Studies on the Thermal Energy Storage (TES) based Photobioreactor for Algal Biomass Production and its use for Different Biofuel Production

THESIS

SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL SCIENCE

Submitted by

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Enrolment No: 675/14

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2019

Every Challenging work needs self efforts

As well as guidance of elder's

Specially those who are very close to my heart

My Humble effort I dedicate to

My revered Father & Mother,

Whose affection, love, encouragement

And prays of day and night

Make me able to get success and honour

Declaration

This is to certify that material embodied in the present work entitled **"Studies on the Thermal Energy Storage (TES) based Photobioreactor for Algal Biomass Production and its use for Different Biofuel Production"** is based on candidate's original research work. It has not been submitted in part or full for any other diploma or degree of any university. The indebtedness of the candidate to others has been duly acknowledged at relevant places. I hereby also undertake that the thesis is essentially free from all kinds of plagiarism.

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CERTIFICATE

This is to certify that the thesis titled "**Studies on the Thermal Energy Storage (TES) based Photobioreactor for Algal Biomass Production and its use for Different Biofuel Production**" submitted by **Ms. Arya Pandey** 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.) regulation-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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ACKNOWLEDGEMENT

Firstly, I bow to The Almighty, without His Blessings, this work could never have been blossomed and completed.

I feel immensely happy to owe my most sincere thanks and gratitude to my supervisor Dr. Richa Kothari, Assistant Professor, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, and co-supervisor Dr. Vineet Veer Tyagi, Assistant Professor, School of Energy Management, Shri Mata Vaishnodevi University, Katra, J&K, for their persistent encouragement, prudent suggestions, and being an inspiration throughout the period of my lab work and compilation of this manuscript. It would not have been possible to present this work in its present form without their perpetual and devoted help and support. I feel extremely indebted to them for being meticulously involved throughout my study.

I am grateful to our Acting Vice Chancellor Prof. M.N.P. Verma, Ex Vice Chancellor Prof. R.C. Sobti and Registrar of Babasaheb Bhimrao Ambedkar University, Lucknow for their constant encouragement and support.

Indeed, the words at my command are not adequate to express my deep sense of gratitude to Prof. S.K. Dwivedi, Head of the Department, Department of Environmental Science for his appreciable interest and involvement, valuable advices and furnishing me with the research facility and excellent infrastructure which is responsible for the accomplishment of this target. I am immensely grateful to Prof. D.P. Singh and other faculties Prof. R.P. Singh, Prof. Naveen K. Arora, Mr. N.K.S. More, Dr. Shikha, Dr. Venkatesh Dutta, Dr. Narendra Kumar, and Dr. Jiwan Singh of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow. Their critical analysis and helpful suggestions have contributed significantly to complete this work.

I express my deep sense of gratitude and regards to Mr. Kapil Chopra, Assistant professor, School of Mechanical Engineering and Dr. Adarsh Pandey, for their cordial help, support, and innovative ideas to complete this work successfully.

Words are not enough to express my grateful indebtedness to my father Mr. Raj Kumar Pandey, mother Mrs. Lalli Pandey, and other family members Mr. Rashmi

Ranjan Pandey, Mr. Alok Ranjan Pandey, Manisha Pandey, Advik Pandey. Their dreams and ambitions made me strong to pursue further studies. Their endless efforts, sacrifices, support, patience, inspiration, affection and encouragement have made it possible.

I take this privilege with great pleasure to express profound and sincere sense of gratitude to my lab members Dr. Virendra Kumar, Dr. Vinayak Vandan Pathak, Mr. Shamshad Ahmad, Anita Verma, Pradeep Kumar Majhi, Riffat Azam, Harmohan Singh, and Atin Kumar Pathak for their cordial help, encouragement, and their innovative ideas throughout the course of my study.

I am thankful to Mr. Anchal K. Jain, Mr. Aviral, Mr. Ranjeet, Mr. Nagesh and Shri Ramsagar, who have been an integral part of Department of Environmental Science, B.B.A. University, Lucknow, for encouraging and taking interest in the progress of my research work.

I would reserve my heartiest thanks for my friends Ananya, Anita, Pawan, Anju, Urvashi, Arpana, Neha, Baqir, Nisha, Pradeep and my childhood friends Shiblu, Anjul for their support, cordial help, innovative ideas, encouragement, as well as entertainment throughout the journey of my study.

Though, all cannot be mentioned, but none is forgotten.

Date: 08|01|2019 **Arya Pandey**

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PREFACE

The global energy consumption increased rapidly with increase in world population, high living standard, and energy using patterns of the society. At present, the basic feedstock to produce commodity fuel is basically crude oil. The rapid depletion of this crude oil is now creating a pressure on transportation and aviation industry as well as environment in terms of pollution (CO_2, CH_4, and N_2O). Around the world noteworthy stepladder are being taken for a paradigm shift from today's petro-based refinery to biomass-based biofuel to sustain the green environment and global economy efficiently across the world. But, most of these emerging approaches are under technical risk due to large capital cost, less efficient, insignificant output. Researchers are focusing on advanced biomass cultivation technologies for biofuel production using different raw materials to produce a broad spectrum of main and co-products. In this context, the significant properties of algal biomass *i.e.* biochemical compounds (protein, carbohydrate, pigments, carotenoids, lipid, and fatty acids) and biofuels production (biodiesel, biohydrogen, biogas, and bioethanol) gained attention to focus on enhanced production of algal biomass. Algae as a production organism are being popular worldwide due to its multi-benefit approach and the functional bimolecular substances of algal biomass are of much concern due to its nutritional, medical, cosmetics, and pharmaceutical applications too. A relevant increment in algal based food and feed market has been seen due to the presence of high value chemical compounds *i.e.* fatty acids, colorants and vitamins, as a competitive with similar components produces from other sources. Therefore, large scale cultivation of algae is of great magnitude to increase the algal based green economy. In natural cultivation system as well as in artificial media, algae exposed to a number of environmental factors *i.e.* pH, light, temperature, nutrient, and carbon sources *etc.* out of which the accessible biogenic element concentrations are the most important parameters that affect algal growth. These variables (chemical and physical conditions) directly influence all the aspects of algal activity such as biomass productivity, specific growth rate, doubling time, metabolism intensity *etc.* The chemical and physical conditions of the algal culture system can be altered, which in turn have an effect on quantitative and qualitative growth of algal biomass. Additionally, there is a strong interrelationship between environmental factor and algal biomass growth rate. Therefore, process optimization of culture conditions efficiently enhances the yield of algal biomass production with desired metabolic products. The relative amounts of algal biomass production are directly linked to designing of photobioreactor, environmental, and nutritional condition including: the intensity of solar radiation, photoperiod, CO_2 concentration, pH, temperature, nutrients available (nitrate, phosphate, carbon), salinity, and hindrance of other microorganisms. In order to understand the synergistic interface between environmental/physical and chemical factors, optimization with multiple variables of these factors is required to develop high microalgal productivity. An effective PBR endow with all necessary conditions for algal biomass growth and development for efficient end products is one of the major objective for this study. Apart from the shape of PBR, temperature is another key parameter affecting the productivity, this is also the part of present investigation, studied in coupling with designed reactor using wastewater as a substrate for algal growth nutritive material. Both type of macro/micro nutrients in wastewaters are found suitable for algal growth and simultaneously treating the wastewater also including heavy metal removal, as reported by various researchers in their recent researches. Benefits of this type integrated system includes: reducing the cost of wastewater treatment and scale-up the biomass production on annual scale, less burden on freshwater resources and chemical media. Algal potential for synthesis of energy products can be enhanced by effective cultivation and harvesting system. Harvesting of algal biomass using advanced flocculants is also a significant part of this study. Effects of various parameters on harvesting efficiency of algal biomass (dose concentration, contact time, temperature, and pH) have been studied critically. Techno-economic assessment of designed bioreactor is compared with conventional reactor system using selected industrial wastewater from Common Effluent Treatment Plant (CETP) after primary treatment. Application of produced algal biomass for biofuel production also investigated in this research work. Therefore, keeping all these challenges in mind, designed objectives have been formulated in order to prove the concepts by experimental validations. Therefore, this research work has been divided into following chapters:

Chapter 1: Introduction and review of literature

In this chapter, an overview of various process parameters and their effect on algal biomass, biochemical compounds, and biofuel production have been described. Designing and fabrication of various photobioreactors, types of photobioreactors, construction materials, advantage and disadvantages, are also described. The wide application of algal biomass in various fields *i.e.* biofuel and value-added products (protein, carbohydrate, pigments, and lipid) production is also discussed critically.

Chapter 2: Materials and methods

The present chapter deals with experimental methodology and analytical techniques used to execute the experimental plane. This chapter explains about the microorganism, experimental plan with methodology used in experimental study. The analytical techniques used in present study are explained and basic information about microalgal culture and its growth optimization under different process parameters is also outlined. The fabrication of thermal energy storage based photobioreactor to enhanced algal production for biofuel/bio-oil production is also discussed in this chapter. Advanced harvesting techniques by using different flocculants to harvest produced algal biomass with analytical and kinetic tools are also a significant part of this chapter.

Chapter 3: Phycoremediation of industrial wastewater and their impact on algal biochemical compounds using *Chlorella pyrenoidosa* with correlation study

The present chapter deals with optimization of selected process parameters (pH, light photoperiod, nitrate, phosphate, carbon in salt form, and carbon in gaseous form) and their impact on algal growth/production and biochemical compounds (protein, carbohydrate, lipid) has been performed with BG-11 medium. Optimization of different concentrations (25%, 50%, 75%, and 100%) of wastewater collected from common effluent treatment plant has been done to investigate the optimum growth of algal biomass with efficient pollution reduction (organic, inorganic, and heavy metals) and algal biochemical compounds production. The relationship between different variables of process parameters and their effect on biochemical compound has also been studied by the application of statistical analysis *i.e.* Pearson correlation co-efficient analysis, which clearly describes the positive and negative correlation between parameters and biochemical compounds. The effect of CO_2 with optimised concentration wastewater has also been investigated to examine algal biomass productivity and its impact on algal biochemical compounds.

Chapter 4: Feasibility of thermal energy storage based photobioreactor for algal cultivation and biofuel production: a lab scale study

In order to investigate the efficiency of PBR for algal biomass production, two different types of reactors have been designed and fabricated *i.e.* horizontal tubular

PBR (TPBR) and thermal energy storage based vertical column PBR. Capric acid has been used as phase change material to maintain the temperature of PBR. Temperature plays an important role to enhance the algal biomass production, pollution reduction, and algal based bio-oil production. It is very important to find the most suitable way to enhance the temperature of the medium without algal cell structural deformities. The present chapter is focused to enhance the temperature of the medium in PBRs for biomass cultivation and bio-oil production.

Chapter 5: Comparative assessment of bioflocculant and chemical flocculants for algal biomass harvesting

The present chapter mainly emphasised to investigate the effect of various flocculants (bio and chemical flocculant) with respect to other parameters (dose concentration, contact time, temperature, and pH) to harvest algal biomass significantly. The obtained experimental data has been supported by Pseudo-second order rate kinetics, thermodynamic functions and zeta potential analysis.

Chapter 6: Techno-economic analysis of TES based PBR for algal biomass and biofuel production: a comparative study

Techno-economic assessment was done on the basis of selected wastewater (CETP wastewater) as nutrient substrate for algal biomass production and fabricated TES based PBR for enhanced algal production. A theoretical assessment has been made using CETP wastewater on the basis of per day and annual wastewater discharge capacity for algal biomass and biofuel production potential. Comparative assessment has been also made in between TES based PBR and electric-coil and gas-based PBR system to maintain the temperature for algal growth during low/unfavourable climatic conditions particular to ambient temperature. Significant results support the designed system in long-term sustainable economy in reference of fuel cost and environmental cost.

Chapter 7: Conclusions and future recommendations

This chapter clearly summarises the concluding remarks of major findings obtained from all chapters. Future recommendations also discussed to support the concept of bio-economy in an integration with wastewater treatment, large biomass cultivation and PCM based PBRs to save high grade electricity, may also be the part of future research designs for lab to land concept.

CONTENTS

Comparative assessment of bioflocculant and chemical flocculants for algal biomass harvesting

LIST OF FIGURE

Chapter-4

Feasibility of Thermal energy storage based photobioreactor for algal cultivation and biofuel production: a lab scale study

Chapter-5

Comparative assessment of bioflocculant and chemical flocculants on harvesting of algal biomass

LIST OF TABLE

ABBREVIATIONS

OPS	Open Pond System
PBR	Photobioreactor
CPBR	Closed Photobioreactor
FP-PBR	Flat panel photobioreactor
VC-PBR	Vertical column photobioreactor
CO_2	Carbon Dioxide
S/V	Surface Area to Volume
LDPE	Low Density Polyethylene
HDPE	High Density Polyethylene
PMMA	Rigid Acrylic
PVC	Poly Vinyl Chloride
TAG	Triacylglycerides
GHG	Green House Gas
VAP	Value-added Products
TDS	Total Dissolve Solid
TSS	Total Suspended Solid
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
PPM	Parts Per Million
TES	Thermal Energy Storage
CGF	Chlorella Growth Factor
NCIM	National Collection of Industrial Microorganism
UV	Ultra Violate

CETP	Common Effluent Treatment Plant
MLD	Million Litre per Day
V/V	Volume by Volume
L/D	Light/Dark
LED	Light Emitting Diode
OD	Optical Density
ARR	Average Removal Rate
TPBR	Tubular Photobioreactor
VC-PBR	Vertical Column Photobioreactor
PCM	Phase Change Material
LACC	Laboratory available $CaCO_3$
HE	Harvesting efficiency
FTIR	Fourier transform infrared spectroscopy
SEM	Scanning electron microscope
EDS	energy-dispersive X-ray
ATP	Adenosine triphosphate
NADPH	Nicotinamide adenine dinucleotide phosphate
LPG	Liquefied Petroleum Gas
GS-PBR	Gas based Photobioreactor
EC-PBR	Electric Coil based Photobioreactor
TES-PBR	Thermal Energy storage based Photobioreactor
ACM	Annual cost method
INR	Indian Rupee

Chapter 1

Introduction and Review of Literature

1.1. Introduction

The supply of clean and renewable source of energy has always been big challenges confronting human civilization. Long before, petrochemical based fossil fuels were deemed as foremost resource of energy, but due to its some negative prospects *i.e.* non renewability, imminent finishing, increase in green house gases (CO_2, SOx, NOx *etc.*) and other harmful impacts made the researchers to look forward for alternative source of energy (Richard et al., 2018; Ahmad, 2017; Williamson, 2016). Therefore, biomass based bioenergy has numerous unique capabilities with significant potential to eradicate the above mentioned harmful consequences. Hence, these capabilities drew the attention of research groups and scientific communities to move on biofuel based energy options rather than petrochemical based fuels. Although, various biofuel generating sources are present *i.e.* palm oil, sunflower, jatropha, corn, soybean, rapeseed, canola, barley, beets, bran *i.e.* (first generation sources of fuel), fuel containing cellulose, lignin, pectin *i.e.* (second generation sources of fuel), and microorganism *i.e.* microalgae, cyanobacteria, bacteria, and fungi *i.e.* (third generation sources of fuel) (Mohr and Raman, 2013, Chisti, 2012; Sims et al., 2010; Havlik et al., 2011). Among these, microalgae is potentially significant due to its fast growth rate, small life cycle, ability to cultivate by the use of nutrient rich wastewater (dairy, tannery, textile, domestic, and municipal wastewater), efficient bio-fixation rate of carbon, higher lipid content with production of end number of value added products, flexibility and potential ability to cultivate under modified conditions of cultivation (*i.e.* pH, nutrients, light, and temperature). These noteworthy points seem to be very desirable for mass cultivation of algal biomass. But, large scale production of algal biomass is controlled by various factors that are still act as an obstacle in the path of algae to fuel production *i.e.* nutritional factors (nitrate, phosphate, and carbon *i.e.*), environmental factors (water, light, and temperature) and physical factors (pH, salinity, and electrical conductivity). In simulated as well as natural cultivation system, algae biomass experienced numerous environmental factors *i.e.* pH, light, nutrient, and carbon sources *etc.* Apart from these factors, the available concentrations of biogenic elements are the most essential parameters that affect algal growth and development (Daliry et al., 2016; Velu et al., 2015; Al-Qasmi et al., 2012). All these variables (chemical and physical) have significant potential as it can directly influence the algal biomass activity in terms of maximum biomass productivity, specific growth rate, generation time, enzymatic activity, metabolism intensity *etc.* (Babu et al., 2015; Blair et al., 2014; Juneja et al., 2013). Therefore, it is possible to alter chemical and physical conditions (variation in pH ranges, intensity/photoperiod of light to which algae exposed, nutrient concentration, stressed condition) of the algal cultivation system, which in turn have direct effect on quantitative and qualitative growth of algal biomass (Velu et al., 2015). The overall process from algal cultivation to product generation basically divided into two main divisions *i.e.* upstream process and downstream process. In order to enhance the algal production, various process parameters can be optimised to achieve maximum algal biomass production, simultaneously, effect of process parameters can also be investigated to achieve desired algal biochemical compound. Whereas, downstream process of algal biomass involves harvesting, flocculation, dewatering, drying, cell disruption, extraction (lipid and bio-diesel production) and hydrolysis (carbohydrate and bioethanol production). So, it is clear that to get a desired product in downstream process, process parameters must be optimised in upstream process. Additionally, there is a strong interrelationship between environmental factor and algal biomass growth rate. Table 1.1 describes different optimized parameters for algal biomass production, as cited by various researchers in the available literature. According to the literature available, various findings in reference of parameter with improvement/enhancement for algal biomass are given below:

1.1.1. pH

pH is a noteworthy parameter of algal biomass growth and development as it investigates the solubility of necessary available nutrients (nitrate, phosphate, potassium) with CO_2,with remarkable impact on algal metabolism. Usually algae exhibit a clear and direct relationship with pH of medium in which has to be grown (Daliry et al., 2017). Algal physiology states that at high pH, trace metal and nutrient absorption altered. Similarly, as the pH becomes low, enzymatic inhibition takes place in photosynthetic medium which increases the contamination chances (Rai et al., 2015). The optimum pH for growth and development of *Spirulina platensis* was found to be best between 7.0-8.0 and maximum growth rate associated with 8.0 (Fagiri et al., 2013). pH of algal culture media is directly subjected to various key factors *i.e.* composition and buffering capacity of the medium, amount of dissolved CO_2, temperature of media and metabolic activity of the algal cells *etc.*(Gong et al., 2014). The optimum range of pH for maximum growth of microalgal biomass varies from strain to strain. Most of the algal strains are comfortably growing on neutral pH or slight base medium (Rai et al., 2015). Hence, the optimization of pH for particular algal strain is necessary in order to obtain optimum growth of algae.

1.1.2. Light

Light in terms of photoperiod as well as intensity is a critical factor and can bring significant change in biochemical composition of algae. Light intensities ranges from 100-200 $\mu E/m^2/s$ are usually applicable for algal biomass production while as the intensity increases from 200-400 $\mu Em^{-2}s^{-1}$microalgal growth rate increases. Various algal sp. (*Mychonastes*, *C. vulgaris*, *Scenedesmus* sp. *etc.*) shows higher lipid production rate as the light intensity increases. The growth and development of algae is affected by the three main conditions of light *i.e.* light limitations, light saturation, light inhibition. A photoperiod based study on the growth of *Dunaliella salina* CCAP 19/30 showed that longer photoperiods led to enhanced the cell density of microalgae (Xu et al., 2016) The level of light intensity is very significant in terms of algal biomass cultivation and its batter growth and development because at certain level algal cell would experience higher solar light saturation but this solar light saturation can be dilute by maximize surface area to volume ratio (Zhu et al., 2015; Mandotra et al., 2016). Solar radiation/intensity play significant role in algal photosynthetic process to obtain its metabolic energy for growth and development. A lot of photobioreactors (artificial condition) are also fabricated at specific angel (45°C) to cultivate algal biomass with inside or outside illumination to harvest maximum solar radiation subsequently, enhance the algal biomass (Sun et al.,2014) Light intensity directly affects the growing capacity and photosynthetic ability of algal biomass. Therefore, light is significant parameter with specific interest to enhance the algal biomass.

1.1.3. Temperature

Temperature is the most significant parameter for optimum growth and development of algal biomass. It strongly affects the cellular chemical composition, nutrients uptake, fatty acid profile, bio-fixation of carbon dioxide, growth rate, specific growth rate, and doubling time of algal biomass (Serra-Maia et al., 2016; Singh and Singh, 2015). Algal growth, development, and metabolic pathway alters with variation in temperature, therefore, advance knowledge and practical approach for biochemical response to temperature promotes the useful insights to generate the proficient systems for biofuel production. Usually high temperature (25-35°C) supports the maximum algal growth. Investigations have reported that both (high and low) temperature can boost algal lipid productivity. As the temperature increases more than 35°C, 15-20°C, 20-30°C, and 14-30°C, net lipid productivity decreases in *Monoraphidium* sp. *Nannochloropsis, oculata, Scenedesmus* sp. and *Nannochloropsis* sp. respectively. It has been reported that microalgae (freshwater) of genus *Chlorella requires* optimum 25°C temperature with growth rate of 1.099 D^{-1} and cell concentration of 5.814 after 6 days of experiment (Singh and Singh, 2015). The optimum temperature range for *C. minutissima* lies between 10°C and 35°C with irradiances ranges from 30 $mmolm^{-2}$ s^{-1} to 550 $mmolm^{-2}s^{-1}$. The observation of experiment revealed that *C. minutissima* required minimum irradiance to carry on net growth and specific growth rate of microalgae increased from 0.12 d^{-1} at 10°C, to 0.66 d^{-1} at 30°C whereas, specific growth rate of *C. minutissima* decreased as the temperature increased up-to 35°C (Aleya et al., 2011). Therefore, temperature is a significant parameter that directly affects the physiology of algal biomass due to change in chemical reaction. Various algae exhibit an amplified exponential growth rate/specific growth rate at optimal temperature but as the temperature increased to its optimum value, algal structural integrity occurs (Dvoretsky et al., 2015). Therefore, temperature is a very noteworthy factor in algae biomass cultivation in bioreactors. Thermal stability in a temperature controlled cultivation system (open pond/closed photobioreactor) for large scale cultivation of algal biomass is a considerable challenge and designing of a temperature controlled bioreactor system may have a high operational cost. An increased in optimum temperature, potentially enhance algal growth and further increase in temperature leads to a swift decline in growth rate.

Algal cultures heated by intense solar radiation are a big problem especially in humid climates where evaporation is inhibited.

1.1.4. Water

To complete the life cycle of algal biomass aquatic system provides habitat to which algal biomass has to be cultivated. Aquatic system delivers nutrients (nitrates, phosphate, water dissolved carbon, and other micro nutrients and trace metals), for proper growth and development of algal biomass. Water bodies help to remove waste products generated by the produced algal biomass and maintain thermal regulation of the system. In order to produce one kg of algal biomass approximately 5-10 L of water is consumed (Jagathese and Farid, 2014). On the basis of desired final product and by-products, water consumption varies in upstream and downstream steps involved in processing routs. So, it is clear that from algal cultivation to biofuel production, water is utilized in each step of process routs. Water serves as a medium (nutrient source) for algal biomass growth and development. It has been reviewed that various industrial wastewater (dairy wastewater, textile wastewater, wastewater taken from coal-fired power generation, municipal wastewater, industrial wastewater) can be utilised as substrate for algal biomass growth (Kothari et al., 2015; Santiago et al., 2013; Saunders et al., 2012; Menger-Krug et al., 2012; Kothari et al., 2012) Kothari et al., (2012) reported 18.8 gL^{-}1 biomass productivity (fresh weight) of *Chlorella pyrenoidosa* with dairy industry wastewater used a as substrate (nutrient source). Therefore wastewater as nutrient source play significant role to enhance algal production without compromising the availability of fresh water.

1.1.5. Nutrients

Nutrient ratio (C: N: P= 106:16:1) often utilized as an indicator for optimal algae biomass cultivation. Recently, literature has cited that C: P ratios ranging from 34:1 to

418:1 and N: P ratios from 3.5:1 to 38:1 are required for cultivation of different microalgal species (Boelee et al., 2012). In case of diatoms, carbon, nitrogen, phosphorous and silicon are key elements for optimum growth and development (Field et al., 2014). At large-scale production, it is possible to set up instrumentation to measure selected nutrients and controlling by automated compensation of them. Beside macro-nutrients (N, P, K) algae requires micronutrients too for its growth and development. These micronutrients magnesium (Mg), iron (Fe), cobalt (Co), zinc (Zn), copper (Cu), and nickel (Ni) are six most significant metals for algal growth usually found in algal cells (Zatkova et al., 2011; Wang et al., 2010). Hence, micro and macro nutrient are important to conduct various enzymatic reactions as well as to execute the metabolic activity of algal biomass.

1.1.5.1. Nitrogen

Nitrogen is a significant nutrient responsible for algal structural and functional protein formation in algal cells and contributes up to 7-20% of dry algal cell weight. Algal biomass takes nitrogen (inorganic in the form of nitrate) and it is being assimilated by biochemically active compounds which in turn recycled within the algal cells and to fulfil physiological demand. Growth of algal biomass is directly interrelated to the rate of uptake of limiting nutrients. Nitrogen sources for algal biomass are considered to be base for protein and nucleic acid formation. Paes et al., (2016) reported a sharp increase in lipid synthesis if the nitrogen supply decreases for *C. vulgaris* and *Nannochloropsis oculata*, while no change on growth pattern was observed. Recently, various research works has been focused and performed to increase the amount of lipid content of algal biomass and most of them have been conducted by stress in nutrient concentrations of culture. Numerous research work have been focused on nitrogen stress due to its crucial role in regulating algal cell growth and metabolism for lipid production under nutrient stress conditions (Daliry et al., 2016; Millan-Oropeza et al., 2015; Kong et al., 2010). A wide range of nitrogen sources (potassium nitrate, urea, ammonium sulfate, ammonium nitrate, peptone, extracted meat) have been applied for *C. vulgaris* simultaneously to study the effect of pH on growth and development of algae under mixotrophic condition. The obtained results found to be best with potassium nitrate with maximum specific growth rate (0.87 day^{-1}), biomass production (3.43 gL^{-1}), biomass productivity (0.57 $gL^{-1}day^{-1}$), and lipid production (47.1 $gL^{-1}day^{-1}$) (Kong et al., 2010). The research finding supports the nitrogen limiting conditions for maximum lipid production, but on the opposing, it minimises the total algal biomass production. Hence, it is clear from the above discussions that nitrate play an important role to enhance biomass and biochemical productivity of algae.

1.1.5.2. Phosphorous

Phosphorous is a crucial component to sustain the growth and development of alga and contribute to less than 1% of total algal biomass *i.e.* 0.03 to 0.06 %. It is important to note that phosphorous is the primary limiting nutrient after nitrogen for algae. 1% of total dry weight of algal biomass is constituted by phosphorous. Effect of phosphorous limitation can be observed in the form of reduction in substrate synthesis and regeneration in Calvin-Benson cycle. Repression of photosynthesis in algal biomass usually takes place in the absence of phosphorous that directly affect the growth of algal biomass. Although, phosphorous has potential impact on algal growth and metabolism but it was observed that phosphorous stressed conditions resulted in increased of algal lipid content (Baiee and Salman, 2016). Algal biomass growth is basically supported by the transfer of soluble inorganic phosphate (SIP) which consists of single phosphate residue, across the algal cell membrane. The phosphate uptake of algal biomass is usually influenced by the environmental/physical factor (light intensity, photoperiod, temperature, pH salinity *etc.*) and state of algal cell (stressed conditions, starvation, and growth) (Li et al., 2010). Phosphate uptake is influenced by the growth of algal cell which is called as metabolic uptake. On the dissimilar, when phosphate stressed condition of algal cells exposed to phosphate rich medium, algal cells are capable to accumulating excess phosphate for growth and development of cells (Babu and Binnal, 2015; Blai et al., 2013). Therefore, phosphate is an important source of nutrient to perform algal physiological as well as metabolic activity.

1.1.5.3. Carbon

Carbon is major nutrient essential for photosynthesis, algal growth and reproduction. Fixed carbon by algal biomass can be used in respiration, energy source, and raw material in formation of additional cells. Reduction in carbon fixation rate entails reduction in algal growth rate. Carbon is also a most vital source of nutrient required by microalgae for structural component formation as it is an elementary element of all biochemical compounds *i.e.* protein, carbohydrate, pigment, carotenoid, and fatty acids *etc.* (Lin and Wu, 2015). Carbon source in the form of CO_2 is a key input for the growth and development of algae as it is a indispensable parameter in photosynthesis that convert it into carbohydrate (Choi and Wu, 2015). Therefore, nutrients are (micro and macro) an essential factor and its optimised concentration is required for the maximum growth of algal biomass. The overall composition of algal biomass lipid directly affected by the concentration and sources of carbon. The accumulation of fatty acids (saturated and unsaturated) can be indueced by the low as well as high concentration of carbon. Carbon sources *i.e.* glucose, acetate, and glycerol are the significant sources that lead the production of lipids and other biochemcal compounds. A diverse range of microalgal species prefer glucose as a corbon source as it can be eaisly assimilated and leads to produce biochemical compounds. A sharp increase in growth rate and energy (2.8 kjmol^{-1}) production has be obtained by using glucose as a source of energy in comparison to acetate. Various research report suggests that 30 and 20 gL^{-1} of glucose as a carbon source increase the net lipid productivity tremendously in *A. protothecoides* and *C. vulgarisv* (Velu et al., 2015). An experimental study reported that the *Chlorella gracilis* increase in cell density as the CO_2 concentration increases (from 280, 385, 550, 750, and 1050 ppm) up to 385ppm but as the concentration increases from 550 ppm a decrease in cell density was found (Khairy et al., 2014). The effects of various parameters on algal growth are clearly described in Table 1.2.

Hence, it can be concluded that all the process parameters plays an important role in algal growth from lab-scale to commercial scale. But, their growth rate totally depends on the type of culture, type of media, types of bioreactor, and geographical locations where it is present *etc.* Similarly, use of algal biomass for different also very much influenced by different environmental factors including geographical location. Harvesting process for algal biomass is also very much influenced by end-use of algal biomass. These all are taken into consideration here in this research study and critically analysed and evaluated for better findings in further section of the chapter.

1.2. Climatic conditions of the world

A biome is defined as a community of flora and fauna having common characteristics feature for the environment they exist in. In comparison to habitat, biome is broader term usually found over a range of continents and established in response to the mutual physical climate.

Table 1.1: Optimum value of parameters to enhance biomass production

Process parameters	Significance	Optimum range	Ref.
pH	It important parameter because several enzymatic activities take place at particular pH only.	7-7.5	(Chinnasamy et al., 2010)
Light	Light is the primary requirement for algal growth and which facilitate photosynthesis process to obtain metabolic	400-700 nm	(Blair et al., 2014)
Temperature	It plays an important role by affecting the biochemistry and physiology due to change in rate of chemical reaction. Most of the algae demonstrate an increased exponential growth rate up to optimal temperature but after cross of this optimal point there is a turn down in structural integrity	25-30˚C	(Chen, 2016)
Water	It provides an aquatic environment and habitat for survival of algal life cycle. It work as medium to deliver nutrients as well as thermal regulator.	Species-specific	(Jagathese and Farid, 2014)
Nitrogen	7-10% of algal biomass is comprised of Nitrogen, making it an essential nutrient. Higher concentrations increase biomass growth.	>1% for 1 gm of dry algal biomass	(Chen and Chen, 2015)
Phosphorus	Phosphorus is a second essential nutrient for algae, and its higher concentrations increase biomass	>10 % for 1 gm of dry algal biomass	(Chen et al., 2012)
CO_2	CO_2 along with bicarbonate(HCO_3^-) forms the primary carbon sources for algae	1.63-1.84%	(Cheirsiip and Torpee, 2012)

Table 1.2: Effect of various parameters on algal biomass productivity

Algal biomass	**Cultivation strategy**	**Findings**	**Ref.**
Physical stressed conditions			
Chlorella pyrenoidosa	pH 7.5	Optimum growth of algal biomass obtained	(Kothari et al., 2017)
Chlamydomonas acidophila	pH 4.4	Denaturation of V-lysin	(Visviki and Palladino, 2001)
Coccochlorispeniocystis	pH decreased from 7.0 to 5.0 and 6.0	Decrease in total accumulated carbon and oxygen evolution	(Coleman and Colman, 1981)
Chlorella vulgaris	High light intensity 2664–9324	27.0 g/L of lipid was produced under provided condition	(Gonçalves et al., 2013)
Scenedesmusalmeriensis	30◦C	7.4 g/L of lipid productivity was obtained with 30°C	(Xin et al., 2011)
Selenastrumminutum	35°C and 420 mmol m^{-2} s^{-1}	Optimum growth rate achieved 1.73 per day	(Singh and Singh, 2015)
C. vulgaris UTEX 259	27°C temperature and 200750 mmolm^{-2} s^{-1} light intensity	1.8 kg dry weight (algal biomass) m^{-3}	(Yun and Park, 2001)
Nutrient stress conditions			
Chlorella sorokiniana	Nitrogen stress condition	Nitrogen stressed condition promotes enzymatic activity with enhanced biomass productivity	(Babu et al., 2017)
Nannochloropsis oceanica	Nitrogen deprived	Act as positive regular to ptomote algal growth	(Lu et al., 2014)

	condition		
Chlorella vulgaris	Oxidative stress	Auxins can suppress lipid peroxidation and hydro- gen peroxide accumulation in response to oxidative stress	(Piotrowska-Niczyporuk and Bajguz, 2014)
Chlamydomonas	Nitrogen starvation	Nitrogen induced triacylglycerol biosynthesis	(Gargouri et al., 2015)
Chlorella ellipsoidea	Nitrogen starvation	Enhanced algal biomass growth with lipid sysnthesis	(Zhang et al., 2014)
Chlamydomonas reinhardtii	Phosphorus starvation	PSR1 gene is an important determinant of lipid and starch accumulation in response to phosphorus starvation but not nitrogen starvation	(Bajhaiya et al., 2015)
Chlamydomonas reinhardtii	CO2-limiting stress	CO_2 –limiting stress induces carbon-concentrating mechanism	(Yoshioka et al., 2014)
Dunaliella bardawil	Salt stress	Carotenogenic genes can be recognized transcription factors	(Liang and Jiang, 2014)

World’s biomes usually controlled by climatic conditions and climate of a particular region determines which type of flora will grow there, and what type of fauna will inhabit it. These three components (climate, flora and fauna) are interwoven to create the fabric of a biome. World’s biome/geographical locations play a significant role for farming of flora. Biomass farming is completely governed by the environmental factor of a particular geographical location to which biomass has to be grown. In general, plants (primitive or developed) are considered as key indicator of a particular biome with direct interrelationship with environment.

The three major climatic conditions are: (i) tropical climate; (ii) temperate climate; and (iii) tundra as illustrated in Fig.1.1. Tropical climatic conditions are most favourable for algal biomass cultivation, as we move from tropical to temperate and tundra, productivity of algal biomass decreases due to the increase of environmental harsh conditions particular to temperature. The given world map gives an idea about the world’s climatic conditions. The most significant factor regarding algal biomass production in climate, associated with temperature, solar radiation, and seasonal changes. Tropical climate of the world consists of semi-arid, rainforest, savannas areas having an average temperature more than 18°C. The area situated near equatorial belt characterised by the presence of tropical climate, which is having a hot and humid weather conditions with excess rainfall. Such type of climate is highly efficient in order to achieve luxuriant vegetation due to the occurrence of excessive rainfall along with plenty of sunshine. Area such as Amazon basin in Brazil, the Congo basin in West Africa, Indonesia, some parts of India and Australia are tropical division of the world. Therefore, tropical and temperate climate is favourable for the growth and development of algal biomass but some time high solar radiation intensity provide a harsh condition for algal growth as a consequence algal cells become dead

(Hannon et al., 2010).Algal biomass harness solar radiation (wavelength ranges from 400-700nm) called as photosynthetic active radiation (PAR) in tropical and sub-tropical regions. In other word increase in solar irradiance (intensity), algal biomass experience high rate of photosynthesis. Photosynthesis in algal biomass also occurs underwater as long as enough light is available. Even in ocean, PAR can penetrate up to 200 metres below from the surface. Photosynthesis process of algae is basically a series of chemical reaction takes place by the acting of several enzymes. Enzymes are a type of catalysts to boost up the chemical reactions in biological process. Therefore, high solar radiation speeds up chemical reactions to initiating the process. Geographical locations of world with low sun light intensity does not support algal biomass production as the metabolic and physiological activity of algae becomes suppressed due to cold shock and rate of photosynthesis also decreased. When solar radiation decreased algal enzymatic activity becomes dormant, denatured, and some time lose their original shape and size. These denatured algal enzymes no longer boost up chemical reactions; subsequently slow down/discontinue photosynthesis. Thus, perfect solar radiation is required by algal biomass to maintain the photosynthesis in terms of activating and maintaining the bio-chemical process.

Keeping this in mind, designing and fabrication of suitable and favourable bioreactor is important with association of all optimised process parameters to enhance the algal biomass production. A wide range of cultivations system has been adopted for algal biomass production (Huang et al., 2018; Kumar et al., 2015). Bioreactor, types of bioreactor, material used for bioreactors, and their pros and cons are discussed in detail in further sections of this chapter.

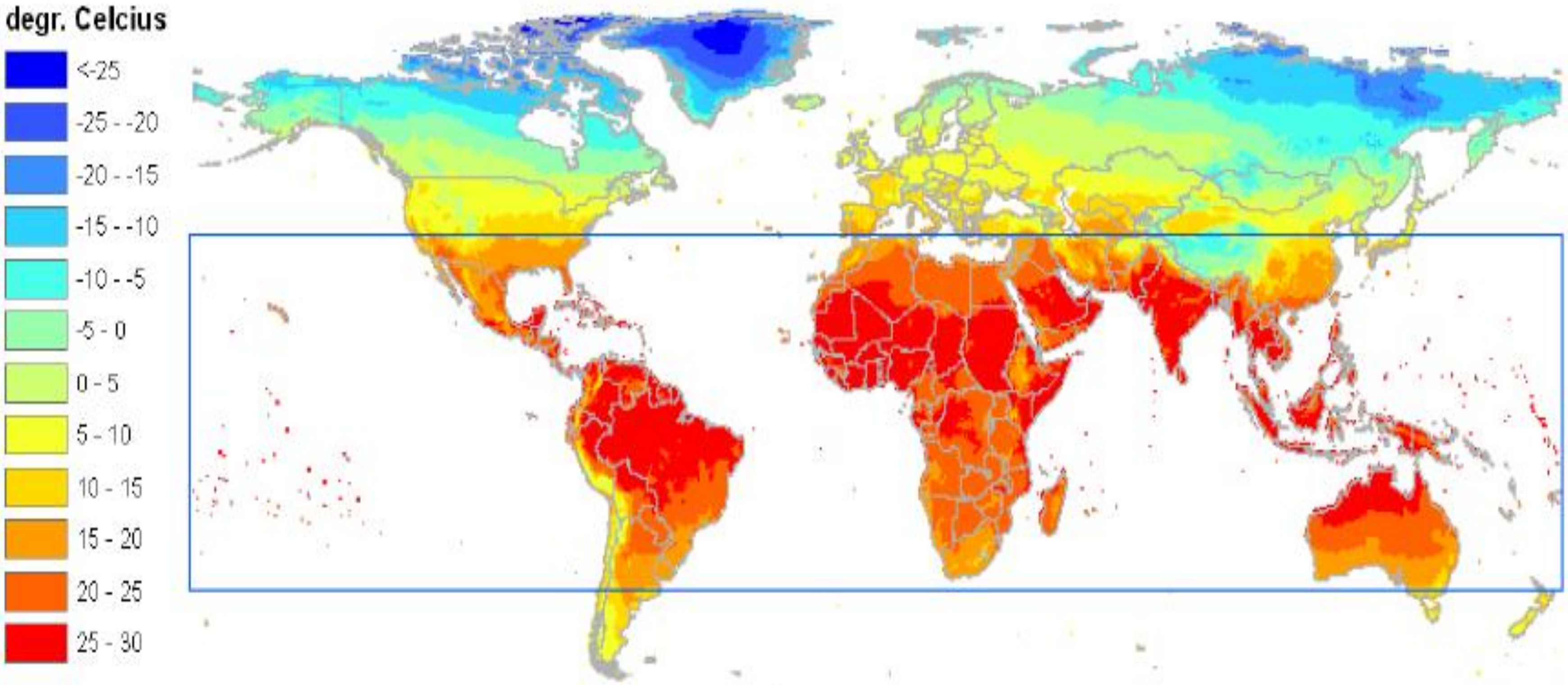


Fig. 1.1: Favourable climatic conditions for algal

1.3. Bioreactor

Bioreactor is defined as an open, closed or semi-closed vessel/reactor made by transparent waterproof materials, and able to provide an ideal/favourable/control growing condition for photosynthetic microorganisms (Wang et al., 2012). Bioreactors are basically used to carry out biochemical processes associated with microbes (microalgae, fungus, plant cells) to produce biological products *i.e.* biofuels (bio-oil, biodiesel, biohydrogen, biogas, and bioethanol), value-added chemical compounds (protein, pigment, carbohydrate, lipid, fatty acids, *etc.*) and other uses (fertilizer, food and fodder) (Kothari et al., 2017). Microalgal biomass requires a suitable photobioreactor with optimised conditions for its growth and development.

Therefore, the working parameters play an important role in algal biomass cultivation as it directly associated with algal growth and developments, whether it is grown in open pond system/outdoor cultivation or closed bioreactors/indoor or outdoor conditions. From the past few decades, different types of bioreactors have been designed and fabricated for algal biomass cultivation (Kumar et al., 2015) and few of them have been applied for commercial scale too. An ideal bioreactor should have following parts-

1.3.1. Agitator

Agitator has property of mixing the contents (medium in which microalgae has to be grow) of the bioreactor which ultimately keeps the microalgal biomass in the suitable homogenous conditions for an optimum transport of nutrients (nitrate, phosphate) and oxygen in order to support an adequate metabolism of microalgal cells.

1.3.2. Sparger

The purpose of the sparger is to supply the oxygen to microalgal cells which has to be grown in bioreactor with aerobic cultivation system. The sparger provides adequate oxygen to the growing microalgal cells via air bubbling and also helps in uniform distribution of reactor contents to make a proper homogeneity in the culture medium.

1.3.3. Jacket

In order to maintain the constant temperature of bioreactor in which algal biomass has to be cultivated, the applied jacket provides the annular area to maintain the passage of constant temperature water buffer. A separate chilled water circulator maintained the desired temperature of the circulating water to maintain low/high temperature in a reservoir of bioreactor. The contact area of jacket favours an adequate transfer of heat while, the constant circulation of desired temperature water maintain the particular temperature to the bioreactor.

1.4. Types of bioreactor

Microalgal cultivation pattern basically associated with two major types of production systems *i.e.* open-pond technology (especially for outdoor microalgal cultivation that harness maximum advantages of natural sunlight) (Kumar et al.,2015) and closed photobioreactor technology which can be either located outdoor to receive natural sunlight or indoor, basically associated with artificially controlled environment (Gao et al., 2014). Scientifically the term “photobioreactor” is applied for algal biomass growth and development particularly with respect to photosynthetic mode of cultivation. There are various types of reactors/bioreactors are used for algal biomass cultivation but designing and fabrication of suitable bioreactors in context with efficient algal biomass production is a tedious task (Ahmad et al., 2017). Algae are of particular interest, due to its multifaceted applications at commercial scale, which increased biofuel production and other value added products by cultivation in these bioreactors under controlled conditions (Kothari et al., 2017). CO_2 removal at global level, pollutant load removal from wastewater, nutritional feed, animal feed, aquaculture, pigments and cosmetics (Lara-Gill et al., 2013; Kumar et al., 2010). Hence, for algal growth system bioreactor, play a crucial role in order to enhance the biomass density.

1.4.1. Open pond system

In open culture system, raceway pond is used for cultivation of alga at mercantile level. It engrosses low capital investment and operational cost with effectual mixing (via paddle wheel) of nutrients and gaseous exchange (Yoo et al., 2013). In raceway pond only mixing is the energy intensive process. There are several shortcomings of the raceway pond such as low productivity, high light intensity, contamination of algal suspension by rapid growing microorganism (Brennan and Owende, 2010).In order to get better light intensity (causes cell mortality) and operating condition some modified raceway ponds were also developed with artificial light but this modification failed as it was not economical feasible for industrial production (Ahmad et al., 2017). Various type of open pond systems (OPS) are being used previously (Fig. 1.2) *e.g.* open raceway pond, shallow pond, and circular pond for algal biomass cultivation. Size of OPS is restricted to 10,000 m^2 as the mixing of nutrients in open pond system by rotating arm is not possible in larger area occupied ponds (Kumar et al., 2015). OPS are usually designed by the use of closed loop and re-circulation channel which is functioned by paddled-wheel in order to maintain the homogenous distribution of nutrients into the system. Depth of OPS ranges from 0.2 to 0.5. meter. OPS is basically based on low power consumption, consequently it is economically feasible and easy to retain, handled, and clean (Yoo et al., 2013). Hence, it is the cheapest method to produce large amount of microalgal biomass at commercial scale to enhance green economy.

1.4.1.1. Unstirred open stytem

In general, most of the natural water system is devoid of stirred unit as a consequence, it leads to a poor mixing, and provides low cost for commercial scale culture. Natural lakes, lagoons, and open ponds are the common open photobioreactor. Such types of culture system provide an economic feasible, easy to operate, and convenient way for monitoring the microalgal culture process (Haan et al., 2017; Vonshak and Richmond, 2012). The natural open pond system is usually less than a half meter in depth to ensure the maximum light pen*et*ration within the system to absorb by the microalgal cells. In order to amplify the receiving of sunlight by microalgal cells and for b*et*ter temperature control, som*et*imes surface water is covered by the plastic films. Basically, *Dunaliella salina* is cultured in this type of unstirred open system).

1.4.1.2. Circular pond system

It is mainly used to culture the *Chlorella* sp. in Asia. Such types of rounded ponds are associated with long rotating arm which resembles with circular reactor associated with wastewater treatment plant. Such type of plants is 20-30 cm in depth and 40-50 cm in diameters. The centre of the circular pond is incorporated with long rotating arm which act as a clock dial and performs as a paddlewheel function (Prussi et al., 2014). The incorporation of paddlewheel enhance the uniform distribution and mixing rate of culture media with algal cells which is lacking in unstirred open pond system, but as the algal cells exposed to the surroundings, the contamination is inescapable which is unhealthy for algal culture. The biomass productivity of algal cell in circular pond system ranges between 8.5 gm^2d^{-1} to 21 gm^2d^{-1} (Haan et al., 2017).

1.4.1.3. Raceway pond system

It is the most popular and widely used system for various algal sp. like *Chlorella, Spirulina, Dunaliella,* and *Haematoccocus etc.* (Kumar et al., 2015). Raceway pond system is either associated with pure culture medium or wastewater culture. It is also possible to integrate raceway pond system with CO_2 capture technology in some power plants. The depth of the raceway pond system is ranges from 15-50 cm. Raceway pond system are associated with paddlewheel, baffle, and channels. Paddlewheel maintains the flow of culture medium which intern maintains the suspension of algal cells and avoids sedimentation and baffles rule the direction of flow of culture medium. Therefore, algal cells are sufficiently blends with medium and keep a continuous flow to harness maximum sunlight and CO_2 from atmosphere. Algal biomass productivity can be achieved up to 60-100 $mgL^{-1}d^{-1}$ in raceway pond (Tradici, 2014). Raceway pond system is the most favourable and economically viable system to sustain the large-scale commercial production algal biomass.

1.4.2. Closed photobioreactor

Close photobioreactor sustain up to five fold higher productivity of algal biomass in respect to volume of reactor over OPS (Duan and Shi, 2014). The other rewards in the close system are water conservation, less energy and chemical requirement and less or no chance of contamination of algal suspension by the pathogenic microorganisms (Usher et al., 2014). Light intensity and culture conditions can be manually adjusted in order to amplify the biomass production, thus close culture system is favourable for biomass production for biodiesel production as suggested by Singh & Sharma (2012). Vertical flat plate annular, plastic bags and air lifted glass or plastic tubular reactor have been in focus in closed photobioreactors (PBR) as illustrated in Fig 1.3. Solar collector is usually attached to the closed photo-bioreactor to ensure the enough light intensity for photosynthesis process in order to achieve high algal biomass.

