)} = (S_{po} – S_{pt}/\Delta t) \times 100 \qquad (1)$$

Where, $S_{po}$ (mg $L^{-1}$) and $S_{pt}$ (mg $L^{-1}$) are initial and final concentrations BOD, COD, $NO_3^-$ and $PO_4^{3-}$ at the time of $\Delta t$.

### 4.2.5. Biochemical characterization of algal biomass

The biochemical profile consists of (chlorophyll, chlorophyll a, chlorophyll b, protein, carbohydrate, lipid and carotenoids). The analytic methods used for the biochemical profile of SHWW have been given in 3.5 of Chapter-3.

### 4.2.6. Optimization of algal growth on SHWW

The selection and characterization of SHWW have been described in section 3.4 of Chapter-3. The algal strain of *C. pyrenoidosa* was grown at different concentrations

(25%, 50%, 75%, and 100%) using SHWW. Whereas, as per the findings and optimized results obtained only 50% concentration of SHWW is further investigated with *C. vulgaris* to get comparative assessment experimentally. The experiment was started in 500 ml of the conical flask. The algal strain was used for the inoculation (10 ml) of each flask. The exponential phase of algal species was determined with the help of optical density at 680 nm on an alternate day by using a double beam spectrophotometer (Model no. Systronics 2203). For the observation of the calibration curve, the initial optical density of 0.1 cells was dried at 60 $^{o}$C for up to 24 hours and the regression equation obtained was y =298.23x-3.8 ($R^2$ = 0.98), where y is the dry weight (mg/l) and x is the absorbance at 680 nm. Best optimized findings were discussed in the result and discussion section of this Chapter.

**4.2.7. Kinetics of algal growth**

For the measurement of microalgal growth kinetics, the specific growth rate was observed by using equation: 2 and 3 which have been discussed in detail in section 3.8.5., of Chapter-3

**4.2.8. Correlation analysis**

Correlations analysis was conducted between physicochemical parameters of SHWW and biochemical parameters of algal strains with help of Metabo Analyst, Statistical Analysis software (student version) with both strains of *Chlorella (C. pyrenoidosa* and *C. vulgaris)* to know the positive correlation between two variables which is also discussed in detail in section 3.8.7., of Chapter-3.

**4.2.9. Kinetics models**

The kinetics model is used for the estimation of the growth of *C. pyrenoidosa* and *C. vulgaris* which is cultivated in SHWW, was simulated using two different sigmoid functions viz., logistic and modified Gompertz models. For the better results of the growth of *C. pyrenoidosa* and *C. vulgaris* studies, the kinetics models are used.

Generally, algae growth follows an "S-shape" curve which is characteristically non-linear. Non-linear growth can be easily modeled using the sigmoid functions. The forms of the models are given below in section 3.8.8., of Chapter-3.

**4.3. Results and Discussions**

The growth and algal biomass production were optimized with various concentrations of wastewater, and this experiment was conducted for 15 days. During the growth period, the biochemical parameters were assessed at every 5 days interval, in terms of optical density (680 nm) with organic load (COD, BOD), nutrient load (nitrate and phosphate). Besides this, the treated wastewater was analyzed thoroughly before it releases to the environment. Co-relational analysis between the physicochemical and bio-chemical profile of SHWW of algal biomass also has been studied in both *Chlorella* sp.

**4.3.1. SHWW characteristics before treatment**

SHWW looks reddish with alkaline pH (10.16), and an offensive smell. The BOD and COD were found to be in a higher range in comparison to discharge limits by Indian standards. Whereas the TDS range for discharge of SHWW was not prescribed by any one of the standard limits as given in Table 4.1. Similarly, COD (3429 mg/l) was also more than 10 times Indian standard (250 mg/l), the same trend was observed with BOD (1798 mg/l) of SHWW before treatment. The concentration of nitrate (331 mg/l) was found to be six times greater than the approved limit (50 mg/l). Similarly, the phosphate concentration was found to be again on a higher side (34 mg/l) in comparison to the permissible range (5-9 mg/l). Whereas, in *C. vulgaris* the range of physicochemical parameters have been given in Table 4.1 which is compared to the discharge limits of various national and international standards. Inorganic and organic forms of nitrogen are present in the wastewater and it includes ammonia, nitrite and nitrate respectively. In the case of SHWW produced nitrates which are the most stable form of nitrogen in the water. An algal bloom is a harmful consequence of excessive nitrates level in the water bodies on the earth (Al-Gheeti *et al.*, 2016). Eriksson *et al.*, (2002) reported that the most common form of orthophosphate comes from the use of soap and detergent products. Thus, it can be seen that high concentrations of nitrate and phosphate in wastewater are the main cause of eutrophication. The reason for high values of BOD and COD could be due to the presence of blood residues, fats, proteins, and fibers present in wastewater. Most of the organic content of wastewater is available in the form of COD. So, the higher concentration of COD manifests the various chemical reactions among organic and inorganic substances of SHWW. On the other side, BOD indicates the presence of biochemical oxidation of organic compounds available in complex forms of selected wastewater. Hence, the higher concentration of BOD is directly proportional to the higher microbial load in the SHWW. The release of raw slaughter wastewater to water bodies affects the quality of water particularly by causing a reduction of dissolved oxygen which may cause the death of aquatic life. Moreover, macronutrients, such as nitrogen and phosphorus, may cause eutrophication in the environment. The release of these nutrients triggers excessive algae growth and subsequent decay. Thus, the mineralization of the algae may lead to the deterioration of aquatic life due to the depletion of dissolved oxygen levels. Finally, SHWW may contain compounds, such as chromium and unionized ammonia, which are directly toxic to aquatic life. Similar results for SHWW composition were also found with successive experimental treatment by anaerobic baffle reactor-based aerobic activated sludge reactor and UV/$H_2O_2$ photoreactor (Bustillo-lecompte *et al.,* 2016). Therefore, the COD/BOD ratio, an indicator for the degradable scale of wastewater also assessed by the initial parameters. Its value must be below 10 (Myra *et al.,* 2015), while in the present study, it is 1.9, indicating that the compounds in the wastewater are relatively degradable and thus the algae can utilize the nutrient for their growth. The untreated wastewater discharge into the water bodies causes health problem and also pollute the environment. Therefore, selecting the algal strain which has the high potential for the treatment of SHWW with optimization of concentration, observations and findings have been discussed in detail in the next section of this Chapter.

### 4.3.2. Algal growth and nutrient removal

#### 4.3.2.1. Algal growth

The growth condition was monitored using various concentrations of SHWW, and it was assessed by optical density (680 nm). The growth of *C. pyrenoidosa* was optimized on the BG-11 growth medium (distilled water) as well as in SHWW. The growth of *C. pyrenoidosa* with different concentration of SHWW was analysed by using the kinetic models in Figure 4.2. During the period of cultivation, the exponential phase was observed between the $5^{th}$ – $9^{th}$ days. Figure 4.1 shows that the algal growth increased with increasing concentration of wastewater from 25% to 75% and the same trend was observed with 100% concentration of SHWW. In the log and lag phase, the trend of algal growth was found similar in all four concentrations. Whereas, in the stationary phase the algal growth was significantly found lower in the concentrations that are 25%, 75%, and 100% compared to 50% concentration. Although the survival rate of selected algal strain is observed well in all the concentrations of wastewater, however, 50% concentration found to be the best for the growth of algae of *C. pyrenoidosa,* based on observation 50% is further investigated for experimental study to obtain the comparative analysis between two variables with *C. vulgaris.* Figure 4.3 represents the algal growth with *C. vulgaris* with 50% concentration of wastewater, for the production of algal biomass. It may be due to the existence of optimized values of nutrients (nitrate and phosphate) availability for algal biomass. There are two main limiting factors for algae growth which are nitrogen and phosphorus as suggested by various researchers (Kothari *et al.,* 2012; Kothari *et al.,* 2013; Singh *et al.,* 2019; Yakoob *et al*., 2021). In the absence of SHWW *i.e*. in control 1(only distilled water) the algae did not show any significant improvement with growth parameters. The results indicate that the algal growth without SHWW did not support the high biomass production is because of lack of nutrients. But in the controlled condition *i.e.* with BG-11 media (control 2), wastewater-dependent growth of algae was also compared with BG-11 growth medium. The growth of algal biomass at 100% concentration was observed slow, and this may be due to higher level contaminants. Thus, it inhibits the growth and presence of some nutrients in SHWW, which are not used by *C. pyrenoidosa* efficiently. However, the growth of algae was stimulated and continued up to the 12$^{th}$ day of treatment, very much noticed with 50% concentration of wastewater may be due to the availability of nutrients in the optimized range required for growth, whereas, 25% concentration did not support the algal growth compared to control 2, that may be due to low level of nutrients in wastewater. Therefore, this study found that 50% concentration of SHWW could be the ideal condition for achieving the highest biomass production using *C. pyrenoidosa*. As per the best finding in *C. pyrenoidosa* and optimized results found with 50% concentration of SHWW is further investigated with *C. vulgaris* with 50% concentration to get comparative assessment experimentally. The growth was observed with 50% concentration with the help of optical density at the wavelength of 680 nm. The growth of *C.vulgaris* was observed with BG-11 growth medium and SHWW during the cultivation days the exponential phase was observed as compared to *C. pyrenoidosa* between the 5$^{th}$ to 9$^{th}$ day of the experiment. In *C. pyrenoidosa* the maximum algal biomass yield was observed with 430 mg/l biomass as per (dry weight) with 50% concentration of SHWW. The Logistic and modified Gompertz models are used in this study to predict the algal biomass production, specific growth rate. Figures 4.2 (a) shows the comparative prediction of the algal growth which also confirmed that the experimental and modeled data points were best fitted for the logistic model ascompared to modified Gompertz in Figure 4.2 (b). Based on two sigmoids, the Logistic model gives more precise results as compared to the modified Gompertz model which is given in Table 4.2. In *C. vulgaris* the maximum algal biomass yield was observed 390 mg/l biomass as per (dry weight) with 50% concentration of SHWW. Moreover, amongst the two sigmoid functions, the logistic model gave more precise results in terms of predicted algal biomass, maximum biomass production potential, maximum specific growth rate, and lag phase Table 4.3. In this study, the fitness of both models was within the acceptable range i.e., >0.9923. Moreover, the lag phase was observed at 6.99 and 7.61 days using the logistic model, which is slightly greater than in the case of the modified Gompertz model, which showed lag phases of 6.64 and 5.94 for tap water and SHWW, respectively. The simulated maximum specific biomass production rate was high in the case of wastewater for the logistic model, whereas, it was uncertain for the modified Gompertz model. Figure 4.4 shows the comparative prediction of the algal growth which also confirmed that the experimental and modeled data points were best fitted for the logistic model as compared to modified Gompertz.

Therefore, in the stage of stationary phase, the growth of algae was observed high with 50% concentration. Based on observation without SHWW, the growth of algae did not support high biomass due to the absence of nutrients. A study reported by Ajala and Alexander, (2020) by using two mathematical models (logistic and Gompertz model) with three strains of algae (*S. obliqus, O. minuta and C. vulgaris)* that showed the growth stimulation of algal strain, when grown with wastewater for the removal of nutrients and found that these two models are fit for the growth condition of algal strains.

**Table 4.2. Comparative evaluation of logistic and modified Gompertz models for *C. pyrenoidosa* growth kinetics**

| Model | Variables | Value | | | | |
|---|---|---|---|---|---|---|
| | | **25%** | **50%** | **75%** | **100%** | **Control** |
| Experimental | OD | 1.033 | 1.320 | 1.016 | 0.500 | 0.240 |
| Logistic | Y | 1.030 | 1.322 | 1.015 | 0.499 | 0.239 |
| | P | 1.139 | 1.468 | 1.139 | 0.601 | 0.300 |
| | Λ | 7.06 | 10.71 | 10.36 | 10.79 | 10.19 |
| | $\mu_m$ | 0.456 | 0.512 | 0.456 | 0.380 | 0.290 |
| | $R^2$ | 0.9990 | 0.9993 | 0.9999 | 0.9993 | 0.9999 |
| Modified Gompertz | Y | 1.057 | 1.349 | 1.039 | 0.510 | 0.244 |
| | P | 1.456 | 1.900 | 1.506 | 0.930 | 0.479 |
| | $\mu_m$ | 0.214 | 0.237 | 0.207 | 0.152 | 0.115 |
| | Λ | 9.69 | 10.49 | 10.22 | 11.65 | 11.58 |
| | $R^2$ | 0.9977 | 0.9980 | 0.9979 | 0.9982 | 0.9986 |

**Table 4.3. Comparative evaluation of logistic and modified Gompertz models for algal growth kinetics for *C. vulgaris***

| Model | Variables | Value | |
|---|---|---|---|
| | | Control | 50% SHWW |
| Experimental | OD | 0.691 | 0.923 |
| Logistic | Y | 0.731 | 0.951 |
| | P | 0.965 | 0.741 |
| | Λ | 6.99 | 7.61 |
| | $\mu_m$ | 0.526 | 0.578 |
| | $R^2$ | 0.9981 | 0.9964 |
| Modified Gompertz | Y | 0.747 | 0.973 |
| | P | 0.788 | 1.030 |
| | $\mu_m$ | 0.347 | 0.315 |
| | Λ | 6.64 | 5.94 |
| | $R^2$ | 0.9923 | 0.9954 |

Another study was also reported that by using *C. vulgaris* grown with domestic wastewater these two models are also suitable for the prediction of different phases of algal growth. In that study, they used three models (Logistic, Gompertz and Richards model), but the logistic and Gompertz is best after the assessment for the analysis of algal growth (Lam *et al*., 2017). Thus, this study concluded that 50% slaughterhouse concentration of wastewater may be the perfect and optimized condition for obtaining maximum algal growth, biomass productivity, and lipid extraction using *C. vulgaris* too and much better than nutrient growth media used as a control (tap water).

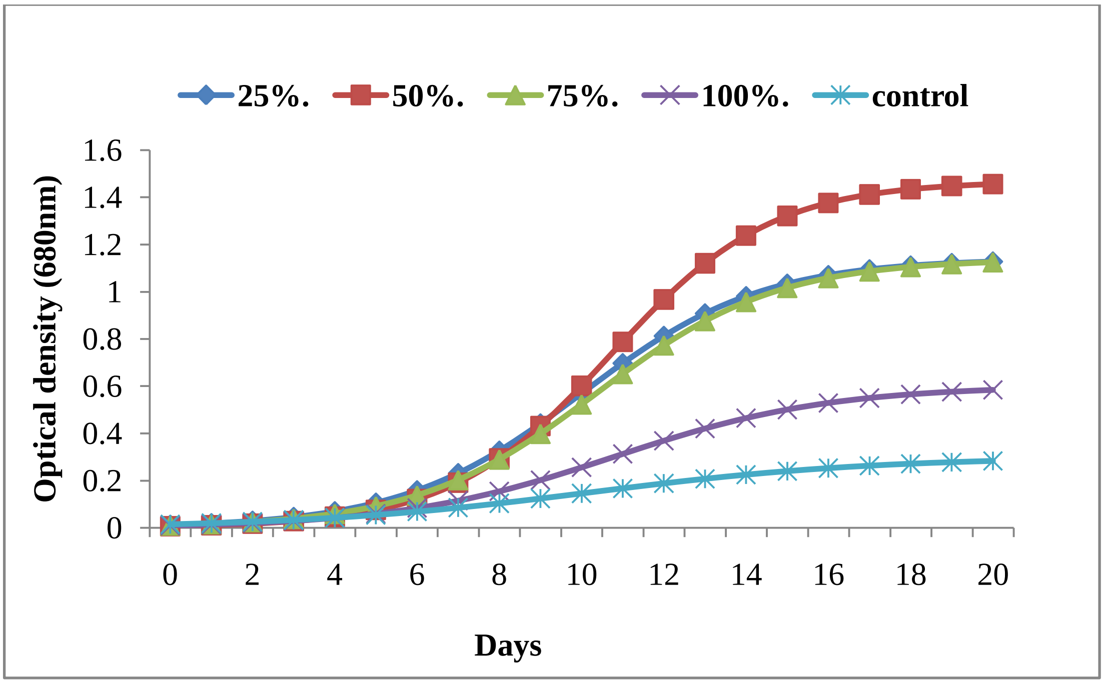



**Figure 4.1. Growth of *C. pyrenoidosa* in SHWW**

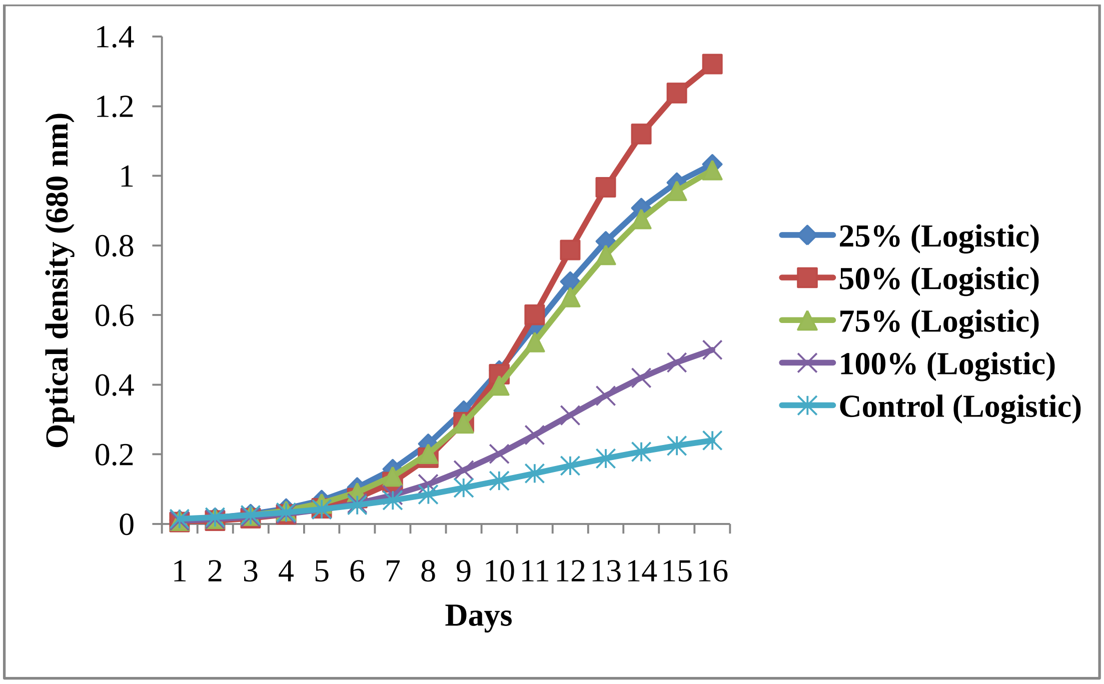



**Figure 4.2 (a). Growth of *C. pyrenoidosa* with different concentrations of SHWW by using the kinetic model**

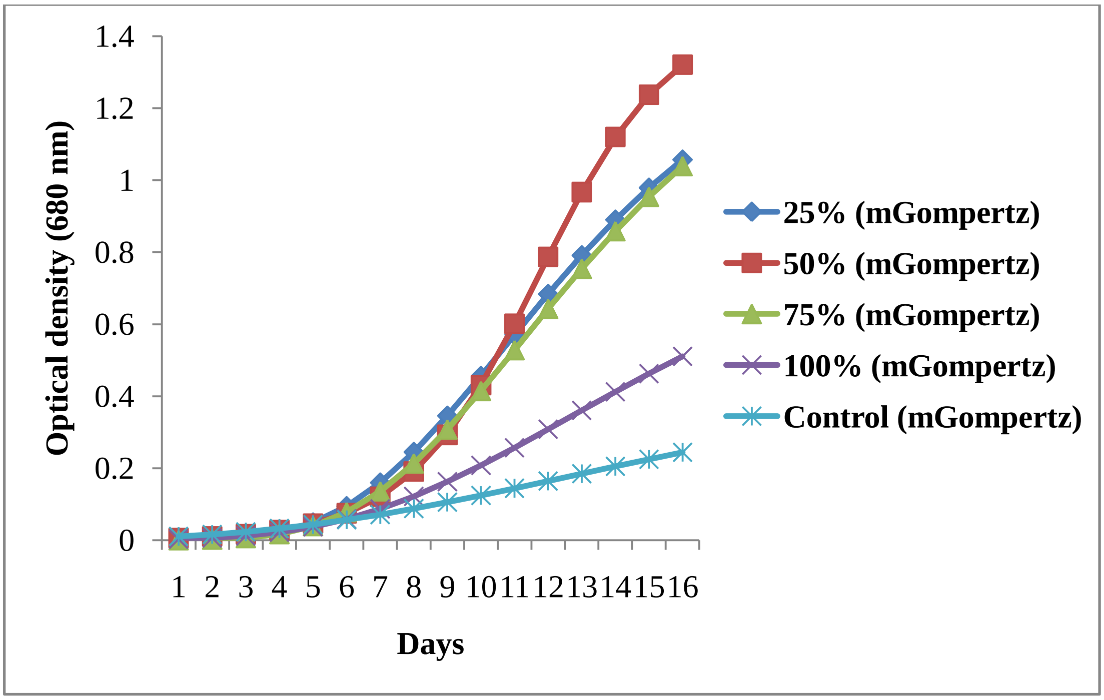



**Figure 4.2 (b). Growth of *C. pyrenoidosa* with different concentrations of SHWW by using the kinetic model**

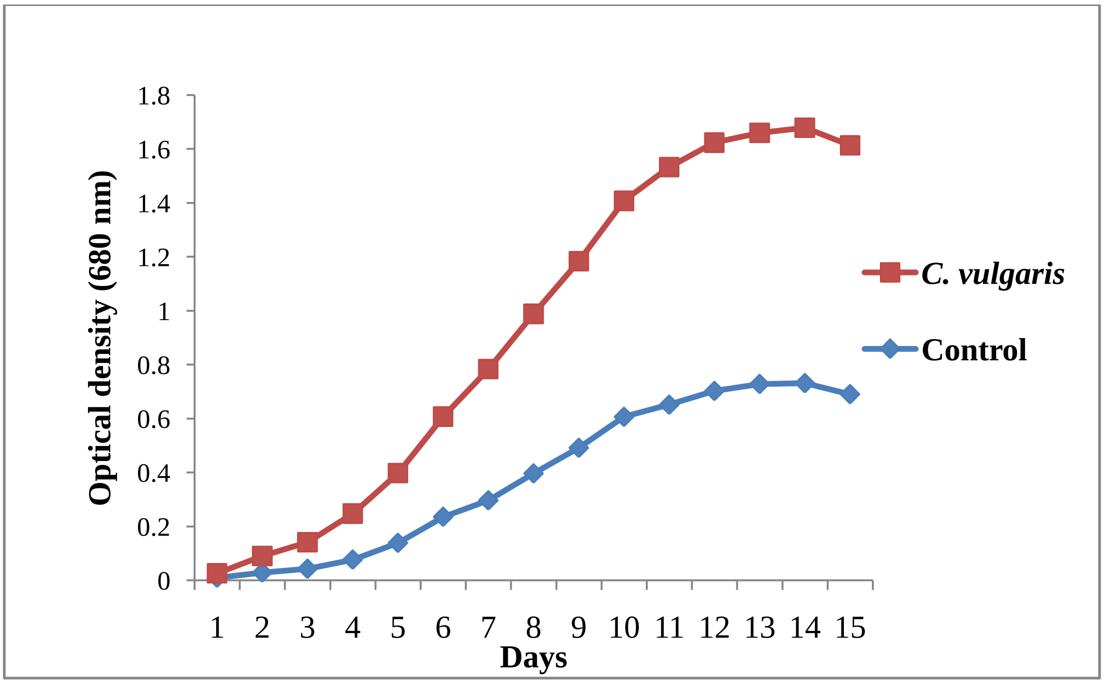



**Figure 4.3. Growth of *C. vulgaris* with 50% concentration of SHWW**

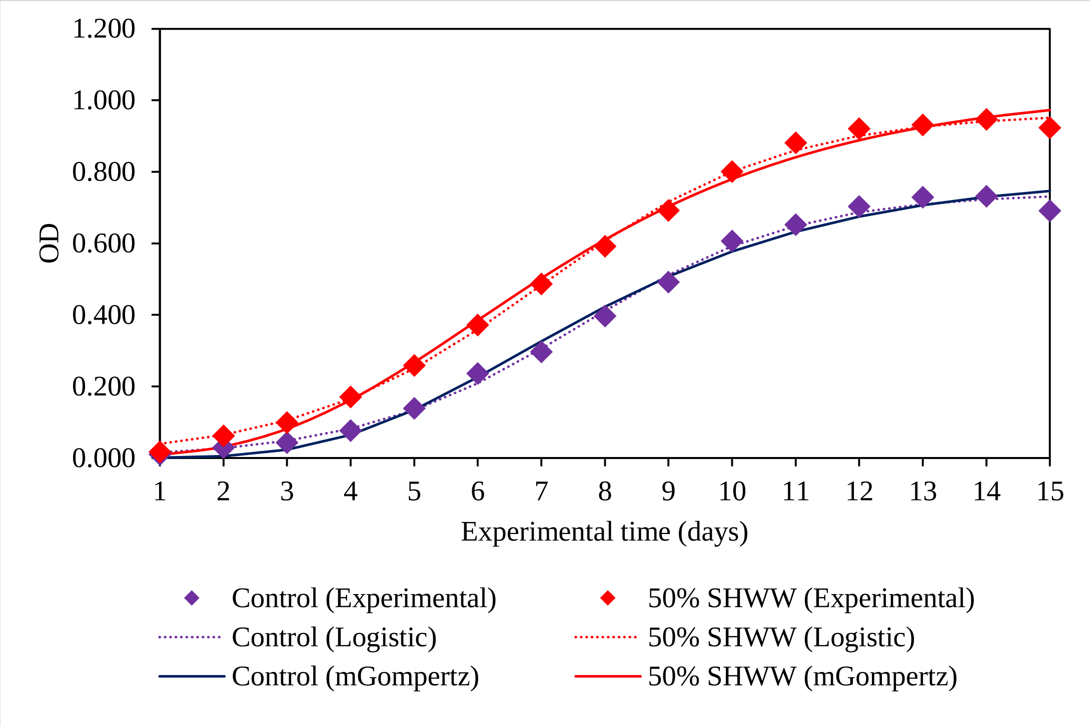



**Figure 4.4. Growth of *C. vulgaris* with SHWW of 50% concentration by using the kinetic model**

#### 4.3.2.2. Nutrient removal efficiency and kinetics studies

Phycoremediation based advanced stream of science using algal sp., for wastewater treatment and ultimately decreases the nutrient levels from the wastewater. For the study of pollutant removal the 50% concentration of SHWW, is selected for this research work. Algae favor orthophosphate for their food material, which is easy for the binding of ions for algal growth. The initial value of pH, total dissolved solids, chemical oxygen demand, biological oxygen demand, including nitrate and phosphate were observed and found to be in a higher amount than the allowable limit. After the inoculation of algal cells, the pollution load is measured from $0^{th}$ day to $15^{th}$ day of the experiment. Analysis of physicochemical parameters for $5^{th}$ day of observation indicates an increase in a reduction level of total dissolved solids, chemical oxygen demand, biological oxygen demand, nitrate and phosphate concentration from 325 mg/l (18%), 1528 mg/l (17%), 839 mg/l (7%), 133 mg/l (22%), and 13.4 mg/l (17%)

respectively as compared to the initial value obtained from uninoculated wastewater. The pollutant reduction load was investigated with different concentrations by using *C. pyrenoidosa* in percentage, which is shown in Figure 4.5. Furthermore, $10^{th}$ day of experimental investigation for total dissolved solids, chemical oxygen demand, biological oxygen demand, nitrate and phosphate concentration were found in the ranges as 211 mg/l (35%), 1243 mg/l (18%), 778 mg/l (7%), 84 mg/l (36%), and 8.6 mg/l (35%) respectively. The algal cell absorbed the nutrients from wastewater for their growth, and increase the level of algal biomass, obtained result indicates that the nutrient load is decreasing in the selected concentration of wastewater. However, the same trend was observed in the reduction of pollutant load up to the $15^{th}$ day of the experiment.

As per the experimental investigation with *C. pyrenoidosa* the best results obtained with the best concentration were investigated for the further analytical procedure. In *C. vulgaris* after the inoculation of algal cells, the pollutant load was measured from $0^{th}$ to $15^{th}$ day of the experiment. The physicochemical analysis of $5^{th}$ day of the experiment indicates that the total dissolved solids, chemical oxygen demand, biological oxygen demand, nitrate and phosphate concentration increased from 225 mg/l (29%), 1325 mg/l (32%), 670 mg/l (32%), 124.6 mg/l (33.6%) and 12.7 mg/l (29%) respectively as compared to the initial value which was without cell suspension of algae wastewater. Furthermore, $10^{th}$ day of analysis was indicate that total dissolved solids, chemical oxygen demand, biological oxygen demand, nitrate and phosphate was 135.7 mg/l (46%), 858 mg/l (35%), 420 mg/l (37%), 67.6 mg/l (45%) and 7.5 mg/l (40%) respectively. Therefore, the same findings were followed up to the $15^{th}$ day of the experimental observation based on the reduction of pollutant load. Figure 4.6 represents the pollutant load in percentage with 50% concentration of wastewater with *C. vulgaris* from the 0th to 15th day of the analysis. The high concentrations of biological oxygen demand and chemical oxygen demand in SHWW might be due to the presence of blood and complex mixtures of fats, proteins, and fibers that contribute to the increase of organic matter. The high concentrations of nutrients in SHWW may provide a suitable environment for algae growth. Nevertheless, the similarity between SHWW and wet market wastewater lies in the level of nutrients which is within the range required for algae growth (Pahazri *et al.*, 2016). The population increased the production and consumption rate of slaughtering is also increased due to the discharge of wastewater to the environment that causes pollution (Kundu *et al.*, 2013). So, all the analysis shows that the algal-based wastewater treatment is a valuable alternative method for the remediation of pollutants from wastewater. Because the algae feed, the nutrient from wastewater and result indicates that the level of pollution decreases. Although, many researchers find that algae can efficiently cultivate in nitrate and phosphate-rich nutrient wastewater environment (Kothari *et al.*, 2012) for various types of industrial effluents/influents the treatment of SHWW with *C. pyrenoidosa* and *C. vulgaris* make this approach novel and eco-friendly to treat the wastewater with low-cost investment. Table 4.4 represents the pollutant reduction load from 0th to 15th day of analysis of both strains of *Chlorella (C. pyrenoidosa* and *C. vulgaris*). SHWW discharges are harmful to the environment because nutrient level (nitrate and phosphate) is very high (Bustillo-Lecompte *et al.*, 2016).

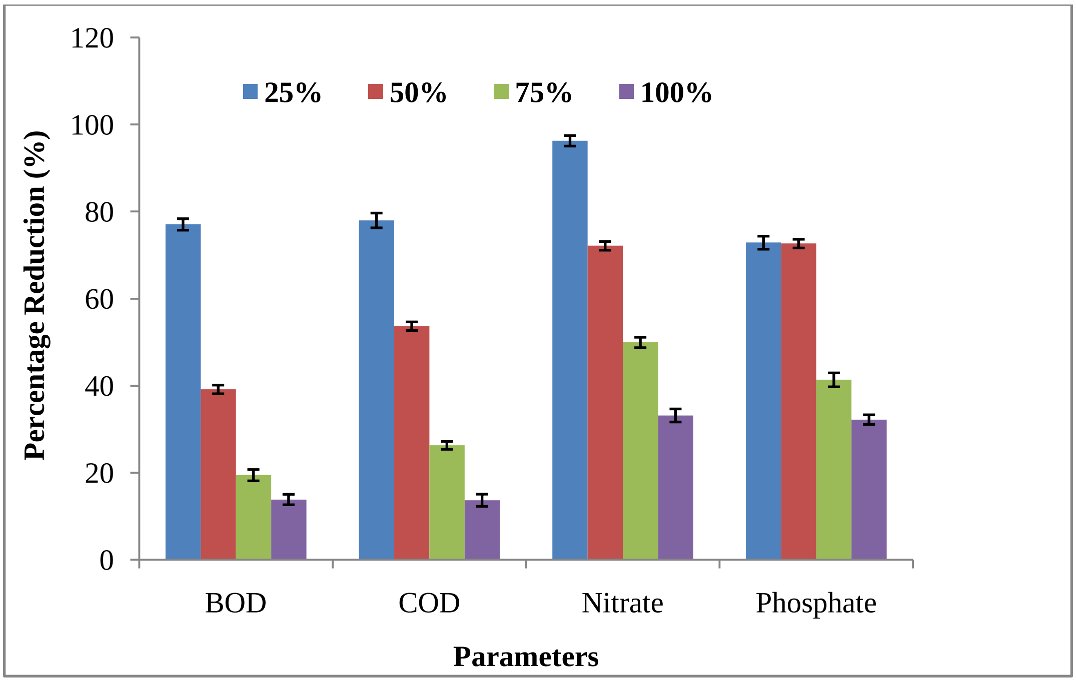



**Figure 4.5. Percent reduction in pollutant load of SHWW with selected concentrations of *C. pyrenoidosa***

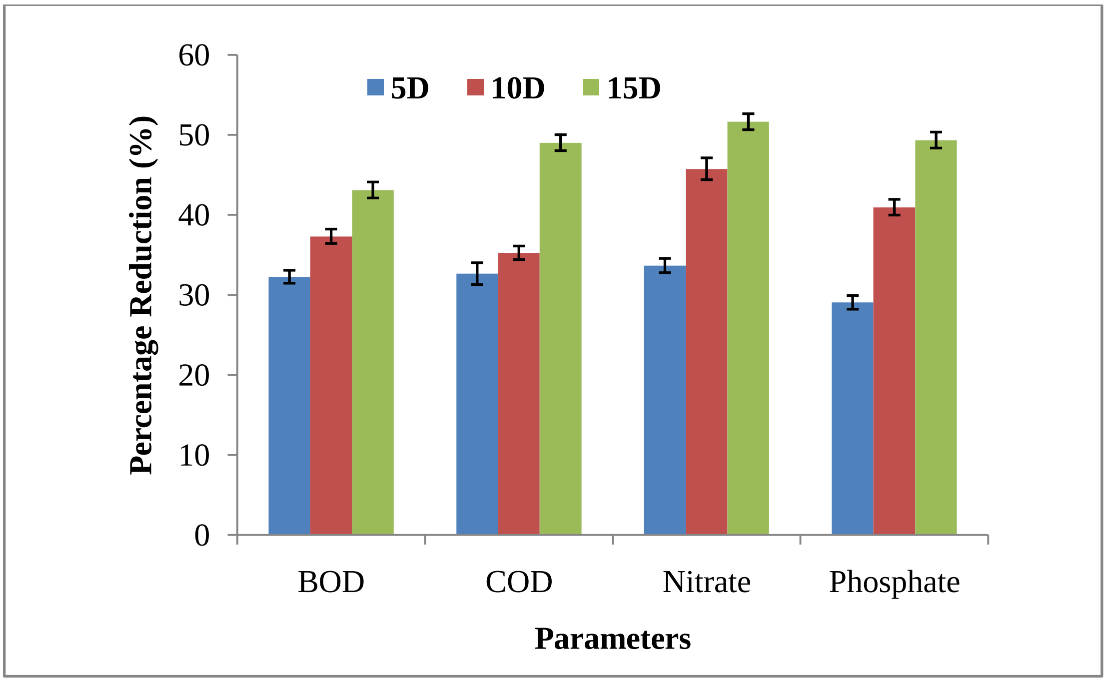



**Figure 4.6. Percent reduction in pollutant load of SHWW with *C. vulgaris* of 50% concentration**

The discharged wastewater has high organic matter that contributes to the reduction of dissolved oxygen in the water. Thus, fish and other aquatic biota cannot survive in water with low dissolved oxygen content (Mohamed *et al.,* 2016). Besides, wastewater has high nutrient levels such as phosphorus and nitrogen that can lead to eutrophication which can be toxic to aquatic organisms. In addition, eutrophication also depletes oxygen levels, destroys nursery grounds and leads to the extinction of certain species (Maizatul *et al.,* 2017). Algal growth plays an important role in the treatment of such types of wastewater. During the algal growth cycle, low-cost nutrients were used by the algal biomass which reduced the pollution load in SHWW. Nitrate is obtained from algae as a source of nitrogen only when the concentration of nitrate is useless. Based on nutrients, it has been reported that SHWW possesses a high amount of nitrogen and phosphorous (Jaish *et al.,* 2017).

From this study, it is evident that the use of algal biomass for treatment of SHWW is a viable alternative in this water and energy crisis scenario because produced biomass has a biofuels potential also as reported by various researchers (Ahmad *et al.,* 2013; Kitrungloadjanaporn *et al.,* 2017; Kumar *et al.,* 2018). The findings indicate that the pollution load of SHWW in terms of selected physicochemical and nutrient parameters was significantly ($P < 0.5$) reduced. Overall, the net removal of total dissolved solids (49.8%), chemical oxygen demand (51.6%), biological oxygen demand (49.3%), nitrate (43.0%) and phosphate (49.0%) was achieved after 15 days of the experimental period. The removal was noted gradually during the lag phase but it started increasing exponentially up to the $10^{th}$ day resulting in the maximum pollution load reduction. In *C. pyrenoidosa* the assessment of pollutant load through kinetic models by using *C. pyrenoidosa* that indicates the linear trend of pollutant reduction with the help of first-order kinetics reaction.

The rate constant of pollutant reduction were recorded as 0.0030 for pH, 0.0294 mg/l/d for phosphate, 0.0376 mg/l/d for nitrate, 0.0382 mg/l/d for TDS, 0.0612 mg/l/d for BOD, 0.0216 mg/l/d for COD, respectively shown in Table 4.5. The First-order fitness plots [Log (C) vs. t] for time-course change in pollutants reduction with *C. pyrenoidosa* were given in Figure 4.7 where the model indicates the acceptable range of fitness i.e., $R^2$>0.9498. Moreover, the kinetic assessment of pollution load reduction by *C. vulgaris* showed that the removal followed a linear trend which can be modeled using the first-order reaction. For the different parameters, the rate constants of pollutant load reduction were recorded as 0.0023 for pH, 0.0450 mg/l/d for phosphate, 0.0508 mg/l/d for nitrate, 0.0491 mg/l/d for TDS, 0.0411 mg/l/d for BOD, and 0.0429 mg/l/d for COD, respectively Table 4.6. The time-course changes in the pollutant parameters in terms of first-order fitness plots [Log(C) vs. t] were given in Figure 4.8. The model showed an acceptable range of fitness i.e., $R^2$>0.8818. A study reported by Laowansiri *et al.,* (2018) by using chicken SHWW for the implementation of the first-order model to assess the kinetics of COD removal and found that the rate constants ranged as 0.84 and 0.93 having good $R^2$ values were found in this studies that indicate high coefficient (Kaplan *et al.,* 2018).

**Table 4.4. Pollutant reduction at 0th day to 15th day by using both *Chlorella sp.* with 50% concentration of wastewater**

| | **Parameters (*C. pyrenoidosa*)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Time period** | **Color** | **pH** | **Odor** | **TDS (mg/l)** | **Nitrate (mg/l)** | **Phosphate (mg/l)** | **BOD (mg/l)** | **COD (mg/l)** |
| 0th day | Reddish | 10.2 | Offensive meat smell | 398 | 172.3 | 16.3 | 900 | 1846.6 |
| Percent reduction (%) | | - | - | - | - | - | - | - |
| 5th day | Light reddish green | 9.9 | Offensive meat smell | 325 | 133.3 | 13.4 | 839 | 1528.3 |
| Percent reduction (%) | | - | - | 18.3 | 22.6 | 17.7 | 6.7 | 17.2 |
| 10th day | Light reddish green | 9.6 | algal smell | 211 | 84 | 8.6 | 778 | 1243.3 |
| Percent reduction (%) | | - | | 35.1 | 36.9 | 35.8 | 7.3 | 18.7 |
| 15th day | Green | 9.3 | algal smell | 148 | 48 | 4.4 | 547.6 | 855.6 |
| Percent reduction (%) | | - | | 29.9 | 42.8 | 48.8 | 29.6 | 31.2 |
| | **Parameters (*C. vulgaris*)** | | | | | | | |
| 0th day | Reddish | 10.4 | Crying foul | 362 | 187.8 | 17.9 | 989 | 1967 |
| Percent reduction (%) | | - | - | - | - | | - | - |
| 5th day | Light reddish green | 10.1 | Crying foul | 225 | 124.6 | 12.7 | 670 | 1325 |

| Parameters (*C. vulgaris*) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Time period** | **Color** | **pH** | **Odor** | **TDS (mg/l)** | **Nitrate (mg/l)** | **Phosphate (mg/l)** | **BOD (mg/l)** | **COD (mg/l)** |
| Percent reduction (%) | | - | - | 29.5 | 33.6 | 29.0 | 32.2 | 32.6 |
| 10th day | Light reddish green | 9.8 | Algal smell | 135.7 | 67.6 | 7.5 | 420 | 858 |
| Percent reduction (%) | | - | - | 46.7 | 45.7 | 40.9 | 37.3 | 35.2 |
| 15th day | Light reddish green | 9.6 | Algal smell | 68 | 32.7 | 3.8 | 239 | 437.5 |
| Percent reduction (%) | | - | - | 49.8 | 51.6 | 49.3 | 43.0 | 49.0 |

**Table 4.5. First-order kinetic parameters for pollutant reduction by *C. pyrenoidosa***

| Parameter | Variable | Value |
|---|---|---|
| pH | Equation | y = -0.0030x + 1.0075 |
| | k | 0.0030 |
| | $R^2$ | 0.9498 |
| Phosphate | Equation | y = -0.0294x + 2.6223 |
| | k | 0.0294 |
| | $R^2$ | 0.9805 |
| Nitrate | Equation | y = -0.0376x + 2.2748 |
| | k | 0.0376 |
| | $R^2$ | 0.9701 |
| TDS | Equation | y = -0.0382x + 1.2666 |
| | k | 0.0382 |
| | $R^2$ | 0.9393 |
| BOD | Equation | y = -0.0612x + 3.1379 |
| | k | 0.0612 |
| | $R^2$ | 0.4540 |
| COD | Equation | y = -0.0216x + 3.2822 |
| | k | 0.0216 |
| | $R^2$ | 0.9720 |

**Table 4.6. First-order-kinetic parameters for pollutant reduction by *C. vulgaris***

| Parameter | Variable | Value |
|---|---|---|
| pH | Equation | y = -0.0023x + 1.0163 |
| | K | 0.0023 |
| | $R^2$ | 0.8818 |
| Phosphate | Equation | y = -0.0450x + 1.2915 |
| | K | 0.0450 |
| | $R^2$ | 0.9775 |
| Nitrate | Equation | y = -0.0508x + 2.3099 |
| | K | 0.0508 |
| | $R^2$ | 0.9848 |
| TDS | Equation | y = -0.0491x + 2.6005 |
| | K | 0.0491 |
| | $R^2$ | 0.9805 |
| BOD | Equation | y = -0.0411x + 3.0138 |
| | K | 0.0411 |
| | $R^2$ | 0.9932 |
| COD | Equation | y = -0.0429x + 3.3197 |
| | K | 0.0429 |
| | $R^2$ | 0.9828 |

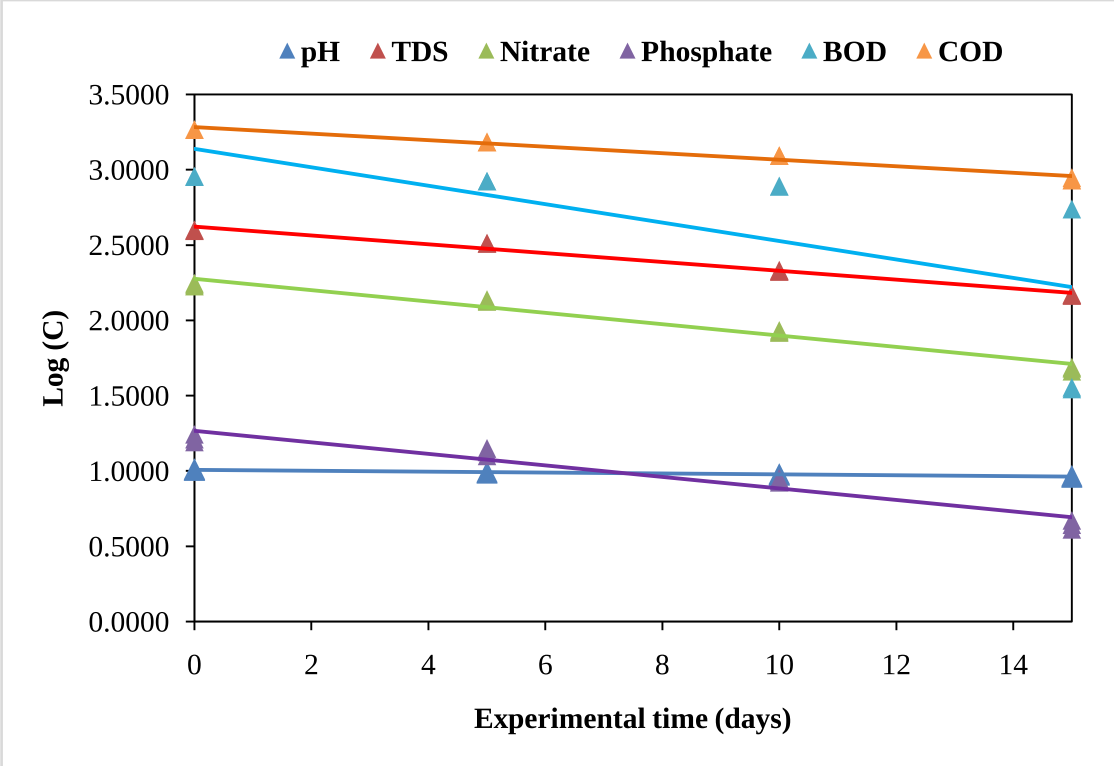



**Figure 4.7. First-order fitness plots [Log(C) vs. t] for time course pollutants reduction in *C. pyrenoidosa***

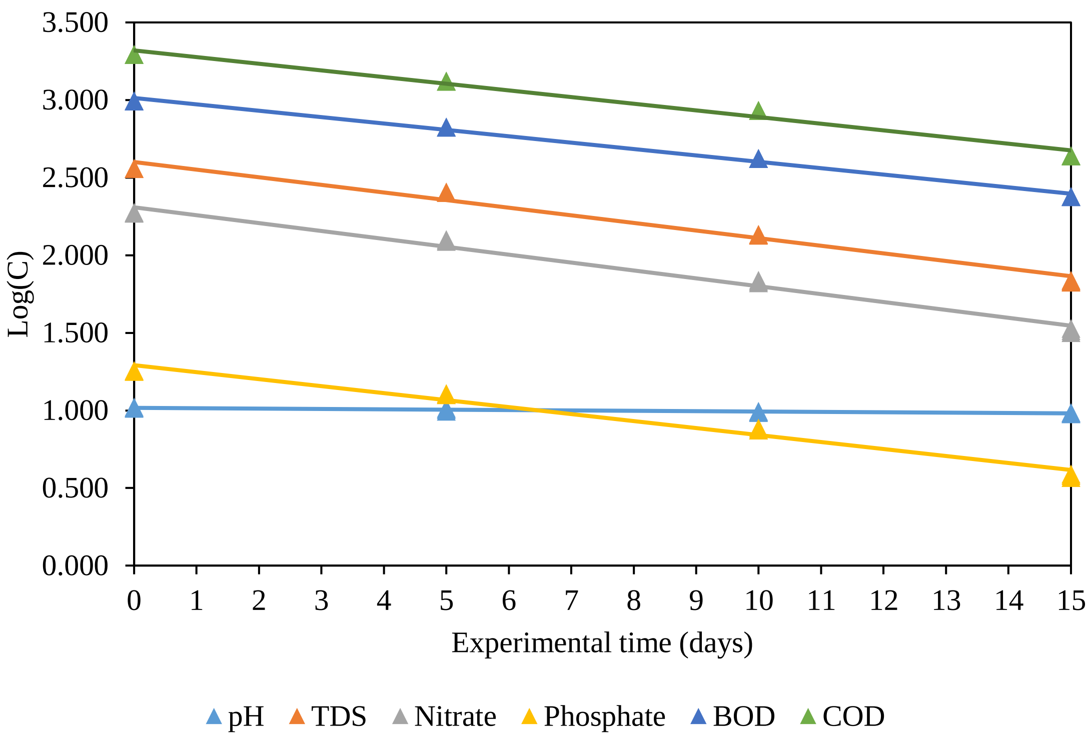



**Figure 4.8. First-order fitness plots [Log(C) vs. t] for time course pollutants reduction in *C. vulgaris***

### 4.4. Biochemical profile of algae grown in SHWW

Biochemical profiles (chlorophyll, protein, carbohydrate and lipid) of algal biomass are the source for the production of bio-based high-quality products and their productivity with both strains (*C. pyrenoidosa* and *C. vulgaris*) with SHWW. Along with light, temperature and nutrients are the essential factors for biochemical compounds,biomass growth and development. Only 50% concentration of wastewater was selected for the changes in chlorophyll, protein, lipid and carbohydrate along with growth dynamics monitored during the uptake of nutrients from wastewater. Based on growth, 50% concentration is the best for the production of biomass (430 mg/l on dry weight basis) in SHWW. For the analysis of chlorophyll, the concentration increases from $0^{th}$ day to $15^{th}$ days of the experiment are 0.31µg /ml, 2.94µg /ml, 9.50 µg /ml and 15.31µg/ ml respectively. Therefore it can be noted that the concentration of protein in SHWW with *C. pyrenoidosa* found to be increased from $0^{th}$ to $15^{th}$ days are10 mg/l (2.32%), 32 mg/l (7.44%), 42.4 mg/l (9.86%), and 53.2 mg/l (12.37%). Thus, the percentage of protein in dry algal biomass was analyzed to increase from 2.32% to 12.37% during the culture period with dry biomass. However, after inoculation, the carbohydrate content in SHWW was measured to uninterruptedly increase up to the $15^{th}$ day of the experiment 10.2 mg/l (2.37%), 22.3 mg/l (5.18%), 36.4 mg/l (8.46%) and 41.2 mg/l (9.58%). The lipid content was estimated 34.8 % in *C. pyrenoidosa* treated with wastewater.