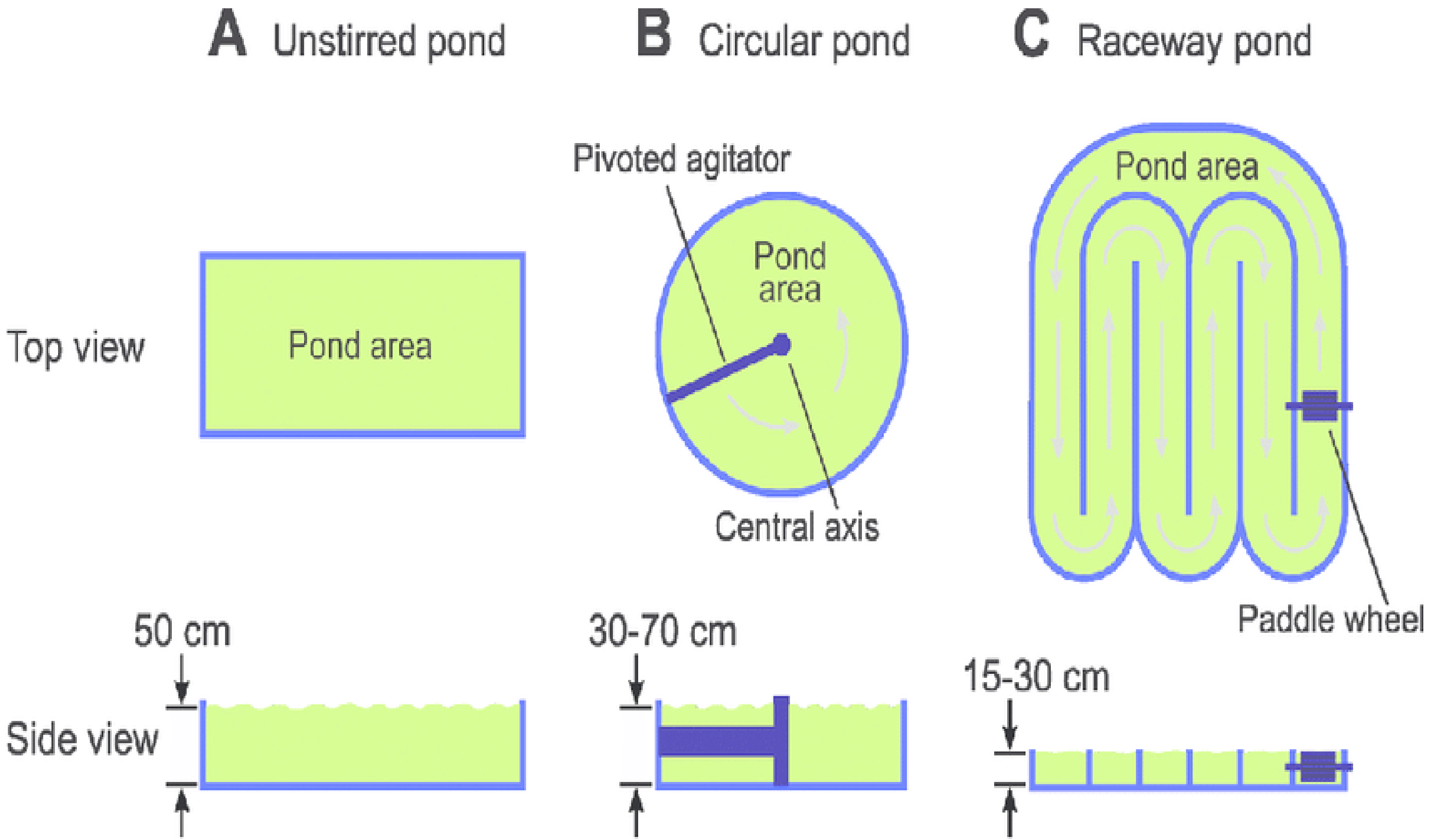


Fig. 1.2: Open pond system (a) unstirred pond; (b) circular pond; (c) raceway pond

Utilization of adequate light sources (in terms of intensity and wavelength), increased efficiency of photon conversion, and maintenance of algae culture condition with uniform distribution of nutrients, are three fundamental principles that directly governed designing and construction of CPBR (Dasgupta et al., 2010). The term close in PBR refers to free from contamination/infection within the algal culture condition. CPBR is extremely proficient for enhanced biomass productivity as it is designed and fabricated to gain optimum solar radiation with suitable tilt angle (Zhang et al., 2013).Table 1.3clearly describes the pros and cons of various open and closed photobioreactors. Different forms of CPBR are clearly described below-

1.4.2.1. Flat panel photobioreactor

Flat panel is common PBR with rectangular box which is basically used for pure algal cultivation or algae with wastewater cultivation. It can be placed either indoors subjected to artificial light sources or placed outdoor exposed to natural light. It is fabricated by transparent or semitransparent materials glass, polycarbonate, and plastic bags *etc.* (Huang et al., 2015). The light path of flat panel is very short which promotes the easy penetration of artificial/natural lights. Mixing or uniform distribution of nutrient medium is maintained by the aeration or air bubbles which are generated by the air sparger. A motor based pump is assembled with flat panel to supplement air bubbles via sparger. In flat plate PBR (FP-PBR) thickness or width of plates are very important parameter because it determines the surface area/volume ratio (Dasgupta et al., 2010). As the width of glass plate decreases it allows better solar light infiltration and allocation of light in FP-PBR as a consequence it supports enhanced optimal cell density and increased biomass productivity. Tilt angle is also very important factor during fabrication of FP-PBR (Issarapayup et al., 2009). The glass angle vary from in its position from summer to winter depending on light intensity as in summer when light intensity is very high then the angle of FP-PBR positioned at 10˚C and 30˚C but in cold climate or in winter it is located at 60˚C to obtain higher algal biomass production.

1.4.2.2. Vertical column photobioreactor

In general vertical column PBR (VC-PBR) is cylindrical in structure having radius close to 0.2 m and length of cylindrical tube is generally up to 4m (Wang et al., 2012). It possesses small radius to increase surface area and volume ratio (Soman and Shastri, 2016). The main downside of VC-PBR is its increased column length which increases the residence time of oxygen, generated during photosynthesis that inhibits the culture growth (Huang et al., 2016).

1.4.2.3. Tubular photobioreactor

Tubular PBR is usually made up of glass tube which should have diameter of about 0.1m or less in order to achieve best possible solar radiation to enhance photosynthetic activity simultaneously higher algal biomass production but it should also taken under consideration that surplus solar light penetration through thin glass tubes may lead to photoinhibition (Vree et al., 2015). It has been reported that in tubular PBR when diameter of tubes increased from 3.8cm to 12.5cm than the volumetric productivity of biomass decreased but it has been noted that there is an increase in areal productivity (Iluz and Abu-Ghosh, 2016). Hence, photoinhibition may be preventing by increasing diameter of the tubes of tubular PBR. Depending upon the orientation, tubular PBR is of various types such as horizontal, vertical, incline and helical *etc.* It has seen that horizontal PBR have better surface area to volume ratio then vertical tubular PBR (Dasgupta et al., 2010; Asumathi et al., 2012; Zhu et al., 2013), therefore, it is significant in terms of achieving solar radiation and algal biomass production. Horizontal PBR have horizontal transparent tube. These bioreactors have perfect angle at 45° which makes it more feasible to harness maximum solar radiation for algal growth and development. Horizontal PBR is free from contamination and feasible for mass cultivation of algal biomass (Zhu et al., 2013).

1.4.3. Construction materials for bioreactor

The construction materials used in fabrication of bioreactor represents an important practical issue with respect to investment/capital cost and performance of microalgal biomass. There are various materials being used in fabrication of bioreactors *i.e.* glass, acrylic or poly vinyl chloride, plexiglass, and polyethylene *etc.* (Ahmed et al., 2017).Therefore, it is critical to select the best suited materials that fulfil the criteria of an efficient bioreactor design and fabrication. These material used in fabrication of bioreactors could have some inhibiting effect on algal biomass. Therefore, it requires protective coating which have shielding effects to avoid inhibiting effects. Various construction materials used in photobioreactors with their durability, advantages and disadvantages are described in Table 1.4.

1.5. Harvesting of algal biomass

Dewatering of algal biomass from the medium is significant step in downstream process of algal biomass as 20 to 30% expenditure evolves to complete the process. Various physical, chemical and biological methods are now being applied to complete the harvesting process of algal biomass (Kothari et al., 2017). Various harvesting process involves in dewatering of algal biomass has numerous drawbacks *i.e.* economically unfeasible, flocculant toxicity, and inefficient *etc.* Various researchers have investigated that dilute culture and minute size of microalgae must be handled with large volume therefore, algal biomass harvesting is a major challenge in order to shift the algal applications from lab scale to commercial scale.

Fig. 1.3: Closed photobioreactor (a) flat panel photobioreactor; (b) vertical column photobioreactor; (c) tubular photobioreactor

Table 1.3: Pros and cons of open and closed system for algal cultivation

Working parameter	Open system (open pond)	Closed photobioreactors (PBRs)	Ref.
Contamination	Very high	Low	(Alexander, 2014))
Space required	Very high	Low	(Milledge, 2011)
Water losses	Dependent on climate condition	Practically none	(Shen et al., 2009)
Carbon dioxide(CO_2) losses	High	Low	(Shen et al., 2009)
Number of cultivable species	Low or few	High grow all type of cultivable species	(Singh and Sharma, 2012)
Production Flexibility	Cleaning Easily the pond and restarting with new alga	Substantial amount of biofouling in the closed PBRs of a photo-stage can make PBR cleaning more costly	(Alexander, 2014)
Degree of process controlling	Very Low	High with different stages of cultivation	(Davis, 2011)
Biomass concentration	Low, 0.1-0.2 $gL^{-1}{}_{approx}$	High, 2-8 $gL^{-1}{}_{approx}$	
Initial investment cost	Low	High	
Downstream processing cost	High (very dilute culture)	Low (higher density culture)	(Grobbelaar, 2009)
Surface Area-to-volume (S/V) ratio	Large (4-10 times higher than closed counterpart)	Small	(Pires et al., 2012)
Efficiency of harvesting	Low	High	(Harun et al., 2010)
Light utilization efficiency	Poor	Excellent	(Harun et al., 2010)
Main limitation for biomass productivity	Surface area	Volume	(Singh and Sharma, 2012)

Conventional methods of algal biomass harvesting *i.e.* centrifugation, filtration, membrane filtration, ultra filtration, ultrasonic separation, foam fractionation, pH adjustment, flotation, and chemical flocculation are energy intensive process (Chen et al., 2011; Zhang et al., 2011; Uduman et al., 2010). At present, various types of organic and inorganic coagulating and flocculating agents are being used to harvest algal biomass. Coagulation is the process involves the use of chemical coagulants to form agglomerates suspended algal biomass in medium. Whereas, flocculation process involve the aggregation of unstablised charge particle present in the medium to make flocs. The produced flocs of algal biomass are easy to recover by solid-liquid separation techniques. Various chemical based flocculants (ferric alum, calcium carbonate, magnesium) are being used to harvest algal biomass efficiently but these chemicals some time deformed and denatured the structural and algal cell surface morphology of algal biomass (Gerde et al., 2014). Whereas, bioflocculant (egg shell, seed extract of *Moringa oleifera*, seed extract of *Strychnos potatorum, Phaseolus vulgaris*, sugar and red maize, modified soil of chitosan) in process for efficient algal biomass harvesting without structural and morphological deformities (Pandey et al., 2018). An advanced method of harvesting algal biomass was proposed by Salama et al., (2015) in which the author has suggested the application of acid mine drainage to coagulate the algal biomass and achieved around 89% and 93% of flocculation efficiency for *S. obliquus* and *C. vulgaris* respectively. Therefore, acid mine drainage was found to the efficient method to harvest algal biomass in significant amount. An experimental investigation was performed by Zhang et al., 2016, and 99.5% *Chlorella zofingiensis* was recovered by the medium using coagulation process with Mg.

Table 1.4: Common construction materials for photobioreactor

Construction Material	**Energy content of materials used in PBRs (Mg/Kg)**	**Durability (year)**	**Advantages**	**Disadvantages**
Glass	25-28	18-20	Tubular PBRs, have notably higher NERs (Net Energy Ratio) than rigid polymers such as polymethyl methylacrylate (acrylic) as it requires less energy content.	Glass materials are usually subtle in nature and require proper handling various connection fittings. It is difficult to transport and assemble.
Low Density Polyethylene (LDPE)	78-80	3-5	Thickness of LDPE PBRs varies from material to material as it depends on algal species.	Various environmental factors directly affect the life span of LDPE such as temperature, solar radiation and atmospheric pressure.
High Density Polyethylene (HDPE)	131-135	20-22	Opacity of HDPE help to prevent damage or photoinhibition and biofouling associated from photo oxidation.	It is quite hard to weld and hence show less tensile strength.
Rigid acrylic (PMMA)	-	-	It supports enhanced biomass productivity with optimum control over the algal biomass culture.	Economically infeasible, cost is too high.
Poly vinyl chloride (PVC)	-	-	It is beneficial to use in photobioreactors as it enhance the algal density in PBR, resistance to corrosion, non-conductivity and light weight in nature.	Quite difficult for light penetration in algal medium

Harvesting process of algal biomass is a method used for removing the water from the algal growth culture and increasing the solid content from >1.0% to up to 20% of solid *i.e.* it a process of gaining solid algal biomass avoiding water (Zhang et al., 2011). Harvesting of algal biomass is energy intensive process and contributes to one third of the cost of biomass production. Most of the harvesting process has many drawbacks such as high cost, flocculent toxicity and non-feasibility (Harun et al., 2010). Various researchers (Letelier-Gordo et al., 2014; Wan et al., 2015; Udhaya et al., 2014) observed that dilute culture and small size of microalgae must be handled with large volume and it is energy intensive process. Therefore, harvesting algal biomass is a major challenge for the commercial scale biofuel production. Various harvesting techniques are clearly described in Table 1.5. Algal biomass harvesting is also being done by the application of chemicals such as multivalent metal salts commonly include aluminium sulfate/alum ($Al_2(SO_4)_3$, ferric chloride ($FeCl_3$), and ferric sulfate ($Fe_2(SO_4)_3$) *etc.* The potential of electrolytes to provoke coagulation is measured by the critical value of coagulation concentration, or the actual concentration required inducing the rapid coagulation.

1.6. Application of algal biomass

Microalgae are recognized as one of the oldest life form on the earth and distributed worldwide having various varieties, which are living in a wide range of environmental conditions. They can nurture well in saline, brackish, marine and fresh water and wastewater that make them promising feedstock than terrestrial crops as a third generation biofuel (Farooq et al., 2015). Microalgae possess necessary genetic, metabolic and enzymatic characteristics to produce biofuel like biodiesel, biogas and bio-hydrogen *etc.* Microalgae can produce lipid, carbohydrate, protein in large amount with very short period of time and their products can be processed and converted in to biofuels and valuable co-products (Kothari et al., 2017) (as demonstrated in Fig. 1.4 and 1.5). Microalgae can fix carbon dioxide from three different sources *i.e.* atmosphere, discharge gases and soluble carbonate (Kumar et al., 2010). Hence, algae have a multi-dimensional potential to use it for various applications. These multiple applications are discussed in detail in upcoming sections:

1.6.1. Wastewater treatment: new remedial approach

Application of microalgae for wastewater treatment is a new idea, and several researchers have developed techniques for exploiting the algae's fast growth and nutrient removal capacity (Batista et al., 2015). The nutrient removal by algae is basically as a result of effective absorption of nutrients by algae for its growth and development; beside this some nutrient stripping phenomena also occur *e.g.* ammonia volatilisation and phosphorus precipitation as a result of the high pH induced by the algae. Some investigation has revealed that a huge part, occasionally up to 90 %, of the phosphorus removal is done due to this effect (Sturm et al., 2011). Alga is also well explored for metal removal efficiency for instance *Chlorella pyrenoidosa* is well known for uptake of Cd metal (Ajayan et al., 2014). Thus algae provide complete treatment of wastewater and provide a clean environment. Table 1.6 described potential of various algae to remove pollutant load from different wastewater.

1.6.2. Biofuel production: green fuel approach

One of the most significant applications of algal biomass is to use it as biomass based biofuel instead of petro-chemical fuel. Algal biomass has significant potential to replace the fossil fuels. Algal biomass based biofuel is renewable and clean fuel for the use without harming environment. Harvested algal biomass can be utilized for synthesis of various biofuel products such as biodiesel, bioethanol, biohydrogen and biogas.

Table 1.5: Harvesting techniques and its specifications

Harvesting techniques	Specifications
Gravity sedimentation	Sedimentation rate of algae influenced by its settling velocity Flocculants are added to form larger algal flocs It is not very effective for routine cultivation of microalgae It is not applicable in algae farm to generate energy feedstock
Centrifugation	It resembles with sedimentation process but centrifugation acceleration replaced gravitational Particle size and density difference are the key factors in separation process This is a reliable method of recovery Some author reported 90 to 100% harvesting efficiency It is efficient method of harvesting but not sustainable due to its high operational cost
Filtration	Most competitive method in comparison to other harvesting technique Mostly used for large sized algae such as filamentous algae or agglomerates and not suitable for smaller size algae Membrane and ultra filtration are used for small size microalgae
Flotation	It is a separation process based on the attachment of air bubble to the solid surface Resulting flocs float to the liquid surface and easily harvested The process efficiency depends upon the nature of suspended particle
Flocculation	Separation of microalgal cells from broth by addition one or more chemical Microalgal cell carry a negative charge which is countered by addition of polyvalent ions called flocculant Inorganic agents (Fe^{+3} and Al^{+3}) and polymeric flocculants are two main group of flocculants.

Each product follow different pathways such as to produce algal biodiesel, transesterification of algal oil is required using acid or base catalyst (Brennan and Owende, 2010). The following subsections discuss about conversion of algal biomass into various energy products. Table 1.7 clearly explains different biofuel production from various algal biomasses.

1.6.2.1 Biodiesel

Microalgae have significant potential to produce 100 times more oil/ acre than other plant even better than soya beans (Quinnn and Davis, 2014). Various microbial species are able to accumulate substantially high content of lipid content then other plant seed producing lipid. These are food source for many animals due to presence of polyunsaturated fatty acids, vitamins, minerals and oil and come in the bottom of food chain being main producer of oxygen on the earth. Algae have the potential to synthesize Triacylglycerides (TAGs) are considered as a resourceful feedstock for biodiesel production (Amaro et al.,2011).Microalgae are predicted to have lower cost per yield, and have the potential to reduce green house gas (GHG) emission through the substitute of fossil fuels (Brennan and Owende, 2010; Amaro et al., 2011). Hena et al., (2015) reported 21.82±2.06, 13.64±0.84, 21.34±1.26 (%) biodiesel production from *Chlorella saccharophila, Scenedesmus* sp., *Consortium* by using dairy industry wastewater. Similarly, Zhou et al., (2012), reported 28.9 % biodiesel production from *Auxenochlorella protothecoides* by using concentrated municipal wastewater.

1.6.2.2. Biohydrogen

Deoiled-algal biomass (oil extracted or residual algal biomass) having wide application in biohydrogen production. Oil extracted algal biomass can be used as a feedstock for anaerobic digestion to produce biohydrogen.

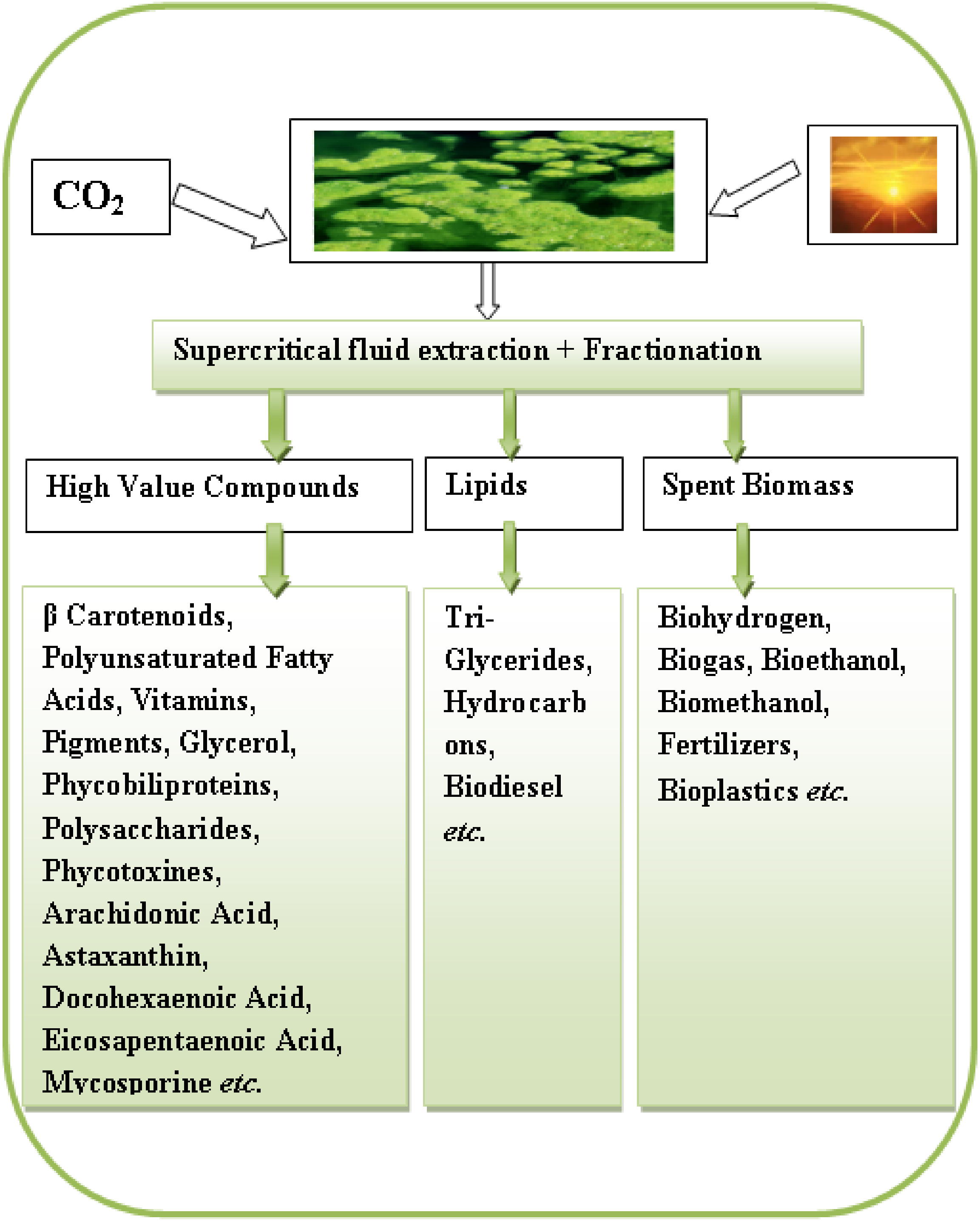


Fig. 1.4: Algal based value-added products

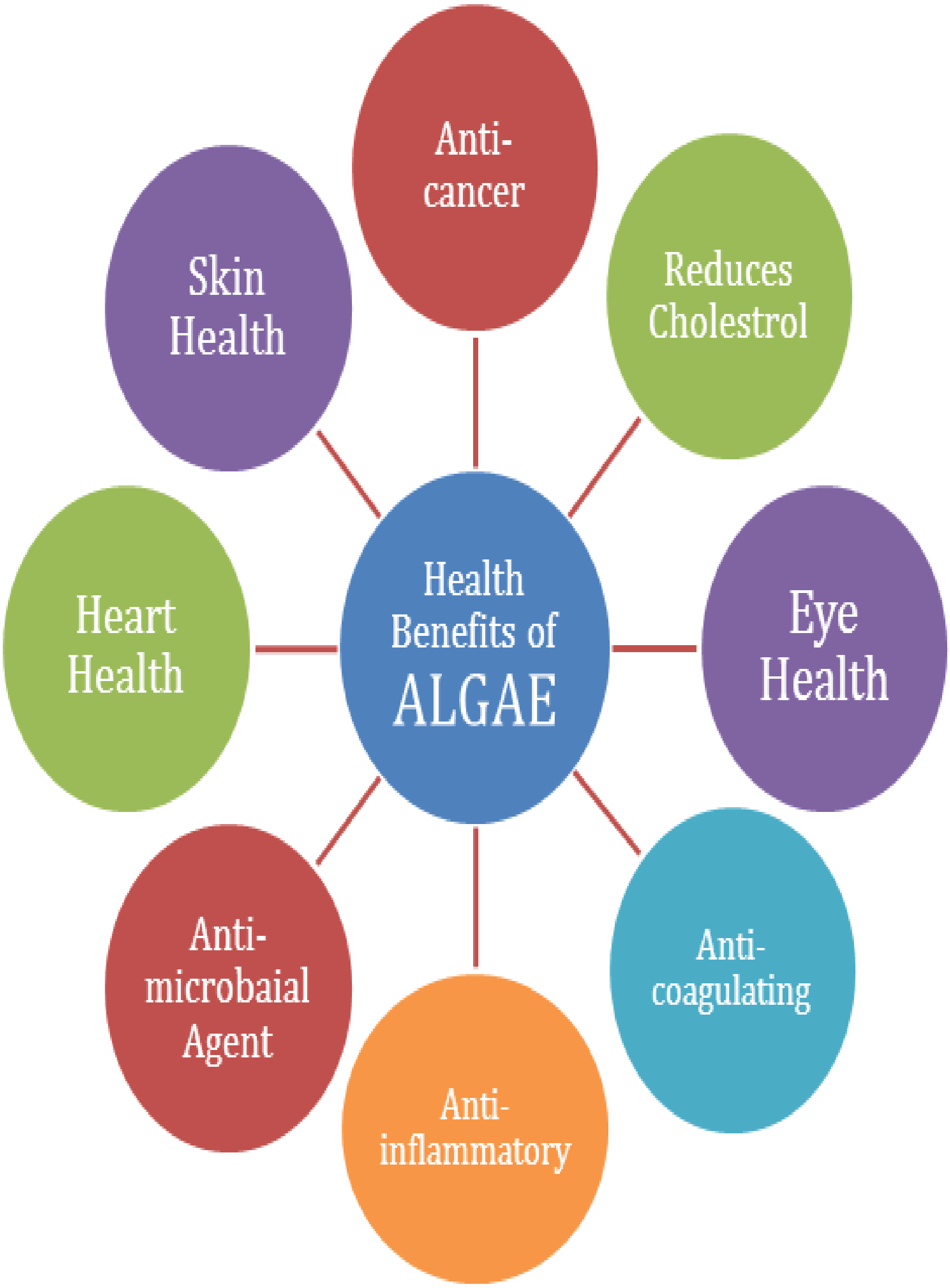


Fig. 1.5: Multiple health benefits of algal biomass

Table 1.6: Phycoremediation for different wastewater

Algal species	Wastewater	Phosphate (ppm)	Nitrate (ppm)	COD (ppm)	BOD (ppm)	Ref.
Chlorella vulgaris	Rubber latex concentrate processing	---	51.1	503	---	(Bich et al., 1999)
Chlorella, Ankistrodesmus and Scenedesmus sp.	Olive-oil mill wastewater	---	0.62–2.1	67–178	46–94	(Roig et al., 2006)
Chlamydomonas, Chlorella, Scenedesmus sp.	Wastewater containing 85–90% carp*et* industry effluents	17.59–21.95	3.97–5.53	106–183	2–21	(Chinnasamy et al., 2010)
Chlamydomonas reinhardtii	Industry effluent (undiluted)	6.92	64	---	---	(Kong et al., 2010)
Chlorella sp. *227*	Effluent after secondary Treatment	1.7	18.9	11.2	6.9	(Cho et al., 2011)
Nannochloropsis sp.	Municipal wastewater	5.3	110.2	---	---	(Jiang et al., 2011)
Chlorella sp.	Centrate (Municipal Wastewater)	183	---	1060	110	(Min et al., 2011)

It has been investigated that composition of deoiled algal biomass consists of lignin free cellulose along with accumulated starch makes it an ideal feedstock for dark fermentative H_2 production (Bruno et al., 2009). In dark fermentation process, mixed anaerobic consortia act as biocatalyst which makes it favourable for biohydrogen (H_2) production. Organic compound, wastewater, different organic waste, and insoluble cellulosic material are widely used as favourable feedstock for biohydrogen production (Reeta et al., 2013; Mohanakrishna et al., 2013; Kothari et al., 2016). Ho et al., (2013), reported 25.1 mL of H_2 production from per gm of dry biomass power by using enriched functional consortia with microalgae. Hence, biohydrogen production by anaerobic digestion process is significant and economically feasible as algae is cheap and easily available and can use waste material as a substrate.

1.6.2.3. Bioethanol

Fermentation of starch rich algal biomass is used for bioethanol production. The starch and starch type materials such as polysaccharides are formed in algal biomass mainly as a food reserve material among the genera of chlorophyta, rhodophyta, cryptophyta *etc.* (John et al., 2011). Therefore, biotechnological process for bioethanol production follows the starch production and its conversion through anaerobic pathway using an anaerobic microorganism (Branyikova et al., 2011). These types of conversion pathways involve various intermediates steps which increases the complexity of the process (Ho et al., 2013). Hence, it fermentation process of starch rich algae a profitable process to produce bioethanol that can be used as a green fuel having no environmental harm.

1.6.2.4. Biogas

The foremost feedstock involve in anaerobic digestion process include biomass from crop residue, forest residue, lignocelluloses and dedicated energy crops, however recent advancement involve alga (fresh water, salt water, and cyanobacteria) as a biomass feedstock for biogas production process. Experimental research has been shown that *Chlamydomonas reinhardtii* was efficient with a production of 587 ml (±8.8 slandered error) biogas, whereas fermentation of *Scenedesmus obliquus* was inefficient with only 287 ml (±10.1 slandered error) biogas production and it has been observed that, methane content of biogas was 7 to 13 % higher than methane content present in biogas produced from maize (Mussgnug et al., 2010).The methane content of biogas from microalgae was 7–13% higher compared to biogas from maize. The residual algal biomass can be used for methane generation (Deeswasmongkol and Paoprasert, 2016).

1.6.3. Carbon sequestration agent

Use of fossil fuel as commercial fuel represents an imbalance of carbon flux because combustion of fossil fuel generate CO_2 which is not further going to be fixed by the fossil fuel reserve but when we talk about the biofuel production by using algal biomass, it generates CO_2 which is further used by the biomass feedstock consequently, it represents a balance carbon flux and can be used in clean development mechanism and CO_2 sequestration to alleviate the problem of climate change and global warming and this effort is also important to cope with energy crisis (Posten, 2009; Richmond, 2004; Chen et al.,2012). There are various researches going on related to bioremediation of CO_2 at lab scale but there is need to evaluate and find out the way to introduce this technology at pilot scale. Table 1.8 describes the potential application of CO_2 for algal growth.

Table 1.7: Biofuels derived from different algal species

Algae	**Different types of biofuel produced from algal biomass**	**Ref.**
Biodiesel (%)		
Chlorella protothecoides	29.4 ˙ 1.5	(Damirbas, 2008)
Botryococcusbraunii	17.85	(Órpez et al., 2009)
		(Sydney et al., 2011)
Chlorella protothecoides	55.2	(Xu et al., 2006)
Biohydrogen (mmolL^{-1}hr^{-1})		
Gloeocapsa alpicola	1.6	(Troshina et al., 2002)
Spirulina platensis	0.18	(Aoyama et al.,1987)
Chlamydomonas reinhardtii	0.13	(Gfeller and Gibbs, 1984)
Chlamydomonas reinhardtii cc124	0.094	(Kosourov et al., 2002)
Platymonas subcordiformis	0.002	(Guan et al., 2004)
Chlamydomonas reinhardtii cc1036	0.48	(Laurinavichene et al., 2006)
Bio-ethanol (%)		
Palmaria	38-74	(Ross et al., 2008)
Porphyra	40-76	(Jensen, 1993)
Ascophyllum	42-70	(Becker, 1994)
Ulva lactuca	55-60	(Inan, 2014)

Tetraselmis sp.CS-362	26.0			(Brown et al.,1998)
Chlorococum sp.	32.5			(Ike et al.,1997)
Chlamydomonas reinhardtii UTEX 90	60.0			(Hirano et al.,1997)
Bio-oil (%DW)				
Botryococcusbraunii		29-75		http://www.oilgae.com/algae/oil/yield/yield.html
Hantzschia DI-160		66		
Scenedesmus TR-84		45		
Neochlorisole oabundans		35-54		
Schizochytrium		50-77		
Phaeodactylum tricornutum		20–30		(Chisti, 2007)
Schizochytrium sp.		50–77		
Nitzschia sp.		45–47		
Chlorella sp.		28–32		
Botryococcusbraunii		25–75		
Biogas ($m^{-3}m^{-3}d$)				
Chlamydomonas reinhardtii			587 ± 9	(Mussgnug et al., 2010)
Chlorella kessleri			335 ± 8	(Mussgnug et al., 2010)
Spirogyra neglecta			0.23 m^3/m^3d	(Baltrėnas and Misevičius 2015)

Table 1.8: CO_2 of sequestration on algae species

Algae strains	Experimental studies	Ref.
Dunaliella	Ratio of CO_2 absorption and desorption rate constant (k_1/k_2) was reported highest. In comparison with ambient CO_2, an addition of 1% volume of CO2 shows best result with respect to algal growth.	(Eloka-Eboka and Inambao, 2017)
*Phomidium valderianum*BDU *2*0041	Reported 56.4 mg $L^{-1}D^{-1}$ of CO_2 bio-fixation in an open tank with the rate of 30 mg $L^{-1}D^{-1}$ of algal growth	(Dineshbabu, 2017)
Scenedesmus dimorphous	Performed an on-off feeding of pure flue gas to algal biomass and obtained growth rate 889 mg $L^{-1}D^{-1}$ with 75.6 g $L^{-1}D^{-1}$ of CO_2 fixation rate in a bubble column photobioreactor, using flue gas with 15% of $CO_{2.}$	(Yadav and Sen, 2017)
Chlorella vulgaris	The percentage efficiency of carbon fixation by algal biomass was reported 80% in an airlift photobioreactor with 0.245 g L^{-1} D^{-1} of algal growth rate	(Sadeghizadeh et al., 2017)
Chlorella sp.	Reported that algal biomass is efficient to fix 96.89 mg $L^{-1}D^{-1}$ of carbon dioxide from the flue gas having 5-15% of CO_2 in an incubator with maximum growth rate 0.64 g L^{-1}	(Kassim and Meng, 2017)
Chlorella vulgaris	Obtained that in a pilot-scale photobioreactor algal biomass is able to fix 0.8 kg CO_2 D^{-1} from the flue gas with 5-30% of CO_2 with maximum growth rate of 0.40 g L^{-1} D^{-1}	(Pavlik et al., 2017)
Chlorella vulgaris	Reported the optimum range of CO_2 sequestration lie between 10-15% of flue gas. Hence the industries emits 10-15% of flue gas can be best utilized for algal growth.	(Songolzadeh et al., 2014)
*Scenedesmus (*KC7337)	Performed a lab-scale study in closed photobioreactor, using flue gas with 13.8% of CO_2 with the efficiency of 252 g L^{-1} of CO_2 bio fixation rate and 4.97 g L^{-1} of algal growth	(Basu, 2014)
Scenedesmus sp.	Reported the bio fixation rate of CO_2 (368 mg $L^{-1}D^{-1}$) by using coal flue gas having 2.5% of CO_2 with maximum algal growth 196 mg $L^{-1}D^{-1}$ in an Airlift photobioreactor with domestic wastewater as nutrient medium.	(Nayak et al., 2016)
Chlorella sp.	Reported 85.6 % of algal based bio fixation efficiency of CO_2	(Aslam and Mughal, 2016)

Algal based bioenergy generation with capturing emitted CO_2 is an integrated approach, which can replace fossil fuel energy, simultaneously reduces atmospheric $CO_{2.}$ Nine groups of algae has been classified *i.e.* (*Cyanophyceae*), (*Chlorophyceae*), (*Bacillariophyceae*), (*Xantophyceae*), (*Chrysophyceae*), (*Rhodophyceae*), (*Phaeophyceae*), (*Dinophyceae*), and (*Prasinophyceae*, *Eustigmatophyceae*) which has potential to detain atmospheric carbon in noteworthy amount. Therefore, it is clear that large scale algal cultivation is highly significant as it has multiple benefits in various fields such as wastewater treatment, carbon sequestration, and biofuel production *etc.*

1.7. Conclusion

Algal biomass production depends on various working parameters such as parameters optimization (pH, light photoperiod, nutrients *etc.*), photobioreactor design (PBR shape, Glass width, Glass transparency) and culture conditions *etc.* An effective PBR endow with all necessary conditions for algal biomass growth and development for efficient end products. Apart from the shape of PBR, temperature is another key parameter affecting the productivity; however favourable temperature depends on the type of alga strain. Alga has multidimensional uses such as biofuel production, wastewater treatment, carbon sequestration, bio-fertilizer, nutrient supplements, animal feed and fodder, cosmetic industry and medicine sector. Algal potential for synthesis of energy products can be enhanced by effective cultivation and harvesting system. Techno-economic feasibility of selected approach (wastewater as nutrient source, biofuel production and cost analysis of fabricated TES based PBR) is significant to scale-up the biomass productivity.

Therefore, keeping all these challenges in mind, following objectives have been formulated in order to prove the concepts by experimental validations:

1. To design and fabrication of suitable TES based PBR for algal cultivation and biomass production.
2. To investigate the effect of various working parameters like pH, temperature, light, carbon source *etc.* which influence algal growth.
3. To study the parametric characterization of selected wastewater for enhancement of algal biomass/growth.
4. To investigate the possibilities to mitigate CO_2 emission and simultaneously providing wastewater nutrient removal using fabricated TES-PBR system.
5. To investigate the multifaceted application of produced/harvested algal biomass.
6. Techno-economic feasibility of proposed TES-PBRs for algal biomass growth.

After extensive review of literature, cited in above sections, each parameters associated with growth of algal biomass including physical and chemical parameters, type of bioreactors with optimised ranges were discussed with different algal strains but specific study with *Chlorella pyrenoidosa* and thermal energy storage based photobioreactor using real type wastewater is not a part of literature till date. Hence, to reduce fresh water consumption, real wastewater taken as nutrient media for algal growth and thermal energy storage based photobioreactor coupled with this to enhance the algal biomass with less-energy consumption. Furthermore, this research work is trying to provide detailed informations regarding all the challenges like material and methodology adopted to carry out experimental plans, divided in three phases as discussed in Chapter-2. Effect of various process parameters (pH, light, temperature, nutrients) on algal growth with media and with wastewater is discussed in Chapter-3, whereas, Chapter-4 explained about fabrication and designing of temperature controlled photobioreactors to obtain dense algal biomass with biofuel/bio-oil production. The application of advanced harvesting technologies to harvest algal biomass is discussed in Chapter-5. Techno-economic feasibility of wastewater used and photobioreactor used to conduct the experimental study is discussed in Chapter-6. Chapter-7 described concluding remark for research problem selected in this research work with future recommendation for research in concern area.

Chapter 2

Materials and Methods

2.1. Introduction

This chapter explains about the microorganism, experimental plan with methodology used in experimental study. The analytical techniques used in present study are explained and basic information about microalgal culture and its growth optimization under different parameters such as pH, light photoperiod, nutrients (nitrate, phosphate, and carbon), and wastewater concentration is also outlined. Experimental procedure adopted for wastewater treatment and parametric observations with heavy metal removal are also discussed. The fabrication of thermal energy storage based photobioreactor to enhanced algal production for biofuel/bio-oil production is also discussed in this chapter. Advanced harvesting techniques by using different flocculants (bioflocculant and chemical flocculant) to harvest produced algal biomass with analytical and kinetic tools are also a significant part of this chapter, which all are experimentally investigated and discussed in chapter-3, 4, and 5 respectively.

2.2. Reagents, chemicals and glasswares

In order to avoid the chances of contaminations, all 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.

2.3. Characteristic features of algal species

Chlorella pyrenoidosa is a single-celled freshwater alga (as illustrated in Fig. 2.1) with higher chlorophyll concentration other then foodstuffs such as green vegetable and fruit used in this research study. Chlorophyll plays an important role to fix solar radiation in other energy forms (sugar) which can be used by human beings. Furthermore, *Chlorella* contains highly concentrated active substances such as the CGF (*Chlorella* Growth Factor), chlorelline and *Chlorella* supported by the body's own substance "sporopollein", present in the central cell wall, and able to bind most of the toxic heavy metals, radioactive substances, drug residues formaldehydes and wood preservers (Byoung-Ki et al., 2016). Classification of *Chlorella pyrenoidosa* has been given in Table 2.1.

2.3.1. Nutrient media and culture conditions

The sample of *Chlorella pyrenoidosa* was acquired from National Collection of Industrial Microorganism (NCIM 2738), Pune, India and retained in BG-11's growth medium as summarizes in Table 2.2. 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 placed 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 665nm 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 at 665 nm by using UV visible spectrophotometer (HALO-DB 20, Thermo Scientific, Schimadzu model). All experimental flasks were inoculated with an initial optical density of 0.05 algal cell densities taken from mother culture of *Chlorella pyrenoidosa*.

2.3.2. Biochemical composition of *Chlorella pyrenoidosa*

Biochemical compounds of algal biomass play a crucial role to produce multiple end products. The sustainable cultivation and processing of algal biomass make a broad spectrum of products generated by pure and raw algal biomass.

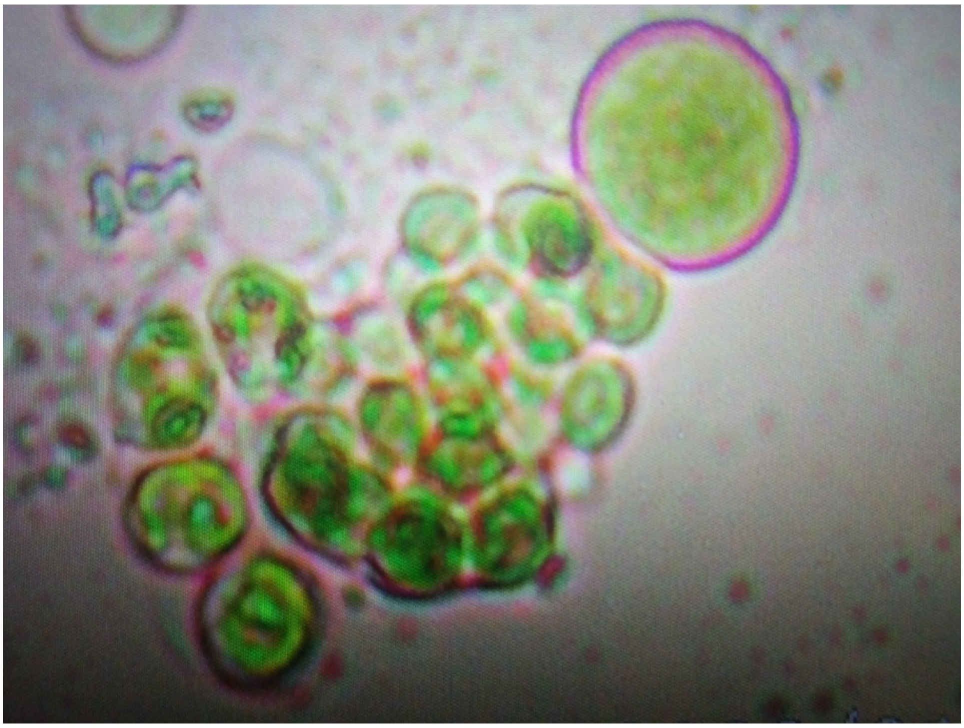


Fig. 2.1: Microscopic image of *Chlorella pyrenoidosa*

Table 2.1: Scientific classification of *Chlorella pyrenoidosa*

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

Table 2.2: Media composition of BG-11

Stock solutions 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
*$MnCl_2.4H_2O$	1.81 g
*$ZnSO_4.7H_2O$	0.22 g
*$Na_2MoO_4.2H_2O$	0.39 g
*$CuSO_4.5H_2O$ 0.08 g	0.08 g
*$Co(NO_3)_2.6H_2O$	0.05

*per litre; ** per 500 mL

The broad spectrum of algal based main products and by-products is economically feasible to support the green economy based on algal biomass. Therefore, it is easy to produce high value (biodiesel, bioethanol, biohydrogen, and biogas) and low value products (cosmetics, pharmaceuticals or nutraceuticals, feed and fodder, fertilizer *etc.*) simultaneously by the application of pure and raw algal biomass. 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, immunomodulatory agents, inflammatory agents *etc.*) (Kothari et al., 2017). The characteristic features regarding biochemical compounds of *Chlorella pyrenoidosa* were observed and found 56% protein, 24.5% carbohydrate, 10.3% lipid, and 0.45µgmL^{-1}of total chlorophyll by using methods *i.e.* phenol sulphuric acid, Lowry method, Bligh and Dyer, and 90% acetone respectively as per our study with BG-11 media. Therefore, it is possible to produce value-added products by using *Chlorella pyrenoidosa,* as it has significant amount of biochemical compounds.

2.4. Wastewater: selection and characterization

In order to establish green and sustainable approaches for algal cultivation system, wastewater has been selected as a source of nutrient for algal growth and development. After extensive literature survey, wastewater from Common Effluent Treatment Plant was selected as a source of nutrient for algal cultivation. Earlier, researchers have used biological agents (bacteria) and aerated lagoons for CETP wastewater treatment (Chandra et al., 2011). But till date very few studies on CETP wastewater treatment has been performed. Furthermore, wastewater in CETP place is also being treated by following conventional methods in sequence *i.e.* screening and grid removal, equalization tank, chemical dosing (flash mixer), primary clarifier, anaerobic and aerobic bacterial treatment, secondary clarifier, sludge thickener, sludge drying, and discharge of effluent at last.

2.4.1. Sampling site

The wastewater after primary treatment (separation of floating material and solid particles) at Common Effluent Treatment Plant, Banthar, Kanpur, U.P., (26.48°N, 80.43°E), India, was used for this experimental study as illustrated in Fig. 2.2. Final discharge (effluent) of wastewater from CETP was collected for this experimental study. CETP wastewater consisted of some heavy metals also, as per chemicals used as raw material in industrial processing units, such as Cr, Cu, Cd, Zn *etc.* which are highly toxic in nature. This treatment facility receives about 1.9 MLD tannery effluents from 21 tannery industries according to official data of that place. The CETP wastewater was collected in plastic cans (20 L) and subsequently stored at 4ºC for further use in laboratory of Department of Environmental Science. Fig. 2.3 clearly illustrates the generalized process scheme of Common Effluent Treatment Plant, followed by authorities.

Optimization of CETP wastewater concentration has been done to examine maximum algal growth. This experimental plan consists of physico-chemical and heavy metal concentration assessment of CETP wastewater. Pollution removal efficiency of microalga was analyzed in this section. Effect of CO_2 with optimized concentration of CETP wastewater is also an important part of this phase.

2.4.2. Physico-chemical characterization

Physico-chemical parameters of wastewater were analyzed by following the standard analytical procedures prescribed by APHA (2014).

Fig. 2.2: Sampling site of CETP wastewater

Analytical details for wastewater (CETP) characterization (TDS, TSS, BOD, COD, Nitrate, Phosphate) have been clearly described in Annexure 1. Heavy metals are also investigated in collected wastewater sample, which are discussed in Phase-I of study. All the protocols for experimental analysis in Phase-I, II, III has been given in annexure section 1.

2.4.3. Concentrations of CETP wastewater

Different concentrations of CETP wastewater was prepared to observe the optimized concentration of CETP wastewater that supports maximum algal growth. Its applicability as nutrient medium for cultivation of *Chlorella pyrenoidosa* has also been studied. Therefore, different concentrations (25%, 50%, 75% and 100% V/V) of CETP wastewater were selected for optimization process using *Chlorella pyrenoidosa*. Recommended nutrient medium (BG-11) was taken as a control for this experimental study.

2.5. Bioreactor

Bioreactor plays significant role to enhance algal biomass for multiple uses. Application of bioreactor to algal cultivation is significant, as all the optimized process parameters can be controlled in it, which is not possible with open pond systems. Details of bioreactors, types, and fabrication materials, with advantages and disadvantages are discussed in Chapter-1, which was used by various researchers in their experimental studies. Various review on bioreactors also taken into consideration, while working on designing part of PBR.

2.6. Experimental plan

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. To enhance the algal biomass production with low-cost harvesting techniques simultaneously treatment of CETP wastewater with designed photobioreactor is taken as general objective for this study, whereas, parametric characterization of selected wastewater with treatment using algal strain with and without designed PBR is studied in particular. The overall process of algal cultivation for biofuel generation of this experimental study has been illustrated in Fig. 2.4.

2.6.1. Experimental set-up

The whole experimental set-up was divided into three main phases. Phase-I consists of phycoremediation of industrial wastewater and their impact on algal bio-chemical compounds using *Chlorella pyrenoidosa*, with special emphasis to heavy metal reduction. Phase-II consists of photobioreactor based studies (tubular and vertical) for algal cultivation and biofuel production on the basis of results obtained from Phase-I, whereas, Phase-III consists of application of advanced harvesting techniques to harvest algal biomass, using bioflocculant and its significance with respect to chemical flocculants. Techno-economic assessment with respect to wastewater (as nutrient media for algal growth) and TES based photobioreactor for enhanced algal cultivation as low cost-approach is also included in this phase of study as described in Table 2.3.

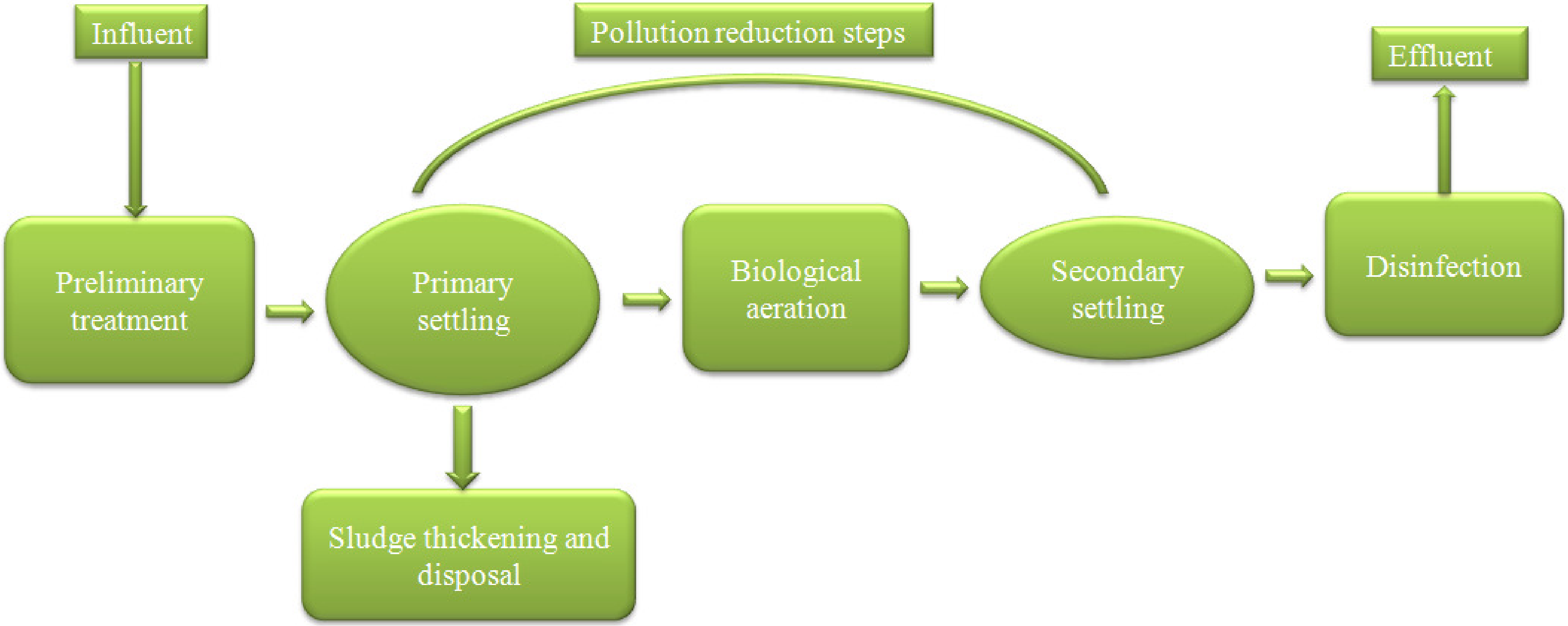


Fig. 2.3: Treatment process of common effluent treatment plant

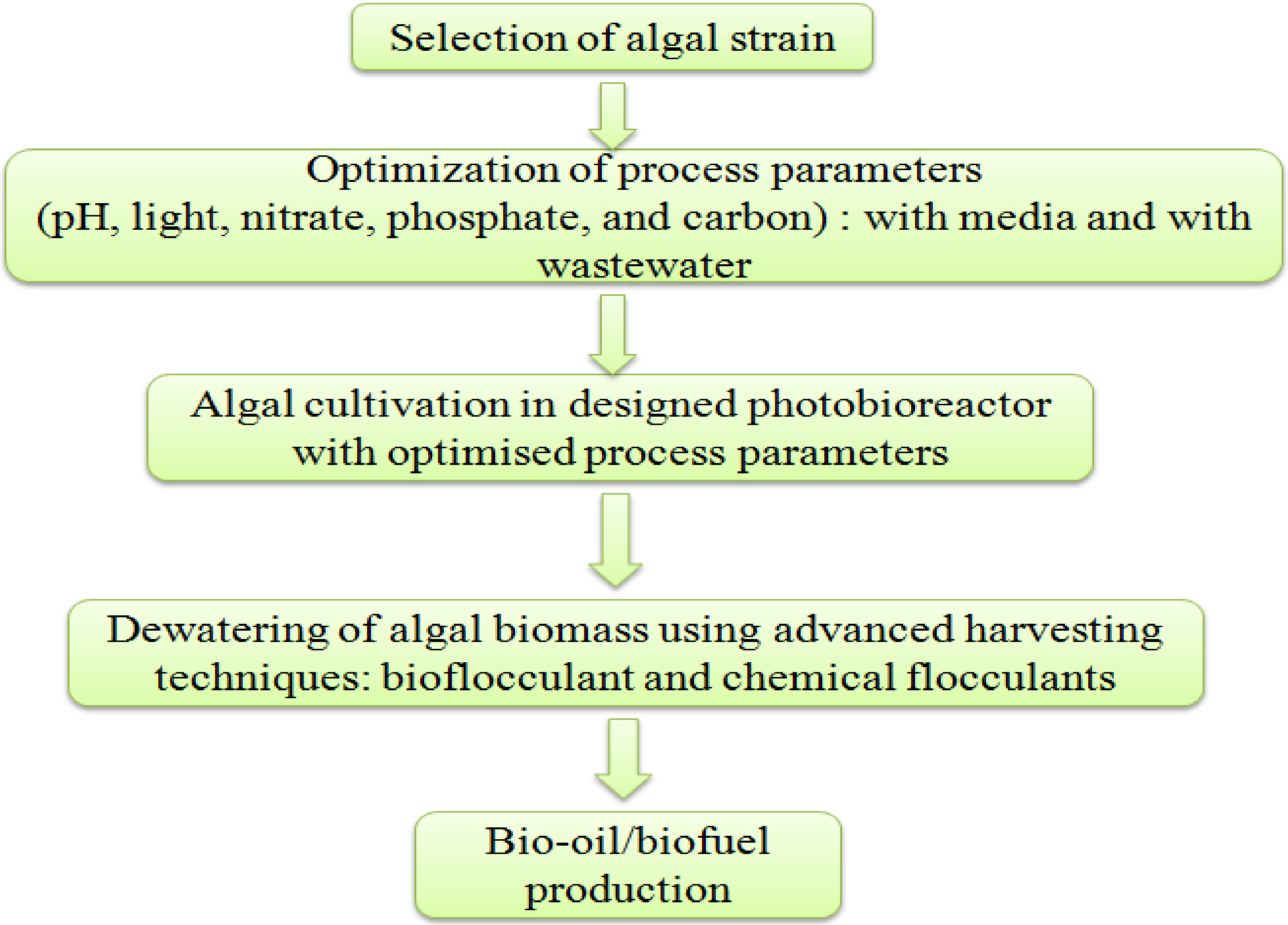


Fig. 2.4: Process steps from algal cultivation to biofuel production

Table 2.3: Experimental plan for research work

Phase-I (Chapter-3) **Phycoremediation of industrial wastewater and their impact on algal biochemical compounds using *Chlorella pyrenoidosa* with correlation study**	**Phase-II (Chapter-4)** **Feasibility of thermal energy storage based photobioreactor for algal cultivation and biofuel production: a lab scale study**	**Phase-III (Chapter-5 and 6)**

Optimization of selected process parameters and their correlation for algal growth with BG-11 media. Parameters selected are as:
• pH
• Photoperiod
• Nutrients
  • Nitrate
  • Phosphate
  • Carbon
Phycoremediation of CETP wastewater using microalgae *Chlorella pyrenoidosa*:
• Optimization of different wastewater concentrations to obtain maximum algal growth
• Impact of heavy metals and physico-chemical parameters on algal growth
• Impact of CO_2
  • Co-relation study between biomass productivity and biochemical composition (protein, carbohydrate, chlorophyll)
	Algal cultivation in designed photobioreactors to enhance algal biomass production and biofuel production with optimized concentrations of parameters with CETP wastewater (obtained from Phase-I).
	• Horizontal tubular photobioreactor
	• Vertical column photobioreactor
		Comparative assessment of bioflocculant and chemical flocculants for algal biomass harvesting (Chapter-5)
		• Impact of flocculants, dose, temperature, pH, on harvesting efficiency
		  • Thermodynamic studies
		  • Zeta potential analysis
		Techno-economic analysis of TES based PBR for algal biomass and biofuel production: a comparative study
		(Chapter-6)
		• Wastewater
		• Biofuel
		• Bioreactor

2.6.1.1. Phase-I: Phycoremediation of industrial wastewater and their impact on algal biochemical compounds using *Chlorella pyrenoidosa* 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 to optimize the physical (pH and photoperiod) and chemical (nitrate, phosphate, and carbon) parameters to obtained desired/ enhanced algal biomass production. The main focus of this experimental study are given on process parameters optimization (pH, light, nutrients) with respect to multiple variables that directly affects algal biomass productivity and algal biochemical compounds (protein, carbohydrates, and lipid). Application of growth kinetics *i.e.* specific growth rate, biomass productivity, and doubling time is also a significant part of this experiment to understand the cross-interaction between parameters and microalgal growth rate. The relationship between different variables of process parameters and their effect on biochemical compound has also been studied by the application of statistical analysis *etc.* Pearson correlation co-efficient analysis, clearly describes the positive and negative correlation between parameters and biochemical compounds.

2.6.1.1.1. 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 pyrenoidosa*, different variables of pH in culture media were investigated. To examine the effect of different ranges of pH on algal cell density, pH buffer was prepared by using 0.1 N of HCl and 0.1 N of NaOH. BG-11 media of algal culture was subjected by acid and base solution to maintain the different concentrations of pH ranges from 6.0, 6.5, 7.0, 7.5, and 8.0. Although maximum algal growth occurs at neutral pH, but optimum pH is the initial pH of algal culture at which microalgae is adopted to grow. Therefore, to investigate the optimum pH, different variables of pH were examining ranges from acid, neutral, to base. Optimized pH value is used with CETP wastewater in experimental set-up for algal biomass growth.

2.6.1.1.2. Light photoperiod

In order to know the best photoperiod to achieve maximum algal biomass growth with respect to metabolic compound (protein, carbohydrate, and lipid) algal biomass was subjected to different exposure of photoperiods *i.e.* light (L) and dark (D) cycle. The experiment was run for 15 days with respect to different photoperiods *i.e.* 24L, 18L:6D, 16L:8D, and 12L:12D by using white LED lights (LEXCO LED strip). The intensity of LED light was measured by solarimeter (KUSRM-MECO-SPM-350) and obtained 10wm^{-2}. The growth of *Chlorella pyrenoidosa* of each culture with different photoperiod was monitored by using spectrophotometric measurements of the optical density (OD-665 nm), typically for live algal cells. The optimized photoperiod was used further with CETP wastewater in experimental set-up for algal biomass growth.

2.6.1.1.3. Nutrients

Nutrients (nitrate, phosphate, carbon, and other micronutrients) play a significant role for better growth and development of algal biomass. 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.

2.6.1.1.3.1. Nitrate

Nitrate is a key limiting factor for algal biomass growth with one of the first nutrient to be drained first. Algal biomass subjected to nitrate stressed condition is significant to investigate the effect of stress conditions on algal growth and biochemical compounds. Therefore, different concentrations (0.05gL^{-1}, 0.1gL^{-1}, 0.2 gL^{-1}, 0.4 gL^{-1}), of nitrogen in form of nitrate salt *i.e.* sodium nitrate ($NaNO_3^-$) were applied to investigate the results based on algal growth and biochemical compounds.

2.6.1.1.3.2. Phosphate

Phosphorous is important fundamental macronutrients and play significant role for the formation of protein/nucleic acid and DNA/RNA respectively. The availability of phosphorous is always a limiting factor for aquatic plants. Some microalgae lead to accumulate organic carbon in the form of triacylglycerols with respect to nutrient starvation conditions. Different concentrations (0.05gL^{-1}, 0.1gL^{-1}, 0.2 gL^{-1}, 0.4 gL^{-1}) of phosphorus source in the form of phosphate salt *i.e.* di-potassium dihydrogen phosphate (K_2HPO_4) were taken to investigate the effect on algal growth.

2.6.1.1.3.3. Carbon

Carbon is an essential nutrient for photosynthesis, and it can be either used in respiration, or as an energy source and as a row material in additional cells formation. Carbon as a source of nutrient plays an important role for algal growth and food formation.

2.6.1.1.3.3.1. Carbon (salt form)

Different concentrations (0.05gL^{-1}, 0.1gL^{-1}, 0.2 gL^{-1}, 0.4 gL^{-1}), of salt of sodium carbonate (Na_2CO_3) as carbon source were taken to investigate the effect of carbon on algal growth.

2.6.1.1.3.3.2. Carbon (gaseous form)

As per literature reviewed, it is well known that algae have significant potential to capture and assimilate CO_2 emitted from various industrial sources *i.e.* flue gas for its growth and development. Therefore, in this experimental study carbon in the form of gas (CO_2) were also used and supplied to investigate the effect on algal growth. *Chlorella pyrenoidosa* were inoculated in 1 L of Erlenmeyer flasks containing 50% test solution (50% wastewater + distilled water) of CETP wastewater with initial 10% concentration of algal biomass. Triplicate bottles were equilibrated at four different concentrations (5%, 10%, 15%, and 20%) of CO_2 mediated experiment (Fig. 2.5). CO_2 gas from a pressurized cylinder was provided to each conical flask having *Chlorella pyrenoidosa*. pH of each flasks were maintained at 7.5 with room temperature ±25°C, and intensity of incident light were fixed at $10w/m^2$. Optimized values of nitrate, phosphate, and light photoperiod, obtained in prior experimental work were used here to co-ordinate this step of research work. The initial concentration of nutrients *i.e.* nitrate ($0.05\ mgL^{-1}$), phosphate ($0.05\ mgL^{-1}$) and light photoperiod were maintained by 16L:8D cycle. pH adjustment in each bottles was maintained by acid base addition. pH adjustment was done only at the beginning of the experiment. The pH was measured by using pH meter. The pH was calibrated using 0.1 M sodium hydroxide solution. The pH level, temperature, and CO_2 concentration was evaluated every day of the experiment.

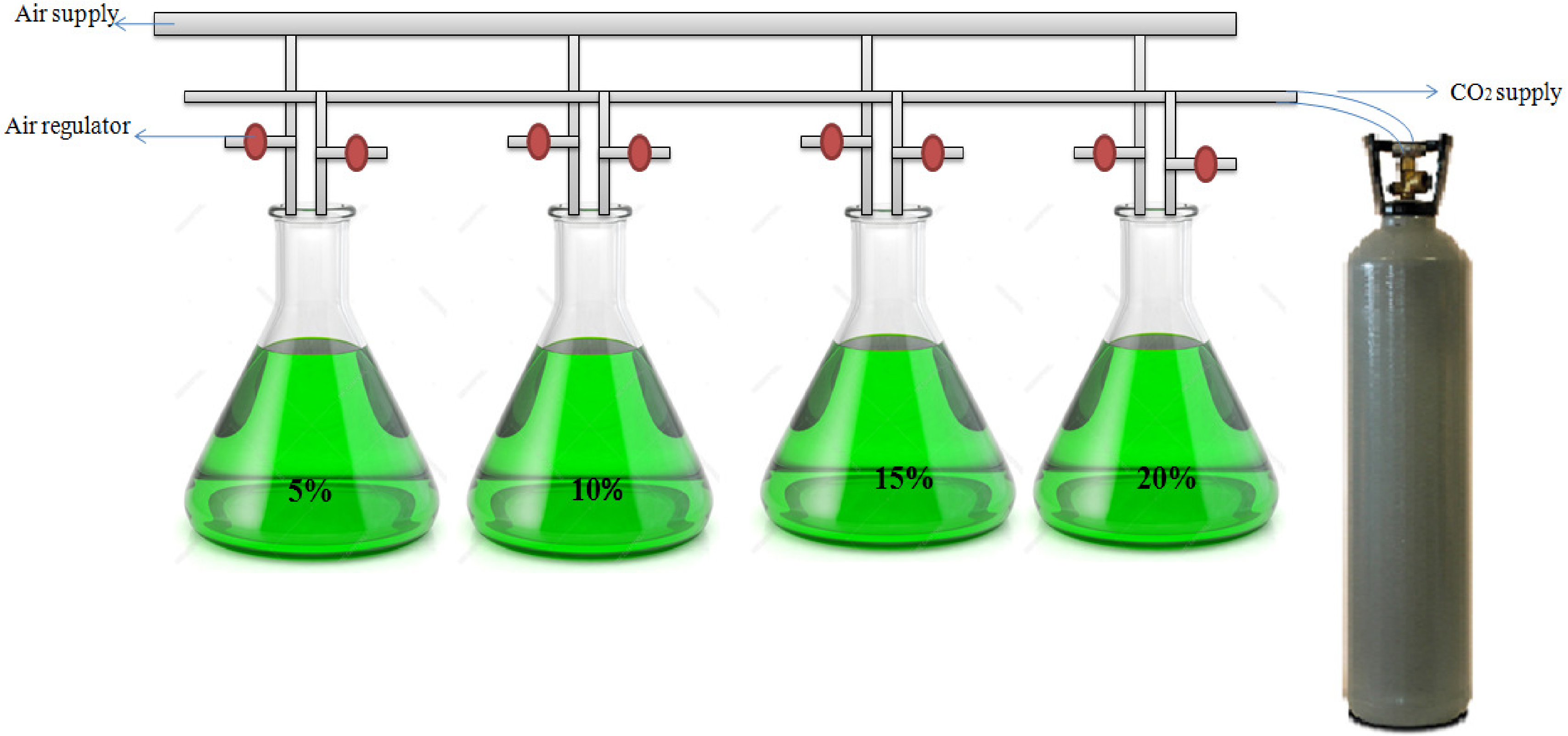


Fig. 2.5: Experimental set-up for CO_2 gas experiment

The total amount of carbon dioxide consumed by the *Chlorella pyrenoidosa* was considered in the form of carbohydrate which has been estimated by Anthrone method (Annexure 1). It is clear from the equation that algal biomass converts the amount of CO_2 consumed in carbohydrates as given in following equation:

$$6CO_2 + 6H_2O = C_6H_{12}O_6 + 6O_2 \tag{2.1}$$

2.6.1.1.3.3.2.1. CO_2 sequestration by algae

Subsequently, 20 ml of algal culture was taken off from the medium and placed in a centrifuge machine. The algal sample was centrifuged for 20 min at 4°C temperature with 8000 rpm. The centrifugation process enabled separation of algal biomass with 99% efficiency. After centrifugation, supernatant was poured out and the obtained algal biomass was dried in oven at 50°C temperature. After drying, algal biomass was weight at weighing balance and measurement ranges from 0.02 to 1.63 g. The obtained value of dried algal biomass weight was applied to know the bio fixation of CO_2 through algal biomass by using equation:

$$CO_2 \text{ sequestration} = C \text{ x } P \text{ x } (M_{CO2}/M_C) \tag{2.2}$$

Where, C = Carbon content in biomass, P = algal biomass productivity, MCO_2= molar mass of carbon dioxide, and MC = molar mass of carbon.

2.6.1.1.4. Heavy metals analysis

The culture of *Chlorella pyrenoidosa* were incubated for 15 days under controlled (pH- 7.5, temperature 25°C) condition and from each flask *i.e.* 25%, 50%, 75%, 100%, 5 mL culture was taken out under sterilized condition at every 5days of experiment for heavy metal analysis. The algal culture was centrifuged at 5000 rpm for 10 minutes. The supernatant was used for different heavy metal estimation, carried out by Atomic Absorption Spectrometry (AA240FS, Fast Sequential Atomic

Absorption Spectrometer) of different test solutions (25%, 50%, 75%, and 100% V/V) at 1st, 5th, 10th, and 15th day of experiment. To understand the percentage reduction of heavy metals concentration in wastewater of different test solution, equation (2.3) was applied and pollution reduction efficiency of *Chlorella pyrenoidosa* was calculated by equation:

$$\text{Pollution reduction (\%) of Heavy Metals} = [(C_i - C_f)/ C_i] \times 100 \qquad (2.3)$$

Where, C_i and C_f are the initial and final concentration of heavy metals of CETP wastewater

The affinity of algal biomass for uptake of heavy metals was evaluated by applying the average removal rate (ARR) kinetics equation (2.4):

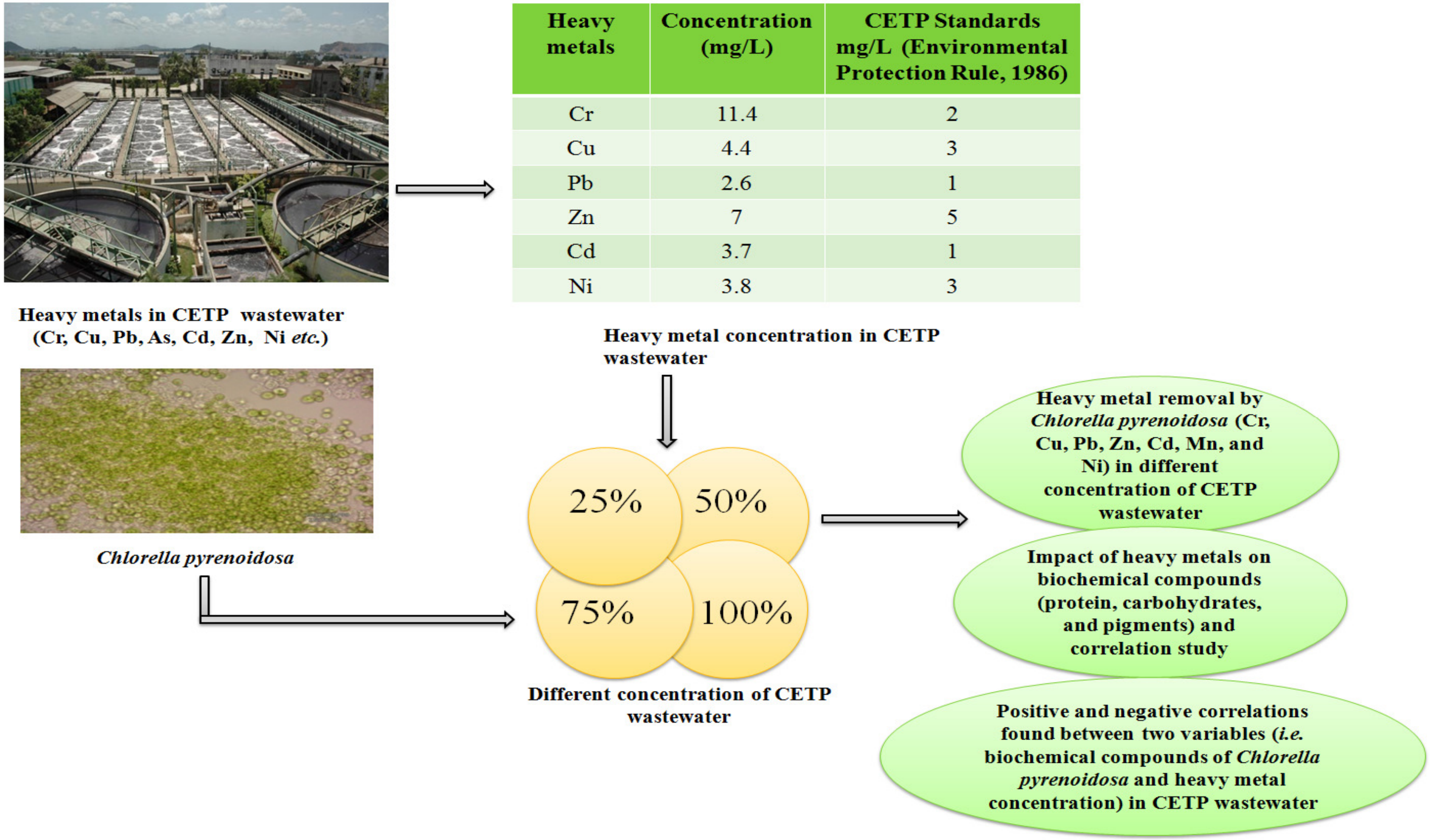
$$\text{ARR (mg heavy metals } L^{-1}D^{-1}) = [X]_{initial} - [X]_{final}/\text{Biomass}_{final} - \text{Biomass}_{initial} * 1/days \qquad (2.4)$$

Where, [X] = concentration of heavy metals.

The whole process of wastewater optimization with *chlorella pyrenoidosa*, pollution reduction, and heavy metal removal has been illustrated in Fig. 2.6.

2.6.1.1.5. Growth Kinetics

Algal growth can be studied under various growth phases such as lag phase, exponential phase, declining phase, stationary phase and death phase. The lag phase of alga occurs if the inoculums are transferred from one set of growth conditions to other. Generally, algal growth in exponential phase is considered to calculate the growth rate. The duration of exponential phase depends on the size of inoculum and the capacity of culture conditions to support the algal growth. Biomass concentration (gL^{-1}) of alga was plotted against time (days) and exponential phase of the algal growth was carefully determined (straight line) by



Heavy metals	Concentration (mg/L)	CETP Standards mg/L (Environmental Protection Rule, 1986)
Cr	11.4	2
Cu	4.4	3
Pb	2.6	1
Zn	7	5
Cd	3.7	1
Ni	3.8	3

Fig. 2.6: Heavy metal removal from different concentration of wastewater

selecting two points N_1 and N_2 at the extreme of this line to calculate the growth rate. The biomass concentrations (gL^{-1}) of algal biomass were plotted against time (days) and exponential phase of algal growth. The algal growth pattern was monitored through kinetic analysis using following equations:

$$\text{Biomass productivity} = (N_2-N_1)/t_2-t_1 \tag{2.5}$$

Specific growth rate and doubling time were also determined by using following equations:

$$\text{Specific growth rate } (\mu) = \ln (N_2-N_1)/t_2-t_1 \tag{2.6}$$

$$\text{Generation time} = 1/\ \mu \tag{2.7}$$

Where, N_2 and N_1 are algal biomass at different time interval t_2 and t_1 respectively.

The other phases such as declining, stationary and death phase can be predicted by observing the growth curve of alga. The declining of algal biomass generally occurs when either a specific requirements for cell division is limiting or any factors inhibiting the reproduction. Stationary phase of alga is characterized with 0 (zero) net growth rates. Stationary phase indicates that alga is 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 alga on varying culture conditions. The value of growth rate obtained in this model was used as a response to growth factors like pH and nutrient for experimental design model, which is explained in subsequent section. Growth kinetic is also studied and used for Phase-II of experimental plan.

2.6.1.1.6. Biochemical analysis

Algal growth characteristics were observed by analysis of its biochemical compositions such as protein, carbohydrate, pigment, and lipid. These parameters were analyzed on every alternate day of growth optimization experiment. Protein estimation of algal biomass was done by Lowry method (Lowry et al., 1951). Phenol sulphuric acid test was originally described as a nonspecific quantitative test for carbohydrate (Dubois et al. 1956). The interaction of phenol solution with carbohydrate produces a finite absorbance, which is measured at 490 nm. Photosynthetic pigments *i.e.* chlorophyll *a*, chlorophyll *b*, and total chlorophyll *a+b* was measured by Mackinncy method (all the methods used in biochemical analysis of algal biomass are discussed in Annexure 1). Lipid productivity was analyzed by following equation:

$$\text{Lipid productivity (\% dry weight)} = (\text{wt. of lipid/ wt. of alga}) \times 100 \qquad (2.8)$$

2.6.1.1.7. Pearson correlation co-efficient analysis

Pearson correlation coefficient is a statistical tool also called as Pearson product-moment correlation coefficient or bi-variate correlation, used to measure linear correlation between two variables. Pearson correlation coefficient value ranges from +1, 0 to -1, where, 0 indicates no linear co-relation, +1, and -1, is perfect positive and negative correlation respectively, between two variables (Raghuvanshi et al., 2014). In order to understand the positive or negative cross interaction/correlation between different variables of process parameters *i.e.* pH, light, and nutrient and algal biochemical compounds (protein, carbohydrate, and lipid), as well as effect of heavy metals in CETP wastewater and algal biochemical compounds (protein, carbohydrate, pigments), Pearson correlation co-efficient (a statistical analysis) has been applied with the obtained data. The applied statistical analysis provides an accurate data to understand the positive or negative impact between these variables.

2.6.1.2. Phase-II: Feasibility of thermal energy storage based photobioreactor for algal cultivation and biofuel production: a lab scale study

Mass cultivation of algae in indoor condition was carried out in various types of closed photobioreactor such as bubble column, air lift and submersible reactor. These reactors have several defects such as high cost, adherence of algal cell due to small size of water droplets, uneven mixing, poor gaseous exchange, uncontrolled reactor temperature *etc.* In order to overcome these defects, the present research work is focused to design and fabricate two different photobioreactor *i.e.* horizontal tubular photobioreactor and vertical column photobioreactors. The present bioreactor was designed to overcome the practical limitations with easy operation to enhance the algal biomass production with low-cost and less energy intensive technology.

2.6.1.2.1. Horizontal tubular photobioreactor

A closed horizontal Tubular Photobioreactor (TPBR) was fabricated for this experimental study. The bioreactor consisted of six transparent acrylic glass tubes, conjoined to each-other with a reservoir (storage tank) of 20L capacity. An electric motor pump was incorporated in the system for continuous circulation of algal cell suspension. An aerator was also inserted in the reservoir for gaseous exchange and to maintain the uniform distribution of nutrients.

2.6.1.2.2. Vertical column photobioreactor

A three chambered vertical column Photobioreactor (VC-PBR) setup with 10L capacity of algal medium were fabricated by using transparent glass material. Each chamber was equipped with thermometer and motor based-aerator to check the temperature of the PBR and uniform distribution of nutrient. Phase change materials (PCMs) are a special type of thermal energy storage materials and generally use chemical bonds to store and release heat energy to a required system. Transfer of thermal energy takes place when material changes from solid to liquid or vice-versa, which is known as change in state or phase. Tyagi and Buddhi, (2008), reported the promising applications of phase change material in heating and cooling for builings. The aurthor has lso reporated the application of calcium chloride hexahydrate (phase change material) as latent heat storage system. Three separated PBR setup were prepared and run in parallel to investigate the temperature of the medium with PCMs and without PCMs. The effect of PCM on PBR temperature profile with respect to control (without PCMs) is a significant part to be studied.

Table 2.4 clearly describes the specifications of closed photobioreactor used in this research work. The present experimental setup (as illustrated in Figure 2.7) categorized into three chambers out of which one contains only algal medium without PCM, the other two contained algal growth medium with PCM *i.e.* capric acid and thermostat for marinating the temperature. Side and back portion of the system were insulated by using 2 cm expandable polystyrene sheets to prevent the transfer of heat to and from the surroundings.

2.6.1.2.3. Experimental set-up

Three separated PBR setup were prepared and run in parallel to investigate the temperature of the medium *i.e.* uncontrolled PBR/without PCM or thermostat (PBR-I), controlled PBR with PCM (PBR-II), and controlled PBR with thermostat/electric heating (PCR-III). The PCMs was selected on the basis of temperature range required for *Chlorella pyrenoidosa* with respect to weather temperature profile of Lucknow during winter season where the experimental work was carried out. The temperature was maintained in winter season with minimum 10°C and maximum 25°C recorded in a day. The temperature of PBR system was obtained near ambient temperature during experiment in PBR-I. So, there is a need to select the PCM having melting temperature in range of 30-35°C. Based on these considerations, an organic PCM was selected as suitable candidate *i.e.* capric acid ($CH_3(CH_2)_8COOH$) of analytical grade to maintain the temperature of the PBR to enhance the algal biomass. The melting point of this PCM was 31.5°C, and latent heat was 36.23 Jg^{-1} respectively. The optimized value of pH, photoperiod, and nutrients (Nitrate and phosphate), 50% test solution of CETP wastewater obtained from phase-I study was fixed with photobioreactor to investigate the effect on algal biomass growth and development. The initial pH of 50% test solution was 8.9, hence the pH of the medium was maintained by using 0.1N of HCl solution to make it 7.5 to achieve maximum algal growth. In order to optimized the photoperiod, algal biomass was subjected with various photoperiods (24L, 18L:6D, 16L:8D, and 12L:12D) and found the maximum algal growth with 18L:6D photoperiod of light and dark cycle. Hence, the PBR was artificially maintained with 16L: 8D condition.

2.6.1.2.4. PCM for TES based PBR

A three chambered vertical column PBR was setup with 5L working volume (50% test solution50% CETP wastewater and 50% distilled water) of algal medium were fabricated by using transparent glass material. Each chamber was equipped with thermometer and motor based-aerator to check the temperature of the PBR to maintain uniform distribution of nutrient.

Table 2.4: Specifications of photobioreactor

Horizontal Tubular Photobioreactor	
Specifications	Description
Material	Glass
Number of tubes	Six (06)
Diameter	0.1m
Length	1m
Total volume	20L
Working volume	10L
Motor	0.5 Horsepower
Vertical Column Photobioreactor	
Material	Glass
Total volume	10L
Working volume	5L
Aeration	Aqua air pump motor
Light source	LED of 20 watt
Temperature sensor	Thermometer
Radiation measurement	Solar power meter (Thermo-scientific) (wm^{-2})
pH measurement	Handy pH meter (Hanna instruments)
PCM (phase change material)	Capric acid
control condition	Thermostats (maintained 30°C in PBR)

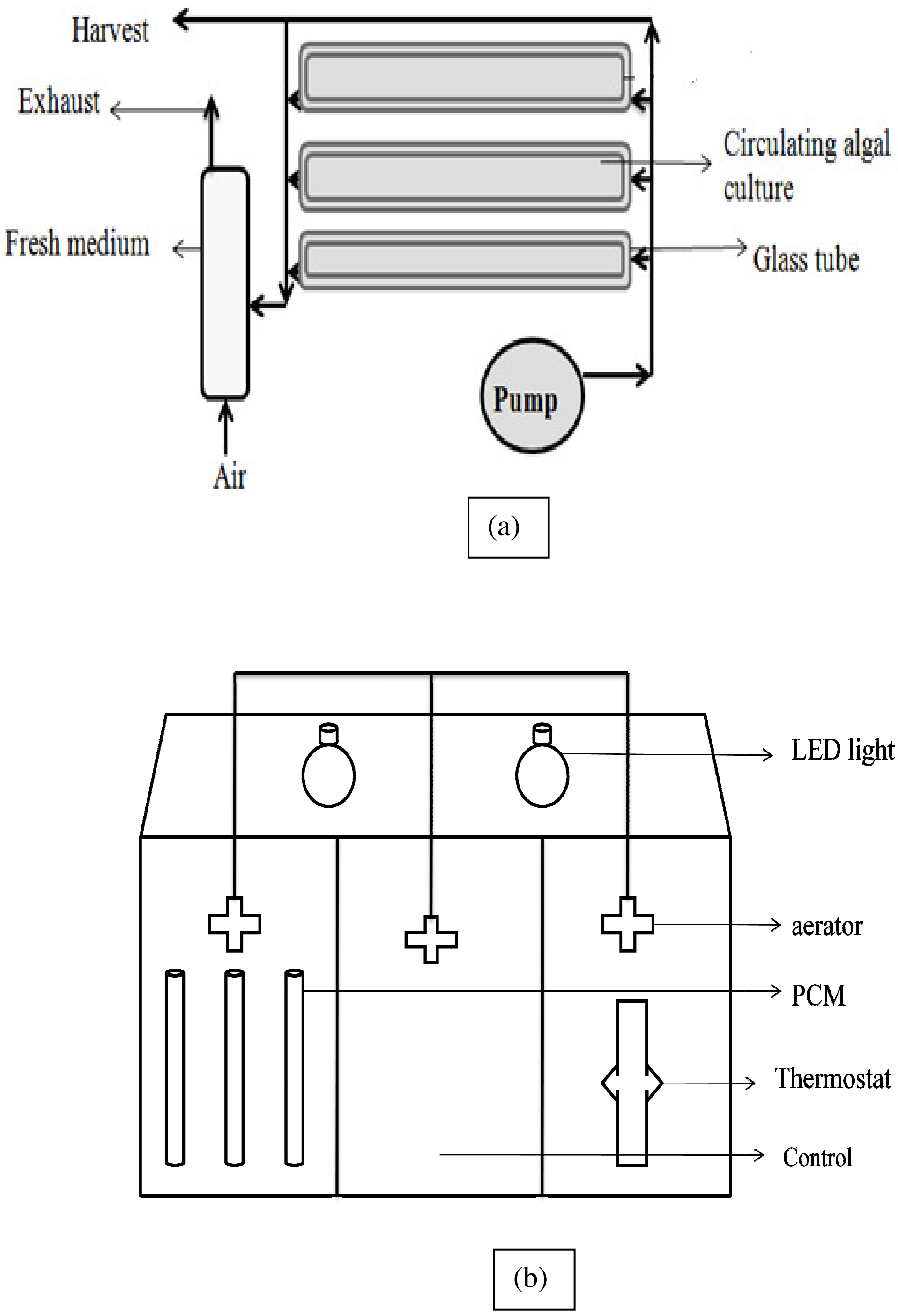


Fig. 2.7: Schematic diagram of photobioreactor: (a) horizontal tubular photobioreactor; (b) vertical column TES based photobioreactor

Three separated PBR setup were prepared and run in parallel to investigate the temperature of the medium with PCMs and without PCMs. Side and back portion of the system were insulated by using 2 cm expandable polystyrene sheets to prevent the transfer of heat to and from the surroundings. The changes in temperature inside PBR-I, II, and III were recorded continuously from 6.00 am to 12.00 pm at regular interval. The average temperature of the medium in PBR during experiment was 20°C in which *Chlorella pyrenoidosa* was cultivated. The favourable temperature for growth and development of *Chlorella pyrenoidosa* ranges from 25-30°C. Therefore, to maintain this temperature range in PBR system in which *Chlorella pyrenoidosa* was cultivated, phase change material based PBR system was designed to store heat energy for temperature maintenance. The amount of required heat energy in PCM was calculated on the basis of latent heat of material through these equations:

$$Q = \int_{T_i}^{T_f} mC_p \mathrm{d}T \tag{2.9}$$

$$= \mathrm{mC_{ap}}\ (\mathrm{T_f} - \mathrm{T_i}) \tag{2.10}$$

Where, Q is heat energy, m is mass of the substance (kg), s is specific heat (units j/kg*K), Δt is change in temperature/temperature difference.

$$Q = \int_{T_i}^{T_m} mC_p dT + ma_m \Delta h_m + \int_{T_m}^{T_f} mC_p dT \tag{2.11}$$

$$Q = \mathrm{m}\left[\mathrm{C_{sp}}(\mathrm{T_m} - \mathrm{T_i}) + \mathrm{a_m}\Delta\mathrm{h_m} + \mathrm{C_{Ip}}\ (\mathrm{T_f} - \mathrm{T_m})\right] \tag{2.12}$$

$$Q = \mathrm{a_r} m \Delta h_r \tag{2.13}$$

Where, Q is heat energy, m is mass of the substance, l is latent heat.

For lab scale experimental work, to maintain 5L medium of *Chlorella pyrenoidosa*, total 3 kg of PCM was used in this work. Three metallic cylindrical shape cans were used to encapsulate the PCM in PBR system. Each can have 500 gm of PCMs which was kept inside the PBR chamber. Solar energy was used to change the phase of PCM used in cylindrical shape cans.

2.6.1.2.5. Algal biofuel production

Algal biomass harvested from 15th day batch experiments was used for extraction of algal oil by following the solvent extraction method as well as by modified Bligh and Dyer methods to investigate the feasibility for biofuel applications (Annuxure-1). Both these methods have been applied for comparative analysis to know the best and efficient method with respect to biofuel production.

2.6.1.3. Phase-III: Comparative assessment of bioflocculant and chemical flocculants for algal biomass harvesting

Algae have the characteristic feature of various value-added products such as oil, ethanol, methanol, biodiesel, biohydrogen, biogas, long-chain hydrocarbon, carbohydrate, phycobiliproteins (a blue food dye), yellow-white protein, β-carotene, phycobiliproteins, phycocyanin and phycoerythrin protein**, v**itamins (*e.g.* A, B1, B2, B6, B12, C, E, nictitate, biotin, folic acid and pantothenic acid), sulphated polysaccharides, antihelmintic (a drug that expels parasitic worms), gama-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, omega-3 fatty acid (Kothari et al., 2017). However, regardless of these advantages, main challenge is associated to harvesting of algal biomass. Therefore, harvesting of algal biomass is a significant part of this experimental work. The quantity of end product is completely depending on dewatering process of algae. Various conventional techniques (centrifugation, gravity sedimentation, filtration, flotation, flocculation, and electro-coagulation) are being used to harvest algal biomass. These methods are economically unviable and consume high electricity which further increases the cost of algal based products. Therefore, to overcome this problem, a low-cost approach has been taken into consideration to achieve maximum harvesting efficiency. In order to conduct this experiment waste egg shell has been used as a source of bioflocculant to harvest the algal biomass efficiently. Impact of dose concentrations, contact time, different temperature with optimized dose concentration, and pH has also been studied in Phase-III of experimental plans. Comparative analysis of bioflocculants with chemical based flocculants is a significant part of this study. Whereas, temperature and pH based data's are clearly supported by kinetic and thermodynamic functions and zeta potential analysis respectively.

2.6.1.3.1. Type of flocculants

2.6.1.3.1.1. Bioflocculant (egg shell)

The collected egg shells (bioflocculant) were washed with distilled water and dried at 40 °C in an oven. Dried egg shells were grind to obtain the fine powder and sieved manually using micro sieve. The eggshell powder (100 mg) was dissolved in 10 ml of 0.1 mol/L acid solution with continuous stirring for 30 min. The acid solution was then diluted to 100 ml using deionised water to make a final eggshell concentration (bioflocculant) of 1000 mgL-1.

Harvesting of algal biomass was performed by applying various concentrations (0, 20, 40, 60, 80, and 100 mgL-^{1}) of bioflocculants (egg shell) in algal cell suspension to optimize the significant bioflocculant dose concentration. The process of was carried out with 60 minute of contact time to investigate the optimum harvesting efficiency with respect to time as illustrated in Fig. 2.8.

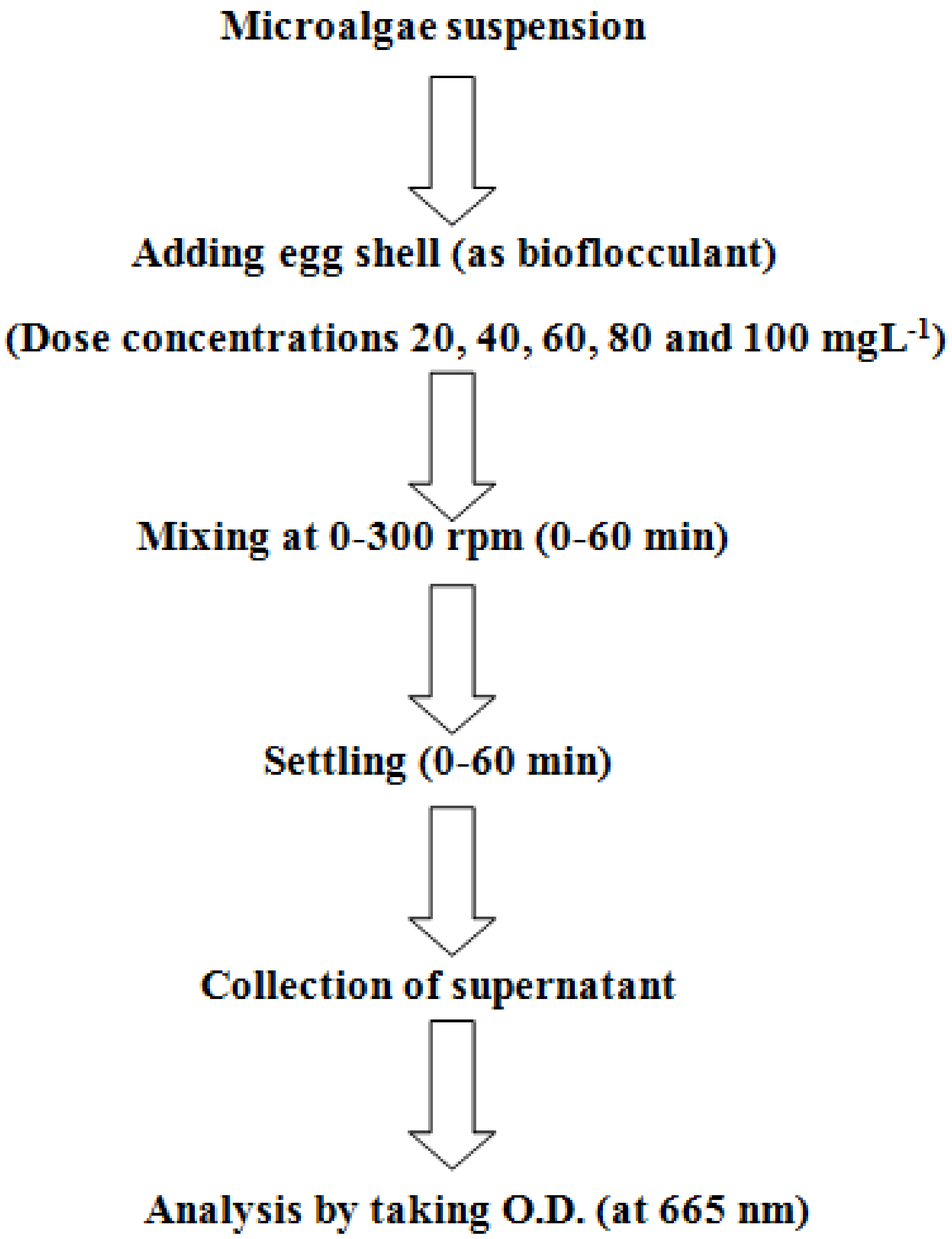


Fig. 2.8: Process for algal biomass harvesting using flocculant

2.6.1.3.1.2. Laboratory available $CaCO_3$ (LACC)

Laboratory available $CaCO_3$ (LACC) of (100 mgL^{-1}) was dissolved in 10 ml of 0.1 mol/L acid solution (hydrochloric acid) with continuous stirring for 30 min. The acid solution was then diluted to 100 ml using deionised water to make a final LACC concentration (chemical flocculant) of 1000 mgL^{-1}.The selected chemical (LACC) has been chosen for comparative analysis with waste material *etc.* waste egg shell based bioflocculant as both of them have same chemical composition with 99.9% elemental Ca^{++} ion.

2.6.1.3.1.3. Alum ($Al_2 (SO_4)_3$)

Alum is a common chemical that have been proved to be an efficient chemical based flocculant (Gerde et al., 2014). This flocculant was directly introduced (100 mgL^{-1}) to algal culture to investigate the HE. In this study alum has been taken as standard reference material to examine the viability of designed flocculant material on comparative basis. These flocculants (egg shell, LACC, and alum) were used with optimized concentration (100 mgL^{-1}) (Kothari et al., 2017) and mixed at 300 rpm, using a mini orbital shaker (VWR Advanced Orbital Shaker, Model 15000). The supernatant from the surface was taken to measure the optical density (OD) at 665 nm wavelengths after the incorporation of flocculant doses into the algal culture.

2.6.1.3.2. Harvesting efficiency (HE)

The flocculants (bio-flocculant and chemical flocculants) was added at a selected/optimized concentration and mixed at 300 rpm, using a mini orbital shaker (VWR Advanced Orbital Shaker, Model 15000). The supernatant from the surface was taken to measure the optical density (OD) at 665 nm wavelengths after the incorporation of bioflocculant doses into the algal culture.

In order to know the harvesting efficiency (equation 2.13) of algal cells with respect to time, on every five minute, optical density of the algal suspension was taken.

$$HE (\%) = [1- \{ODa_{665} (t)/ ODa_{665} (t_0)\} / \{ODb_{665} (t)\ ODb_{665} (t_0)\} \times 100] \quad (2.13)$$

Where, ODa_{665} (t_0), ODa_{665} (t) and ODb_{665} (t_0) and ODb_{665} (t) are the turbidities of algal cell suspension without and with treated bioflocculant at time zero and time *t*, respectively. Data represented in table and figures are mean values from three replicates.

2.6.1.3.3. Impact of temperature on harvesting efficiency

After optimization of bioflocculant dose concentration, the optimum concentration of bioflocculant dose was further proceeded to investigate the effect of temperature on harvesting efficiency. The optimized dose were subjected to different temperature ranges 30°C, 35°C, 40°C, 45°C, 50°C to investigate the change in harvesting efficiency. The required ranges of temperature were maintained by hot water bath.

2.6.1.3.3.1. Kinetic model

Kinetic study by pseudo-second ordermodel is mainly used for adsorption process (Nuhoglu and Malkoc 2009; Ho and McKay 1999). However, various authors have used this model to evaluate the rate of reaction other than adsorption process such as biomass production and oil production (Mauryaet al., 2014). Therefore, surface binding of bioflocculant can be also evaluated by following this model. The rate of pseudo-second-order reaction usually depends on binding of flocculants on the surface of algal biomass, which can be expressed by following equation:

$$t/q_t= 1/k_2\ q_e^2+t/q \quad (2.14)$$

Where,K_2= Rate constant ($gmg^{-1}min^{-1}$); q_e = Amount of biomass ($mg\ g^{-1}$) flocculated at equilibrium; q_t = Amount of biomass ($mg\ g^{-1}$) flocculated at time t.

Initial variables such as q_e and $q_t$are quantified by following equation:

$$q = (C_i - C_f) * V / m \quad (2.15)$$

Where, q = Bioflocculant adsorption capacity; C_i, = Initial algal biomass concentration; C_f = Biomass concentration after flocculation; V = Volume of the solution (L); m = Amount of bioflocculant (mg l^{-1}).

The pseudo second order kinetic model is expressed by plot between t/q versus t. Kinetic variables such as K_2 (rate constant), h (initial flocculation rate), q_e (calculated bioflocculation capacity) can be calculated from the slope and intercept of the straight line equation.

2.6.1.3.3.2. Determination of thermodynamic functions

In this phase of experimental study, the Eyring and Arrhenius equation were applied to describe the adsorption behaviour of bioflocculant by the *Chlorella pyrenoidosa*

2.6.1.3.3.2.1. Eyring equation

The effect of temperature on time-dependent flocculation of algal biomass can be expressed by the thermodynamic parameters. Eyring type plot between $\ln k_2/T$ versus 1/T was assessed to calculate the thermodynamic parameters. Eyring equation is used in chemical kinetics to describe the variance of the rate of reaction with temperature. The change in enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG) after adsorption of bioflocculant has been investigated in flocculation process at different temperature by using Von't Hoff equation (Yao et al., 2010; Shivaraj et al., 2001). The thermodynamic parameters such as standard free energy changes (ΔG), the standard enthalpy changes (ΔH) and the standard entropy change (ΔS) is obtained from experiments at various temperatures using the following equations:

$$\text{Intercept} = [\ln (kb/h) + \Delta S/R] \quad (2.16)$$

$$\text{Slope} = [-\Delta H/R] \quad (2.17)$$

Where, k_b= Boltzmann constant; h= Plank's constant; and R= Gas constant.