The experimental investigation of *C. pyrenoidosa* treated with SHWW as per the finding obtained in lab-scale with best results of best concentration is further applied for the potential of SHWW with other strain (*C. vulgaris*). The biomass, lipid, carbohydrates and protein productivity of *C. pyrenoidosa* and *C. vulgaris* compared to other studies is given in detail in Table 4.8. As compared to *C. vulgaris* the chlorophyll was estimated on 0th to 5th day of the experiment is increased from day 0th to 15 day of analysis is 0.19 µg/ ml, 4.94 µg/ ml, 7.50 µg/ ml, 17.31 µg/ ml. Similarly in protein the concentration is increased from 0th to 15thday of the observation was 2.14 mg/l (14%), 3.54 mg/l (23%), 4.97 mg/l (32%) and 6.24 mg/l (41%). The same trend was followed with carbohydrate concentration. The total biomass productivity (26 mg/l/d) and lipid content (30.7%) with *C. vulgaris* was obtained. The lipid content of both *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) is shown in Figure 4.11 of this Chapter. The biochemical profile of algae with 50% concentration of *C. pyrenoidosa* and *C. vulgaris* are illustrated in Figures 4.9 and 4.10 respectively. Similarly, the lipid content with *C. vulgaris* obtained from 50% concentration of SHWW was found to be on a higher side as reported by Kitrungloadjanaporn *et al.,* (2017) by using swine slaughter wastewater for the production of lipid content (20.9%) with *C. vulgaris TISTR 8580*. This might be due to the high stress posed by a high level of pollutants that served as nutrients for algal biomass, present in wastewater. Algal biomass is known as a good source for biofuels production, due to a large amount of lipid present in the cell. In *Scenedesmus sp.,* the lipid indicates 22% of total biomass yield with low nutrient concentration (Zhang *et al.,* 2014). Algae contain maximum lipid content as compared to other terrestrial crops due to their simple structure and high potential for the production of oil (Kumar*et al.,* 2020). Therefore, the carbohydrate contents in their cells are normally high (Xin, 2011; Cassidy 2011; Baharuddin *et al.,* 2016). Algae can grow in an environment with pH ranging from 7 to 9.5 at which the algae exhibit a higher efficiency in capturing carbon dioxide in the atmosphere which then induces the high production of biomass (Zang *et al.,* 2011). However, chlorophyll content in algae decreases when the pH values increase from pH 8.5 to 9.5. The decrease in chlorophyll content is associated with low algae activity and the low removal of nutrients from wastewater. Rai *et al.,* (2015) revealed that the maximum production of the biomass was recorded at pH 7 (1.3 g /l), while the lowest production was noted at pH 8 (0.9 g /l).

Therefore, the pH of wastewater during the phycoremediation process needs to be within the optimal pH range. Table 4.7 indicates the biochemical profile of *C. pyrenoidosa* and *C. vulgaris* with SHWW compared with other studies. The present experimental investigated results clearly show the potential for algal biomass production with favorable biochemical profile with SHWW at 50% concentration. It indicates the potential of *Chlorella* sp. with other wastewater also but in comparison with SHWW for the biochemical profile of *C. pyrenoidosa,* not as high in meat wastewater (CUT), (Lu *et al.,* 2015). As per our findings, the dry weight of algal biomass is 430 mg/l with (50%) concentration of SHWW. Approximately similar results were reported by Wang *et al.,* (2010), Taskan, (2016) for *Chlorella* sp. and a mixed consortium of algae (18 species), respectively, with high nutrient-rich wastewater. So, the best findings are obtained on the ground of algal biomass productivity with the half-percentage concentration of wastewater and use of a single strain only. Similarly, Taskan, (2016) was taken an initial cell concentration of 0.2 g/l but in the present Chapter, the experiment proceeds with 0.1 g/l concentration of cell at the initial level. Hence, biomass productivity and biochemical profile are significant in our studies in reference to the treatment of wastewater, which may be responsible for unhygienic and unhealthy conditions in the surrounding, if discharged without any treatment.

In addition, many researchers used the photobioreactor method for the culturing of algae, which is a very costly process for the production of algal biomass, but in this study, lab-scale findings look to be eco-friendly as compared to other studies. It has the capability for the production of animal feed, biofuels, and also value-added products. Whereas, Ghosh *et al.,* (2016) stated that there is an inverse relationship between the production of biomass and lipid accumulation it may be due to environmental stresses. On the other hand, Ge *et al.,* (2018) reported that there is fluctuation in lipid content between the open pond and photobioreactor based harvesting also, this difference again due to environmental stress like increase in temperature of open pond system by the sunlight and it has been reported that algal biomass accumulates maximum lipid during stressful conditions and stored them in the form of TAG form in the lipid body. Similarly, Madadi *et al.,* (2021) reported the utilization of *C. vulgaris* with wastewater for the production of lipid and biomass, they found that the highest lipid content was produced by using Duncan's method with 50% concentration of wastewater. Followed by Chinnasamy *et al.,* (2010) obtain (18.10%) lipid by using industrial wastewater with *C. saccharophila.* Shen *et al.,* (2017) also reported that the lipid content of *Chlorella* sp. approx 9-35% was obtained when cultivated with different wastewater. So, for the observation according to literature provided by different researchers confirming *C. vulgaris* can be grown on wastewater for the production of lipid content and the best result was obtained in our present study as per the lipid content and biomass production.

Additionally, Sharma *et al.,* (2020) also observed the municipal wastewater treated with *Chlorella* sp. for the promotion of lipid content (30.33%), they found that 75% concentration of wastewater was found best lipid accumulation. The observed results support that the lipid content was estimated with percentage is comparatively same as per the present work. The comparative assessment of biomass and lipid, protein and carbohydrate productivity with different algal strains with the finding of the present study is described in Table 4.8 of this Chapter.

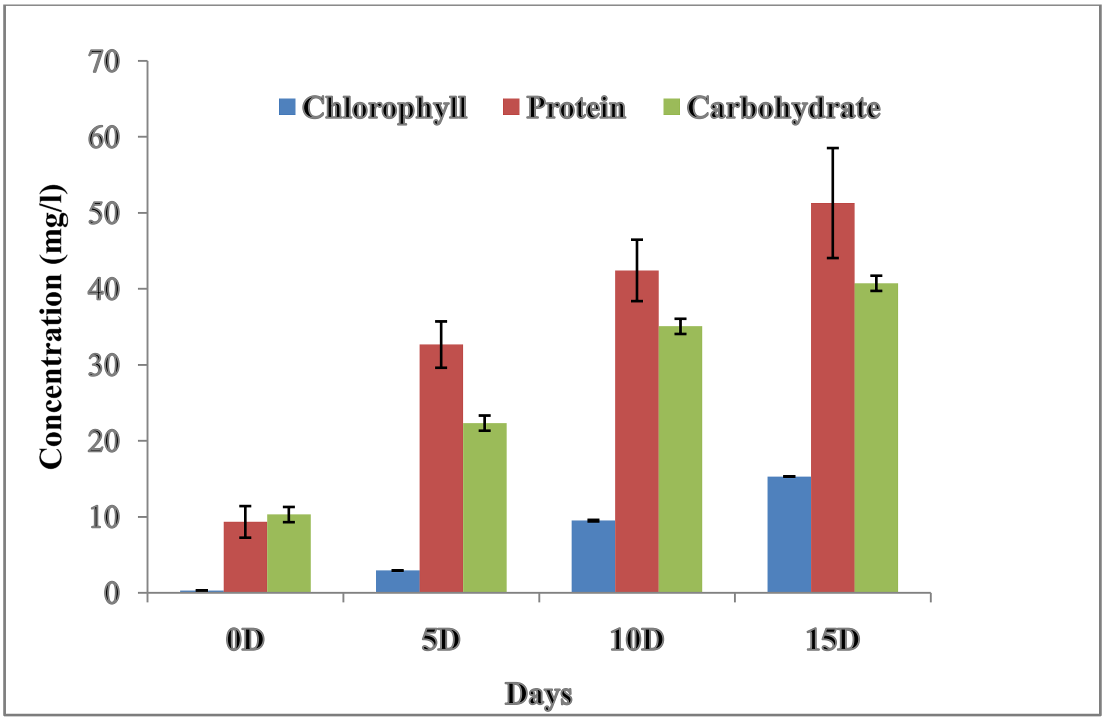



**Figure 4.9. Biochemical profile of SHWW with 50% concentration of *C. pyrenoidosa***

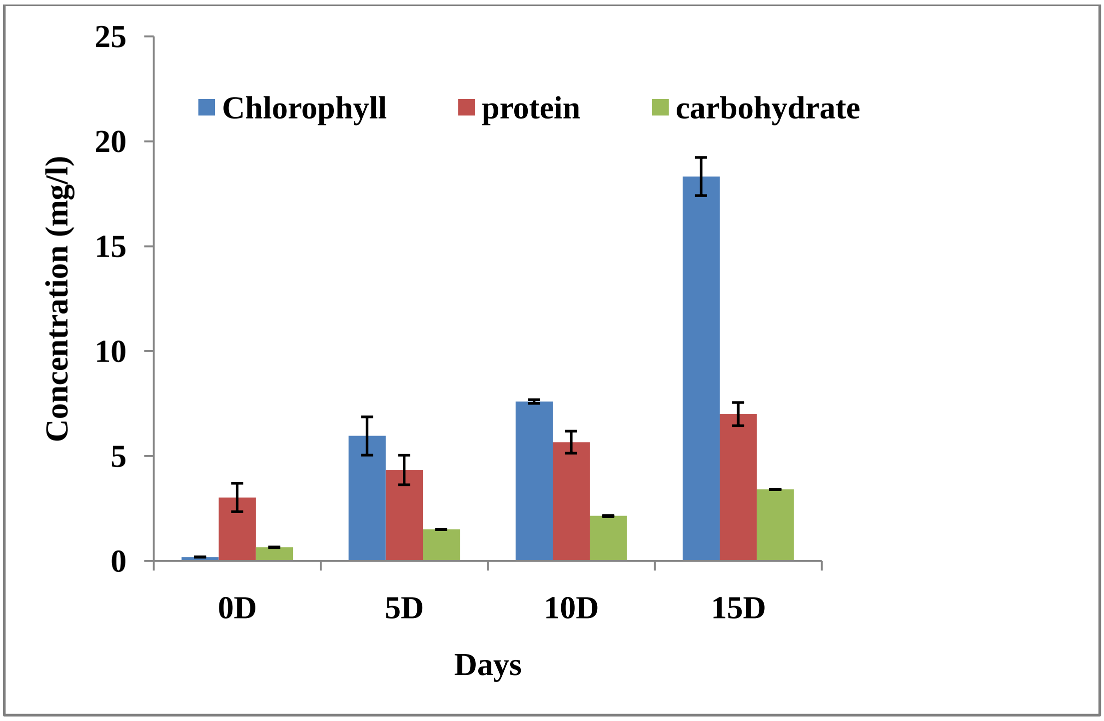



**Figure 4.10. Biochemical profile of SHWW with 50% concentration of *C. vulgaris***

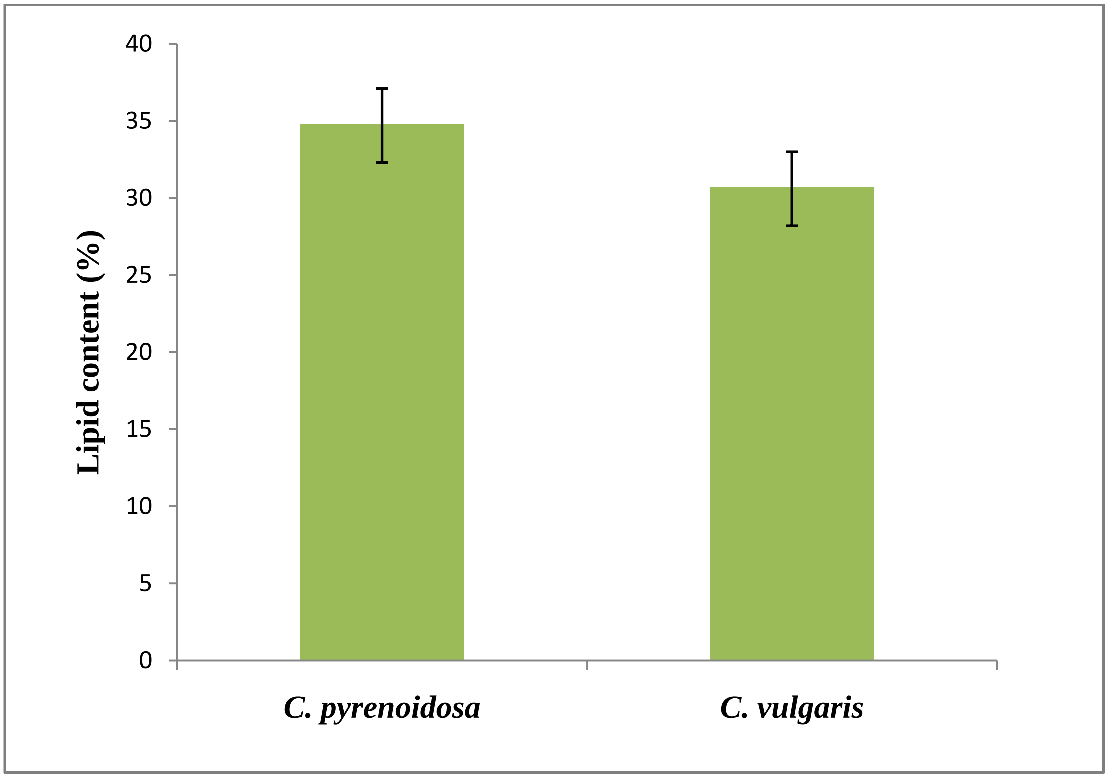



**Figure 4.11. Lipid estimation (%) of both *Chlorella sp* (*C. pyrenoidosa* and *C. vulgaris***

**Table 4.7. Comparative analysis of biochemical profile of algae with various types of wastewater with current study**

| Wastewater | Algal Strains | Biochemical Parameters | | | Culture Period (Days) | Reference |
|---|---|---|---|---|---|---|
| | | Carbohydrate (%) | Protein (%) | Chlorophyll µg/ml | | |
| Meat wastewater (CUT) | *Chlorella sp* | 27 (including nucleic acid) | 51.58 | | 9 | Lu *et al.*, 2015 |
| Industrial process water | *Chlorella pyrenoidosa* | NA | 65.2% | 5.41 mg/ gDW | 16 | Safafar *et al.*, 2016 |
| Industrial process water | *Chlorella vulgaris* | NA | 55.1 % | 32.44 mg/ gDW | 16 | Safafar *et al.*, 2016 |
| Artificial anaerobic effluents | *Chlorella pyrenoidosa* | 25.7 % | 63.7 % | 3.7 % | 15 | Zhao *et al.*, 2019 |
| Starch processing wastewater | *Chlorella pyrenoidosa* | 25.4% | 62.3% | 3.9 % | 12 | Tan *et al.*, 2019 |
| SHWW | *C. pyrenoidosa* | 9.58% | 12.37% | 15.31 µg /ml | 15 | Present study (50%) |
| SHWW | *C. vulgaris* | 22.64% | 41.32% | 17.31 µg /ml | 15 | Present study (50%) |

**Table 4. 8. Comparative study of bio-chemical compound productivity of both strains of *Chlorella* in the previous and present research**

| **Algal strain** | **Biomass productivity (g/l)** | **Lipid productivity (mg/l/d)** | **Carbohydrate productivity (mg/l/d)** | **Protein productivity (mg/l/d)** | **Metabolism mode wastewater type** | **References** |
|---|---|---|---|---|---|---|
| *C. vulgaris*<br>*Scenedesmus sp* | 4.9<br>0.75 | 80<br>- | NA<br>- | NA<br>- | Salinity+Nitrogen Starvation/wastewater | Mirizadeh *et al.*, (2020)<br>Visca *et al.*, 2017 |
| *C. vulgaris* | 2.92 | 163 | - | - | Wastewater+glycerol addition | Ma *et al.*, (2016) |
| *C. vulgaris* | 1.89±0.07 | 24.7±1 | - | - | Centrate wastewater | Ge *et al.*, (2018) |
| *C. sorokiana* | ≥7 | 83.5% | - | - | Three algae cultivation/farm wastewater | Hena *et al.*, (2015) |
| *Phaeodactylum Trocornutum* | 54.76 | 54.76 | - | - | Mixed municipal wastewater + seawater | Wang *et al.*, (2019) |
| *C. pyrenoidosa* | 28.6 (mg/l/d) | 9.9 (mg/l/d) | 2.73 (mg/l/d) | 3.53(mg/l/d) | SHWW | Present study (50%) |
| *C. vulgaris* | 26 (mg/l/d) | 7.8 (mg/l/d) | 5.8 (mg/l/d) | 10.7 (mg/l/d) | SHWW | Present study (50%) |

### 4.5. Correlation analysis of SHWW

The correlational analysis of SHWW was comprised of physicochemical and biological parameters of SHWW and in the present study, the correlation between the different concentrations of SHWW parameters, which consists of 25%, 50%, 75%, and 100% have been analyzed. The highest positive correlation was observed in 25% and 50% concentration of SHWW of TS, BOD, COD, nitrate and phosphate between the chlorophyll, protein, and carbohydrate are highly correlated which is also present in Figure 4.12. In 75% and 100% concentration, the correlation of TS, BOD, COD, nitrate and phosphate between the chlorophyll, protein, and carbohydrate was not found highly correlated, because, due to the concentration of wastewater decrease. In the case of 75% the chlorophyll, protein, and carbohydrate are not positively correlated to BOD, COD, nitrate and phosphate except TS and the same results were found in 100% concentration. The best positive correlation was found between the 25% and 50% concentrations of wastewater. There is a strong correlation reported with the case of municipal wastewater between nitrogen and phosphorous and in the context of protein, carbohydrates in the biomass (Venckus *et al.*, 2017) and in this study, a similar correlation was found in SHWW. Thus, the analysis and finding of the coefficient assist in rapid analysis in wastewater treatment and management. The significant correlation between the algal biomass and the nutrients that indicate the importance of microalgal communities can be used as an important tool for the various phases of wastewater treatment. In *C. vulgaris* the correlation analysis was comprised between the physicochemical parameters and biological parameters of SHWW with 50% concentration and the highest positive correlation was shown between the TDS, nitrate and phosphate as compared to protein, carbohydrates and chlorophyll which is shown in Figure 4.13.

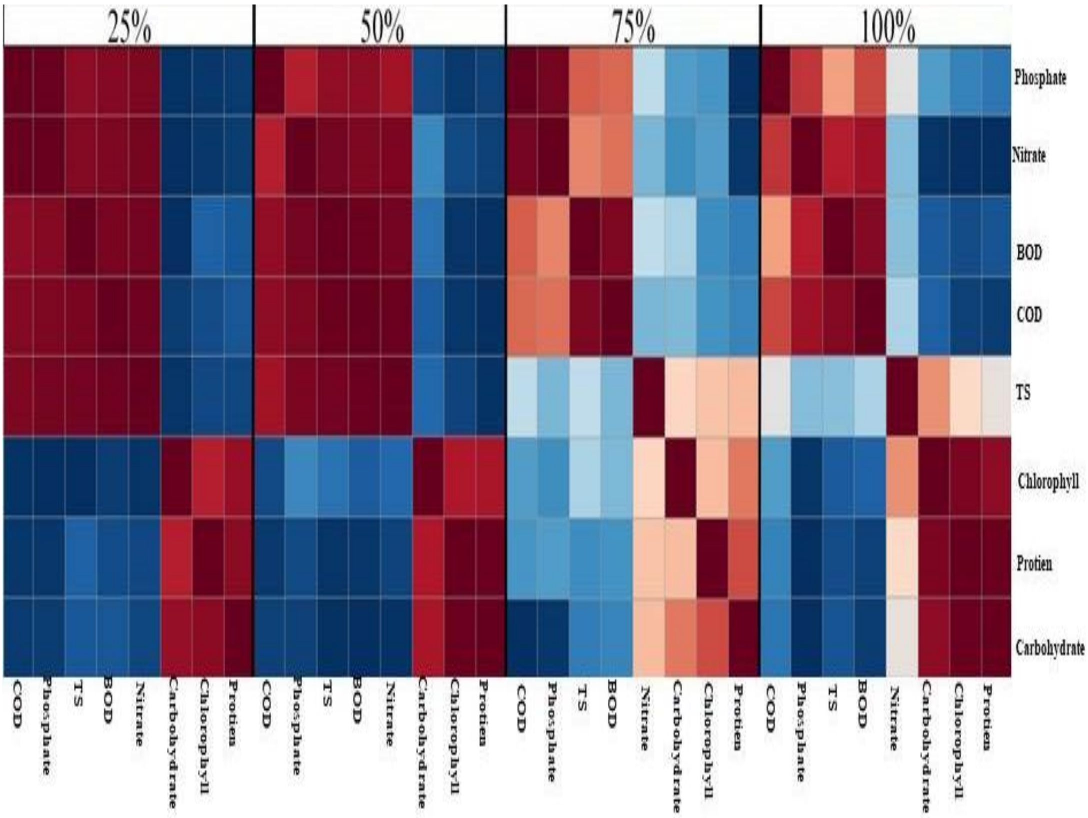



**Figure 4.12. Correlation analysis for physico-chemical parameters of SHWW and biochemical profile of *C. pyrenoidosa* with different concentration**

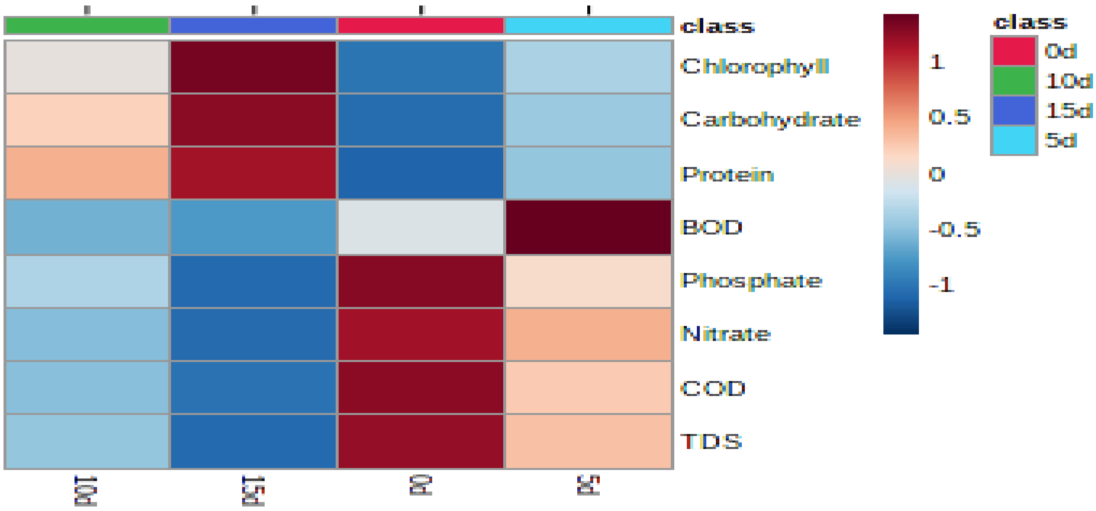
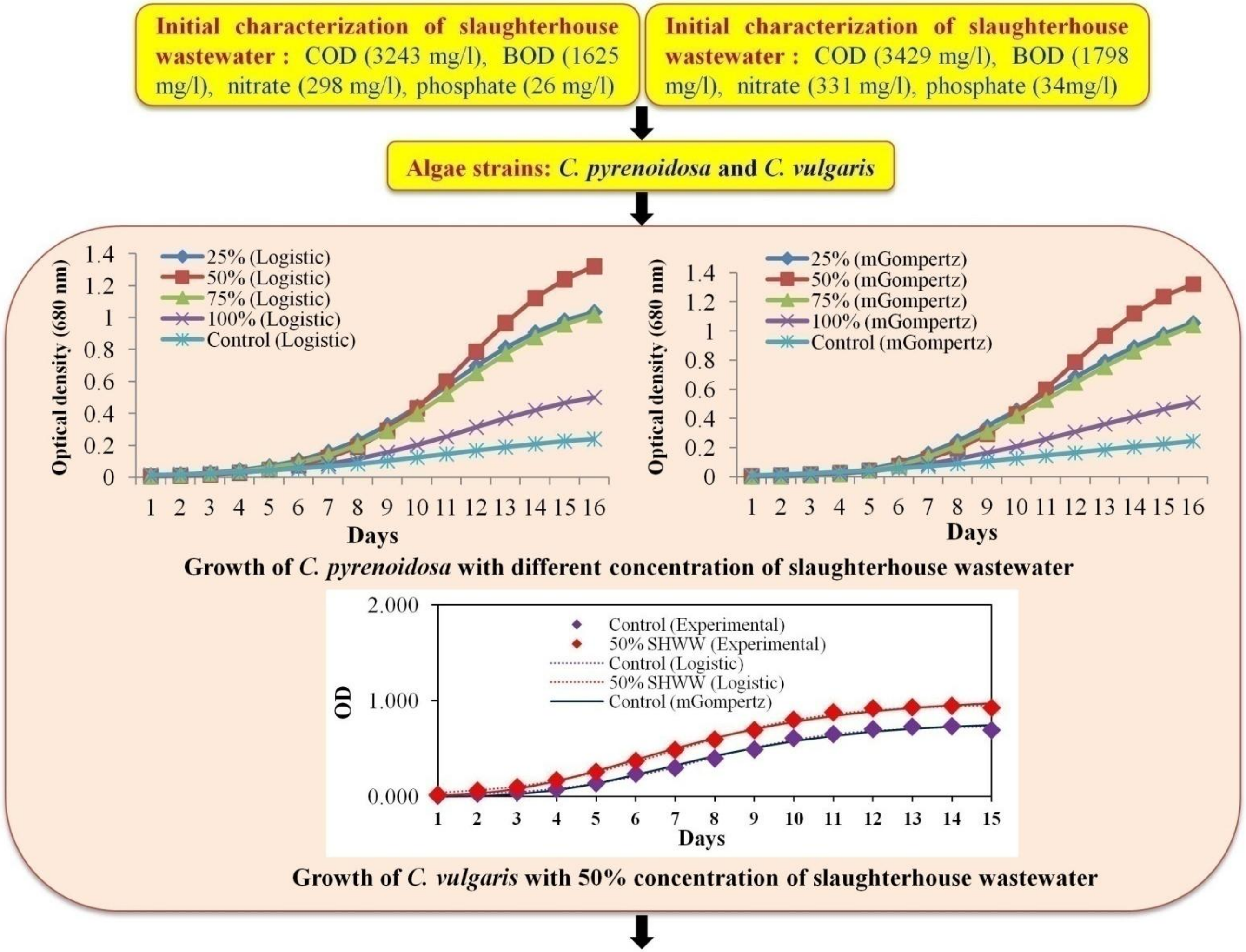



**Figure 4.13. Correlation analysis for physico-chemical parameters of SHWW and biochemical profile of *C. vulgaris* with 50% concentration**

This novel approach provides a sustainable solution for SHWW treatment because discharges of wastewater without treatment are always responsible for the deterioration of the surrounding environment and the spread of pathogenic bacteria from slaughter waste materials. In SHWW 50% concentration was the best for high biomass production (430 mg/l) and removal of pollutant load (17-31%, 7-29%) from wastewater in *C. pyrenoidosa* as compared to *C. vulgaris* with 50% concentration the biomass was yield (390 mg/l) is low compare to *C. pyrenoidosa.* In this Chapter, 50% concentration of *C. vulgaris* is investigated for biomass production (390 mg/l) and also removal of pollutant load (32-49%, 32-43%). In comparison to the modified Gompertz model, the logistic model produced better results. Figure 4.14 indicate the overall view of the experimental setup for the utilization of slaughterhouse wastewater, to reduce the freshwater footprinting, reuse of wastewater in a very low approach. Furthermore, using the concept of construction of on-site pits in combination with algal biomass near slaughterhouses may be able to solve the problem of wastewater discharges without treatment for healthy, clean and commercial solutions in favor of bio-economy. Another study reported that SHWW as a production media of algae biomass as well as the potential of biomass yields as fish feed have been explored. Moreover, *Chlorella* sp. has a high potential for their nutrient sources like protein, carbohydrate and lipid. Therefore, the phycoremediation-based process of SHWW is used for value-added products and its main aim is to reduce pollutants from waste such as sewage. Therefore, it represents a very good resource for the production of biomass.


Initial characterization of slaughterhouse wastewater : COD (3243 mg/l), BOD (1625 mg/l), nitrate (298 mg/l), phosphate (26 mg/l)
Initial characterization of slaughterhouse wastewater : COD (3429 mg/l), BOD (1798 mg/l), nitrate (331 mg/l), phosphate (34mg/l)
Algae strains: C. pyrenoidosa and C. vulgaris
25% (Logistic)
50% (Logistic)
75% (Logistic)
100% (Logistic)
Control (Logistic)
Optical density (680 nm)
Days
25% (mGompertz)
50% (mGompertz)
75% (mGompertz)
100% (mGompertz)
Control (mGompertz)
Optical density (680 nm)
Days
Growth of C. pyrenoidosa with different concentration of slaughterhouse wastewater
Control (Experimental)
50% SHWW (Experimental)
Control (Logistic)
50% SHWW (Logistic)
Control (mGompertz)
OD
Days
Growth of C. vulgaris with 50% concentration of slaughterhouse wastewater

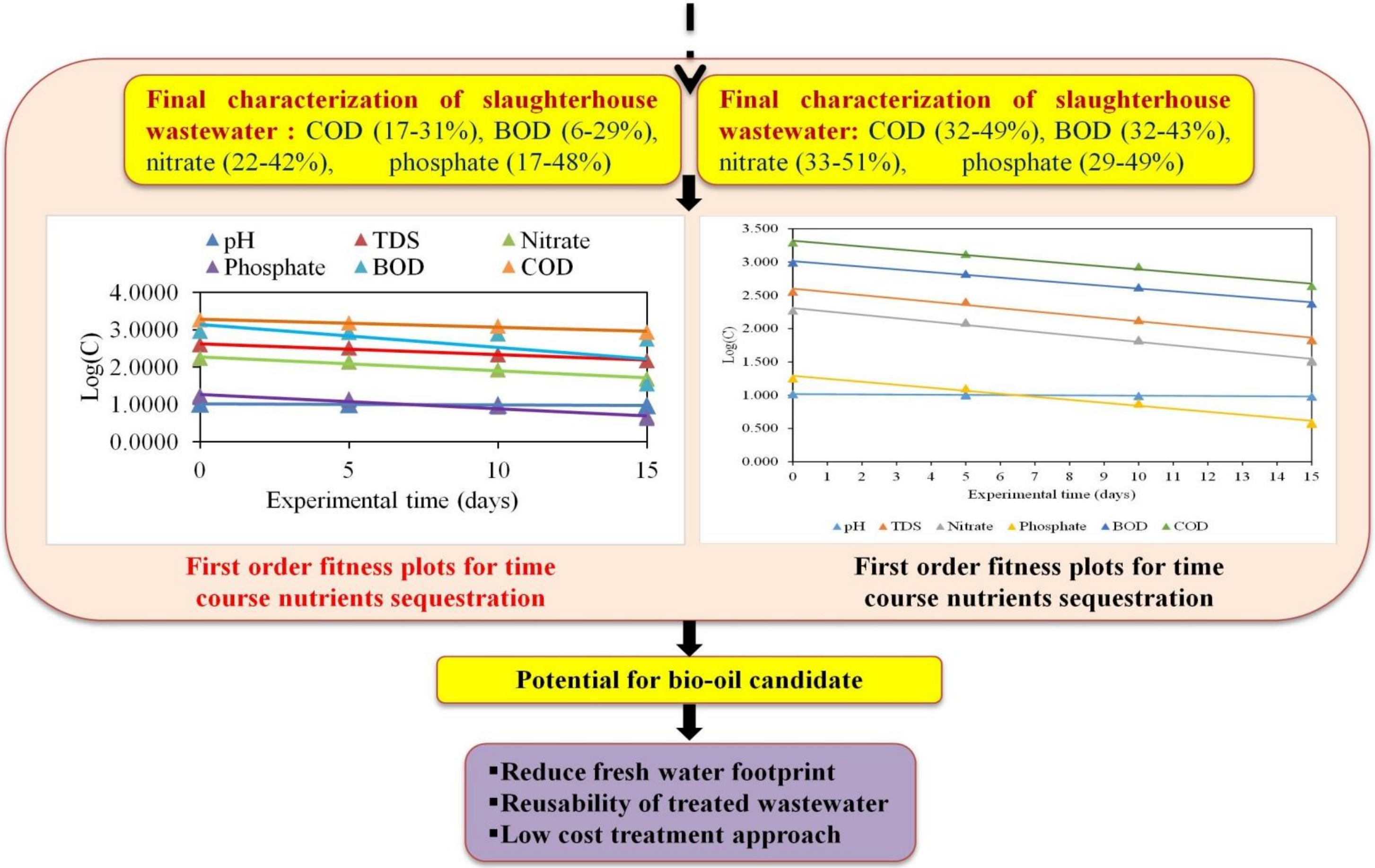



**Figure 4.14. Experimental set-up with SHWW**

# Chapter 5

## Phycoremediation of open sewage contaminated channel wastewater and their impact on algal bio-chemical compounds using both Chlorella species with correlation study

### 5.1. Introduction

Currently, global environmental issues raise unavoidable challenges for the utilization of natural resources. An increase in human population has affected the clean water bodies, which has emerged as a global issue. The biomass production via algae using industrial wastewater offers the possibility of recycling industrial residues to create new sources of raw materials for energy and material use. However, the contamination of freshwater is a global problem. Water contaminants are constantly released into the environment as a result of natural and anthropogenic activities, demands a better awareness about the chemical contamination in the surface water. The organic and inorganic impurities present in municipal, industrial and agricultural waters range from microplastics to high nutrient loads and heavy metals, nutrition and health (Wollmann *et al*., 2019). Open sewage-contaminated channel wastewater (OSCCW) usually come from mixture of toilets waste, kitchens, and bathrooms at a central wastewater treatment plant without closing material flow (Tang *et al*., 2020). The nutrients present in municipal wastewater are discharged into water bodies which can lead to eutrophication ultimately deteriorating the aquatic environment (Huang *et al*., 2020). The two key nutrients in municipal wastewater are nitrogen and phosphorous which if reused could diminish not only eutrophication and potential pollution but their recovery could act as a sustainable fertilizer source (Kehrein *et al*., 2020). The water quality degrades when the untreated wastewater is discharged into the receiving water body (lakes, streams and rivers) which create a problem for the consumption of clean water for human being.

In addition, the wastewater discharge that contains an excess amount of nitrate and phosphate into the received water body leads to eutrophication resulting from lack of oxygen in the water body (Collins *et al*., 2018). Presently, there is an exponential growth rate in the urban population leading to the high amount of wastewater, so the reuse of water is a matter of concern for sustainable development. The municipal and industrial wastewater released into the freshwater bodies also poses a serious threat to environmental challenges. Wastewater is rich in various forms of nutrients hydrocarbon and heavy metals. In wastewater phosphorous and nitrogen are especially found in the form of (phosphorous, ammonium/ammonia nitrite and nitrate) causing eutrophication. Algae, the integral part of biodiversity no doubt plays a pivotal role in the purification of wastewater. The world is facing severe scarcity of water reusing and recycling wastewater can be revolutionizing. In most cases, untreated wastewater is discharged into the ground and the natural drainage system that causes pollution and eutrophication in downstream areas. The wastewater treatment has recently shifted from pollution control to resource exploitation in view of technological viability, economic, societal needs and sustainable development. There is an urgent requirement of phycoremediation process that can treat the wastewater sustainably, and further can be exploited for fuel and other value-added products. The idea of using algae for bioremediation and simultaneous fuel production requires a high level of effort. Many researchers have been carried out using suitable algal species for the research purpose is an integrated approach (Menegazzo *et al*., 2019; Aziz *et al*., 2020; Shokravi *et al*., 2020) for conventional wastewater treatment systems. Enormous volumes of freshwater are required to transport the small volume of human excreta from the toilet to the wastewater treatment plant. Furthermore, nutrients from the toilet are diluted by rainwater, groundwater intrusion, and industrial wastewater (Jasim *et al*., 2020). Therefore, the current challenge is to rethink the present wastewater treatment system and provide the technology not only remove organic and inorganic compounds but also sustainably recover them. Population growth has increased the demand for synthetic fertilizers while concurrently depleting non-renewable phosphate rock. Therefore, the exploitation of alternative resources is required to produce new fertilizers (Jastrzebska *et al*., 2019). Algae represent a sustainable and chemical-free option for wastewater treatment due to their ability to uptake nutrients, reduce biochemical oxygen demand, heavy metals, pathogens and their ability to tolerate high load wastewater. They also represent an environmental friendly alternative to the energy-intensive chemical and physical removal processes for phosphate.

Furthermore, remediation with algae using wastewater as a low-cost nutrient source for algae cultivation, harvesting, may prove to be a source for the production of different valuable byproducts, such as lipids, fertilizers, and biofuels. Despite many studies that have shown the promise of algae for enhanced nutrient uptake and biomass production (Pacheco *et al*., 2020). Therefore, additional research is required to apply algae-based systems in real-life applications. The use of algae for bioremediation of wastewater may be an effective and reasonable approach for the developing countries as well as physical and chemical remediation approaches are expensive. Moreover, if poisonous substances are not remediated before the discharge of wastewater it may contaminate the environment and natural water bodies and also affects human health (Luka *et al*., 2014). The major composition of OSCCW is sewage and it comprises major pathogens like viruses, bacteria, and parasitic worms and non-pathogenic bacteria. Carbohydrates, fats, proteins, amino acids, and volatile acids can make up to three-quarters of organic carbon in sewage. As compared to other organisms, algae are unique, having the ability to grow mixotrophically in wastewater and utilizing organic/inorganic carbon substrate to produce as well as efficiently remediate the wastewater. Algal utilization in wastewater treatment (secondary and tertiary) processes could be a unique solution for the removal of substances. Also, algal mitigation of nutrients present in OSCCW has shown great applicability toward the production and biofuels feedstock. However, all algae are not able to tolerate wastewater environments that are toxic due to multiple factors depending on the source and type of wastewater.

Thus, screening for suitable algal strains is a prerequisite for algal-based phycoremediation of wastewater. The strain is selected based on the chemical and biological composition of the wastewater. Keeping in view this, Chapter is based on the production of algal biomass and lipid extraction for value-added products. Growth of *Chlorella* sp. is the main objective of this Chapter by using OSCCW to evaluate the treatment efficiency of physico-chemical properties of wastewater and their assessment for the production of biomass and other biochemical compounds. Kinetic study for algal growth also investigated.

**5.2. Material and methods**

All the experimental materials and methods in reference of algae and media are discussed in Chapter-3 with details.

**5.2.1. Reagent, chemical and glassware**

All the detailed procedure of chemical, glassware and reagents are given in section 3.2., of Chapter-3.

**5.2.2. Physico-chemical characterization of OSCCW**

The physico-chemical parameters of OSCCW for this experimental work by using the standard method (APHA, 2012) and their discharge limits are shown in Table 5.1 based on Indian standards.

**Table 5.1. Physico-chemical composition of OSCCW with permissible discharge limit**

| Parameters | Present Study (OSCCW) | CPHEEO |
|---|---|---|
| pH* | 9.1±0.089 | 6.5-9 |
| Color* | Greyish | - |
| Odor* | Offensive smell | - |
| TDS | 2.36±0.503 | - |
| TSS | 239±0.513 | - |
| COD | 225.42±9.3 | 20 |
| BOD | 98.29±7.17 | - |
| Nitrate | 35.05±0.32 | - |
| Phosphate | 16.39±0.32 | - |
| Sulphate | 3.09±0.48 | - |
| Ammonia | 0.041±0.003 | - |
| Nitrite | 1.46±0.032 | |

*All the parameters were in (mg/l) except pH, Color and Odor (Values indicates mean ± standard deviation (n=3)**

### 5.2.3. Optimization of algal growth in OSCCW

The algal strain of *C. pyrenoidosa* was grown at different concentrations (25%, 50%, 75% and 100%) by using OSCCW. Whereas, as per optimized results obtained with maximum growth rate 50% concentration of OSCCW is further investigated with *C. vulgaris* to get comparative assessment experimentally. Best optimized findings were discussed in the result and discussion section of this Chapter.

### 5.2.4. Nutrient removal from OSCCW using both *Chlorella* sp.

The nutrient removal efficiency was investigated in 2 L conical flask with working volumes of different concentrations (25%, 50%, 75% and 100%) by using OSCCW. The experiment was carried out in 300 ml of the conical flask, which consists of different concentrations of OSCCW. The cell suspension was inoculated for the reduction of pollutants in OSCCW. All the samples were centrifuged with 5000 rpm up to 10 minutes at $4^{o}$C and 0.45 mm filter paper was used for the filter process. The filtrate was used to analyze BOD, COD, nitrate and phosphate. The nutrient load was measured by using equation (1) after removal efficiency of pollutant load (APHA, 2012).

### 5.2.5. Biochemical analysis

Analytical protocols for biochemical profile of chlorophyll, carbohydrate, protein and lipid from harvested algal biomass are already described in section 3.5., of Chapter-3.

### 5.2.6. Kinetics of algal growth

For the measurement of kinetics of algal growth, the following equation is described in detail in section 3.9.5., of Chapter-3

### 5.2.7. Correlation analysis

The correlation analysis was conducted between physicochemical profile and biochemical parameters of both *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) with the help of Metabo Analyst, statistical Analysis software (student version).

### 5.3. Results and Discussion

In this Chapter, OSCCW treated with algae (*C. pyrenoidosa* and *C. vulgaris)* is used for the production of algal biomass and bio-chemical compounds (chlorophyll, protein, carbohydrates and lipids). For the production of algal biomass 50% concentrations of both algal strains were used in the experiment for 15 days. During the growth period, physico-chemical parameters were assessed at every 5 days gap, based on optical density (680 nm) with organic load (COD, BOD), nutrient load (nitrate and phosphate), for the reduction of pollutants. The color of OSCCW looks grayish and pH (9.1) with an offensive smell. Based on observation, the initial pH increased from day 1 to day 15 (9.1 to 9.8) of the experiment in *C. pyrenoidosa*. The same trend was observed *in C. vulgaris* pH increased from 1 to 15 days of the experiment (9.1 to 9.9). Increase in pH from day 1 to day 15 is may be due to the photosynthetic activity of the algae. The pH can affect the potential of algae to incorporate nutrients either by a change in the algal cell physiology or by a change in the availability of nutrients. However, the predominant impact of pH on nutrient removal is due to an indirect way of phosphate precipitation and ammonium volatilization (Whitton *et al*., 2015), followed by Filippino *et al*., (2015). The results of Abdel Hameed *et al*., (2007) demonstrate that the pH increased around 9 in the initial stages of treatment with various configurations of immobilized *C. vulgaris.* Same finding was observed by (Zhang *et al*., 2014; Infant *et al*., 2013) with varied initial pH from 5 to 11 for a culture of *Chlorella* sp. and the removal of both nitrate and phosphate was not significantly affected. This may imply that the initial pH of wastewater has little influence on treatment performance it was found that algal growth increased alkalinity to a similar level regardless of the initial pH. The BOD and COD were found to be higher in range in comparison to discharge limits. The nutrient availability (nitrate and phosphate) has the potential to treat algae. Therefore, there are two main limiting factors for algal growth *i.e,* nitrogen and phosphorus as suggested by various researchers (Kothari *et al*., 2012, 2013; Singh *et al*., 2019). The phycoremediation process is very effective to treat different types of wastewater that ultimately decreases the nutrient levels from the wastewater. The 50% concentration of OSCCW is selected for the production of algal biomass and bio-chemical compounds of both strains. The initial value of pH, COD, BOD, including nitrate and phosphate, were observed to be higher than the allowable limit.