The slop and intercept of strait line equation is used to calculate the thermodynamic parameters ΔH and ΔS. The Gibb's free energy (ΔG) (Equation 2.18) has been calculated by the obtained value of ΔS and ΔH for different temperature in Calvin.

$$\Delta G= \Delta H-T\Delta S \tag{2.18}$$

Where, ΔG = Gibbs free energy; ΔH = Enthalpy; ΔS = Entropy.

The above said equation had been applied to know the mathematical relationship between bioflocculant adsorption by the algal cell at different temperature and time. Thermodynamic parameters obtained from Van't Hoff graph, which is also known as Eyring type equation is the most widely accepted models have been taken into consideration to know the rate of reaction after absorbance of bioflocculant by the algal cell.

2.6.1.3.3.2.2. Arrhenius equation

Arrhenius equation has been used to investigate the activation energy *i.e.* maximum energy required to start the chemical reaction after incorporation of bioflocculant. The Arrhenius equation expresses that flocculation is a function of temperature in pseudo-second-order rate constant. The Arrhenius equation can be expressed by the following equation:

$$\text{Slope} = -Ea/R \tag{2.19}$$

Where, -Ea = Arrhenius activation energy; R = Gas constant which is equal to 8.314 j/ mol $^{-1}$ k^{-1}

2.6.1.3.4. Impact of pH on harvesting efficiency

To investigate the effect of pH on HE of algal cell, a flocculation experiments were performed with best obtained result of dose concentration in four small beakers (1000 ml). The dense algal cultures were kept 1000 ml in each beaker. pH of bioflocculant were maintained (2.0, 4.0, 6.0, 8.0, and 10.0) by adding 1N of HCl and NaOH (Fig. 2.9). The maintained pH of bioflocculant were tested to investigate the efficiency of flocculation by taking OD at 665 nm, using UV visible spectrophotometer (HALO-DB 20, Thermo Scientific).

2.6.1.3.4.1. Zeta potential analysis

An instrument, Nanoplus Zeta /nano particle analyzer (Model: Nano Plus-3, Serial no. 405613, made in Japan), was used to investigate the zeta potential of the system (bioflocculant) to analyze the harvesting efficiency. The zeta potential of *Chlorella* sp. in growth medium (BG-11) was measured within the pH ranges (2.0, 4.0, 6.0, 8.0, and 10.0). Zeta potential was performed in triplicate at 25°C and the average values have been taken into account.

2.6.1.4. Techno-economic analysis of TES based PBR for algal biomass and biofuel production: a comparative study

Techno-economic study for algal biofuel production process was carried out on the basis of industrial discharge capacity and rate of algal production. Growth rate of alga and its lipid productivity on per day basis was selected for calculation of profit in terms of biodiesel production per day. On the other hand, wastewater treatment capacity of alga was also considered to reduce the cost involve in complete biofuel production process.

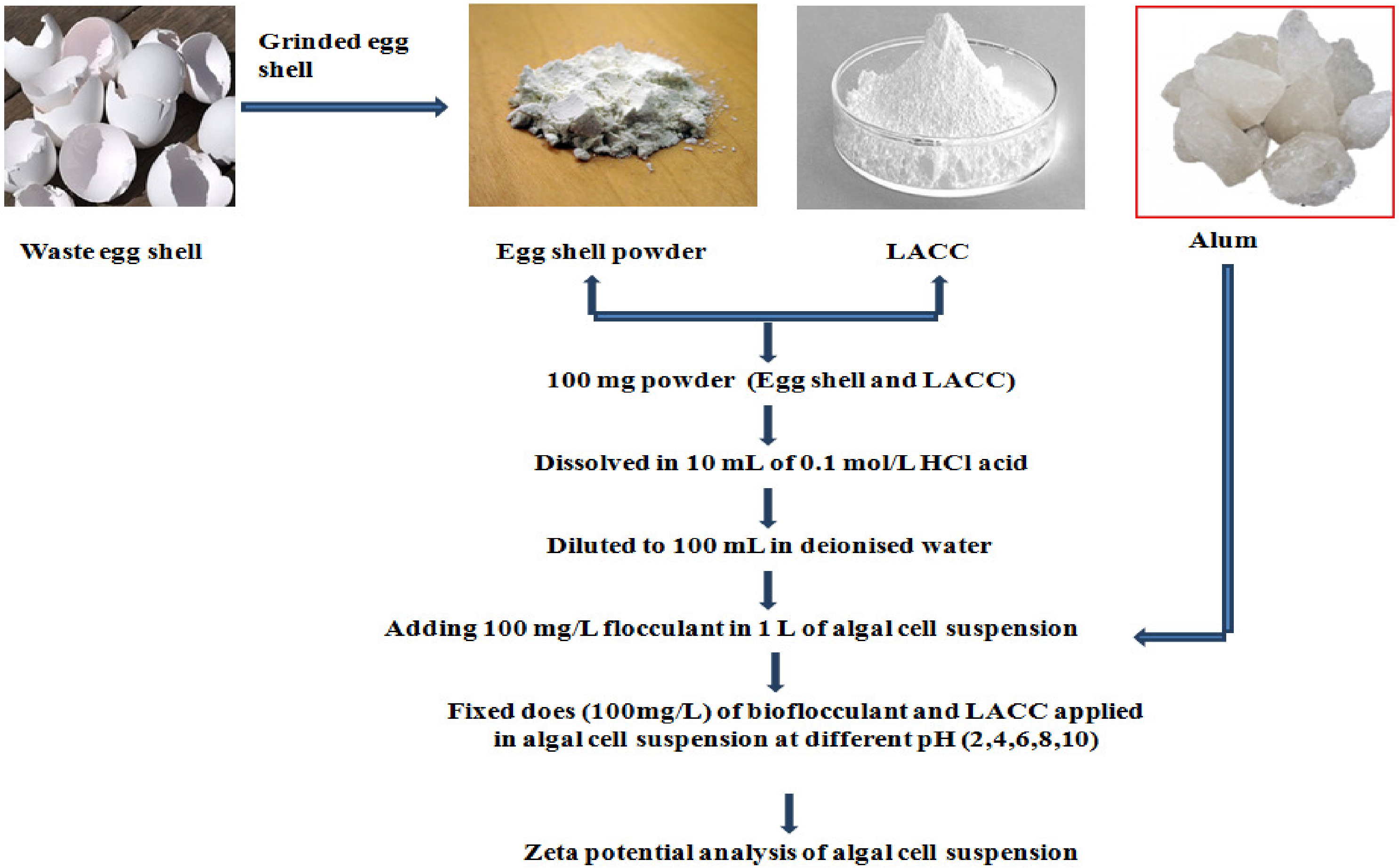


Fig. 2.9: Process route of algal biomass harvesting

Cost analysis of TES based PBR with other photobioreactor has been predicted. Annual cost method, saving per day and payback period has also been critically discussed in techno-economic analysis of Chapter-6.

2.6.1.4.1. Annual cost method (ACM)

Annual cost method (ACM) is implemented due to the fact that systems under our consideration for evaluation did not have same life times. The system annual cost (A_C) by considering time value of money concept may be evaluated as:

$$A_C = A_{FC} - S_{FF} * SV + I_C * C_{RF} + A_{MC} \tag{2.20}$$

WhereA_{FC}, annual cost of fuel, [INR]; SV, salvage value of system; I_C, system initial cost, [INR]; A_{MC}, annual cost for maintenance, [INR/year].

The termsS_{FF}, C_{RF} are the sinking fund factor and capital recovery factor respectively. These two terms can be written as:

$$S_{FF} = \frac{i}{[(i+1)^j - 1]} \tag{2.21}$$

$$C_{RF} = \frac{(i+1)^j * i}{[(i+1)^j - 1]} \tag{2.22}$$

Where$i$, interest rate, [INR]; j, operational life of system.

The annual fuel cost of TES-PBR is the total cost of electrical energy consumption for heating of water during cloudy or rainy days. Where, the annual cost of fuel for EC-PBR and GS-PBR is the total cost of electricity and LPG gas used to meet hot water requirement for desired temperature range (Kablan 2004). For evaluation of annual cost of fuel, daily hot water need for growth of algae has to be calculated. The energy required to meet daily hot water requirement may be evaluated as:

$$E_{DHR} = m_w * C_{pw} * \left(T_{wf} - T_{wi}\right) \tag{2.23}$$

Where, m_w, mass of hot water requirement per day, [Litre/day];C_{pw}, specific heat of water [kJ/kg. K];T_{wf}andT_{wi} are the initial and final temperature of water respectively.

Thus annual cost of fuel of TES-PBR and EC-PBR could be calculated as per according to following equation:

$$A_{FC} = \frac{N * E_{DHR} * E_C}{\eta_{el}} \tag{2.24}$$

Where,N, number of days electricity used to fulfill the need of hot water requirement [days] ;E_C,electricity cost [INR/kWh];η_{el}, electrical coil efficiency.

The annual cost of fuel of GS-PBR can be calculated as:

$$A_{FC} = \frac{P_{LPG} * E_{DHR} * 365}{CV_{LPG} * \eta_{GB}} \tag{2.25}$$

Where,P_{LPG}, price of LPG [INR./kg];CV_{LPG}, calorific value of LPG [kWh/kg];η_{GB}, efficiency of gas burner.

The salvage value of system is varying with operational time (j) of the system. Therefore, by considering that system depreciation with respect to time is linear in nature, hence salvage value can be calculated as:

$$SV(j) = j * I_C \left[\frac{1}{j} - \frac{1}{j_{max}}\right] \tag{2.26}$$

Wherej_{max}, is the maximum time after that system is totally discarded [Year]

For making the analysis simpler, from market survey it is noted that annual cost for maintenance for EC-PBR and GS-PBR is 5% of annual capital cost whereas in case of TES-PBE, no maintenance is required.

2.6.1.4.2. Saving per day

The total saving/day for TES-PBR may be calculated by evaluation of savings by production of hot water by EC-PBR, GS-PBR, and TES-PBR at desired temperature per litre and then this saving multiply by total hot water requirement in a day.

$$S_{hw/l} = C_{cs/l} - C_{PCM-PBR/l} \tag{2.27}$$

Where, $S_{hw/l}$ savings in hot water production per liter [INR];$C_{cs/l}$ cost of production of hot water per liter by conventional systems (EC-PBR and GS-PBR) [INR];$C_{PCM-PBR/l}$ cost of production of hot water per litre by TES-PBR [INR]

Where,

$$C_{cs/l} = \frac{C_{CS/A}}{P_{cs/A}} \tag{2.28}$$

$$C_{PCMWHS/l} = \frac{C_{PCM-PBR/A}}{P_{PCM-\mathrm{PBR}/A}} \tag{2.29}$$

$$S_{day} = S_{hw/l} m_w \tag{2.30}$$

Where,$C_{cs/A}$ and $C_{PCM-\mathrm{PBR}/A}$cost of production of hot water [INR] by conventional system and TES-PBR per annum respectively; $P_{CS/A}$ and $P_{PCM-PBR/A}$ hot water production per year by conventional and TES-PBR respectively [Litre]; S_{day}savings in hot water production per day [INR].

2.6.1.3. Payback period

Payback period is the period of time required for an investment to recover its initial investment in terms of savings (Sreekumar 2010). In order to calculate the economic feasibility of TES-PBR payback period is evaluated by following equation:

$$PP_N = \frac{ln\left[1-\frac{C_I}{S_1}(i-f)\right]}{ln\left(\frac{1+f}{1+i}\right)} \tag{2.31}$$

WherePP_N, payback period for N years;S_1 benefit during first year (INR)

2.7. Instrumental analysis

2.7.1. Spectroscope

Growth measurement of algal cell was carried out every day using spectrophotometer (HALO-DB 20, Thermo Scientific) in terms of absorbance taken at 665nm.

2.7.2. Atomic absorption spectrometer

Heavy metals analysis of CETP wastewater has been done by using Atomic Absorption Spectrometry (AA240FS, Fast Sequential Atomic Absorption Spectrometer).

2.7.3. Fourier transform infrared spectroscopy (FTIR)

FTIR analysis has been done on algal based biofuel analysis particularly algal lipid and oil. A Perkin Elmer spectrum RX/FTIR system has been used to obtain IR spectrum within the range of 4000 cm^{-1} to 500 cm^{-1}.This technique works on the principle of interaction between infrared radiation and algal sample usually in form of solid liquid and gas. This instrument basically measures the frequencies to which sample absorbs with absorption intensities too. These frequencies are significant to identify the chemical functional groups of sample.

2.7.4. Scanning electron microscope (SEM) and Energy-dispersive X-ray spectroscopy (EDS)

Algal cell surface characterizations were analyzed by SEM-EDS (SAM: model: JSM-6490LV, Make: JEOL, Japan) to know the algal surface structural morphology and cell wall composition with respect to elements. A scanning electron microscope is based on characteristic feature of electron microscope that enabled to produce sample images by scanning cell surface area with focused beam of electrons. Whereas, EDS works on the principle of interaction of X-ray sources (excited energy) and sample. The fundamental of this analysis is that each element has unique atomic structure and allows forming a unique set of peaks on its electromagnetic emission spectrum. The basic mechanism of analysis consists of taking dried algal cell coated with thin layer of gold using a sputter coater to incorporate into SEM and EDS unit to investigate the algal cell surface structural and compositional changes.

2.7.5. Zeta potential analysis

An instrument, Nanoplus Zeta /nano particle analyzer (Model: Nano Plus-3, Serial no. 405613, made in Japan), was used to investigate the zeta potential of the system (bioflocculant) to analyze the harvesting efficiency.

2.8. Conclusion

This chapter summarizing the basic requirements for execution of experimental objectives in general and statistical as well as thermodynamic functions, and analytical instruments to investigate for Phase-I, II, and III, whereas, experimental findings from these phases on individual basis will part of upcoming chapters 3, 4 and 5.

Chapter 3

Phycoremediation of industrial wastewater and their impact on algal biochemical compounds using Chlorella pyrenoidosa with correlation study

3.1. Introduction

The significant properties of algae *i.e.* biochemical compounds (protein, carbohydrate, pigments, carotenoids, lipid, and fatty acids) and biofuels production (biodiesel, biohydrogen, biogas, and bioethanol) gained attention to focus on enhanced production of algal biomass (Kothari et al., 2017; Tong et al., 2014). Algae can be cultured by the application of various conditions. By the change in process parameters, enhanced algal biomass production can be obtained. Therefore, large scale cultivation of algae is of great magnitude to increase the algal based green economy. In natural cultivation system as well as in artificial media, algae exposed to a number of environmental factors *i.e.* pH, light, and nutrient sources *etc.* out of which the accessible biogenic elements concentrations are the most important parameters that affect algal growth (Daliry et al., 2016; Velu et al., 2015). These variables (chemical and physical conditions) directly influence all the aspects of algal activity such as biomass productivity, specific growth rate, doubling time, metabolism intensity *etc.* (Babu and Binnal, 2015; Blair et al., 2013). Therefore, optimization of process parameters of algal culture efficiently enhanced the yield for algal biomass production with desired metabolic products. Earlier scientists have demonstrated that under specific/optimised/selected conditions, algae have momentous possibility to produce bio-oil/lipid for biodiesel production forty times higher than oil seed crops such as soy and canola (Juneja et al., 2013). An alga produces protein, carbohydrate, and lipid by using light and nutrient in photosynthesis. In order to understand the synergistic interface between environmental/physical and chemical factors, optimization with multiple variables of these factors is required to develop high algal productivity. Hence, it is significant to investigate the effect of parameters on algal biomass growth and development. The methods adopted to investigate the impact of parameters on algal growth have been clearly discussed in Phase-I of chapter 2. The present chapter deals with optimization of selected process parameters and their correlation for algal growth with real wastewater and media as control. Impacts of process parameters with CETP wastewater concentrations have been also studied to investigate the growth rate of *Chlorella pyrenoidosa* simultaneously with pollution reduction (physico-chemical and heavy metals in CETP wastewater). In order to understand the correlation study between parameters and algal growth and productivity, Pearson correlation co-efficient has been applied. The present chapter is divided into three main sections:

3.1.1. Optimization of selected process parameters and their correlation with algal growth in BG-11 media

3.1.2. Optimization of different concentrations of wastewater using *Chlorella pyrenoidosa* in integration with remediation, impact of heavy metals on algal growth

3.1.2. Effect of CO_2 on algal growth by using optimised CETP wastewater concentration

3.2. Materials and methods

3.2.1. Algal species

Chlorella pyrenoidosa has been selected for this experimental work and all relevant informations regarding the collection of algae, media used *etc.* are discussed in section 2.6.1.1., of Chapter 2.

3.2.2. Impact of process parameters

Various physical parameters *i.e.* pH, light photoperiod and chemical parameters *i.e.* nitrate, phosphate and carbon source (as salt and gaseous form) are responsible for growth and productivity of *Chlorella pyrenoidosa.* Various parameters and their ranges are used to investigate the effect of working parameters on algal biomass growth, discussed in section 2.6.1.1., of Chapter-2

3.2.3. Biochemical analysis

The analytical methods used for algal biochemical compounds are discussed in Annexure 1.

3.2.4. Wastewater collection and characterization

Wastewater from common effluent treatment plant has been selected for this experimental study. Detailed information about selected CETP wastewater has been provided in Section 2.4., of Chapter-2.

3.2.4. Optimization of different concentrations of CETP wastewater

Four different concentrations (25%, 50%, 75% and 100% V/V) have been used to investigate the potential growth of *Chlorella pyrenoidosa* on wastewater.

3.2.5. Pearson correlation co-efficient

In order to understand the positive or negative cross interaction/correlation between different variables of process parameters *i.e.* pH, light, and nutrient and algal biochemical compounds (protein, carbohydrate, and lipid), as well as effect of heavy metals in CETP wastewater and algal biochemical compounds (protein, carbohydrate, pigments), Pearson correlation co-efficient (a statistical analysis) has been applied with the obtained data. The applied statistical analysis provides an accurate data to understand the positive or negative impact between these variables.

3.3. Results and discussion

Optimization of various process parameters and wastewater concentrations with respect to algal biomass growth and biochemical compounds has been assessed and analysed here in section 3.3.1. Furthermore, phycoremediation, heavy metals reduction, and effect of various concentrations of CO_2 gas on algal biomass productivity with CO_2 sequestration capability has also been given in section (3.3.2.) with finding.

3.3.1. Optimization of selected process parameters and their correlation with algal growth in BG-11 media

3.3.1. Effect of process parameters on algal growth and biochemical compounds

Different parameters (pH, photoperiod, nitrate, phosphate, and carbon) and their effect on algal biomass production, specific growth rate, doubling time with biochemical compound (protein, carbohydrate and lipid) have been enlisted in Table 3.1.

3.3.1.1. pH

Effect of initial pH on algal growth was investigated with different ranges of pH from 6.0, 6.5, 7.0, 7.5, and 8.0. pH is an important parameter in algal biomass cultivation, which plays a significant role to determine the solubility and availability of CO_2 and nutrients (nitrate, phosphate and carbon) and has a direct impact on algal metabolism too. Optimum growth of algal biomass occurs around neutral pH. Changing pH in algal media may limit algal growth through the metabolic inhibition. At acidic pH (6.0, 6.5) algae slowly enters into acceleratory experimental phase followed by a 5 days of lag phase. On the other hand, growth of alga at pH 7 enters into exponential phase earlier than the pH 6.0. The best and fast growth was achieved at pH 7.5 where a short lag phase was observed of 3 days and alga enters into acceleratory exponential phase after 3rd day and achieved highest growth on 15th day as illustrated in Fig. 3.1. (a). Maximum algal biomass production was obtained at pH 7.5 *i.e.* 1.6±0.02 gL^{-1} whereas, at pH 6.0 algae showed minimum productivity 0.62±0.03 gL^{-1}.In terms of biochemical compounds (protein and carbohydrate), the present experimental results examined that maximum protein and carbohydrate contents (1.64 mgL^{-1} and 1.18 mgL^{-1}) were obtained at pH 7.5 as demonstrated in Fig 3.1 (b and c). Although, effect of different pH have also been studied on algal biomass protein and carbohydrate by Khalil et al., (2010). Similar to this context, author has reported maximum carbohydrate content of *C. ellipsoidea* was obtained at pH 9 and decreased significantly at pH 10, while the lowest value of carbohydrate was reported on pH 4. In order to support the present experimental result Kalil et al., (2010) and Gong et al., (2015) also reported the alkaline environment ranges from 8-10 pH for optimum growth of *Chlorella vulgaris*. Pruder and Bolton (2017), observed that *T. Pseudonana* adapted to low pH 6.5 showed a slow growth rate. Similar to this context, Visviki and Santikul, (2000) reported the optimum growth of *C. applanata* at pH (7.4). This variation in pH directly affects on distribution of CO_2 species and carbon availability, whereas higher pH level directly affects the physiological process of algal cells. Relative concentrations of CO_2, HCO_3^- and CO_3^{-2} of the carbon forms varies according to pH ranges. Higher the pH, CO_3^{-2} increases but bicarbonate and molecular CO_2 decreases. Hence, obtained data suggest a generalised pattern for growth at variation in pH, but it may be a species-specific response. So, pH may an important abiotic factor affecting the ecology and physiology of algal biomass. The lipid accumulation property of algal biomass with different variables of pH indicates higher lipid accumulation 26.5±0.02% (161.2±0.21 mgL^{-1}) at low pH (6.0). Therefore, *Chlorella pyrenoidosa* shows a higher lipid production under acidic condition. As pH of the medium moved from acid to base, lipid production decreased. The lowest amount of lipid was obtained at higher pH 7.5. Similar to this study, Moheimani (2013) reported 99±17.2 mgL-1D-1 and 92±13.1 $mgL^{-1}D^{-1}$ lipid production by *T. Suecica* CS-187 and *Chlorella* sp. at pH 7.0 and 7.5 respectively. Hence, it can be concluded that optimization of pH ranges for maximum algal production and lipid are 7.5 and 6 respectively.

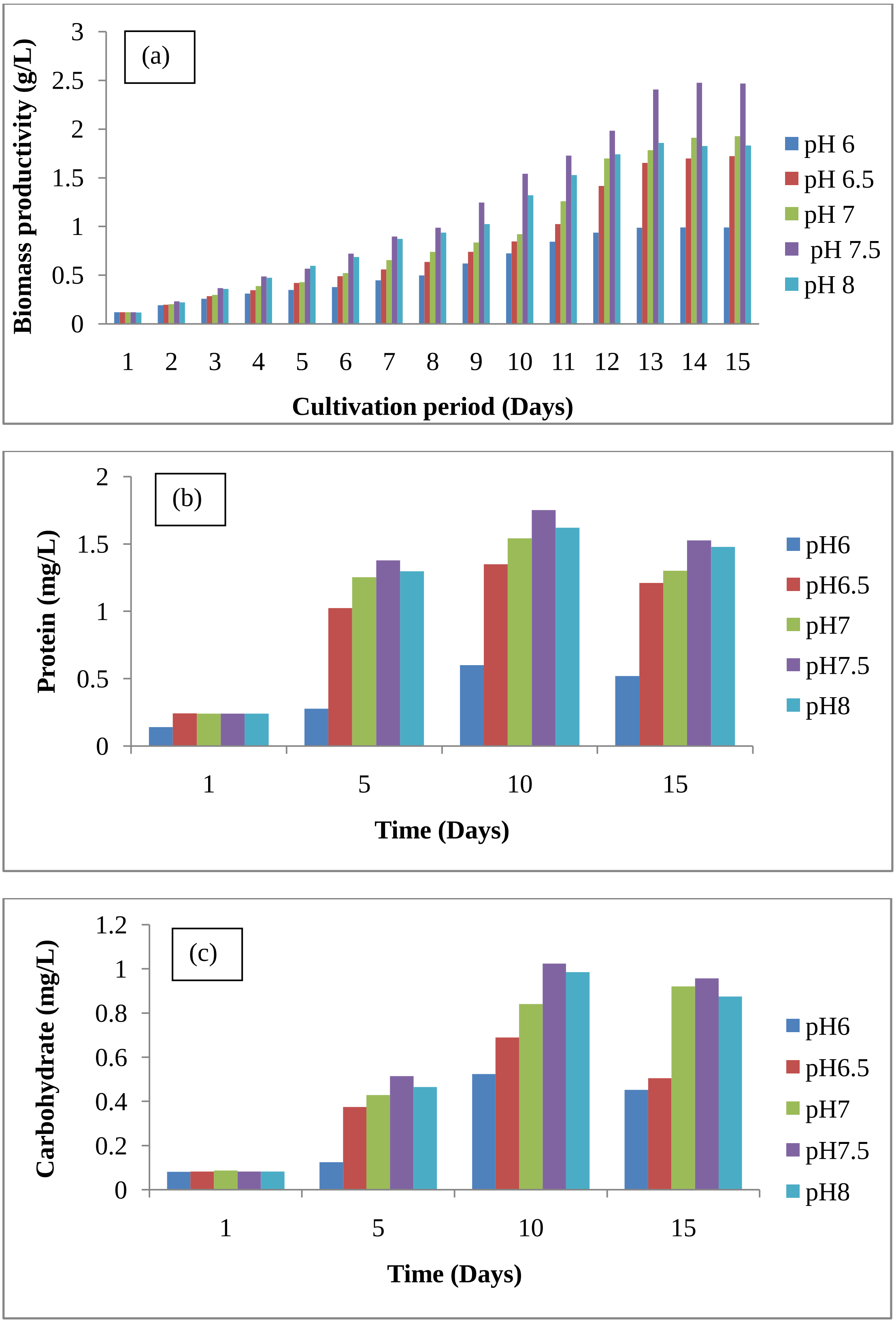


Fig. 3.1: Effect of pH on algal biomass: (a) productivity: (b) protein; and (c) carbohydrate

Therefore, pH fluctuation either due to controlled/uncontrolled in medium also changes the lipid quantity. Our results also asses that pH has inverse relationship with membrane lipid quantity as TAG accumulation increase/decrease as per the conditions provided to the medium.

3.3.1.2. Light photoperiod

The growth of stationary culture of *Chlorella pyrenoidosa* in BG-11 media, was monitored for 15 days at four different light photoperiods (24L, 18L:06D, 16L:08D, and 12L:12D) of light and dark cycle. It is a crucial factor for *Chlorella pyrenoidosa* that determine the autotrophic growth and photosynthetic activity. The present experimental work was based on to provide four different light photoperiods for *Chlorella pyrenoidosa* to check the best photoperiod for algal growth and development in terms of biomass productivity and biochemical compounds. The result revealed that the optimized photoperiod was obtained with 16L: 08D for *Chlorella pyrenoidosa* with maximum biomass productivity (1.52±0.03gL^{-1}) and minimum (0.62±0.07 gL^{-1}) at 24h duration of light as illustrated in Fig. 3.2 (a). It might be possible that the low algal biomass productivity is due to prolong period of exposure towards the light of *Chlorella pyrenoidosa*. Although, photosynthesis and cell photo-acclimatization both are light driven process, but the response for photoperiod is vary with different algal species. Similar type of findings is also cited by Gammanpila et al., (2015), reported that 0.63 gL^{-1} of algal biomass (*Chlorella vulgaris*) was recovered with light and dark cycle of 16:08 hour. Therefore, the best growth of algal biomass supports the 16:08h and minimum at 24h of light and dark cycle respectively. Apparently, this is due to the photoperiod (24L:0D), *Chlorella pyrenoidosa* perform photo reduction that absorbs light energy and stored it in ATP and NADPH (energy-carrying molecules). These energy pool molecules can be used in biomolecules synthesis that promotes the growth of microalgae. In light and dark cycle (18L:06D, 16L:08D, and 12L:12D), *Chlorella pyrenoidosa* exposed to the dark period too, in which light independent reaction can also be performed through Calvin cycle that operates during the dark phase of photosynthesis. This chemical reaction converts CO_2 and other compounds into glucose by using ATP and NADPH from photo reduction. The effect of different photoperiod have also been studied on biochemical compounds and the maximum protein, carbohydrate, and lipid was obtained (1.6 mgL^{-1}, 0.98 $mgL^{-1}$and 27.6±0.11%) at 16L:8D, 24L:0D, 16L: 08D conditions respectively as demonstrated in Fig. 3.2 (b and c). Rai et al., (2015) have reported maximum lipid production (26.8%) when exposed to 24 hour light to *Chlorella* sp. Lipid content was found maximum with 16L:8D (light and dark) cycle (present study) whereas, Rai et al., was found maximum lipid 24h exposure to light of algal species. Although, algal photosynthesis and cell photo-acclimatization are light driven process, therefore, responses to photoperiods are species-specific process. Algal biomass and lipid production in various algae increased under high light and low dark conditions, which may be the response of algae for enhanced lipid production until saturation point was observed, afterward photo-inhibition (excess light cannot be received by photosynthetic apparatus) takes place, subsequently, algae limits further production.

3.3.1.3. Nutrients

Considerable changes in biochemical compounds of algal biomass can be observed under the conditions of nutrient limitations. In general, the growth rate of algal biomass is directly related to the uptake rate of most limiting nutrient under optimal condition (favourable temperature 25-30°C and pH 7-9).

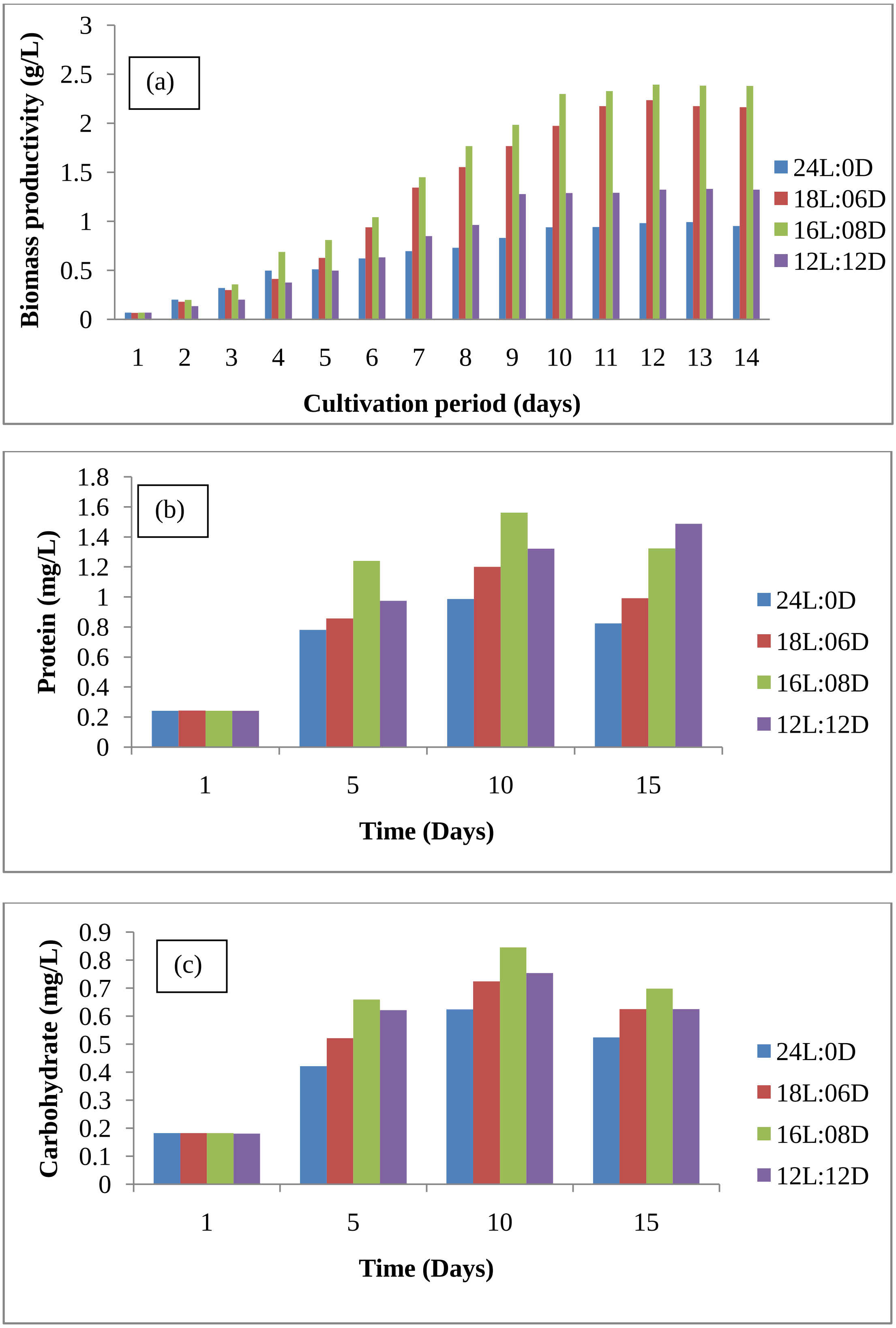


Fig. 3.2: Effect of light on algal biomass: (a) productivity; (b) protein; and (c) carbohydrate

3.3.1.3.1. Nitrate

Effect of nitrate on algal growth was investigated with variable of nitrate ranges from 0.05gL^{-1}, 0.1gL^{-1}, 0.2gL^{-1}, and 0.4gL^{-1}concentrations in culture medium. Nitrate is a significant constituent of all structural as well as functional proteins in algal cells and contributes around 7-20% of dry algal biomass. At lower nitrate concentration (0.05 gL^{-1}), alga shown a longer lag phase of 4 days, whereas, log phase was achieved on 8th day of cultivation period. In case of 0.1 gL^{-1} nitrate concentration, lag phase of alga become shorter, though, exponential phase of algal growth were similar to the lag phase obtained with lowest nitrate concentration. Maximum biomass productivity was achieved with 0.4 gL^{-1} of nitrate concentration1.72±0.02 gL^{-1}, which is characterized by a short lag phase with highest biomass productivity on 14th day of cultivation period as depicted in Fig. 3.3 (a). Taziki et al., (2015), reported maximum biomass productivity of *Chlorella vulgaris* 1.03±0.13gL^{-1} at 3 gL^{-1}of nitrate concentrations in BG-11 media. The protein and carbohydrate content in *Chlorella pyrenoidosa* was obtained maximum 1.9 and 1.2 mgL^{-1} with optimum concentration of nitrate 0.4 gL^{-1} as demonstrated in Fig. 3.3 (b and c). The maximum amount of lipid (28.5 %) was obtained with 0.05 gL^{-1}of nitrate concentration. Millan-Oropeza et al., (2015) have also found the 49.7% of lipid production with 250 mgL^{-1}of nitrate concentration. Higher lipid synthesis showed follow similar trends as reported in *P. Tricornutum* cultivated under nitrate stressed condition containing 0.5 mM sodium nitrate (Ge et al., 2014). The algal lipid synthesis increased in nitrate deprived conditions. It happens due to the degradation of stored starch, particularly being converted into lipid. Nitrogen deprived condition affects algal biomass productivity by limiting protein synthesis rate consequently, citric acid cycle inhibited. Furthermore, it causes inadequate protein synthesis in photosynthesis, resulting decrease in carbon fixation

(Valenzuela et al., 2013; Cataldo et al., 1975). Therefore, it is clear that as nitrate play an influential role to enhance protein and carbohydrate content of algal biomass. In terms of lipid production it has been observed that reduction in nitrate concentration enhance the lipid production in *Chlorella pyrenoidosa*.

3.3.1.3.2. Phosphate

Effect of phosphate on algal growth was investigated at a range of (0.05 gL^{-1}, 0.1 gL^{-1}, 0.2 gL^{-1}, and 0.4 gL^{-1}) concentration. At lower phosphate concentration (0.05 gL^{-1}) alga slowly enters into acceleratory exponential phase followed by a 5 days of lag phase and produce lowest biomass concentration. The growth of alga at 0.1 gL^{-1} phosphate concentration, lag phase is shorten by one day 4 days, and produce more biomass concentration than achieved at 0.05 gL^{-1} concentration of phosphate. Significant growth was observed with phosphate concentration 0.2 gL^{-1} and 0.4 gL^{-1}. Algae in 0.1 gL^{-1} phosphates medium enter in exponential phase earlier than other treatments. The best growth was achieved with 0.2 gL^{-1} whereas, a short lag phase was observed of 3 days and alga enters into acceleratory exponential phase after 3rd day and achieved highest biomass productivity 1.45±0.03gL^{-1}. Fig. 3.4 (a) clearly showing biomass productivity with selected phosphate concentration for 15 days of batch experimental set-up. Mahat et al., (2014), reported maximum biomass production 0.426 gL^{-1}at 2.0 gL^{-1} of nitrate concentration with *Nannochloropsis oculata*. Whereas, maximum lipid productivity 5.7% was obtained as the concentration decreased from 2-0.1 $gL^{-1}$of phosphate. Maximum protein and carbohydrate content was obtained 1.8 $mgL^{-1}$and 1.1 mgL^{-1} at 0.4 $gL^{-1}$of phosphate concentration in algal culture medium as illustrated Fig. 3.4 (b and c).

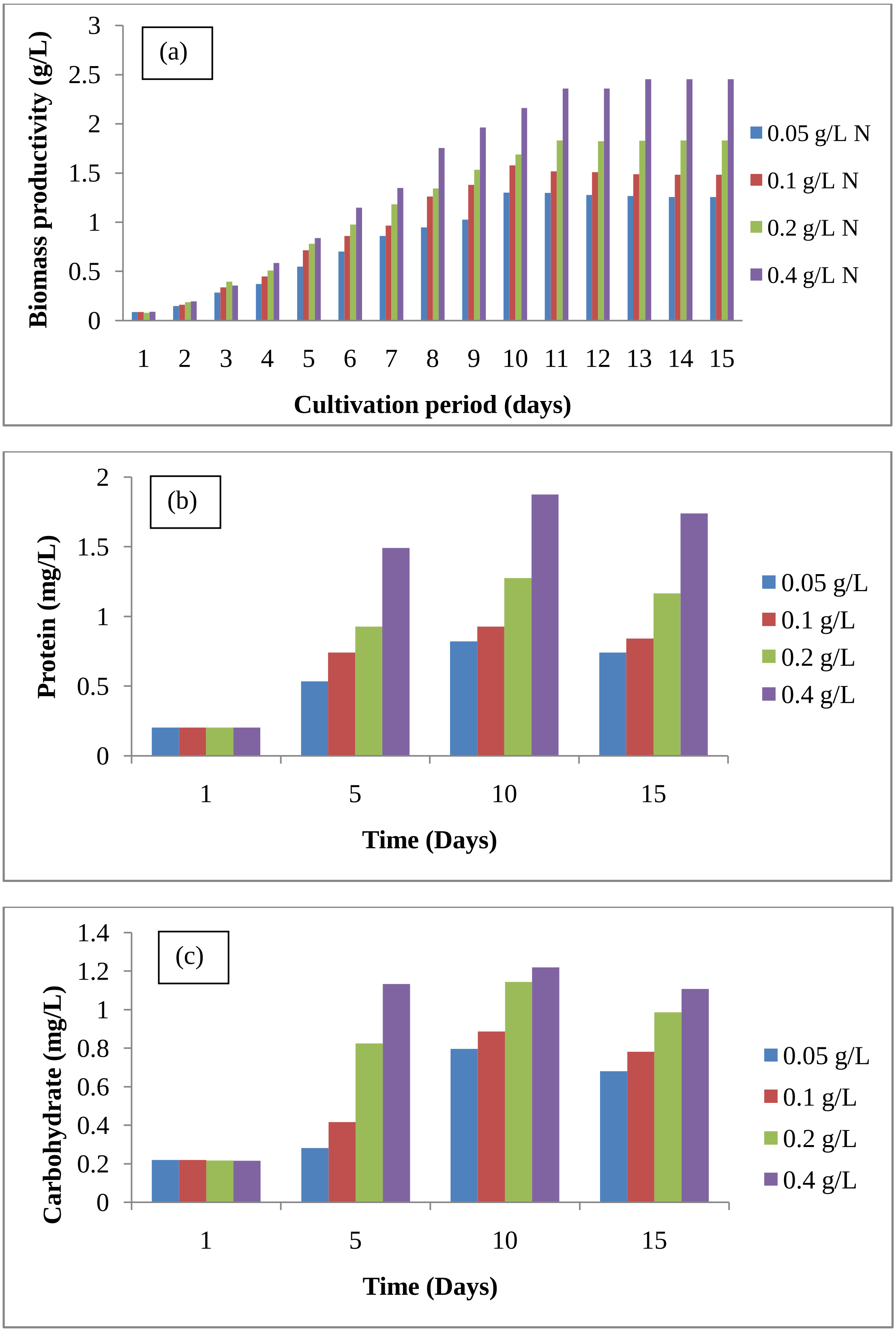


Fig. 3.3: Effect of nitrate on algal biomass: (a) productivity; (b) protein; and (c) carbohydrate

Lipid content of algal biomass was almost same at 0.05 and 0.1 gL^{-1}phosphate concentrations 25.5 and 25.1%. As the concentration of phosphate increased the lipid production decreased whereas, the protein and carbohydrate content of algal biomass increased. Li et al., (2010), reported an increase in total lipid content from 23 to 53% in *Scenedesmus* sp. with reduction in phosphate concentration from 0.1 to 2.0 mgL^{-1}. Phosphate uptake is a function of pH, which induced phosphate uptake activity under non starved condition. In comparison to other nutrient (carbon, nitrogen, and hydrogen) phosphorus is not needed for growth in large quantity, but it is one of most common limiting elements for algal growth. According to literature, phosphate uptake by algal cells follows three types of mechanism such as (i) luxury consumptions (ii) ability to use phosphate at low concentration (iii) alkaline phosphatage production (Subramanian et al., 2009). Subsequently, decrease in biomass production with higher consumption of phosphate may be due to "luxury consumption" of phosphate by algal cells resulting, storage of polyphosphate granules in cells.

3.3.1.3.3. Carbon

The present experimental work was subjected to provide different concentration (0.05 gL^{-1}, 0.1gL^{-1}, 0.2gL^{-1}, and 0.4 gL^{-1}) of Na_2CO_2. The experiment was run for the 15 days and found the maximum biomass productivity (1.76±0.07 gL^{-1}) with 0.2 $gL^{-1}$of carbon concentration. Carbon is one of the other essential nutrients that must be supplied in sufficient quantity for photosynthesis. The fixed carbon by algal biomass has three dimensions of uses (i) Respiration (ii) energy source (iii) raw material in the formation of additional algal cells. An alga requires an inorganic source to perform photosynthesis, which can be utilized in the form of CO_2, carbonate, and bicarbonate autotrophic algal growth. Algal growth at higher concentration of carbon has already been reported. But algal biomass has potential to tolerate CO_2 up to it maximum *i.e.*

carrying capacity to enhance algal biomass production. Kong et al., (2011), reported that the increase in glucose concentration from 1 to 20 gL^{-1} also increases the lag phase of cell growth with maximum biomass productivity 2.24 g L^{-1}as illustrated in Fig. 3.5 (a). The maximum protein and carbohydrate content was obtained 1.1 mgL^{-1} and 2.2 mgL^{-1}at 2$gL^{-1}$of carbon concentration in terms of Na_2CO_3 Fig. 3.5 (b and c). Whereas, the total lipid content (maximum) was obtained 26.4% at 0.05 gL^{-1}. Ogbonna and Ogbonna, (2018), reported maximum biomass production of *Dictyosphaerium* sp. 2.89±0.03 gL^{-1} with 30.0 gL^{-1} of glucose concentration, whereas, maximum lipid production was obtained 42.3±1.33 % with 30.0 gL^{-1} of glucose concentration. Therefore, it may be concluded that biomass productivity was maximum with optimised concentration of carbon but lipid content decreased with high concentration of carbon source. Minimum lipid production was obtained at higher carbon concentration. It is due to the presence of excess carbon source resulting inhibition of lipid biosynthesis *i.e.* saturation limit in carbon uptake using photosynthesis, which links to low lipid production.

3.3.1.4. Correlation analysis

Table 3.2 describes interaction between different parameters (pH, photoperiod, nitrate, phosphate and carbon) and their affect on protein and carbohydrate of *Chlorella pyrenoidosa* by the application of Pearson correlation coefficient analysis. The Pearson correlation co-efficient (r) measures the strength and direction of a linear relationship between two variables. The effect of different parameters *i.e.* pH, light, and nutrients (nitrate, phosphate, and carbon) shows a strong positive linear relationship for protein and carbohydrate. The statistical data collected from Pearson correlation co-efficient analysis shows a strong correlation between all the variables (parameters) and algal biochemical compounds.

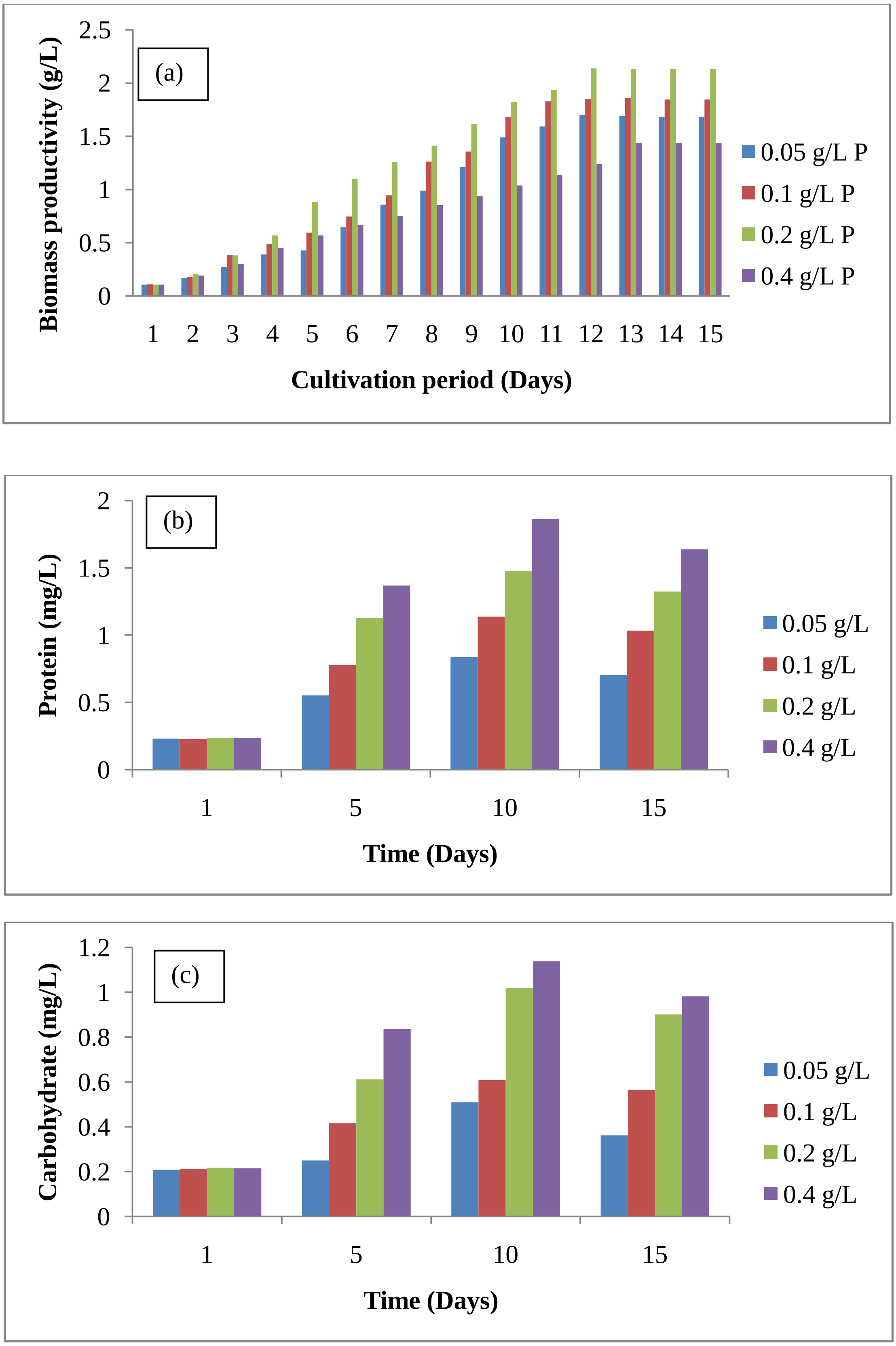


Fig. 3.4: Effect of phosphate on algal: (a) biomass productivity; (b) protein; and (c) carbohydrate

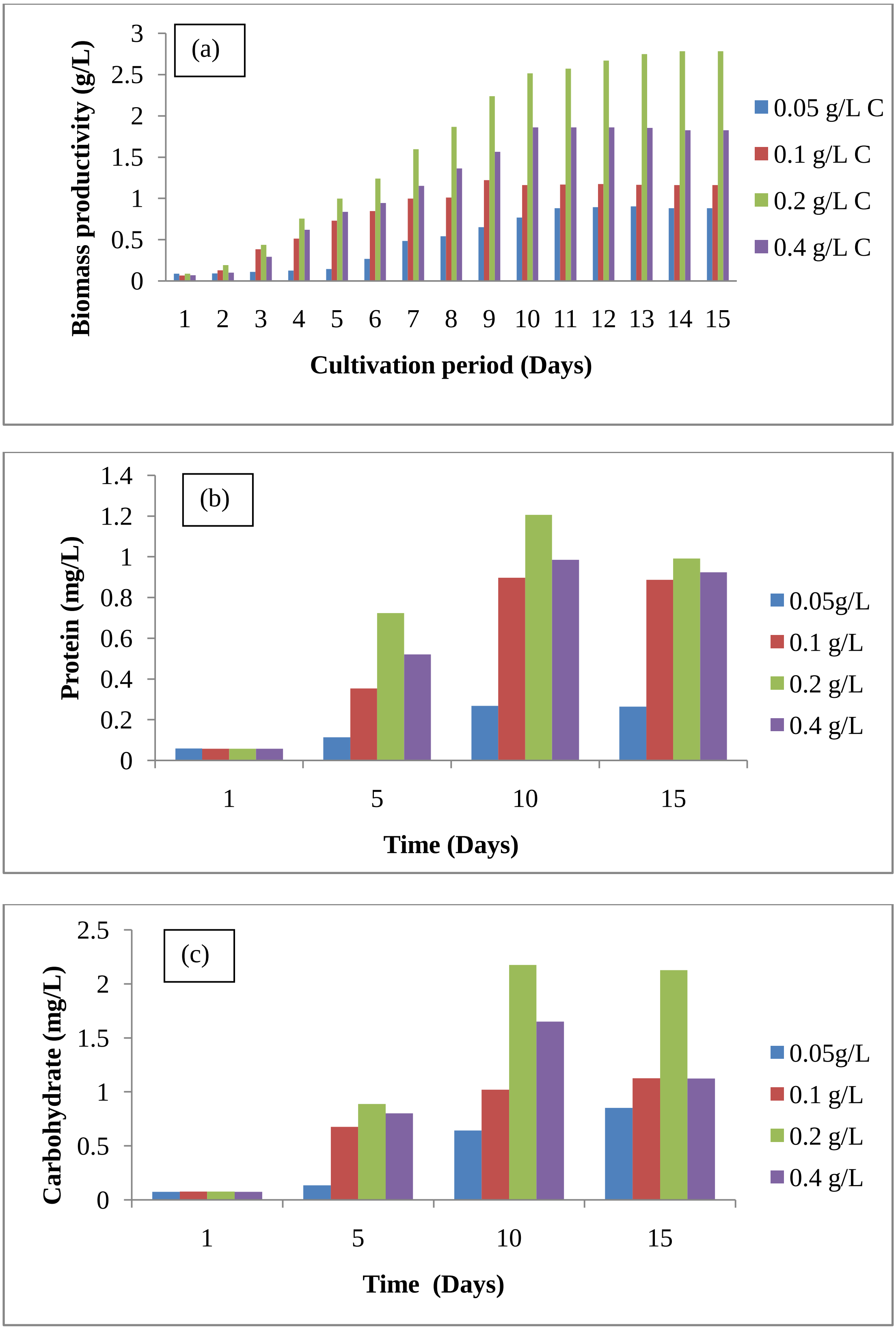


Fig. 3.5: Effect of carbon on algal biomass: (a) productivity; (b) protein; and (c) carbohydrate

Table 3.1: Process parameters, biomass productivity, specific growth rate, generation time, and lipid content

S.No.	Parameters	Ranges	Maximum biomass productivity (gL^{-1})	Biomass productivity ($mgL^{-1}D^{-1}$)	μ (Specific growth rate, Day^{-1})	Generation time (day^{-1})	Lipid content (%)
1.	pH	6	0.62±0.03	95.0±0.04	4.5±0.08	0.15±0.02	26.5±0.02
		6.5	1.05±0.01	161.3±0.05	5.1±0.05	0.16±0.02	25.6±0.09
		7	1.2±0.08	174.4±0.1	5.2±0.02	0.16±0.07	21.5±0.07
		7.5	1.6±0.02	231.1±0.31	5.5±0.07	0.17±0.07	19.2±0.08
		8	1.12±0.04	181.2±0.09	5.2±0.01	0.16±0.04	19.5±0.04
2.	Light	24L:0D	0.62±0.07	92.4±0.08	4.5±0.02	0.22±0.05	19.5±0.01
		18L:6D	1.42±0.02	218.3±0.11	5.4±0.07	0.19±0.03	25.6±0.07
		16L:8D	1.52±0.03	231.4±0.34	5.4±0.03	0.18±0.03	27.2±0.11
		12L:12D	0.84±0.06	129.7±0.02	4.9±0.04	0.21±0.07	15.5±0.01
3.	Nitrate (gL^{-1})	0.05	0.82±0.04	122.5±0.01	4.8±0.04	0.21±0.03	28.5±0.03
		0.1	1.0±0.02	143.8±0.07	4.9±0.02	0.2±0.08	26.4±0.21
		0.2	1.15±0.01	177.2±0.05	5.2±0.04	0.19±0.09	23.2±0.02
		0.4	1.62±0.04	238.8±0.04	5.5±0.03	0.18±0.04	22.6±0.07
4.	Phosphate (gL^{-1})	0.05	0.81±0.02	164.72±0.09	5.1±0.04	0.2±0.03	25.5±0.06
		0.1	1.21±0.09	180.4±0.08	5.2±0.01	0.19±0.07	25.1±0.07
		0.2	1.45±0.03	206.9±0.087	5.3±0.03	0.18±0.04	23.2±0.08
		0.4	0.98±0.05	138.7±0.07	4.9±0.04	0.20±0.03	21.9±0.06
5.	Carbon (gL^{-1})	0.05	0.57±0.07	89.4±0.08	4.4±0.07	0.22±0.05	26.4±0.09
		0.1	1.01±0.03	115.5±0.09	4.7±0.04	0.21±0.03	20.4±0.07
		0.2	1.76±0.07	272.98±0.04	5.6±0.03	0.17±0.07	23.6±0.04
		0.4	1.16±0.01	114.58±0.06	4.7±0.07	0.21±0.07	19.2±0.05

In case of lipid, as the pH increases, the amount of total lipid decreases. It clearly indicates that low pH supports high production of total lipid. The statistical data also shows a perfect downhill (negative) linear relationship between these two valuables (pH and lipid). pH fluctuation either due to controlled/uncontrolled in medium also changes the lipid quantity. Our results also assessed that pH has inverse relationship with membrane lipid quantity as TAG accumulation increase/decrease as per the conditions provided to the medium. In case of different photoperiods subjected to the algal biomass, indicates low production of lipid when the light provided for 24L:0D and 12L: 12D, while at 18L: 6D, maximum lipid production was obtained.

Therefore, a sharp decrease in total lipid production has been obtained when the photoperiod was less. *Chlorella pyrenoidosa* perform photo reduction that absorbs light energy and stored it in ATP and NADPH (energy-carrying molecules). These energy pool molecules can be used in biomolecules synthesis that promotes the growth of microalgae. The statistical graph shows a weak uphill linear relationship between these two variables (photoperiod and lipid). In case of nitrate and phosphate, as the concentration of these two nutrient increases, the amount of total lipid decreases. The low concentration of nitrate and phosphate supports the high production of lipid. Higher lipid synthesis showed follow similar trends as reported in *P. Tricornutum* cultivated under nitrate stressed condition containing 0.5 mM sodium nitrate (Ge et al., 2014). The algal lipid synthesis increased in nitrate deprived conditions. It happens due to the degradation of stored starch, particularly being converted into lipid. Nitrogen deprived condition affects algal biomass productivity by limiting protein synthesis rate consequently, citric acid cycle inhibited. Phosphate uptake is a function of pH, which induced phosphate uptake activity under non starved condition. In comparison to other nutrients (carbon, nitrogen, and hydrogen) phosphorus is not needed for growth in large quantity, but it is one of most common limiting elements for algal growth. According to literature, phosphate uptake by algal cells follows three types of mechanism such as: (i) luxury consumptions (ii) ability to use phosphate at low concentration (iii) alkaline phosphatage production (Subramanian et al., 2009). While in case of carbon source, as the concentration increases, the amount of total lipid also increases. The statistical data also indicates a strong downhill linear relationship with nitrate and phosphate while it is a strong positive linear relationship with carbon source. However, minimum lipid production was obtained at higher carbon concentration. It is due to the presence of excess carbon source resulting inhibition of lipid biosynthesis saturation limit in carbon uptake using photosynthesis, which links to low lipid production (Chiu et al., 2009; Lepage et al., 1984). Hence, it is clear from the above discussions that stress condition of pH and nutrient (nitrate and phosphate) promotes the high production of lipid.

Various authors have reported the biomass productivity under varying concentration of pH, nitrate, phosphate and carbon sources. The results from present study revealed that significant dosage of nutrients with optimal pH and photoperiods are required to achieve higher biomass concentration. Optimised value of process parameters obtained in section 3.3.1 of this chapter. pH, light photoperiod used in section 3.3.2., of Phase-I where wastewater has been taken as nutrient medium and Phase-II to obtain maximum algal biomass productivity and lipid content for biofuel production.

Table 3.2: Correlation analysis between process parameters and biochemical compounds of algae

S.N.	Parameters	Ranges	Protein	Carbohydrate
1.	pH	6	0.90*	0.90*
		6.5	0.82*	0.90*
		7	0.67**	0.89*
		7.5	0.69**	0.86*
		8	0.81*	0.91*
2.	Light	24h	0.89*	0.96*
		18:06h	0.84*	0.88*
		16:08h	0.67*	0.85*
		12:12h	0.81*	0.83*
3.	Nitrate (gL^{-1})	0.05	0.97*	0.79*
		0.1	0.97*	0.94*
		0.2	0.97*	0.98*
		0.4	0.99*	0.97*
4.	Phosphate (gL-1)	0.05	0.88*	0.84*
		0.1	0.92*	0.95*
		0.2	0.90*	0.95*
		0.4	0.83*	0.82*
5.	Carbon (gL^{-1})	0.05	0.97*	0.99*
		0.1	0.96*	0.99*
		0.2	0.96*	0.99*
		0.4	0.94*	0.99*

***strong positive correlation, ** week positive correlation**

3.3.2. Optimization of different concentrations of wastewater using *Chlorella pyrenoidosa* in integration with remediation, impact of heavy metals on algal growth with correlation study

3.3.2.1. Initial characterization

The collected sample was dark brown in colour with pungent odour with high rate of pollutant contamination. Due to this high concentration of pollutant load, direct use of CETP wastewater for algal cultivation is not possible. The initial concentrations of TDS, TSS, BOD, COD, nitrate, and phosphate were obtained 13887±6.0, 9588±3.6, 539.3±1.5, 1284.6±3.7, 26.0±1.0 and 7.53±0.35 in CETP wastewater respectively. The heavy metal concentration in selected wastewater shows a high concentration of Cr (VI) (11.4 mgL^{-1}) followed by the Cu (4.4mgL^{-1}), Pb (2.6 mgL^{-1}), Zn (7.1 mgL^{-1}), Cd (3.7 mgL^{-1}), Mn (3.8 mgL^{-1}), and Ni (3.7 mgL^{-1}) in CETP wastewater. Various researchers have also reported higher concentration of organic and inorganic pollutants in wastewater from common effluent treatment plant (Chandra et al., 2011; Hangargkar and Takpere, 2015; Shukla et al., 2007). In this experimental set-up, two controls were considered *i.e.* BG-11 media ($Control_{BG}$) to achieve algal growth curve (section-3.1.2.2) while optimising different concentrations of wastewater. Wastewater (without algal treatment) has been taken as control while it was treated with *Chlorella pyrenoidosa* for heavy metal removal.

3.3.2.2. Algal growth

On the basis of four different concentrations (25%, 50%, 75%, and 100%) of CETP wastewater, algal growth and wastewater optimization have been done. Algal growth was determined Growth characteristics of *Chlorella pyrenoidosa* showed a lag phase of 4 initial days after that alga enters to acceleratory log phase as illustrated in Fig 3.6. The maximum biomass productivity was obtained with 25 and 50% test solution of

CETP wastewater1.4±0.07 gL^{-1} which is almost equal to the $control_{BG}$ medium. The maximum specific growth rate 4.9±0.06 $mgL^{-1}d^{-1}$ and biomass productivity 139±0.07 $mgL^{-1}d^{-1}$ was obtained in 50% test solution of CETP wastewater. An increase in the specific growth rate and biomass productivity was observed on increasing the concentration from 25% to 50%, while further increase in the concentrations (75 to 100%) was found to inhibit the algal growth. Thus, 50% concentration of CETP wastewater supported highest algal growth, subsequently, maximum specific rate and biomass productivity (Table 3.3). Hence, the sequence of different concentrations of wastewater favoured maximum algal growth are 50>25>75>100 % with maximum biomass production *i.e.* 1.4±0.07>1.3±0.08>0.6±0.03>0.3±0.04 gL^{-1}. The results indicated that alga tolerate pollution load up to 50% in a better extent, thus heavy metal resistant mechanism can play a crucial role for the growth and development of algae in wastewater solution. Similar to the present study, Lim et al., (2010), also optimized the different concentration of industrial effluent (Textile industry) and reported 60% wastewater concentration for higher biomass yield. In another study Pathak et al., (2015) observed 25% concentration of textile industry wastewater for higher biomass yield (14 µg/ml/day) of *C. pyrenoidosa*. The potential to tolerate high pollution load depends on type of algal strains. Algal growth is directly influenced by various biotic and abiotic factors. As the algal biomass transferred from BG-11 based media to different concentrations of CETP wastewater based environment, algae usually experiences a wide change in nutrient profile of medium. Algae faces slow growth rate in 75% and 100% concentrations of CETP wastewater than other diluents. It may be possible due to the presence of higher concentrations of heavy metals in the medium that reduced algal productivity. Whereas, algae showed maximum growth in 25% and 50% concentration, is due to their heavy metal resistant mechanism at maximum tolerance level. Therefore, maximum tolerance level/capacity of this alga can be applied for algal based treatment system due to its potential growth in wastewater (CETP).

3.3.2.3. Pollution removal by algal biomass

Algal biomass showed its potential for removal of organic as well as nutrient (inorganic) load from the different concentration of CETP wastewater. The pollution removal efficiency was found to be influenced by the strength of wastewater as well as growth potential of algae. The pH of selected concentration of CETP wastewater was changed from 6.9±0.11 to 6.4±0.30, 7.6±0.2 to 6.8±0.3, 7.9±0.3 to 7.8±0.3, and 8.4±0.25 to 7.6±0.5for 25, 50%, 75% and 100% respectively as described in Table 3.4. Reduction in BOD and COD was obtained maximum in order with 25>50>75>100% of CETP wastewater *etc.* 82.3%, 71.9%, 52.2%, and 48.4% for BOD and 75.4%, 68.5%, 53.9%, and 46.5% for COD. The highest reduction in inorganic pollutant obtained for nitrate was 90.3%, 82.12%, 64.8%, and 48.3% whereas, reduction in phosphate concentration was obtained 85.3%, 78.4%, 51.4%, and 46.8% with 25, 50, 75, and 100% of CETP wastewater concentration respectively. Ajayan et al., (2014) reported 35% and 37.1% reduction in BOD and COD whereas, 44.3% and 95% reduction was observed with nitrate and phosphate in tannery wastewater treated with *Scenedesmus* sp. Kothari et al., (2012), reported 60% and 87% reduction in nitrate and phosphate using *Chlorella pyrenoidosa* with dairy industry wastewater. Therefore, it is clear that algal biomass uptake nitrate in significant amount for its growth and development.

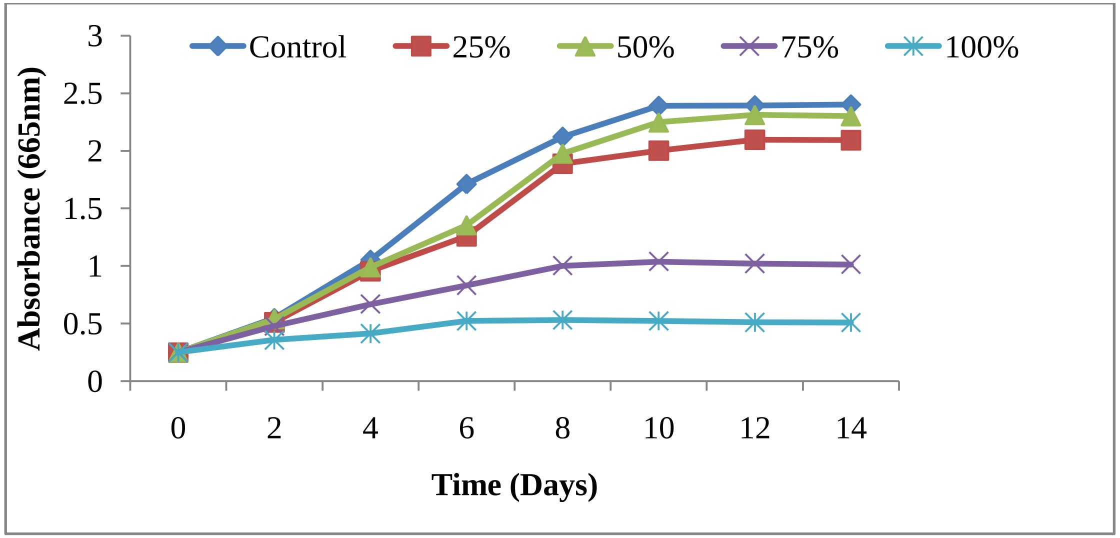


Fig. 3.6: Growth curve of *Chlorella pyrenoidosa* in CETP wastewater

Table 3.3: Growth kinetics of *Chlorella pyrenoidosa*

Concentrations	**Maximum biomass production (gL^{-1})**	**Biomass productivity ($mgL^{-1}d^{-1}$)**	**µ (Specific growth rate, Day^{-1})**	**Generation Time (Day^{-1})**	**Lipid (%)**
Control	1.5±0.03	143±0.4	4.9±0.07	0.20±0.05	23.6±0.04
25%	1.3±0.08	125±0.06	4.8±0.05	0.20±0.09	26.3±0.11
50%	1.4±0.07	139±0.07	4.9±0.06	0.20±0.01	27.6±0.15
75%	0.6±0.03	45±0.04	3.8±0.09	0.26±0.03	17.1±0.09
100%	0.3±0.04	27±0.05	3.3±0.07	0.30±0.07	12.3±0.14

It might be due to higher uptake of nutrients for the survival of cell growth under stress condition (Pathak et al., 2015). Algae require nutrients (nitrate, phosphate, and carbon) for it growth, development and biochemical synthesis. Various industrial wastewater (textile, dairy, tannery, and CETP wastewater) are important source of nutrients too that acts as substrate for algal growth. Therefore, this industrial wastewater can be directly or after dilution can be used as source of nutrient for algae. Essential nutrients (nitrate, phosphate, carbon) are required by algae for growth and development, subsequently, significant reduction is obtained after treatment with algae. Hence, it is clear that algal biomass potentially significant to remove organic and inorganic pollutant load from wastewater.

3.3.2.4. Heavy metal removal by *Chlorella pyrenoidosa*

In addition to removal of nutrient and organic load, heavy metals were also found to be reduced by *Chlorella pyrenoidosa* from different concentration of CETP wastewater. Collected wastewater was consisted of various heavy metals such as Cr (VI), Cu, Pb, Zn, Cd, Mn, and Ni. The heavy metal concentration in selected wastewater shows a high concentration of Cr (VI) (11.4 mgL^{-1}) followed by the Cu (4.4 mgL^{-1}), Pb (2.6 mgL^{-1}), Zn (7.0 mgL^{-1}), Cd (3.7 mgL^{-1}), Mn (3.6 mgL^{-1}), and Ni (3.8 mgL^{-1}) in wastewater. After treatment with algal biomass *i.e. Chlorella pyrenoidosa*, heavy metals were reduced significantly as described in Table 3.5. Heavy metals (Cr (VI), Cu, Pb, Zn, Cd, Mn, and Ni) have been removed very effectively from the wastewater, with the removal rates ranging from 80%, 64%, 88%, 82%, 50%, 64%, and 81% in 25% wastewater concentration, 73%, 60%, 75%, 66%, 87%, 83%, 74% and 47% in 50% wastewater concentration, 57%, 59%, 70%, 56%, 72%, 66%, and 62% in 75% wastewater concentration, and 47%, 55%, 56%, 71%, 61%, 77%, and 72% respectively in 100% wastewater concentration. Maximum concentration of Cr (VI) in was identified (11.34 mgL^{-1}) with 100% concentration of CETP wastewater. After treated with *Chlorella pyrenoidosa* Cr (VI) concentration of 100% concentration of wastewater was removed to 5.99 mgL^{-1} (47.2 % reduction). Hence, dilution of wastewater with distilled water played a crucial role to enhance the percentage reduction. The removal efficiency of Cr (VI), Cu, and Pb increased rapidly with lower concentrations of wastewater. Generally, removal rate kinetics shows inverse relationship with the increase in concentration of heavy metals. But, *Chlorella pyrenoidosa* did not follow the usual trend in this experimental work for some metals. In case of Zn and Ni maximum removal efficiency was allied with 100% test solution. Mn shows maximum removal efficiency (77%) with 100% test solution in comparison with 75% test solution with obtained removal efficiency of 66%. It might be possible due to stress condition faced by algal biomass in 100% concentration of CETP wastewater with low nutrient availability and maximum heavy metals load. Many studies have clearly demonstrated the potential of metal removal from wastewater by algal biomass. A comprehensive study has been conducted by Malla, (2012) on CETP wastewater and found the initial concentration of Cd (0.15-0.2), Fe (0.44-5.8), Pb (0.16-0.2), Zn (0.04-0.5), and Cu (0.4-0.77) mgL^{-1} with reduction 9-30%, 68-77%, 100%, 15-93%, and 80-90% respectively by using *Chlorella minutissima.* Chauhan and Khambholja, (2017) reported the initial concentration of Zn (4.18 mgL^{-1}), Cu (9.38 mgL^{-1}), and Ni (1.18 mgL^{-1}) in CETP wastewater. By the treatment of *Cladophora*, the final concentration of metal ions reduced to Zn (0.08 mgL^{-1}), Cu (0.72 mgL^{-1}), Ni (1.182 mgL^{-1}).The potential of algal biomass with heavy metal removal have been reported by various researchers (Ayangbenro et al., 2017; Lee et al., 2016; Ungureanu et al., 2015).

Table 3.4: Pollution reduction (%) of different concentrations of CETP wastewater treated with *Chlorella pyrenoidosa*

Parameters		**25(%)**	**50(%)**	**75(%)**	**100(%)**
pH		6.9±0.11	7.6±0.2	7.9±0.3	8.4±0.25
		6.4±0.30	6.8±0.3	7.8±0.3	7.6±0.5
TDS (mg/L)	Initial	3385.3±9.0	7213.3±4.9	10386±5.03	13887±6.0
	Final	1020±8.5	2867±5.5	4534±5.0	6237±7.0
	(%) Removal	69.8	60.1	56.3	55.1
TSS (mg/L)	Initial	1959.6±9.0	4291.6±5.0	7539±8.3	9588±3.6
	Final	763±5.5	1935±3.6	3817±5.2	4993±9.5
	(%) Removal	61.0	54.9	49.3	47.9
BOD (mg/L)	Initial	132.3±4.16	270.6±1.5	328.3±3.0	539.3±1.5
	Final	23.3±2.0	76±2.0	157±5.5	278±3.6
	(%) Removal	82.3	71.9	52.2	48.4
COD (mg/L)	Initial	265±4.0	584.3±5.0	749.3±1.5	1284.6±3.7
	Final	65±3.6	183.6±3.0	345±2.0	687±5.2
	(%) Removal	75.4	68.5	53.9	46.5
Phosphate (mg/L)	Initial	2.0±0.26	4.43±0.41	6.5±0.3	7.53±0.35
	Final	0.29±0.015	0.95±0.03	3.15±0.03	4.0±0.11
	(%) Removal	85.3	78.4	51.4	46.8
Nitrate (mg/L)	Initial	6.6±0.26	12.5±0.35	18.6±0.36	26.0±1.0
	Final	0.63±0.03	2.24±0.04	6.5±0.03	13.4±0.25
	(%) Removal	90.3	82.12	64.87	48.3

The composition of algal cell wall is basically carbohydrates and polysaccharides with negative charged groups of other components *etc.* hydroxyl, amino, carboxyl or sulfohydryl group. Cell wall of algae and cyanobacteria are composed of carbohydrates and polysaccharides with negatively-charged groups (e.g. hydroxyl, amino, carboxyl or sulfohydryl group). Most positively-charged metals can tightly bind to the negatively-charged ligand groups, which is the basis of metal removal from metal containing wastewater.

Micronutrient transporters play significant role to transport heavy metals into algal cell membrane. Binding of heavy metals takes place to the specific cellular compounds/compartments resulting detoxification of heavy metals from the medium (wastewater) to algal cells occur. Seldom algal based chelation process also takes place to minimise the wastewater metal toxicity. Algal biochemical compounds *etc.* polysaccharides, protein, and lipid posses' functional group (amino hydroxyl, carboxyl and sulfate) acting as binding sites for heavy metals. The biosorption process of heavy metals by algal cells takes place in two steps (a) instant physical adsorption among heavy metal ions of wastewater and cell surface and then slow chemical adsorption. Therefore, it is clear that CETP wastewater is heavily loaded with numerous heavy metals and algal biomass (cellular or filamentous) play a crucial role to minimise the concentration of these heavy metals. The concentration of different heavy metal varies from place to place and depends on the industries situated around the CETP as it is a mixture of different industrial wastewater. Similarly, concentration of heavy metal ions in the wastewater also varied from one study to other study due to dilutions at that collection point via other type of wastewater.

Table 3.5: Heavy metal concentration (mgL^{-1}) and percentage removal in CETP wastewater

Treatment concentration		Cr (VI)	Cu	Pb	Zn	Cd	Mn	Ni
Control (CETP wastewater 100%)	Initial	11.34	4.37	2.58	7.11	3.65	3.81	3.76
	Final	11.08	4.13	2.51	7.11	3.59	3.79	3.69
	% Removal	2.3	5.5	3.1	0.14	1.4	0.78	2.12
25%	Initial	3.08	0.89	0.09	1.78	0.05	0.68	0.99
	Final	0.61	0.11	0.01	0.32	0.03	0.24	0.18
	% Removal	80.2	64.0	88.5	82.02	50	64.7	81.8
50%	Initial	5.94	1.43	0.89	2.99	0.98	1.36	1.83
	Final	1.58	0.56	0.22	1.01	0.11	0.23	0.46
	% Removal	73.4	60.8	75	66.2	87.7	83.0	74.8
75%	Initial	8.83	3.02	1.76	4.03	2.04	2.75	2.11
	Final	3.71	1.21	0.53	1.77	0.57	0.91	0.79
	% Removal	57.9	59.9	70.5	56.1	72.05	66.9	62.6
100%	Initial	11.34	4.37	2.58	7.11	3.65	3.81	3.76
	Final	5.99	1.94	1.13	2.06	1.39	0.87	1.04
	% Removal	47.2	55.6	56.2	71.0	61.9	77.2	72.3

3.3.2.5. Average removal rate (K) kinetics for metal uptake

The K value was found different for observed heavy metals, which revealed that alga posses different level of affinity to uptake the heavy metal. In present study, *Chlorella pyrenoidosa* showed highest affinity for uptake of Cr followed by Zn, Cu and Pb. The K value is also found to be influenced by hydraulic retention time *etc.* contact time as well as concentration of effluent. Increase in the concentration of effluent was tending to increase the affinity of metal on to algal biomass. Thus the metal uptake rate (mg heavy metal $L^{-1}D^{-1}$) for Cr was varied from (5th day to 15th day) 2.62 to 3.0.3 for 25%, 0.57 to 5.8 for 50%, 5.78 to 8.12 for 75% and 10.8 to 11.2 for 100% concentration of CETP wastewater. Metal uptake rate for Cu varied from 0.73 to 0.88 for 25%, 1.2 to 1.39 for 50%, 1.46 to 2.79 for 75% and 4.13 to 4.33 for 100% concentration of CETP wastewater. Similar trend of increase in the uptake rate (K) was found with remaining metals as shown in the Table 3.6. Similar to the present study various researchers have also investigated the uptake rate of heavy metals by using a number of biological agents such as aquatic plant, algal biomass *etc.* Thus, algal biomass in wastewaters provides a simple, durable method for reduction of metals pollutants. Dried algal biomass have also potential to remove heavy metals from metal containing industrial wastewaters, as metals and action exchanger can be regained by desorption with acids or desorbing agents (Sbihi et al., 2012; Ayangbenro and Babalola, 2017). Thus, algal biomass in wastewaters provides a simple, durable method for reduction of metal pollutants. The affinity of algal biomass for heavy metal uptake is due to the large surface area and high binding affinity. Obtaining higher algal biomass even in presence of heavy metal (particularly Cr (VI) due to its high concentration) with significant concentration of Cr (VI) uptake are attributes of special interest in present algal strain *Chlorella pyrenoidosa* which is potential in phycoremediation programs for wastewater treatment containing metals as well as salts.

3.3.2.6. SEM and EDS analysis

SEM and EDS analysis was done on before and after treatment of algal biomass obtained from different concentrations (25%, 50%, 75%, and 100%) of CETP wastewater as illustrated in Figure 3.7 and 3.8. SEM images and EDS graph of algal biomass (before treatment) shows smooth and clear surface with devoid of heavy metals presence before exposure to heavy metals. Whereas, SEM analysis revealed the change in cell surface structural morphology of *Chlorella pyrenoidosa* treated with different concentrations of CETP wastewater. The alteration in cell surface morphological characterization of *Chlorella pyrenoidosa* after interaction with heavy metals showed structural deformities, surface modifications and size enlargement. Such deformities were possible due to characteristic change in algal cell morphology in response to heavy metals stress. The EDS analysis were done to analyse the heavy metal concentration in algal cell surface and observed the peaks of Cr with other trace elements in EDS spectra. Presence of trace and absence of peaks of other metals in EDS spectra shows that heavy metal removal by *Chlorella pyrenoidosa* might be attributed to intracellular mechanism, instead of heavy metal adsorption by cell surface.

3.3.2.7. Correlation analysis

Table 3.7 presented the interaction of heavy metal with biochemical composition (carbohydrate, protein and pigments) of *Chlorella pyrenoidosa* by employing the Pearson correlation coefficient analysis.

Table 3.6: Average removal rate (mg heavy metal L^{-1}D^{-1}) kinetics of metal by *Chlorella pyrenoidosa*

Heavy metals	Days	25%	50%	75%	100%
Cr (VI)	5th	2.62	0.57	5.78	10.81
	10th	2.93	5.68	6.26	11.12
	15th	3.03	5.84	8.12	11.25
Cu	5th	0.73	1.23	1.46	4.13
	10th	0.83	1.33	2.37	4.27
	15th	0.88	1.39	2.79	4.33
Pd	5th	0.08	0.79	1.14	2.46
	10th	0.09	0.84	1.50	2.53
	15th	0.095	0.87	1.65	2.55
Zn	5th	1.60	2.54	2.26	6.74
	10th	1.74	2.80	3.40	6.98
	15th	1.75	2.92	3.69	7.06
Cd	5th	0.05	0.85	1.46	3.50
	10th	0.64	0.92	1.80	3.58
	15th	0.05	0.97	1.93	3.62
Mn	5th	0.57	1.13	1.46	3.63
	10th	0.64	1.26	2.39	3.74
	15th	0.66	1.34	2.57	3.79
Ni	5th	0.84	1.64	1.27	3.63
	10th	0.96	1.74	1.82	3.708
	15th	0.97	1.80	1.96	3.739

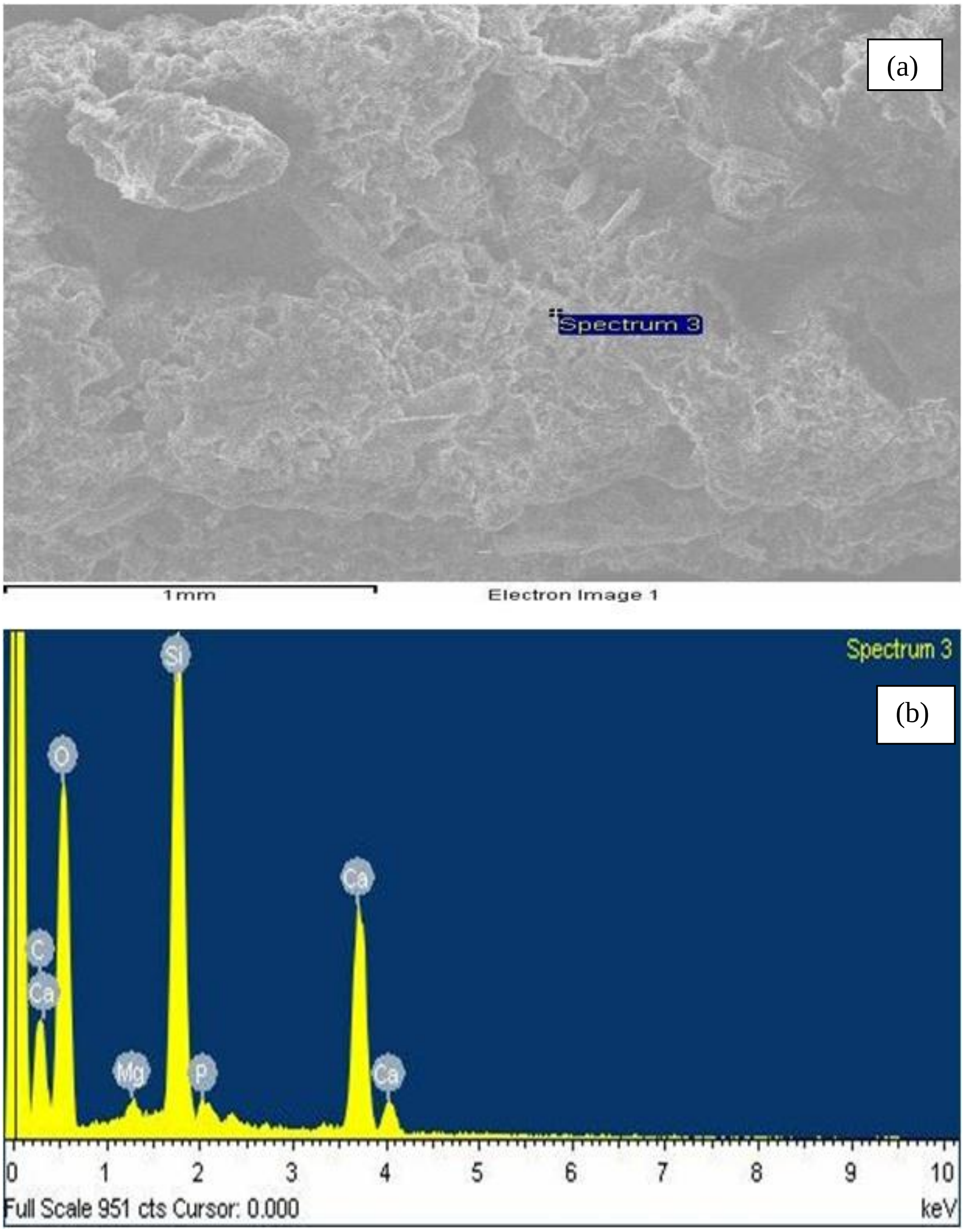


Fig. 3.7: Images of algal cells before treatment: (a) SEM; (b) EDS

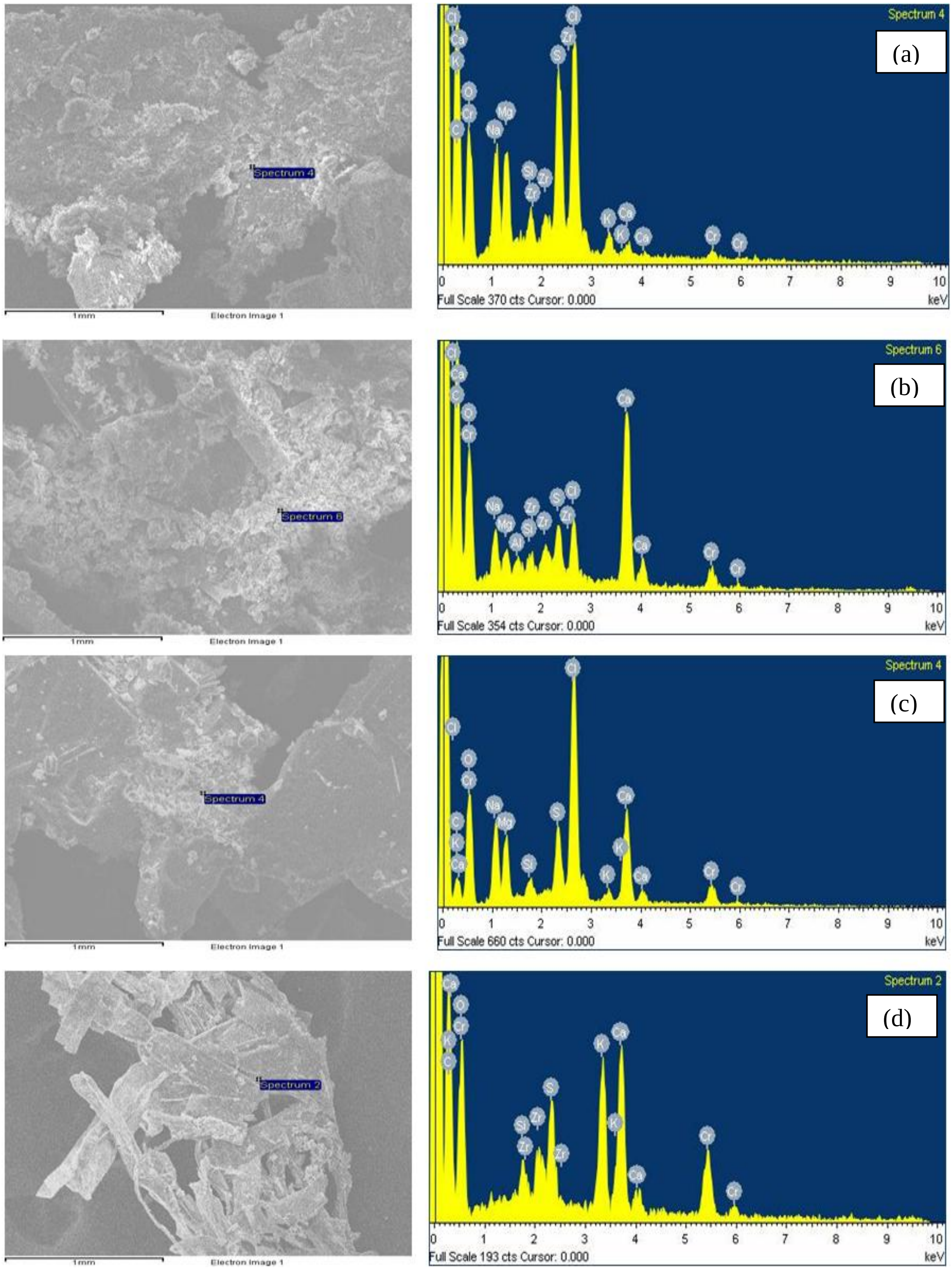


Fig. 3.8: SEM and EDS images of algal cells after treatment: (a) 25%; (b) 50%; (c) 75%; and (d) 100% concentrations of CETP wastewater

Correlation analysis revealed strong downhill linear relationship between all selected metal and protein content of alga at 50, 75% and 100%concentration CETP wastewater, while moderate and weak downhill relationship found for the Zn and Mn in relation to the algal protein at 25% concentration CETP wastewater. Correlation between metal and carbohydrate content of algal biomass revealed strong downhill relationship at all selected concentration CETP wastewater (*i.e.* 50 to 100%). In this case at 75% concentration, Cr, Pb and Cd showed almost perfect downhill relationship with carbohydrate content of alga. Correlation between chlorophyll '*a*' and metal revealed strong downhill relationship at 50% CETP wastewater. While weak downhill relationship was found at 100% CETP wastewater concentration. At 75% concentration, strong downhill relationship was found with all selected metal except Cu. Correlation of chlorophyll '*b*' and total chlorophyll in relation with selected metals was found strong downhill at all selected CETP wastewater concentration. A remarkable change in carbohydrate content with positive correlation in 100% test solution has also been noticed. It may be due to the strong counteracting mechanism of alga under highly stressed condition *i.e.* higher concentration of heavy metals. Microalgae react to heavy metal stress through various defence mechanism systems (exclusion, making complexes, compartmentalization, synthesis of binding proteins like metallothioneins (MTs), phytochelatins (PCs) and their translocation into cellular vacuoles play a vital role to avoid metal toxicity. Higher concentration of metal toxicity is usually tolerated by the presence of legends such as oxalate, histidine, nicotianamine, phosphate derivatives, carboxylic acid and citrate. At higher level of exposure of algal cells with various heavy metals present in wastewater, may suppress the expression of biochemical compound and photosynthetic pigments including stress and anti-oxidative proteins. A sharp decrease in tissue protein content with increasing Cu has been reported by Melea et al., (2006). Metal toxicity is highly related to the production of oxygen free radicals or reactive oxygen species, which is responsible to induce proteotoxicity and some other major protein expression changes in living organisms against the oxidative stress. Therefore, it is possible that *Chlorella pyrenoidosa* can grow in metal stress condition through various active legends and metal defence mechanism system. Algal cells are composed of thiol (-SH) pools, when metals are bond to this thiol pools it starts to deplete. As a consequence, antioxidant level present inside the cells makes it vulnerable to an oxidative stress condition. Therefore, algal cells passes through the higher concentration of metal-induced oxygen disruption, which intern magnify the lipid per-oxidation followed by membrane disruption of algal cells. As a result of which lifetime of algal cells become shorter. Hence, phycoremediation of heavy metal is possible but due to their insignificant counteracting mechanism on higher concentration of heavy metals it does not support a positive correlation between the biochemical compound and photosynthetic pigments. In this context, Zhang et al., (2015) has also reported a negative correlation between photosynthetic pigments and algal density of *Chlorella pyrenoidosa* in aqueous solution of Zn and Cu. The same study has also performed on *S. obliquus* and found that no significant correlations exist between carotenoid content and dissolve copper contents. In this context Zhou et al., (2012) has also reported a negative correlation between photosynthetic pigments and algal density of *Chlorella pyrenoidosa* in aqueous solution of Zn and Cu. The same study has also performed on *S. obliquus* and found that no significant correlations exist between carotenoids content and dissolve copper contents.

Table 3.7: Correlation analysis between heavy metals and biochemical compounds of *Chlorella pyrenoidosa*

Concentration (%)	**Biochemical compounds**	**Heavy metals**						
		Cr (VI)	**Cu**	**Pd**	**Zn**	**Cd**	**Mn**	**Ni**
25	Protein	-0.83229	-0.9408	-0.7743	-0.6655	-0.3130	-0.7965	-0.8264
50		-0.9258	-0.8488	-0.8888	-0.8662	-0.8329	-0.736	-0.8934
75		-0.987	-0.7521	-0.9581	-0.9564	-0.9817	-0.913	-0.9738
100		-0.77003	-0.6299	-0.7882	-0.6547	-0.8818	-0.7164	-0.9190
25	Carbohydrate	-0.9265	-0.82417	-0.89536	-0.9202	-0.90329	-0.946	-0.93507
50		-0.8791	-0.89507	-0.86971	-0.95505	-0.92152	-0.894	-0.85698
75		-0.9936	-0.83797	-0.99454	-0.95181	-0.99532	-0.8945	-0.96891
100		0.96728	0.965192	0.989658	0.978099	0.949645	0.97905	0.92909
25	*Chlorophyll*	-0.8701	-0.8701	-0.9114	-0.9634	-0.9868	-0.8974	-0.8683
50		-0.93084	-0.88597	-0.90419	-0.91527	-0.88227	-0.806	-0.90291
75		-0.86996	-0.4359	-0.79589	-0.69186	-0.88355	-0.5804	-0.74375
100		-0.28467	-0.17975	-0.3833	-0.2325	-0.43217	-0.2659	-0.4871
25	Chlorophyll *b*	-0.90107	-0.76428	-0.9178	-0.96106	-0.97092	-0.9260	-0.90283
50		-0.93564	-0.85343	-0.89876	-0.86259	-0.83079	-0.7252	-0.9048
75		-0.83728	-0.36748	-0.75138	-0.64864	-0.85085	-0.5353	-0.70356
100		-0.85381	-0.85877	-0.91093	-0.88496	-0.85568	-0.8776	-0.84334
25	Total Chlorophyll	-0.905	-0.76964	-0.92361	-0.96546	-0.97011	-0.9293	-0.90618
50		-0.9462	-0.87234	-0.91309	-0.88027	-0.85097	-0.7490	-0.91788
75		-0.97773	-0.6632	-0.92946	-0.90248	-0.97673	-0.8396	-0.93095
100		-0.82597	-0.78953	-0.88828	-0.82206	-0.86963	-0.8321	-0.87521

3.3.3. Effect of CO_2 on algal growth by using optimised CETP wastewater concentration

3.3.3.1. Algal growth on different concentration of CO_2

The carbon parameter in CO_2 experimental system was representative of the artificially induced CO_2 via CO_2 gas cylinder. *Chlorella pyrenoidosa* was subjected to four different concentrations (5%, 10%, 15%, and 20%) of carbon (gaseous form) where BG-11 and 50% concentration of CETP wastewater was taken as control and nutrient substrate respectively for algae. It was found that as the flow rate of CO_2 gas increased from 5% to 20% for *Chlorella pyrenoidosa*, the dissolved inorganic carbon also increased. A remarkable decrease in pH was found as the flow rate of CO_2 gas increases. The standing biomass of *Chlorella pyrenoidosa* increased as the concentration of CO_2increased from 5% to 15% but at 20% alga showed saturation behaviour with decrease biomass productivity as illustrated in Fig. 3.9.

Maximum biomass productivity was obtained (2.09±0.08 gL^{-1}) in 50% test solution of CETP wastewater with 15% CO_2 supply (gaseous form). Whereas, algal culture subjected to 5% of CO_2 showed less biomass productivity 0.75±0.05 gL^{-1}. Algal culture without CO_2 supply showed less biomass productivity0.5±0.04 gL^{-1} as given in Table 3.8. Hence, it is clear that CO_2 plays significant role to amplify algal biomass growth and development. Similar to this study, effect of different concentration of CO_2on algal biomass has also been studied by Rendon et al., (2013). The author has reported maximum biomass production of *Chlorella vulgaris* 1.59±0.021 gL^{-1} at 8.5% CO_2concentration. An experiment was performed by Sung et al., (1998), and obtained an increase in biomass production from 0.5-5.7 gL^{-1} as the CO_2 concentration increased to 10%.

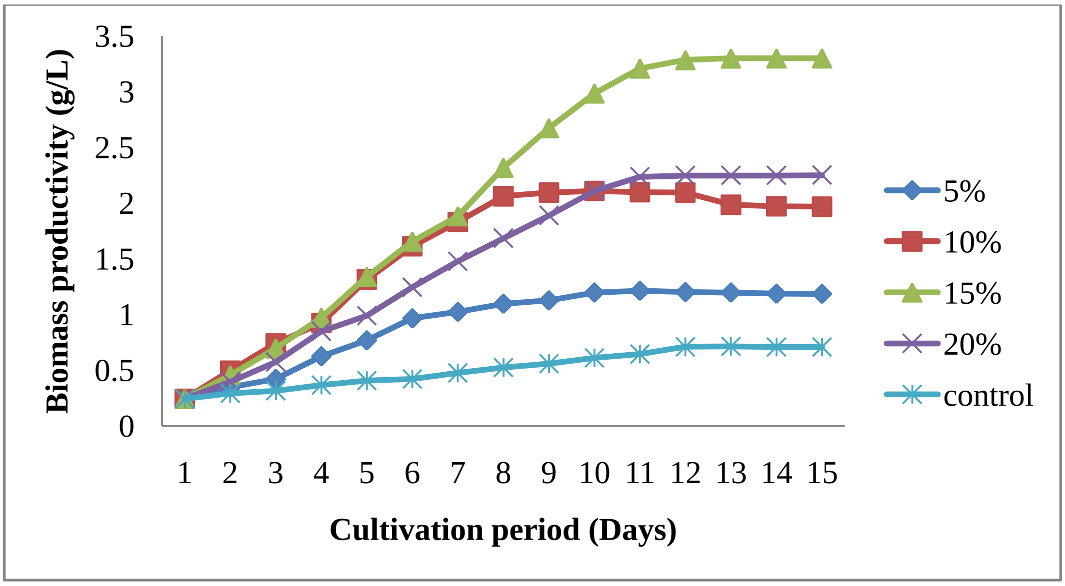


Fig. 3.9: Growth curve of *Chlorella pyrenoidosa* at different concentrations of CO_2

Algal biomass subjected to 2% and 10% of CO_2 resulted 1.6 to 1.5 gL^{-1} of biomass after 6 day of incubation period (Chiu et al., 2011). Concentration of CO_2 plays a significant role in photosynthesis. As its level increases, it leads to increase in the mass transfer mechanism from the gas mixture to the medium, as a consequence, decrease in pH. Due to the decline in pH, there is a drastic reduction in algal cell growth. Cyanobacteria (blue-green algae) and eukaryotic algae use bicarbonate as a carbon source with pH between ~6.4 -10.3. CO_2 rapidly gets captured into algal cells via bicarbonate transporters present in both the plasma membrane and in the chloroplast envelope of eukaryotic algae. Inside the chloroplast, bicarbonate is converted into CO^+ that can be fixed by RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase, carboxylase-oxygenase) to produce two molecules of 3-phosphoglycerate. Hence, it is clear that an increase in concentration of carbon would be able to enhance algal biomass productivity. Algae are potentially capable to perform photosynthesis process by using green house gas, $CO_2$and growing photo-autotrophically. Therefore, in order to reduce atmospheric pollutants ($CO_2$from different sources *etc.* industries) algal based systems can be integrated to use the produced $CO_2$from various point/non-point sources.