**5.3.1. Algal growth and nutrient removal in OSCCW**

**5.3.1.1. Algal growth and kinetics study**

The algal strain of both *Chlorella* sp. was grown at 50% concentrations by using OSCCW. The experiment was performed in 500 ml conical flask and incubated at 30 ± 1 °C in a 12 h light (10 $W/m^2$)/dark cycle. The algal strain of *Chlorella* sp was used for the inoculation (10 ml) of each flask. The exponential phase was observed between the $5^{th}$ to $9^{th}$ days of the experiment. In the log and lag phase, the trend of growth was observed on the basis of selected concentrations of both *Chlorella* sp. whereas, in the stationary phase the growth of algae was declined in both strains of *Chlorella*. The result showed that the growth of algae in absence of OSCCW did not give the best result due to lack of nutrients. But in controlled conditions with BG-11 medium the growth of algae was observed in both *Chlorella* sp. and the best result was found in *C. pyrenoidosa* based on the growth curve as compared to *C. vulgaris.* However, the growth of *C. vulgaris* was much noticed between on $13^{th}$ – $15^{th}$ days of the experiment with 50% concentration of OSCCW may be due to the level of nutrients being high as compared to *C. pyrenoidosa*. Therefore, this study indicates that 50% concentration of OSCCW with *C. pyrenoidosa* is the best for the production of algal biomass which is given in Figure 5.1. The highest biomass content analyzed in *C. pyrenoidosa* was 0.902 g/l whereas 0.848 g/l biomass content was analyzed in *C. vulgaris* at $15^{th}$ day of the experiment. Like biomass content, biomass productivity was higher in *C. pyrenoidosa* as compared to *C. vulgaris* that was 60.13 mg/l/d and

56.53 mg/l/d respectively. Moreover, amongst the two sigmoid functions, the logistic model gave more precise results in terms of predicted algal biomass, maximum biomass production potential, maximum specific growth rate, and lag phase in Table 5.2. In this study, in the case of *C. pyrenoidosa* both models are fit within the acceptable range i.e., >0.9774. Moreover, the lag phase was observed at 8.23 and7.20 days using the logistic model, which is slightly greater than in the case of the modified Gompertz model, which showed lag phases of 0.353 and 0.816 for tap water and OSCCW, respectively. The simulated maximum specific biomass production rate was high in the case of wastewater for the logistic model, whereas, it was uncertain for the modified Gompertz model. Figure 5.2 showed the comparative prediction of the algal growth which also confirmed that the experimental and modeled data points were best fitted for the logistic model as compared to modified Gompertz. As well as in *C. vulgaris* both models are fit within the acceptable range i.e., >0.9933. Moreover, the lag phase was observed at 8.13 and 8.16 days using the logistic model, which is slightly greater than in the case of the modified Gompertz model, which showed lag phases of 0.262 and 0.389 for tap water and OSCCW, respectively. The maximum specific biomass production rate was high in the case of wastewater for the logistic model, whereas, it was uncertain for the modified Gompertz model. Figure 5.3 showed the comparative prediction of the algal growth which also confirmed that the experimental and modeled data points were best fitted for the logistic model as compared to modified Gompertz. Moreover, amongst the two sigmoid functions, the logistic model gave more precise results in terms of predicted algal biomass, maximum biomass production potential, maximum specific growth rate, and lag phase Table 5.3.

**Table 5.2. Comparative evaluation of logistic and modified Gompertz models for *C. pyrenoidosa* growth kinetics**

| Model | Variables | Value | |
|---|---|---|---|
| | | BGCP | WWCP |
| Experimental | OD | 1.690 | 0.902 |
| Logistic | Y | 1.791 | 0.925 |
| | P | 1.823 | 0.925 |
| | Λ | 8.23 | 7.20 |
| | $\mu_m$ | 0.596 | 1.089 |
| | $R^2$ | 0.9861 | 0.9672 |
| Modified Gompertz | Y | 1.830 | 0.929 |
| | P | 1.956 | 0.929 |
| | $\mu_m$ | 7.34 | 6.67 |
| | Λ | 0.353 | 0.816 |
| | $R^2$ | 0.9774 | 0.9504 |

**Table 5.3. Comparative evaluation of logistic and modified Gompertz models for *C. vulgaris* growth kinetics**

| Model | Variables | Value | |
|---|---|---|---|
| | | BGCV | WWCV |
| Experimental | OD | 1.490 | 0.988 |
| Logistic | y | 1.442 | 1.165 |
| | P | 1.525 | 1.178 |
| | λ | 8.13 | 8.16 |
| | $\mu_m$ | 0.482 | 0.638 |
| | $R^2$ | 0.9930 | 0.9740 |
| Modified Gompertz | y | 1.513 | 1.185 |
| | P | 1.723 | 1.244 |
| | $\mu_m$ | 7.24 | 7.25 |
| | λ | 0.262 | 0.389 |
| | $R^2$ | 0.9933 | 0.9644 |

Based on observation without OSCCW, the growth of algae did not support high biomass due to the absence of nutrients. A study reported by Ajala and Alexander, (2020) using two mathematical models (logistic and Gompertz model) in three strains of algae (*Scenedesmus obliqus, Oocytis minuta* and *Chlorella vulgaris*) showed the growth stimulation of algal strain, when grown with wastewater for the removal of nutrients and found that these two models are fit for the growth condition of microalgal strains. Another study also reported that by using *Chlorella vulgaris* grown with domestic wastewater these two models are also suitable for the prediction of different phases of algal growth. In this study, they used three models (Logistic, Gompertz and Richards model), but the Logistic and Gompertz are best after the assessment for the analysis of algal growth (Lam *et al.*, 2017). Thus, this study concluded that 50% slaughterhouse concentration of wastewater may be the perfect and optimized condition for obtaining maximum algal growth, biomass productivity, and lipid extraction using *C. vulgaris* too and much better than nutrient growth media used as a control (tap water).

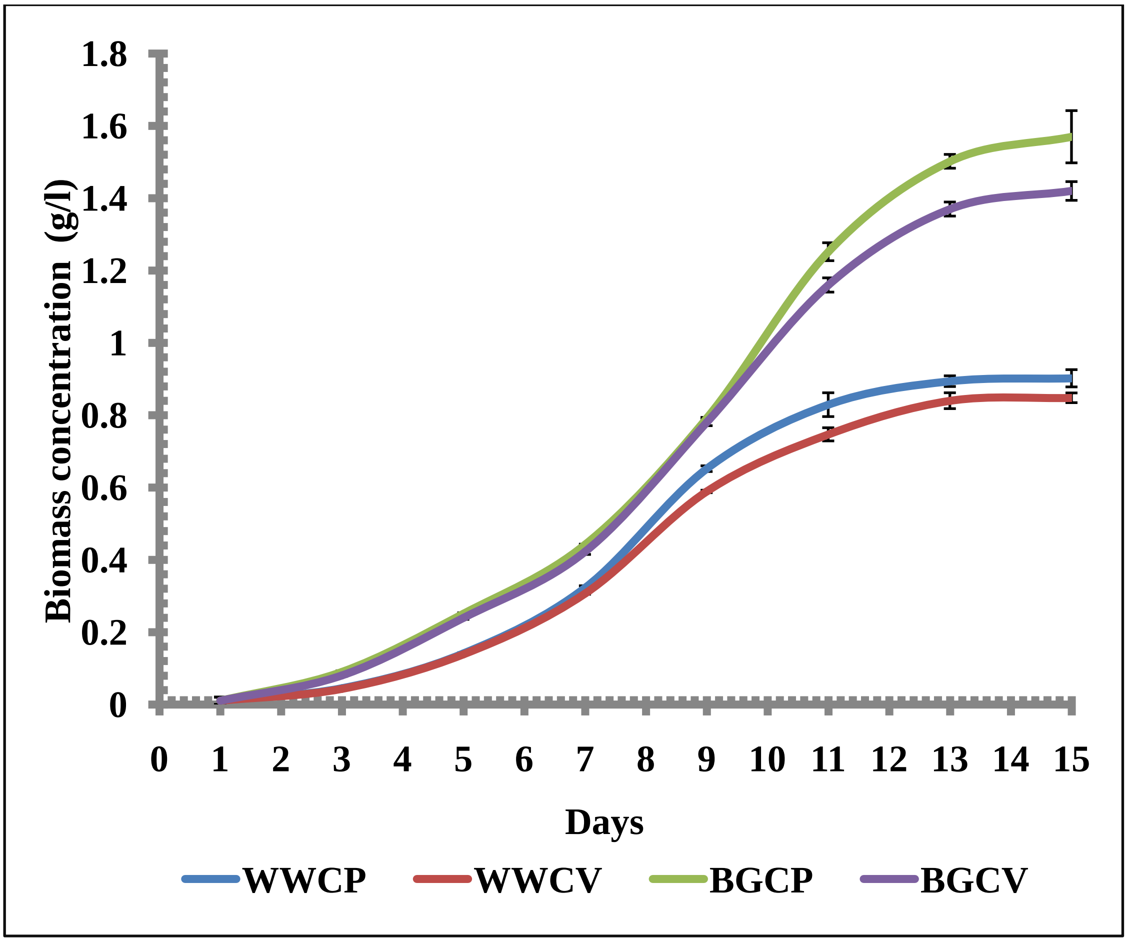



**Figure 5.1. Growth of both strains of *Chlorella* sp. in 50% concentration OSCCW**

(Note: WWCP=*C. pyrenoidosa* in wastewater; WWCV=*C. vulgaris* in wastewater; BGCP= *C. pyrenoidosa* in BG11; BGCV= *C. vulgaris* in BG11)

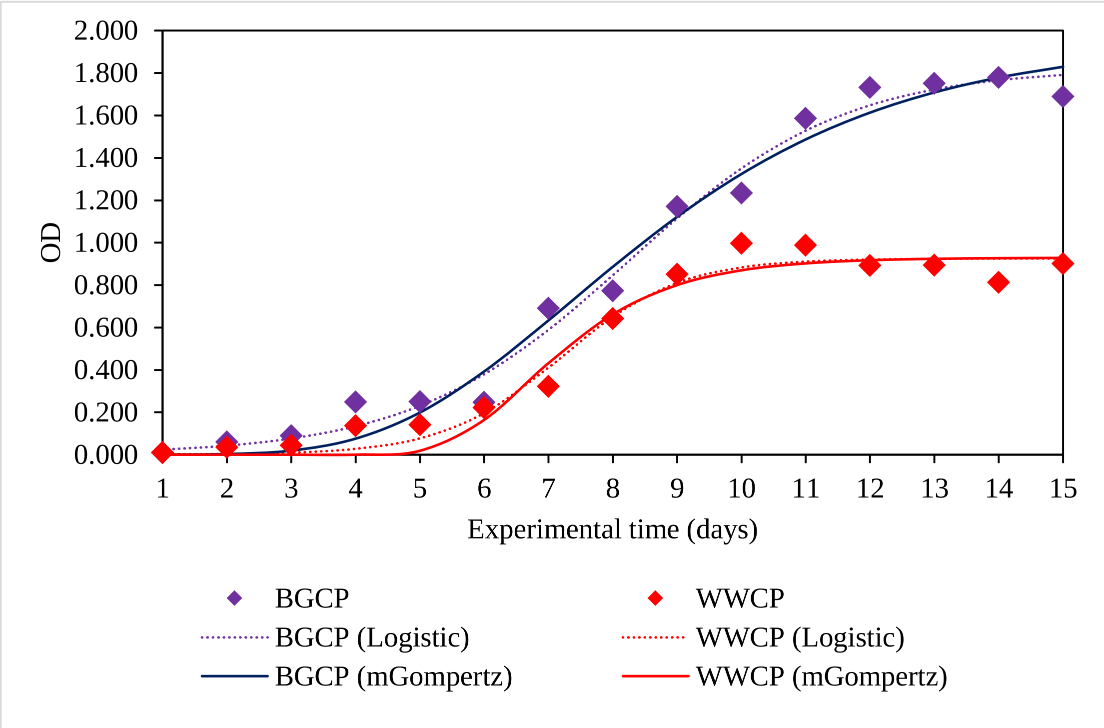



**Figure 5. 2. Growth of *C. pyrenoidosa* with OSCCW of 50% concentration**

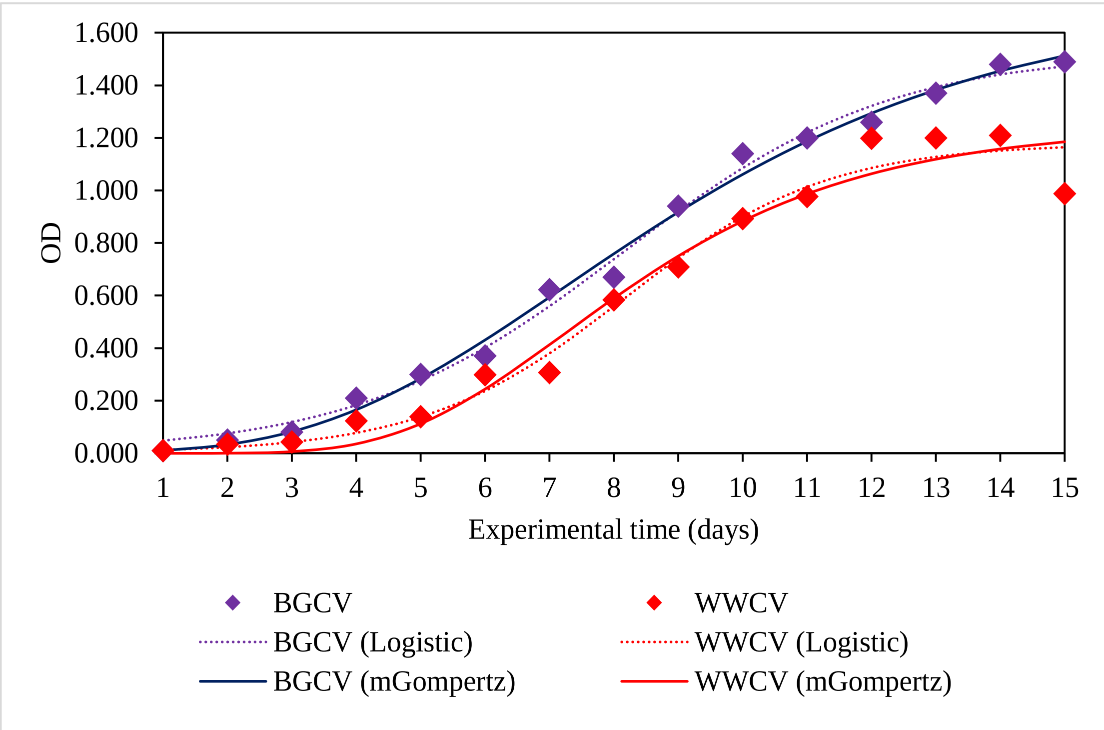



**Figure 5.3. Growth of *C. vulgaris* with OSCCW of 50% concentration**

### 5.3.1. 2. Nutrient removal by using OSCCW

Phycoremediation is a process in which algal-based wastewater treatment is used to decrease the nutrient levels in the wastewater. For best algal growth 50% concentration of both *Chlorella* sp. are selected for study for the reduction of pollutant. The initial value of pH, COD, BOD, nitrate and phosphate were observed and found to be in a higher amount than the allowable limit, as shown in Table 5.1. After inoculation of algal cells with both *Chlorella* sp. the pollution load is measured on $0^{th}$ to $15^{th}$ day of the experiment. Analysis of physicochemical parameters of *C. pyrenoidosa* on the $5^{th}$ day showed reduction in level of COD, BOD, nitrate and phosphate concentration from 199.10 mg/l (11.67%), 74.41 mg/l, (24.29%), 22.02 mg/l, (37.12%) and 11.2 mg/l, (31.66%) as compared to the initial values obtained from inoculated wastewater.Same trend was followed with $5^{th}$ day of physicochemical analysis of *C. vulgaris* 178.09 mg/l (20.99%), 84.42 mg/l, (14.11%), 24.7 mg/l, (29.46%) and 13.43 mg/l, (18.05%) respectively. Furthermore, the same tendency was followed on the $10^{th}$ day of the experiment in *C. pyrenoidosa* on analysis COD, BOD, nitrate and phosphate concentration were found in the ranges 121.31 mg/l (46.18%), 36.96 mg/l (57.37%), 13.07 mg/l (62.6%), and 7.9 mg/l, (51.7%) respectively. Therefore, the same tendency was followed on the $10^{th}$ day of the experiment with *C. vulgaris* on analysis of COD, BOD, nitrate and phosphate were found in range 109.11 mg/l (51.5%), 47.9 mg/l (51.2%), 11.9 mg/l (66%), 5.65 mg/l (65.5%) respectively, which is given in Table 5.4. (Ahmad *et al*., 2016; Zhou *et al*., 2012; Guldhe *et al*., 2017) reported that 100% of nitrate was reduced when *S. obliquus* was cultured in OSCCW, and 60% nitrate removal was reported during growth of *A. protothecoides* in OSCCW after 6 days same observation was reported. 76% of nitrate was removed by using *C. sorokiniana* in aquaculture wastewater respectively. The algal cells absorbed the nutrients from wastewater for their growth and development result showed that the nutrient load is declining in the selected concentration of both algal strains with wastewater.

However, the same tendency was observed in the reduction of pollutant load up to the 15$^{th}$ day of lab-scale observation. On the basis of experimental observation the nitrate and phosphate was reduced from day 5$^{th}$ to day15$^{th}$ of the experiment in both *Chlorella* sp. and the same reduction rate for nitrate and phosphate was reported by Kiran *et al*., (2014).

Based on the finding of Ajala *et al*., 2020 in OSCCW the result was found in their study is the same as our finding the nitrate was removed by 68% and phosphate was 84% by using *Chlorella* sp. The observation value that is reported in the literature is very helpful for the comparative study. So, all the analysis which is based on algal-based wastewater treatment is a valuable treatment for the removal of pollutants from OSCCW. For the analysis of different parameters like COD, BOD, nitrate and phosphate with 50% concentration of both *Chlorella* sp. is given in Figures 5.4 and 5.5 for the reduction of pollutant load in (%) with *C. pyrenoidosa* and *C. vulgaris* respectively. After the inoculation of the algal cells, pollutant load reduction of OSCCW was evaluated on the 15$^{th}$ day. The findings indicated that the pollution load of OSCCW in terms of selected physicochemical and nutrient parameters was significantly ($P$< 0.5) reduced.

Overall, the net removal of COD (72.4%), BOD (62.3%), nitrate (76.9%) and phosphate (67.6%) was achieved after 15 days of the experimental period Figure 5.6. The removal was noted gradually during the lag phase but it started increasing exponentially up to the 10$^{th}$ day resulting in the maximum pollution load reduction. Moreover, the kinetic assessment of pollution load reduction by *C. pyrenoidosa* showed that the removal followed a linear trend which can be modeled using the first-order reaction.

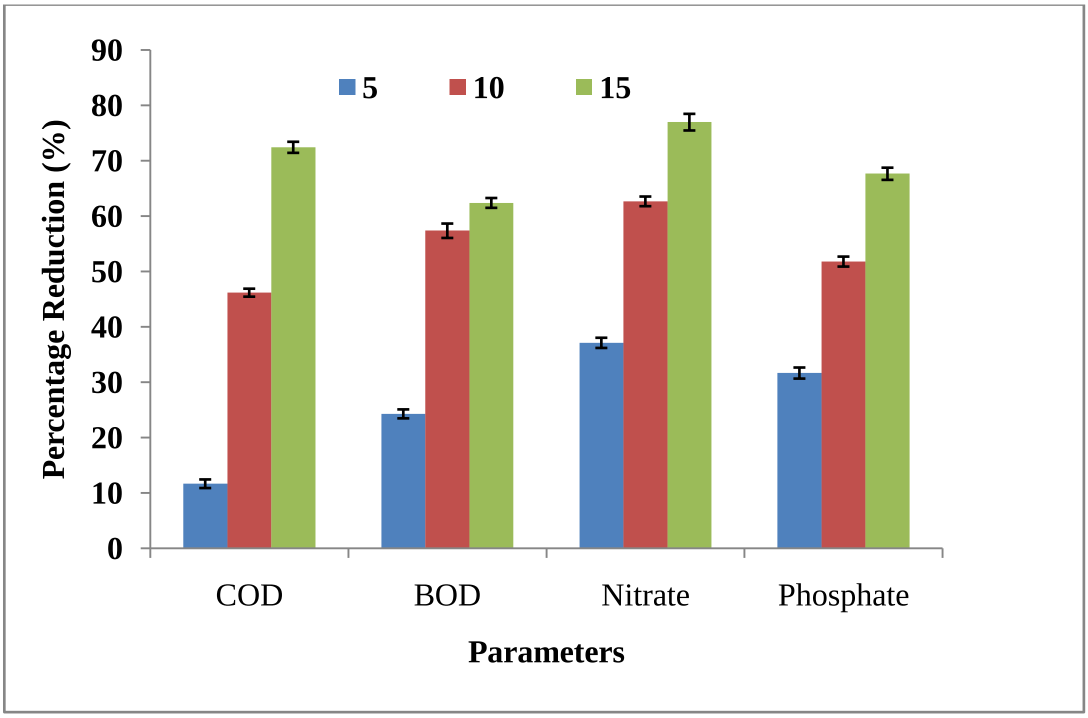



**Figure 5.4. Percentage reduction of pollutant load in 0th to 15th day of experiment with *C. pyrenoidosa***

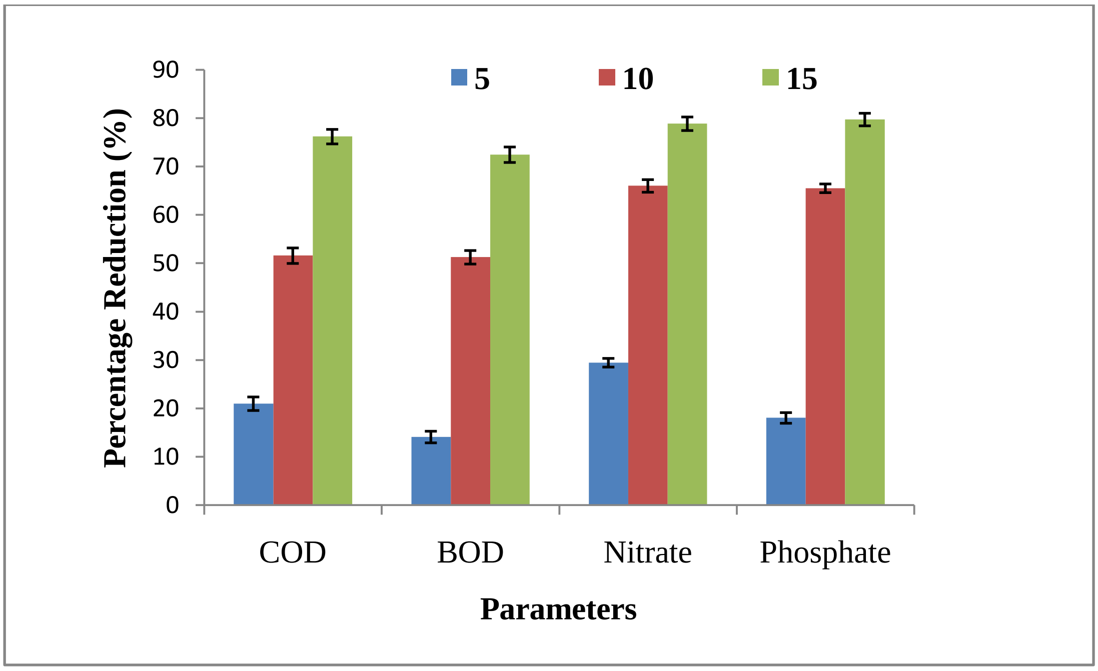



**Figure 5.5. Percentage reduction in pollutant load in 0th to 15th day of experiment with *C. vulgaris***

For the different parameters, the rate constants of pollutant load reduction were recorded as 0.0019 for pH, 0.0449 mg/l/d for phosphate, 0.0466 mg/l/d for nitrate, 0.0509 mg/l/d for TDS, 0.0376 mg/l/d for BOD, and 0.0414 mg/l/d for COD, respectively Table 5.5. The time-course changes in the pollutant parameters in terms of first-order fitness plots [Log(C) vs. t] were given in Figure 5.6. The model showed an acceptable range of fitness i.e., $R^2$>0.8818. The same trend was followed with *C. vulgaris* showed that the removal followed a linear trend which can be modeled using the first-order reaction. For the different parameters, the rate constants of pollutant load reduction were recorded as 0.0017 for pH, 0.0331 mg/l/d for phosphate, 0.0439 mg/l/d for nitrate, 0.0441 mg/l/d for TDS, 0.0291mg/l/d for BOD, and 0.0375 mg/l/d for COD, respectively Table 5.6. The time-course changes in the pollutant parameters in terms of first-order fitness plots [Log(C) vs. t] were given in Figure 5.7.

**Table 5.4. Pollutant removal using *C. pyrenoidosa* and *C. vulgaris* with 50% concentration of OSCCW between 0th to 15th days of experiment**

| **Time period** | ***C. pyrenoidosa*** | | | | | | | ***C. vulgaris*** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Parameters** | | | | | | | **Parameters** | | | | | | |
| | Color* | pH* | Odor* | Nitrate | Phosphate | BOD | COD | Color* | pH* | Odor* | Nitrate | Phosphate | BOD | COD |
| 0th day | Grayish | 9.1 | Bad smell | 35.0 | 16.3 | 98.2 | 225.4 | Grayish | 9.1 | Bad smell | 35.0 | 16.3 | 98.2 | 225.4 |
| Percentage reduction (%) | | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5th day | Light Grayish | 9.3 | Bad smell | 22.0 | 11.2 | 74.4 | 199.1 | Light Grayish | 9.5 | Bad smell | 24.7 | 13.4 | 84.4 | 178.0 |
| Percent reduction (%) | | - | - | 37.1 | 31.6 | 24.2 | 11.6 | - | - | - | 29.4 | 18.0 | 14.1 | 20.9 |
| 10th day | Light Grayish green | 9.7 | Algal smell | 13.0 | 7.9 | 36.9 | 121.3 | Light Grayish green | 9.7 | Algal smell | 11.9 | 5.6 | 47.9 | 109.1 |
| Percent reduction (%) | | - | - | 62.6 | 51.7 | 57.3 | 46.1 | - | - | - | 66.0 | 65.5 | 51.2 | 51.5 |
| 15th day | Green | 9.8 | Algal smell | 8.0 | 5.3 | 36.9 | 62.1 | Green | 9.9 | Algal smell | 7.4 | 3.32 | 27.0 | 53.6 |
| Percent reduction (%) | | - | | 76.9 | 67.6 | 62.3 | 72.4 | - | - | - | 78.8 | 79.7 | 72.4 | 76.2 |

***All the parameters were in (mg/l) except pH, Color and Odor**

**Table 5.5. First order kinetic parameters for pollutant reduction by *C. pyrenoidosa***

| Parameter | Variable | Value |
|---|---|---|
| pH | Equation | y = 0.0019x + 0.9632 |
| | K | 0.0019 |
| | $R^2$ | 0.7638 |
| Phosphate | Equation | y = -0.0449x + 1.2803 |
| | K | 0.0449 |
| | $R^2$ | 0.8887 |
| Nitrate | Equation | y = -0.0466x + 1.5721 |
| | K | 0.0466 |
| | $R^2$ | 0.9823 |
| TDS | Equation | y = -0.0509x + 2.9942 |
| | K | 0.0509 |
| | $R^2$ | 0.9881 |
| BOD | Equation | y = -0.0376x + 2.044 |
| | K | 0.0376 |
| | $R^2$ | 0.9516 |
| COD | Equation | y = -0.0414x + 2.4019 |
| | K | 0.0414 |
| | $R^2$ | 0.9567 |

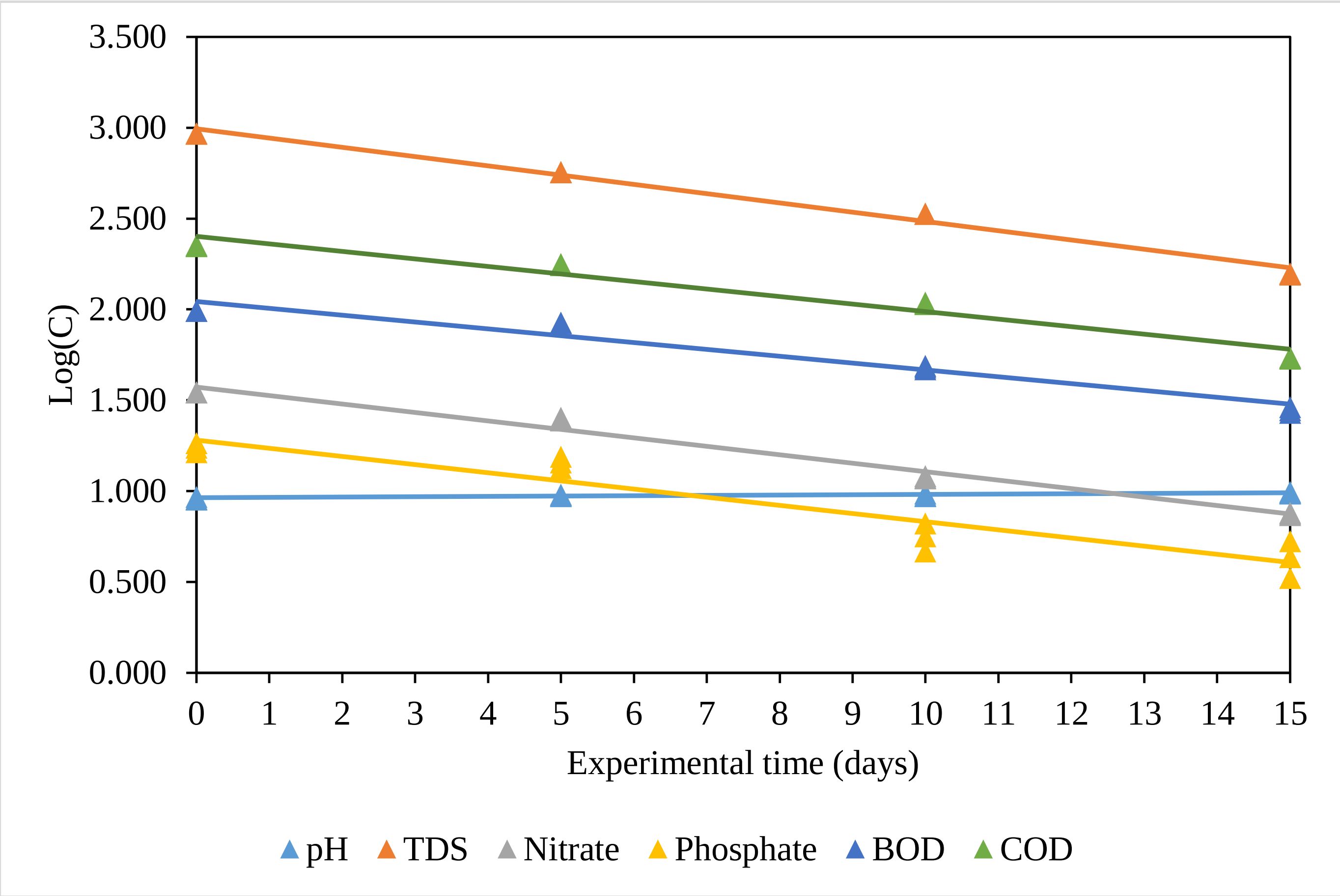



**Figure 5.6. First-order fitness plots [Log(C) vs. t] for time course pollutants reduction**

**Table 5.6. First-order kinetic parameters for pollutant reduction by *C. vulgaris***

| **Parameter** | **Variable** | **Value** |
|---|---|---|
| pH | Equation | y = 0.0017x + 0.9648 |
| | K | 0.0017 |
| | $R^2$ | 0.8413 |
| Phosphate | Equation | y = -0.0331x + 1.2218 |
| | K | 0.0331 |
| | $R^2$ | 0.9927 |
| Nitrate | Equation | y = -0.0439x + 1.5562 |
| | K | 0.0439 |
| | $R^2$ | 0.9937 |
| TDS | Equation | y = -0.0441x + 2.9993 |
| | K | 0.0441 |
| | $R^2$ | 0.9817 |
| BOD | Equation | y = -0.0291x + 2.0034 |
| | K | 0.0291 |
| | $R^2$ | 0.9935 |
| COD | Equation | y = -0.0375x + 2.4135 |
| | K | 0.0375 |
| | $R^2$ | 0.9268 |

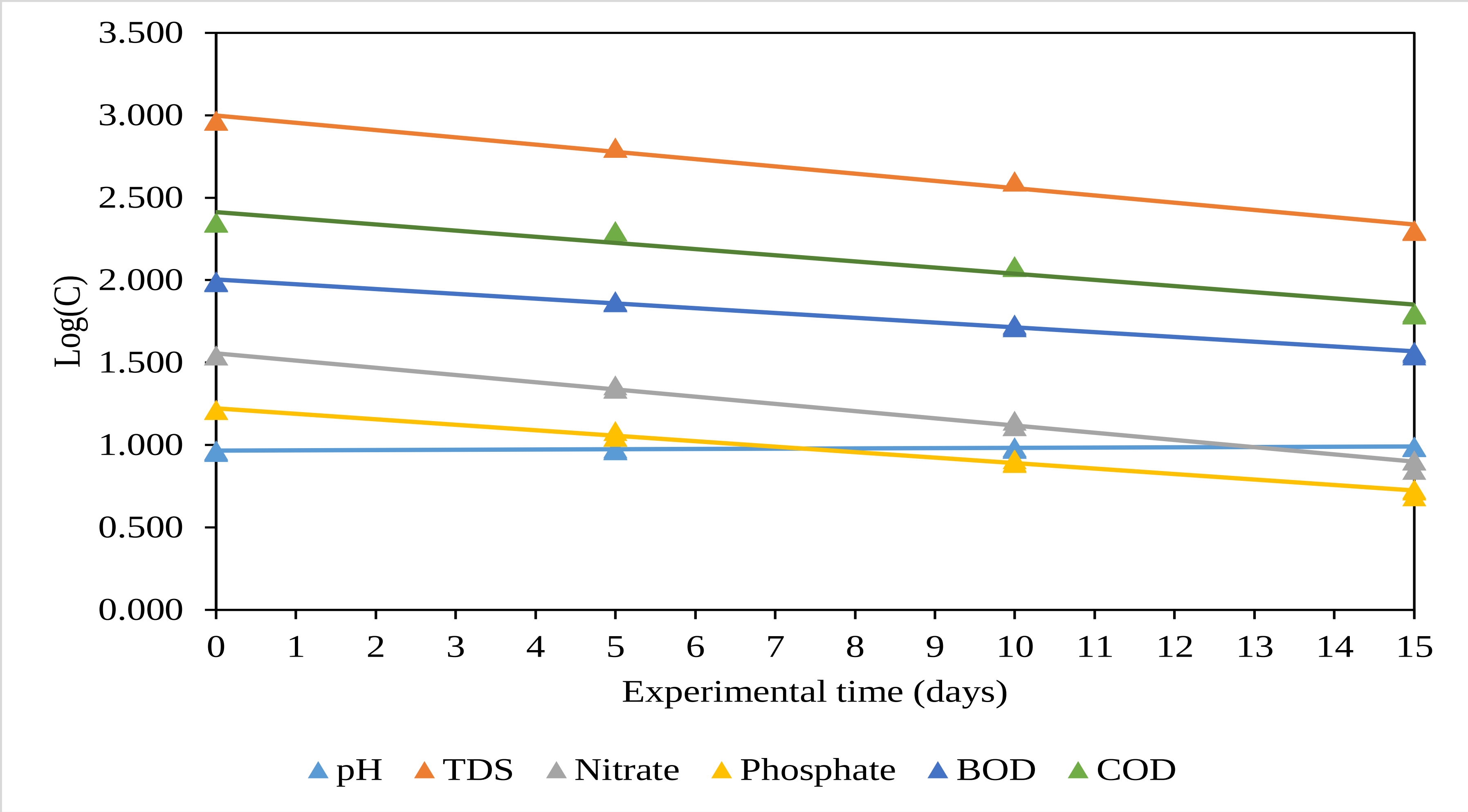



**Figure 5.7. First-order fitness plots [Log(C) vs. t] for time course pollutants reduction**

### 5.4. Biochemical profile of algae grown in OSCCW

The biochemical profile (chlorophyll, protein, carbohydrate and lipid) are microalgal bio-based products. Along with temperature and light and nutrients are the essential sources for the development of biochemical profile and their growth. In both *Chlorella* sp. only 50% concentration of wastewater is selected for the change in biochemical profile, nutrient uptake level and their growth. On the basis of observation in OSCCW with *C. vulgaris* the analysis of chlorophyll noticed from 5th to 15$^{th}$ day of the experiment was increased 2.7 µg/ml, 6.2 µg/ml and 10.9 µg/ml. The concentration of protein and carbohydrates were analyzed (24.43 mg/l, 170 mg/l and 235.6 mg/l) (24.7 mg/l, 151 mg/l, and 274.4 mg/l) from 5th to 15$^{th}$ days of experiment respectively. The lipid was estimated by (34.6%). Figure 5.9 represent the OSCCW treated with algal biomass for the production of biochemical compounds. There is a major difference between the values obtained from carbohydrate and protein in the strains of algae under the growth condition, as well as the lipid extraction. The observed carbohydrate content of different algal strains is similar to what has been reported in the literature, as shown in Table 5.7 of this Chapter. Meanwhile, a much higher value of carbohydrate content of about 53% was reported by Jiang *et al.,* 2016. Whereas, in *C. pyrenoidosa* the chlorophyll was observed in the same trend increase from 5$^{th}$ to15$^{th}$ day of the experiment are 2.7 µg/ml, 7.1µg/ml, and 11.9 µg/ml respectively. Similarly, the concentration of protein from 5$^{th}$ to 15$^{th}$ days of the experiment was increased 13.96 mg/l, 100 mg/l and 168 mg/l respectively. Therefore carbohydrate content is also increased up to the 15$^{th}$ day are 11.81 mg/l, 22.97 mg/l and 26.13 mg/l respectively. Therefore, Lipid is extracted from both *Chlorella* sp. with 50% concentration of OSCCW for the production of bio-based products which is given in Figure 5.8. In *C. pyrenoidosa* the lipid was estimated (34.6%) on the 15$^{th}$ day of the experiment in lab scale. Similarly, in the case of *C. vulgaris* with the same concentration 50% the lipid was yielded (31.1%). So, on the basis of the high rate of lipid estimation (34.6%) in *C. pyrenoidosa* as compared to *C. vulgaris* the lipid was found (31.1%) with the same concentration (50%) of OSCCW. Therefore, *C. pyrenoidosa* has a high potential for algal biomass production as compared to *C. vulgaris*. But based on the profile of protein and carbohydrate in *C. vulgaris* were found best result due to the increased level of biochemical profile from 5$^{th}$ to 15$^{th}$day of the analysis. The biochemical profile of OSCCW compared with other author studies,by using different strain with different wastewater are given in Table 5.7. This result indicates that the algae have a potential for the production of biomass and lipid extraction. The highest growth rate had been reported by Harshita *et al*., (2020) followed by Sharma *et al*., (2016).

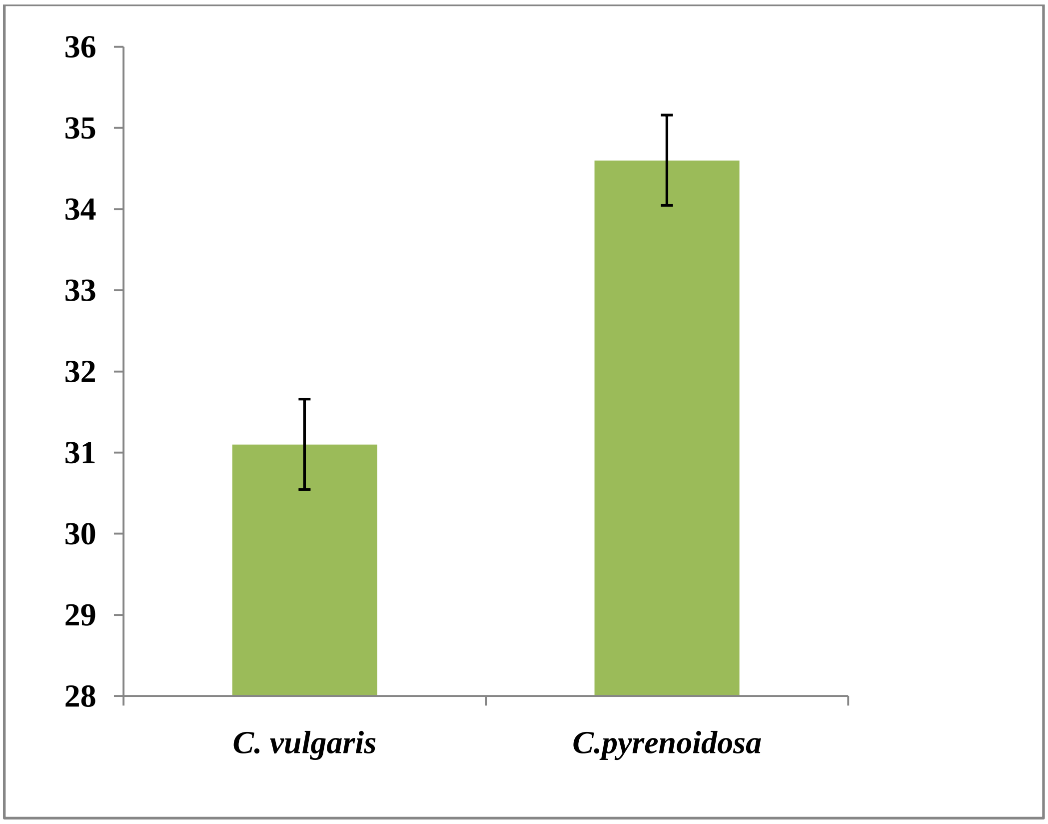



**Figure 5.8. Lipid content of both *Chlorella strain* with 50% concentration of OSCCW**

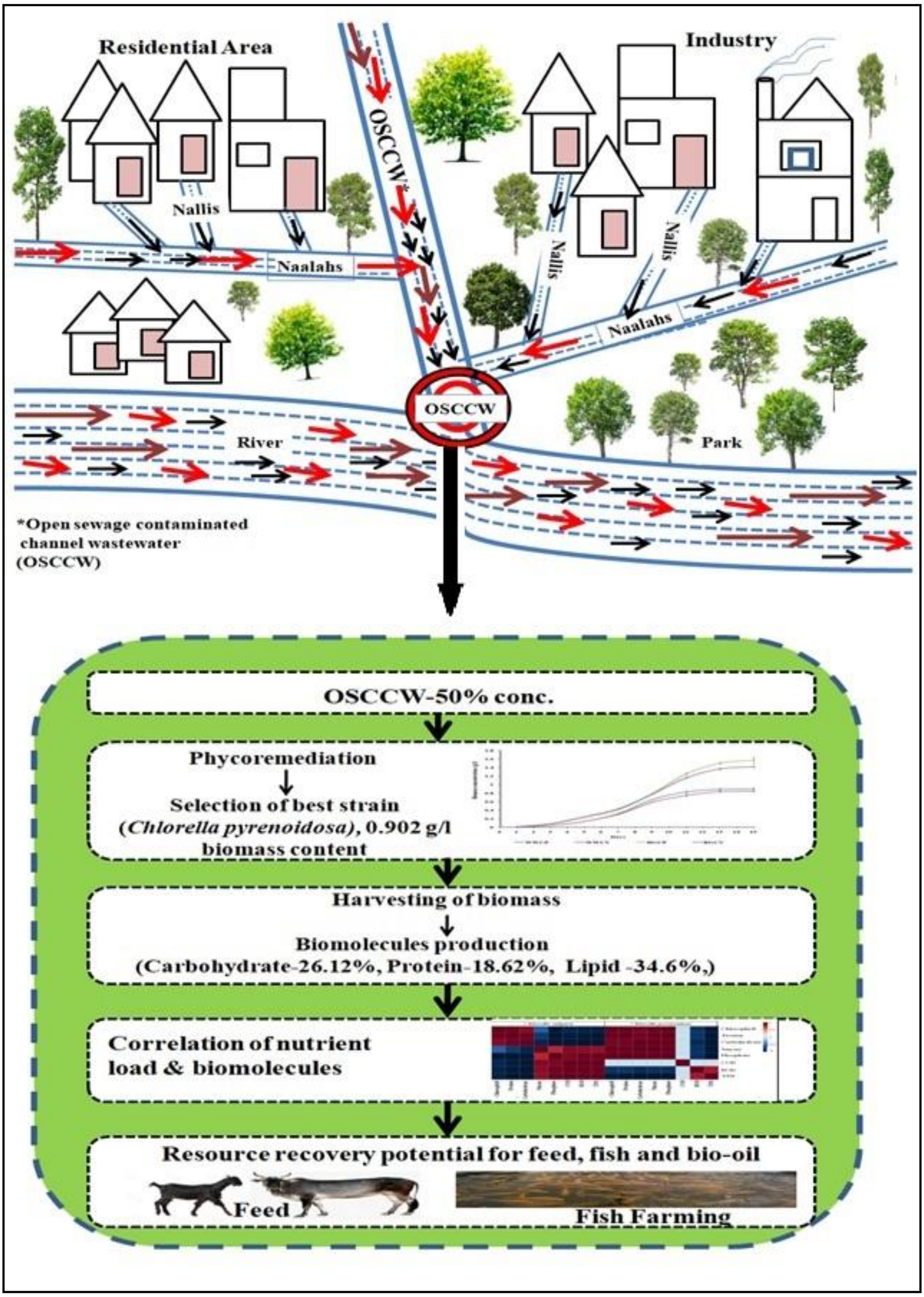



**Figure 5.9. Experimental representation of OSCCW an overview**

**Table 5.7. Comparison of Carbohydrate, protein and lipid contents of algal strains in present study with other authors**

| Wastewater type | Algal strains | Biochemical profile (%) | | | Culturing period | References |
|---|---|---|---|---|---|---|
| | | Protein | Carbohydrate | Lipid | (Days) | |
| Aquaculture ww | *S. obliquus* | 19.52 | 35.05 | 30.85 | 14 | Zhou *et al*., 2012 |
| BG-11 medium | *S. obliquus* | 44.40 | 19.70 | 20.30 | 16 | Ahmad, *et al*., 2019 |
| Aquaculture ww | *C. sorokiniana* | 28.81 | 35.43 | 31.85 | 14 | Ansari *et al*., 2019 |
| Municipal ww | *Scenedesmus sp* | 56.00 | 25.00 | 13.00 | - | Mohammadi *et al*., 2018 |
| Municipal ww | *S. obliquus* | 28.5 | 27.5 | 26.5 | 16 | Ahmad, *et al*., 2019 |
| Municipal ww | *C. vulgaris* | 30.14 | 23.39 | 11.47 | 14 | Ajala and Alexander 2020 |
| Municipal ww | *C.vulgaris* | 29.60 | 29.33 | 7.53 | 14 | Ajala and Alexander 2020 |
| Wastewater | *C. vulgaris* | 15.7 | 30.87 | 27.22 | 11 | Peng *et al.*, 2019 |
| OSCCW | *C.pyrenoidosa* | 18.65 | 26.13 | 34.6 | 15 | Present study |
| OSCCW | *C. vulgaris* | 28.0 | 32.36 | 31.1 | 15 | Present study |

## 5.5. Correlation analysis of OSCCW

The correlational analysis of municipal wastewater consists of physicochemical and biological parameters of OSCCW. In the present study, the correlation between two *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) with 50% concentration with physicochemical parameters and biochemical profile of algal strain in OSCCW. In this section, the co-relation between two variables indicates the high differences among them. In *C.pyrenoidosa* correlation coefficient analysis of parameters was given in Figure 5.10.