3.3.3.2. CO_2 sequestration by *Chlorella pyrenoidosa*

As the concentration of CO_2 supply increased from 5% to 20%, the amount of biologically fixed CO_2 increased *i.e.* 2.15±0.04, 2.74±0.04, 3.76±0.05, 5.5±0.07 g CO_2 L^{-1} D^{-1} with 5%, 10%, 15%, and 20% respectively at 15th day of experiment as given in Table 3.9. In case of control_{BG}, the algal culture was put devoid of CO_2 supply and covered by cotton plug so that it can receive natural CO_2. The culture of *Chlorella pyrenoidosa* in control obtained only 0.08±0.02 gCO_2 L^{-1} D^{-1}at 15th day of experiment. It has been reported that to produce 1 g of algal biomass, 1.83 g of CO_2 can be recycled. *Chlorella vulgaris ARC1* is able to biologically fixed 0.0183 and 0.0384 gCO_2 L^{-1} D^{-1}at ambient and elevated CO_2 supply0.036% and 6% respectively (Chinnasamy et al., 2009). *Spirulina platensis* is also potentially significant to fix 0.318 gCO_2 L^{-1} D^{-1}as reported by Bittencourt et al., (2010). *Synechocystis aquatiliscan* able to fixed highest concentration of $CO_2$with 1.5 gCO_2 L^{-1} D^{-1}(Singh and Singh, 2014). The atmospheric CO_2 gets sequestered by algal biomass during the process of photosynthesis. Basically two mechanisms are involved in CO_2sequestrationC3 and C4 pathways. C3 plants posses around 250, 000 species whereas, C4 plants have 7500 species. Most of the algal biomass uses Calvin Cycle (C3 pathway) for CO_2 sequestration. In Calvin Cycle pathway, CO_2 and 5-carbon compound bind with each-other to yield 3-carbon compounds and this reaction is catalysed by Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) enzyme. Hence, the experimental data reveals that *Chlorella pyrenoidosa* is a significant algal species having potential to receive high amount of CO_2under artificial condition.

Table 3.8: Growth kinetics of *Chlorella pyrenoidosa* with different concentrations of CO_2

CO_2 supply	5%	10%	15%	20%	control
Maximum productivity (gL^{-1})	0.75±0.05	1.26±0.06	2.09±0.08	1.4±0.07	0.5±0.04
Biomass productivity ($mgL^{-1}d^{-1}$)	85.0±0.09	163.1±0.21	281.7±0.05	186.2±0.08	42.5±0.07
Specific growth rate ($\mu gL^{-1}d^{-1}$)	1.9±0.07	4.1±0.11	5.3±0.09	4.3±0.03	1.1±0.05
Generation time (Day^{-1})	0.46±0.03	0.36±0.08	0.29±0.07	0.34±0.07	0.69±0.01
Lipid (%)	26.5±0.05	21.1±0.05	19.8±0.04	17.5±0.07	23.2±0.02

Table 3.9: CO_2 sequestration ($gCO_2\ L^{-1}\ D^{-1}$) by *Chlorella pyrenoidosa*

	CO_2 supply to *Chlorella pyrenoidosa*				
Days	**5%**	**10%**	**15%**	**20%**	**Control**
1st	0.005±0.02	0.005±0.02	0.005±0.02	0.005±0.01	0.005±0.02
5th	0.75±0.05	1.05±0.08	1.81±0.08	2.35±0.04	0.11±0.07
10th	2.34±0.07	3.07±0.03	4.27±0.04	5.89±0.06	0.11±0.04
15th	2.15±0.04	2.74±0.04	3.76±0.05	5.5±0.07	0.08±0.08

3.3.3.3. Effect of CO_2 on biochemical compound

Various concentrations of CO_2 treatment significantly affect the protein and carbohydrate content of *Chlorella pyrenoidosa*. It has been observed that the maximum protein content was obtained 4.3 mg L^{-1} at 15% of CO_2treatment. It was observed that as the concentration of CO_2 treatment for algal cells increased from 5-20%, a remarkable increase in protein content was obtained but at 20% of CO_2treatment a downfall in protein content was noticed. The maximum protein content of algal biomass was eight times higher than $control_{BG}$ (without CO_2) treatment. The experimental results found a substantial effect on algal carbohydrate content by the treatment of different concentrations of CO_2 (5-20%). The carbohydrate content of *Chlorella pyrenoidosa* was obtained maximum at 10% *i.e*. 6.2 mgL^{-1} and minimum at 15% *i.e.* 4.6 mgL^{-1} of CO_2 treatment. The optimum value of obtained carbohydrate was 10.5 fold higher than $control_{BG}$ (without CO_2) treatment. A slow rate of increment in algal carbohydrate content was observed in initial phase because the algal culture shifted from lag phase to exponential phase. The algal carbohydrate content was obtained maximum at late exponential to late stationary phase. The total amount of lipid produced by *Chlorella pyrenoidosa* was obtained maximum *etc.* 26.4±0.05 % at 5% of CO_2supply. In case of 10%, and 15% of CO_2 supply, lipid was obtained 21.1±0.05 %, and 19.8±0.04 % respectively. 17.5±0.07 % of lipid was obtained with 20% of CO_2 supply. In case of control (without CO_2) 23.2±0.02 of lipid was obtained with minimum amount of algal biomass. Tamarys et al., (2011), has also reported increase in lipid content of *Chlorella protothecoides* up to 55.2% due to additional supply of carbon. Therefore, low concentration of CO_2 promotes lipid productivity in higher amount. It might be possible due to the stress of

CO_2concentration in algal culture that leads to enhanced lipid synthesis rather than algal biomass productivity for survival of algal biomass in unfavourable conditions.

3.4. Conclusion

For the sustainability of any algal biomass based products (biofuel or value-added products), biomass in bulk amount is required. Therefore, parameter optimization is a very significant tool to enhance the algal biomass to know the effect on its particular biochemical compounds. The present experimental result supports higher lipid production with low pH, nitrate, and phosphate, whereas high carbon concentration and 16L: 08D cycle of photoperiod supports higher lipid production. pH (7.5) was found as best medium for high biomass production. Lower pH was found suitable to induce higher lipid content (26.5±0.02 %). Optimized photoperiod 16L: 08D with maximum biomass (1.52±0.03 gL^{-1}) and lipid content (27.2±0.11 %).0.4 gL^{-1} nitrate was found as best medium for high biomass (1.62±0.04 gL^{-1}) production. Lower nitrate concentration (0.05 gL^{-1}) was found suitable to induce higher lipid content (28.5±0.03 %). 0.1 gL^{-1} phosphate was found as best medium for high biomass production. Lower phosphate concentration (0.05 gL^{-1}) was found suitable to induce higher lipid content (25.5±0.06%). 0.2 gL^{-1} carbon concentration was found as best medium for high biomass production (1.76±0.07 gL^{-1}). Lower carbon concentration was found suitable to induce higher lipid content (26.4±0.09%).

Microalgae based CETP wastewater treatment was found to as effective way for reduction of pollutant load and heavy metal. Among various selected concentration of CETP wastewater, alga showed its best growth at 50%, however, *Chlorella pyrenoidosa* also found to tolerate the higher concentration (75% and 100%). Alga showed its best growth at 25% and 50% concentrations, however algal biomass also found to tolerate the higher concentration (75% and 100%) with less productivity. Algal affinity to uptake metal was found highest for Cr (VI) followed by the Zn, Cu and Pb.50% test solution was found with maximum biomass productivity (1.4±0.07 gL^{-1}) and lipid production (27.6±0.015 %). The Pearson correlation co-efficient analysis supports with positive correlation with carbohydrate in 100% test solution whereas, it was found negative with protein and pigments (chlorophyll '*a*', chlorophyll '*b*', and total chlorophyll) with these concentrations50%, 75%, and 100%.The experimental results found significant with different concentrations of CO_2 treatment to *Chlorella pyrenoidosa*. Maximum biomass productivity was obtained (2.09±0.08 gL^{-1}) with 50% test solution of CETP wastewater with 15% CO_2 supply (gaseous form). Lipid productivity obtained maximum at 5% CO_2 supply. Maximum rate of CO_2 bio fixation was obtained at 10th day with 15% of CO_2 supply 5.8 $gCO_2\ L^{-1}\ D^{-1}$ by *C. pyrenoidosa.*

Chapter 4

Feasibility of thermal energy storage based photobioreactor for algal cultivation and biofuel production: a lab scale study

4.1. Introduction

Cultivation of algae in photobioreactor is significant to enhance biomass productivity under controlled/optimised conditions such as pH, temperature, light photoperiod and nutrients *etc*. There are various external and internal factors affect the algal biomass growth pH, carbon source, nutrients and temperature *etc.* out of these factors, temperature difference is a widely measured environmental factor that directly affects the performance of algal growth. Algal biomass production increases with an increase in optimum required temperature range (25-35°C) whereby, above the optimum required temperature, the algal biomass growth inhibited. For an efficient algal growth, below then optimal temperature is strictly not conducive as the viscosity of cytoplasm is affected by the low temperature. The most favourable temperature for psychrophilic, mesophilic, and thermophilic strains are 15-17°C, 20-25°C and 40-45°C respectively. In general, algae are grown with optimal growth rate at 20-25°C temperature. Gomes and Juneau, (2017) studied the temperature effect on biomass production with nutrient uptake on some algal sp. *Chlorella vulgaris, Pseudokirchneriell asubcapitata*. The author reported an increment in biomass productivity, growth rate and efficient nutrient up-take with increase in temperature. Mass cultivation of algal biomass is strictly inhibited by the temperature of the associated area (Chalifour et al., 2014; Chalifour and Juneau, 2011; Chen et al., 2010). The temperature controlled system for algal biomass is hypothetical. Various artificial measures (hot water bath, electric heaters) have been adopted by the researchers to maintain the temperature of the system to enhance the algal biomass (Wen et al., 2016). But these adopted methods are highly energy intensive approaches, which enhance the total expenditure of processing routes of algal cultivation. So, many heating and cooling systems are being incorporated in PBR system but most of them are not economically feasible. Present available photo-bioreactor systems are not having temperature controlled system so, there is huge requirement to design a temperature controlled PBR system for its application at commercial scale. Therefore, a novel approach has been initiated by using thermal energy storage (TES) based PBR system to maintain the temperature of PBR in which algal biomass has to be cultivated. Temperature control through TES based system is a novel approach to maintain the temperature in PBR system for efficient algal growth. The use of TES based PBR system make it more attractive and energy efficient device to enhance algal biomass. Around the globe, extreme weather (hot/cold) conditions are found throughout the year. Solar radiation is available around the clock in every country. Presently, phase change materials (PCMs) are using solar heat energy for several applications in different temperature ranges. Extensive research work is ongoing to utilize the phase change materials for heat/cold storage with the temperature range of 20-30°C for building heating/cooling applications (Tyagi et al., 2016; Tyagi et al., 2014; Waqas and Din, 2013; Soares et al., 2013). Therefore, potential of these phase change materials can be used in PBR system for efficient temperature maintain. Tyagi et al., (2008) has been developed $CaCl_2.6H_2O$ as a PCM for space cooling applications. The result of the study showed that six hours temperature can be maintained in night of experimental room after the heat storage in HDPE based TES system. To maintain the temperature of PBR, phase change material has been used in this present experimental work. Phase change materials (PCMs) are a special type of thermal energy storage materials and its use in photobioreactor is a novel approach as a reliable source of heat energy to sustain required temperature in PBRs. In order to conduct the present research work two different types of closed photobioreactor have been fabricated to complete the research work. Detailed descriptions of these two closed photobioreactor have been provided in Chapter 2. On the basis of fabrication of two different closed photobioreactors, the present chapter is divided into two sections:

4.1.1. Fabrication of horizontal tubular photobioreactor to enhance algal cultivation and biofuel/bio-oil production

4.1.2. Fabrication of thermal energy storage based photobioreactor for algal biomass cultivation and its use for biofuel/bio-oil production

4.2. Materials and methods

4.2.1. Algal culture

The culture of *Chlorella pyrenoidosa* was obtained from National Collection of Industrial Microorganism (NCIM 2738), Pune, Maharastra, India and maintained in recommended BG-11 growth medium. Detailed descriptions of selected algal sp. have been given in section 2.3 of Chapter-2.

4.2.2. Wastewater

Experimental results obtained from Chapter-3 revealed that 50% concentration of CETP wastewater was potentially significant to support for enhanced algal biomass production. Therefore, in this phase of experimental study 50% concentration of CETP wastewater has been used as substrate for algal biomass growth and biofuel production.

4.2.3. Study area

The experimental work was performed in Lucknow (27°40’N 80°00’E) at the campus of BBA University. Lucknow region are basically divided among three different seasons *i.e.* winter, summer, and monsoon. Temperature during winter season (December to February) falls up to 7-8°C at night whereas; it fluctuates in the range of 12-25°C at day time. Lucknow is characterised by hot summers (March to June) temperature goes up to 40-48°C at day time whereas; at night temperature falls up to 25-30°C. Monsoon seasons (July to September) are usually having 25°C temperature.

4.2.4. Bioreactor

The present chapter used two different photobioreactor to investigate the effect on algal biomass productivity and bio-fuel production.

4.2.4.1. Horizontal tubular photobioreactor

A closed horizontal Tubular Photobioreactor (TPBR) was fabricated for this experimental study. The bioreactor consisted of six transparent glass tubes, conjoined to each-other with a reservoir (storage tank) of 20L capacity. An electric motor pump was incorporated in the system for continuous circulation of algal cell suspension. An aerator was also inserted in the reservoir for gaseous exchange and to maintain the uniform distribution of nutrients. The Tubular Photobioreactor (TPBR) for cultivation of algal biomass was designed and fabricated to enhance/dense production of biomass whereas, 50% concentration of CETP wastewater taken as nutrient media for algal biomass. BG-11 medium was taken as control to analyse the algal productivity and growth rate comparatively.

4.2.4.2. Vertical column photobioreactor

Three separated PBR setups were prepared and run in parallel to investigate the temperature of the medium uncontrolled PBR/without PCM or thermostat (PBR-I), controlled PBR with PCM (PBR-II), and controlled PBR with thermostat/electric heating (PBR-III). The experiment was run for 13 days to get maximum biomass production of selected algae. The PCM was selected on the basis of temperature range required for *Chlorella pyrenoidosa* with respect to weather temperature profile of Lucknow (study area) during winter season where the experimental work was carried out. The temperature was maintained in winter season with minimum 10°C and maximum 25°C recorded in a day. The temperature of PBR system was obtained near ambient temperature during experiment in PBR-I. So, there is a need to select the PCM having melting temperature in range of 30-35°C. Based on these considerations, an organic PCM was selected as suitable candidate *i.e.* capric acid ($CH_3(CH_2)_8COOH$) (Fig. 4.1) of analytical grade to maintain the temperature of the PBR to enhance the algal biomass. The melting point of this PCM was 31.5°C, and latent heat was 36.23 Jg^1 respectively. A three chambered vertical column PBR was setup with 5L working volume (50% concentration of CETP wastewater) of algal medium were fabricated by using transparent glass material. Each chamber was equipped with thermometer and motor based-aerator to check the temperature of the PBR to maintain uniform distribution of nutrient. Three separated PBR setup were prepared and run in parallel to investigate the temperature of the medium with PCMs and without PCMs.

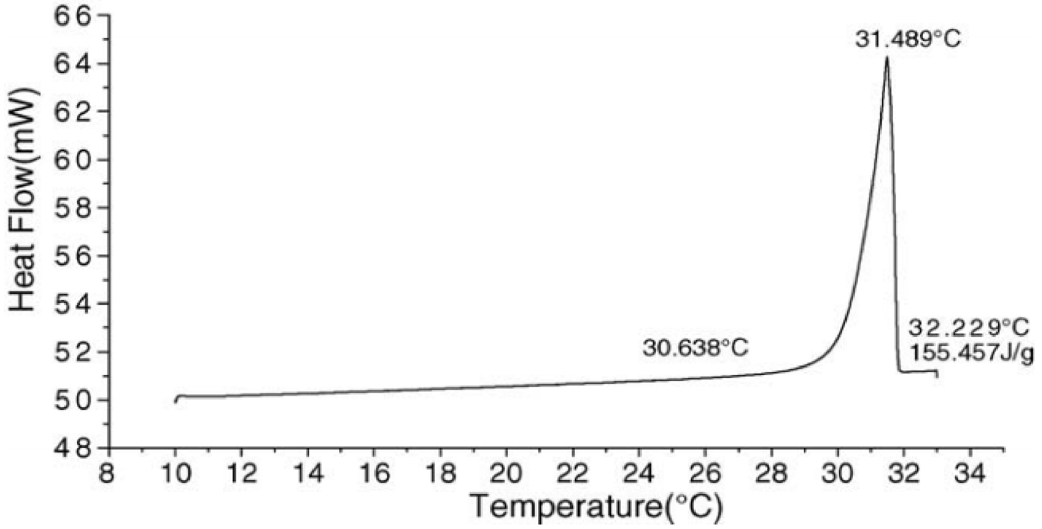


Fig. 4.1: Differential scanning calorimetry (DSC) graph of Capric acid

Table 4.1: Specifications of photobioreactor

Horizontal Tubular Photobioreactor	
Specifications	Description
Material	Glass
Number of tubes	Six (06)
Diameter	0.1m
Length	1m
Total volume	20L
Working volume	10L
Motor	0.5 Horsepower
Vertical Column Photobioreactor	
Material	Glass
Total volume	10L
Working volume	5L
Aeration	Aqua air pump motor
Light source	LED of 20 watt
Temperature sensor	Thermometer
Radiation measurement	Solar power meter (Thermo-scientific) (wm^{-2})
pH measurement	Handy pH meter (Hanna instruments)
PCM (phase change material)	Capric acid
control condition	Thermostats (maintained 30°C in PBR)

Side and back portion of the system were insulated by using 2 cm expandable polystyrene sheets to prevent the transfer of heat to and from the surroundings. The changes in temperature inside PBR-I, II, and III were recorded continuously from 6:00 am to 12:00 pm at regular interval. The PCMs were needed to charge thrice times a day from 6:00 am to 12:00 pm.

The average temperature of the medium in PBR during experiment was 20°C in which *Chlorella pyrenoidosa* was cultivated. The favourable temperature for growth and development of *Chlorella pyrenoidosa* ranges from 25-30°C. Therefore, to maintain this temperature range in PBR system in which *Chlorella pyrenoidosa* was cultivated, phase change material based PBR system was designed to store heat energy for temperature maintenance. The amount of required heat energy in PCM was calculated on the basis of latent heat of material through these equations:

$$Q = \int_{T_i}^{T_f} mC_p \mathrm{d}T \tag{4.1}$$

$$= \mathrm{mC_{ap}}\ (\mathrm{T_f} - \mathrm{T_i}) \tag{4.2}$$

Where, Q is heat energy, m is mass of the substance (kg), s is specific heat (units j/kg*K), Δt is change in temperature/temperature difference.

$$Q = \int_{T_i}^{T_m} mC_p dT + ma_m \Delta h_m + \int_{T_m}^{T_f} mC_p dT \tag{4.3}$$

$$Q = \mathrm{m}\left[\mathrm{C_{sp}}(\mathrm{T_m} - \mathrm{T_i}) + \ \mathrm{a_m}\Delta\mathrm{h_m} + \mathrm{C_{Ip}}\ (\mathrm{T_f} - \ \mathrm{T_m})\right] \tag{4.4}$$

$$Q = \mathrm{a_r} m\Delta h_r \tag{4.5}$$

Where, Q is heat energy, m is mass of the substance, l is latent heat. Where, m is the mass, $C_{p,s}$, $C_{p,l}$ is the specific heat of PCM in solid and liquid phase, *i, m*&$_f$ are initial, melting and final temperature, dT is the temperature rise.

For lab scale experimental work, to maintain 5L medium of *Chlorella pyrenoidosa*, total 3 kg of PCM was used in this work. Three metallic cylindrical shape cans were used to encapsulate the PCM in PBR system. Each can have 500 gm of PCMs which was kept inside the PBR chamber. Solar energy was used to change the phase of PCM used in cylindrical shape cans.

4.2.5. Growth kinetics

Growth measurement of algal cell was carried out at every day using spectrophotometric method and growth in terms of absorbance was taken. Algal growth pattern was also monitored through kinetic analysis using following equations:

$$\text{Biomass productivity} = (N_2\text{-}N_1)/t_2\text{-}t_1 \tag{4.6}$$

Where N_2 and N_1 algal biomass at different time interval t_2 and t_1 respectively Specific growth rate and biomass and doubling time were also determined by using following equations-

$$\text{Specific growth rate } \mu = \ln (N_2 - N_1)/t_2 - t_1 \tag{4.7}$$

$$\text{Generation time} = 1/\mu \tag{4.8}$$

4.2.6. Heavy metal analysis

The culture of *Chlorella pyrenoidosa* were incubated for 12 days under controlled conditions (in fabricated PBRs) and 10 mL culture was taken out under sterilized condition at every 4 days of experiment for Cr (VI) analysis. The algal culture was centrifuged at 5000 rpm for 10 minutes. The supernatant was used for different heavy metal estimation carried out by Atomic Absorption Spectrometry (AA240FS, Fast Sequential Atomic Absorption Spectrometer). To understand the percentage reduction of heavy metals concentration in wastewater of different test solution, equation (4.8) were applied and pollution reduction efficiency of *Chlorella pyrenoidosa* was calculated by following equation:

$$\text{Pollution reduction (\%) of Heavy Metals} = [(C_i - C_f)/ C_i] \times 100 \tag{4.8}$$

4.2.7. SEM and EDS analysis

Algal cell surface characterizations were analyzed by SEM-EDS (SAM: model: JSM-6490LV, Make: JEOL, Japan) to know the algal surface structural morphology and cell wall composition with respect to elements.

4.2.8. Fourier transforms infrared spectroscopy

FTIR analysis has been done on algal based biofuel analysis particularly algal lipid and oil. A Perkin Elmer spectrum RX/FTIR system has been used to obtain IR spectrum within the range of 4000 cm^{-1} to 500 cm^{-1}.This technique works on the principle of interaction between infrared radiation and algal sample usually in form of solid liquid and gas.

4.3. Results and discussion

4.3.1. Fabrication of horizontal tubular photobioreactor to enhance algal cultivation for biofuel/bio-oil production

4.3.1.1. Algal growth and lipid production in TPBR

Culture of *Chlorella pyrenoidosa* in TPBR was cultivated with batch mode of operation for 15 days to investigate the efficiency of algal production. The growth pattern of *Chlorella pyrenoidosa* in BG-11 medium were observed and found that during the first 7 days algal growth was swift afterwards it conquered almost a constant speed (Fig. 4.2).The present study demonstrates the significant biomass productivity 2.98±0.057 and 3.65±0.062 gL^{-1} in BG-11 media and 50% concentration of CETP wastewater respectively (Table 4.2). Lipid productivity of *Chlorella pyrenoidosa* has also been observed at each five day of experiment and found that a sharp increase in lipid profile of algae. The amount of lipid obtained in media and wastewater was 21.2±0.022 %, 24.4±0.026 %, 23.4±0.21 % and 23.7±0.27 %, 26.9±0.09 %, 26.4±0.23 % at 5th, 10th, 15th day of cultivation respectively (Fig. 4.3 and Table 4.3). The experimental results showed that wastewater is proficient to produce algal based lipid in significant amount as compared to media. The total biomass obtained at final day of experiment was 23.3 gm and 21.9 gm in media and 50% concentration of CETP wastewater. The amount of bio-oil produced from algae was 31.9 and 33.6 % in media and wastewater respectively by using n-hexan method. Mulumba and Farag (2012), have reported 1.1 gL^{-1} of algal biomass productivity with 11% of lipid production in tubular photobioreactor having reservoir of 22.5 L capacity. Although, the production of algal biomass was obtained higher in TPB as the tilt angle of reactor's glass tubes put at 45°, resulting, harnessing of solar radiation enhanced. Yet, tubular photobioreactor does not offer high algal biomass production due to the lack of high area and volume ratio of the glass tubes of reactor. Due to the low area and volume ratio of photobioreactor tubes, efficient gas transfer (CO_2) cannot take placed subsequently, decreased rate of photosynthesis occurred. In terms of substrate volume (10 L) taken as nutrient medium algal biomass production was not significant. Total expenditure of TPBR was higher than the production of algal biomass. The main drawback of this bioreactor was associated to the harvesting of algal biomass from the tubes of reactor. Around 20-25 gm of algal biomass was immovable on the walls of reactors tube. Therefore, due to the lack of harvesting of total amount of algal biomass from the reactors tube, TPBR was not preceded in further experimental work. In order to overcome the lacking aroused in TPBR, vertical column PBR was used to enhance algal production.

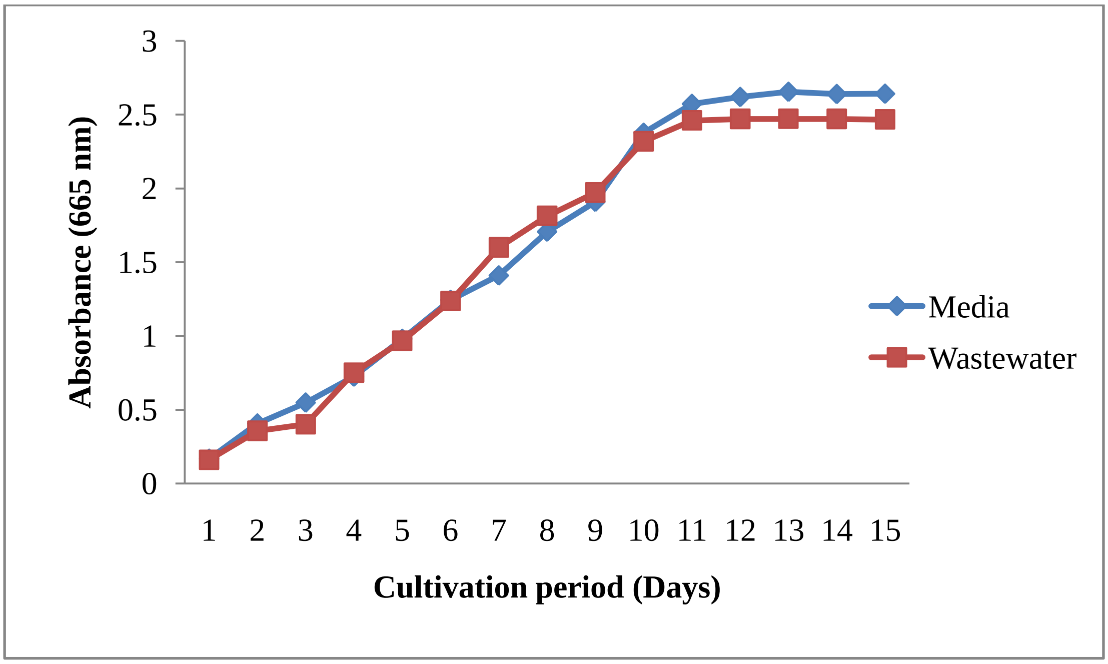


Fig. 4.2: Growth of *Chlorella pyrenoidosa*

Table 4.2: Growth kinetics of algae in tubular photobioreactor

Culture condition	Maximum biomass productivity (gL^{-1})	Biomass productivity (mgL^{-1})	μ(Specific growth rate day^{-1})	Doubling time (day^{-1})
Media	2.98±0.057	249.2±0.075	5.5±0.032	0.181±0.062
Wastewater (CETP 50%)	3.65±0.062	287.9±0.041	5.8±0.052	0.187±0.047

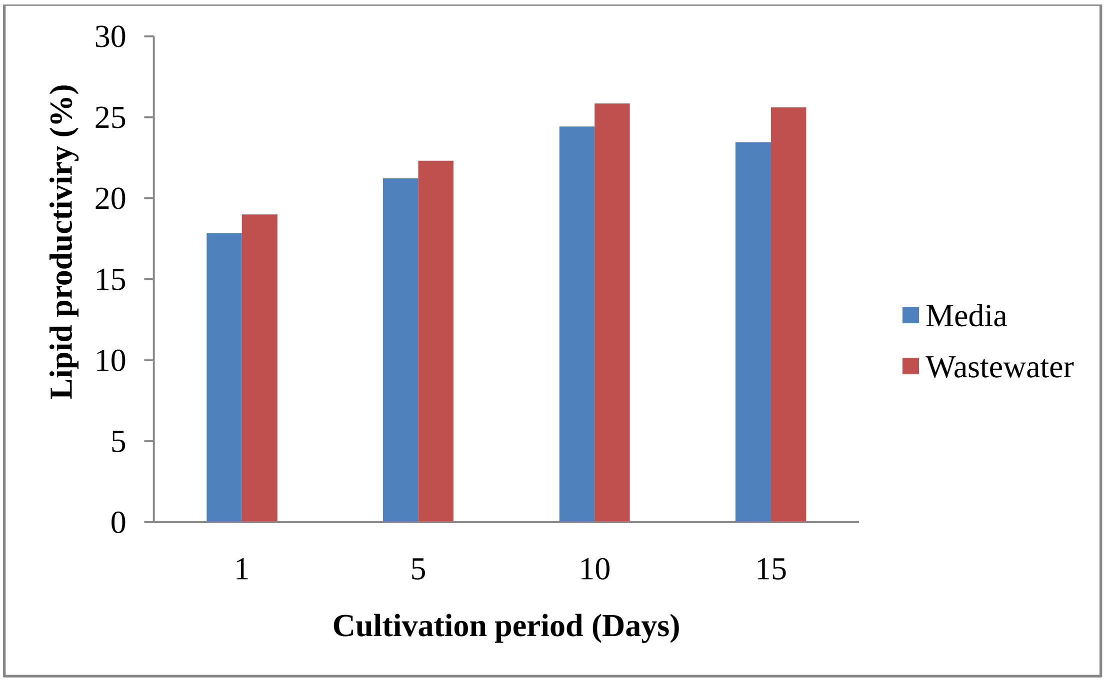


Fig. 4.3: Lipid productivity of *Chlorella pyrenoidosa* in TPBR

Table 4.3: Lipid productivity in media and wastewater

Cultivation period	Media	Wastewater
1st	17.8±0.047%	19.0±0.11%
5th	21.2±0.022%	23.7±0.27%
10th	24.4±0.026%	26.9±0.09%
15th	23.4±0.21%	26.4±0.23%

4.3.2. Fabrication of thermal energy storage based photobioreactor for algal biomass cultivation and its use for biofuel/bio-oil production

4.3.2.1. Algal growth in TES based PBR

Investigation of algal biomass growth and development with different PBR system carried out to know the effect of temperature on algal biomass productivity as illustrated in Fig. 4.4. The biomass productivity of *Chlorella pyrenoidosa* was found 0.85±0.14, 4.8±0.27, 5±0.09 gL^{-1}in PBR-I, II, and III respectively with 50% concentration of wastewater as given in Table 4.4.The total dry weight of *Chlorella pyrenoidosa* was obtained 15.4 gm 15.9 gm in PBR-I and II whereas, total dry biomass of *Chlorella pyrenoidosa* was obtained around 1.3 gm in PBR-I. Therefore, it is clear that temperature maintained by using phase change material is efficient and novel approach to enhance the algal biomass productivity as temperature directly affects the growth and development of algal biomass. Temperature is a foremost ecological parameter that robustly regulates algal growth in terms of biological growth rate and photosynthesis.

4.3.2.2. Effect of temperature on algae growth

The ambient temperature during the experiment was ranges between 5-24°C as illustrated in Fig. 4.5. The maximum temperature (24-25°C) lies only for 1-3 hours. Low temperature is not favourable to achieve maximum algal growth as in low temperature the metabolic rate and physiological activity of algal biomass also becomes slow due to cold shock. The experimental results revealed that the temperature of PBR-I was ranged between 3-18°C which almost equal to the ambient temperature Fig. 4.6.

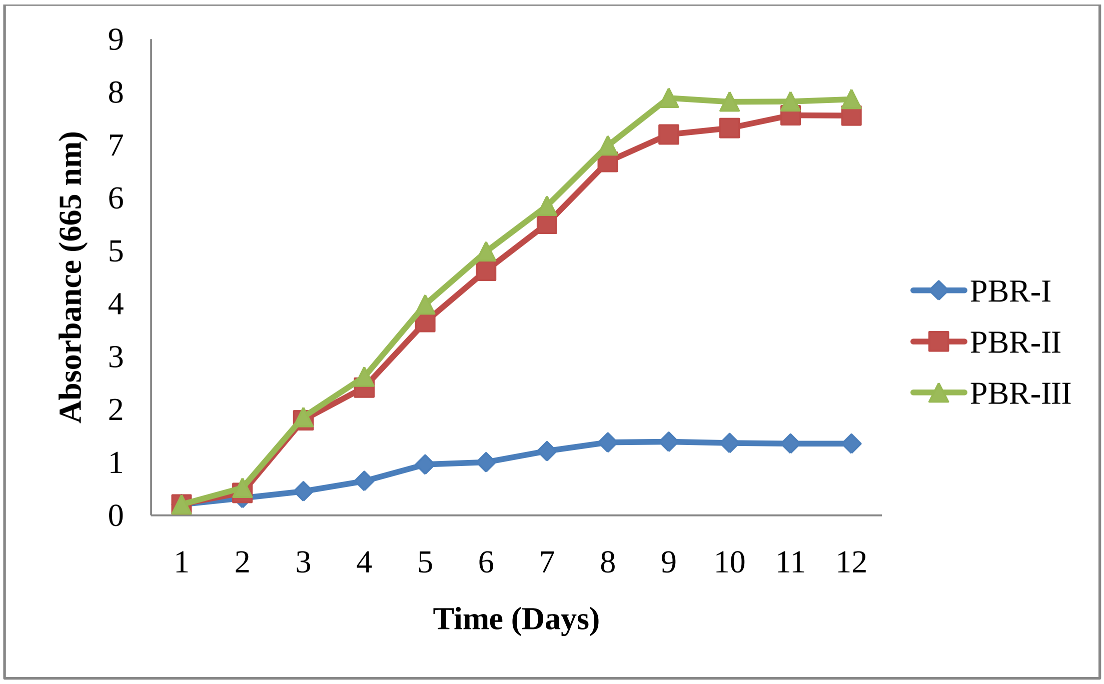


Fig. 4.4: Growth curve of *Chlorella pyrenoidosa* in PBRs

Table 4.4: Growth kinetics of algae in PBRs

PBR	**Maximum biomass production (gL^{-1})**	**Biomass productivity ($mgL^{-1}d^{-1}$)**	**μ (Specific growth rate d^{-1})**	**Generation Time (Day^{-1})**
PBR-I	0.85±0.14	64.08±0.11	4.16±0.74	0.24±0.44
PBR-II	4.8±0.27	410.7±0.19	6.01±0.21	0.16±0.51
PBR-III	5±0.09	462.2±0.40	6.12±0.14	0.16±0.32

Capric acid was used as a phase change material to maintain the temperature of PBR-II. Latent heat stored in PCMs was capable to enhance the temperature of the algal media up to 30±2°C than the surroundings Fig. 4.7. Therefore, the temperature of PBR-II having PCM was able to maintain the temperature of the medium in cold condition with required ranges of *Chlorella pyrenoidosa* 25-30°C. It clearly indicates that medium in which *Chlorella pyrenoidosa* was inoculated (without PCM) is less supportive for its (algal biomass) growth and development. PBR-III was able to maintain the temperature 30°C constantly (Fig. 4.8) for algal growth and development. Therefore it is clear that capric acid is able to maintain the temperature of the medium in cold climatic conditions up to 30±2°C from 18±2°C (average temperature). The increase in biological growth rate or photosynthesis can be describes as a function of temperature co-efficient (Q_{10}) or Arrhenius functions *i.e.* the ecological factor by which biological growth rate of algal biomass increased as temperature rise 10°C. Therefore, phase change materials (PCMs) are a special type of thermal energy storage materials and its use in photobioreactor is a novel approach as a reliable source of heat energy to sustain required temperature in PBRs. They generally use chemical bonds to store and release heat energy to the system required. Transfer of thermal energy takes place when a material changes from solid to a liquid or from a liquid to a solid which is known as change in state or ‘phase’. In general, every material act as a phase change material as it can change its cumulative state at such as solid, liquid and gas at certain pressure and temperature. When the PCM material alters itself from one state to other it releases the stored energy which is known as latent heat energy. The release of energy takes place at constant temperature.

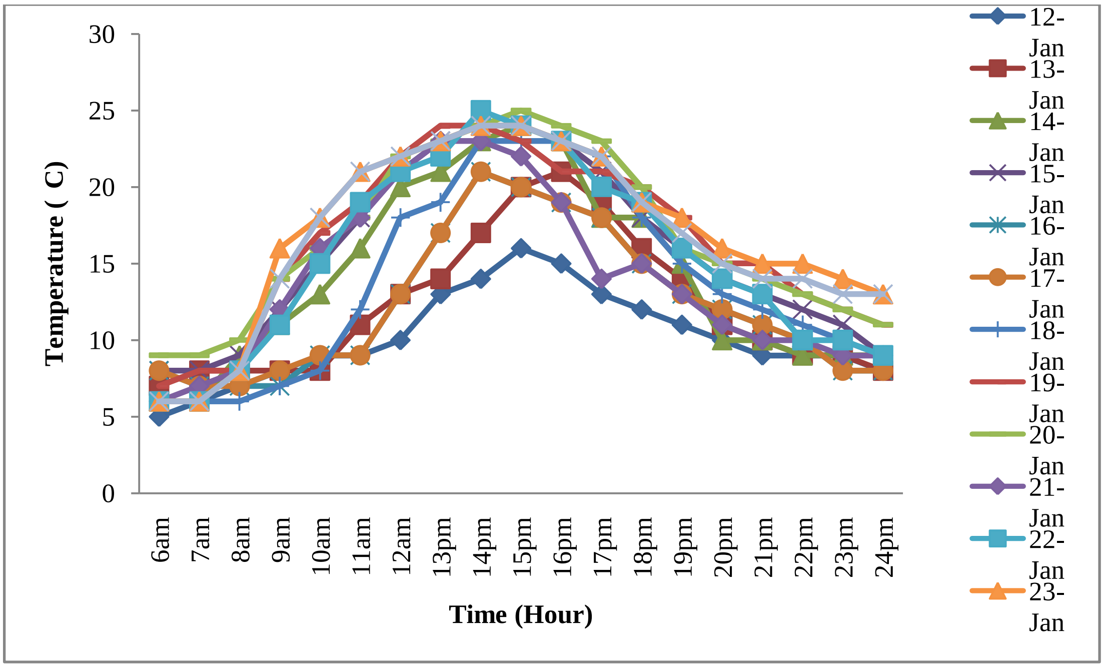


Fig. 4.5: Ambient temperature profile during experiment

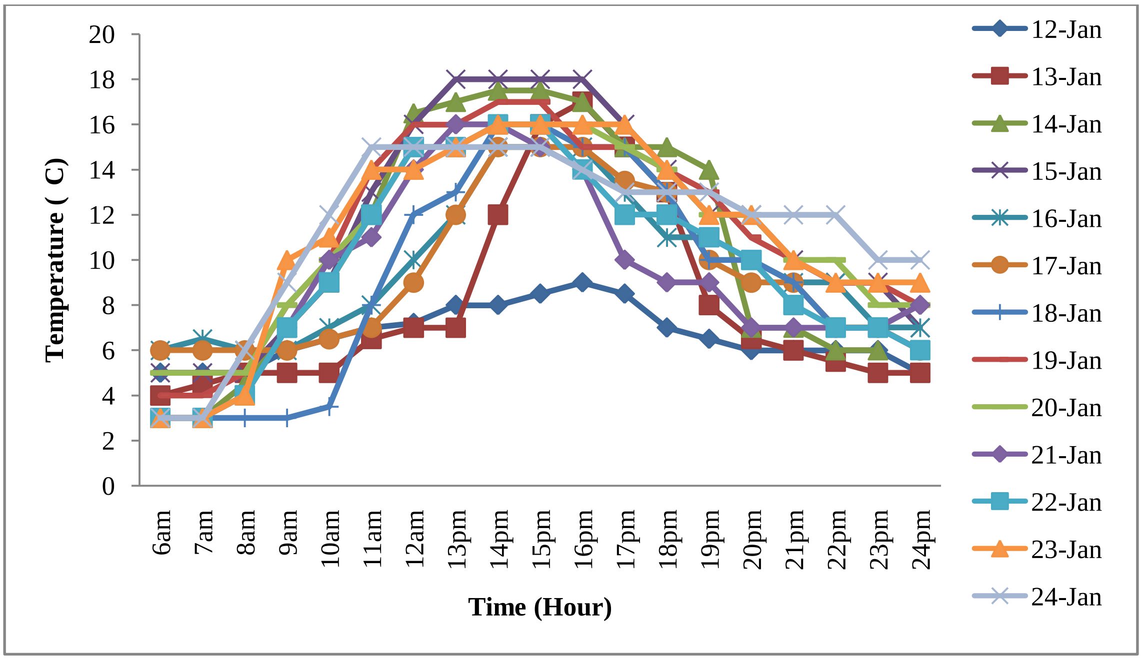


Fig. 4.6: Temperature profile of PBR-I

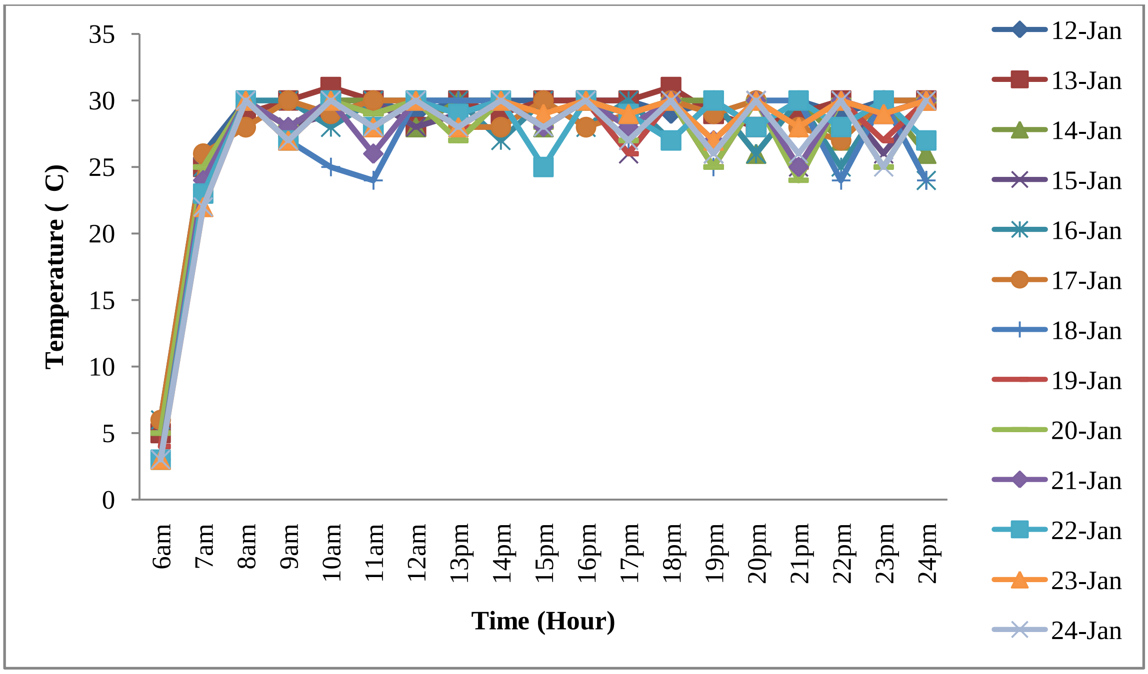


Fig. 4.7: Temperature profile of PBR-II

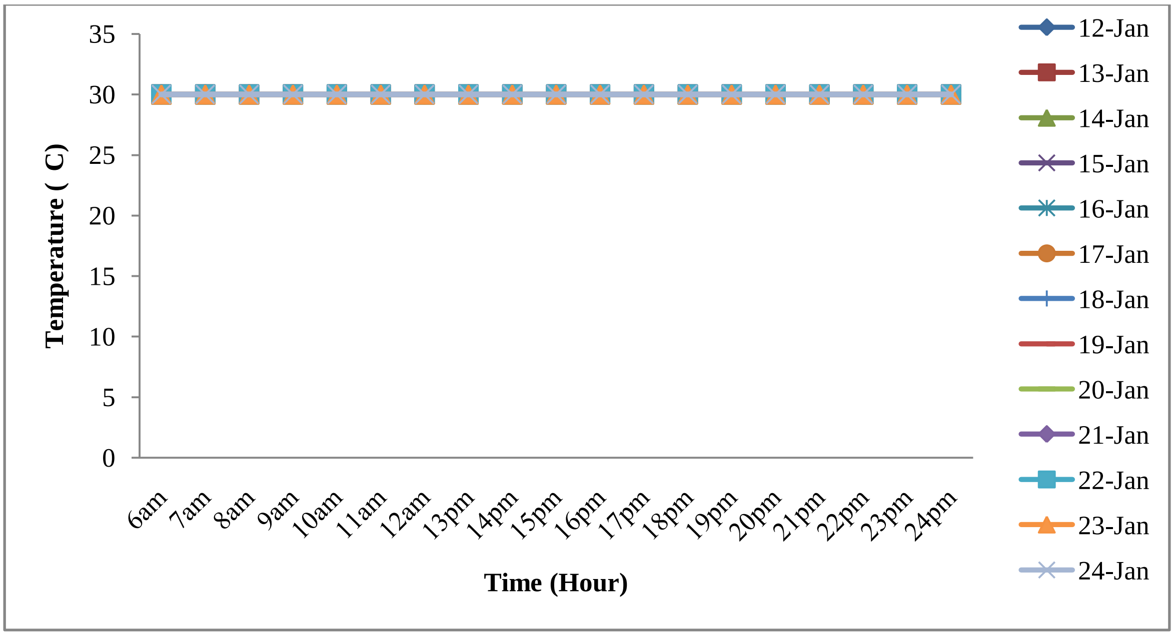


Fig. 4.8: Temperature profile of PBR-III

Therefore, PCM of PBR-II was able to provide optimum temperature required for optimum algal growth. Algal biomass growth at non-optimal temperature is responsible for higher biomass losses (as in PBR-I). It is due to the reason that low temperature affects photosynthesis rate by decreasing carbon assimilation activity. Low temperature directly affect the rate of photosynthesis resulting, decline in ribulose-1,5-bisphosphate activity (RuBisCO). Higher temperature decreases photosynthesis rate by inactivate photosynthetic proteins and distributing balance of energy in the cells of algal biomass subsequently, reduction in photosynthesis take place which in turn decreases biomass productivity. Higher temperature also affects the algal cell size and respiration rate.

4.3.2.3. Heavy metal removal by algal biomass in PBRs

The efficiency of live algal biomass (*Chlorella pyrenoidosa*) to remove heavy metal Cr(VI) from 50% test solution of in PBR-I, II, and III were investigated to get more critical findings regarding heavy metal removal. The concentration of Cr (VI) in PBR-I, II, and III at a regular interval has been presented in Table 4.5. The maximum initial concentration of Cr in 50% concentration of CETP wastewater was 5.67 mgL^{-1} and after treatment with *Chlorella pyrenoidosa* it was reduced to 2.94 mgL^{-1}, 0.83 mgL^{-1}, 0.79 mgL^{-1} in PBR-I, II, and III respectively. The experimental study revealed that maximum percentage removal of Cr(VI) obtained in PBR-II and III with 85.3±0.074%, 86.0±0.085% at final day of experiment. A significant decrease in percentage reduction was obtained with 48.1±0.03% in PBR-I where PCM was not applied. It may be possible that Cr(VI) ion bind to active algal biomass or passive algal biomass cell surface and are also transported within the cell, whereas the adsorptions process does not depend on metabolic process, requiring several metal transporters (Barakat, 2011). Several algal cell derived biomolecules (*i.e.*

polyphosphates, phytochelatins, metallothioneins, and metllaoprotiens *etc.*) help in the sequestration of Cr(VI) from the wastewater. Besides this, the enzymatic reaction can alter the oxidation number of Cr(VI) and change into less toxic forms. Micro-precipitation of Cr(VI) removal in the form of phosphates and sulphates is a potential approach to remove Cr from wastewater (Ungureanu et al., 2015). The algal cell wall made up of micro-fibrillarexo-polysaccharides has typical chemical composition, contain functional groups such as –COOH$^-$, -OH$^-$, -PO_4^{-3}, -RSH, SO_4^{-2}.These functional groups produce anionic nature to the cell wall and microfibrils. Since, heavy metals ion in wastewater is cationic in nature they are absorbed by the cell and microfibrils surface. Cyanobacterial cell wall consists of mainly peptidoglycan, polymer of N-acetylglucosamine and β 1, 4-N-acetylmuramic acid, which provide mostly –COOH functional group for Cr adsorption. Few cyanobacterial cell walls bear capsule wall which is anionic in nature due to acid in nature, and thus help in metal adsorption. *Sargassummuticum* has been found to remove 50% Sb^{++} metal ion at 23°C (Ungureanu et al., 2015). Yalcin et al., (2012), has observed 68% reduction in Cd^{++} at 20°C by using algal biomass *i.e. Cystoseira barbata*. The potential of *Spirogyra* sp. has been investigated with Pb^{++} metal ion reduction from 200 to 140mgL^{-1} *i.e.* 30% at 25°C (Gupta and Rastogi, 2008). Ali et al.,(2013), reported that metal uptake by *S. platensis* increased gradually with increasing in temperature and it was found that metal (Cu) uptake was maximum (90.61%) at temperature 37°C. Hence algal biomass has significant capability for metal reduction, where temperature plays an important role.