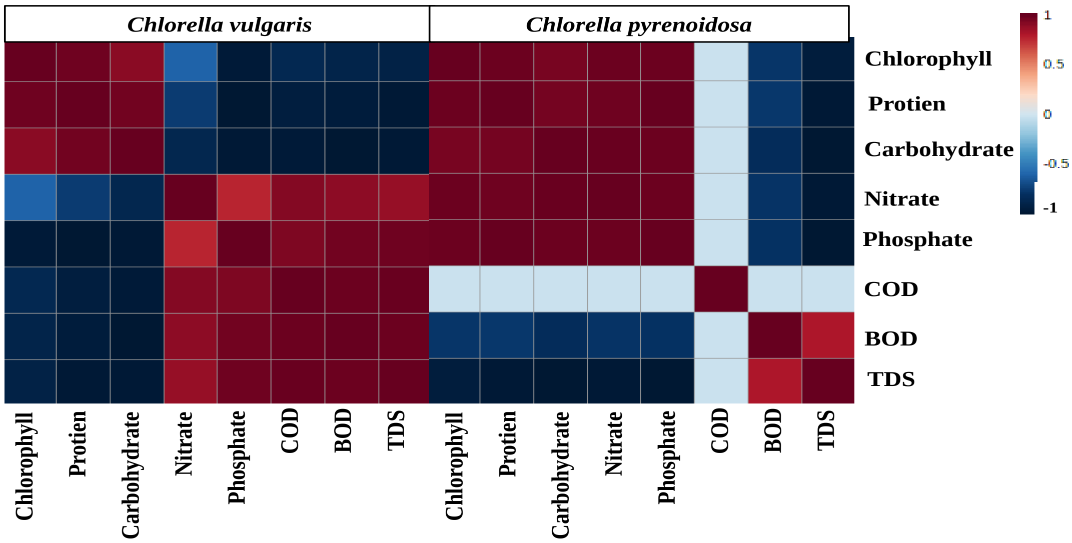



**Figure 5.10. Analysis of correlation between physico-chemical parameters and biochemical profile of both *Chlorella* sp.**

The highest positive correlation was observed with the $0^{th}$ to $5^{th}$ day of TDS, COD, BOD, nitrate and phosphate as compared with carbohydrate, chlorophyll and protein, was observed with $10^{th}$ to $15^{th}$ day was observed, that is highly correlated with each other. In *C. vulgaris* correlation TDS, BOD, COD, nitrate and phosphate between two variables are not positively correlated with chlorophyll, protein, and carbohydrate was not found highly correlated which is shown in given below. There is a strong correlation reported with the case of OSCCW between the nitrogen and phosphorous and in the context of protein, carbohydrates in the biomass (Venckus *et al*., 2017). In our study, the same positive correlation was found in OSCCW with *C. pyrenoidosa*. Thus, the analysis and observation on the basis of correlation with positive results indicated a very effective method for wastewater treatment and their management. The significant correlation between the algal biomass and the nutrients that indicate the importance of microalgal communities can be used as an important tool for the various phases of wastewater treatment.

The algal-based OSCCW has high potential for the removal of nutrients from wastewater. The *C. pyrenoidosa* achieved a highly efficient amount of nutrient removal rate. Despite, the removal efficiency of nitrate and phosphate by the algae after the stationary growth phase were 76.9% and 67.9% are found in *C. pyrenoidosa,* and as compared to *C. vulgaris* the removal efficiency was found in nitrate and phosphate after the stationary growth phase was 78.8% and 79.7%. The algal-based OSCCW treatment is a very low and environmental friendly method to treat the *Chlorella* sp. with wastewater for the extraction of bio-chemical compounds. The remediation of wastewater using *Chlorella* sp. provides an environmentally acceptable and effective option for wastewater remediation, which are not only recycles valuable nutrients but also improves the quality of water. Therefore it may be regarded as an efficient nutrient removal process for the removal of nutrients from wastewater.

# Chapter 6

# Algal derived bio-chemical compounds using wastewater: Theoretical Assessment for value-added products and their applications

## 6.1. Introduction

The main objective of this experimental study is for culturing of algal biomass under various parameters for the production of maximum biomass to explore its maximum. Algae cultured in large scale can boost the green technology economically. In natural or artificial process, it exposeda number of environmental factors like pH, nutrient and light. Despite all of this, the biogenic accessible elements concentration is the main parameter that affects the growth of algae. In this work, the bio-chemical compounds are derived from algae by treating with different wastewater (SHWW, OSCCW) for the estimation of valuable results, which is based on value-added products. Algae play an important role in the daily diet and future life. It is considered as a rich source of proteins, carbohydrates and lipids for value-added products and it is an essential food for human beings as well as animal feed all over the world. Barkia *et al.,* (2019) reported that algae have the capability for the production of good value-added products like methanol, ethanol, oil, biogas, diesel, biohydrogen, long-chain hydrocarbons, phycobiliprotein, phycocyanin, phycoerythrin, vitamins (A, B1, B2, B6, B12, C, E, nictitate, folic acid, biotin and pantothenic acid), protein, anthelmintic, polysaccharides, eicosapentaenoic acid, arachidonic acid, docosahexaenoic acid and omega-3 fatty acid. Algae in addition to biofuels resources are also considered as an excellent source of a variety of biologically active compounds or metabolites which can offer infinite applications in different sectors. Such bio-chemical compounds exhibit exceptional pharmaceutical as well neutraceuticals values that have markedly benefited mankind (Kothari *et al.,* 2017). However, regardless of these challenges is associated with the completely harvesting of algal biomass. Whereas, harvesting of algal biomass is an important part of this experimental research for value-added products. Therefore, various conventional treatment processes (flocculation, centrifugation, sedimentation, electrocoagulation and gravity) are used for the cultivation, dewatering and harvesting of algal biomass. To understand the synergistic interface between the physical, chemical and environmentalfactors for the optimization ofbiomass, is the main factor which affects the productivity of algal biomass. The parametric impact on algal growth was investigated, which has been discussed in detail in phase-1 of Chapter-4., and 5. The present Chapter is focused on algal-based bio-chemical compounds using both wastewaters (SHWW, OSCCW) with both strains of *C. pyrenoidosa* and *C. vulgaris* based on theoretical assessment (obtained from phase-1 of Chapter-4 and 5) for the production of value-added products and their application for further commercial use investment.

## 6.2. Materials and methods

### 6.2.1. Algal Species

*C. pyrenoidosa* and *C. vulgaris* have been selected for this experimental investigation and all important information related media used, collection of algae which are discussed in detailed in Chapter-3 of section 3.3.1.

### 6.2.2. Parametric impact

Several physical and chemical parameters (pH, light, temperature, nitrate and phosphate) are accountable for the growth and productivity. There are several parametric ranges which are used to investigate the effect of working parameters on algal growth and their biomass is discussed in detailed in Chapter-3 of section 3.7.1.2.

### 6.2.3. Biochemical analysis

The process used for the analysis of bio-chemical compounds in this experimental study is given in Annexure-1.

### 6.2.4. Collection of wastewaters and their characterization

The wastewaters collection and their characteristics for the completion of this experimental work is given in detail in Chapter-3 of section 3.4.1.1 and 3.4.1.2.

### 6.2.5. Correlation analysis between physicochemical and biochemical profile

The correlation analysis between two variables in both species (*C. pyrenoidosa* and *C. vulgaris)* is clearly given in detailed with description of section 3.7.1.5., of Chapter-3.

## 6.3. Results and Discussion

For the investigation of biochemical compounds, with wastewater concentration has been analyzed for the production of biomass and their productivity. The bio-chemical compounds are (carbohydrates, protein, chlorophyll a, b, carotenoids, pigments and lipid) with best results obtained with best concentration of both strains (*C. pyrenoidosa* and *C. vulgaris)* with both wastewater (SHWW, OSCCW) obtained from phase -1 of Chapter-4 and 5.

### 6.3.1. Parametric effect on algal growth and bio-chemical compounds of SHWW

In this experimental study the biochemical profiles (Carbohydrates, protein, lipid, pigments and carotenoids) are the main source for the production of high quality value-added products. The light and temperature are the main factors for the improvement of biochemical profile, biomass growth and their development. For the investigation of this study, only 50% concentration was taken for analysis of SHWW and OSCCW with both strains (*C. pyrenoidosa* and *C. vulgaris)* for the theoretical assessment for value-added products and their applications. There are various applications of algae, which are obtained from algal biomass are used as a feed supplements, feedstock for fish towards farm animals. As per the experimental observation reported by resesrcher a study revealed that *Spirulina platens* is used as a diet which help to gain the weight in chicks. About 2.0 to 2.5% of *Spirulina platensis* biomass have the potential to change the color of the egg yolk without any effect. Another investigation was assessed with *Chlorella* used as a diet supplement for the feeding of poultry, which shows only 1% of fresh liquid in chicks that improve their growth and performance (Selim, *et al.*, 2018; Alfaia *et al.*, 2021).

### 6.3.2. Influencing processing parameters: Mechanism and optimization

In order to maximize the efficiency of wastewater treatment via algal biomass mechanism, are supposed to be optimized. Such process parameters are described as below. These are mainly characterized as carbohydrates, protein, chlorophyll, carotenoids, lipid and pigments.

#### 6.3.2.1. Carbohydrates

Carbohydrates occurred in the form of dropping sugars like sucrose, lactose, glucose, fructose and polysaccharides. For the production of bioethanol, algal cells are composed of several complex carbohydrates that is cellulose, glycogen, starch and agarose. Without any modification bioethanol can be produced from algal carbohydrates. Carbohydrates are monosaccharides, oligosaccharides and polysaccharides have both comprised of metabolic and functional structure. In addition, algae manufacture glucose and starch like energy products, which are known as carbon containing photosynthesis products are obtained from carbohydrates. Infact, algal polysaccharides are gaining more attention as hygroscopic agents in cosmetic industry and as an antioxidant. It is a natural source of highly valuable, fascinating and biologically active compounds. These compounds have conventional awareness from researchers towards the companies in these years due to the presence of possible applications in different areas of life sciences. Its applications ranges indicate biomass used as food and feed for the production of bioactive compounds for the utilization of value-added products. In our study the carbohydrates content (9.58%) observed the range between the 15th day of experiment by using SHWW treated with *C. pyrenoidosa* and in *C. vulgaris* the carbohydrates content (22.66%) was obtained, all the detailed obtained results are given in Chapter 4 of section 4.5. In OSCCW the carbohydrates content obtained in *C. pyrenoidosa* (26.13%) and in *C. vulgaris* (32.36) was found in our present study. The obtained result as per our study is best for the utilization of nutritional purposes for fish and animal feed, followed by different experimental investigation as per available literature and their values obtained. According to Gbadamosi and Lupatsch, (2017) the carbohydrates content was found (28.8%) which are used for fish meal and oil by using *Nanochloropsis* sp. Additionally, *S. almeriansis* and *A. platensis* have been tested as a poultry feed, pigs and ruminants without any side effect in animal health observed a better improvement in their quality of meat (Madeira *et al.,* 2017). So, for the best of our knowledge the result obtained in our study by using SHWW and OSCCW by treating with both strains *(C. pyrenoidosa* and *C. vulgaris)* compare to previous study is best and it can be utilized as fish meal as well as animal feed. The main applications of carbohydrates are antioxidants, anticoagulant, antiproliferative and immunostimulatory.

#### 6.3.2.2. Protein

Algal biomass depending upon environmental factors and the strain utilized, have been recognized as potential candidate for rich protein content availability. These molecules consist of several functions which are used for nutritional, pharmaceuticals and cosmetics products. In developing countries particularly some species like *Chlorella* sp. and *Dunaliella salina,* have high protein content for human being. In our study, the protein content observed the range in between 12.37% observed with 15th day of experiment by using SHWW treated with *C. pyrenoidosa* and in *C. vulgaris* the protein content (30.32%) was obtained, all the detailed obtained results are given in Chapter-4. In OSCCW the values obtained in protein with *C. pyrenoidosa* are (18.65%) as well as in *C. vulgaris* of protein contents are (27.78%) with 15$^{th}$ day of analysis. The obtained result as per our study is best for the utilization of nutritional purposes for fish and animal feed, followed by different experimental investigation as per available literature and their values obtained. Various algal sp. which are rich in protein ranges *Nannochloropsis oculata* (22-37), *Arthrospira platensis* (50-70%), *P. creuntum* (8-56%) *and Haematococcus pluvialis (*45-50%) which are rich in protein and are utilized as following cell disruption techniques (Safi *et al.*, 2013). There are many nutritional benefits of algae as per the literature reviewed, by various researchers reported (Montalvo *et al.,* 2019; Lupatini *et al.*, (2017). So, that's why phycoremediation process is the best option for the extraction of protein and its utilization for food products. Further applications as per the findings of present study are diet of athletes, build muscle tissue which are broken during workout. Algal cells are consumed in the form of tablets, pills and powder forms. However, they have been incorporated into a number of functional foods, which are noodles, biscuits, bread, drinks, beer and sweets (Bleakley and Hayes 2017). Therefore, as per mentioned previous studies the protein content of seaweeds varies vary as per different factor. Moreover, algal carbohydrates like alginates and carrageen are industrially commercialized for thickening and gelling agents for various applications of biotechnological, food, textile and biomedical industries. These are considered as functional food ingredients. The seaweed carbohydrates like alginates, carrageen, and fucoidan indicate a broad range of biological activities which consist of anticoagulant, antioxidant, anti-inflammatory and immunostimulatory activities. Furthermore, the consumption of dietary fiber has a positive influence on human health with needful impacts are reduced risk from preventing of colon cancer as well as constipation reduce hypercholesterolemia, diabetes and obesity. It can be used for human nutrition, animal feed, aquaculture, poultry feed, pig and ruminants (Bleakley and Hayes 2017).

**6.3.2.3. Chlorophyll**

Chlorophyll is considered an omnipresent green pigment which is occurred naturally. It has potential because it is significantly used as a dyeing agent for food and medicinal purposes. It is extensively used as a constituent in health and hygiene products such as air fresheners, antiperspirants, lozenges, and against foul smells. These are the algal strains that are used for the extraction of chlorophyll and utilized for various applications, *Chlorella* sp., *Sanropus androgynous*, green algae are used as an antioxidant, food additives, immune activators, cytotoxic as well as tumoral. In our study, the chlorophyll content observed the range in between (15.31µg/ml) with 15$^{th}$ day of experiment by using SHWW treated with *C. pyrenoidosa.* In *C. vulgaris* the chlorophyll content (17.31%) was obtained, all the detailed obtained results are given in Chapter-4. In OSCCW the values obtained in chlorophyll with *C. pyrenoidosa* are (11.9 µg/ml)as well as in *C. vulgaris* of chlorophyll contents (10.9 µg/ml) with 15$^{th}$ day of observation respectively. Chlorophyll is considered an omnipresent green pigment which is occurred in naturally. Due to the presence of high chlorophyll content *Chlorella* sp. is popularly called as “Emerald food” (Khanra*et al.,* 2018). It has potential because it is significantly used as a dyeing agent in food and medicinal purposes. It is extensively used in health and hygiene products such as air fresheners, antiperspirants, lozenges and against foul smells. These are the algal strains that are used for the extraction of chlorophyll and utilized as various applications: *Chlorella* sp., *Sanropus androgynous*, green algae are used as an antioxidant, food additive, immune activators, cytotoxic as well as tumoral cells (Odjadjare *et al.*, 2017; Khanra *et al.*, 2018).

**6.3.2.4. Lipid**

Algae consist of a valuable source of lipids, polyunsaturated fatty acids like arachidonic acids, omega-3 and omega-6 fatty acids and eicosapentaenoic acid, *etc*. Due to the presence of antifungal, antibacterial, and antioxidative properties, polyunsaturated fatty acids are considered as a significant source for the function of human body. There are many positive health benefits of polyunsaturated fatty acids of omega-3 and omega-6 fatty acids that prevent our health, which are as type 2 diabetes, coronary heart disease, pulmonary disease and hypertension. In addition, it is a promising technique for lipid and Sterols extraction from algae which are used for various applications of pharmaceuticals and neutraceuticals products as well as cosmetics. Therefore, another use of lipid extraction from algae is the production of biodiesel, and it is considered a candidate for an inspiring future marketing value with immediate production of value-added based products. At present time due to the requirement of biodiesel, it can be easily produced from algae in a large scale after the cultivation process and it is used as value-added products (Gallego *et al.*, 2018). In our study, the lipid content observed in the range between (34.8%) with 15$^{th}$ day of experiment by using SHWW treated with *C. pyrenoidosa* and in *C. vulgaris* the lipid content (30.7%) was obtained, all the detailed obtained results are given in Chapter-4. In OSCCW the values obtained in lipid with *C. pyrenoidosa* are (34.6%) as well as in *C. vulgaris* of lipid contents (31.1%) with 15$^{th}$ day of observation respectively.

The lipid extraction method is an important process for the production of algae and biofuels. Figure 6.1 indicate algal biomass utilization for value-added products. There are several techniques, which are used for the extraction of lipid are discussed in detail in Table 6.1. Whereas the biochemical profile of both strain (*C. pyrenoidosa* and *C.vulgaris*) is described in Table 6.6 and 6.7 with both wastewaters (SHWW and OSCCW). As per the value obtained with both strains the algal biomass are utilized as a food. On the basis of literature different strains are used for the production of bio-chemical compounds for value-added products which are described in Table 6.2 of this Chapter.

Algae is a promising tools which increase the demand of bioactive compounds like carotenoids, carbohydrates, proteins, flavonoids and phenolic. Identification and isolation of novel bio-chemical compounds from algae will provide a valuable platform for the development of new therapeutic agents for neutraceuticals and pharmaceuticals products. Table 6.3 indicates different applications of bio-chemical compounds utilized for various applications. Therefore, numerous compounds extracted from algae are widely used as cosmetic industry as water-binding agents, thickening agents, and antioxidants in skin and facial care products. It is well known that algae contain valuable products that are proteins, carbohydrate, lutein, β-carotene, phenolic and flavonoids compounds that are useful for several industrial applications. Despite of these excellent and advanced benefits, there are several limitations that should be focused as well. Therefore, to overcome these issues some of these bio-chemical active compounds are presently particulated in proper vectors to relieve for purification and hence for the reduction of purification as well as production costs.

**Table 6.1. Mechanical and chemical techniques used for the extraction of lipids (Sharma *et al.*, 2015)**

| Extraction method | Advantages | Key methodology |
|---|---|---|
| Bead beating | Moderate efficiency | cells disrupted by using high-speed spinning beads |
| Electroporation | High efficiency | Cell walls and cell membranes altered to improve the lipid extraction |
| Ultrasound-assisted extraction | Economical and eco-friendly | Cavitation produces heat shock waves for the disruption of algal cell |
| Expeller press | The simple and effective crushing method | High pressure to crush and squeeze the cell for extraction of oil from algae |
| **Bligh and Dyer method** | **Easy processing for number of samples** | **Extraction of lipid from algae by using chloroform and methanol ratio of 2:1 by volume** |
| Folch method | Easy processing for number of samples | Combination of different solvents like chloroform-methanol 2:1 by volume |
| Pressurized solvent extraction | High efficiency | Utilized nitrogen and solvent for extraction |
| Soxhlet extraction method | Moderate efficiency | Normally quantify the high-quality lipids like triglycerides and fatty acid hexane is used as a solvent. |

**Table 6.2. Biochemical profile of algae for value-added products (Katiyar *et al.*, 2017; Shubha and Kifle, 2018; Ge *et al.*, 2018; Ji *et al.*, 2015; Do, *et al.*, 2019; Andreotti *et al.*, 2020)**

| **Algal strains** | **wastewater** | **Lipid (%)** | **Carbohydrates (%)** | **Protein (%)** |
|---|---|---|---|---|
| *Chaetoceros calcitrans* | - | 39 | 10 | 58 |
| *Anabaena cylindrical* | - | 4-7 | 25-30 | 43-56 |
| *Chlorella protothecoides* | - | 55 | 10-15 | 10-52 |
| *Scenedesmus quadricauda* | - | 1-9 | - | 47 |
| *Spirullina maxima* | - | 6-7 | 13-16 | 60-71 |
| *Poryphyridium cruentum* | - | 9-1440 | - | 5728-39 |
| *Chlorella sorokiniana* | - | 19-22 | - | - |
| *Chlorella pyrenoidosa* | - | 2 | 26 | 57 |
| *Chaetoceros muellerii* | - | 33 | 11-19 | 44-65 |
| *Botryococcus braunii* | - | 25-75 | - | - |
| *Chlamydomonas rheinhardii* | - | 21 | 17 | 48 |
| *Dunaliella salina* | - | 6 | 32 | 57 |
| *Scenedesmus dimorphus* | - | 16-4 | 21-52 | 8-18 |
| *Spirullina platensis* | - | 4-9 | 8-14 | 46-63 |
| *Spirulina sp* | Palm factory wastewater | 9.09 | 17.4 | 60.34 |
| *Micratinium reisseri* | Municipal ww | 20 | - | - |
| *Tetraselmis suecia* | Aquaculture ww | 25.06 | 10.62 | 50.2 |
| *Desmodesmas sp* | Municipal ww | 23.51 | 24.41 | 39.66 |
| *Scenedesmus sp* | Food ww | 20.8 | 22.1 | - |
| *Chlorella sp* | Centrate water | 14.8 | 10.5 | 64.2 |
| *Phormidium sp* | Swine wastewater | 11 | 16 | 62 |
| *Chlamydomonas sp* | Textile | 11 | 19.5 | 51.9 |

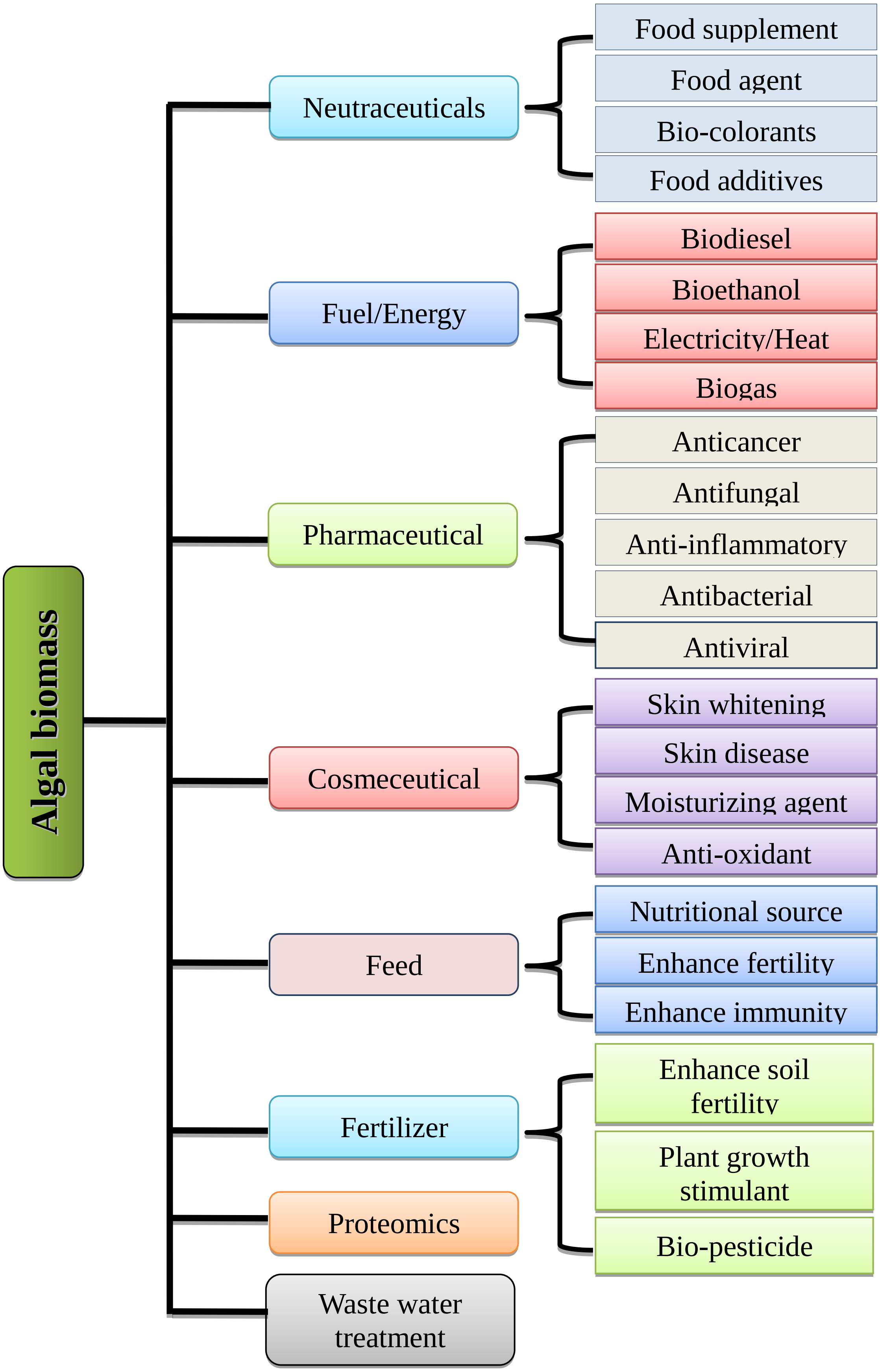



**Figure 6.1. Algal biomass utilized for value-added products**

**Table 6.3. Different applications of bio-chemical compounds obtained from algae**

| **Algal strains** | **Compounds** | **Neutraceutical/Pharmaceutical products** | **References** |
|---|---|---|---|
| *Chlorella sp*<br>*Spirullina*<br>*Haematococcs pluvialis*<br>*C.zofingenisis* | Astaxanthin | Tablets, powders, nectar, noodles, Powder extracts, beverages, extracts Tablets, chips, Anti-inflammatory, and Antioxidants | Hu *et al*., 2016<br>Sowjanya and Manjula, 2016<br>Ciccone *et al.*, 2013 |
| *Crypthecodiuimu sp*<br>*Pyramimonas sp*<br>*Schizochytrium sp* | Polyunsaturated fatty acid | Anti-inflammatory, anticancer, used as a food for pregnant, | Long *et al.*, 2018<br>Teng *et al.*, 2015 |
| *Pyropiayezoensis*<br>*Haematococcus pluvialis* | Carotenoids | Food applications, anti-inflammatory activity, antioxidative activity, Human nutrition and weather influence | Choi *et al*., 2015<br>Thomas *et al*., 2017 |
| *Porphyridium cruentum* | Polysaccharides | Pharmaceuticals, cosmetics | Santhosh *et al*., 2016 |
| *Nanochloropsis sp*<br>*Nannochloropsis*<br>*Porphydium*<br>*Phaeodactylum tricomulum* | Eicosapentaenoic acid (EPA) | Cardiovascular benefits<br>Anti-inflammatory | Asgharpour *et al*., 2015<br>Asgharpour *et al*., 2015<br>Kavitha *et al.*, 2016<br>Xue *et al*., 2014 |
| *Arthrospira platensis* | Phycocyanin | Antioxidants | Wollina *et al.*, 2018 |
| *Scenesdesmus sp*<br>*Muriellopsis sp*<br>*C. sorokiniana*<br>*Ankistrodesmus brunii*<br>*Dunaliella salina*<br>*Chlorella sp* | Lutein | Anti-inflammatory<br>Antioxidants<br>Diabetic, retinopathy<br>Antioxidant and anticancer activity, Neutraceuticals | Saha *et al.*, 2020<br>Molino *et al.*, 2020<br>Sansone *et al.*, 2017<br>Liu *et al.*, 2017<br>Fu *et al.*, 2014<br>Santhosh *et al.*, 2016 |
| *Chlamydomonas nivalis*<br>*Chlorella zofingiensis* | Canthaxanthin | Antioxidants, anti- inflammatory | Sathasivam *et al.*, 2014 |

| | | | |
|---|---|---|---|
| *Poryphyridium sp*<br>*Chlorella stigmatophora*<br>*Phaeodactylum tricornutum* | Polysaccharides | Anti-inflammatory, Antiviral, Antioxidant, reduce free radicals | Paliwal *et al.*, 2017<br>Chen *et al.*, 2018 |
| *Odontella aurita*<br>*Salpingoeca marina* | Fucoxanthin | Anticancer activity<br>Prevention of osteoporosis, Cholesterol, gastric, ulcer | Gong *et al.*, 2016<br>Liu *et al.*, 2017 |
| *Phaeophyta,*<br>*Rhodophyta*<br>*Chlorophyta*<br>*Rhodophyta*<br>*Chaetoceros calcitrans*<br>*Isochrysis galbana*<br>*Nanochloprosis sp*<br>*Pavlova salina* | Polysaccharides | Antifungal, anticancer, Anti-inflammatory, Antiprotozoal, reduce coronary heart disease, Antihelminthic, anti-inflammatory,<br>Decrease occurrence of chronic diseases like obesity, arthritis, cardiovascular diseases, Diabetes, improve brain potential, Cardiovascular disease, improve the function of brain | Lekshmi and Kurpa, 2019<br>Morokutti- kurz *et al.*, 2017<br>Gaikwada *et al.*, 2020<br>Olasehinde *et al.*, 2017<br>Balamurugan *et al.*, 2018<br>Katiyar and Arora, 2020<br>Mourella *et al.*, 2017 |
| *P.cruentum*<br>*Tetraselmis*<br>*Odonthaliacorymbifera*<br>*Chlorella pyrenoidosa* | Vitamins<br>Bromophenols<br>Polyunsaturated fatty acids | Reproduction, Cholesterol control, used as anticancer, Antioxidant, tyrosine's inhibitors, Antimicrobial, Anti-diabetic | Koyande *et al.*, 2019<br>Delasoie *et al.*, 2018<br>Islam *et al.*, 2017<br>Wan *et al.*, 2019 |
| *Symphyocladialatiuscula,* | Bromophenol derivative | Antidiabetic | Paudel *et al.*, 2019 |

**Table 6.4. Algal composition (% w/w on dry ± SD *) as per the dietary basis**

| **Algal strains** | **Protein** | **Carbohydrates** | **lipids** | **Total dietary fibers** | **Sources** |
|---|---|---|---|---|---|
| *Arthrospia platensis* | 50-63 | 7.7-22.2 | - | - | Alessandro *et al.*, 2016 |
| *Scenedesmus almeriansis* | 49.4-55 | - | - | - | Chen *et al.*, 2015 |
| *Haematococcus pluvialis (green phase)* | 32.59±1.20 | 0.13±0.01 | 3.24±0.1 | 34.56±0.90 | Molino *et al.*, 2018 |
| *Haematococcus pluvialis (red phase)* | 10.2-17 | - | - | - | Shah *et al.*, 2016 |
| *Chlorella vulgaris* | 45.64±1.20 | 5.30±0.50 | 3.13±0.2 | 35.04±1.60 | Molino *et al.*, 2018 |
| *Dunaliella salina* | - | - | 17 | - | Bonnefond *et al.*, 2016 |
| *Nanochloropsis sp* | 41.6-42.1 | 167-18.6 | - | - | Matos *et al.*, 2016 |

*** SD = standard deviation**

**Table 6.5. Isolation and extraction of algae for bio-chemical compounds**

| **Bioactive compounds** | **Algal species** | **Isolates/Wastewater/Extraction** | **References** |
|---|---|---|---|
| Fucoxanthin | *Phaeodactylum tricornutum* (UTEX 640) | Pressurized liquid extraction | Neumann *et al.*, 2019 |
| Phycocyanin | *Spirulina platensis* | Freeze thawing followed by solvent extraction | Hao *et al.*, 2019 Prabakaran *et al.*, 2020 |
| Phycocyanin | *Spirulina platensis* | Super critical fluid extraction | Deniz *et al.*, 2016 |
| Phycocyanin | *Limnothrix sp* | Four purification step that includes adsorption of impurities with chitosan, charcoal ammonium sulphate precipitation and ion exchange chromatography | Safaei *et al.*, 2019 |
| Eicosapentanoic acid | - | Enriched EPA oil | Rao *et al.*, 2020 |

**Table 6.6. Biochemical profile of SHWW of both strains**

| **Slaughterhouse wastewater (SHWW)** *(C. pyrenoidosa)* | | | |
|---|---|---|---|
| **Carbohydrates (mg/l)** | **Protein (mg/l)** | **Chlorophyll µg/ml** | **Lipid (%)** |
| 10.2 | 10 | 0.31 | - |
| 22.3 | 32 | 2.94 | - |
| 36.4 | 42 | 9.5 | - |
| 41.2 | 53 | 15.31 | 34.8 |
| **Slaughterhouse wastewater (SHWW)** *(C. vulgaris)* | | | |
| **Carbohydrates (mg/l)** | **Protein (mg/l)** | **Chlorophyll (µg/ml)** | **Lipid (%)** |
| 0.67 | 2.14 | 0.19 | - |
| 1.507 | 3.54 | 4.94 | - |
| 2.17 | 4.97 | 7.5 | - |
| 3.42 | 6.24 | 17.31 | 39.7 |

**Table 6.7. Biochemical profile of OSCCW of both strains**

| **Open sewage contaminated channel wastewater (OSCCW)** *(C. pyrenoidosa)* | | | |
|---|---|---|---|
| **Carbohydrates (mg/l)** | **Protein (mg/l)** | **Chlorophyll (µg/ml)** | **Lipid (%)** |
| - | - | - | - |
| 16.65 | 13.96 | 2.7 | - |
| 170 | 100 | 7.1 | - |
| 235.6 | 168 | 11.9 | 34.6 |
| **Open sewage contaminated channel wastewater (OSCCW)** *(C. vulgaris)* | | | |
| **Carbohydrates (mg/l)** | **Protein (mg/l)** | **Chlorophyll µg/ml** | **Lipid (%)** |
| - | - | - | - |
| 24.7 | 24.43 | 2.7 | - |
| 151 | 170 | 6.2 | - |
| 274.4 | 235.6 | 10.9 | 31.1 |

These novel approaches provide an innovative approach by using the algae to obtain the valuable bio-chemical compounds for the treatment of various disease. Furthermore, in genetic engineering the algae have high metabolites towards natural organism for drug delivery has been explored. Inspite, of all these more efforts required to develop the multifunctional ranges of products, that are affordable and also inter-related with nutritional science that give a more health benefits in current market. These finding give a good plateform to assess and explored the valuable algal biomass for further utilization of value-added products for a sustainable environment. The main aim of this Chapter is completely focused on algal based biomass for value-added products. Major finding can be concluded that the value-added products production is an integrated approach for commercialized application, but the main challenges cannot be ignored at the part of environment. Therefore algal based wastewater treatment is a zero investment on the basis of cost and has high potential on market level.

# Chapter 7
# Techno-Economic Assessment: Comparative Study

### 7.1. Introduction

In the current scenario, renewable energy is the best option to solve the environmental issues and to attract attention on both the academic and industrial sectors. Algae, as a renewable energy feedstock, extensively studied and offer several advantages like fast growth, year-round cultivation, the requirement of lower quality land, high oil content as per weight, utilization of carbon dioxide and value-added by-products. The techno-economical assessment system depends on its research, development, commercial aspects and environmental elements. It covers all the lab scale to the land project, and the social development for societal growth and fuel cost as an eco-friendly sustainable feedstock. In this pattern, various generation of fuel ($1^{st}$, $2^{nd}$ and $3^{rd}$) are very effective, but the algal-based biofuels process give a potential for sustainable development globally. Keeping in view of all the objectives, algal biomass-based biofuels in addition with wastewater, bioreactors analysis and its assessment are evaluated theoretically and experimentally. Therefore, the process, which is done through a laboratory scale, is unable to compile the requirements on a pilot scale. Hence, the utilization of *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) is the best option for the integrated approach up to final products from biomass.

The cultivation of algae plays an important role for the development of urban and rural areas on the basis of their socio-economic status. Algae help in the bioremediation of wastewater, thus reducing the water pollution from different water bodies to clean the environment. It increases the chances of labor and the settlement of the small scale industries as a purpose of business in different sectors. This may also help to provide several opportunities especially pertaining to agriculture for crop production and aquaculture industries that may help to develop the rural areas. Therefore, it has a lot of by-products that may be widely used by humans, feeds for aquaculture, cattle feeds, poultry farming, and it is used as a cosmetics product. Taken together the algae refinery thus supports the socio-economic development for the maintenance of a green and clean environment. In addition, the algae refinery encompasses a number of advantages, on the basis of increased biomass productivity for bio-products revival, removal of pollutants from the wastewater and reduction in the overall cost. Therefore, algae have the potential for the production of bioenergy based valuable products and their applications utilized for different purposes. In order to overcome the high-cost issue, extensive research has been in progress for the improvement of available technologies. In spite of all this, the study is focused on comparing and improving the viable approach applied for the cultivation method. However, algal biomass harvested from the bioreactor process for the production of different biofuels has been critically assessed. On the basis of the concluding remarks from Chapter-4, 5 and 6, it has been justified that algal-based wastewater treatment is an integrated approach for the production of bio-chemical compounds for sustainable environment very particularly for algal biomass.

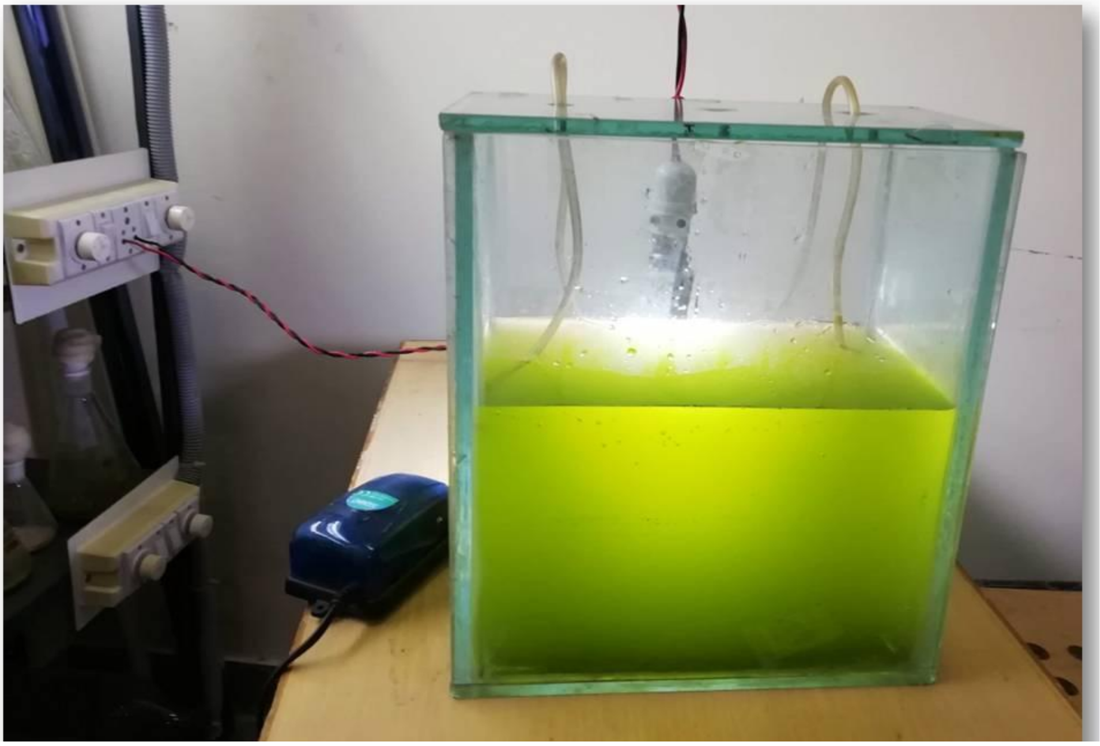
### 7.2. Material and methods

In this Chapter both *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) has been utilized for the experimental study and the physical and chemical parameters of 50% concentration of both wastewaters (SHWW and OSCCW) as are mentioned in the Chapter-3, 4 and 5 with bioreactor.

#### 7.2.1. Bioreactor

In this study, the vertical flat panel bioreactor was utilized to run the experiment, which was investigated with *Chlorella* sp. in both wastewaters (SHWW and OSCCW) and working volume was used 5 L.

**(a)**



**(b)**

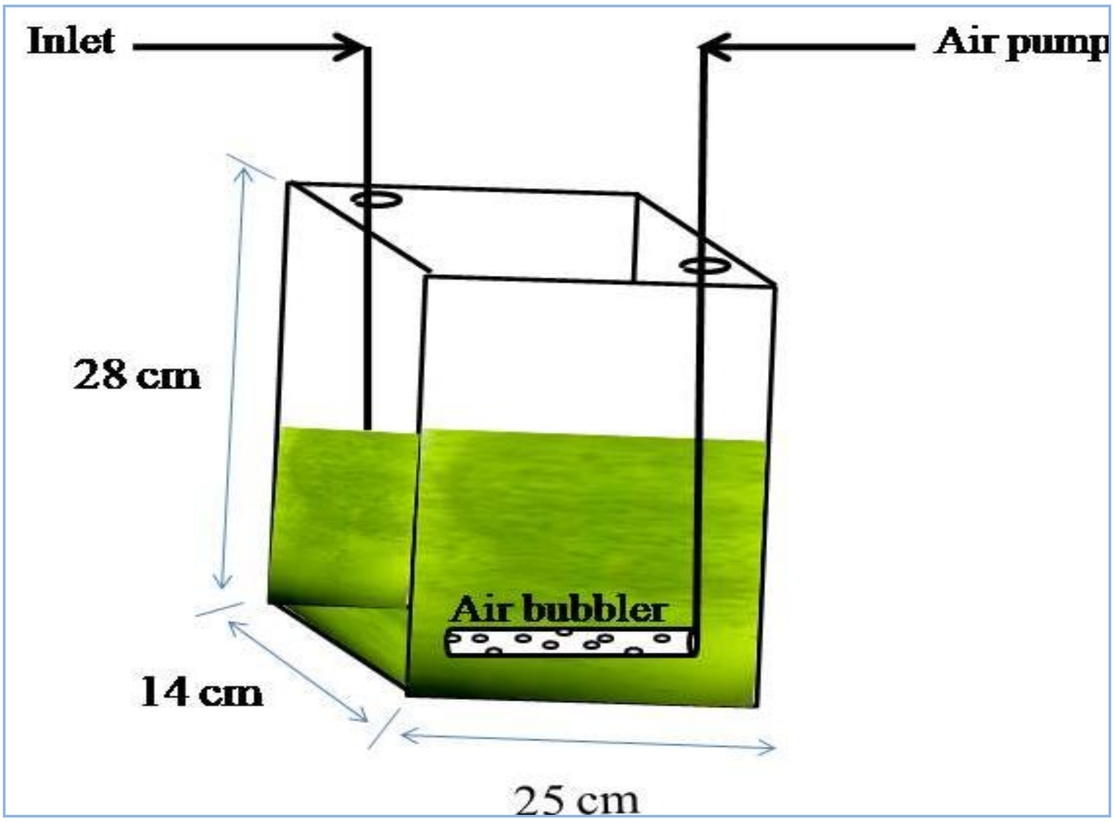



**Figure 7.1. Experimental setup (a) Actual picture of bioreactor; (b) Dimensions of bioreactor**

The flat panel bioreactor dimensions were 28 cm height, 25 cm length, and 12 cm width. It was made by toughened glass, which has a thickness of 8 mm. The air-bubbler was used for agitation at the bottle of the flat panel bioreactor in Figure 7. 1. The aeration was used for the supply of air pump (SEBO SB-548A) which has the capacity of 3.5 L $min^{-1}$ with an air bubbler having a size 4.5" x 0.8" x 0.5"/11.5 x 2 x 1.2 cm (Jardin Air Bubble).

**7.2.2. Biomass growth and biomass productivity**

For the objective of this Chapter, there were two *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) used in this experiment for cultivation of algal biomass in bioreactor by using SHWW and OSCCW. The characteristics feature of algal species is given in Chapter-3 of section 3.3. The algal growth and biomass productivity of SHWW and OSCCW are given in Chapter-3 of section 3.7.1.4 and 3.7.2.4 respectively. The analytical method of biochemical profile is given in Annexure-1. The selection and characterization of SHWW and OSCCW are given in section 3.4 of Chapter-3 is discuss in detailed.

**7.2.3. Bio-chemical compounds/composition analysis**

The bio-chemical compounds (proteins, carbohydrates, lipids, chlorophyll and carotenoids) of both strains (*C. pyrenoidosa* and *C. vulgaris)* in SHWW and OSCCW is given in Chapter-3 of section, 3.7.1.4 and section, 3.7.2.4 is elaborated in detail respectively.

**7.3. Results and discussions**

In this experimental study, two different *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) were used to grow in the SHWW and OSCCW. The biomass growth and biomolecules profile of both the *Chlorella* sp. were analyzed in the bioreactors for 15 days.

### 7.3.1. Algal growth in SHWW and OSCCW

The biomass growth of *C. pyrenoidosa* and *C. vulgaris* was analyzed in SHWW for 15 days of the cultivation time period and best concentration was optimized at 50%, of both strains which is given in Chapter-4 of section 4.4.2.1. The similar 50% concentration of OSCCW was also utilized for biomass growth of *C. pyrenoidosa,* and *C. vulgaris* which is given in Chapter-5 of section 5.3.1.1. of both strains. The algal biomass growth was optimized in the 500 ml conical flask with 300 ml working volume of SHWW and OSCCW. After optimization of algal growth, both algae were grown in a vertical flat plate bioreactor with 5 L working volume of both wastewaters to estimate the large scale cultivation of both *Chlorella* sp. *C. pyrenoidosa* and *C. vulgaris* produced 1290.75 mg biomass in 300 ml of SHWW which is shown in Table 7.1 of this Chapter, whereas 21512.5 mg biomass was recorded in vertical flat plate bioreactor which has working volume 5 L (5000 ml). *C. vulgaris* was generated 1170 mg biomass in 300 ml culture medium of SHWW and 19500 mg biomass was observed 5 L culture medium in vertical flat plate bioreactor. The biomass content of *C. pyrenoidosa* was observed 2706 mg in 300 ml OSCCW and 45100 mg in vertical flat plate bioreactor. *C. vulgaris* was produced 1344 mg biomass in 300 ml OSCCW culture medium and 22400 mg in flat plate bioreactor. It has shown that both species of *Chlorella* were followed similar trends with both wastewaters (50% concentration of SHWW and OSCCW) and *C. pyrenoidosa* was shown superiority over *C. vulgaris* in terms of biomass production. Same trend is observed on bioreactor part as with lab-scale growth patterns. Table 7.1 shown the values observed with bioreactor. The parameters that influence the large scale production of algae cultivation are pH, light intensity and temperature. Algae were grown in different variables temperature like 20 $^oC$, 24 $^oC$, 28 $^oC$ and 32 $^oC$ for the observation of temperature effect on the basis of different variables, as well as the pH also effect the algal growth and the optimal temperature required for culture productivity is 27 ˚C (Munir *et al.,* 2015).

As per the available literature up and down of temperature in any reactor, it effects the algal growth and their productivity (Chiranjeevi and Mohan, 2016; Lap and Ta, 2020). Sukacova *et al.,* (2021) reported that *C. pyrenoidosa* was utilized for the production of algal growth and biomass productivity, but the parameters like temperature fluctuation affect the algal growth. Loomba *et al.*, (2021) reported that the algal growth is depend upon the concentration of algal, photobioreactor interior design as well as light incidents are the major factors for determining the algal growth and their biomass productivity. Gatamaneni *et al.*, (2018) investigated that the algae cultivation in batch mode obtained results of lipid content were around 8 mg/l, whereas in algae grown in flat plate bioreactor the lipid obtained was 22 mg/l. As per the available literature, the algae cultivated in bioreactor at pilot scale for any commercial purpose, bioreactor is the best option for the cultivation of algae for the production of biomass for value-added products (Kalavrouziotis *et al.,* 2018).

**Table 7.1. Algal growth in SHWW and OSCCW**

| **Wastewater** | **Algae** | **Biomass production** | |
|---|---|---|---|
| | | **Conical flask (300 ml)** | **Vertical flat plate bioreactor(5000 ml)** |
| SHWW | *C. pyrenoidosa* | 1290.75 (mg) | 21512.5 (mg) |
| | *C. vulgaris* | 1170 (mg) | 19500 (mg) |
| OSCCW | *C. pyrenoidosa* | 2706 (mg) | 45100 (mg) |
| | *C. vulgaris* | 1344 (mg) | 22400 |

### 7.3.2. Bio-chemical compounds of algae

The bio-chemical compounds of algae consist of (protein, chlorophyll, carbohydrates and lipid) which are considered as good source of biomass production for value-added products. In this study, only 50% concentration of SHWW and OSCCW was investigated with *C. pyrenoidosa* for investigation. For the analysis of biochemical profile from 5$^{th}$ to 15$^{th}$ day of the experiment in SHWW and OSCCW were found best results on the basis of lab-scale analysis which is discussed in detailed in Chapter-4 of section 4.4., and Chapter-5 of section 5.4., respectively. Therefore, for the analysis of SHWW for chlorophyll, carbohydrates and protein content, values obtained are (0.31 µg/ml to 15.31 µg/ml), (2.37% to 9.58%) and (2.32% to 12.37%) respectively. The lipid content obtained on the 15$^{th}$ day of the investigation period in our study was 34%. Various studies evaluated that the *Chlorella* sp. is best for the production of lipid content which are as Wang *et al.,* (2016); Lin., *et al.,* (2015); Bhushan *et al.,* (2020) by using bold basal medium (BBM), for the cultivation of *Chlorella* sp. and obtained 28.2% of lipid content as well as another study reported that aquaculture wastewater media used for cultivation of algae and obtained maximum lipid content (23.7%). As per the observation with different studies, our lipid content results were found best as compared to other studies. Whereas in OSCCW the 50% concentration of wastewater were used with *C. pyrenoidosa* to run the experiment on lab scale. The value obtained at the time of analysis of chlorophyll, protein and carbohydrates from 5$^{th}$ to 15$^{th}$ day of investigation are (2.7 to 11.9 µg/ml), (9.9% to 18.65%) and (11.81 % to 26.13 %) respectively. The lipid content was obtained (34.6%) in *C. pyrenoidosa*. As per the literature reviewed, the highest lipid content was observed by using *C.*

*sorokiniana* when it grown with aquaculture wastewater than the synthetic medium reported by Guldhe *et al.,* (2017).

The bio-chemical compounds (chlorophyll, protein, carbohydrates and lipid) of *C. vulgaris* were analyzed in SHWW and OSCCW. Only 50% concentration of wastewater were taken for the analysis of this experimental approach where the chlorophyll, protein, carbohydrates and lipid content were investigated with SHWW with *C. vulgaris* are (0.19 µg/ml to 17.31 µg/ml), (23.44% to 30.32%) and (9.98% to 16.64%) respectively, as well as lipid content was obtained (39.7%) which is discussed in detailed of Chapter-4. Whereas in OSCCW with *C. vulgaris* the chlorophyll, protein, carbohydrates and lipid content were investigated and is discussed in Chapter-5 and the value obtained in OSCCW are (2.7 µg/ml to 10.9 µg/ml), (17.58% to 27.78%) and (17.77% to 32.36%). Similarly in lipid the value obtained are (31.1%) respectively.

The carbohydrates content of *C. pyrenoidosa* and *C. vulgaris* obtained was 123.6 mg and 194.64 mg in 300 ml and 2060 mg and 3244.5 mg in 5000 ml of SHWW respectively. Similarly, the protein content was obtained 159 mg, 354.6 mg in 300 ml of SHWW and 2650 mg, 5910 mg in 5000 ml of SHWW respectively. The lipid content was recorded about 449.10 mg, 462 mg with 300 ml of SHWW and 7486.35 mg, 7700 mg in 5000 ml of SHWW respectively. The production of chlorophyll in *C. pyrenoidosa* and *C. vulgaris* was recorded 45.93 µg/ml in 300 ml SHWW medium, 765.5 µg/ml in 5L vertical flat plate bioreactor and 51.93 µg/ml and 865.5 µg/ml in 300 ml and 5000 ml medium respectively. The bio-chemical compounds of algae grown in 300 ml and 5000 ml of SHWW and OSCCW with both strains of *Chlorella* is given in detailed in Table 7.2.

In OSCCW, *C. pyrenoidosa* and *C. vulgaris* were quantified for the biomolecules production, the carbohydrates content was found 706.8 mg, 832.2 mg in 300 ml wastewater medium and 11780 mg, 13720 mg in 5000 ml medium respectively. However, the protein content was noticed 504 mg, 706.8 mg in 300 ml and 5000 ml OSCCW culture medium respectively, and at the end of experiment the lipid content was recorded 936 mg, 791.18 mg in 300 ml medium respectively whereas 15600 mg, 13720 mg lipid was found with 5000 ml of OSCCW respectively. *C. pyrenoidosa* and *C. vulgaris* were produced 35.7 µg/ml, 595 µg/ml and 32.7 µg/ml, 545 µg/ml chlorophyll in 300 ml and 5000 ml OSCCW medium respectively which is given in Table 7.2. Thus, the present experimental study indicates that the *C. pyrenoidosa* and *C. vulgaris* are the prominent strains that produce biomass and bio-chemicals compounds at large scale in bioreactor by utilizing the various type of wastewater (SHWW and OSCCW). Harvesting of algal biomass is a tedious process because algae are micro-size (1-30 µm) organism that is suspended in the liquid medium and dewatering of algae has various operational challenges that enhanced the cost of algae cultivation. Various physical, chemical and biological routes are applied for the harvesting of algae. Physical methods such as centrifugation require high energy consumption and chemical contamination is the main challenge with chemical harvesting methods because organic polymers and inorganic salts are used for harvesting of algae and high cost is another problem with chemical harvesting methods. However, biological methods are considered as low cost but purity of harvested algal biomass is another challenge in which bacteria and fungi are utilized for harvesting of algal biomass from liquid medium (Li, 2020).

**Table 7.2. Bio-chemical compounds of *C. pyrenoidosa* and *C. vulgaris* in vertical flat plate bioreactor with SHWW and OSCCW**

| Wastewater | Algae | Bio-chemical compounds | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Protein** | | **Carbohydrate** | | **Lipid** | | **Chlorophyll** | |
| | | Conical flask (300 ml) | Vertical flat plate bioreactor (5000 ml) | Conical flask (300 ml) | Vertical flat plate bioreactor (5000 ml) | Conical flask (300 ml) | Vertical flat plate bioreactor (5000 ml) | Conical flask (300 ml) | Vertical flat plate bioreactor (5000 ml) |
| SHWW | *C. pyrenoidosa* | 159 mg | 2650 mg | 123.6 mg | 2060 mg | 449.10 mg | 7486.35 mg | 45.93 µg | 765.5 µg |
| | *C. vulgaris* | 354.6 mg | 5910 mg | 194.64 mg | 3244.5 mg | 462 mg | 7700 mg | 51.93 µg | 865.5 µg |
| OSCCW | *C. pyrenoidosa* | 504 mg | 8400 mg | 706.8 mg | 11780 mg | 936 mg | 15600 mg | 35.7 µg | 595 µg |
| | *C. vulgaris* | 706.8 mg | 11780 mg | 823.2 mg | 13720 mg | 791.18 mg | 13186.9 mg | 32.7 µg | 545 µg |

Therefore, the harvesting and dewatering processes of algae is important process for total costs. Several studies reported that the harvesting costs at 20% to 30% of the total production costs (Fasaei *et al.,* 2018). Scalability of biomolecules from algae is also a major problem because extraction of pure bio-chemical compounds (such as protein) is an obstacle, though quantification of the biomolecule is easy (Bleakley and Hayes, 2017) and large bimolecular production enhances techno-economical cost. Therefore, sustainable utilization of algal biomass and biomolecules is needed to address the techno-economical assessment that analyzed the operational cost of biomass recovery, energy requirement and cost of biomolecules extraction.

### 7.3.3. Impacts of bioreactor's design and fabrication on growth and bioactive compounds

During real-time conditions optimization of light and temperature is a technical challenge. So, here in this advanced stage laboratory to reactor scale wastewater treatment (SHWW and OSCCW), we focused on ambient temperature (ranges 27-32 °C) and natural day-night light cycle (12:12 h) to carry out the experimental study with selected algal strains. Bioreactor is considered as the best approach for the treatment of different wastewater for cultivation of algae in pilot scale. It was observed that different types of bioreactors have been developed by various researchers on the basis of their attractive characteristics and their industrial adaptation must be strongly recommended (Narayanan and Narayan, 2019; Narala *et al.*, 2016). It can be cultivated through open and closed bioreactors system. Raceway ponds are the common open pond system, for the growth of algae, whereas, the bioreactor is very popular system for the cultivation of algae (Huang *et al.,* 2017). The detailed process of bioreactors, types, fabrication materials advantages and disadvantages is discussed in detail in Chapter-2, which was used by several researchers in their experimental studies. There are several factors which effect the growth of algae are: nutrients, carbon sources and temperature. So, several heating and cooling systems are included in conventional type of bioreactors, but all these type of systems are economically not feasible. Bioreactors maintain the harvesting environment of algae which help in synthesis of neutraceuticals, cosmetics and pharmaceutical products. This technology permits for the strict control of environmental factor and biomass quality as well as photosynthetic efficiency and their productivity as reported by (Narala *et al.,* 2016; Acien *et al.,* 2017). Bioreactor based algal cultivation techniques are best known for the production of biomass, while as, still there are some technical issues that require to be solved to unfold its potential in up scaling of algal biomass production for biofuels. There are many developed method, which are used for high productivity, biomass quality and its density, to prevent the contamination of algal culture. Algae production has proven environmental reimbursement but also potential unpleasant impacts related to the high water and energy demand, emission control, management of wastewater, land use and risk of microbial contamination (Costa *et al.,* 2019; Barros *et al.,* 2019; Bechet *et al.,* 2017). Hence, there is a need of technological and material innovation, a need for adapted strains as well as a rethinking about the use of wastewater and its impact on production costs. In the same way, nutrition monitoring would also help to decrease the production cost as well as the environmental load caused by the amount of nutrition-loaded wastewater (Jaywant and Arif, 2019). The different type of bioreactorsare compared on the basis of their flow rate, merits and their applications which are given in Table 7.3.

**Table 7.3. Impacts of Bioreactor's with merits and applications (Narayanan and Narayan, 2019; Ahmad *et al.*, 2017; Kothari *et al.*, 2017**

| **Type** | **Flow rate** | **Merits** | **Applications** |
|---|---|---|---|
| Ideal bioreactor | Medium | Suitable for large capacity wastewater treatment plant | Wastewater treatment plants |
| Fed batch reactor | Medium | Suitable for batch process operations | Fermentation processes<br>Baker's yeast production |
| Continuous stirred tank reactor | High | Suitable for catalyzed reactions | Treatment of tannery effluents<br>Penicillin production |
| Plug flow tubular reactor | Medium | Suitable for constant velocity operations | Petrochemical industries and refineries |
| Membrane bio reactor | Medium | Suitable for higher concentrated effluents | Sewage sludge treatment plants<br>Reverse osmosis water purification plants wastewater treatment plants |
| Photobioreactor | Low | Suitable for algal culture | Algae extracted from sea weeds and growth of micro-organisms |

### 7.3.4. Techno-economics evaluation with challenges in lab-to land approach

The on-site treatment of wastewater in the bioreactor has various potential advantages that are cost-saving, resource recovery, reuse of water and nutrient-rich fertilizer. The wastewater treatment is a cost-intensive process due to the involvement of machinery in the conventional treatment plant, wastewater transportation from one place to the treatment facility uses pumping sets that have need energy in the form of petroleum fuels or electricity. However, onsite wastewater treatment does not need cost-intensive machinery and water transport systems. Recovery of water by onsite treatment can provide clean water to industries and although clean water does not match the standards of drinking use but industrial activities such as washing, cleaning and irrigation standard can be achieved by algal treatment of wastewater. Treatment of wastewater and production of bio-chemical compounds by algae are mentioned in Chapter-6, which is a dual benefits process in which algae utilized nutrients of wastewater and produced valuable biomass and provides economic benefits.

Utilization of bioreactor for wastewater treatment and production of algal biomass is an important process at large scale, because algae have the ability to convert pollutants into valuable biomass that have economic importance. In laboratory conditions, vital process parameters such as pH, temperature and solar radiation in controlled conditions, treatment of wastewater and biomass growth of algae in the conical flask is obtained in optimum level, when utilized in bioreactor for wastewater treatment and production of algal biomass at large scale also provided similar results as laboratory conditions where the source of light is sun. Therefore, the cost of conventional light sources is reduced by natural sunlight as compared to laboratory conditions. Similarly, real-time conditions are supported to temperature control into the bioreactor, by which the temperature ranges between 27°C- 32°C in day-night time and maintenance of optimum temperature into bioreactor does not need, because this algae can grow easily in this range of temperature, though not ideal but it suitable range of temperature for algal cultivation. Therefore, electricity does not need for temperature control in the bioreactor that saves energy and cost. Coupling of carbondioxide supply with bioreactor can mobilize the algal growth and treatment of wastewater, because researchers suggested that coupling of carbondioxde or flue gas with bioreactor is influenced in the carbon, hydrogen and nitrogen of algal biomass (Posadas *et al.*, 2015) that can alter the biochemical composition of algae. Algae sequestrate the carbondioxide into valuable biomass and also mitigate greenhouse gas emissions. Wastewater (SHWW and OSCCW) have high pH values ($10.16 \pm 0.32$ and $9.1 \pm 0.089$) which are mentioned in Chapter-4 and Chapter-5, can easily manage to reduce the pH by coupling of carbondioxide with algae in bioreactor because the nature of carbondioxide is acidic. Thus, it may be reduced the cost and chemicals for pH maintenance. Wastewater is a potential source of nutrients that can be converted into fertilizer. It is estimated that worth 480, 420 and 400 USD per Ton of di-ammonium phosphate, urea and potash can be obtained from wastewater that is lost during wastewater is untreated or partially treated. The algae-based fertilizers have the ability to retain moisture in the soil and slow-release property of fertilizer that avail nutrients (N and P) to plant when they have need (Molazadeh *et al.,* 2019). Thus, the recovery of nutrients by algal treatment of wastewater at a large scale in the bioreactor can be the cost-cutting process that can also be enhanced the sustainability of wastewater treatment.

Apart from the advantages of the onsite treatment of wastewater, many limitations have also been associated with it such as the requirement of land for onsite treatment is one of the main challenges when scarcity of land is already a problem. However, algal treatment of wastewater is still in the developing phase *i.e.* not a mature technology, so utilization at the industrial level involves high risk. Similarly, wastewater treatment at a large scale into bioreactor has various environmental low-cost considerations such as almost negligible emissions, carbondioxide sequestration, water reusability but infrastructural cost such as installation of the bioreactor, availability of land and operation and maintenance cost are high. Therefore, more research and development is required to reduce the cost that will make more reliable and adaptive technology for future requirements.

The results of present study can be easily reproducible at commercial level because the pre-optimization of biomass growth and bio-chemical compounds of both *Chlorella* sp. are followed similar trends at large scale in vertical flat plate bioreactor (5L). The 50% concentration of SHWW and OSCCW was utilized as nutrient medium that can reduce load on freshwater sources and chemicals. Therefore, algae can be produced high amount of biomass and biomolecule with simultaneous treatment of wastewater at large scale that can be clean and cost cutting technique for cotemporary world. The main finding in this experimental study by using two *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) cultivated with SHWW and OSCCW with bioreactor for the production of biomass in pilot scale for further commercial application. In SHWW, the bio-chemical compounds (protein, carbohydrates, lipid and chlorophyll) with (*C. pyrenoidosa* and *C. vulgaris*) investigated that the values obtained with a working volume of 300 ml in conical flask and 5000 ml in bioreactor of SHWW and OSCCW. The best results were found in OSCCW of *C. pyrenoidosa* compared to SHWW of *C. pyrenoidosa* results which are given in detail in Table. 7.2. Therefore, same finding was obtained with OSCCW of *C. vulgaris* compared to SHWW of *C. vulgaris*. So, the OSCCW found best results, treated with both strains of

*Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*). The algae can be cultivated in these types of wastewater at large scale for commercial application as well as value-added products. OSCCW is an eco-friendly treatment for clean and green environment for future research of innovation work. Furthermore, the harvested algal biomass required to be valorized in a multi-product for biorefinery approach, for the enhancement of economic viability and environmental sustainability of wastewater bioremediation. Hence, more research and development is required to make it sustainable technology in future.

- ➢ The results of this study are shown that the type of bioreactor and their specifications in real time situations, is the important parameter that support the algal biomass production and value-added products.
- ➢ The 50% concentration of both wastewater with both strains of *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) is best as per our investigation on lab-to-land approach.
- ➢ The present study provides an innovative approach, which is economically viable, proficient as well as expedient green approach for the cultivation of algal biomass for further valuable applications.
- ➢ It is a low-cost technique for the production of algal biomass-based biofuels and other for value-added products that are used in cosmetics, nutrient-supplements, medical and fertilizer, as per techno-economic assessment in between conventional and advanced remediation approaches.

# Chapter 8
# Conclusion

## 8.1. Conclusion

The objectives decided for this thesis, are successfully completed on the basis of their experimental validation. Optimization of different concentrations (25%, 50%, 75% and 100%) of wastewater were assessed with two strains of *Chlorella (C. pyrenoidosa* and *C. vulgaris)* for efficient treatment for the production of maximum algal growth for value-added products is an important part of this research work. The experimental results were assessed through algal growth by using growth kinetic models (Logistic and Gompertz) and co-relational analysis between the process parameters (TDS, BOD, COD, nitrate and phosphate) with algal bio-chemical profile (protein, carbohydrates, chlorophyll and lipid) for the analysis of positive and negative correlation between two variables. Designing and the specific characteristics of bioreactor for the enhancement of algal growth and its biomass as well as biofuels coupled with wastewater is an integrated approach, which is also a part of Phase-I study, all the parameters which are assessed in Phase-1 study carried out on the basis of experimental investigation for Phase-II. Phase-III of experimental study is emphasized on advancement of algal biomass harvesting process and their optimization with different concentrations. Therefore, the concluding remarks of each Chapter, have been given at the end of each Chapter but the overall conclusion of this thesis work is provided here with elaboration.Several practical challenges faced during the various stages of experiment *i.e.* physical and chemical changes at the time of processing for the production of algal biomass and pilot scale assessments for value-added products. Inspite of all these challenges, to remove the burden on fresh water sources, wastewater from different sources has been selected as a substrate for algal biomass (*C. pyrenoidosa* and *C. vulgaris*) growth and their development. Effect of several parameters has been taken into consideration for algal growth, biomass and biochemical productivity. Specific concluding remarks as per findings are given below:

Due to high contamination in water resources, types of pollutants, and their impacts different treatment option are available (conventional and advanced). Some alternative strategies with an integrated approach for wastewater treatment with algal biomass and its valuable applications are also available, which is part of this research study. After an extensive survey of the literature and on the basis of bio-based value-added products and their influencing parameters as well as the residual biomass, which are applied for future applications, provides a new view for bio-economy. Therefore, algal biomass has high potential to solve all these problems that are associated with wastewater to fuel conversion. Whereas, per the investigation after the experimental analysis with both strains (*C. pyrenoidosa* and *C. vulgaris*) with selected wastewaters *i.e.* SHWW and OSCCW by using BG-11 media (control) as a source of nutrient for the enhancement of algal biomass production in an ecofriendly way for clean and sustainable environment. This harvested biomass required to volarized in multiproduct for biorefinery approach, for the enhancement of economically viable and sustainable development. SHWW are used for the production media of algal biomass as well as the potential of biomass yields have been explored. This novel approach provides a sustainable solution for SHWW treatment because discharges of wastewater without treatment are always responsible for the deterioration of the surrounding environment and the spread of pathogenic bacteria from slaughter waste materials. Furthermore, using the concept of construction of on-site pits in combination with algal biomass near slaughterhouses may be able to solve the problem of wastewater discharges without treatment for healthy, clean and commercial solutions. Moreover, *Chlorella* sp. has a high potential for their nutrient sources like protein, chlorophyll, carbohydrate and lipid.

OSCCW, is large contaminated source in semi-urban, rural and urban areas with characteristics of high load of organic and inorganic pollutants. Therefore, it represents a very good resource for the production of biomass as well as the algal-based OSCCW has high potential for the removal of nutrients from wastewater and extraction of bio-chemical compounds. The remediation of wastewater using *Chlorella* sp. provides an environmentally acceptable and effective option for wastewater remediation, which are not only recycles valuable nutrients but also improves the quality of water. Therefore it may be regarded as an efficient nutrient removal process for the removal/recovery of nutrients from wastewater. Due to the increasing demand algae is a promising tool which can be applied for the production of bioactive compounds like (carotenoids, carbohydrates, proteins) for value-added products. Identification and isolation of novel bio-chemical compounds from algae will provide a valuable platform for the development of new therapeutic agents for neutraceuticals and pharmaceuticals products, theoretical evaluation of this concept assessed in Chapter-6 and summarized with potential and challenges. Therefore, to overcome these issues some of these bio-chemical active compounds are presently articulated in proper vectors to relieve for purification and hence for the reduction of purification as well as production costs.

The 50% concentration of SHWW and OSCCW was utilized as nutrient medium that can reduce load on fresh water sources and chemicals. Therefore, algae can be produced high amount of biomass and bio-chemical compounds with simultaneous treatment of wastewater at large scale that can be clean and cost cutting technique for cotemporary world. Furthermore, the harvested algal biomass required to be valorized in a multi-product for biorefinery approach, for the enhancement of economic viability and environmental sustainability of wastewater bioremediation. The bioreactor based wastewater treatment provides a good platform and their specifications in real time situations, is the important parameter that support the algal biomass production and value-added products. It provides an innovative approach, which is economically viable, proficient as well as expedient green approach for the cultivation of algal biomass for further valuable applications. Figure 8.1 representing the overall process of wastewater treatment by using algae and their utilization for various processes and its benefits for the future very much belongs to this research work only in graphical format.The techno-economic assessment approach is discussed in detailed in Chapter-7 of this thesis. For the benefits of these types of production system an integrated approach for the minimization of wastewater, cost analysis, good amount of biomass in bulk, less requirement of chemical media and freshwater for the completion of this process taken into account. From the experimental evaluation, findings clearly state that research efforts, particularly with low cost, operating cost and their lifespan of reactor is a great demand for pilot scale cultivation.

Hence, utilization of wastewater as a media for the cultivation of algal growth is a good alternative for treatment process, which is further investigated for bioenergy options (biohydrogen, biodiesel and biogas) and value added end products. Simultaneously, algal biomass used for treatment of wastewater can be processed for biodiesel (bio-oil), bio-chemical products and can be used for value-added products with clean and green environment. This lead to the conclusion that the utilization of wastewater with (*C. pyrenoidosa* and *C. vulgaris)* have a potential. Whereas, upstream and downstream processing require an optimization steps for “system”

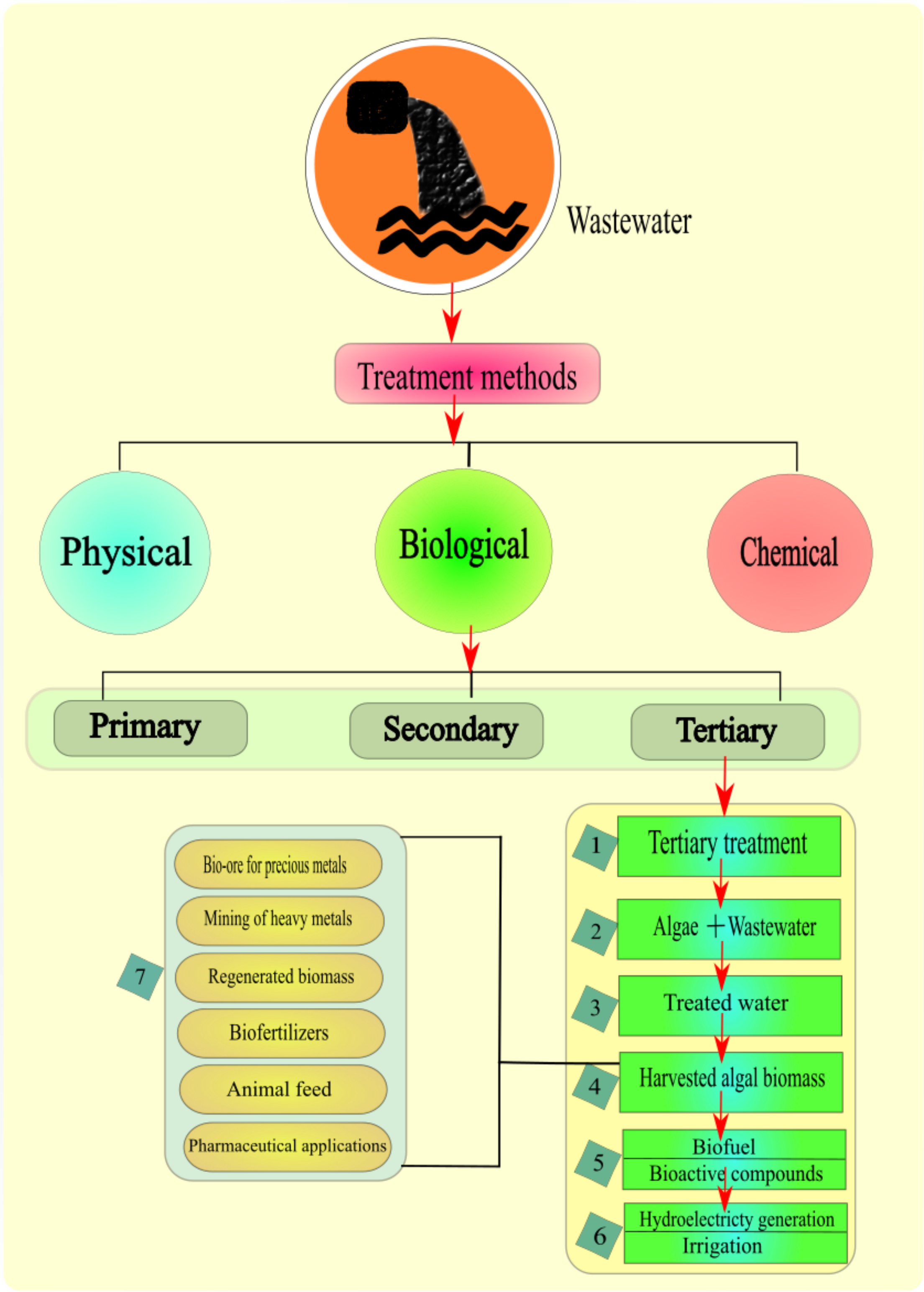



**Figure 8.1. Systematic representation of wastewater treatment using algae for future applications**

**Note: In this Figure (1-5) is the part of this research work and (6, 7) is for future recommendation.**

Working in efficient way with any types of algal strain with wastewater. The work done in this thesis indicates a relevant answer to the problems associated with clean efficient production of biofuels and other value-added products too. It also present a new way forward for liquid waste management approach to solve the energy crisis, freshwater crisis and control of environmental pollution from local to national to regional level.

**8.2. Future scope of work**

- A similar study could be conducted to optimize the different conditions for the various other nutrient rich wastewaters by the same/other algal species for treatment and for exploration of various bio-active compounds of value-added character from biomass.
- The phycoremediation study for wastewater treatment could be carried out by immobilizing the biomass of the algal species may also be a good alternative and for exploration of various bio-active compounds of value-added character from biomass.
- Genetic engineering approach for improvement of yields with wastewater may also a new field to explore for clean and green aspect.
- Furthermore, techno-economic feasibility of each adopted technology should also be assessed to make it commercially accessible in sustainable way with long-term aspect.

# *Summary*

Due to the global population increase the energy consumption also increased rapidly on the basis of high living standard, and energy utilization. The process by which renewable source of energy can be supplied has become a big challenge. Nowadays researchers are focusing on advanced biomass cultivation technologies for the production of biofuels utilizing different raw material for the production of main products. In this framework the important properties of algal biomass *i.e.* biochemical profile (carbohydrate, protein, lipid, chlorophyll, chlorophyll a, chlorophyll b, carotenoids and pigment) as well as biofuels production (biodiesel, biohydrogen, bioethanol and biogas) gained attention to fulfill the biomass production. Algae are popular worldwide, due to their multiple applications such as nutritional value, cosmetic products and pharmaceutical based products. Therefore, pilot scale cultivation of algae contains good quality of food due to their chemical compounds for valuable products. In natural as well as artificial cultivation system, algae are exposed to a number of factors such as temperature, pH, nutrient and carbon sources. All these parameters affect the growth of algal biomass, biomass productivity, metabolisms activity, specific growth rate and doubling time. The physical and chemical conditions of algae depends upon the culture system of algae, which in turn have an effect on qualitative and quantitative analysis of algal biomass. Additionally, algae received much attention, for the production of biofuels to replace fossil fuel, and carbon dioxide, due to their high photosynthetic efficiency. Several of these applications require a photobioreactor system in which monoculture of algal biomass, can be developed for high amount of biomass productivity. There are various open ponds, outdoor and enclosed photo bioreactor systems which have been developed for the growing of phototrophic based algae like cyanobacteria and algae. The photobioreactor facilitate the maximum solar energy capture and conversion, preferably using low cost operational activities. To understand the synergetic relationship between physical/chemical and environmental factor, optimized with multiples variables of these factor need for the development of algal productivity. The macro and micro nutrient present in wastewater are suitable for algal growth and their development. This types of low cost integrated approach contains: reduction of wastewater cost treatment, biomass production on annually, reduction of freshwater burden, reduction of media cost. Therefore, keeping in views, the designed objectives have been taken to prove the concepts which is based on experimental validations.

**Chapter 1: Introduction**

In this chapter, an overview of wastewater treatment mechanism and their effect on environment is discussed in detail. The wastewater directly discharging into the environment creates a risk for ecosystem and human health. Therefore, this Chapter is designed with the objective to provide basics on types of water resources, sources of water contamination and pollution, types of pollutants, and their impacts. Different treatment options (conventional and advanced) are also discussed in this Chapter. In turn, biological wastewater treatment may prove to be good to fulfill the biological remediation process for the reduction in consumption of natural resources towards preventing the environment. The classification of algae is given in Figure 1 of this Chapter. Attention is warranted for the use of advanced cutting-edge techniques in the treatment of wastewater with an aim of resource recovery and low costs too. Impacts of these in terms of water crisis and the major industrial sources responsible for large wastewater discharges are also discussed. Further, some major wastewater contributors are also discussed in this Chapter. Alternative strategies with an integrated approach for wastewater treatment are also part of this chapter, very particularly regarding the use of algal biomass for wastewater treatment and their wide range of applications.

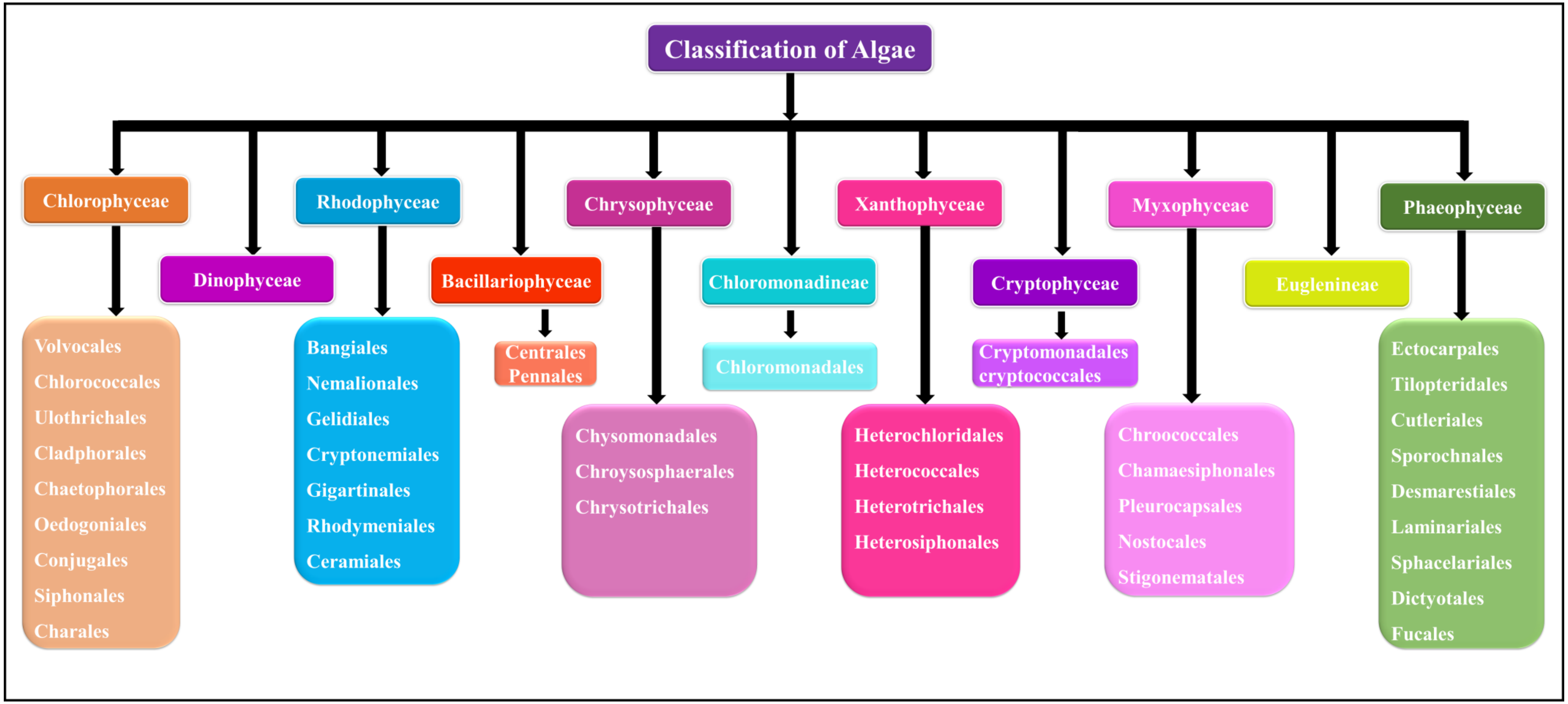



**Figure 1. Classification of algae (Fritsch 1935)**

**Chapter 2: Review of literature**

This Chapter deals with the chemical and biological contamination in water which has become a major problem in the last several years and affecting society significantly. Despite these techniques, the contamination is still present in wastewater so, requires some advanced treatment options. Algal-based wastewater treatment represents cost-effective and efficient treatment techniques, by which the algae biomass is the only renewable resource which has high uptake capability of pollutants removal. After an extensive survey of the literature, on the basis of bio-based value-added products, and their influencing parameters and the residues biomass, which are applied for future applications, which provide a new view for bio-economy. Designing and fabrication different types of photobioreactors, materials used for construction, and its advantages and disadvantages are also discussed. Algal biomasses have high potential to solve all these problems, which is associated with wastewater to fuel conversion. Therefore, the wide range of valuable applications of algal biomass for various fields are: biofuels and value-added products (carbohydrates, proteins, pigments, carotenoids and lipid) is also discussed in detailed.

**Chapter 3: Materials and methods**

This Chapter is explaining about the experimental plans and their methodology, algal species and wastewater treatment process for the production of end-products. The analytical techniques used in present study are explained with basic information of algal sp., characteristics of wastewater and algal growth optimization with selected wastewaters (different concentrations) at organic pollutant loads with different physical parameters are also outlined. Experimental procedures adopted with phycoremediation approach for wastewater treatment and parametric observations are also discussed.

Harvesting of known algal biomass with highest growth rate in coupling with wastewater at lab scale is also assessed with designed bioreactor to scale-up the biomass at large scale as per objective designed. The co-relational analysis between bio-chemical compounds (chlorophyll, carbohydrate, protein, lipid and carotenoids) with selected water samples using selected algal strains was also investigated. Application of bio-compounds is also assessed for neuracaeutical value products on theoretical basis as per the findings. Techno-economic assessment also investigated for the approaches selected to complete the objectives of this research study.

**Chapter 4: Phycoremediation of slaughterhouse wastewater and their impact on algal biochemical compounds using both *Chlorella* species with correlation study**

Therefore, the objective of this chapter is focused on a novel approach for bioremediation. Presently, the algal biomass production at the commercial level uses the existing freshwater resources which are not sustainable due to their demand for drinking and other purposes and is available at a high cost. It is also an alarming condition for the environmentally sustainability and contributes as one of the factors in capital production cost. Thus, the utilization of wastewater as an alternative source for freshwater and to reduce the cost in a dual way *i.e.* as a medium for biomass and providing low-cost source nutrients from those instead of high-cost chemicals, decrease the production cost and break the limitations with its wide applications. Hence, the present study explores to assess the impact of variation in the concentration of SHWW for the growth of *C. pyrenoidosa* and *C. vulgaris* for high biomass production. Keeping in view, *C. vulgaris* was taken in this experiment study for the assessment of algal growth and lipid production potential using SHWW with an aim for treatment and one of the important value-added products *i.e.* bio-oil. The main important part of this work is based on the growth of both strains of *C. pyrenoidosa* and *C. vulgaris* was done by using different kinetic models by nutrient availability in SHWW via phycoremediation and conceptualization of freshwater footprinting is also investigated on the part of reusability of treated water and freshwater crisis. The SHWW was treated with two *Chlorella* sp. (*C. vulgaris* and *C. pyrenoidosa).* The growth and algal biomass production were optimized with various concentrations of wastewater, and this experiment was conducted for 15 days. During the growth period, the biochemical parameters were assessed at every 5 days interval, in terms of optical density (680 nm) with organic load (COD, BOD), nutrient load (nitrate and phosphate), reduction of pollution load, and nutrient load. Besides, the treated wastewater was analyzed thoroughly before it releases to the surroundings. Co-relational analysis between the physicochemical and biochemical profile of SHWW of algal biomass is also studied in both *Chlorella* sp. In the present study, it is concluded that algal-based wastewater treatment is an effective and low-cost method without any harm to the environment. Therefore, different concentration (25%, 50%, 75% and 100%) of wastewater have been taken for this experimental investigation. In comparison to the modified Gompertz model, the logistic model produced better results. SHWW treatment is an effective and best alternative nutrient source to enhance the biochemical profile of algal biomass for their products. Only 50% concentration of wastewater was selected for the changes in chlorophyll, protein, lipid and carbohydrate along with growth dynamics monitored during the uptake of nutrients from wastewater. Based on growth, 50% concentration is the best for the production of biomass (430 mg/l on dry weight basis) in SHWW. Therefore, the pH of wastewater during the phycoremediation process needs to be within the optimal pH range. The present experimental investigation results clearly show as the potential for algal biomass production with favorable biochemical profile with SHWW at 50% concentration. the correlation between two variables indicates the high differences among them. The highest positive correlation was observed in 25% and 50% concentration of SHWW of TS, BOD, COD, nitrate and phosphate between the chlorophyll, protein, and carbohydrate are highly correlated which is also present. The best positive correlation was found between the 25% and 50% concentrations of wastewater. In *C. vulgaris* the correlation analysis was comprised between the physicochemical parameters and biological parameters of SHWW with 50% concentration. Furthermore, using the concept of construction of on-site pits in combination with algal biomass near slaughterhouses may be able to solve the problem of wastewater discharges without treatment for healthy, clean and commercial solutions in favor of bio-economy. The SHWW has a high potential to treat algae for the production of algal biomass and lipid extraction. Therefore, the phycoremediation based process of SHWW indicates that is used for value-added products and its main aim is to reduce pollutants from waste such as sewage. Therefore, it represents a very good resource for the production of biomass.

**Chapter 5: Phycoremediation of open-sewage channel contaminated wastewater and their impact on algal biochemical compounds using both *Chlorella* species with correlation study**

The present chapter deals with the current global environmental issues raise unavoidable challenges for the utilization of natural resources. Increasing the human population is a challenge that affects the clean water body becomes a global problem. The biomass production from algae using industrial wastewater offers the possibility of recycling industrial residues to create new sources of raw materials for energy and material use. However, the contamination of freshwater is a global problem. Therefore, different concentration (25%, 50%, 75% and 100%) of wastewater have been taken for this experimental investigation. For the production of algal biomass 50% concentrations of both algal strains were used to experiment for 15 days. During the growth period, physico-chemical parameters were assessed at every 5 days gap, based on optical density (680 nm) with organic load (COD, BOD), nutrient load (nitrate and phosphate), for the reduction of pollutants. When varied initial pH from 5 to 11 for a culture of *Chlorella* sp., the removal of both nitrate and phosphate was not significantly affected. This may imply that the initial pH of wastewater has little influence on treatment performance, which was found that algal growth increased alkalinity to a similar level regardless of the initial pH. The BOD and COD were found to be a higher range in comparison to discharge limits on the basis of general standard discharge limit. The nutrient availability (nitrate and phosphate) has the potential to treat with algae. The high impact of natural and anthropogenic activities which are constantly released into the environment that demands a better awareness about the chemical contamination in the surface water. Furthermore, following wastewater remediation with algae using wastewater as a low-cost nutrient source for algae cultivation, harvesting the algae may prove to be a source for the production of different valuable byproducts, such as lipids, fertilizers, and biofuels. For many algae, wastewater contains essential nutrients for their growth such as nitrogen, phosphorus, and organic compounds like carbohydrates, amino acids, and vitamins. However, all algae are not able to tolerate wastewater environments that are toxic due to multiple factors depending on the source and type of wastewater. Thus, screening for suitable algal strains is a prerequisite for algal-based phycoremediation of wastewater. The strain is selected based on the chemical and biological composition of the wastewater. But in addition, there is a need of information on the kinetic assessment of lipid production for algae cultivated in nutrient-limited wastewater.

Keeping in view this Chapter aims on the the production of algal biomass and lipid extraction for value-added products. The *Chlorella* sp. is the main objective of this chapter by using OSCCW to evaluate the physico-chemical properties, for the production of biomass and lipid. Algae is a rich source of crude oil and high value-added products that are not only applied to food, feed but diverse new fields such as photovoltaic. Algae produced various photosynthetic pigments, secondary metabolites such as chlorophylls, carotenoids and phycobiliproteins are a good source of sensitizers. The result shows that the growth of algae in absence of OSCCW did not give the best result due to lack of nutrients. But in controlled conditions with BG-11 medium the growth of algae was observed with both *Chlorella* sp. and the best result was found in *C. pyrenoidosa* based on growth curve as compared to *C. vulgaris.* However, the growth of *C. vulgaris* was much noticed between on $13^{th}$ – $15^{th}$ days of the experiment with 50% concentration of OSCCW may be due to the level of nutrients being high as compared to *C. pyrenoidosa*. The highest biomass content was analyzed at 0.902 g/l in *C. pyrenoidosa* whereas 0.848 g/l biomass content was analyzed in *C. vulgaris* at $15^{th}$ day of experiment. In this study, they were used three models (Logistic, Gompertz and Richards model), but the Logistic and Gompertz are best after the assessment for the analysis of algal growth. Therefore, *C. pyrenoidosa* has high potential for algal biomass production as compared to *C. vulgaris*. But based on the profile of chlorophyll, protein and carbohydrate in *C. pyrenoidosa* were found best result due to the increased level of biochemical profile from $0^{th}$ to $15^{th}$ day of the analysis. In *C. pyrenoidosa* the highest positive correlation was observed with the $0^{th}$ to $5^{th}$ day of TDS, COD, BOD, nitrate and phosphate as compared with carbohydrate, chlorophyll and protein, was observed with $10^{th}$ to $15^{th}$ day was observed, that is highly correlated with each other. In *C. vulgaris* correlation TDS, BOD, COD, nitrate and phosphate between two variables are not positively correlated with chlorophyll, protein, and carbohydrate was not found highly correlated. Thus, the analysis and observation on the basis of correlation with positive results indicate it is a very effective method for wastewater treatment and their management. The significant correlation between the algal biomass and the nutrients that indicate the importance of microalgal communities can be used as an important tool for the various phases of wastewater treatment. The algal-based OSCCW has high potential for the removal of nutrients from wastewater. In this study, the *C. pyrenoidosa* achieved the high efficient amount of nutrient removal rate and high lipid production. The algal-based OSCCW treatment is a very low and environmental friendly method to treat the *Chlorella* sp. with wastewater for the extraction of lipid. The remediation of wastewater using *Chlorella* sp. provides an environmentally acceptable and effective option for wastewater remediation, which not only recycles valuable nutrients but also improves the quality of water. Therefore it may be regarded as an efficient nutrient removal process for the removal of nutrients from wastewater.

**Chapter 6: Algal derived Bio-chemical compounds using wastewater: Theoretical Assessment for value added products and their applications**

The present chapter deals with the investigation of culturing of algal biomass under various parameters for the production of maximum biomass. Algae are cultured by the application of various conditions. Due to large scale of cultivation of algae increase the green technology economically. In the natural or artificial process, it is exposed to a number of environmental factors like pH, nutrient sources as well as light. The bio-chemical compounds (carbohydrates, protein, lipid, carotenoids and pigments) are the main compounds which help to produce maximum biomass for value-added products. Despite all of this, the biogenic accessible elements concentrations are the main parameters that affect the growth of algae. In this work, the bio-chemical compounds are derived from algae by treating with different wastewater (SHWW, OSCCW) for the estimation of valuable results, which is based on value-added products. Algae play an important role in the daily diet and future life. It is considered as a rich source of proteins, carbohydrates and lipids for value-added products and it is an essential food for human beings as well as animal feed all over the world. However, regardless of these challenges is associated with the completely harvesting of algal biomass. Whereas, harvesting of algal biomass is an important part of this experimental research for value-added products. Therefore, various conventional treatment processes (flocculation, centrifugation, sedimentation, electrocoagulation and gravity) are used for the cultivation and dewatering for the harvesting of algal biomass. Numerous compounds extracted from algae are widely used as cosmetic industry as water-binding agents, thickening agents, and antioxidants in skin and facial care products. It is well known that algae contain valuable products that are proteins, carbohydrate, lutein, β-carotene, phenolic and flavonoids compounds that are useful for several industrial applications. Despite of these excellent and advanced benefits, there are several limitations that should be focused as well. Therefore, to overcome these issues some of these bio-chemical active compounds are presently articulated in proper vectors to relieve for purification and hence for the reduction of purification as well as production costs. These novel approaches provide a innovative approach by using the algae to obtain the valuable bio-chemical compounds for the treatment of various disease. Furthermore, in genetic engineering the algae have high metabolites towards natural organism for drug deliveries have been explored. As per the experimental observation light and temperature are the main factor for the improvement of biochemical profile, biomass growth and their development. There are various applications of algae, which are used as a feed supplements. The obtained biomass from algae has multifunctions which are used as a feedstock for fish towards farm animals. So for the best of our knowledge the result obtained in our study by using SHWW and OSCCW by treating with both strains *(C. pyrenoidosa* and *C. vulgaris)* compare to previous study is best and it can be utilized as fish meal as well as animal feed. The main application of carbohydrates are antioxidants, anticoagulant, antiproliferative and immunostimulatory. Inspite, of all these more efforts required to develop the multifunctional ranges of products, that are affordable and also inter-related with nutritional science that give a more health benefits in current market. The main aim of this chapter is completely focused on algal based biomass for value-added products. Major finding can be concluded that the vaue-added products production is an integrated approach for commercialized application, but the main challenges cannot be ignored at the part of environment. Therefore algal based wastewater treatment is a zero investment on the basis of cost and have high potential on market level.

**Chapter 7: Techno-economic assessment: comparative study**

Due to the current environmental issues, renewable energy is the best option to solve the environmental issues, to attract increasing attention on both the academic and industrial sectors. The techno-economical assessment system depends on its research, development, commercial aspects and environmental elements. Keeping in view of all the objectives, algal biomass-based biofuels in addition with wastewater, bioreactors analysis and its assessment are evaluated theoretically and experimentally. Therefore, the process, which is done through a laboratory scale, is unable to compile the requirements on a pilot scale. Hence, the utilization of *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) is the best option for the integrated approach up to final products from biomass. The 50% concentration of SHWW and OSCCW was utilized as nutrient medium that can reduce load on fresh water sources and chemicals. Therefore, algae can be produced high amount of biomass and biomolecule with simultaneous treatment of wastewater at large scale that can be clean and cost cutting technique for cotemporary world. The main finding in this experimental study by using two *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) cultivated with SHWW and OSCCW with bioreactor for the production of biomass in pilot scale for further commercial application.