Table 4.5: Heavy metal removal by algae in PBRs

Days	PBR-I (20±2°C)	PBR-II (30±2°C)	PBR-III (30°C)
		Percentage reduction of Cr (VI)	
4th	29.1±0.017	52.2±0.041	56.6±0.034
8th	47.9±0.021	85.3 ±0.089	86.2±0.024
12th	48.1±0.03	85.3±0.074	86.0±0.085

4.3.2.3.1. SEM and EDS analysis

SEM and EDS analysis was performed to study the cell surface structural and morphological changes to point out the impact of temperature and wastewater composition on algal cells. Fig. 4.9 and 4.10 illustrated surface morphology of *Chlorella pyrenoidosa* cultivated in PBR-I, PBR-II, and PBR-III before and after treatment of 50% concentration of CETP wastewater. Algal cell surface structural morphology was found shrink as cultivated in PBR-I it is due to the cold shock as the temperature of PBR-I was uncontrolled. At low temperature alga exhibited stunted growth. EDS analysis of algal biomass also showed nominal amount of metal reduction as the pick of Cr was very minute. Therefore, low temperature is not favourable for efficient algal growth as well as metal removal. Algal biomass cultivated in PBR-II was found with smooth surface which indicated that optimum temperature favoured algal biomass production without cell surface structural deformities. EDS analysis of algal cells were found with high and sharp peck of Cr metal ion which indicated that temperature played a positive role not only to produce algal biomass but efficient metal removal was also noticed. Algal biomass was also found with efficient metal removal from EDS analysis but with algal cell surface structural deformities with PBR-III. It may be possible due to the electric heating from thermostats algal medium.

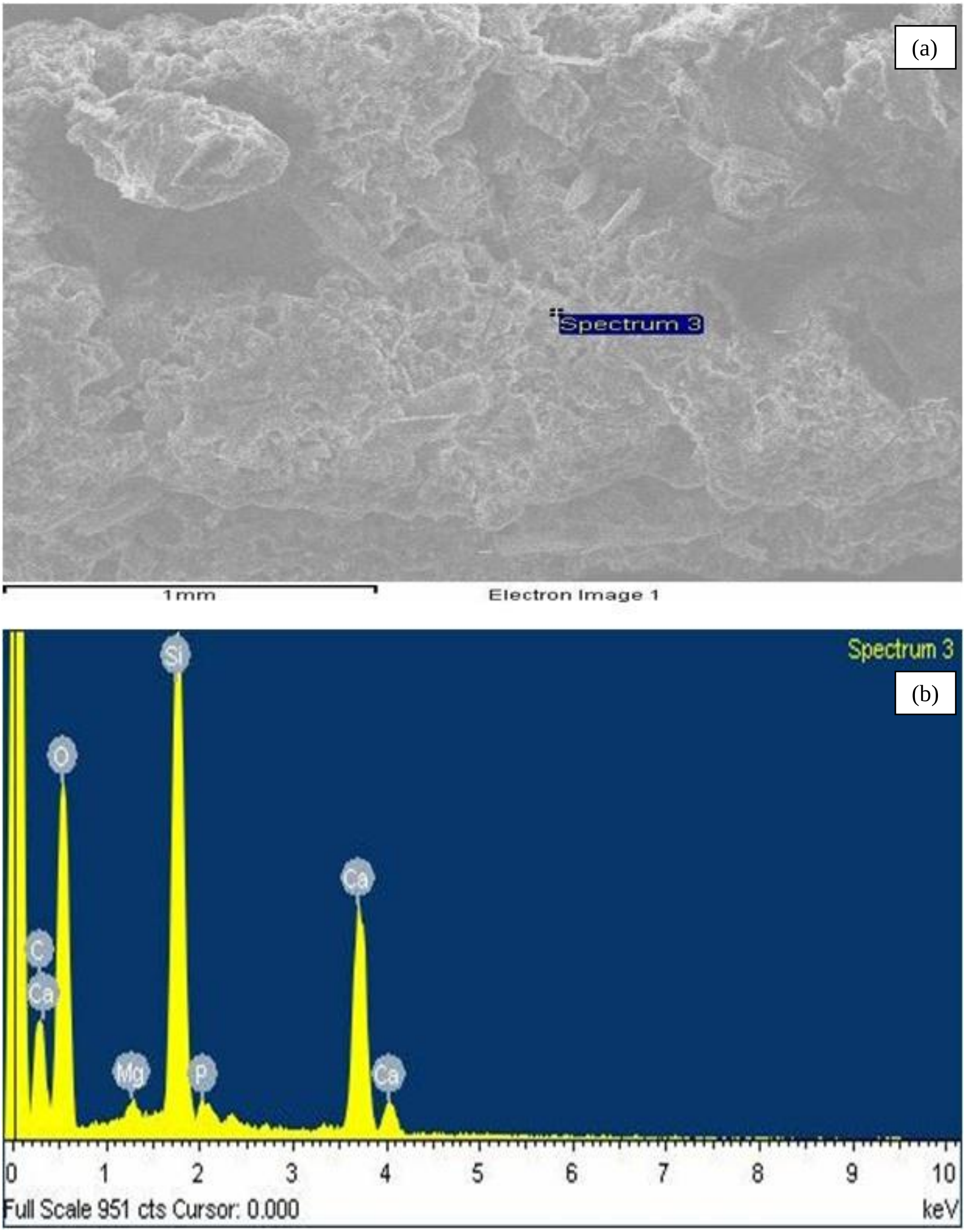


Fig. 4.9: Images of algal biomass before treatment: (a) SEM; (b) EDS

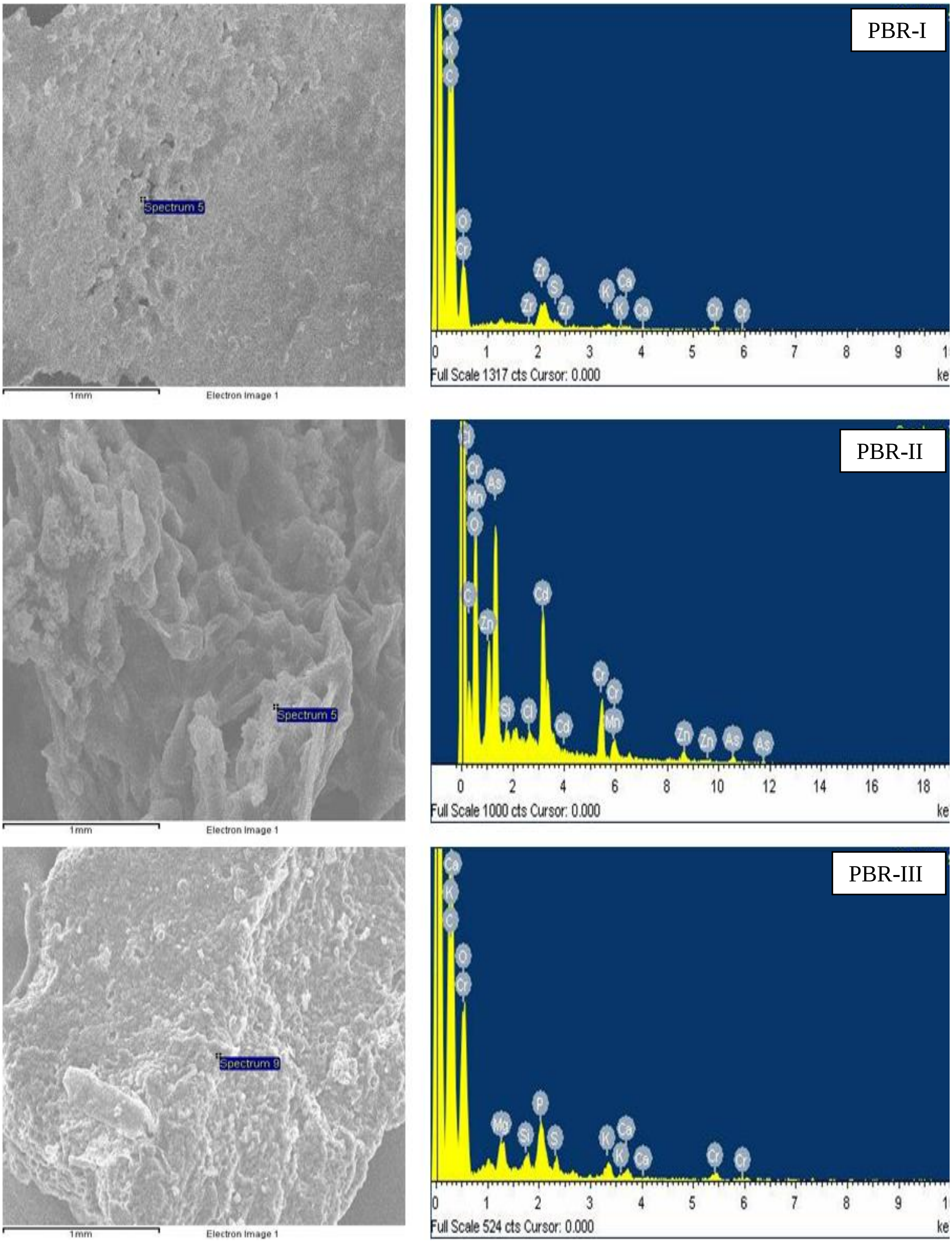


Fig. 4.10: SEM and EDS of algal biomass after treatment of wastewater: (a) PBR-I; (b) PBR-II; (c) PBR-III

4.3.2.4. Biofuel production

The amount of biomass harvested from batch experiment using CETP wastewater (50% concentration) was about 15.4 gm and 15.9 gm in PBR-II and PBR-III Whereas, 1.6 gm biomass was obtained from PBR-I. As the amount of total algal biomass was almost same in PBR-II, and III, therefore, two different methods were used to produce algal oil. n-hexane method and Modified Bligh and Dyer (MB&D) method to investigate the best suited method for maximum production. 41% of algal oil was obtained with single solvent system (n-hexane) using Soxhlet method (Fig. 4.11). The total oil content obtained by using Modified Bligh and dyer method was 35% (Fig. 4.12). Whereas, only 11.4% oil was produced from the algal biomass collected from PBR-I (without temperature adjustment). Temperature is a sensitive parameter for algal biomass productivity and metabolic activity, particularly for algal-oil production. Therefore, it is possible to enhance algal biomass and boil-oil production by temperature adjustment in photobioreactor. In our experimental study, the dependency of bio-oil content of *Chlorella pyrenoidosa* completely related with temperature of photobioreactor. Hence, it is clear from the above study that a wide range of algal species are found with specific value-added products. But, due to temperature fluctuation (extreme hot/cold, cold/warm climate area) these algal biomass are not being produced throughout the year. Therefore, applications of PCMs for heating or cooling are a novel approach to maintain the desired temperature of photobioreactor in which algal biomass has to be cultivated as well as to produce bio-oil.

4.3.2.3.4.1. FTIR analysis of algal oil

FTIR spectroscopy has been extensively applied for characterization of functional groups present in mixture of several compounds and is a significant tool to analyze the functional group of algal oil. The amount of total algal biomass obtained after final day of experiment from PBR-II and PBR-III was almost same15.4 and 15.9 gm respectively. Therefore, the obtained algal biomass (dry) was used to investigate the biofuel application by using two methods *i.e.* Bligh and Dyer method and Soxhlet method using n-hexen. The FTIR spectra exhibited the typical bands and peaks characteristics for algal oil. The spectra of algal oil obtained with Soxhlet method using n-hexane from algal biomass grown on 50% test solution of CETP wastewater illustrated in figure. The FTIR band occurred at 3420.0 cm^{-1} result from –CH bond stretching. The bands occurred at 2922.8 cm^{-1}, resultant from the =C-H bond stretching related to the functional class of alkenes. The peak obtained at 1713.1 cm^{-1}, results from the presence of C=O group due to presence of carboxylic acid. The short peak such as 1463.4 cm^{-1}, 1380.3 cm^{-1}, 1261.3 cm^{-1}, 1178.9 cm^{-1}, and 804.4 cm^{-1} occurred due to NH_2 scissoring, NH_3^+ symmetric bending , O-C stretch, carboxylic acid derived compound, C-OH stretch and CH_2=CH_2 stretch respectively. NH_2 scissoring represented the present of primary amine while symmetric bending of ammonium shows the possibility of ammonium salt. Whereas, the peak obtained at 1713.1 cm^{-1}, in Bligh and Dyer method was not as sharp as the peak obtained n-hexen method. Therefore, to find best solvent system to achieve higher algal bio-oil yield is very significant.

Table 4.6: FTIR analysis of algal-oil obtained with different methods

n-hexen method	**Modified Bligh and Dyer method**	**Assignment**	**Functional Class**
3441.96	3452.2	-OH and –CO stretch	
2923.29	2956.0	CH_3 and $–CH_2$- in aliphatic compounds(antisym and sym stretching	Asymetical and symmentrical stretcning vibration of methylene (CH2) group
-	2923.0	CH_3 and $–CH_2$- in aliphatic compounds(antisym and sym stretching	Asymetical and symmentrical stretcning vibration of methylene (CH2) group
2852.55	2853	-CH_3 attached to O or N(CH stretching modes)	Asymetical and symmentricalstretcning vibration of methylene (CH2) group
2754.94	-	C-H stretching (Aldehyde C-H)	Aldehyde and ketones
	2062.9	-C=O	Ester conbonyl functional group of the triglycerides
1712.69	1737.4	C=O in carboxylic acid (C=O fairly broad)	Ester carbonyl functional group of the triglycerides
-	1635.8	NH_2 scissoring	Primary amines
1481.81	1461.7	NH_3^+ symmetric bending	Free amino acid/ammonium salt
1380.3	1377.7	O-C stretch	Carboxylic acid derivatives
1261.6	-	T butyl in hydrocarbons (skeletal vibration ,second band near $1200cm^{-1}$	C-O stretching
1178.9	1185.7	C-OH in alcohols (C-O Stretch)	C-O stretch (str)
1096.2	1079.7	C-OH in alcohols (C-O Stretch)	Stretching vibration of the C-O ester group
-	969.5	C-OH in alcohols (C-O Stretch)	Stretching vibration of the C-O ester group
804.2	-	$CH=CH_2$ in vinyl plane compounds (CH2out of plane wag)	C-O stretching Bending vibration of CH functional groups of isolated transolefin
723.3		$CH=CH_2$ in vinyl plane compounds (CH2out of plane wag)	C-O stretching Bending vibration of CH functional groups of isolated transolefin

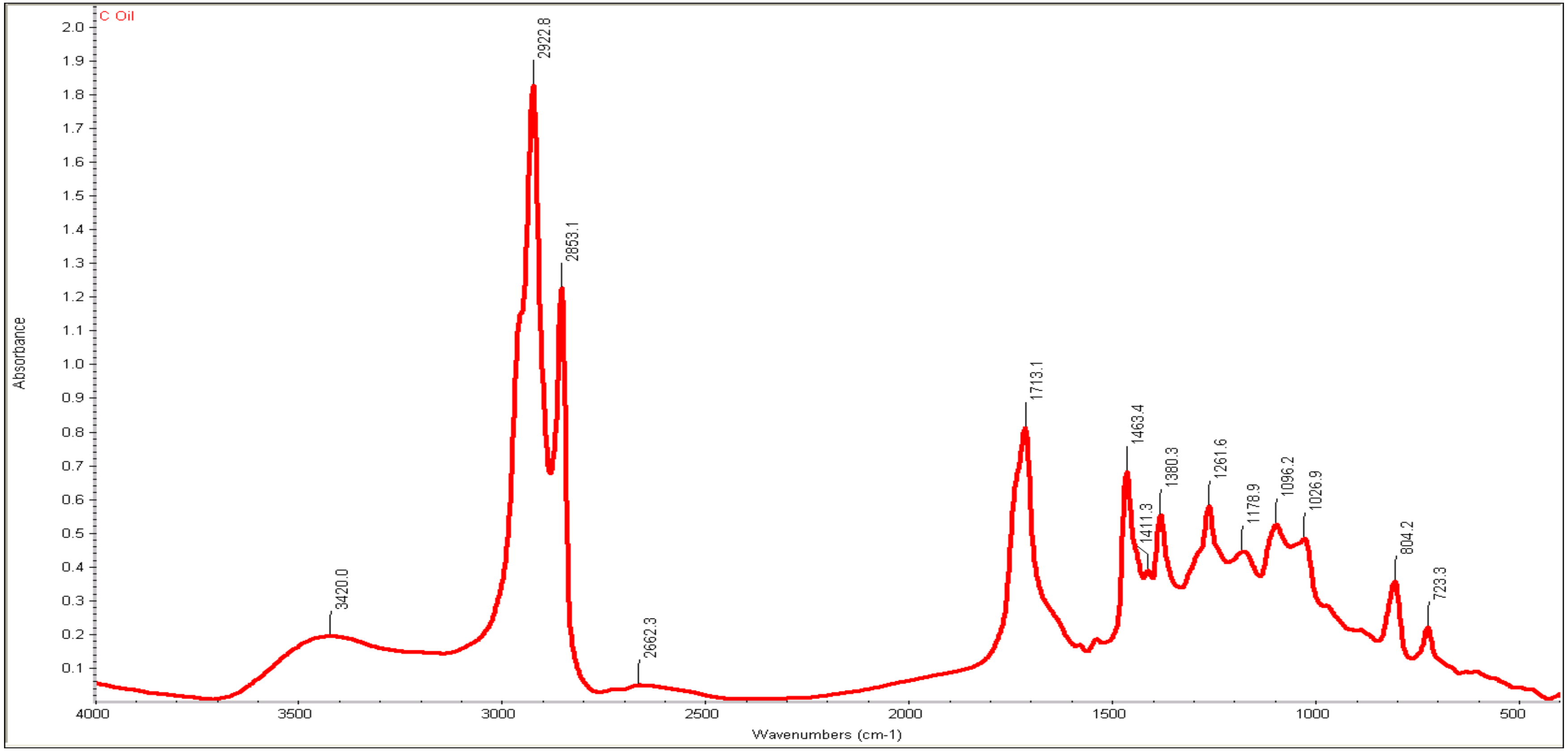


Fig. 4.11: FTIR spectra of algal bio-oil with n-hexane method

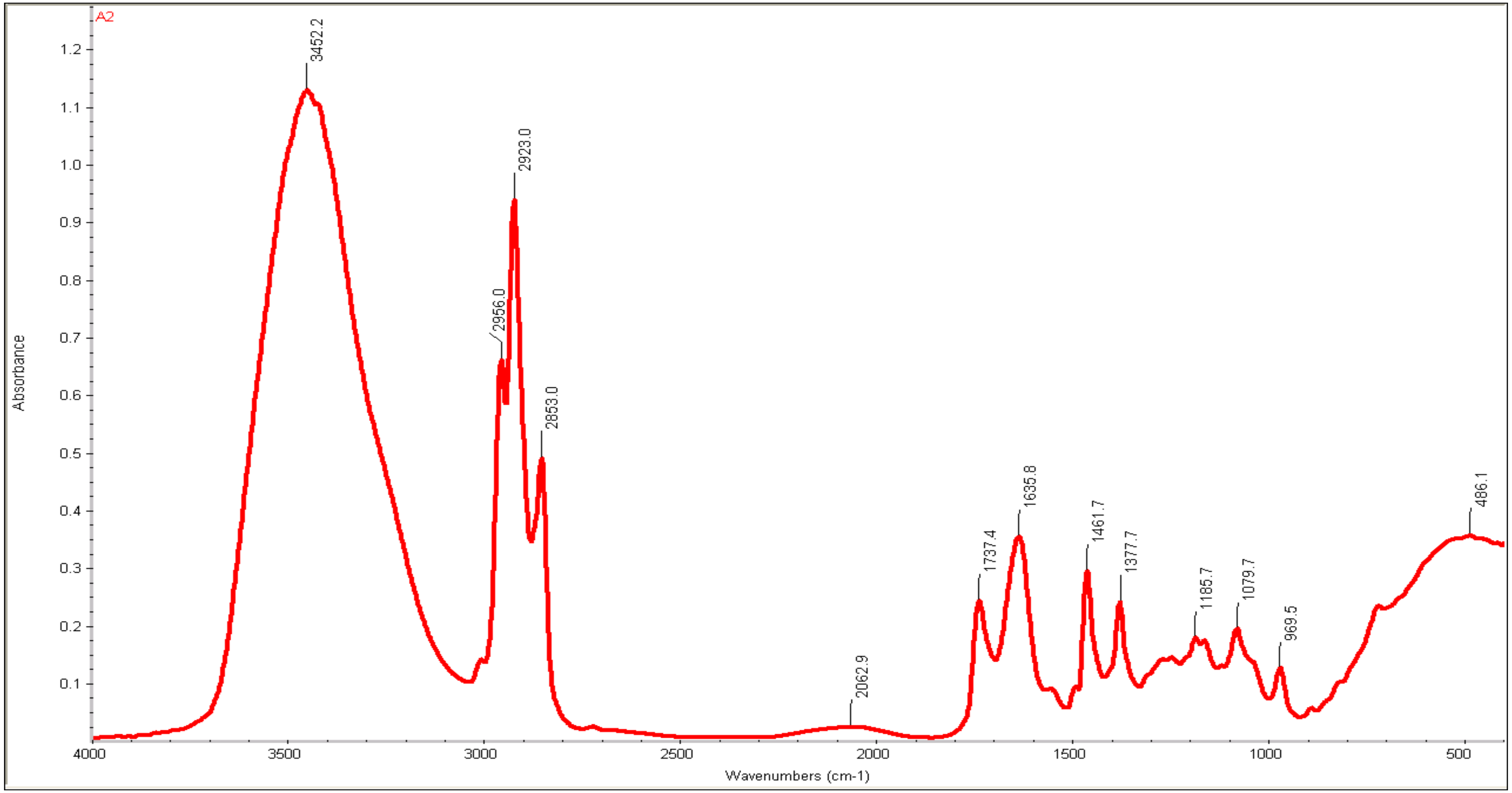


Fig. 4.12: FTIR spectra of algal bio-oil with MB&D method

4.4. Conclusion

Hence, it is clear that temperature plays an important role to enhance the algal biomass production, pollution reduction, and algal based bio-oil production. It is very important to find the most suitable way to enhance the temperature of the medium without algal cell structural deformities. The present chapter is focused to enhance the temperature of the medium in PBRs for biomass cultivation as well as heavy metal removal with bio-oil production.

- TPBR was efficient to produce (≈22 gm) dry algal biomass but the total amount of produce biomass cannot be harvested as some amount of biomass (20-25 wet biomass) remain stuck on reactors tube wall.
- In PBR-I algal biomass was not obtained in significantly amount (1.3 gm) as the optimum temperature was not provided to the system resulting, decrease biomass and bio-oil (11.4%) production with nominal metal removal (48.1±0.03).
- In PBR-II, significant amount of algal biomass (15.4 gm) was produced as the temperature of system was maintained by phase change material to its optimum required temperature. 41% algal oil was obtained from PBR-III using n-hexane method.
- In PBR-III, 15.9 gm of total biomass was obtained with significant amount (35 %) algal oil production using Modified Bligh and Dyer method.

Chapter 5

Comparative assessment of bioflocculant and chemical flocculants for algal biomass harvesting

5.1. Introduction

Downstream process for algal biomass usually associated with algal biomass harvesting and oil extraction which contributes to 60% of total production cost. Cost effective harvesting technology is a bottleneck in the research and development of algal based bioenergy and value-added compounds production at commercial level (Zhu et al., 2013; Min et al., 2011). Currently, various harvesting methods are in practice for harvesting of algal biomass from their suspension such as centrifugation, gravity sedimentation, natural and pressurized filtration, chemical flocculation, electro-flocculation, and vacuum filtration, *etc.* (Chen et al., 2011; Zhang et al., 2011; Knuckey et al., 2006; Uduman et al., 2010). Among these filtration process centrifugation, was reported with the highest yield in terms of percentage solid content 22%. However, pressurized filtration process was found with more efficiency (27%) than centrifugation (Grima et al., 2003; Semerjian and Ayoub 2003). The other centrifugation processes range between 1.5 to 18% regarding separation efficiency. In general, chemical flocculants have been known for commercial applications. These chemicals are highly efficient regarding harvesting algal biomass, but the main shortcoming related to these chemicals is their high cost that makes the techniques economically unviable. Therefore, selection of a cost effective harvesting method is critical, although, chemical coagulants have been widely used for harvesting of algae from water. Their use for harvesting become problematic as they reduce the quality of algal biomass, water and increase the complexity in lipid extraction and its conversion process. Hence, application of bioflocculant can be cost effective and efficient option for biomass harvesting in an eco-friendly method. Egg shells have the characteristic feature of biodegradability, biocompatibility, significant adsorption properties, and noteworthy flocculation ability. Itpossesses tremendous cationic charge density and can easily adsorb and destabilize the negative charges present over microalgal cells. Harvesting of algae using bioflocculant has a great potential to decrease algal biomass production costs at commercial level. Hence, the present chapter mainly emphasised to investigate the effect of various flocculants (bio and chemical flocculant) with respect to other parameters (dose concentration, contact time, temperature, and pH) harvest algal biomass significantly. The present chapter mainly divided into two subsections.

5.1.1 Impact of bioflocculant (egg shell) with respect to dose, time and temperature for algal biomass harvesting efficiency.

5.1.2. Impact of pH on harvesting efficiency of flocculants and its zeta potential analysis

5.2. Materials and methods

5.2.1. Flocculant preparation

5.2.1.1 Bioflocculant (egg shell)

The collected egg shells (bioflocculant) were washed with distilled water and dried at 40°C in an oven. Dried egg shells were grind to obtain the fine powder and sieved manually using micro sieve. The eggshell powder (100 mg) was dissolved in 10 ml of 0.1 molL^{-1} acid solution with continuous stirring for 30 min. The acid solution was then diluted to 100 ml using deionised water to make a final eggshell concentration (bioflocculant) of 1000 mgL^{-1}. Harvesting of algal biomass was performed by applying various concentrations (0, 20, 40, 60, 80, and 100 mgL^{-1}) of bioflocculants (egg shell) in algal cell suspension to optimize the significant bioflocculant dose concentration. The process of was carried out with 60 minute of contact time to investigate the optimum harvesting efficiency with respect to time.

5.2.1.2. Laboratory available $CaCO_3$ (LACC)

Laboratory available $CaCO_3$ (LACC) of (100 mgL^{-1}) was dissolved in 10 ml of 0.1 mol/L acid solution (hydrochloric acid) with continuous stirring for 30 min. The acid solution was then diluted to 100 ml using deionised water to make a final LACC concentration (chemical flocculant) of 1000mgL^{-1}. The selected chemical (LACC) has been chosen for comparative analysis with waste material waste egg shell based bioflocculant as both of them have same chemical composition with 99.9% elemental Ca^{++} ion.

5.2.2.3. Alum ($Al_2(SO_4)_3$)

Alum is a common chemical that have been proved to be an efficient chemical based flocculant (Wyatt et al., 2012). This flocculant was directly introduced (100 mgL^{-1}) to algal culture to investigate the HE. In this study alum has been taken as standard reference material to examine the viability of designed flocculant material on comparative basis. These flocculants (egg shell, LACC, and alum) were used with optimized concentration (100 mgL^{-1}) (Kothari et al., 2017) and mixed at 300 rpm, using a mini orbital shaker (VWR Advanced Orbital Shaker, Model 15000). The supernatant from the surface was taken to measure the optical density (OD) at 665 nm wavelengths after the incorporation of flocculant doses into the algal culture.

5.2.2. Harvesting efficiency (HE)

The flocculants (bio-flocculant and chemical flocculants) was added at a selected/optimized concentration and mixed at 300 rpm, using a mini orbital shaker (VWR Advanced Orbital Shaker, Model 15000). The supernatant from the surface was taken to measure the optical density (OD) at 665 nm wavelengths after the incorporation of bioflocculant doses into the algal culture.

In order to know the harvesting efficiency (equation 5.1) of algal cells with respect to time, on every five minute, optical density of the algal suspension was taken.

$$HE\ (\%) = [1- \{ODa_{665}\ (t)/\ ODa_{665}\ (t_0)\}\ /\ \{ODb_{665}\ (t)\ ODb_{665}\ (t_0)\} \times 100] \qquad (5.1)$$

Where, ODa_{665} (t_0), ODa_{665} (t) and ODb_{665} (t_0) and ODb_{665} (t) are the turbidities of algal cell suspension without and with treated bioflocculant at time zero and time *t*, respectively. Data represented in table and figures are mean values from three replicates.

5.2.3. Impact of temperature on harvesting efficiency

After optimization of bioflocculant dose concentration, the optimum concentration of bioflocculant dose was further proceeded to investigate the effect of temperature on harvesting efficiency. The optimized dose were subjected to different temperature ranges 30°C, 35°C, 40°C, 45°C, and 50°C to investigate the change in harvesting efficiency. The required ranges of temperature were maintained by hot water bath.

5.2.3.1. Kinetic model

Kinetic study by pseudo-second ordermodel is mainly used for adsorption process (Nuhoglu and Malkoc 2009; Ho and McKay 1999). However, various authors have used this model to evaluate the rate of reaction other than adsorption process such as biomass production and oil production (Maurya et al., 2014). Therefore, surface binding of bioflocculant can be also evaluated by following this model. The rate of pseudo-second-order reaction usually depends on binding of flocculants on the surface of algal biomass, which can be expressed by following equation:

$$t/q_t = 1/k_2\ q_e^2 + t/q \qquad (5.2)$$

Where, K_2= Rate constant ($gmg^{-1}min^{-1}$); q_e = Amount of biomass (mg g^{-1}) flocculated at equilibrium; q_t = Amount of biomass (mg g^{-1}) flocculated at time t.

Initial variables such as q_e and q_t are quantified by following equation:

$$q = (C_i - C_f)*V / m \quad (5.3)$$

Where, q = Bioflocculant adsorption capacity; C_i, = Initial algal biomass concentration; C_f = Biomass concentration after flocculation; V = Volume of the solution (L); m = Amount of bioflocculant (mg l^{-1}).

The pseudo second order kinetic model is expressed by plot between t/q versus t. Kinetic variables such as K_2 (rate constant), h (initial flocculation rate), q_e (calculated bioflocculation capacity) can be calculated from the slope and intercept of the straight line equation.

5.2.3.2. Determination of thermodynamic functions

In order to support the temperature based experimental data of harvesting efficiency of algal biomass, the Eyring and Arrhenius equation were applied to describe the adsorption behaviour of bioflocculant by the *Chlorella pyrenoidosa*

5.2.3.2.1. Eyring equation

The effect of temperature on time-dependent flocculation of algal biomass can be expressed by the thermodynamic parameters. Eyring type plot between $\ln k_2/T$ versus 1/T was assessed to calculate the thermodynamic parameters. Eyring equation is used in chemical kinetics to describe the variance of the rate of reaction with temperature. The change in enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG) after adsorption of bioflocculant has been investigated in flocculation process at different temperature by using Von't Hoff equation (Yao et al., 2010; Shivaraj et al., 2001). The thermodynamic parameters such as standard free energy changes (ΔG), the standard enthalpy changes (ΔH) and the standard entropy change (ΔS) is obtained from experiments at various temperatures using the following equations 5.4 and 5.5:

$$\text{Intercept} = [\ln (kb/h) + \Delta S/R] \quad (5.4)$$

$$\text{Slope} = [-\Delta H/R] \quad (5.5)$$

Where, k_b= Boltzmann constant; h= Plank's constant; and R= Gas constant.

The slop and intercept of strait line equation is used to calculate the thermodynamic parametersΔH and ΔS. The Gibb's free energy (ΔG) (Equation 5.6) has been calculated by the obtained value of ΔS and ΔH for different temperature in Calvin.

$$\Delta G= \Delta H-T\Delta S \tag{5.6}$$

Where, ΔG = Gibbs free energy; ΔH = Enthalpy; ΔS = Entropy.

The above said equation had been applied to know the mathematical relationship between bioflocculant adsorption by the algal cell at different temperature and time. Thermodynamic parameters obtained from Van't Hoff graph, which is also known as Eyring type equation, the most widely accepted models taken into consideration to know the rate of reaction after absorbance of bioflocculant by the algal cell.

5.2.3.2.2. Arrhenius equation

Arrhenius equation has been used to investigate the activation energy *i.e.* maximum energy required to start the chemical reaction after incorporation of bioflocculant. The Arrhenius equation expresses that flocculation is a function of temperature in pseudo-second-order rate constant. The Arrhenius equation can be expressed by the following equation:

$$\text{Slope} = -Ea/R \tag{5.7}$$

Where, -Ea = Arrhenius activation energy; R = Gas constant which is equal to 8.314 j/ mol $^{-1}$ k^{-1}

5.2.4. Impact of pH on harvesting efficiency

To investigate the effect of pH on HE of algal cell, a flocculation experiments were performed with best obtained result of dose concentration in four small beakers (1000 ml). The dense algal cultures were kept 1000 ml in each beaker. pH of bioflocculant were maintained (2.0, 4.0, 6.0, 8.0, and 10.0) by adding 1N of HCl and NaOH. The maintained pH of bioflocculant were tested to investigate the efficiency of flocculation by taking OD at 665 nm, using UV visible spectrophotometer (HALO-DB 20, Thermo Scientific).

5.2.4.1. Zeta potential analysis

An instrument, Nanoplus Zeta /nano particle analyzer (Model: Nano Plus-3, Serial no. 405613, made in Japan), was used to investigate the zeta potential of the system (bioflocculant) to analyze the harvesting efficiency. The zeta potential of flocculants (bioflocculant and chemical flocculant) was measured within the pH ranges (2.0, 4.0, 6.0, 8.0, and 10.0) as maintained by acid base solutions. Zeta potential was performed in triplicate at 25°C and the average values have been taken into account.

5.3. Results and discussion

In order to investigate the effect of various flocculants associated with other parameters *i.e.* dose concentration, temperature, and pH on harvesting efficiency of algal biomass have been critically discussed in this chapter. The obtained data from different parameters are supported by kinetic model, thermodynamic functions, and zeta potential analysis.

5.3.1 Impact of bioflocculant (egg shell) with respect to dose, time and temperature for algal biomass harvesting efficiency

5.3.1.1. Effect of bioflocculant's dose on harvesting efficiency

The suspension of *Chlorella pyrenoidosa* was subjected to the different concentration of bioflocculant (egg shell) ranges from the 0, 20, 40, 60, 80, and 100 mgL^{-1} with respect to different time intervals (every five minutes OD has been taken at 665 nm) to investigate the maximum harvesting efficiency of best-suited doses with minimum time. The highest harvesting efficiency was obtained (≈95.6%) at highest (100 mgL^{-1}) as illustrated in Fig. 5.1. The experimental data shows that maximum biomass was being settled down with 100 mgL^{-1} concentration of bioflocculant at ≈45 minute. The algal biomass culture (without bioflocculant) had harvesting efficiency with ≈15.6%. Therefore, the present bioflocculation process is more effective and economically viable as reported in traditional harvesting methods such as centrifugation, filtration, and flotation that have been applied to various algal species (Grima et al., 2003; Semerjian and Ayoub 2003). Unlike organic flocculants (cationic polyacrylamide, cationic starch and chitosan) and inorganic (alum) are degradable and less effect on algal cell but still they are highly expensive. Quite the reverse, egg shell (as bioflocculant) is cheap and best method to harvest algal biomass as fully supported by the results obtained in this study. Papazi et al., (2010) showed a harvesting efficiency of 60% for *Chlorella minutissima* by addition of 1 g L^{-1} of $Al_2(SO_4)_3$ and $ZnCl_2$ in, respectively, 1.5 and 6 h. According to Lee et al., (1998), and McGarry, (1970) used up to 300 and 125 mg L^{-1} of Al^{3+} respectively to harvest algal biomass. However, the present study is based on the cheap and easily available waste material which is highly efficient regarding separation efficiency with less time, and lowest energy input for further utilization of algal-based bioproducts. In the case of chemical flocculation, although significant separation efficiency was reported by Udhaya et al., (2014), but sometimes it damages algal cell structure. On the other hand, microalgae flocculated with chemical flocculants have already reported for commercial level application, but their frequent release in aquatic environment may cause toxicity to the aquatic organism. Thus, use of biowaste as bioflocculant seems to be significant for algal harvesting process with no toxic effects.

5.3.1.2. Effect of temperature on HE

The effect of temperature (30°C, 35°, 40°C, 45°C, and 50°C) was also observed on harvesting efficiency of algal biomass, to get it more critical findings, best concentration result (100 mgL^{-1}) obtained by using different concentration of bioflocculant, is taken here as a part of investigations as represented in Fig. 5.2.

The highest harvesting efficiency was obtained (≈99.3%) with highest temperature range 50°C, whereas, the lowest harvesting efficiency was obtained (≈97.09%) at room temperature (30°C), with the use of bioflocculant concentration of 100 mgL^{-1}. It is also known that as the temperature increases, rate of reaction also increases. Therefore, it may be possible that temperature provoked the harvesting efficiency of algal biomass, when increases up to some extent *i.e.* 30°C to 50°C. Some researchers had already reported that flocculation can also be induced by changing the culture conditions by applying temperature changes (Lei et al., 2015). Effect of temperature to harvest algal biomass by using bioflocculant was also observed by Salim et al., (2011). Hence, temperature plays a significant role to enhance the rate of reaction and binding capacity of bioflocculant by the algal biomass. The experimental data also exposed that there is reduction in time as the temperature increases. At 40°C and 50°C temperature, harvesting of algal biomass has taken place approximately within half an hour. Therefore, harvesting efficiency of bioflocculant showed great differences when, algal biomass exposed to different temperature ranges. Most of the bioflocculants usually belongs to protein, extracellular polymeric substances, and polysaccharides, subsequently; at high temperature these substances lost bioactivity to achieve thermal stability (Kim et al., 2011). It has been reported that flocculation activity of different bioflocculants enhanced at 40°C whereas, limited flocculation activity was noticed at low temperature (Wan et al., 2013).

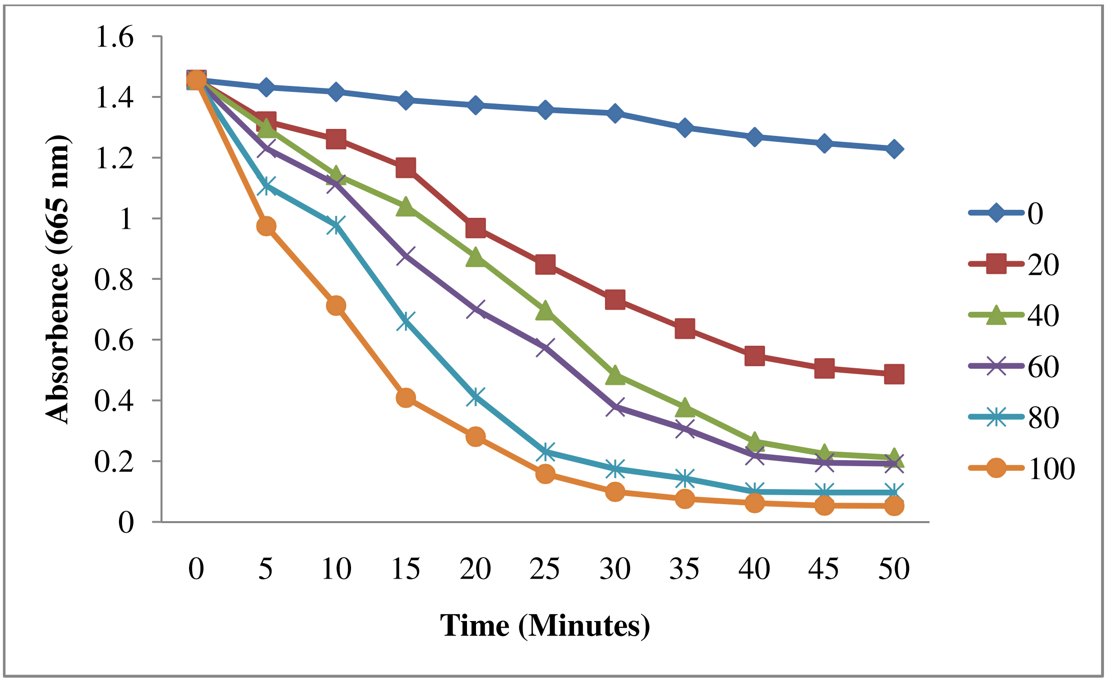


Fig. 5.1: HE of *Chlorella pyrenoidosa* with different concentration of bioflocculant

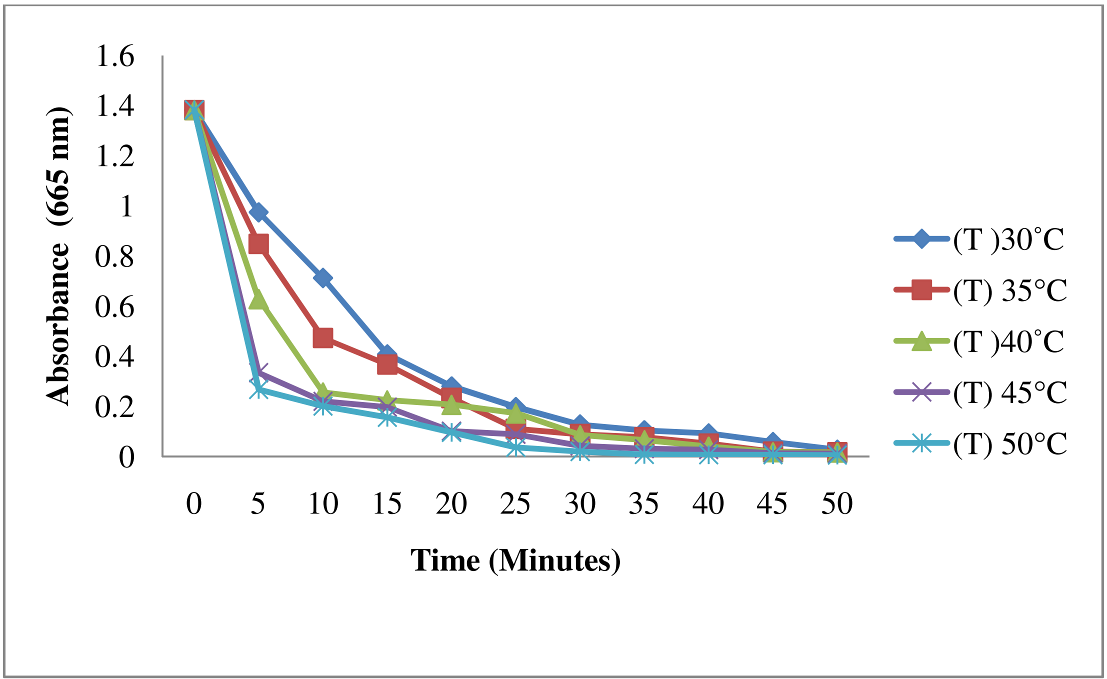


Fig. 5.2: HE of *Chlorella pyrenoidosa* with different temperature

Hence, the chemical components of bioflocculants play a significant role to enhance the harvesting efficiency. To support the experimental data regarding harvesting efficiency of algal biomass, kinetics and thermodynamics model has been applied and described in further section by this study.

5.3.1.2.1. SEM and EDS analysis

Effect of bioflocculant concentration were observed with only lowest (0 concentration) and maximum (100 mg/L)concentration to depict the changes in algal cell surface structures during experimentation using microscopic, SEM and EDS analysis (Fig. 5.3). The microscopic images showed that there is a remark increase in algal biomass harvesting as the bioflocculant concentration increases with no changes in structural morphology of the cell. Although, effects of temperature (30°C, 40°C, 50°C) on cell surface was also observed by using SEM analysis with 100 mg/L concentration of bioflocculant as exhibited in Fig. 5.4. The result obtained from the SEM micrograph showed a normal and smooth shape prior to the flocculation process while after treatment with bioflocculant algal cell deposition of calcium with minute changes in cell surface at 40°C, however, major changes in cell surface were observed with flocculation process at 50°C temperature. Hence, this experiment shows that temperature has a marked effect on cells surface structure due to its effect on binding of bioflocculants with cell surface. A slight whitening of the algal cell has been obtained after treatment with bioflocculant at each temperature. It is due to the calcium deposition of the egg shell. Although, SEM images shows a minute changes but EDS analysis confirmed the presence of calcium in the cell wall of algal biomass.

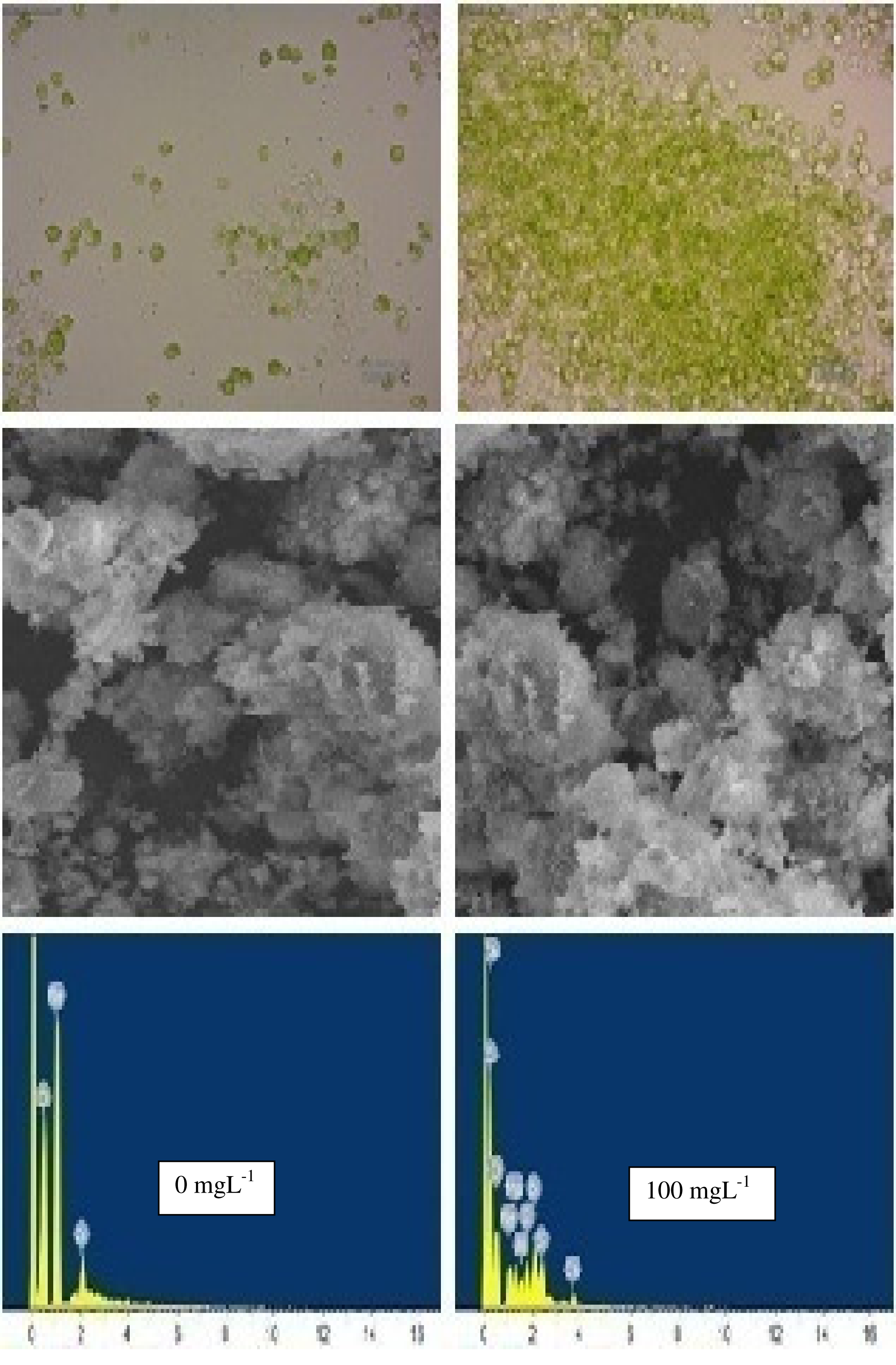


Fig. 5.3: Microscopic, SEM, and EDS images of algal cells: (a) 0; and (b) 100 mgL^{-1} bioflocculant concentration

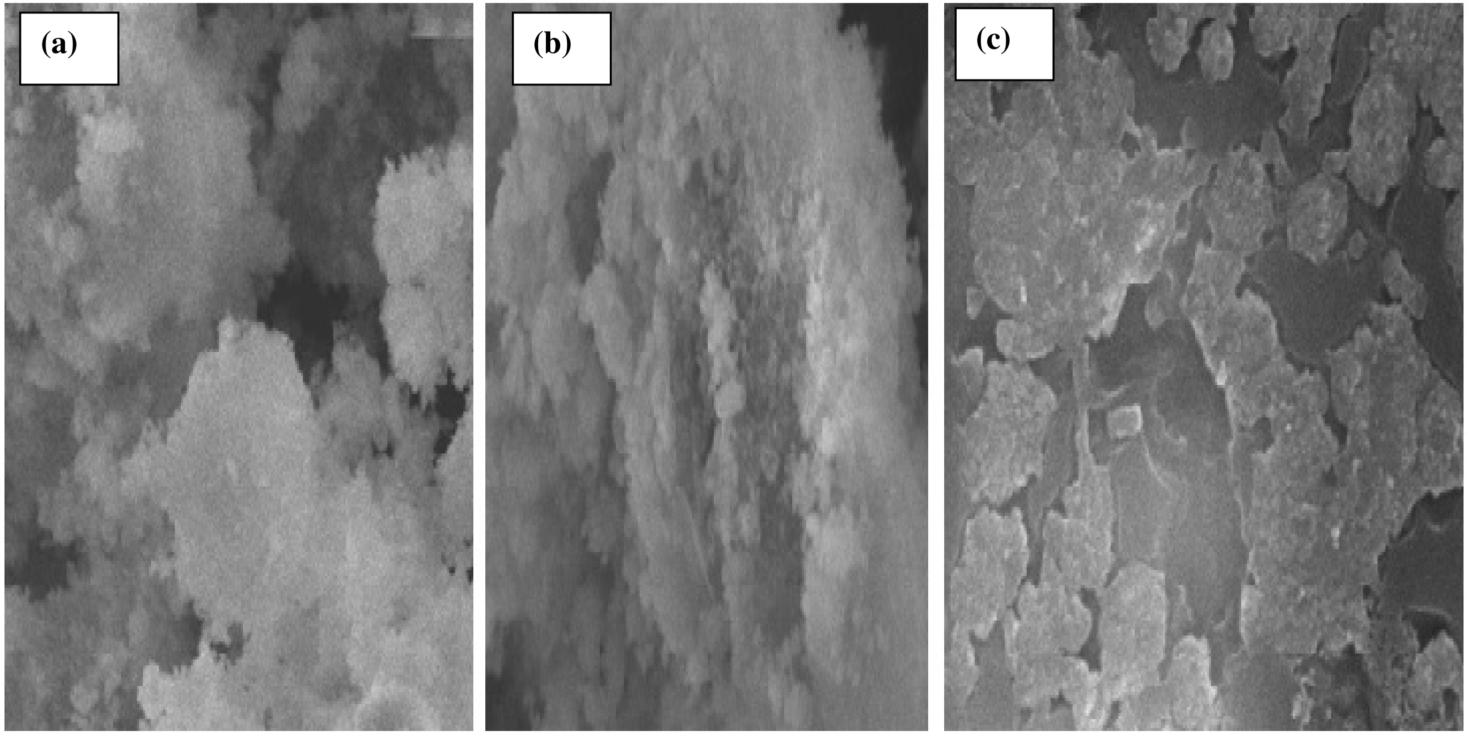


Fig. 5.4: Effect of temperature on surface structure of *Chlorella pyrenoidosa*: (a) 30°C; (b) 40°C; (c) 50°

5.3.1.2.2. Kinetic model and thermodynamic functions

Experimental data for flocculation of algal biomass by using bioflocculant was found best fitted with pseudo second order kinetics model. The plot t/q versus t was found significant over all temperature and shows high extent of correlation (R^2 = >0.90) as illustrated in Fig. 5.5. The rate constant derived from straight line equation was found increasing with increase in temperature and ranges from (0.002-0.041 mg/g) as shown in Table 5.1. It clearly confirms that effective flocculation can be achieved at higher temperature. The values of initial flocculation rate *i.e.* 'h' also resemble the same observation and found to be increase with increase in temperature. The maximum value of 'h' was 8.33 mg $g^{-1}min^{-1}$at 50°C. The feasibility of flocculation process at different temperature was observed through evaluation of thermodynamic variables. The Eyring plot (lnK_2/T versus 1/T) is illustrated in Fig. 5.6. The ΔH and ΔS value havebeen calculated from the slope and intercept of Eyring plots. The positive value of ΔH shows the endothermic nature of adsorption, and there may be a possibility of physical adsorption. In the case of physical adsorption, an increase in temperature of the system will enhance the bioflocculant adsorption up to some extent. The positive value of ΔS shows an increased disorder and randomness in bioflocculant adsorption by algal culture. The negative value of ΔG depicts that adsorption is highly favourable for flocculants *i.e.* the reaction is spontaneous as given in Table 6.2. The positive value of ΔH indicates that interaction of flocculent by algal biomass is an endothermic process, and the positive value of ΔS reveals that, there is an increase in randomness during adsorption process (Nuhoglu et al., 2009). Activation energy was obtained from the Arrhenius plot (lnk verses 1/T) as exemplify in Fig. 5.7, and the calculated value is 113.04kj/mol. Activation energy is the minimum required energy to start a chemical reaction. Therefore, at 50°C harvesting efficiency was obtained maximum.