The biomass growth of *C. pyrenoidosa* and *C. vulgaris* was analyzed in SHWW for 15 days of the cultivation time period and best concentration was optimized at 50%, of both strains. The 50% concentration of OSCCW was also utilized for biomass growth of *C. pyrenoidosa,* and *C. vulgaris* of both strains. The algal biomass growth was optimized in the 500 ml conical flask with 300 ml working volume of SHWW and OSCCW. After optimization of algal growth, both algae were grown in a vertical flat plate bioreactor with 5 L working volume of both wastewaters to estimate the large scale cultivation of both *Chlorella* sp. *C. pyrenoidosa* and *C. vulgaris* produced 1290.75 mg biomass in 300 ml, whereas 21512.5 mg biomass was recorded in vertical flat plate bioreactor which has working volume 5 L (5000 ml). *C. vulgaris* generated 1170 mg biomass in 300 ml culture medium of SHWW and 19500 mg biomass was observed 5 L culture medium in vertical flat plate bioreactor. The bio-chemical compounds (chlorophyll, protein, carbohydrates and lipid) of *C. vulgaris* were analyzed in SHWW and OSCCW. Only 50% concentration of wastewater were taken for the analysis of this experimental approach where the chlorophyll, protein, carbohydrates and lipid content were investigated with SHWW with *C. vulgaris.* Thus, the present experimental study indicates that the *C. pyrenoidosa* and *C. vulgaris* are the prominent strains that produce biomass and bio-chemicals compounds at large scale in bioreactor by utilization of various types of wastewater (SHWW and OSCCW). The algae can be cultivated in these types of wastewater at large scale for commercial application as well as value-added products. OSCCW is an eco-friendly treatment for clean and green environment for future research. The 50% concentration of both wastewater with both strains of *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) is best as per our investigation on lab-to-land approach. It provides an innovative approach, which is economically viable, proficient as well as expedient green approach for the cultivation of algal biomass for further valuable applications. It is a low-cost technique for the production of algal biomass-based biofuels and other for value-added products that are used in cosmetics, nutrient-supplements, medical and fertilizer, as per techno-economic assessment in between conventional and advanced remediation approaches. Bioreactor based algal cultivation techniques is best for the  production of biomass for valuable applications, while as, still there is some technical issue that require to be solved to unfold its potential in up scaling of algal biomass production for biofuels. Thus, the recovery of nutrients by algal treatment of wastewater at a large scale in the bioreactor can be the cost-cutting process that can also enhance the sustainability of wastewater treatment. There are many developed method, which are used for high productivity, biomass quality and its density, to prevent the contamination of algal culture. Algae production has proven to be environmental reimbursement and also reduce potential unpleasant impacts related to the high water and energy demand, emission control, management of wastewater, land use and risk of microbial contamination.

**Chapter 8: Conclusions and future recommendations**

The experimental results were assessment through algal growth by using growth kinetic models (Logistic and Gompertz) and correlational analysis between the process parameters (TDS, BOD, COD, nitrate and phosphate) with algal biochemical profile (protein, carbohydrates and lipid) for the analysis of positive and negative correlation between two variables. Optimization of different concentration (25%, 50%, 75% and 100%) of wastewater were assessed with two strain of *Chlorella* for the efficient treatment for the production of maximum algal growth for value added products is a important part of this research work. Correlational analysis have been taken as the variables between two process parameters and biochemical profile for the negative and positive correlation of both strains. Hence, utilization of wastewater as a media for the cultivation of algal growth is a good alternative for treatment process, which is further investigated for bioenergy options (biohydrogen, biodiesel and biogas). It also presents a new way forward for future energy options and waste management approach to solve the energy crisis, freshwater crisis and control of environmental pollution from local to national to regional level. The various applications of algal biomass for the treatment of wastewater to produce biofuels is an integrated approach for clean and ecofriendly sustainable environment. Phycoremediation based treatment is low cost process for the generaton of bioenergy. But there are some practical challenges during the implementation of this process. Several practical challenges are faced during the stage of experiment *i.e.* physical and chemical changes at the time of processing for the production of algal biomass in pilot scale for value added products. Inspite of all these challenges, to remove the burden on our freshwater sources, wastewater from different sources have been selected as substrate for algal biomass (*C. pyrenoidosa* and *C. vulgaris*) growth and their development. Effect of several parameters has been taken into consideration for algal growth, biomass and biochemical productivity. Hence, utilization of wastewater as a media for the cultivation of algal growth is an good alternative for treatment process, which is further investigated for bioenergy options (biohydrogen, biodiesel and biogas). The bioreactor plays an important role for the cultivation of biomass. Simultaneously, algal biomass used for treatment of wastewater can be processed for biodiesel (bio-oil) and its biomass can be used for value-added products. Implication of algal biomass for bioenergy (biofuel) as well as value-added bio-chemical products provide a integral approach for the treatment of wastewater with clean and green environment. This leads to the conclusion that the use of wastewater with (*C. pyrenoidosa* and *C. vulgaris)* have a potential for biofuels production. Whileas, upstream and downstream processing steps require an optimization for “system” working in efficient way with any types of algal strain with wastewater. The work done in this thesis indicates a relevant answer to the problems associated with clean efficient production of biofuels. It also presents a new way forward for future energy options and waste management approach to solve the energy crisis, freshwater crisis and control of environmental pollution from local to national to regional level.

# References

Acien, F.G., Molina, E., Reis, A., Torzillo, G., Zittelli, G.C., Sepulveda, C., Masojidek, J. (2017). Photobioreactors for the production of microalgae. In Microalgae-based biofuels and bioproducts, 1-44.

Adamczyk, M., & Sajdak, M. (2018). Pyrolysis behaviours of microalgae *Nannochloropsis* gaditana. Waste and biomass valorization, 9, 2221-2235.

Adarme-Vega, T.C., Lim, D.K.Y., Timmins, M., Vernen, F., Li, Y., Schenk, P.M. (2012). Microalgal biofactories: A promising approach towards sustainable omega-3 fatty acid production. Microbial Cell Factories, 11: 96

Addams, L., Boccaletti, G., Kerlin, M., Stuchtey, M. (2009). Charting our water future: economic frameworks to inform decision-making. McKinsey & Company, New York.

Adesra, A., Srivastava, V.K., Varjani, S. (2021). Valorization of dairy wastes: integrative approaches for value added products. Indian Journal of Microbiology, 1-9.

Adewale, P., Dumont, M. J., Ngadi, M. (2016). Enzyme-catalyzed synthesis and kinetics of ultrasonic assisted methanolysis of waste lard for biodiesel production. Chemical Engineering Journal, 284, 158-165.

Agarwal, A.K., Dhar, A., Gupta, J.G., Kim, W.I., Choi, K., Lee, C.S., Park, S. (2015). Effect of fuel injection pressure and injection timing of Karanja biodiesel blends on fuel spray, engine performance, emissions and combustion characteristics. Energy Conversion and Management, 91, 302–314.

Agha Kouchak, A., Chiang, F., Huning, L.S., Love, C.A., Mallakpour, I., Mazdiyasni, O., Moftakhari, H., Papalexiou, S.M., Ragno, E., Sadegh, M. (2020). Climate Extremes and Compound Hazards in a Warming World. Annual Reviw of Earth and Planatery Science, 48, 519–548.

AghaKouchak, A., Chiang, F., Huning, L.S., Love, C.A., Mallakpour, I., Mazdiyasni, O., Sadegh, M. (2020). Climate extremes and compound hazards in a warming world. Annual Review of Earth and Planetary Sciences, 48, 519-548.

Ahmad Kamaroddin, M.F. (2017). In-situ Disinfection and Algal Lipid Extraction Using Ozonation in Novel Microbubble Bioreactor for Biofuel Production.

Ahmad, F.A.U.K.Y., Khan, A.U., Yasar, A. (2013). The potential of *Chlorella vulgaris* for wastewater treatment and biodiesel production. Pakistan Journal of Botany, 45, 461-465.

Ahmad, M., Liu, S., Mahmood, N., Mahmood, A., Ali, M., Zheng, M., Ni, J. (2017). Effects of porous carrier size on biofilm development, microbial distribution and nitrogen removal in microaerobic bioreactors. Bioresource Technology, 234, 360-369.

Ahmad, S., Pathak, V.V., Kothari, R., Kumar, A., Krishna, S.B.N. (2018). Optimization of nutrient stress using *C. pyrenoidosa* for lipid and biodiesel production in integration with remediation in dairy industry wastewater using response surface methodology. 3 Biotech, 8, 1-13.

Ahmad, T., Aadil, R.M., Ahmed, H., ur Rahman, U., Soares, B.C., Souza, S.L., Cruz, A.G. (2019). Treatment and utilization of dairy industrial waste: A review. Trends in Food Science & Technology, 88, 361-372.

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Thomaidis, N.S., Xu, J. (2017). Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review. Journal of Hazardous Materials, 323, 274-298.

Ajala, S.O., & Alexander, M.L. (2020). Assessment of *Chlorella vulgaris, Scenedesmus obliquus*, and *Oocystis minuta* for removal of sulfate, nitrate, and phosphate in wastewater. International Journal of Energy and Environmental Engineering, 11, 311-326.

Aleksic, N., Nesovic, A., Sustersic, V., Gordic, D., Milovanovic, D. (2020). Slaughterhouse water consumption and wastewater characteristics in the meat processing industry in Serbia. Desalin Water Treat, 190, 98-112.

Alfaia, C.M., Pestana, J.M., Rodrigues, M., Coelho, D., Aires, M.J., Ribeiro, D. M., Prates, J.A.M. (2021). Influence of dietary *Chlorella vulgaris* and carbohydrate-active enzymes on growth performance, meat quality and lipid composition of broiler chickens. Poultry Science, 100, 926-937.

Al-Gheethi, A.A., Mohamed, R.M., Jais, N.M., Efaq, A.N., Abd Halid, A., Wurochekke, A.A., Amir-Hashim, M.K. (2017). Influence of pathogenic bacterial activity on growth of *Scenedesmus* sp. and removal of nutrients from public market wastewater. Journal of Water and Health, 15, 741-756.

Al-Gheethi, A.A., Mohamed, R.M.S.R., Afaiz, A.R.M., Mas, R.J., Amir, H.K. (2016). Treatment of wastewater from car washes using natural coagulation and filtration system. Material Science and Engineering, 136, 12–31.

Ali, J., Wang, L., Waseem, H., Sharif, H.M.A., Djellabi, R., Zhang, C., Pan, G., (2019). Bioelectrochemical recovery of silver from wastewater with sustainable power generation and its reuse for biofouling mitigation. Journal of Cleaner Production, 235, 1425–1437.

Al-Jabri, H., Das, P., Khan, S., Thaher, M., AbdulQuadir, M. (2021). Treatment of Wastewaters by Microalgae and the Potential Applications of the Produced Biomass—A Review. Water, 13, 27.

Amid, S., Aghbashlo, M., Peng, W., Hajiahmad, A., Najafi, B., Ghaziaskar, H.S., Tabatabaei, M. (2021). Exergetic performance evaluation of a diesel engine powered by diesel/biodiesel mixtures containing oxygenated additive ethylene glycol diacetate. Science of the Total Environment, 792, 148435.

Amid, S., Aghbashlo, M., Tabatabaei, M., Hajiahmad, A., Najafi, B., Ghaziaskar, H. S., Mohammadi, P. (2020). Effects of waste-derived ethylene glycol diacetate as a novel oxygenated additive on performance and emission characteristics of a diesel engine fueled with diesel/biodiesel blends. Energy Conversion and Management, 203,112245.

Amin, L.R., ABO-EL-ENEIN, S.A., ABDEL-GHANY, A.M., SAMY, E.B.S., AMIN, T.R. (2018). Gas chromatography combined to mass spectrometry analyses of some citrus peels bioactive compounds against the cotton leafworm, Spodoptera littoralis (boisd.). Egyptian Journal of Agricultural Research, 96, 15-29.

Andrade, L.H., Mendes, F.D.S., Espindola, J.C., Amaral, M.C.S. (2014). Nanofiltration as tertiary treatment for the reuse of dairy wastewater treated by membrane bioreactor. Separation and Purification Technology, 126, 21-29.

Andreotti, V., Solimeno, A., Rossi, S., Ficara, E., Marazzi, F., Mezzanotte, V., Garcia, J. (2020). Bioremediation of aquaculture wastewater with the microalgae Tetraselmis suecica: Semi-continuous experiments, simulation and photo-respirometric tests. Science of the Total Environment, 738, 139859.

Ansari, F.A., Ravindran, B., Gupta, S.K., Nasr, M., Rawat, I., Bux, F. (2019). Techno-economic estimation of wastewater phycoremediation and environmental benefits using *Scenedesmus obliquus* microalgae. Journal of Environmental Management, 240, 293-302.

Ansari, F.A., Singh, P., Guldhe, A., Bux, F. (2017). Microalgal cultivation using aquaculture wastewater: integrated biomass generation and nutrient remediation. Algal Research, 21, 169-177.

APHA, A.W. (2012). Standard Methods for the Examination of Water and Wastewater, twenty-sec. Washington, DC.

Artabe, A.E., Cunha-Silva, H., Barranco, A. (2020). Enzymatic assays for the assessment of toxic effects of halogenated organic contaminants in water and food. A review. Food and Chemical Toxicology, 145, 111677.

Arun, J., Gopinath, K.P., Sivaramakrishnan, R., SundarRajan, P., Malolan, R., Pugazhendhi, A. (2021). Technical insights into the production of green fuel from CO2 sequestered algal biomass: A conceptual review on green energy. Science of the Total Environment, 755, 142636.

Arun, S., Sinharoy, A., Pakshirajan, K., Lens, P.N. (2020). Algae based microbial fuel cells for wastewater treatment and recovery of value-added products. Renewable and Sustainable Energy Reviews, 132, 110041.

Asgharpour M., Rodgers B., Hestekin J.A. (2015). Eicosapentaenoic acid from Porphyridium Cruentum: Increasing growth and productivity of microalgae for pharmaceutical products. Energies, 8, 10487–10503.

Ashokkumar, V., Chen, W.H., Ngamcharussrivichai, C., Agila, E., Ani, F.N. (2019). Potential of sustainable bioenergy production from *Synechocystis* sp. cultivated in wastewater at large scale–a low cost biorefinery approach. Energy Conversion and Management, 186, 188-199.

Aslam, M., Charfi, A., Lesage, G., Heran, M., Kim, J. (2017). Membrane bioreactors for wastewater treatment: a review of mechanical cleaning by scouring agents to control membrane fouling. Chemical Engineering 307, 897-913.

Aslam, M., McCarty, P.L., Shin, C., Bae, J., Kim, J. (2017). Low energy single-staged anaerobic fluidized bed ceramic membrane bioreactor (AFCMBR) for wastewater treatment. Bioresource Technology, 240, 33-41.

Aziz, M.M.A., Kassim, K.A., Shokravi, Z., Jakarni, F.M., Liu, H.Y., Zaini, N., Shokravi, H. (2020). Two-stage cultivation strategy for simultaneous increases in growth rate and lipid content of microalgae: A review. Renewable and Sustainable Energy Reviews, 119,109621.

Baharuddin, N.N. D.E., Aziz, N.S., Sohif, H.N., Karim, W.A.A., Al-Obaidi, J.R., Basiran, M. (2016). Marine microalgae flocculation using plant: the case of *Nannochloropsis oculata* and *Moringa oleifera*. Pakistan Journal of Botany, 48, 831-842.

Baker, B.R., Mohamed, R., Al-Gheethi, A., Aziz, H.A. (2021). Advanced technologies for poultry slaughterhouse wastewater treatment: A systematic review. Journal of Dispersion Science and Technology, 42, 880-899.

Barik, S., Paul, K.K., Priyadarshi, D. (2018, June). Utilization of kitchen food waste for biodiesel production. In IOP conference series: Earth and Environmental Science, 167, 012036.

Barros, A.C., Gonçalves, A.L., Simoes, M. (2019). Microalgal/cyanobacterial biofilm formation on selected surfaces: the effects of surface physicochemical properties and culture media composition. Journal of Applied Phycology, 31, 375-387.

Barros, A., Pereira, H., Campos, J., Marques, A., Varela, J., Silva, J. (2019). Heterotrophy as a tool to overcome the long and costly autotrophic scale-up process for large scale production of microalgae. Scientific Reports, 9, 1-7.

Bartolomeu, M., Neves, M.G.P.M.S., Faustino, M.A.F., Almeida, A. (2018). Wastewater chemical contaminants: Remediation by advanced oxidation processes. Photochemical & Photobiological Sciences, 17, 1573-1598.

Bazrafshan, E., Moein, H., Kord Mostafapour, F., Nakhaie, S. (2013). Application of electrocoagulation process for dairy wastewater treatment. Journal of Chemistry.

Beacham, T.A., Sweet, J.B., Allen, M.J. (2017). Large scale cultivation of genetically modified microalgae: A new era for environmental risk assessment. Algal Research, 25, 90–100.

Bechet, Q., Laviale, M., Arsapin, N., Bonnefond, H., & Bernard, O. (2017). Modeling the impact of high temperatures on microalgal viability and photosynthetic activity. Biotechnology for Biofuels, 10, 1-11.

Bechet, Q., Moussion, P., Bernard, O. (2017). Calibration of a productivity model for the microalgae *Dunaliella salina* accounting for light and temperature. Algal Research, 21, 156-160.

Behl, J.E., Brown, C.L., Conlon, K., Herring, S., Kunkel, K.E., Lawrimore, J., Uejio, C. (2018). Changes in extreme events and the potential impacts on human health. Journal of Air and Waste Management Association, 68, 265-287.

Behl, K., Sesha Charan, P., Joshi, M., Sharma, M., Mathur, A., Kareya, M.S., Nigam, S. (2020). Multifaceted applications of isolated microalgae *Chlamydomonas* sp. TRC-1 in wastewater remediation, lipid production and bioelectricity generation. Bioresource Technology, 304, 122993.

Beneois, K., Dahmani, S., Berrebah, K. (2016). Efficiency and limits of physicochemical treatment of dairy wastewater. (Case Study:Dairy industry in Western Algeria). International Journal of Chemical, Environmental and Biological Sciences, 4, 9-11.

Bernaerts, T.M., Gheysen, L., Foubert, I., Hendrickx, M.E., Van Loey, A.M. (2019). The potential of microalgae and their biopolymers as structuring ingredients in food: A review. Biotechnology Advances, 37, 107419.

Bhalamurugan, G.L., Valerie, O., Mark, L. (2018). Valuable bioproducts obtained from microalgal biomass and their commercial applications: A review. Environmental Engineering Research, 23, 229-241.

Bharagava, R.N., Saxena, G., Mulla, S.I., Patel, D.K. (2018). Characterization and identification of recalcitrant organic pollutants (ROPs) in tannery wastewater and its phytotoxicity evaluation for environmental safety. Archives Environmental Contamination Toxicology, 75, 259-272.

Bhatia, S.K., Mehariya, S., Bhatia, R.K., Kumar, M., Pugazhendhi, A., Awasthi, M.K., Yang, Y.H. (2021). Wastewater based microalgal biorefinery for bioenergy production: Progress and challenges. Science of the Total Environment, 751, 141599.

Bhushan, S., Kalra, A., Simsek, H., Kumar, G., Prajapati, S.K. (2020). Current trends and prospects in microalgae-based bioenergy production. Journal of Environmental Chemical Engineering, 8, 104025.

Bikker, P., van Krimpen, M.M., van Wikselaar, P., Houweling-Tan, B., Scaccia, N., van Hal, J.W., López-Contreras, A.M. (2016). Biorefinery of the green seaweed Ulva lactuca to produce animal feed, chemicals and biofuels. Journal of Applied Phycology, 28, 3511-3525.

Biller, P., & Ross, A. B. (2014). Pyrolysis GC–MS as a novel analysis technique to determine the biochemical composition of microalgae. Algal Research, 6, 91-97.

Biller, S.J., Berube, P.M., Berta-Thompson, J.W., Kelly, L., Roggensack, S.E., Awad, L., Chisholm, S.W. (2014). Genomes of diverse isolates of the marine cyanobacterium Prochlorococcus. Scientific Data, 1, 1-11.

Bleakley, S., & Hayes, M. (2017). Algal proteins: extraction, application, and challenges concerning production. Foods, 6, 33.

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37, 911-917.

Bonnefond, H., Moelants, N., Talec, A., Bernard, O., Sciandra, A. (2016). Concomitant effects of light and temperature diel variations on the growth rate and lipid production of *Dunaliella* salina. Algal Research, 14, 72-78.

Bose, A., O'Shea, R., Lin, R., Murphy, J.D. (2020). A perspective on novel cascading algal biomethane biorefinery systems. Bioresource Technology, 304, 123027.

Botebol, H., Lesuisse, E., sutak, R., Six, C., Lozano, J.C., Schatt, P., Bouget, F.Y. (2015). Central role for ferritin in the day/night regulation of iron homeostasis in marine phytoplankton. Proceedings of the National Academy of Sciences, 112, 14652-14657.

Branyikova, I., Prochazkova, G., Potocar, T., Jezkova, Z., Branyik, T. (2018). Harvesting of microalgae by flocculation. Fermentation, 4, 93.

Braz, C.A., Carvalho, A.K., Bento, H., Reis, C.E., De Castro, H.F. (2020). Production of value-added microbial metabolites: oleaginous fungus as a tool for valorization of dairy by-products. BioEnergy Research, 13, 963-973.

Breuer, G., Lamers, P.P., Martens, D.E., Draaisma, R. B., Wijffels, R.H. (2012). The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. Bioresource Technology, 124, 217-226.

Budhiary, K.N.S., & Sumantri, I. (2021). Langmuir and Freundlich isotherm adsorption using activated charcoal from banana peel to reduce total suspended solid (TSS) levels in tofu industry liquid waste. In IOP Conference Series: Materials Science and Engineering, 1053, 1-012113.

Bule, M.H., Ahmed, I., Maqbool, F., Bilal, M., Iqbal, H.M. (2018). Microalgae as a source of high-value bioactive compounds. Frontier. Bioscience, 10, 197-216.

Burek, P., Satoh, Y., Fischer, G., Kahil, M.T., Scherzer, A., Tramberend, S., Wiberg, D. (2016). Water futures and solution-fast track initiative.

Bustillo-Lecompte, C., & Mehrvar, M. (2017). Slaughterhouse wastewater: treatment, management and resource recovery. Physico-chemical wastewater treatment and resource recovery, 153-174.

Bustillo-Lecompte, C., Mehrvar, M., Quinones-Bolanos, E. (2016). Slaughterhouse wastewater characterization and treatment: an economic and public health necessity of the meat processing industry in Ontario, Canada. Journal of Geoscience and Environment Protection, 4, 175-186.

Bustillo-Lecompte, C.F., Mehrvar, M. (2017). Treatment of actual slaughterhouse wastewater by combined anaerobic–aerobic processes for biogas generation and removal of organics and nutrients: An optimization study towards a cleaner production in the meat processing industry. Journal of Cleaner Production, 141, 278-289.

Caporgno, M.P., & Mathys, A. (2018). Trends in microalgae incorporation into innovative food products with potential health benefits. Frontiers in nutrition, 5, 58.

Carvalho, F., Prazeres, A.R., Rivas, J. (2013). Cheese whey wastewater: Characterization and treatment. Science of the total environment, 445, 385-396.

Cassidy, KO. (2011). Evaluating algal growth at different temperatures.

Cataldo, D.A., Maroon, M., Schrader, L.E., Youngs, V.L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Communications in soil science and plant analysis. 6, 71-80.

CEA (Central Electricity Authority), (2016). All India Electricity Statistics: General Review. Ministry of Power, Government of India, New Delhi, India.

Cecal, A., Humelnicu, D., Rudic, V., Cepoi, L., Ganju, D., Cojocari, A. (2012). Uptake of uranyl ions from uranium ores and sludges by means of *Spirulina platensis*, *Porphyridium cruentum* and *Nostok* linckia algae. Bioresource Technology, 118, 19-23.

Chang, H., Quan, X., Zhong, N., Zhang, Z., Lu, C., Li, G., Yang, L. (2018). High-efficiency nutrients reclamation from landfill leachate by microalgae *Chlorella vulgaris* in membrane photobioreactor for bio-lipid production. Bioresource Technology, 266, 374-381.

Chen, C.Y., Kao, C.L., Liu, C.M. (2018). The cancer prevention, anti-inflammatory and anti-oxidation of bioactive phytochemicals targeting the TLR4 signaling pathway. International Journal of Molecular Sciences, 19, 2729.

Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J., Chang, J.S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresource Technology, 102, 71-81.

Chen, F., Zeng, S., Luo, Z., Ma, J., Zhu, Q., Zhang, S. (2020). A novel MBBR–MFC integrated system for high-strength pulp/paper wastewater treatment and bioelectricity generation. Separation Science and Technology, 55, 2490-2499.

Chen, G., Zhao, L., Qi, Y. (2015). Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: a critical review. Applied Energy, 137, 282-291.

Chen, L., Yu, Z., Liang, J., Liao, Y., Ma, X. (2018). Co-pyrolysis of chlorella vulgaris and kitchen waste with different additives using TG-FTIR and Py-GC/MS. Energy conversion and management, 177, 582-591.

Cheng, D., Liu, Y., Ngo, H H., Guo, W., Chang, S. W., Nguyen, D.D., Liu, Y. (2020). A review on application of enzymatic bioprocesses in animal wastewater and manure treatment. Bioresource Technology, 313, 123683.

Cheng, P., Osei-Wusu, D., Zhou, C., Wang, Y., Xu, Z., Chang, T., Huo, S. (2020). The effects of refractory pollutants in swine wastewater on the growth of *Scenedesmus* sp. with biofilm attached culture. International Journal of Phytoremediation, 22, 241-250.

Chew, K.W., Yap, J.Y., Show, P.L., Suan, N.H., Juan, J.C., Ling, T.C., Chang, J.S. (2017). Microalgae biorefinery: high value products perspectives. Bioresource Technology, 229, 53-62.

Chia, S.R., Ong, H.C., Chew, K.W., Show, P.L., Phang, S.M., Ling, T.C., Chang, J.S. (2018). Sustainable approaches for algae utilisation in bioenergy production. Renewable Energy, 129, 838-852.

Chinnasamy, S., Bhatnagar, A., Claxton, R. Das, K.C. (2010). Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. Bioresource Technology, 101, 6751-6760.

Chiranjeevi, P., & Mohan, S.V. (2016). Critical parametric influence on microalgae cultivation towards maximizing biomass growth with simultaneous lipid productivity. Renewable Energy, 98, 64-71.

Choi, Y.E., Rhee, J.K., Kim, H.S., Ahn, J.W., Hwang, H., Yang, J.W. (2015). Chemical genetics approach reveals importance of cAMP and MAP kinase signaling to lipid and carotenoid biosynthesis in microalgae. Journal of Microbiology and Biotechnology, 25, 637-647.

Choi, Y.H., Kim, E.Y., Mikami, K., Nam, T.J. (2015). Chemoprotective effects of a recombinant protein from Pyropia yezoensis and synthetic peptide against acetaminophen-induced Chang liver cell death. International Journal of Molecular Medicine, 36, 369-376.

Chowdury, K.H., Nahar, N., Deb, U.K. (2020). The growth factors involved in microalgae cultivation for biofuel production: A Review. Computational Water, Energy, and Environmental Engineering, 9, 185-215.

Ciccone, M.M., Cortese, F., Gesualdo, M., Carbonara, S., Zito, A., Ricci, G., Riccioni, G. (2013). Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. Mediators of inflammation.

Cointet, E., Wielgosz-Collin, G., Bougaran, G., Rabesaotra, V., Gonçalves, O., Meleder, V. (2019). Effects of light and nitrogen availability on photosynthetic efficiency and fatty acid content of three original benthic diatom strains. PLoS One, 14, e0224701.

Collins, A.L., Price, J.N., Zhang, Y., Gooday, R., Naden, P.S., Skirvin, D. (2018). Assessing the potential impacts of a revised set of on-farm nutrient and sediment ‘basic’control measures for reducing agricultural diffuse pollution across England. Science of the Total Environment, 621, 1499-1511.

Costa, J.A.V., Freitas, B.C.B., Cruz, C.G., Silveira, J., Morais, M.G. (2019). Potential of microalgae as biopesticides to contribute to sustainable agriculture and environmental development. Journal of Environmental Science and Health, 54, 366-375.

Costa, J.A.V., Freitas, B.C.B., Rosa, G.M., Moraes, L., Morais, M.G., Mitchell, B.G. (2019). Operational and economic aspects of Spirulina-based biorefinery. Bioresource Technology, 292, 121946.

CPHEEO, G. (2013). Manual on sewerage and sewage treatment systems.

Crini, G., & Lichtfouse, E. (2019). Advantages and disadvantages of techniques used for wastewater treatment. Environmental Chemistry Letters, 17, 145-155.

Cui, Y., Thomas-Hall, S.R., Chua, E.T., Schenk, P.M. (2021). Development of High-Level Omega-3 Eicosapentaenoic Acid (EPA) Production from *Phaeodactylum tricornutum.* Journal of Phycology, 57, 258-268.

D'Alessandro, E.B., & Antoniosi Filho, N.R. (2016). Concepts and studies on lipid and pigments of microalgae: A review. Renewable and Sustainable Energy Reviews, 58, 832-841.

Daliry, S., Hallajisani, A., Mohammadi, R.J., Nouri, H., Golzary, A. (2017). Investigation of optimal condition for *Chlorella vulgaris* microalgae growth.

De Alva, M.S., Pabello, V.M.L., Ledesma, M. T. O., Gomez, M.J.C. (2018). Carbon, nitrogen, and phosphorus removal, and lipid production by three saline microalgae grown in synthetic wastewater irradiated with different photon fluxes. Algal Research, 34, 97-103.

De Godos, I., Arbid, Z., Lara, E., Cano, R., Munoz, R., Rogalla, F. (2017). Wastewater treatment in algal systems. Innovative Waste water treatment resource recovery technologies impacts on energy economy and environment.

De Moraes Sa, J.C., Lal, R., Cerri, C.C., Lorenz, K., Hungria, M., de Faccio Carvalho, P.C. (2017). Low-carbon agriculture in South America to mitigate global climate change and advance food security. Environment International, 98, 102-112.

De Morais, M.G., Costa, J.A.V. (2007). Carbon dioxide fixation by Chlorella kessleri, *C. vulgaris, Scenedesmus obliquus and Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors. Biotechnology Letters, 29, 1349-1352.

De Morais, M.G., Vaz, B.S., De Morais, E.G., Costa, J.AV. (2015). Biologically active metabolites synthesized by microalgae. Biomedical Research International.

Deb, U.K., Shahriar, M., Bhowmik, J., Chowdury, M.K.H. (2017). The effect of irradiance related temperature on microalgae growth in a tubular photo bio-reactor for cleaner energy. American Journal of Computational Mathematics, 7, 371-384

Delasoie, J., Rossier, J., Haeni, L., Rothen-Rutishauser, B., Zobi, F. (2018). Slow-targeted release of a ruthenium anticancer agent from vitamin B 12 functionalized marine diatom microalgae. Dalton Transactions, 47, 17221-17232.

Demirel, B., Yenigun, O., Onay, T. T. (2005). Anaerobic treatment of dairy wastewaters: a review. Process biochemistry, 40, 2583-2595.

Deng, X., Li, D., Xue, C., Chen, B., Dong, J., Tetteh, P.A., Gao, K. (2020). Cultivation of *Chlorella sorokiniana* using wastewaters from different processing units of the silk industry for enhancing biomass production and nutrient removal. Journal of Chemical Technology & Biotechnology, 95, 264-273.

Deniz, I., Ozen, M. O., Yesil-Celiktas, O. (2016). Supercritical fluid extraction of phycocyanin and investigation of cytotoxicity on human lung cancer cells. The Journal of Supercritical Fluids, 108, 13-18.

Dennehy, P.H. (2019). Infectious gastroenteritis. In Introduction to Clinical Infectious Diseases. 157-168.

Dhamodaran, G., Krishnan, R., Pochareddy, Y.K., Pyarelal, H.M., Sivasubramanian, H., Ganeshram, A.K. (2017). A comparative study of combustion, emission, and performance characteristics of rice-bran-, neem, and cottonseed-oil biodiesels with varying degree of unsaturation. Fuel, 187, 296–305.

Di Termini, I., Prassone, A., Cattaneo, C., Rovatti, M. (2011). On the nitrogen and phosphorus removal in algal photobioreactors. Ecological Engineering, 37, 976-980.

Do, J.M., Jo, S.W., Kim, I.S., Na, H., Lee, J.H., Kim, H.S., Yoon, H.S. (2019). A feasibility study of wastewater treatment using domestic microalgae and analysis of biomass for potential applications. Water, 11, 2294.

Drahansky, M., Paridah, M., Moradbak, A., Mohamed, A., Owolabi, F., Abdulwahab, T., Asniza, M. (2016). “Application of biosorption for removal of heavy metals from wastewater," in Biosorption, eds J. Derco and B. Vrana (London: Intechopen).

Dreywood, R. (1946). Qualitative test for carbohydrate material. Industrial & Engineering Chemistry Analytical Edition,18, 499-499.

Dwivedi, A.K. (2017). Researches in water pollution: A review. International Research Journal of Natural and Applied Sciences, 4, 118-142.

Edwin, O.K., Alemaw, B.F., Laletsang, K., Tafesse, N.T. (2017). Estimating hydraulic properties of alluvial sand aquifer in motloutse river course, eastern botswana. Asian Review of Environmental and Earth Sciences, 4, 28-35.

Ejike, C.E., Collins, S.A., Balasuriya, N., Swanson, A.K., Mason, B., Udenigwe, C.C. (2017). Prospects of microalgae proteins in producing peptide-based functional foods for promoting cardiovascular health. Trends Food Science Technology, 59, 30-36.

El-Baz, F.K., Abd El Baky, H.H. (2018). Pilot scale of microalgal production using photobioreactor. In photosynthesis from its evolution to future improvements in photosynthetic efficiency using nanomaterials; Intech Open: London, UK, 53.

Elrayies, G.M. (2018). Microalgae: Prospects for greener future buildings. Renewable and Sustainable Energy Reviews, 81, 1175–1191.

El-Shenody, R.A., Ashour, M., Ghobara, M.M.E. (2019). Evaluating the chemical composition and antioxidant activity of three Egyptian seaweeds: Dictyota dichotoma, Turbinaria decurrens, and Laurencia obtusa. Brazilian Journal of Food Technology, 22.

Endo, H., Yoshida, M., Uji, T., Saga, N., Inoue, K., Nagasawa, H. (2016). Stable nuclear transformation system for the Coccolithophorid algae Pleurochrysiscarterae. Scientific Reports, 6, 22252

Eriksson, E., Auffarth, K., Henze, M., Ledin, A. (2002). Characteristics of grey wastewater. Urban water, 4, 85-104.

Fadhil, A.B., Aziz, A.M., Al-Tamer, M.H. (2016). Biodiesel production from Silybum marianum L. seed oil with high FFA content using sulfonated carbon catalyst for esterification and base catalyst for transesterification. Energy Conversion and Management, 108, 255–265.

Fagiri, Y.M.A., Salleh, A., El-Nagerabi, S.A.F. (2013). Impact of physico-chemical parameters on the physiological growth of *Arthrospira* (*Spirulina platensis*) exogenous strain UTEXLB2340. African Journal of Biotechnology, 12.

FAO. (2016). FAOSTAT. Database. (Available at http://faostat3.fao.org/browse/R/RP/E Rome, Food and Agriculture Organization of the United Nations (FAO).

FAO/WHO (World Health Organization). (2016). International code of conduct on pesticide management. Guidelines on highly hazardous pesticides. Rome, FAO.

Fasaei, F., Bitter, J.H., Slegers, P.M., Van Boxtel, A.J.B. (2018). Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Research, 31, 347-362.

Feng, P., Deng, Z., Fan, L., Hu, Z. (2012). Lipid accumulation and growth characteristics of *Chlorella zofingiensis* under different nitrate and phosphate concentrations. Journal of Bioscience and Bioengineering, 114, 405–410.

Filippino, K.C., Mulholland, M.R., Bott, C.B. (2015). Phycoremediation strategies for rapid tertiary nutrient removal in a waste stream. Algal Research, 11, 125-133.

Frumento, A.A. Casazza, S. Al Arni, and Converti, A. (2013). Cultivation of *Chlorella vulgaris* in tubular photobioreactors: A lipid source for biodiesel production. Biochemical Engineering Journal, 81, 120–125.

Fu, W., Paglia, G., Magnusdottir, M., Steinarsdóttir, E.A., Gudmundsson, S., Palsson, B.O., Brynjolfsson, S. (2014). Effects of abiotic stressors on lutein production in the green microalga *Dunaliella salina.* Microbial Cell Factories, 13, 1-9.

Gaikwad, K.K., Singh, S., Negi, Y.S. (2020). Ethylene scavengers for active packaging of fresh food produce. Environmental Chemistry Letters, 18, 269-284.

Gaikwad, S.A., & Nalawade, S.P. (2020). GCMS ANALYSIS FOR SEPARATION AND IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM

FRESHWATER CYANOBACTERIUM NOSTOC FUSCESCENCE. Journal of Advanced Scientific Research, 11.

Galasso, C., Gentile, A., Orefice, I., Ianora, A., Bruno, A., Noonan, D.M., Brunet, C. (2019). Microalgal derivatives as potential nutraceutical and food supplements for human health: A focus on cancer prevention and interception. Nutrients, 11, 1226.

Gangwar, R.K., Singh, J., Singh, A.P., Singh, D.P. (2013). Assessment of water quality index: a case study of river ramganga at bareilly UP India. International Journal of Scientific Engineering Research, 4, 2325-2329.

Garcia, J.L., de Vicente, M., Galan, B. (2017). Microalgae, old sustainable food and fashion neutraceuticals. Microbial Biotechnology, 10: 1017–1024.

Garcia-Martineza, J.B., Urbina-Suarezb, N.A., Zuorroc, A., Barajas-Solanob, A.F., Kafarova, V. (2019). Fisheries wastewater as a sustainable media for the production of algae-based products. Chemical Engineering, 76, 1339-1344.

Gatamaneni, B.L., Orsat, V., Lefsrud, M. (2018). Factors affecting growth of various microalgal species. Environmental Engineering Science, 35, 1037-1048.

Gbadamosi OK, Lupatsch I (2018) Effects of dietary *Nannochloropsis salina* on the nutritional performance and fatty acid profile of Nile tilapia, Oreochromis niloticus. Algal Research 33:48–54.

Ge, S., Qiu, S., Tremblay, D., Viner, K., Champagne, P., Jessop, P.G. (2018). Centrate wastewater treatment with *Chlorella vulgaris*: simultaneous enhancement of nutrient removal, biomass and lipid production. Chemical Engineering Journal, 342, 310-320.

Gedda, G., Kolli Balakrishna, R.U.D., Shah, K.J. (2021). Introduction to Conventional Wastewater Treatment Technologies: Limitations and Recent Advances. Advances in Wastewater Treatment. I, 91, 1-36.

Geilman, W.G., Schmidt, D., Herfurth-Kennedy, C., Path, J., Cullor, J. (1992). Production of an electrolyte beverage from milk permeate. Journal Dairy Science, 75, 2364-2369.

Ghaffar, A. (2015). PARASITOLOGY-CHAPTER FOUR NEMATODES (Round Worms). Microbiology and Immunology On-Line. University of South Carolina, School of Medicine.

Ghosh, A., Khanra, S., Mondal, M., Halder, G., Tiwari, O.N., Saini, S., Bhowmick, T.K. Gayen, K. (2016). Progress toward isolation of strains and genetically engineered strains of microalgae for production of biofuel and other value added chemicals: a review. Energy Conversion and Management, 113, 104-118.

Giwa, A., Ogunribido, A. (2012). The applications of membrane operations in the textile industry: a review. British Journal of Applied Science & Technology, 2, 296.

Gnanasekaran, S., Saravanan, N., Ilangkumaran, M. (2016). Influence of injection timing on performance, emission and combustion characteristics of a DI diesel engine running on fish oil biodiesel. Energy, 116, 1218–1229.

Goli, A., Shamiri, A., Khosroyar, S., Talaiekhozani, A., Sanaye, R., & Azizi, K. (2019). A review on different aerobic and anaerobic treatment methods in dairy industry wastewater. Journal of Environmental Treatment Techniques, 6, 113-141.

Gonçalves, A.L., Pires, J.C., Simoes, M. (2016). The effects of light and temperature on microalgal growth and nutrient removal: an experimental and mathematical approach. RSC Advances, 6, 22896-22907.

Gong, M., & Bassi, A. (2016). Carotenoids from microalgae: A review of recent developments. Biotechnology advances, 34, 1396-1412.

Gonzalez-Delgado, A.D., & Peralta-Ruiz, Y. (2017). Evaluation of In-Situ Transient Simultaneous Cell Disruption and Transesterification of Microalgae. Contemporary Engineering Sciences, 10, 1319-1327.

Gonzalez-Tineo, P.A., Duran-Hinojosa, U., Delgadillo-Mirquez, L.R., Meza-Escalante, E.R., Gortares-Moroyoqui, P., Ulloa-Mercado, R.G., Serrano-Palacios, D. (2020). Performance improvement of an integrated anaerobic-aerobic hybrid reactor for the treatment of swine wastewater. Journal of Water Process Engineering, 34, 101164.

Goswami, R.K., Agrawal, K., Verma, P. (2021). Phycoremediation of nitrogen and phosphate from wastewater using Picochlorum sp. A tenable approach. Journal of Basic Microbiology.

Goswami, R.K., Mehariya, S., Obulisamy, P.K., Verma, P. (2021). Advanced microalgae-based renewable biohydrogen production systems: A review. Bioresource Technology, 320, 124301.

Govt of India (2013) Handbook on drinking water treatment technologies, Ministry of drinking water and sanitation (MDWS); 2013th edition ASIN: B078ZFF91S.

Grahl, S., Strack, M., Weinrich, R., (2018). Consumer oriented product development: the conceptualization of novel food products based on *Spirulina* (*Arthrospira platensis*) and resulting consumer expectations. Journal of Food Quality, 1–11.

Greening, G.E., & Cannon, J.L. (2016). Human and animal viruses in food (including taxonomy of enteric viruses). In Viruses in foods. 5-57.

Guldhe, A., Ansari, F.A., Singh, P., Bux, F. (2017). Heterotrophic cultivation of microalgae using aquaculture wastewater: a biorefinery concept for biomass production and nutrient remediation. Ecological engineering, 99, 47-53.

Guyer, J.P. (2018). An Introduction to preliminary wastewater treatment. Guyer Partners.

Habibi, A., Vahabzadeh, F. (2013). Degradation of formaldehyde in packed-bed bioreactor by kissirisimmobilized Ralstonia eutropha. Biotechnol Bioprocess Engineering, 18, 455–464.

Hamed, I. (2016). The evolution and versatility of microalgal biotechnology: a review. Comprensive Reviews in Food Science and Food Safety, 15, 1104–1123.

Hameed, M.A. (2007). Effect of algal density in bead, bead size and bead concentrations on wastewater nutrient removal. African Journal of Biotechnology, 6.

Han, T., Lu, H.F., Ma, S.S., Zhang, Y.H., Liu, Z.D., Duan, N. (2017). Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: A review. International Journal of Agricultural and Biological Engineering, 10, 1-29.

Hanie Amin, H. (2019). Ulva lactuca as a cheap and safe biopesticide in fields and its chemical composition (in vitro). Egyptian Journal of Aquatic Biology and Fisheries, 23, 415-428.

Hao, S., Li, S., Wang, J., Zhao, L., Yan, Y., Wu, T., Wang, C. (2019). C-phycocyanin suppresses the in vitro proliferation and migration of non-small-cell lung cancer cells through reduction of RIPK1/NF-κB Activity. Marine Drugs, 17, 362.

Hao, S., Yan, Y., Li, S., Zhao, L., Zhang, C., Liu, L., Wang, C. (2018). The in vitro anti-tumor activity of phycocyanin against non-small cell lung cancer cells. Marine Drugs, 16, 178.

Harshitha, G., Sahoo, A., Sethy, R. (2019). Bioelectricity generation from different biomass feed at anode chamber and to study process parameters in microbial fuel cells. Biocatalysis and Agricultural Biotechnology, 20, 101191.

He, J., Chen, J.P. (2014). A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modeling simulation tools. Bioresource Technology, 160, 67-78.

Hemphill, A., Müller, N., Müller, J. (2019). Comparative pathobiology of the intestinal protozoan parasites Giardia lamblia, Entamoeba histolytica, and Cryptosporidium parvum. Pathogens, 8, 116.

Hena, S., Fatimah, S., Tabassum, S. (2015). Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. Water Resources and Industry, 10, 1-14.

Hu, J., Song, Y., Mao, X., Wang, Z.J., Zhao, Q.J. (2016). Limonoids isolated from Toona sinensis and their radical scavenging, anti-inflammatory and cytotoxic activities. Journal of Functional Foods, 20, 1-9.

Hu, L., Li, H., Qin, R., Xu, R., Li, J., Li, L., Wei, P., Yang, J. (2016). Plant phosphomannoseisomerase as a selectable marker for rice transformation. Scientific Reports, 6, 25921.

Huang, X., Guida, S., Jefferson, B., Soares, A. (2020). Economic evaluation of ion-exchange processes for nutrient removal and recovery from municipal wastewater. NPJ Clean Water, 3, 1-10.

Huang, Y., Zhao, S., Ding, Y.D., Liao, Q., Huang, Y., Zhu, X. (2017). Optimizing the gas distributor based on CO2 bubble dynamic behaviors to improve microalgal biomass production in an air-lift photo-bioreactor. Bioresource Technology, 233, 84-91.

Iasimone, F. Panico, A., De Felice, V., Fantasma, F., Iorizzi, M., Pirozzi, F. (2018). Effect of light intensity and nutrients supply on microalgae cultivated in urban wastewater: Biomass production, lipids accumulation and settleability characteristics. Journal of Environmental Management, 223, 1078-1085.

Ibrahim, L.A., & El-Sayed, E.B. (2019). Performance evaluation of Suez Bay industrial wastewater treatment plant case study: Ataqa Region, Egypt. World Journal of Environmental Biosciences 8, 1-11.

Igiri, B.E., Okoduwa, S.I., Idoko, G.O., Akabuogu, E.P., Adeyi, A.O., Ejiogu, I.K. (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. Journal of Toxicology.

İhsan, E.K.İ.N. (2020). Types of microalgae cultivation photobioreactors and production process of microalgal biodiesel as alternative fuel. Acta Biologica Turcica 33, 114-131.

Ilyas, M., Ahmad, W., Khan, H., Yousaf, S., Yasir, M., Khan, A. (2019). Environmental and health impacts of industrial wastewater effluents in Pakistan: a review. Reviews Environmental Health, 34, 171-186.

Infante, C., Leon, I., Florez, J., Zárate, A., Barrios, F., Zapata, C. (2013). Removal of ammonium and phosphate ions from wastewater samples by immobilized *Chlorella* sp. International Journal of Environmental Studies, 70, 1-7.

Islam, M.A., Heimann, K., Brown, R.J. (2017). Microalgae biodiesel: Current status and future needs for engine performance and emissions. Renewable and Sustainable Energy Reviews, 79, 1160-1170.

Iwai, M., Hori, K., Sasaki-Sekimoto, Y., Shimojima, M., Ohta, H. (2015). Manipulation of oil synthesis in *Nannochloropsis* strain nies-2145 with a phosphorus starvation–inducible promoter from *Chlamydomonas reinhardtii*. Frontiers in Microbiology, 6, 912.

Jagtap, S.P., Pawar, A.N., Lahane, S. (2020). Improving the usability of biodiesel blend in low heat rejection diesel engine through combustion, performance and emission analysis. Renewable Energy, 155, 628-644.

Jais, N.M., Mohamed, R.M.S.R., Al-Gheethi, A.A., Hashim, M.A. (2017). The dual roles of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. Clean Technologies and Environmental Policy, 19, 37-52.

Janczukowicz, W., Zielinski, M., Dębowski, M. (2008). Biodegradability evaluation of dairy effluents originated in selected sections of dairy production. Bioresource Technology, 99, 4199-4205.

Jasim, N.A. (2020). The design for wastewater treatment plant (WWTP) with GPS X modelling. Cogent Engineering, 7, 1723782.

Jastrzębska, M., Kostrzewska, M.K. (2019). Using an environment-friendly fertiliser from sewage sludge ash with the addition of Bacillus megaterium. Minerals, 9, 423.

Jastrzębska, M., Kostrzewska, M.K., Saeid, A. (2019). Can Phosphorus from Recycled Fertilisers Replace Conventional Sources. An Agronomic Evaluation in Field-Scale Experiments on Temperate Luvisols. Applied Sciences, 9, 2086.

Jaywant, S.A., & Arif, K.M. (2019). A comprehensive review of microfluidic water quality monitoring sensors. Sensors, 19, 4781.

Ji, M.K., Yun, H.S., Park, S., Lee, H., Park, Y.T., Bae, S., Choi, J. (2015). Effect of food wastewater on biomass production by a green microalga *Scenedesmus obliquus* for bioenergy generation. Bioresource Technology, 179, 624-628.

Jiang, L., Ji, Y., Hu, W., Pei, H., Nie, C., Ma, G., Song, M. (2016). Adjusting irradiance to enhance growth and lipid production of *Chlorella vulgaris* cultivated with monosodium glutamate wastewater. Journal of Photochemistry and Photobiology B: Biology, 162, 619-624.

Johnson, T.J., Katuwal, S., Anderson, G.A., Gu, L., Zhou, R., Gibbons, W.R. (2018). Photobioreactor cultivation strategies for microalgae and cyanobacteria. Biotechnology progress, 34, 811-827.

Joseph, N., Ryu, D., Malano, H.M., George, B., Sudheer, K.P. (2019). Estimation of industrial water demand in India using census-based statistical data. Resources, Conservation and Recycling, 149, 31-44.

Kalavrouziotis, I. K., Soultanopoulos, A., Varnavas, S. P. (2018). Effect of olive core and olive core ash on the growth of Helianthus annuus and soil quality. Global Nest Journal, 20, 169-172.

Kalra, R., Conlan, X.A., Goel, M. (2020). Fungi as a potential source of pigments: harnessing filamentous fungi. Frontiers in Chemistry, 8, 369.

Kang, K., Qiu, L., Sun, G., Zhu, M., Yang, X., Yao, Y., Sun, R. (2019). Codensification technology as a critical strategy for energy recovery from biomass and other resources-A review. Renewable and Sustainable Energy Reviews, 116, 109414.

Kaplan, S., & Gurcan, E.K. (2018). Comparison of growth curves using non-linear regression function in Japanese quail. Journal of Applied Animal Research, 46, 112-117.

Karadag, D., Koroglu, O.E., Ozkaya, B., Cakmakci, M. (2015). A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater. Process Biochemistry, 50, 262-271.

Karas, B.J., Diner, R.E., Lefebvre, S.C., Mc Quaid, J., Phillips, A.P., Noddings, C.M., Brunson, J.K., Valas, R.E., Deerinck, T.J., Jablanovic, J. (2015). Designer diatom episomes delivered by bacterial conjugation. Nature Communication, 6, 6925

Karthikeyan, O.P., Trably, E., Mehariya, S., Bernet, N., Wong, J.W.C., Carrere, H. (2018). Pretreatment of food waste for methane and hydrogen recovery: A review. Bioresource Technology, 249, 1025–1039.

Katiyar, R., & Arora, A. (2020). Health promoting functional lipids from microalgae pool: A review. Algal Research, 46, 101800.

Katiyar, R., Banerjee, S., Arora, A. (2021). Recent advances in the integrated biorefinery concept for the valorization of algal biomass through sustainable routes. Biofuels, Bioproducts and Biorefining, 15, 879-898.

Katiyar, R., Kumar, A., Gurjar, B.R. (2017). Microalgae based biofuel: challenges and opportunities. In Biofuels, 157-175.

Kavitha, M.D., Seema Shree, M.H., Vidyashankar, S., Sarada, R. (2016). Acute and subchronic safety assessment of Porphyridium purpureum biomass in the rat model. Journal of Applied Phycology, 28, 1071-1083.

Kavitha, S., Banu, J.R., Subitha, G., Ushani, U., Yeom, I.T. (2016). Impact of thermo-chemo-sonic pretreatment in solubilizing waste activated sludge for biogas production: Energetic analysis and economic assessment. Bioresource Technology, 219, 479-486.

Kehrein, P., Van Loosdrecht, M., Osseweijer, P., Garfí, M., Dewulf, J., Posada, J. (2020). A critical review of resource recovery from municipal wastewater treatment plants–market supply potentials, technologies and bottlenecks. Environmental Science: Water Research and Technology, 6, 877-910.

Kent, M., Welladsen, H.M., Mangott, A., Li, Y. (2015). Nutritional evaluation of Australian microalgae as potential human health supplements. PLoSONE,10, 0118985.

Khan, M.I., Shin, J.H., Kim, J.D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial Cell Factories, 17, 1-21.

Khanra, S., Mondal, M., Halder, G., Tiwari, O.N., Gayen, K., Bhowmick, T.K. (2018). Downstream processing of microalgae for pigments, protein and carbohydrate in industrial application: A review. Food and Bioproducts Processing, 110, 60-84.

Kim, C., Lee, C.R., Song, Y.E., Heo, J., Choi, S.M., Lim, D.H., Kim, J.R. (2017). Hexavalent chromium as a cathodic electron acceptor in a bipolar membrane microbial fuel cell with the simultaneous treatment of electroplating wastewater. Chemical Engineering Journal, 328, 703-707.

Kim, K.Y., Yang, W., Ye, Y., La Barge, N., Logan, B.E. (2016). Performance of anaerobic fluidized membrane bioreactors using effluents of microbial fuel cells treating domestic wastewater. Bioresource Technology, 208, 58-63.

Kim, Y.J., Hong, H., Yun, J., Kim, S.I., Jung, H.Y., Ryu, W. (2021). Photosynthetic nanomaterial hybrids for bioelectricity and renewable energy systems. Advanced Materials, 33, 2005919.

Kiran, B., Pathak, K., Kumar, R., Deshmukh, D. (2014). Cultivation of *Chlorella* sp. IM-01 in municipal wastewater for simultaneous nutrient removal and energy feedstock production. Ecological Engineering, 73, 326-330.

Kiran, B.R., Venkata Mohan, S. (2021). Microalgal cell biofactory—therapeutic, nutraceutical and functional food applications. Plants, 10, 836.

Kitrungloadjanaporn, P., Sripongpun, G., Triampo, W. (2017). Nutrient removal from the effluent of swine slaughterhouse wastewater by *Chlorella vulgaris* TISTR 8580. International Journal of Advances in Agricultural & Environmental Engineering, 4, 28-32.

Kolev Slavov, A. (2017). General characteristics and treatment possibilities of dairy wastewater–a review. Food Technology Biotechnology, 55, 14-28.

Kong, X., Zhang, L., Lu, X., Zhang, C., Hua, Y., Chen, Y. (2019). Effect of high-speed shearing treatment on dehulled walnut proteins. LWT Food Science and Technology, 116, 108500.

Kothari, R., Ahmad, S., Pathak, V.V., Pandey, A., Kumar, A., Shankarayan, R., Tyagi, V.V. (2019). Algal-based biofuel generation through flue gas and wastewater utilization: a sustainable prospective approach. Biomass Conversion and Biorefinery, 1-24.

Kothari, R., Pandey, A., Ahmad, S., Kumar, A., Pathak, V.V., Tyagi, V.V. (2017). Microalgal cultivation for value-added products: a critical enviro-economical assessment. 3 Biotech, 7, 1-15.

Kothari, R., Pathak, V.V., Kumar, V., Singh, DP. (2012). Experimental study for growth potential of unicellular alga *Chlorella pyrenoidosa* on dairy waste water: an integrated approach for treatment and biofuel production. Bioresource Technology, 116, 466-470.

Kothari, R., Prasad, R., Kumar, V., Singh, D.P. (2013). Production of biodiesel from microalgae *Chlamydomonas, polypyrenoideum* grown on dairy industry wastewater. Bioresource Technology, 144, 499-503.

Kottuparambil, S., Thankamony, R.L., Agusti, S. (2019). Euglena as a potential natural source of value-added metabolites. A review. Algal research, 37, 154-159.

Koyande, A.K., Chew, K.W., Rambabu, K., Tao, Y., Chu, D.T., Show, P.L. (2019). Microalgae: A potential alternative to health supplementation for humans. Food Science and Human Wellness, 8, 16-24.

Kumar, K., Mishra, S.K., Shrivastav, A., Park, M.S., Yang, J.W. (2015). Recent trends in the mass cultivation of algae in raceway ponds. Renewable and Sustainable Energy Reviews, 51, 875-885.

Kumar, M.D., Sivamohan, M. V. K., Narayanamoorthy, A. (2012). The food security challenge of the food-land-water nexus in India. Food Security, 4, 539-556.

Kumar, R., Ghosh, A.K., Pal, P. (2020). Synergy of biofuel production with waste remediation along with value-added co-products recovery through microalgae cultivation: a review of membrane-integrated green approach. Science of the Total Environment, 698, 134169.

Kumar, V., Nanda, M., Joshi, H.C., Singh, A., Sharma, S., Verma, M. (2018). Production of biodiesel and bioethanol using algal biomass harvested from fresh water river. Renewable Energy, 116, 606-612.

Kundu, P., Debsarkar, A., Mukherjee, S. (2013). Treatment of slaughter house wastewater in a sequencing batch reactor: performance evaluation and biodegradation kinetics. Biomedical Research International.

Kusmayadi, A., Suyono, E.A., Nagarajan, D., Chang, J.S., Yen, H.W. (2020). Application of computational fluid dynamics (CFD) on the raceway design for the cultivation of microalgae: a review. Journal of Industrial Microbiology and Biotechnology, 47, 373-382.

Lam, M.K., Yusoff, M.I., Uemura, Y., Lim, J.W., Khoo, C.G., Lee, K.T. and Ong, H.C. (2017). Cultivation of *Chlorella vulgaris* using nutrients source from domestic wastewater for biodiesel production: Growth condition and kinetic studies. Renewable Energy. 103, 197-207.

Lam, M.K., Yusoff, M.I., Uemura, Y., Lim, J.W., Khoo, C.G., Lee, K.T., Ong, H.C. (2017). Cultivation of Chlorella vulgaris using nutrients source from domestic wastewater for biodiesel production: Growth condition and kinetic studies. Renewable Energy, 103, 197-207.

Laowansiri, S., Tharasena, B., Khamhok, S. (2018). Kinetics on anaerobic digestion and wastewater treatment of chicken slaughterhouse. Journal of Food Health and Bioenvironmental Science. 11, 19-24.

Lap, B.Q., & Ta, D.T. (2020). Analysis of the Principal Factors Affecting the Algae Growth in an Urban Eutrophic Shallow Lake by an Ecosystem Model. Water, Air, & Soil Pollution, 231, 1-17.

Latsos, C., Van Houcke, J., Timmermans, K.R. (2020). The effect of nitrogen starvation on biomass yield and biochemical constituents of *Rhodomonas* sp. Frontiers in Marine Science, 900.

Lekshmi, V.S., & Kurup, G.M. (2019). Anticoagulant activities of sulfated polysaccharides from the edible marine algae Padina tetrastromatica and Ulva fasciata: A combined in vitro and in vivo approach. Journal of Pharmacognosy and Phytochemistry, 8, 693-698.

Levasseur, W., Perre, P., Pozzobon, V. (2020). A review of high value-added molecules production by microalgae in light of the classification. Biotechnology Advances, 41, 107545.

Li, D., Liu, R., Cui, X., He, M., Zheng, S., Du, W., Wang, C. (2021). Co-culture of bacteria and microalgae for treatment of high concentration biogas slurry. Journal of Water Process Engineering, 41, 102014.

Li, K., Liu, Q., Fang, F., Luo, R., Lu, Q., Zhou, W., Ruan, R. (2019). Microalgae-based wastewater treatment for nutrients recovery: A review. Bioresource Technology, 291, 121934.

Li, S., Hu, T., Xu, Y., Wang, J., Chu, R., Yin, Z., Zhu, L. (2020). A review on flocculation as an efficient method to harvest energy microalgae: mechanisms, performances, influencing factors and perspectives. Renewable and Sustainable Energy Reviews, 131,110005.

Libutti, A., Gatta, G., Gagliardi, A., Vergine, P., Pollice, A., Beneduce, L., Tarantino, E. (2018). Agro-industrial wastewater reuse for irrigation of a vegetable crop succession under Mediterranean conditions. Agricultural Water Management, 196, 1-14.

Likozar, B., & Levec, J. (2014). Transesterification of canola, palm, peanut, soybean and sunflower oil with methanol, ethanol, isopropanol, butanol and tert-butanol to biodiesel: Modelling of chemical equilibrium, reaction kinetics and mass transfer based on fatty acid composition. Applied Energy, 123, 108-120.

Lin, J.J., Chen, Y.W. (2017). Production of biodiesel by transesterification of Jatropha oil with microwave heating. Journal of Taiwan Institute of Chemical Engineers, 75, 43–50.

Lin, T.S., & Wu, J.Y. (2015). Effect of carbon sources on growth and lipid accumulation of newly isolated microalgae cultured under mixotrophic condition. Bioresource Technology, 184, 100-107.

Liu, H., Lu, Q., Wang, Q., Liu, W., Wei, Q., Ren, H., Ming, C., Min, M., Chen, P., Ruan, R. (2017). Isolation of a bacterial strain, Acinetobacter sp. from centrate wastewater and study of its cooperation with algae in nutrients removal. Bioresource Technology, 235, 59–69.

Liu, H., Zhang, Z., Zhang, H., Lee, D.J., Zhang, Q., Lu, C., He, C. (2020). Evaluation of hydrogen yield potential from *Chlorella* by photo-fermentation under diverse substrate concentration and enzyme loading. Bioresource Technology, 303, 122956.

Liu, Q.M., Xu, S.S., Li, L., Pan, T.M., Shi, C.L., Liu, H., Cao, M.J., Su, W.J., Liu, G.M. (2017). In vitro and in vivo immunomodulatory activity of sulfated polysaccharide from Porphyra haitanensis. Carbohydrate polymers, 1, 189-96.

Liu, T., Liu, W. H., Zhao, J. S., Meng, F. Z., Wang, H. (2017). Lutein protects against β-amyloid peptide-induced oxidative stress in cerebrovascular endothelial cells through modulation of Nrf-2 and NF-κb. Cell Biology and Toxicology, 33, 57-67.

Liu, Z. (2021). Urban sewage treatment odor gas release characteristics and regional differences. Environmental Technology & Innovation, 21, 101190.

Long, S.F., Kang, S., Wang, Q.Q., Xu, Y.T., Pan, L., Hu, J.X., Piao, X.S. (2018). Dietary supplementation with DHA-rich microalgae improves performance, serum composition, carcass trait, antioxidant status, and fatty acid profile of broilers. Poultry Science, 97, 1881-1890.

Loomba, V., von Lieres, E., & Huber, G. (2021). How Do Operational and Design Parameters Effect Biomass Productivity in a Flat-Panel Photo-Bioreactor? A Computational Analysis. Processes, 9, 1387.

Lopez-Pacheco, I.Y., Silva-Nunez, A., Garcia-Perez, J.S., Carrillo-Nieves, D., Salinas-Salazar, C., Castillo-Zacarías, C., Parra-Saldívar, R. (2021). Phyco-remediation of swine wastewater as a sustainable model based on circular economy. Journal of Environmental Management, 278, 111534.

Lu, Q., Zhou, W., Min, M., Ma, X., Chandra, C., Doan, Y.T., Ruan, R. (2015). Growing *Chlorella* sp. on meat processing wastewater for nutrient removal and biomass production. Bioresource Technology, 198, 189-197.

Lu, Y., Zhang, Z., Xu, Y., Liu, Q., Qian, G. (2015). CaFeAl mixed oxide derived heterogeneous catalysts for transesterification of soybean oil to biodiesel. Bioresource Technology, 190, 438-441.

Luka, Y., Usman, F.O., Tya, T.S.K., Joseph, C. (2014). Kinetics of Bioremediation of Lake Gerio in Jimeta-Yola Using Pseudomonas aerigunosa. International Refereed Journal of Engineering and Science, 3, 54-59.

Lupatini, A. L., Colla, L. M., Canan, C., & Colla, E. (2017). Potential application of microalga *Spirulina platensis* as a protein source. Journal of the Science of Food and Agriculture, 97, 724-732.

Lwimbo, Z.D., Komakech, H.C., Muzuka, A.N. (2019). Impacts of emerging agricultural practices on groundwater quality in Kahe catchment, Tanzania. Water, 11, 2263.

Ma, H., Wang, H., Tian, C., Wang, L., Yuan, W., Qi, Y., Lv, W. (2021). An integrated membrane-and thermal-based system for coal chemical wastewater treatment with near-zero liquid discharge. Journal of Cleaner Production, 291, 125842.

Ma, R., Thomas-Hall, S.R., Chua, E.T., Alsenani, F., Eltanahy, E., Netzel, M.E., Schenk, P.M. (2018). Gene expression profiling of astaxanthin and fatty acid pathways in *Haematococcus pluvialis* in response to different LED lighting conditions. Bioresource Technology, 250, 591-602.

Ma, X., Zheng, H., Addy, M., Anderson, E., Liu, Y., Chen, P., Ruan, R. (2016). Cultivation of *Chlorella vulgaris* in wastewater with waste glycerol: strategies for improving nutrients removal and enhancing lipid production. Bioresource Technology, 207, 252-261.

Mackinney, G. (1941). Absorption of light by chlorophyll solutions. Journal of Biological Chemistry, 140, 315-322.

Madadi, R., Zahed, M.A., Pourbabaee, A.A., Tabatabaei, M., Naghavi, M.R. (2021). Simultaneous phycoremediation of petrochemical wastewater and lipid production by *Chlorella vulgaris.* SN Applied Sciences, 3, 1-10.

Madeira, M.S., Cardoso, C., Lopes, P.A., Coelho, D., Afonso, C., Bandarra, N.M., Prates, J.A. (2017). Microalgae as feed ingredients for livestock production and meat quality: A review. Livestock Science, 205, 111-121.

Madhav, S., Ahamad, A., Kumar, A., Kushawaha, J., Singh, P., Mishra, P. K. (2018). Geochemical assessment of groundwater quality for its suitability for drinking and irrigation purpose in rural areas of Sant Ravidas Nagar (Bhadohi), Uttar Pradesh. Geology, Ecology and Landscapes, 2, 127-136.

Maizatul, A.Y., Mohamed, R.M.S.R., Al-Gheethi, A.A., Hashim, M.A. (2017). An overview of the utilisation of microalgae biomass derived from nutrient recycling of wet market wastewater and slaughterhouse wastewater. International Aquatic Research, 9, 177-193.

Manasa, R.L., & Mehta, A. (2020). Wastewater: sources of pollutants and its remediation. In Environmental Biotechnology, 2, 197-219.

Manyatshe, A., Fosso-Kankeu, E., Van der Berg, D., Lemmer, N., Waanders, F., Tutu, H. (2017). Dispersion of inorganic contaminants in surface water in the vicinity of Potchefstroom. Physics and Chemistry of the Earth, 100, 86-93.

Markou, V., Kontogianni, M.C., Frontistis, Z., Tekerlekopoulou, A.G., Katsaounis, A., Vayenas, D. (2017). Electrochemical treatment of biologically pre-treated dairy wastewater using dimensionally stable anodes. Journal of Environmental Management, 202, 217-224.

Marquez-Escobar, V.A., Banuelos-Hernandez, B., Rosales-Mendoza, S. (2018). Expression of a Zika virus antigen in microalgae: Towards mucosal vaccine development. Journal of Biotechnology, 282, 86–91.

Mata, T.M., Martins, A.A., Caetano, N.S. (2010). Microalgae for biodiesel production and other applications: a review. Renewable and Sustainable Energy Reviews, 14, 217-232.

Matos, A.P., Feller, R., Moecke, E.H.S., de Oliveira, J.V., Junior, A.F., Derner, R.B., Sant’Anna, E.S. (2016). Chemical characterization of six microalgae with potential utility for food application. Journal of the American Oil Chemists' Society, 93, 963-972.

Matos, J., Cardoso, C., Bandarra, N.M., Afonso, C. (2017). Microalgae as healthy ingredients for functional food: a review. Food Function, 8, 2672-2685.

Mehariya, S., Iovine, A., Casella, P., Musmarra, D., Figoli, A., Marino, T., Sharma, N., Molino, A. (2019). Fischer–Tropsch Synthesis of Syngas to Liquid Hydrocarbons. In Lignocellulosic Biomass to Liquid Biofuels; Yousuf, A., Pirozzi, D., Sannino, F., Eds.; Elsevier: Amsterdam, The Netherlands, 217–248.

Mehariya, S., Iovine, A., Casella, P., Musmarra, D., Figoli, A., Marino, T., Molino, A. (2020). Fischer–Tropsch synthesis of syngas to liquid hydrocarbons. In Lignocellulosic biomass to liquid biofuels, 217-248.

Mehariya, S., Marino, T., Casella, P., Iovine, A., Leone, G.P., Musmarra, D., Molino, A. (2020). Biorefinery for Agro-Industrial Waste into Value-Added Biopolymers: Production and Applications. In Biorefineries: A Step Towards Renewable and Clean Energy, 1-19.

Mehta, P., Rani, R., Gupta, R., Mathur, A.S., Puri, S.K. (2018). Biomass and lipid production of a novel freshwater thermo-tolerant mutant strain of *Chlorella pyrenoidosa* NCIM 2738 in seawater salinity recycled medium. Algal Research, 36, 88-95.

Melchiors MS, Piovesan M, Becegato VR, Becegato VA, Tambourgi EB, Paulino AT. (2016). Treatment of wastewater from the dairy industry using electroflocculation and solid whey recovery. Journal of Environmental Management, 182, 574-580.

Mendes, L.B.B., Vermelho, A.B. (2013). Allelopathy as a potential strategy to improve microalgae cultivation. Biotechnology for Biofuels, 6, 1-14.

Menegazzo, M.L., Fonseca, G.G. (2019). Biomass recovery and lipid extraction processes for microalgae biofuels production: A review. Renewable and Sustainable Energy Reviews, 107, 87-107.

Metsoviti, M.N., Papapolymerou, G., Karapanagiotidis, I.T., Katsoulas, N. (2020). Effect of light intensity and quality on growth rate and composition of *Chlorella vulgaris*. Plants, 9, 31.

Miller, W.S., Castagna, C.J., Pieper, A.W. (2009). Understanding ion-exchange resins for water treatment systems 1-13.

Mirizadeh, S., Nosrati, M. and Shojaosadati, S.A. (2020). Synergistic effect of nutrient and salt stress on lipid productivity of *Chlorella vulgaris* through two-stage cultivation. Bioenergy Research. 13, 507-517.

Moges, M.E., Heistad, A., Heidorn, T. (2020). Nutrient recovery from anaerobically treated blackwater and improving its effluent quality through microalgae biomass production. Water, 12, 592.

Mohammadi, M., Mowla, D., Esmaeilzadeh, F., Ghasemi, Y. (2018). Cultivation of microalgae in a power plant wastewater for sulfate removal and biomass production: A batch study. Journal of Environmental Chemical Engineering, 6, 2812-2820.

Molazadeh, M., Ahmadzadeh, H., Pourianfar, H.R., Lyon, S., Rampelotto, P.H. (2019). The use of microalgae for coupling wastewater treatment with CO2 biofixation. Frontiers in Bioengineering and Biotechnology, 7, 42.

Molino A., Rimauro J., Casella P., Cerbone A., Larocca V., Chianese S., Karatza D., Mehariya S., Ferraro A., Hristoforou E. (2018). Extraction of astaxanthin from microalga *Haematococcus pluvialis* in red phase by using generally recognised as safe solvents and accelerated extraction. Journal of Biotechnology, 283, 51–61.

Molino, A., Mehariya, S., Iovine, A., Casella, P., Marino, T., Karatza, D., Musmarra, D. (2020). Enhancing biomass and Lutein production from *Scenedesmus almeriensis*: Effect of carbon dioxide concentration and culture medium reuse. Frontiers in Plant Science, 415.

Molino, A., Mehariya, S., Iovine, A., Larocca, V., Di Sanzo, G., Martino, M., Musmarra, D. (2018). Extraction of astaxanthin and lutein from microalga *Haematococcus pluvialis* in the red phase using CO2 supercritical fluid extraction technology with ethanol as co-solvent. Marine drugs, 16, 432.

Molino, A., Rimauro, J., Casella, P., Cerbone, A., Larocca, V., Chianese, S., Musmarra, D. (2018). Extraction of astaxanthin from microalga *Haematococcus pluvialis* in red phase by using generally recognized as safe solvents and accelerated extraction. Journal of Biotechnology, 283, 51-61.

Mondal, M., Goswami, S., Ghosh, A. (2017). Production of biodiesel from microalgae through biological carbon capture: A review. 3 Biotech, 7, 1-21.

Monirul, I.M., Masjuki, H.H., Kalam, M.A., Mosarof, M.H., Zulkifli, N.W.M., Teoh, Y.H., How, H.G. (2016). Assessment of performance, emission and combustion characteristics of palm, Jatropha and Calophyllum inophyllum biodiesel blends. Fuel, 181, 985-995.

Montalvo, G.E.B., Thomaz-Soccol, V., Vandenberghe, L.P., Carvalho, J.C., Faulds, C.B., Bertrand, E., Soccol, C.R. (2019). Arthrospira maxima OF15 biomass cultivation at laboratory and pilot scale from sugarcane vinasse for potential biological new peptides production. Bioresource Technology, 273, 103-113.

Morales, M., Sanchez, L., Revah, S. (2018). The impact of environmental factors on carbon dioxide fixation by microalgae. FEMS Microbiology Letters, 365, 262.

Morin-Crini, N., Crini, G., Roy, L. (2017). Eaux industrielles contaminees. PUFC, Besancon, 513, 37-47.

Morokutti-Kurz, M., Graf, C., Prieschl-Grassauer, E. (2017). Amylmetacresol/2, 4-dichlorobenzyl alcohol, hexylresorcinol, or carrageenan lozenges as active treatments for sore throat. International Journal of General Medicine, 10, 53.

Morote, A.F., Olcina, J., Hernández, M. (2019). The use of non-conventional water resources as a means of adaptation to drought and climate change in Semi-Arid Regions: South-Eastern Spain. Water, 11, 93.

Mourelle, M.L., Gomez, C.P., Legido, J.L. (2017). The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. Cosmetics, 4, 46.

Muhammad, I., Muhammad, A., Niaz, S., Shams, S., Khan, A., Khan, I., Ullah, A. (2016). Physicochemical analysis of water swat at bakhela distt malakand, kpk Pakistan OF RIVER. International Journal of Pure and Applied Zoology, 4, 70-76.

Muhirwa, D., Nhapi, I., Wali, U.G., Banadda, N., Kashaigili, J., Kimwaga, R. (2010). Characterization of wastewater from an abattoir in Rwanda and the impact on downstream water quality.

Munir, N., Imtiaz, A., Sharif, N., Naz, S. (2015). Optimization of growth conditions of different algal strains and determination of their lipid contents. Journal of Animal and Plant Science, 25, 546-553

Munoz-Cupa, C., Hu, Y., Xu, C., Bassi, A. (2021). An overview of microbial fuel cell usage in wastewater treatment, resource recovery and energy production. Science of the Total Environment, 754, 142429.

Musa, M.A., Idrus, S. (2021). Physical and Biological Treatment Technologies of Slaughterhouse Wastewater: A Review. Sustainability, 13, 4656.

Myra, T., David, H., Judith, T., Marina, Y., Ricky, B.J., Reynaldo, E. (2015). Biological treatment of meat processing wastewater using anaerobic sequencing batch reactor (ASBR). International Research Journal of Biological Sciences, 4, 66-75.

Nagarajan, D., Chang, J.S., Lee, D.J. (2020). Pretreatment of microalgal biomass for efficient biohydrogen production–Recent insights and future perspectives. Bioresource Technology, 302, 122871.

Najjar, Y.S., Abu-Shamleh, A. (2020). Harvesting of microalgae by centrifugation for biodiesel production: a review. Algal Research 51, 102046.

Nantha Gopal, K., Thundil Karupparaj, R. (2015). Effect of pongamia biodiesel on emission and combustion characteristics of DI compression ignition engine. Ain Shams Engineering Journal, 6, 297–305.

Narala, R.R., Garg, S., Sharma, K.K., Thomas-Hall, S.R., Deme, M., Li, Y., Schenk, P.M. (2016). Comparison of microalgae cultivation in photobioreactor, open raceway pond, and a two-stage hybrid system. Frontiers in Energy Research, 4, 29.

Narayanan, C.M., & Narayan, V. (2019). Biological wastewater treatment and bioreactor design: a review. Sustainable Environment Research, 29, 1-17.

National Inventory of Sewage Treatment Plants June 2020 (cpcb.nic.in)

Nazari, M.T., Freitag, J.F., Cavanhi, V.A.F., Colla, L.M. (2020). Microalgae harvesting by fungal-assisted bioflocculation. Reviews in Environmental Science and Biotechnology, 19, 369–388.

Neumann, U., Derwenskus, F., Flaiz Flister, V., Schmid-Staiger, U., Hirth, T., Bischoff, S.C. (2019). Fucoxanthin, a carotenoid derived from Phaeodactylum tricornutum exerts antiproliferative and antioxidant activities in vitro. Antioxidants, 8, 183.

Nie, J., Sun, Y., Zhou, Y., Kumar, M., Usman, M., Li, J., Shao, J., Wang, L., Tsang, D.C.W. (2020). Bioremediation of water containing pesticides by microalgae: Mechanisms, methods, and prospects for future research. Science of the Total Environment, 707, 136080.

Olasehinde, T.A., Olaniran, A.O., Okoh, A.I. (2017). The rapeutic potentials of microalgae in the treatment of Alzheimer’s disease. Molecules, 22, 480.

Owolabi, A.O. (2017). Geoenvironmental evaluation of abandoned mine sites in Jos Area, Plateau State, Nigeria (Doctoral dissertation, The Federal University of Technology).

Owolabi, A.O. (2018). Assessment of radioactive contamination in water bodies around mine workings using radiation counter. Journal of Mining and Environment, 9, 795-806.

Pacheco, D., Rocha, A.C., Pereira, L., Verdelhos, T. (2020). Microalgae water bioremediation: trends and hot topics. Applied Sciences, 10, 1886.

Pahazri, N.F., Mohamed, R.M.S.R., Al-Gheethi, A.A., Kassim, A.H.M. (2016). Production and harvesting of microalgae biomass from wastewater: a critical review. Environmental Technology Reviews, 5, 39-56.

Paliwal, C., Mitra, M., Bhayani, K., Bharadwaj, S.V., Ghosh, T., Dubey, S., Mishra, S. (2017). Abiotic stresses as tools for metabolites in microalgae. Bioresource Technology, 244, 1216-1226.

Pandey, A., Pathak, V.V., Kothari, R., Black, P.N., Tyagi, V. (2019). Experimental studies on zeta potential of flocculants for harvesting of algae. Journal of Environmental Management, 231, 562–569.

Park, S., Nguyen, T.H.T., Jin, E. (2019). Improving lipid production by strain development in microalgae: Strategies, challenges and perspectives. Bioresource Technology, 292, 121953.

Parsy, A., Sambusiti, C., Baldoni-Andrey, P., Elan, T., Perie, F. (2020). Cultivation of Nannochloropsis oculata in saline oil & gas wastewater supplemented with anaerobic digestion effluent as nutrient source. Algal Research, 50, 101966.

Parthasarathi, R., & Subha, K.S. (2018). Prospective biosurfactant applications in food industry-opportunities and challenges. International Journal of Current Research Life Science, 7, 1560-1565.

Patel, A., Pruthi, V., Pruthi, P.A. (2017). Synchronized nutrient stress conditions trigger the diversion of CDP-DG pathway of phospholipids synthesis towards de novo TAG synthesis in oleaginous yeast escalating biodiesel production. Energy, 139, 962–974.

Pathak, H., Bhatt, B.P., Gupta, S.K. (2015). State of Indian agriculture 'water'. National academy of agricultural sciences, New Delhi.

Pathak, V.V., Ahmad, S., Kothari, R. (2019). Implication of algal microbiology for wastewater treatment and bioenergy production. Environmental Biotechnology and Sustainable Future, 263-286.

Patziger, M., Gunthert, F.W., Jardin, N., Kainz, H., Londong, J. (2016). On the design and operation of primary settling tanks in state of the art wastewater treatment and water resources recovery. Water Science and Technology.

Pavlidis, G., & Karasali, H. (2020). Natural remediation techniques for water quality protection and restoration. In Methods for Bioremediation of Water and Wastewater Pollution. 327-340.

Peng, K.T., Zheng, C.N., Xue, J., Chen, X.Y., Yang, W.D., Liu, J.S., Li, H.Y. (2014). Delta 5 fatty acid desaturase upregulates the synthesis of polyunsaturated fatty acids in the marine diatom Phaeodactylum tricornutum. Journal of Agricultural and Food Chemistry, 62, 8773-8776.

Peng, L., Fu, D., Chu, H., Wang, Z., Qi, H. (2019). Biofuel production from microalgae: A review. Environmental Chemistry Letters, 18, 285–297.

Peng, Y.Y., Gao, F., Hang, W.J.W., Yang, H.L., Jin, W.H., Li, C. (2019). Effects of organic matters in domestic wastewater on lipid/carbohydrate production and nutrient removal of *Chlorella vulgaris* cultivated under mixotrophic growth conditions. Journal of Chemical Technology and Biotechnology, 94, 3578-3584.

Pereira, A.G., Jimenez-Lopez, C., Fraga, M., Lourenço-Lopes, C., Garcia-Oliveira, P., Lorenzo, J.M., Simal-Gandara, J. (2020). Extraction, properties, and applications of bioactive compounds obtained from microalgae. Current pharmaceutical design, 26, 1929-1950.

Pervov, A., Tikhonov, K., Makisha, N. (2018). Application of reverse osmosis techniques to treat and reuse biologically treated wastewater. In IOP Conference Series: Materials Science and Engineering. 365, 6, 062026.

Peteiro, C. (2018). Alginate production from marine macroalgae, with emphasis on kelp farming. In Alginates and their Biomedical Applications. 27-66.

Piwowar, A., & Harasym, J. (2020). The importance and prospects of the use of algae in agribusiness. Sustainability, 12, 5669.

Płaczek, M., Patyna, A., Witczak, S. (2017). Technical evaluation of photobioreactors for microalgae cultivation. In E3S web of conferences. EDP Sciences, 19, 02032.

Posadas, E., Munoz, A., Garcia-Gonzalez, M.C., Muñoz, R., Garcia-Encina, P.A. (2015). A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters. Journal of Chemical Technology & Biotechnology, 90, 1094-1101.

Posadas, E., Alcantara, C., Garcia-Encina, P.A., (2017). Microalgae-based biofuels and bioproducts. In: Gonzalez-Fernandez C, Munoz R, editors. Microalgae cultivation in wastewater, 67–91.

Poudyal, R.S., Tiwari, I., Najafpour, M.M., Los, D.A., Carpentier, R., Shen, J.R., Allakhverdiev, S.I. (2016). Current insights to enhance hydrogen production by photosynthetic organisms. Hydrogen Science and Engineering: Journal of Materials, Processes, Technology, 461-488.

Prabakaran, G., Sampathkumar, P., Kavisri, M., Moovendhan, M. (2020). Extraction and characterization of phycocyanin from *Spirulina platensis* and evaluation of its anticancer, antidiabetic and anti-inflammatory effect. International Journal of Biological Macromolecules, 153, 256-263.

Prabhakar, Z.N., Lokesh, M., Saidaiah, M., Sai, E.S. (2017). Awareness regarding zoonotic diseases among the butchers of Proddatur, Kadapa Dist., AP, India. Iranian Journal of Health, Safety and Environment, 4, 729-737.

Prasertcharoensuk, P., Bull, S.J., Phan, A.N. (2019). Gasification of waste biomass for hydrogen production: effects of pyrolysis parameters. Renewable Energy, 143, 112–120

Praveen, P., Loh, K.C. (2016). Nitrogen and phosphorus removal from tertiary wastewater in an osmotic membrane photobioreactor. Bioresource Technology, 206, 180-187.

Priyadarshani, I., & Rath, B. (2012). Commercial and industrial applications of micro algae–A review. Journal of Algal Biomass Utilization, 3, 89-100.

Qiu, R., Gao, S., Lopez, P.A., Ogden, K.L. (2017). Effects of pH on cell growth, lipid production and $CO_2$ Addition of Microalgae *Chlorella sorokiniana.* Algal Research, 28, 192-199.

Rad, S.J., & Lewis, M.J. (2014). Water utilisation, energy utilisation and waste water management in the dairy industry: a review. International Journal of Dairy Technology, 67, 1-20.

Raghunath, B.V., Punnagaiarasi, A., Rajarajan, G., Irshad, A., Elango, A. (2016). Impact of dairy effluent on environment—a review. Integrated Waste Management India, 239-249.

Rai, M.P., Gupta, S. (2017). Effect of media composition and light supply on biomass, lipid content and fame profile for quality biofuel production from *Scenedesmus abundans*. Energy Conversion and Management, 141, 85-92.

Rai, M.P., Gautom, T., Sharma, N. (2015). Effect of salinity, pH, light intensity on growth and lipid production of microalgae for bioenergy application. Online Journal of Biological Sciences, 15, 260.

Rajak, U., Verma, T.N. (2018). Effect of emission from ethylic biodiesel of edible and non-edible vegetable oil, animal fats, waste oil and alcohol in CI engine. Energy Conversion and Management, 166, 704-718.

Rajasulochana, P., & Preethy, V. (2016). Comparison on efficiency of various techniques in treatment of waste and sewage water–A comprehensive review. Resource-Efficient Technologies, 2, 175-184.

Raman, L.A., Deepanraj, B., Rajakumar, S., Sivasubramanian, V. (2019). Experimental investigation on performance, combustion and emission analysis of a direct injection diesel engine fuelled with rapeseed oil biodiesel. Fuel, 246, 69-74.

Ramos-Suarez, J.L., Carreras, N. (2014). Use of microalgae residues for biogas production. Chemical Engineering Journal, 242, 86-95.

Rangabashiam, D., Munuswamy, D.B., Duraiswamy Balasubramanian, S., Christopher, D. (2020). Performance, emission, and combustion analysis on diesel engine fueled with blends of neem biodiesel/diesel/additives. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 1-11.

Rao, A., Briskey, D., Nalley, J.O., Ganuza, E. (2020). Omega-3 eicosapentaenoic acid (EPA) rich extract from the microalga nannochloropsis decreases cholesterol in healthy individuals: A double-blind, randomized, placebo-controlled, three-month supplementation study. Nutrients, 12, 1869.

Raouf, M.E.A., Maysour, N.E., Farag, R.K. (2019). Wastewater treatment methodologies, review article. , 3, 18.

Rathore, V., Tyagi, S., Newalkar, B., Badoni, R. (2015). Jatropha and Karanja oil derived DMC–biodiesel synthesis: A kinetics study. Fuel, 140, 597–608.

Rathoure, A. K. (Ed.). (2016). Toxicity and waste management using bioremediation. IGI Global,

Ratnapuram, H.P., Vutukuru, S.S., Yadavalli, R. (2018). Mixotrophic transition induced lipid productivity in *Chlorella pyrenoidosa* under stress conditions for biodiesel production. Heliyon, 4, 1–20.

Reddy, M.V., Kumar, G., Mohanakrishna, G., Shobana, S., Al-Raoush, R.I. (2020). Review on the production of medium and small chain fatty acids through waste valorization and $CO_2$ fixation. Bioresource Technology, 309, 123400.

Ren, H.Y., Zhu, J.N., Kong, F., Xing, D., Zhao, L., Ma, J., Liu, B.F. (2019). Ultrasonic enhanced simultaneous algal lipid production and nutrients removal from non-sterile domestic wastewater. Energy Conversion and Management, 180, 680-688.

Renuka, N., Sood, A., Ratha, S.K., Prasanna, R., Ahluwalia, A.S. (2013). Nutrient sequestration, biomass production by microalgae and phytoremediation of sewage water. International Journal of Phytoremediation, 15, 789-800.

Reyero, I., Arzamendi, G., Zabala, S., Gandía, L.M. (2015). Kinetics of the NaOH-catalyzed transesterification of sunflower oil with ethanol to produce biodiesel. Fuel Process Technology, 129, 147–155.

Rodionova, M.V., Poudyal, R S., Tiwari, I., Voloshin, R.A., Zharmukhamedov, S.K., Nam, H.G., Allakhverdiev, S.I. (2017). Biofuel production: challenges and opportunities. International Journal of Hydrogen Energy, 42, 8450-8461.

Rosales-Mendoza, S., Garcia-Silva, I., Gonzalez-Ortega, O., Sandoval-Vargas, J. M., Malla, A., Vimolmangkang, S. (2020). The potential of algal biotechnology to produce antiviral compounds and biopharmaceuticals. Molecules, 25, 4049.

Roy, A., Dabhi, Y., Brahmbhatt, H., Chourasia, S.K. (2021). Effect of emulsified fuel based on dual blend of Castor-Jatropha biodiesel on CI engine performance and emissions. Alexandria Engineering Journal, 60, 1981-1990.

Roy, P.K., Kumar, D., Ghosh, M., Majumder, A. (2016). Disinfection of water by various techniques–comparison based on experimental investigations. Desalination and Water Treatment, 57, 28141-28150.

Roy, T., Sahani, S., Sharma, Y.C. (2020). Study on kinetics-thermodynamics and environmental parameter of biodiesel production from waste cooking oil and castor oil using potassium modified ceria oxide catalyst. Journal of Cleaner Production, 247, 119166.

Sadegh, H., & Ali, G.A. (2021). Potential applications of nonmaterial in wastewater treatment: nanoadsorbents performance. In Research Anthology on Synthesis, Characterization, and Applications of Nanomaterials, 1230-1240.

Safaei, M., Maleki, H., Soleimanpour, H., Norouzy, A., Zahiri, H. S., Vali, H., & Noghabi, K. A. (2019). Development of a novel method for the purification of C-phycocyanin pigment from a local cyanobacterial strain Limnothrix sp. NS01 and evaluation of its anticancer properties. Scientific reports, 9, 1-16.

Safafar, H., Uldall Nørregaard, P., Ljubic, A., Møller, P., Lovstad Holdt, S., Jacobsen, C. (2016). Enhancement of protein and pigment content in two *Chlorella* sp. cultivated on industrial process water. Journal of Marine Science and Engineering, 4, 84.

Safi, C., Zebib, B., Merah, O., Pontalier, P. Y., Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. Renewable and Sustainable Energy Reviews, 35, 265-278.

Saha, S.K., Ermis, H., Murray, P. (2020). Marine microalgae for potential lutein production. Applied Sciences, 10, 6457.

Sajjad, A.A., Yunus, M.Y.B.M., Azoddein, A.A.M., Hassell, D.G., Dakhil, I.H., Hasan, H.A. (2020). Electrodialysis Desalination for Water and Wastewater: A Review. Chemical Engineering Journal, 380, 122231.

Sajjadi, B., Chen, W.Y., Raman, A.A.A., Ibrahim, S. (2018). Microalgae lipid and biomass for biofuel production: A comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. Renewable and Sustainable Energy Reviews, 97, 200-232.

Salama, E.S., Kurade, M.B., Abou-Shanab, R.A., El-Dalatony, M.M., Yang, I.S., Min, B., Jeon, B.H. (2017). Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renewable and Sustainable Energy Reviews, 79, 1189-1211.

Saleh, I.A., Zouari, N., Al-Ghouti, M.A. (2020). Removal of pesticides from water and wastewater: Chemical, physical and biological treatment approaches. Environmental Technology and Innovation. 101026.

Samarakoon, K.W., O-Nam, K., Ko, J.Y., Lee, J.H., Kang, M.C., Kim, D., Jeon, Y.J. (2013). Purification and identification of novel angiotensin-I converting enzyme (ACE) inhibitory peptides from cultured marine microalgae (*Nannochloropsis oculata)* protein hydrolysate. Journal of Applied Phycology, 25, 1595-1606.

Samdani, P., Choudhary, M.P. (2017). Biological Treatment of Waste Water by Filtration.

Samer, M. (2015). Biological and chemical wastewater treatment processes. Wastewater Treatment Engineering, 150, 212.

San Pedro, A., Gonzalez-Lopez, C.V., Acien, F.G., Molina-Grima, E. (2015). Outdoor pilot production of *Nannochloropsis gaditana*: Influence of culture parameters and lipid production rates in raceway ponds. Algal Research, 8, 205–213.

Sansone, C., Galasso, C., Martire, M.L., Fernandez, T.V., Musco, L., Dell’Anno, A., Brunet, C. (2020). Testing the Halophila Stipulacea Environmental Threat as A Potential Antioxidant Agent.

Sansone, C., Galasso, C., Orefice, I., Nuzzo, G., Luongo, E., Cutignano, A., Ianora, A. (2017). The green microalga Tetraselmis suecica reduces oxidative stress and induces repairing mechanisms in human cells. Scientific Reports, 7, 1-12.

Santal, A.R., Singh, N. (2013). Biodegradation of melanoidin from distillery effluent: role of microbes and their potential enzymes. Biodegradation of Hazardous and Special Products, 5, 71-100.

Santhosh S, Dhandapani R, Hemalatha N. (2016). Bioactive compounds from microalgae and its different applications – A review. Advances in Applied Science and Research, 7, 153-158.

Santos, F.M., Lopes, A.P., Vilar, V.J.P., Pires, J.C.M. (2020). Microalgal growth and nutrient uptake in heavy metal polluted environments. Encyclopedia of Marine Biotechnology, 1, 497-514.

Santosh, O., Bajwa, H.K., Bisht, M.S., Nirmala, C. (2019). Functional biscuits from bamboo shoots: Enrichment of nutrients, bioactive compounds and minerals in bamboo shoot paste fortified biscuits. International Journal of Food Science and Nutrition, 4, 89-94.

Saroha, J. (2017) Water Resource Potential, Utilization and Conservation in India.

Sasakova, N., Gregova, G., Takacova, D., Mojzisova, J., Papajova, I., Venglovsky, J., Kovacova, S. (2018). Pollution of surface and ground water by sources related to agricultural activities. Frontiers in Sustainable Food Systems, 42.

Sathasivam, R., Radhakrishnan, R., Hashem, A., Abd_Allah, E.F. (2019). Microalgae metabolites: A rich source for food and medicine. Saudi Journal of Biological Sciences, 26, 709-722.

Selim, S., Hussein, E., Abou-Elkhair, R. (2018). Effect of Spirulina platensis as a feed additive on laying performance, egg quality and hepatoprotective activity of laying hens. European. Poultry Science, 82, 14-24.

Seon, G., Joo, H.W., Kim, Y.J., Park, J., and Chang, Y.K. (2019). Hydrolysis of lipid-extracted *Chlorella vulgaris* by simultaneous use of solid and liquid acids. Biotechnology Progress 35, 2729.

Sero, E.T., Siziba, N., Bunhu, T., Shoko, R. (2021). Isolation and screening of microalgal species, native to Zimbabwe, with potential use in biodiesel production. All Life, 14, 256-264.

Serra, T., Colomer, J., Pau, C., Marin, M., Sala, L. (2014). Tertiary treatment for wastewater reuse based on the Daphnia magna filtration–comparison with conventional tertiary treatments. Water Science and Technology, 70, 705-711.

Shah, M., Mahfuzur, R., Liang, Y., Cheng, J.J., Daroch, M. (2016). Astaxanthin-producing green microalga Haematococcus pluvialis: from single cell to high value commercial products. Frontiers in Plant Science, 7, 531.

Sharma, A.K., Sahoo, P.K., Singhal, S. (2016). Comparative evolution of biomass production and lipid accumulation potential of Chlorella species grown in a bubble column photobioreactor. Biofuels, 7, 389-399.

Sharma, J., Kumar, V., Kumar, S.S., Malyan, S.K., Mathimani, T., Bishnoi, N.R. and Pugazhendhi, A., 2020. Microalgal consortia for municipal wastewater treatment–Lipid augmentation and fatty acid profiling for biodiesel production. Journal of Photochemistry and Photobiology B: Biology, 202, 111638.

Sharma, N.K., Philip, L. (2016). Combined biological and photocatalytic treatment of real coke oven wastewater. Chemical Engineering Journal, 295, 20-28.

Sharma, P., & Sharma, N. (2017). Industrial and biotechnological applications of algae: a review. Journal Advances Plant Biology, 1, 01.

Shen, L., Ndayambaje, J.D., Murwanashyaka, T., Cui, W., Manirafasha, E., Chen, C., Wang, Y. Lu, Y., 2017. Assessment upon heterotrophic microalgae screened from wastewater microbiota for concurrent pollutants removal and biofuel production. Bioresource Technology, 245, 386-393.

Shivsharan, V.S., Kulkarni, S.W., Wani, M. (2013). Physicochemical characterization of dairy effluents. International Journal Life Sciences Biotechnology and Pharmacaeutical Research, 2, 182-191.

Shokravi, Z., Shokravi, H., Chyuan, O.H., Lau, W.J., Koloor, S.S.R., Petrů, M., Ismail, A.F. (2020). Improving 'lipid productivity'in microalgae by bilateral enhancement of biomass and lipid contents: a review. Sustainability, 12, 9083.

Show, P.L., Tang, M.S.Y., Nagarajan, D., Ling, T.C., Ooi, C.W., Chang, J.S. (2017). A Holistic Approach to Managing Microalgae for Biofuel Applications. International Journal of Molecular Science, 18, 215.

Shrivastava, P., Kumar, A., Tekasakul, P., Lam, S. S., Palamanit, A. (2021). Comparative investigation of yield and quality of bio-oil and Biochar from pyrolysis of woody and non-woody biomasses. Energies, 14, 1092.

Shuba, E.S., Kifle, D. (2018). Microalgae to biofuels: 'Promising' alternative and renewable energy, review. Renewable and Sustainable Energy Reviews 81, 743–755.

Siciliano, G., Urban, F., Tan-Mullins, M., Mohan, G. (2018). Large dams, energy justice and the divergence between international, national and local developmental needs and priorities in the global South. Energy Research & Social Science, 41, 199-209.

Simsek, S., Uslu, S. (2020). Comparative evaluation of the influence of waste vegetable oil and waste animal oil-based biodiesel on diesel engine performance and emissions. Fuel, 280, 118613.

Singh, H.M., Kothari, R., Gupta, R., Tyagi, V.V. (2019). Bio-fixation of flue gas from thermal power plants with algal biomass: Overview and research perspectives. Journal of Environmental Management, 245, 519-539.

Singh, H.M., Pathak, A.K., Chopra, K., Tyagi, V.V., Anand, S., Kothari, R. (2019). Microbial fuel cells: a sustainable solution for bioelectricity generation and wastewater treatment. Biofuels, 10, 11-31.

Singh, M., Sandhu, S.S. (2020). Performance, emission and combustion characteristics of multi-cylinder CRDI engine fueled with argemone biodiesel/diesel blends. Fuel, 265, 117024.

Singh, M., Singh, D. K., Gandhi, S. K., Sarin, A., Saini, S., Mahla, S. K., Sandhu, S. S. (2020). Effect of metal contaminants and antioxidants on the oxidation stability of argemone mexicana biodiesel: experimental and statistical study. Waste and Biomass Valorization, 11, 6189-6198.

Singh, S.P., Singh, P. (2015) Effect of Temperature and Light on the Growth of Algae Species: A Review. Renewable & Sustainable Energy Reviews, 50, 431-444.

Siqueira, S. F., Queiroz, M. I., Zepka, L. Q., Jacob-Lopes, E. (2018). Introductory chapter: Microalgae biotechnology. A brief introduction. Microalgal biotechnology,1.

Slocombe, S.P., Ross, M., Thomas, N., McNeill, S., Stanley, M.S. (2013). A rapid and general method for measurement of protein in micro-algal biomass. Bioresource Technology, 129, 51–57.

Sole-Bundo, M., Garfí, M., Matamoros, V., Ferrer, I. (2019). Co-digestion of microalgae and primary sludge: Effect on biogas production and micro contaminants removal. Science of the Total Environment, 660, 974–981.

Solovchenko, A., Verschoor, A.M., Jablonowski, N.D., Nedbal, L. (2016). Phosphorus from wastewater to crops: An alternative pathinvolving microalgae. Biotechnology Advances, 34, 550–564.

Sowjanya, M., & Manjula, K. (2016). Lifestyle patterns and stress among diabetics. International Journal of Food Science and Nutrition, 1, 23-25.

Sowjanya, M., Manjula, K. (2016). Dietary Spirulina in pharmaceutical and neutraceutical applications. International Journal of Science and Research, 2319-7064.

Srivastava, S.K., Agrawal, B., Kumar, A., Pandey, A. (2020). Phytochemicals of Azadirachta indica source of active medicinal constituent used for cure of various diseases: A Review. Journal of Scientific Research, 64, 385-90.

Staff, R. (2017): Study links pollution to millions of deaths worldwide (available at https://www.reuters.com/article/ushealth-pollution/study-links-pollution-to-millions-of-deathsworldwide-idUSKBN1CO39B).

Su, G., Jiao, K., Chang, J., Li, Z., Guo, X., Sun, Y., Lin, L. (2016). Enhancing total fatty acids and arachidonic acid production by the red microalgae Porphyridium Purpureum. Bioresources and Bioprocessing, 3, 1-9.

Suarez, A., Fidalgo, T., Riera, F.A. (2014). Recovery of dairy industry wastewaters by reverse osmosis. Production of boiler water. Separation and Purification Technology, 133, 204-211.

Suganya, T., Varman, M., Masjuki, H.H., Renganathan, S. (2016). Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: a biorefinery approach. Renewable and Sustainable Energy Reviews, 55, 909-941.

Sukacova, K., Losak, P., Brummer, V., Masa, V., Vicha, D., Zavrel, T. (2021). Perspective design of algae photobioreactor for greenhouses—A comparative study. Energies, 14, 1338.

Sydney, E.B., Sydney, A.C.N., de Carvalho, J.C., Soccol, C.R. (2019). Microalgal strain selection for biofuel production. In Biofuels from algae, 51-66

Takache, H., Pruvost, J., Marec, H. (2015) Investigation of light/dark cycles effects on the photosynthetic growth of *Chlamydomonas Reinhardtii* in condi-tions representative of photobioreactor cultivation. Algal Research, 8, 192-204.

Tan, X.B., Zhao, X.C.,Yang, L.B. (2019). Strategies for enhanced biomass and lipid production by *Chlorella pyrenoidosa* culture in starch processing wastewater. Journal of Cleaner Production, 236, 117671.

Tang, X., Wen, Y., He, Y., Jiang, H., Dai, X., Bi, X., Chen, H. (2020). Full-scale semi-centralized wastewater treatment facilities for resource recovery: operation, problems and resolutions. Water Science and Technology, 82, 303-314.

Tarazona Delgado, R., Guarieiro, M.D.S., Antunes, P.W., Cassini, S.T., Terreros, H.M., Fernandes, V.D.O. (2021). Effect of nitrogen limitation on growth, biochemical composition, and cell ultrastructure of the microalga Picocystis salinarum. Journal of Applied Phycology, 33, 2083-2092.

Taşkan, E. (2016). Performance of mixed algae for treatment of slaughterhouse wastewater and microbial community analysis. Environmental Science and Pollution Research, 23, 20474-20482.

Tawfik, A., Sobhey, M., Badawy, M. (2008). Treatment of a combined dairy and domestic wastewater in an up-flow anaerobic sludge blanket (UASB) reactor followed by activated sludge (AS system). Desalination, 227, 167-177.

Teng, E., Taylor, K., Bilousova, T., Weiland, D., Pham, T., Zuo, X., Cole, G.M. (2015). Dietary DHA supplementation in an APP/PS1 transgenic rat model of AD reduces behavioral and Aβ pathology and modulates Aβ oligomerization. Neurobiology of disease, 82, 552-560.

Teoh, Y.H., How, H.G., Masjuki, H.H., Nguyen, H.T., Kalam, M.A., Alabdulkarem, A. (2019). Investigation on particulate emissions and combustion characteristics of a common-rail diesel engine fueled with Moringa oleifera biodiesel-diesel blends. Renewable Energy, 136, 521–534.

Tibbetts, S.M., Milley, J.E., Lall, S.P. (2015). Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. Journal of Applied Phycology, 27, 1109–1119.

Ting, H., Haifeng, L., Shanshan, M., Zhang, Y., Zhidan, L., Na, D. (2017). Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: A review. International Journal of Agricultural and Biological Engineering, 10, 1-29.

TMofasser Hossain, A.K.M., Brennan, M.A., Mason, S.L., Guo, X., Zeng, X.A. Brennan C.S. (2017). The effect of astaxanthin-rich microalgae "*Haematococcus pluvialis"* and wholemeal flours incorporation in improving the physical and functional properties of cookies. Foods, 6, 57.

Tripathi, R., Singh, J., Thakur, I.S. (2015). Characterization of Microalga Scenedesmus sp. ISTGA1 for potential $CO_2$ sequestration and biodiesel production. Renewable Energy, 74, 774-781.

Turkcan, A. (2020). The effects of different types of biodiesels and biodiesel-bioethanol-diesel blends on the cyclic variations and correlation coefficient. Fuel, 261, 116453.

Tzanakakis, V.A., Paranychianakis, N.V., Angelakis, A.N. (2020). Water supply and water scarcity, 12, 2347.

Udaiyappan, A.F.M., Hasan, H.A., Takriff, M.S., Abdullah, S.R.S. (2017). A review of the potentials, challenges and current status of microalgae biomass applications in industrial wastewater treatment. Journal of Water Process Engineering, 20, 8-21.

Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A. (2010). Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. Journal of Renewable and Ssustainable Energy, 2, 012701.

Uebel, L.D.S., Costa, J.A., Olson, A.C., Morais, M.G.D. (2019). Industrial plant for production of *Spirulina* sp. LEB 18. Brazilian Journal of Chemical Engineering, 36, 51-63.

Um, M.M., Barraud, O., Kerouredan, M., Gaschet, M., Stalder, T., Oswald, E., Bibbal, D. (2016). Comparison of the incidence of pathogenic and antibiotic-resistant Escherichia coli strains in adult cattle and veal calf slaughterhouse effluents highlighted different risks for public health. Water Research, 88, 30-38.

Un, U.T., & Ozel, E. (2013). Electrocoagulation of yogurt industry wastewater and the production of ceramic pigments from the sludge. Separation and Purification Technology, 120, 386-391.

Un, U.T., Koparal, A.S., Ogutveren, U.B. (2013). Fluoride removal from water and wastewater with a bach cylindrical electrode using electrocoagulation. Chemical Engineering Journal, 223, 110-115.

UNESCO-WWAP (United Nations World Water Assessment Programme). (2017). The United Nations world water development report. Wastewater: The untapped resource. Paris, France: United Nations Educational, Scientific and Cultural Organisation (UNESCO).

US EPA annual report 2017 (Office of wastewater management), 830R17006 March 2018 www.epa.gov/OWM.

Van Den Hende, S., Beelen, V., Bore, G., Boon, N., Vervaeren, H. (2014). Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs: from lab reactors to an outdoor raceway pond. Bioresource Technology, 159, 342-354.

Velasco, L.A., Carrera, S., Barros, J. (2016). Isolation, culture and evaluation of Chaetoceros muelleri from the Caribbean as food for the native scallops, Argopecten nucleus and Nodipecten nodosus. Latin American Journal of Aquatic Research, 44, 557–568.

Velazquez-Lucio, J., Rodriguez-Jasso, R.M., Colla, L.M., Saenz-Galindo, A., Cervantes-Cisneros, D.E., Aguilar, C.N., Fernandes, B.D., Ruiz, H.A. (2018). Microalgal biomass pretreatment for bioethanol production: A review. Biofuel Research Journal, 5, 780–791.

Venckus, P., Kostkeviciene J., Bendikienė, V. (2017). Green algae *Chlorella vulgaris* cultivation in municipal wastewater and biomass composition. Journal of Environmental Engineering and Landscape Management, 25, 56-63.

Vialkova, E.I., Sidorenko, O.V., Glushchenko, E.S. (2019, December). Qualitative composition and local pretreatment of dairy wastewaters. In IOP Conference Series: Materials Science and Engineering. 687, 066049.

Victor, S., Rocha-Ferreira, E., Rahim, A., Hagberg, H., Edwards, D. (2021). New possibilities for neuroprotection in neonatal hypoxic-ischemic encephalopathy. European journal of pediatrics, 1-13.

Visca, A., Di Caprio, F., Spinelli, R., Altimari, P., Cicci, A., Iaquaniello, G., Pagnanelli, F. (2017). Microalgae cultivation for lipids and carbohydrates production. Chemical Engineering Transactions, 57, 127-132.

Wan, X. Z., Li, T.T., Zhong, R.T., Chen, H.B., Xia, X., Gao, L.Y., Zhao, C. (2019). Anti-diabetic activity of PUFAs-rich extracts of *Chlorella pyrenoidosa* and *Spirulina platensis* in rats. Food and Chemical Toxicology, 128, 233-239.

Wang, J.H., Zhang, T.Y., Dao, G.H., Xu, X.Q., Wang, X.X., Hu, H.Y. (2017). Microalgae-based advanced municipal wastewater treatment for reuse in water bodies. Applied Microbiology Biotechnology, 101, 2659-2675.

Wang, K.J., Wang, P.S., Nguyen, H.P. (2021). A data-driven optimization model for coagulant dosage decision in industrial wastewater treatment. Computers & Chemical Engineering, 152, 107383.

Wang, Y., He, B., Sun, Z., Chen, Y. F. (2016). Chemically enhanced lipid production from microalgae under low sub-optimal temperature. Algal Research, 16, 20-27.

Wang, Y., Wang, S., Sun, L., Sun, Z., Li, D. (2020). Screening of a Chlorella-bacteria consortium and research on piggery wastewater purification. Algal Research, 47, 101840.

Wei, X., Xue, X., Wu, L., Yu, H., Liang, J., Sun, Y. (2020). High-grade bio-oil produced from coconut shell: A comparative study of microwave reactor and core-shell catalyst. Energy, 212, 118692.

Wellburn, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology, 144, 307-313.

Whitton, R., Ometto, F., Pidou, M., Jarvis, P., Villa, R., Jefferson, B. (2015). Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment. Environmental Technology Reviews, 4, 133-148.

Widowati, I., Zainuri, M., Kusumaningrum, H.P., Susilowati, R., Hardivillier, Y., Leignel, V., Mouget, J.L. (2017). Antioxidant activity of three microalgae *Dunaliella salina*, *Tetraselmis chuii* and Isochrysis galbana clone Tahiti. In IOP Conference Series: Earth and Environmental Science, 55, 1, 012067.

Wolf, J., Stephens, E., Steinbusch, S., Yarnold, J., Ross, I.L., Steinweg, C., Doebbe, A., Krolovitsch, C., Müller, S., Jakob, G. (2016). Multifactorial comparison of photobioreactor geometries in parallel microalgae cultivations. Algal Research, 15, 187–201.

Wollina, U., Voicu, C., Gianfaldoni, S., Lotti, T., França, K., Tchernev, G. (2018). *Arthrospira platensis*–potential in dermatology and beyond. Open access Macedonian Journal of Medical Sciences, 6, 176.

Wollmann, F., Dietze, S., Ackermann, J.U., Bley, T., Walther, T., Steingroewer, J., Krujatz, F. (2019). Microalgae wastewater treatment: Biological and technological approaches. Engineering in Life Sciences, 19, 860-871.

Wong, J.W.C., Kaur, G., Mehariya, S., Karthikeyan, O.P., Chen, G. (2018). Food waste treatment by anaerobic co-digestion with saline sludge and its implications for energy recovery in Hong Kong. Bioresource Technology.

World Health Organization, & UNICEF. (2013). Progress on sanitation and drinking-water. World Health Organization.

Xia, S., Gao, B., Li, A., Xiong, J., Ao, Z., Zhang, C.W. (2014). Preliminary characterization, antioxidant properties and production of Chrysolaminarin from Marine Diatom Odontellaaurita. Marine Drugs, 12, 4883–4897.

Xie, Y., Li, J., Ma, R., Ho, S.H., Shi, X., Liu, L., Chen, J. (2019). Bioprocess operation strategies with mixotrophy/photoinduction to enhance lutein production of microalga *Chlorella sorokiniana* FZU60. Bioresource Technology, 290, 121798.

Xin, L., Hong-Ying, H., & Yu-Ping, Z. (2011). Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. Bioresource Technology, 102, 3098-3102.

Xu, K., Zou, X., Mouradov, A., Spangenberg, G., Chang, W., Li, Y. (2021). Efficient bioflocculation of Chlorella vulgaris with a chitosan and walnut protein extract. Biology, 10, 352.

Xu, K., Zou, X., Wen, H., Xue, Y., Zhao, S., Li, Y. (2018). Buoy-bead flotation harvesting of the microalgae Chlorella vulgaris using surface-layered polymeric microspheres: A novel approach. Bioresource Technology, 267, 341–346.

Yaakob, M.A., Mohamed, R.M.S.R., Al-Gheethi, A.A.S., Kassim, A.H.M. (2018). Characteristics of chicken slaughterhouse wastewater. Chemical Engineering Transactions, 63, 637-642.

Yaakob, Z., Ali, E., Zainal, A., Mohamad, M., Takriff, M.S. (2014). An overview: biomolecules from microalgae for animal feed and aquaculture. Journal of Biological Research-Thessaloniki, 21, 1-10.

Yadav, A., Chowdhary, P., Kaithwas, G., Bharagava, R.N. (2017). Toxic Metals in the Environment: Threats on Ecosystem and Bioremediation Approaches. In Handbook of Metal-Microbe Interactions and Bioremediation. 128-141.

Yadav, G., Dash, S.K., Sen, R. (2019). A biorefinery for valorization of industrial waste-water and flue gas by microalgae for waste mitigation, carbon-dioxide sequestration and algal biomass production. Science of the Total Environment, 688, 129-135.

Yakoob, H., Teoh, Y.H., Din, Z.U., Saba, N.U., Jamil, M.A., Mujtaba, M A., Abid, A. (2021). The potential of sustainable biogas production from biomass waste for power generation in Pakistan. Journal of Cleaner Production, 127250.

Yakoob, M.Y., Shi, P., Hu, F.B., Campos, H., Rexrode, K.M., Orav, E.J., Mozaffarian, D. (2014). Circulating biomarkers of dairy fat and risk of incident stroke in US men and women in 2 large prospective cohorts. The American Journal of Clinical Nutrition, 100, 1437-1447.

Yang, F., Xiang, W., Li, T., Long, L. (2018). Transcriptome analysis for phosphorus starvation-induced lipid accumulation in *Scenedesmus* sp. Scientific Reports, 8, 1-11.

Yen, H.W., Hu, I.C., Chen, C.Y., Nagarajan, D., Chang, J.S. (2019). Design of photobioreactors for algal cultivation. In Biofuels from algae, 225-256.

Yilmaz, N., Atmanli, A., Trujillo, M. (2017). Influence of 1-pentanol additive on the performance of a diesel engine fueled with waste oil methyl ester and diesel fuel. Fuel, 207, 461-469.

Yilmaz, N., Atmanli, A., Vigil, F.M. (2018). Quaternary blends of diesel, biodiesel, higher alcohols and vegetable oil in a compression ignition engine. Fuel, 212, 462-469.

Yodsuwan, N., Sawayama, S., Sirisansaneeyakul, S. (2017). Effect of nitrogen concentration on growth, lipid production and fatty acid profiles of the marine diatom *Phaeodactylum tricornutum*. Agriculture and Natural Resource 51, 190–197.

Yonar T, Sivrioglu O. (2017). Electrochemical degradation of dairy effluent using novel Sn/Sb/ Ni-Ti anodes. Journal of Physical Chemistry and Biophysics, 7.

Zang, C., Huang, S., Wu, M., Du, S., Scholz, M., Gao, F., Dong, Y. (2011). Comparison of relationships between pH, dissolved oxygen and chlorophyll a for aquaculture and non-aquaculture waters. Water, Air, and Soil Pollution, 219, 157-174.

Zarrinmehr, M.J., Farhadian, O., Heyrati, F.P., Keramat, J., Koutra, E., Kornaros, M., Daneshvar, E. (2020). Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, Isochrysis galbana. The Egyptian Journal of Aquatic Research, 46, 153-158.

Zdarta, J., Meyer, A.S., Jesionowski, T., Pinelo, M. (2019). Multi-faceted strategy based on enzyme immobilization with reactant adsorption and membrane technology for biocatalytic removal of pollutants: a critical review. Biotechnology Advances, 37, 107401.

Zhang, J., Hu, Q., Qu, Y., Dai, Y., He, Y., Wang, C.H., Tong, Y.W. (2020). Integrating food waste sorting system with anaerobic digestion and gasification for hydrogen and methane co-production. Applied Energy 257, 113988.

Zhang, Q., Wang, T., Hong, Y. (2014). Investigation of initial pH effects on growth of an oleaginous microalgae Chlorella sp. HQ for lipid production and nutrient uptake. Water Science and Technology, 70, 712-719.

Zhang, T.Y., Wu, Y.H., Hu, H.Y. (2014). Domestic wastewater treatment and biofuel production by using microalga *Scenedesmus* sp. ZTY1. Water Science and Technology, 69, 2492-2496.

Zhang, W., Zhang, W., Zhang, X., Amendola, P., Hu, Q., Chen, Y. (2013). Characterization of dissolved organic matters responsible for ultrafiltration membrane fouling in algal harvesting. Algal Research, 2, 223-229.

Zhang, X.Y., Xu, Y.J., Guan, W.J., Lin, L.F. (2018). Regional, age and respiratory-secretion-specific prevalence of respiratory viruses associated with asthma exacerbation: a literature review. Archives of Virology, 163, 845-853.

Zhang, Y., Ren, L., Chu, H., Zhou, X., Yao, T., Zhang, Y. (2019). Optimization for Scenedesmus obliquus Cultivation: the Effects of Temperature, Light Intensity and pH on Growth and Biochemical Composition. Microbiology and Biotechnology Letters, 47, 614-620.

Zhao, W.Y., Zhou, M., Yan, B., Sun, X., Liu, Y., Wang, Y., Xu, T., Zhang, Y. (2018). Waste conversion and resource recovery from wastewater by ion exchange membranes: State-of-the-art and perspective. Industrial and Engineering Chemistry Research, 57, 6025–6039.

Zhao, X.C., Tan, X.B., Yang, L.B., Liao, J.Y., Li, X.Y. (2019). Cultivation of *Chlorella pyrenoidosa* in anaerobic wastewater: The coupled effects of ammonium, temperature and pH conditions on lipids compositions. Bioresource Technology, 284, 90-97.

Zheng, M., Ji, X., He, Y., Li, Z., Wang, M., Chen, B., Huang, J. (2020). Simultaneous fixation of carbon dioxide and purification of undiluted swine slurry by culturing *Chlorella vulgaris* MBFJNU-1. Algal Research, 47, 101866.

Zhou, W., Li, Y., Min, M., Hu, B., Zhang, H., Ma, X., Ruan, R. (2012). Growing wastewater-born microalga Auxenochlorella protothecoides UMN280 on concentrated municipal wastewater for simultaneous nutrient removal and energy feedstock production. Applied Energy, 98, 433-440.

Zhu, L. (2015). Microalgal culture strategies for biofuel production: a review. Biofuels, Bioproducts and Biorefining , 9, 801-814.

Zhu, S., Huang, W., Xu, J., Wang, Z., Xu, J., Yuan, Z. (2014). Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga *Chlorella zofingiensis.* Bioresource Technology, 152, 292-298.

Zienkiewicz, A., Zienkiewicz, K., Poliner, E., Pulman, J.A., Du, Z.Y., Stefano, G., Benning, C. (2020). The microalga Nannochloropsis during transition from quiescence to autotrophy in response to nitrogen availability. Plant Physiology, 182, 819-839.

# *Appendix*

**Methodology for Wastewater Characterization**

The industrial wastewater were analyzed on each 5$^{th}$ day time interval for various physicochemical parameters such as pH, TDS, BOD, COD, TSS, Nitrate Phosphate and Sulphate by following the standard methods (APHA, 2012).

**(A) Physical parameters**

**(1) Total dissolved solids (mg/l):**

Total dissolved solids of filterable residue are those solids which left after evaporated of the filtered sample.

**Procedure:**

- 100 ml washed and dried crucible was taken and weigh, immediately before used, 100 ml of well-mixed sample was poured and filtered through filter paper (Whatman no. 42).
- Collected the filtrate in the 100 ml weighed crucible. Evaporated the sample in an oven at 105ºC ±1 for 4 to 6 hrs and cooled the crucible and weight.

**Calculation:**

$$\textbf{TDS (mg/l)} = \frac{A-B}{V} \times 10^6$$

Where, A = Final weight of crucible in gm

B = Initial weight of crucible in gm, and V = Volume of sample.

**(2) Total suspended solids (mg/l)**

Total suspended solids are the retained material on Whatman no. 42 filter paper after filtration TSS was determined by taking difference between the total solids and total dissolved solids.

**Calculation:**

$$TSS\ (mg/l) = TS - TDS$$

**(B) Chemical parameters**

**(1) pH (Hydrogen ion concentration):**

pH is the Hydrogen ion concentration in the given water sample. pH equals to negativity $\log_{10}$ of $H^+$ concentration.

$$pH = -\log_{10} (H^+)$$

**Apparatus:**

Digital pH meter.

**Procedure:**

The pH was measured by dipping the pH meter in the samples.

**(2) Biochemical oxygen demand (mg/L)Principle:**

The principle of the method involves measuring the difference of the oxygen concentration in the samples and after incubation for 5 days at 20°C.

**Apparatus and reagents:**

**a).** BOD bottles

**b).** BOD incubator (at 20°C)

**c).** **Phosphate buffer:** 2.1gm $H_2SO_4$, 5.43gm $KH_2PO_4$, 8.35 gm $Na_2HPO_4.7H_2O$ and0.42gm $NH_4Cl$ were dissolved in distilled water to prepare 250 ml of solution.

**d).** **Magnesium sulfate:** 8.25gm $MgSO_4$ was dissolved in distilled water to prepare100 ml of solution.

**e).** **Calcium chloride:** 2.75gm of anhydrous $CaCl_2$ was dissolved in distilled water toprepare 100 ml of solution.

**f).** **Ferric chloride:** 0.25gm $FeCl_3.6H_2O$ was dissolved in distilled water to prepare 1liter of solution.

**g).** **Sodium sulfite solution:** 1.57gm $Na_2SO_4$ was dissolved in 100 ml distilled waterand dilute to 1000 ml.

**Procedure:**

Dilution water was prepared in a glass container by bubbling compressed air in distilled water for about 30 minutes.

- Added 1 ml each of phosphate buffer, magnesium sulfate and calcium, calcium chloride and ferric chloride solutions for each liter of dilution water and mix thoroughly. Neutralize the sample to pH around 7.0.
- Prepared dilutions in a large glass bottle mix the content thoroughly. Fill 2 sets of the BOD bottle. Kept one set of the bottles in BOD incubator at 20°C for 5 days, and determine the DO content in another set immediately.
- DO in the sample bottle was noted immediately after the completion of 5days incubation period. Similarly, a blank was run for dilution water.

**Calculation:**

$$\text{BOD (mg/l)} = (D_0 - D_5) \times \text{dilution factor}$$

Where, $D_0$ = initial DO in the sample and $D_5$ = DO after 5 days.

**(3) Chemical oxygen demand (mg/L):**

The sample is refluxed with $K_2Cr_2O_7$ and $H_2SO_4$ in presence of mercuric sulfate to neutralize the effect of chlorides and silver sulfate. The excess of potassium dichromate is nitrated against of $K_2Cr_2O_7$ used is proportional to the oxidization organic matter in the sample.

**a). Potassium dichromate solution (0.25N):** 6.13 gm of $K_2Cr_2O_7$ was dissolved in distilled water to make 500 ml of solution.

**b). Ferrous ammonium sulfate (0.1N):** 39.2gm of Fe $(NH_4)_2(SO_4).6H_2O$ was dissolved in water adding 20 ml conc. $H_2SO_4$ to make 1 liter of solution.

**c). Ferroin indicator:** 1.48gm of 1-10, phenolphthalein and 0.69gm of ferrous sulfatewas dissolved in distilled water to make 100 ml of solution.

**d). Sulphuric acid** - (Sp.Gr.1.83)

**e).** Mercuric Sulfate Solid**f).** Silver Sulfate- Solid **Procedure:**

- 20 ml of sample or suitable aliquot dilution of the sample was taken in a COD flask 10 ml of 0.25N $K_2Cr_2O_7$, a pinch of $AgSO_4$ and $HgSO_4$ were added and than 30 ml of sulphuric acid was added slowly.
- Refluxed the samples at least for 2 hour on hot plate. The flask was removed, cooled and made the final volume of the aliquot to about 140 ml with doubled distilled water.
- Added 2-3 drops of ferroin indicator. Mixed thoroughly and titrated with 0.1 N Fe $(NH_4)_2(SO_4)_2$ solution. A blank was run with distilled water using same quantity of the chemicals.

**Calculation:**

(b-a) X N of FAS X 1000X 8

COD (mg/L) =

ml of sample Where, a = ml of titrant with sample b = ml of titrant with blank

**(3) Nitrate (mg/L):**

**Principle**

Nitrate and brucine react to produce a yellow colour, the intensity of which can be measured at 410 nm by using spectrophotometer.

**Reagent and apparatus:**

**A).** Spectrophotometer

**B). Brucine-sulphanilic acid solution**

Dissolved 1gm brucine sulfate and 0.1 gm of sulfanilic acid in about 70 ml of hot distilled water. After addition of 3 ml Conc. HCl, the volume was up to the 100 ml. The pink colour develops slowly, does not affect the sensitivity.

**C). Sulphuric acid solution:** 500 ml conc. $H_2SO_4$ was added in 125 ml distilled water and then cooled.

**D). Sodium chloride solution:** 300 gm NaCl was dissolved in distilled water and make up the volume of 1 liter of solution.

**E). Sodium arsenite solution:** 5.0gm $NaAsO_2$ was dissolved in distilled water and diluted to 1 liter of solution.

**F). Standard nitrate solution:** 0.722gm of $KNO_3$ was dissolved in distilled water and make up the volume of 1 liter. The solution contains 100 mg N/L. Diluted it to 100 times to prepare a solution having 1 mg N/L (10 ml-1000ml).

**G). Preparation of standard curve**

A standard curve was prepared between concentration and absorbance by taking the dilution from 0.1 to 1.0 mg N/L at the interval of 0.1, employing the same procedure as for the sample.

**Procedure:**

- 10 ml sample or an aliquot dilution was taken in a 50 ml test tube and put it in wire rack.
- Placed the rack in cool water bath and added 2 ml of NaCl solution. Added 10 ml of $H_2SO_4$ solution after mixing the contents thoroughly swirling by hand.
- Added 0.5 ml brucine reagent and mix thoroughly. Placed the rack in a hot water bath with boiling water, upto 20 minutes.
- Cooled the contents again in cold water bath and the readings were taken at 410 nm by using spectrophotometer. The concentration of $NO_3$-N from the standard curve was found out.

**(4) Phosphate (mg/L)**

Phosphate is measured with the help of ammonical molybdate in an acid medium to form molybdophosphoric acid is then reduced to a pink colour complex and these blue colour compound detected through an absorbance of 680 nm using spectrophotometer.

$$\mathbf{H_3PO_4+12H_2MoO_4 \longrightarrow H_3P(Mo_3O_{10})_4+12H_2O}$$

Total suspended solids are the retained material on Whatman no. 42 filter paper after filtration TSS was determined by taking difference between the total solids and total dissolved solids.

**Reagent:**

1) Ammonium molybdate $(NH4)_2MoO_4$

2) $SnCl_2$

**Procedure:**

- Take 10 ml sample in a test tube
- Add 0.4 ml ammonium molybdate $(NH4)_2MoO_4$ in a test tube • Then add 2 drop $SnCl_2$
- Take OD at 680 nm.

**Calculations:**

Phosphate (mg/l) = K-factor x Absorbance (O.D)K- Factor = Absorbance (O.D) / Concentration.

**(5) Sulfate (mg/l)**

**Principle:** Sulfate ion is precipitated in the form of barium chloride in hydrochloric acid medium. The concentration of sulfate can be determined from the absorbance of the light by barium sulfate and then comparing it with a standard curve.

**Reagent and apparatus:**

**A).** Spectrophotometer and magnetic stirrer.

**B). Conditioning reagent:** 75 gm of NaCl, 30 ml Conc. HCl, 100 ml 95% ethyl or isopropyl alcohol were mixed in 300 ml of distilled water. Add 50 ml of glycerol to this solution and mixed thoroughly.

**C). Barium chloride:** Crystal of $BaCl_2$.

**D). Standard sulfate solution:** 0.1479 gm of anhydrous $Na_2SO_4$ was dissolved in distilled water to make 1 litre of solution. This solution contained 100 mg/l of sulfate.

**E). Preparation of standard curve:** Standard curve was prepared between concentration and absorbance by taking the dilution from 0.0-40.0 mg/l at the interval of 5 mg/l.

**Procedure:**

- 100 ml sample or a suitable aliquot was taken in a conical flask and added 5.0 ml of conditioning reagent.
- Stirred the sample on a magnetic stirrer and added a spoonful of $BaCl_2$ crystals.

Stirred it for only one minute.

- The readings were taken by using Spectrophotometer at an absorbance of 420 nm exactly after 4 minutes. The concentration of sulfate from the standard curve was found out.

**Calculation**

Sulphate (mg/L) = K-factor x Absorbance (O.D.)

K- Factor = Absorbance (O.D.) / Concentration

**(C) Methodology for bio-chemical analysis of microalgae**

Algal growth characteristics were observed by analysis of its biochemical compositions such as protein, carbohydrate, lipid and pigments. These parameters were analyzed on every alternate day of growth optimized experiment.

**(1) Carbohydrate**

Carbohydrate was estimated by Anthrone method which was originally described as a nonspecific quantitative test for carbohydrate (Dreywood, 1946). The interaction of phenol solution with carbohydrate produces a finite absorbance, which is measured at an absorbance of 620 nm.

**Reagent and apparatus:**

**(1)** Phenol Solution (5%): 30 g of phenol dissolved in 1 liter distilled water.

**(2)** Sulphuric Acid: 96% reagent grade.

**Procedure:**

- Mix 0.1 ml of algal sample with 1 ml of 5% phenol solution. • Subsequently add 5 ml of sulphuric acid rapidly.
- Keep the whole content in water bath for 20 minute.
- Cool at room temperature and read the absorbance at 620 nm followed by cooling at room temperature.

**Calculation:**

The carbohydrate concentration can be calculated from the calibration curve of known concentration.

**(2) Protein (mg/ml)**

This method combines the reaction of copper ions with the peptide bond under alkaline condition with the oxidation of aromatic protein residues. The Lowry method is best for the extraction of protein concentrations from 0.01 to 1.0 mg/ml.

**Reagents:**

1. N NaOH
2. 0.5% $CuSO_4.5H_2O$ (1 ml) +Na-K tartrate 1% (1 ml) + $NaCO_3$ 5% (50 ml)
3. Folin-phenol reagent (1N)

**Procedure:**

- Take 0.5 ml cell suspension and add 0.5 ml NaOH and boiled in water bath at 100 $^0$C for 10 minutes.
- After boiling add 2.5 ml reagent 2 and incubate at room temperature for 10 minute.
- Add 0.5 ml of Folin-phenol reagent and incubate for 15 minute at room temperature.
- Take optical density at the wavelength of 660 nm by using UV spectrophotometer

**Calculation:**

The protein concentration can be calculated from the calibration curve of known concentration.

**(3) Chlorophyll**

The chlorophyll in algal cell was determined by the spectrophotometric method prescribed by Mackinney *et al.*, (1941).

**Reagents:**

1. 90% aqueous acetone solution
2. N Hydrochloric acid
3. 1% Magnesium carbonate suspension

Total suspended solids are the retained material on Whatsman no. 42 filter paper after filtration TSS was determined by taking difference between the total solids and total dissolved solids.

**Procedure:**

- Take 10 ml of wastewater sample and centrifuge tubes at 5000 rpm for 10 minutes
- Decant the supernatant and add 10 ml of aqueous acetone solution.
- Centrifuge again at same rpm and time period.
- Store the crushed algal sample at 4 ºC for 20-30 minutes.
- Take the absorbance of sample and blank at 663 nm and 645 nm.

**Calculation:**

Chlorophyll 'a' = $(12.7\times Abs_{663}) - (2.69 \times Abs_{645})$ Chlorophyll 'b' = (22.9×Abs645)-(4.7×Abs663) Chlorophyll 'a+b' = (20.2×Abs645)+(8.0×Abs663)

**(D) Extraction of lipid from algal biomass**

**(1) Modified Bligh and Dyer (MB&D) Method**

A mixture of 0.5 ml of PBS (8 mM $Na_2HPO_4$, 140 mM NaCl, 2mM $NaH_2PO_4$, pH 7.4) and glass beads (0.5mm) was added to test tubes containing the algal cells. Cells were disintegrated by high speed centrifugation for 4 minute. After that 3 ml of extracted solvent (methanol and chloroform, 1:2 v/v) was added to the sample and shaken briefly. Whole content was kept overnight at room temperature. To produce a biphasic layer, 1 ml of distilled water was added to the mixture and centrifuged at 5000 rpm for 10 minute at 20°C. The lower organic phase was drained using pipette and the extraction procedure was repeated with 2 ml of the extracted solution. The collected organic phase was kept in to a pre weighted small petridish. Chloroform and methanol mixture was evaporated at 60°C and the extracted lipid was weighing (Bligh and Dyer, 1959).

**(2) Extraction by n-hexane**

Dried algal biomass was extracted by n-hexane and diethyl ether in 2:1 ratio containing 0.1 molar potassium chloride. The oil extracted appeared in upper layer and the residue of algal cells were settling down at the bottom. Solvent was removed by placing the flask containing algal oil on rotary evaporator (Kothari *et al.*, 2012).

**(3) Transesterification of algal oil**

Mixture of catalyst (NaOH) and methanol was poured into the flask containing algal oil. The whole content was kept for three hours on continuous rotating shaker (200 rpm) to allow the completion of reaction. After three hours, biodiesel formed on upper layer and the pigment along with glycerin settled down at the bottom. Biodiesel was separated with the help of separating funnel.

**(E) Scanning electron microscope**

Surface characteristics of algal cell were analyzed by using SEM facility. The algal samples used for SEM analysis were fixed with osmium tetroxide ($OsO_4$). A 10%

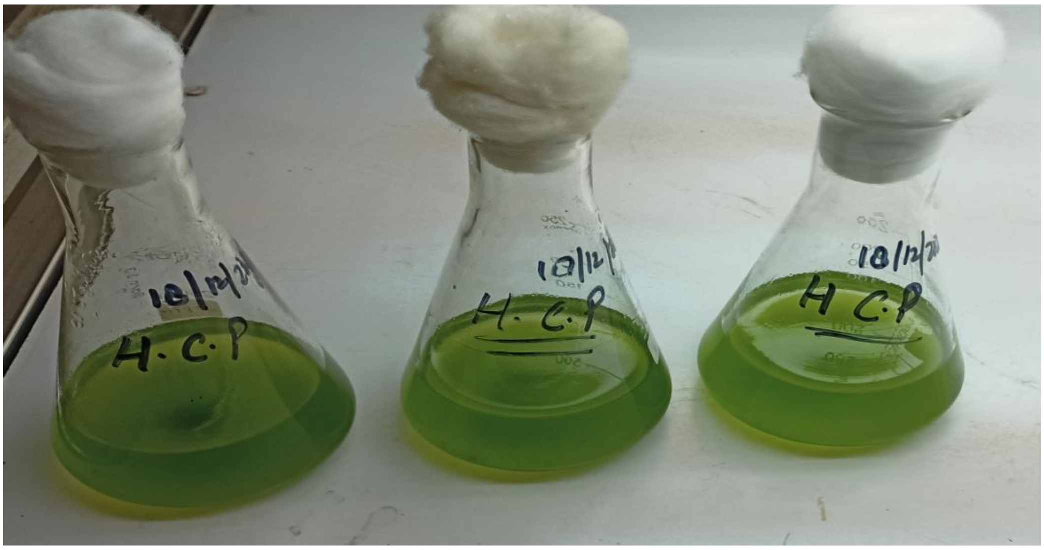
working solution of osmium tetroxide in distilled water was used. Samples were fixed for 10-30 minutes with a final concentration of 1-2% of osmium tetroxide. Following steps were followed for sample preparation:

1. A volume of 200-500 μL of culture was filtered by applying the light pressure on the plunger of the syringe to avoid the damage of sample.
2. Wash the samples about 3 times to remove the salt.
3. Dehydrate the samples by passing through a series of alcohols in increasing concentrations (25%, 50%, 75%, and 100% V/V).
4. The dried material was processed for critical point drying (CPD), in which ethanol is replaced by liquid carbon dioxide under control conditions of pressure and temperature. Pressure is reduced to evaporate the carbon dioxide without causing surface tension on algal cell. Then samples were dried under the atmospheric conditions.
5. Prior to the SEM analysis samples were coated with metal coating.

**(F) Fourier Transform Infrared Spectroscopy**

FTIR analysis was performed to characterize the functional groups of algal oil samples and algal based biosorbents (dried and wet algal biomass). A Perkin Elmer spectrum RX/FTIR system was used to obtain IR spectrum within a range of 4000 cm-1 to 500 cm-1 using a KBr disk for reference. Prior to the FTIR analysis the solid samples were dried enough to avoid any moisture content that can cause additional spectra and problems in interpretation of functional groups. Spectral adsorption bands were identified in relation to the published information. Supporting information on band was also obtained by analyzing a range of pure biochemical standards (protein, nucleic acid, fatty acid and soluble carbohydrate).

## Photos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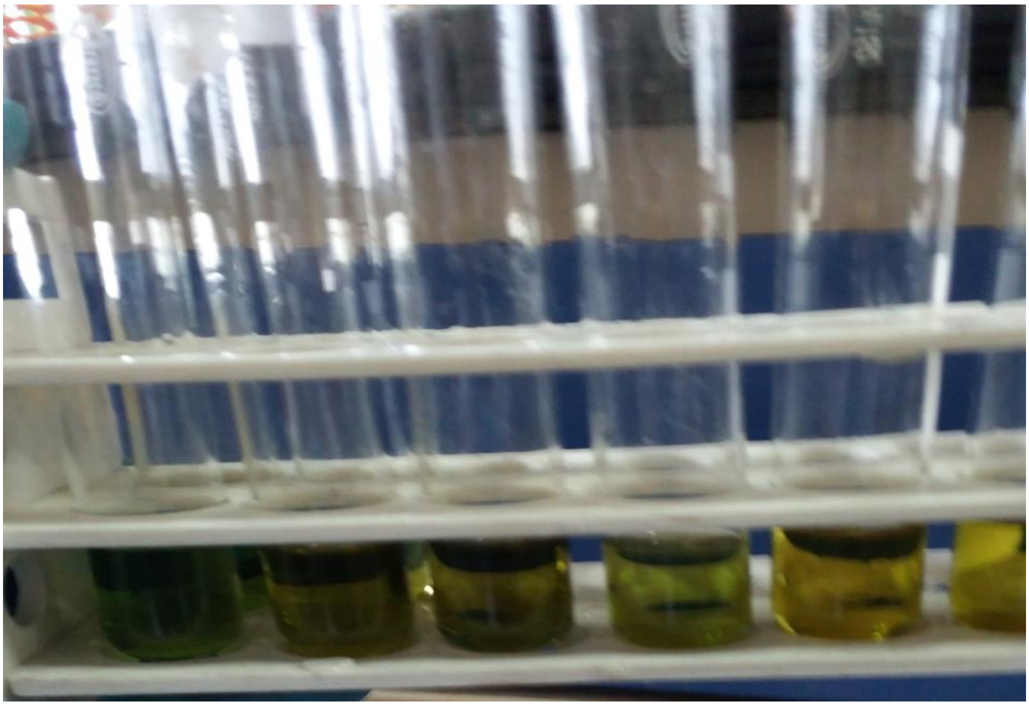

# *Publications*

**Research paper**

1. **Azam, R.**, Kothari, R., Singh, H.M., Ahmad, S., Ashokkumar, V., & Tyagi, V. V. (2020). Production of algal biomass for its biochemical profile using slaughterhouse wastewater for treatment under axenic conditions. Bioresource Technology, 306, 123116.
2. **Azam, R.**,Kothari, R., Singh, H. M., Ahmad, S., Sari, A., & Tyagi, V. V. (2022). Cultivation of two *Chlorella species* in Open sewage contaminated channel wastewater for biomass and biochemical profiles: Comparative lab-scale approach. Journal of Biotechnology, 344, 24-31.
3. Singh, H. M., Tyagi, V.V., Kothari, **R., Azam**, R., Slathia, P.S., & Singh, B. (2020). Bioprocessing of cultivated *Chlorella pyrenoidosa* on poultry excreta leachate to enhance algal biomolecule profile for resource recovery. Bioresource Technology, 316, 123850.
4. Majhi, P.K., **Azam, R**., Kothari, R., Arora, N.K., Tyagi, V.V. (2022). Impact of Flow Rate in Integration with Solar Radiation on Color and COD Removal in Dye Contaminated Textile Industry Wastewater: Optimization Study. Energy Engineering, 119, 419-427.
5. Kothari. R., **Azam, R.**, (2018). The potential for energy from waste. Energy Future in the July-September issue of the magazine, 6,4, 2278-7186.

**Book Chapters**

- ✓ **Azam, R.**, Pandey, A., Black, P. N., Tyagi, V. V., & Kothari, R. (2019). Bioprocesses for wastewater reuse: closed-loop system for energy options. In Water Conservation, Recycling and Reuse: Issues and Challenges. 121-145.
- ✓ Kour, G., Kothari, **R., Azam**, R., Majhi, P. K., Dhar, S., Pathania, D., & Tyagi, V. V. (2021). Conducting Polymer Based Nanoadsorbents for Removal of Heavy Metal Ions/Dyes from Wastewater. In Advances in Hybrid

Conducting Polymer Technology 135-157.

**Workshop and conferences attended**

- Oral presentation of paper in international conference on renewable energy for sustainable environment: Challenges and Remedies on 20$^{th}$-21$^{th}$ March 2017 on the topics of Population dynamics and aquatic avifauna of gharana wetland reserve Jammu: A case study.
- Oral presentation of paper in National conference on renewable energy and sustainable environment: Challenges and Remedies held on 24-25 March, 2018 on the topics of Impact of dried algal biomass on heavy metal in industrial wastewater: A state of art.
- Oral presentation of paper in National conference on climate change, societal consequences and Mitigation: Future vision held on 26-27 April, 2018 on the topics of Bio-products from algal biomass: a viable sustainable approach for bio-economy.
- Attended workshop on Global initiative of academic networks two week Gian course on Principal of Environmental catalysis held on 6-17 August, 2018 organized by department of environmental sciences, central university of Haryana.
- Attended national workshop on advances in clean energy conversion technologies and materials for energy storage applications held on 24$^{th}$-25$^{th}$January, 2019 organized by department of mechanical engineering, Shri Mata Vaishno Devi university Katra and Jawaharlal Nehru technological university Hyderabad.