But *Chlorella pyrenoidosa* grow well at 25-30°C and can tolerate up to 40°C for few hours only. Although, at 50°C cell structure of *Chlorella* shows some variations like cell wall deformation and cell disruption, and change in surface structure up to some extent. Hence, it is concluded that at 40° C temperature with bioflocculant (100 mg/L) is highly efficient to harvest algal biomass from the suspension.

Table 5.1: Value obtained from pseudo second order model

		Pseudo-second-order model		
Temperature (°C)	**K_2 (mg/g)**	**q_e (mg/g)**	**h (mg $g^{-1}min^{-1}$)**	**R^2**
30	0.0023	21.73913	1.06383	0.97
35	0.003	19.23	1.1093	0.99
40	0.01	16.66667	2.7889	0.99
45	0.01	14.9253	3.3191	0.99
50	0.041	14.285	8.333333	0.99

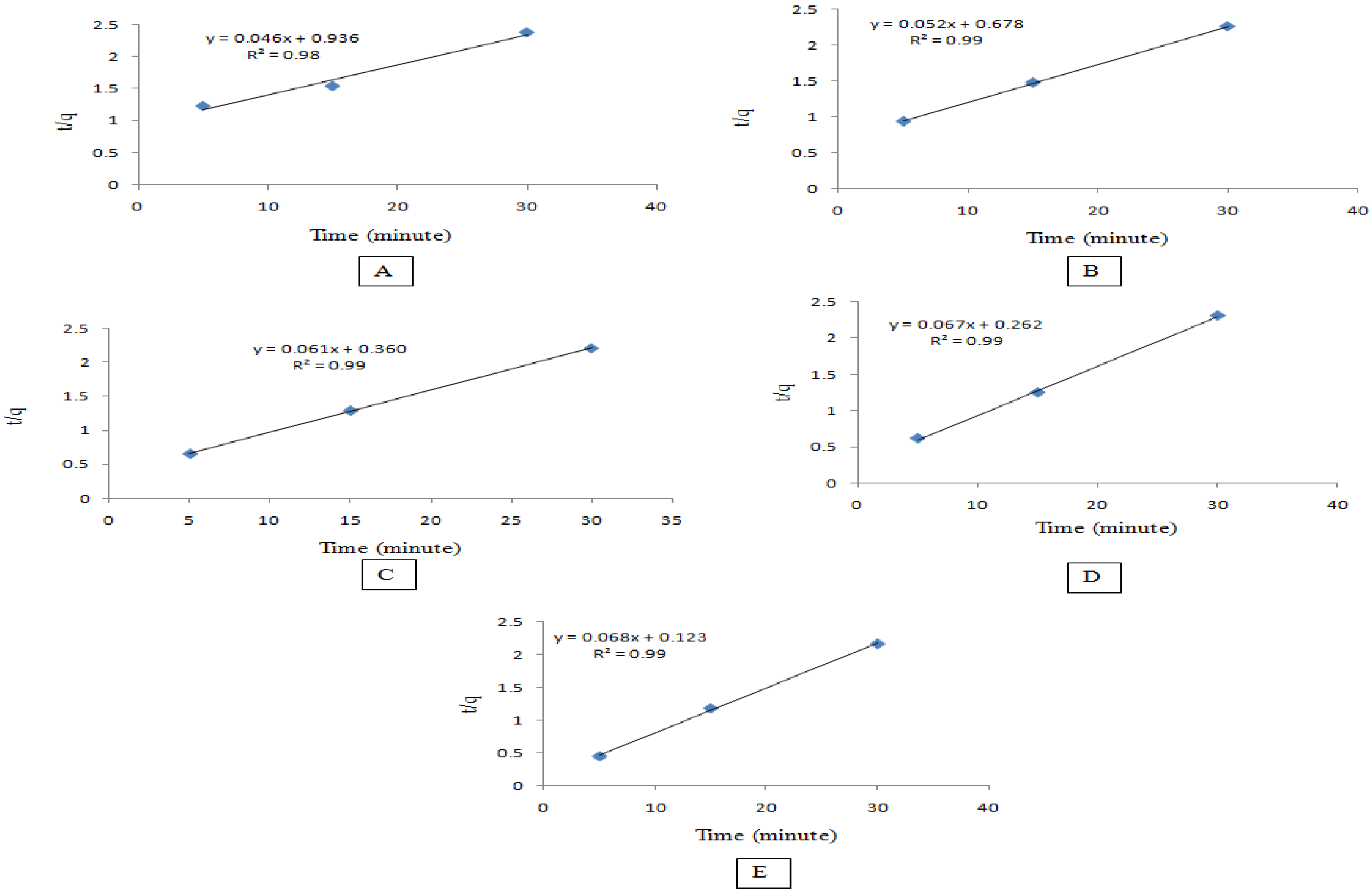


Fig. 5.5: Pseudo-second-order rate constant at different temperature: (a) 30°C (b) 35°C (c) 40°C (d) 45°C (e) 50°C

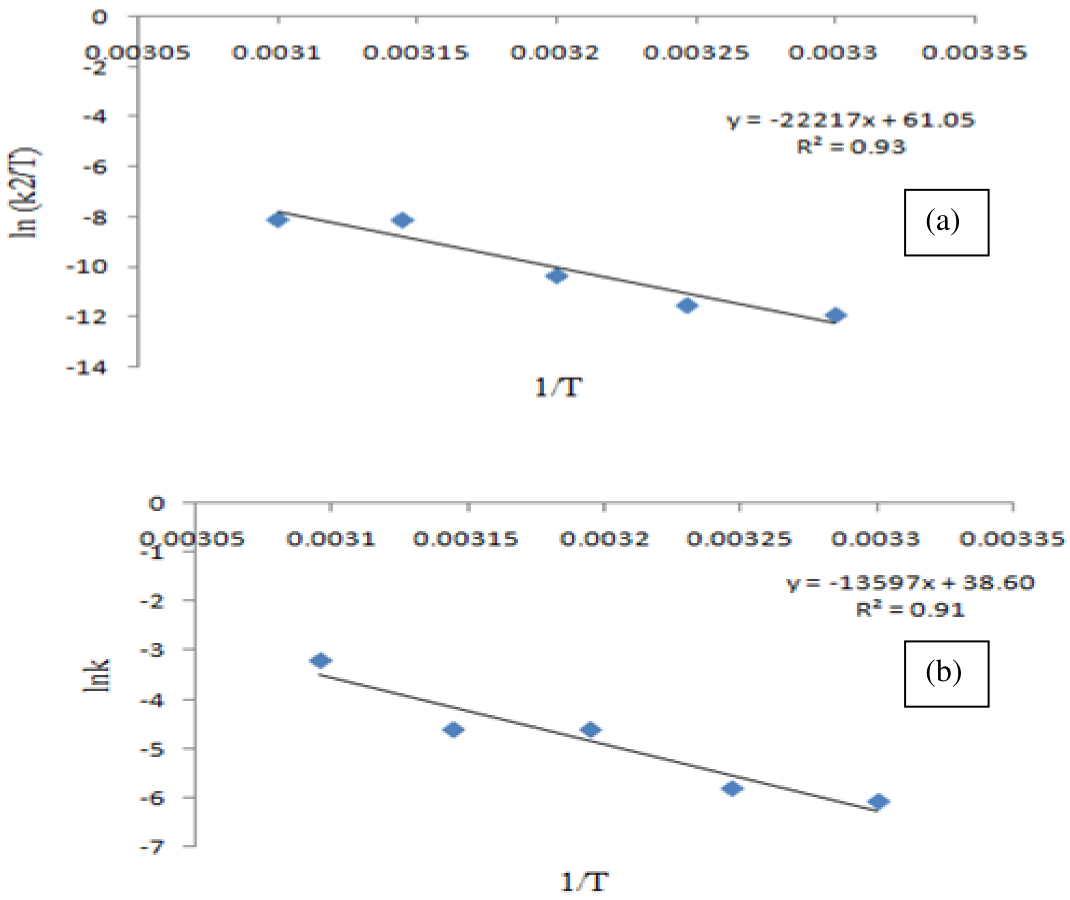


Fig. 5.6: Thermodynamics functions: (a) Eyring and (b) Arrhenius plot at various temperatures

Table 5.2: Thermodynamic functions

Thermodynamic parameters	**Values (KJ/mol)**	
Enthalpy, ΔH	184.7121	
Entropy, ΔS	1.613157	
Activation energy, Ea	113.04	
Gibbs free energy, ΔG	Temp.(Kelvin)	ΔG
	303	-304.074
	308	-312.14
	313	-320.206
	318	-328.272
	323	-336.338

5.3.2. Impact of pH on harvesting efficiency of flocculants and its zeta potential analysis

The effect of pH on harvesting efficiency of algal biomass with flocculants has been investigated. The optimised concentration of bioflocculant (egg shell) has been further investigated with different ranges of pH. To investigate the feasibility of bioflocculant for harvesting of algal biomass, comparative analysis has been done with LACC and alum. The obtained experimental data has been supported by zeta potential analysis.

5.3.2.1. Effect of flocculants on harvesting efficiency

The algal cell suspensions were subjected to 100 mg/L of bioflocculant and LACC for comparative analysis of both flocculants to understand flocculation efficiency. The experiment was performed for 60 minutes. On every 5 minutes OD (665 nm) from the surface has been taken to know the density reduction in the algal cell suspension as illustrated in Fig. 5.8. The highest HE was obtained by using alum *i.e.* 99%. A significant result has also been found by using bioflocculant and LACC *i.e.* 95% and 83% respectively. Although, the percentage of HE is quite less then alum but the main advantage is that bioflocculant does not harm the cell surface structure, while alum play a vital role in deteriorating the cell surface structure and may be restricted for further use. Some study have also reported that divalent metal ions (Mg^{+2}, Ca^{+2}, Al^{+2} *etc.*) are significant in terms of HE of microalgal biomass (Vandamme et al., 2012; Papazi et al., 2010). These metal ions have ability to hydrolyse and formed positive charged precipitations which coagulates negatively charged microbial cells. Papazi et al., (2010), reported the harvesting efficiency of 85% on *Chlorella minutissma* by using ferric salt which is less then this study *i.e.* 99% (alum). Papazi et al., 2010 stated that aluminium salt is more effective in flocculating *Chlorella* sp. than ferric salts. The HE of cationic organic polyacrylamide based flocculent has been studied on

Teraselmis and *Spirulina* with 70% of biomass recovery efficiency by Pushparaj et al., (1993), which is less than this experimental study (95% egg shell bioflocculant).

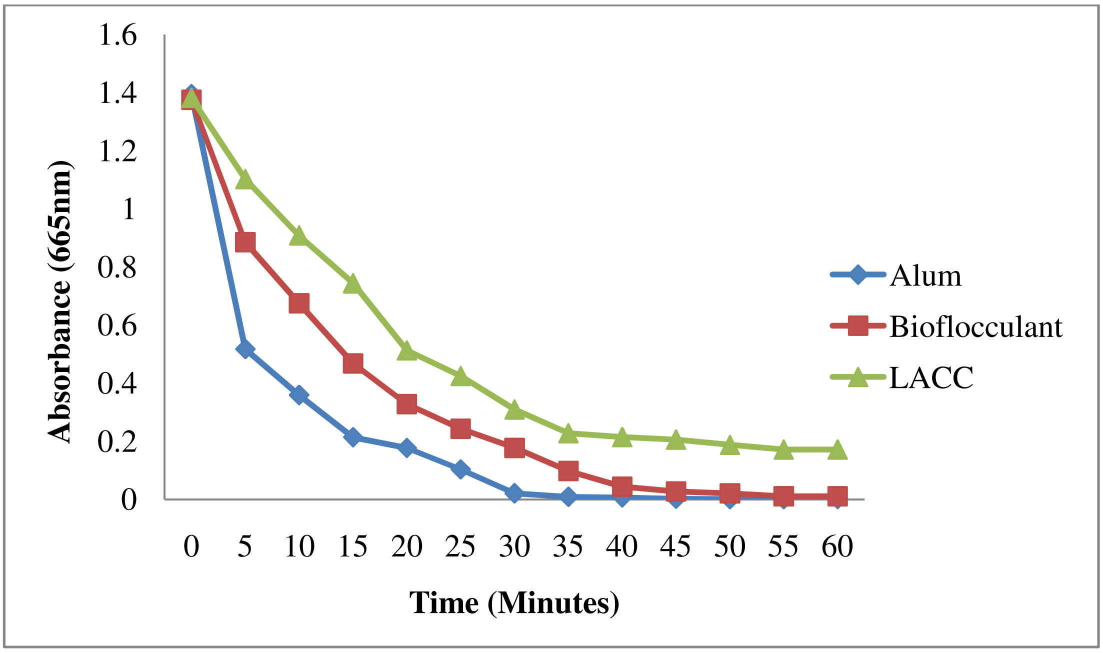


Fig. 5.7: Effect of different flocculants on harvesting of algal biomass

5.3.2.1.1. SEM and EDS analysis

The effect of flocculants (waste egg shell, LACC, and alum) on morphology of *Chlorella pyrenoidosa* during flocculation process was studied by SEM image analysis (Fig. 6.8.). Smooth and fine flocs of algal biomass were obtained with no cell surface structural deformities and cell shrinkage. A slight variation in structural morphology with cell surface shrinkage has been obtained, when algal biomass was subjected with LACC. In case of alum, the scenario was totally changed, as it is a potential chemical based flocculant but imparts a high degree of cell surface and structural deformities. Therefore, the impact of chemical (alum) stress on algal cell surface clearly indicates the potential of harvesting of algal biomass but it cannot be used as livestock feed due to the metal stress. Kim et al., (2011) reported a slow growth rate of *Scenedesmus* sp. when treated by the alum as a flocculating agent. It is clear from the SEM images thata slight whitening of the algal cell has been obtained after treatment with LACC at room temperature. It is due to the deposition of the calcium carbonate, but such whitening was completely absent in algal biomass treated with egg shell based bioflocculant. Although, SEM images shows a minute change, but EDS analysis confirmed the presence of calcium in the cell wall of algal biomass.EDS graph of algal biomass harvested by alum showed the presence of aluminum and harmful elements. Therefore, algal biomass harvested by alum are restricted to further use in various fields such as medicinal, pharmaceutical, cosmetic, nutritional diet *etc.*Hence, it is clear from the SEM images that harvested algal biomass by using bioflocculant is most suitable for using as livestock feed, shrimp feed, poultry feed, animal feed, and aquaculture. Whereas, algal biomass harvested by using bioflocculant are independent over these limitations.

5.3.2.2. Effect of pH on harvesting efficiency with flocculants

5.3.2.2.1. Bioflocculant

In order to know the effect of pH on HE of algal biomass, the experiment was further preceded with optimum concentration (100 mg/L) of bioflocculant and different range of pH (2.0, 4.0, 6.0, 8.0, and 10.0 pH) as illustrated in Fig. 6.9. The experimental data of the experiment demonstrated a sharp increment in HE of *Chlorella pyrenoidosa* at low pH. The microalgal cells were began to flocculate/coagulate when pH decreased from 8.0 to 4.0 pH. Flocculation of algal cells could still be observed at pH decrease from 4.0 pH and increases from 8.0 to 10.0 pH but, the percentage of HE was relatively low. The further decrease in pH from 4.0 to 2.0, a rapid flocculation and fast subsidence of algal cell within few minutes has been noticed in algal cell suspension, but the HE were achieved less (97%) then at pH 4.0 (99%).

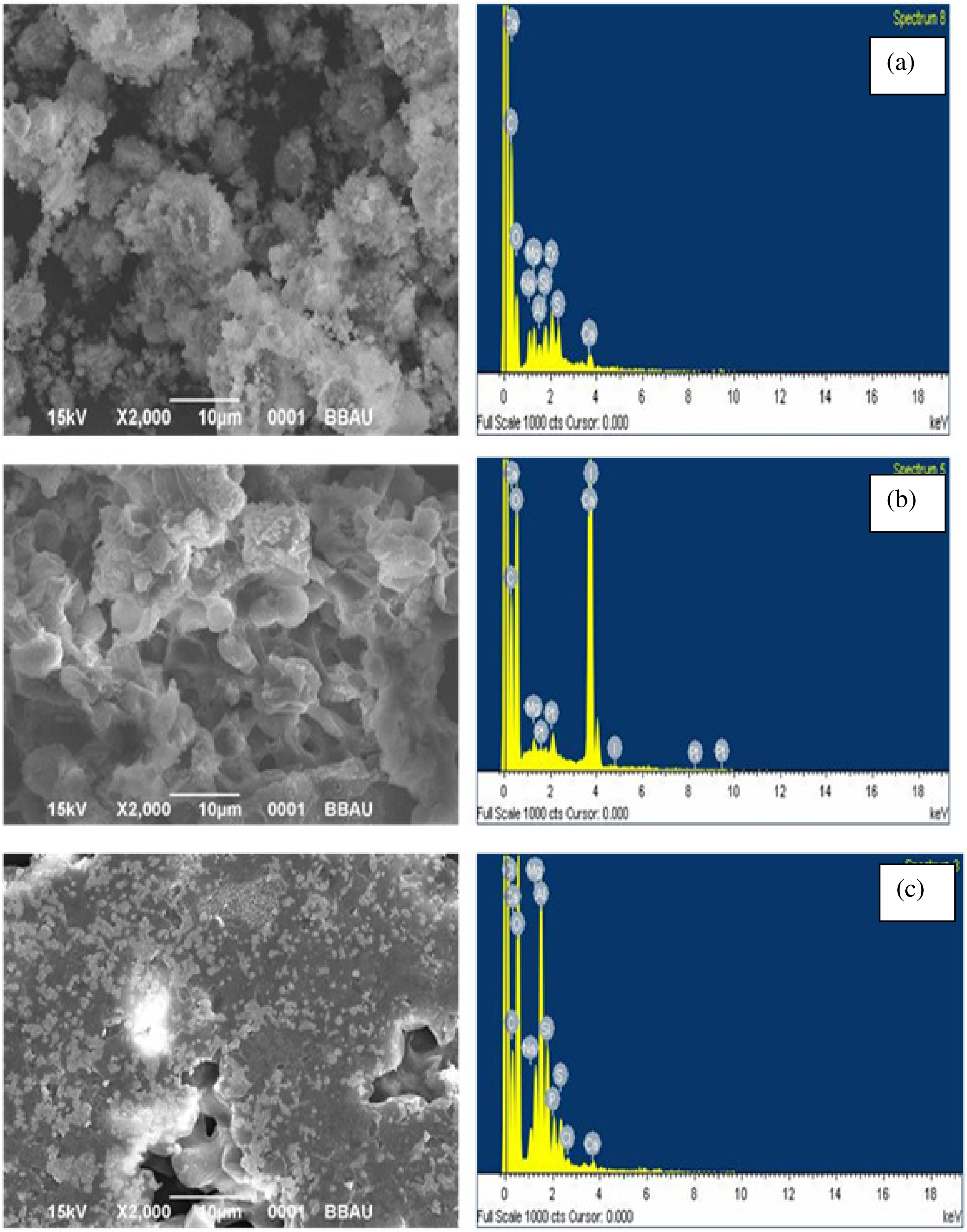


Fig. 5.8: SEM and EDS images of algal cells treated with flocculants (100 mg/L): (a) Egg shell as bioflocculant; (b) LACC; and (c) alum

When the pH increased from 6.0 to 10.0 a remarkable decrease in pH (95%, 91%, and 89%) has been noticed, which indicates that increase in pH is responsible of decrease in HE of algal biomass. Therefore, a favourable flocculation zone was attained when the pH was less than 6.0. These result indicates that pH induced flocculation efficiency is an advantageous method to harvest the *Chlorella pyrenoidosa.* The further decrease in pH from 4.0 to 2.0, a rapid flocculation/coagulation and fast subsidence of algal cell within few minutes has been noticed in algal cell suspension, but the HE were achieved less (97%) then at pH 4.0 (99%). When the pH increased from 8.0 to 10.0 a remarkable decrease in HE *i.e.* 91% and 89% has been noticed. The effect of pH on HE of *Chlorella pyrenoidosa* can be explained by the physical properties of bioflocculant (egg shell) and physico-chemical interactions between microalgal cells and bioflocculants. At low pH value, the algal cell surface becomes protonated while it is deprotonated at high pH value. The interaction between algal cells and bioflocculants as well as HE is affected by the potential charge difference. Therefore, at high pH value (more than 7.0) algal cell surface become negatively charged and bioflocculant (egg shell) also become protonated in acidic medium. As a result of which, a flocculation/coagulation and settling of microalgal cells are greatly hindered by the protonated medium of microalgal cell suspension. Similar to this context at high pH (more than 8.0), the particles present in the suspension are highly negatively/positively charged that causes repulsion, as a result of which decrease harvesting efficiency. It has also been reported that divalent metal ion (Ca^{++}, Mg^{++} *etc.*) play a significant role in flocculation of algal biomass at various pH. These divalent metal ions are hydrolyzed to form positively charged precipitate which bind with the negatively charged microalgal cells. In dense culture of microalgae, pH values are highly variable, it may enhanced up to 10.0 due to intense primary production while it may decreased (less than 7.0) due to the addition of CO_2 by algal respiration. Therefore, a favourable flocculation zone was attained when the pH was 4.0. These result indicates that pH induced harvesting efficiency is an advantageous method to harvest the *Chlorella pyrenoidosa.*

5.3.2.2.2. Laboratory available $CaCO_3$

The influence of pH (ranges from 2.0 to 10.0) on HE was tested in lab scale experiment as illustrated in Fig. 5.10. A remarkable increase in the percentage of HE was observed by using LACC. The optima of HE have been noticed at pH 8 with 95%. However, a minute difference in FE was noticed at pH 10 *etc.* 94%. When the pH value decreased from base to acid, a remarkable decrease in HE of algal biomass was observed. This indicates that the flocculation efficiency of *Chlorella pyrenoidosa* decrease when the pH value of the growth media decrease. The observation of this experimental study is being agreement with the previous experimental study. It may be possible that the bivalent cations of calcium ion pay a significant role to increase the flocculation efficiency of algal biomass, when the pH values increase from acid to base.

5.3.2.3. Interrelation between HE and pH using zeta potential analysis

5.3.2.3.1. Bioflocculant

The zeta potential of charged particles and HE are usually affected by the pH values. Bioflocculant (egg shell) and microalgal cell suspension is considered as a colloidal dispersion. Zeta potential can be determined as an indicator of dispersion stability or the degree of electrostatic repulsion. High value of zeta potential is an indicator of electrically stabilized colloids, while low value of zeta potential follows the perfect flocculation/coagulation trend. Usually, zeta potential values ranges from 0 to ±5 shows a high degree of flocculation/coagulation due to instability, while the zeta potential value ranges from ±10 to ±30 mV shows a high degree of stability resulting, no flocculation/coagulation. In present study, a bioflocculant dose (100 mg/L) corresponded to the ≈0 value of zeta potential with maximum HE 99% at pH 4.0. Zeta potential and flocculation efficiency of algal biomass is a pH dependent process as illustrated in Fig. 5.12. From pH 10.0 to 2.0, zeta potential showed a sharp increase from -0.84 mV to 0.96 mV in algal cell suspension with 100 mgL^{-1} bioflocculant dose concentration. Similar to this context Rashid et al., (2013), also reported an increasing trend in zeta potential value with high concentration of flocculant dose. The corresponding flocculation efficiency of algal biomass drastically increased to the maximum with pH 4.0 at 25°C temperature. Choi (2015), reported an optimal pH *etc.* 6.0-7.0 for maximum harvesting efficiency *i.e.* 99%. However, a sharp decrease in harvesting efficiency (88%) has been noticed while increasing pH (10.0).

Variation in pH values also affect the zeta potential values of negatively and positively charged partials of algal cell suspension (Vandamme et al., 2010; Wu et al., 2012) Liu et at., (2013), reported that microalgal cells began to flocculate when pH decreases from 6.7 to 5.0 and flocculation was still noticed when pH further decreases up to 4.5. In this context, Liu et al., (2013) found a flocculation efficiency of 90% at pH 4.0. Therefore, best flocculation zone has been attained when pH was lower than 5.0. However, a slight decrease in flocculation efficiency was obtained when pH was further decrease from 4.0 to 2.0. This result shows that pH induced flocculation efficiency is a significant method to harvest fresh water microalgae. The algal cells usually receive the charges of flocculent and exhibit stability from ionization of carboxylic groups into carboxylate ions. As the pH decreases from 6.0, carboxylate ions accepts proton, therefore, the surface charge of the algal cell decreases, as a consequence algal cell become instable in media and flocculate to form big flocs.

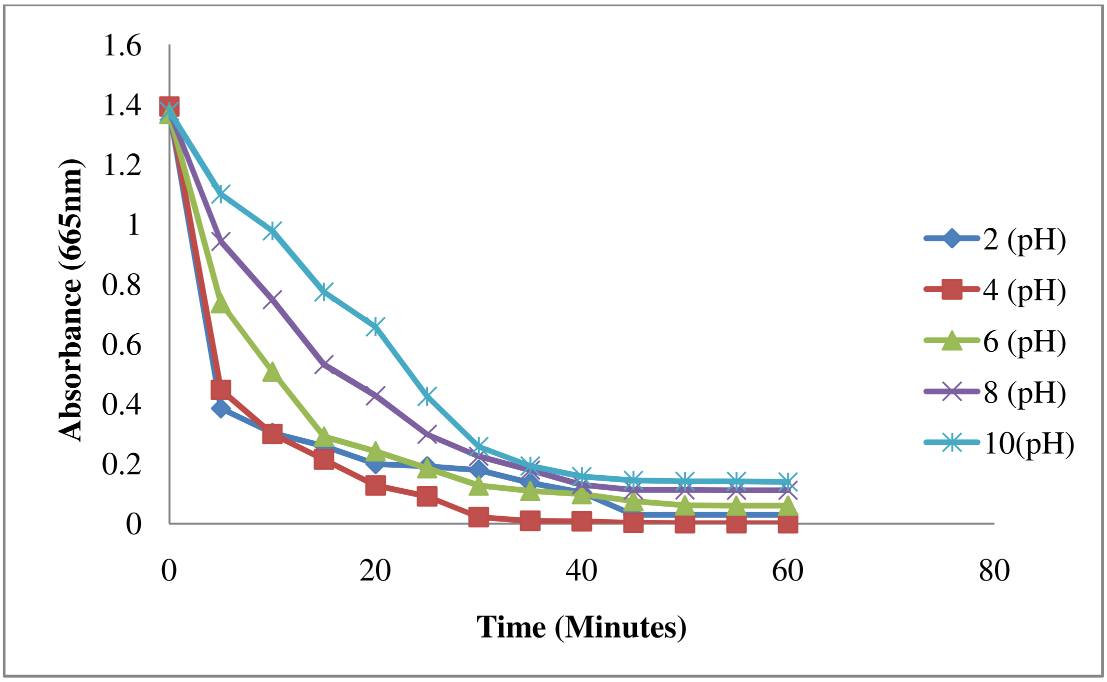


Fig. 5.9: Effect of pH on harvesting efficiency of algal biomass using bioflocculant

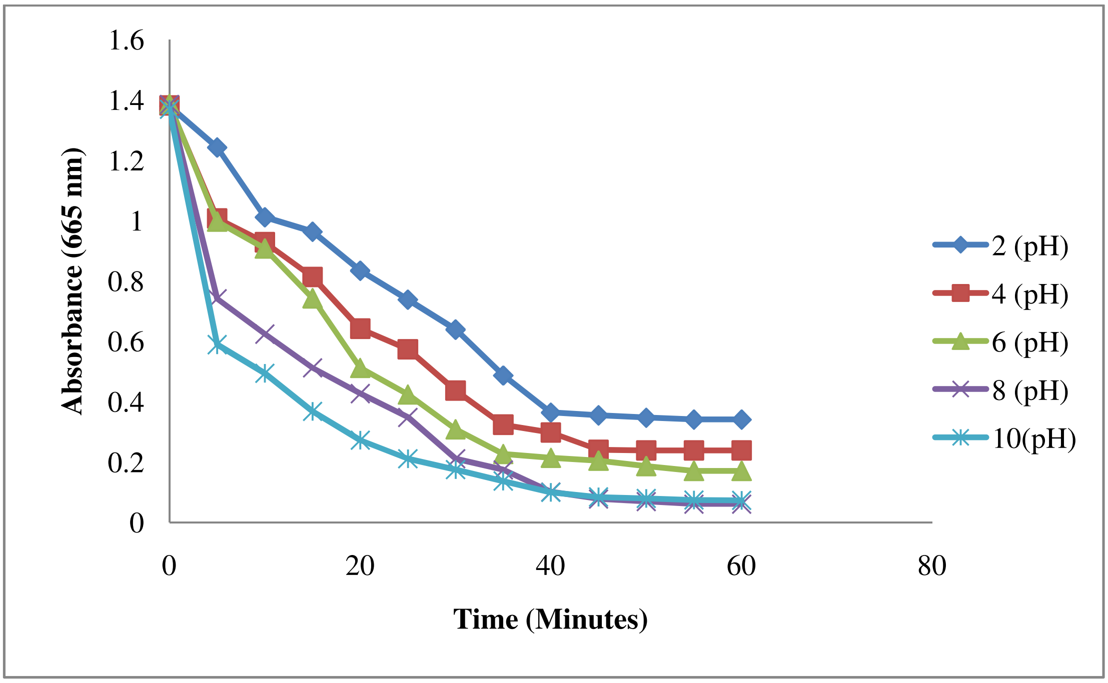


Fig. 5.10: Effect of pH on harvesting efficiency of algal biomass using LACC

At the time, when surface charge become totally neutralized at pH 4.0 FE reached up to maximum *i.e.* 99%.

5.3.2.3.2. Laboratory available $CaCO_3$

In contrast to above, HE of *Chlorella pyrenoidosa* obtained maximum (95%) at high pH (8.0) with zeta potential (*ζ*) (-0.4 mV) by using LACC (Fig. 6.13). Research regarding this experiment previously reported that bivalent cations (Ca^{++} , $Mg^{++}$and, Fe^{+++}) play a significant role in the flocculation process with high pH. At low pH (2.0) HE of *Chlorella pyrenoidosa* was considerably decreased up to 75% with zeta potential (*ζ)* value (1.31 mV). No significant differences in harvesting efficiency were occurred between 8.0 (95%) and 10.0 (94%) with ζ-0.4 and -0.84 mV respectively. The multivalent metal cations play a significant role in flocculation process. At high pH, metal cations might be combined with hydroxyl ions to form metal hydroxide. The positive charges present on metal hydroxides neutralize the negative charges present over microalgal cell surface therefore algal cell suspension began to flocculate (Wu et al., 2012). Zeta potential of algal cell suspension varied with different pH, but algal cell surface charge is negative in alkaline pH. The decrease in zeta potential (*ζ)* with increasing pH indicates the decrease in cell surface charges. It might be possible due to the neutralization of charges in this range (Zhang et al., 2012). Zeta potential (*ζ*) was pH dependent and shown a decrease in zeta value as the pH increases. In this same context, Powell and Hill (2013), reported that flocculation of *N. Oceanic* IMET1 is also a pH dependent process. There was no significant flocculation obtained at the pH of 8.0, but a remarkable increase in flocculation were noticed when the pH was above 9.0. An increase in harvesting efficiency algal biomass at high pH is possibly due to some auto-flocculation of microalgae itself, Vandamme et al., (2012), reported a significant flocculation occurred when CO_2 supply was interrupted in the culture of

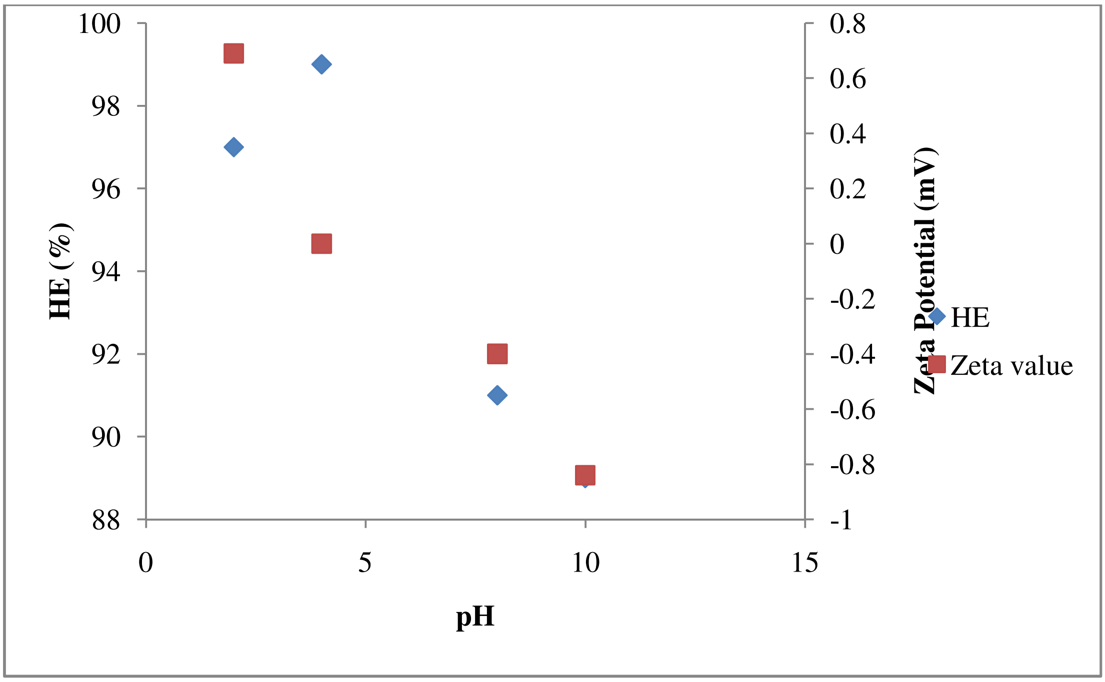


Fig. 5.11: Interrelationship between HE and pH with zeta potential of bioflocculant

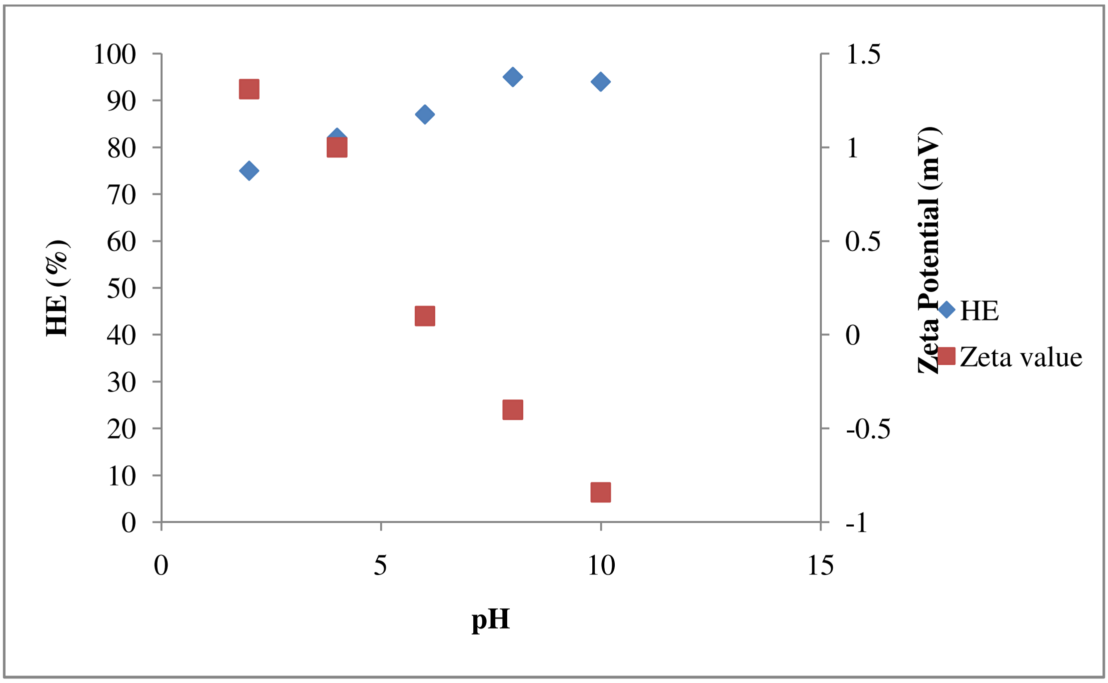


Fig. 5.12: Interrelationship between HE and pH with zeta potential of LACC

Chlorella vulgaris, as a consequence pH level increased to 9.5. They also reported 95% flocculation efficiency of *Chlorella* at pH of 11.5 and 12.Hence, it is clear from the above explanation that bioflocculant based harvesting of algal biomass is significant in compare with LACC in terms of efficiency. The use of bioflocculant is a low-cost approach to sustain the environment. Harvested algal biomass via bioflocculant is free from the degradation and deformities of algal components as well as cell surface structure respectively, and can be best to use as a livestock feed as the egg shell power and acid solution required in preparation of egg shell based bioflocculant is very less in quantity.

5.4. Conclusion

The experimental results of this chapter reveal the significant use of bioflocculant (100 mg/L concentration) with optimized time (30 minutes) and temperature (40°C), which provides better HE with respect to *Chlorella pyrenoidosa* without any cells surface structural deformities. Compared to inorganic flocculants, it is cheap and does not impart harmful impact on the algal shell. These studies also disclose that temperature also plays an important role to achieve better harvesting efficiency. The experimental data is proven by the pseudo second-order kinetics model and thermodynamic functions.

The experiment provides an economically viable, proficient, and expedient green approach to harvest algal biomass *i.e. Chlorella pyrenoidosa* the advantages of this approach has been observed with maximum flocculation efficiency *etc.* 99% without any algal cell surface and structural deformities.

The present method showed sustainability with operational simplicity to overcome the hurdles arising in harvesting of algal biomass with maximum efficiency to promote large scale application (bioenergy and biomaterial) from harvested algae.

Maximum HE (99%) was found to be associated with 0.0 mV of ζ value with pH 4.0 for bioflocculant, which was relatively higher than the LACC (95% at pH 8).This observation concludes highest HE of bioflocculant at its isoelectric point, which represents the least stable colloidal system. Whereas, ζ value for LACC indicated the unstable colloidal system on selected pH ranges.

Furthermore, bioflocculant was found non-toxic without structural deformities in comparison to LACC. Hence, it's an economically viable, proficient, and expedient green approach to harvest algal biomass using waste egg shell with sustainable chemistry for biomass harvesting.

Chapter 6

Techno-economic analysis of TES based PBR for algal biomass and biofuel production: a comparative study

6.1. Introduction

Existing energy and socio-economic scenario have been significantly boosted concern for renewable biofuel production. Biological routes for bioenergy production with technical advancement being emerged as an efficient approach to attenuate the concern regarding petro-chemical based energy generation. In particular, algal based biofuel production seemed promising since last five years as algae may be burned or basified as a crude material to be processed to produce liquid fuel *i.e.* biodiesel, bioethanol, or gaseous fuel *i.e.* biohydrogen or biogas. Techno-economic assessment to achieve optimum algal biomass production to produce biofuel completely depends upon strategies and techniques adopted in various process routes. Techno-economic assessment for any system either natural or mane-made totally depends on its research, development, and commercial aspects including environmental elements. Keeping these objectives in mind, algal biomass based (liquid and gaseous) biofuels in integration with wastewater (CETP) and thermal energy storage based photobioreactor investigated and assessed for techno-economic evaluation, experimentally as well as theoretically with environmental benefits and losses. Therefore, optimization of process parameters (pH, light photoperiod, temperature, nitrate, phosphate, carbon as $CaCO_3$, wastewater concentration, CO_2 in the form of gas, fabrication of photobioreactor, harvesting parameters *i.e.* effect of flocculants (chemical and bioflocculants), flocculants dose, contact time, temperature, and pH involved in each step of algal cultivation (upstream and downstream processing) have been studied minutely to get enhanced algal biomass production. Furthermore, algal biomass harvested from designed photobioreactors processed for production of different biofuels (bio-oil, biodiesel, and biogas) have also been critically assessed. As per concluding remarks from chapter-3, 4, and 5, it has been justified that wastewater treatment with designed photobioreactor in integration with biofuel production and use of flocculants to enhance algal biomass provides an economical and environmental soundness with technical routes of conversion. Table 6.1 describes various forms of energy outputs in terms of heat, electricity, liquid, gaseous, and solid fuels very particular to algal biomass only.

6.2. Wastewater and biofuel: Integrated Approach

For dense cultivation of algal biomass, wastewater (CETP) has been selected as nutrient media for algae. An alga usually requires water (fresh/marine) for its growth and development, whereas, the selected wastewater (CETP) has been provided as source of nutrient for algal growth due to the significant presence of nitrate and phosphate in wastewater (Section 3.1.2.2. of Chapter-3). Thus, this wastewater (nutrient rich) is potential and alternative source for algal cultivation simultaneously minimises burden over distilled water and chemical based nutrient media. On the basis of findings at laboratory experimental plans (Chapter-3, Section 3.1.2.3; Chapter-4, Section 4.1.2.1), a theoretical assessment has been made using CETP wastewater on the basis of per day and annual wastewater discharge capacity for algal biomass and biofuel production potential. Only two different concentrations (50% and 100%) of CETP wastewater were selected for algal biomass production and biofuel production on the basis of wastewater production and treatment simultaneously as presented in Table 6.2 (actual and predicted total biomass and lipid production).

Table 6.1: Energy extraction methods from algal biomass with environmental and economical aspects

Processes	Total algal biomass utilization	Drying after harvesting	Primary energy production	Environmental aspects	Economical aspects	References
Direct combustion	Yes	Yes	Heat energy	Fine particle emission	Consumes high energy in drying biomass	(Milano et al., 2016)
Pyrolysis	Yes	Yes	Liquid by flash Pyrolysis	Sensitive to CO poisoning	Requires extra equipment for fuel purity	(Chiaramonti et al., 2017)
Gasification	Yes	Yes	Gas (primarily)	Produces fine particulate matters	High energy consumption	(Hallenbeck et al., 2016)
Liquefaction	Yes	No	Liquid		High energy consumption	(Chen et al., 2015)
Biohydrogen	Yes	No	Gas	Waste material produced after combustion is water, reduces fossil fuel based pollution	Storage, packaging, and transportation of fuel is expensive	(Debowski et al., 2015)
Fuel cells	Yes	No	Electricity	High power density, no corrosive material	High cost, requires extra equipments for fuel purification	(Le et al., 2008)
Bioethanol	No	No	Liquid	No emissions of harmful gases	Under R&D for Lab to Land Approach	(Hallenbeck et al., 2016)
Biodiesel	No	Yes	Liquid	Eco-friendly and sustainable technology	Under R&D for Lab to Land Approach	(Kothari et al., 20012)
Anaerobic digestion	Yes	No	Gas	Eco-friendly and sustainable technology	Less expensive	(Fasahati et al., 2017)

50% concentration of wastewater was selected on the basis of maximum biomass production (1.4 gL^{-1}) and lipid content (27%) and pollution load removal at lab-scale, although with 100% concentration results were found not significant but predicted values of biomass and lipid suggest that it may be a solution of treatment when we are discharging the wastewater without treatment in surroundings. So, this can be a sustainable integrated approach for waste minimization with biofuel production simultaneously. Here, total algal growth period taken as one cycle, completed in total 15 days (*i.e.* life cycle of selected algal biomass). It means algal biomass was harvested at interval of 15 days in total 300 working days (maximum days of working) of plant. According to some previous and our experimental results minimum to maximum 70 to 75% of lipid can be converted to crude bio-oil (Kothari et al., 2012). This conversion efficiency totally depends on various process parameters (pH, temperature, light, and nutrients) and on growth parameters for species selected for the study. Although theoretical findings of biomass production and lipid productivity were found good enough for Lab to Land approach but storage of wastewater on per day basis is a big challenge on practical scale. So, this area is a part of future research, here we can suggest pits formation for collection of wastewater on industrial discharging sites or at CETP location before treatment.

Furthermore, Table 6.2 describing the predicted total biomass and biofuel production with CETP wastewater if used/coupled with designed TES-PBR at large scale, here experimental findings with TES-PBR-II system (Chapter-4) were used to calculate the results. Here, algal biomass collected/harvested on the basis of 13 days /cycle (*etc.* life cycle of algal biomass) during ambient temperature of 5 to 18°C and 3 to 15°C (PBR-I, without PCM), but reactor temperature was controlled (25 to 30°C) with phase change material to enhance algal growth during adverse temperature condition. TES-PBR is significant to produce optimum algal biomass productivity even in extreme cold condition, as the temperature of the PBR-II is controlled by phase change material. Temperature fluctuation is a big challenge to cultivate algal biomass throughout the year. North region of India faces two to four month of winter season. Whereas, cold climatic regions cross the world (temperate, tundra, and taiga) faces moderate to extreme cold conditions. Therefore, it is difficult to cultivate algal biomass in extreme worst conditions. But, TES based PBR is a novel/efficient/potential approach to enhance algal biomass and biofuel productivity even in unfavourable climatic condition (particularly temperature fluctuations). Similarly, if we plane algal biomass production at commercial scale for cold climatic regions and winter season in tropical regions, TES based PBR will provide an efficient results for biomass based biofuel production. The maximum algal biomass production with PBR-I (without TES based PBR) and II (with TES based PBR) are 0.85 gL^{-1} and 4.8 gL^{-1} respectively with unfavourable conditions (temperature fluctuation). Algal biomass production in PBR-II is 3.5 times higher than biomass produced with favourable condition (temperature range 25°C-30°C). Whereas, if we compared the biomass productivity of PBR-I with PBR-II total production has been increased 5.6 times higher. Therefore, it is possible to enhance the algal biomass productivity in present fabricated PBR with optimum biomass and biofuel production. Residual biomass after bio-oil extraction, also has a potential to produce biogas as a bioenergy option (Kothari et al., 20017), however, dried algal biomass without any processing in integration with wastewater (dairy) also used for biogas production (Kothari et al., 2012).

Table 6.2: Actual and predicted biomass and lipid production with CETP wastewater discharge

CETP wastewater concentration	Biomass productivity	Lipid productivity (%)	Predicted Biomass Production (*MLD)	Annual Predicted Biomass Production (kg)	Predicted Lipid Production (%) (*MLD)	Annual Predicted Lipid Production (*MLD)	Biodiesel production (*MLD) (70 % conversion)	Annual Predicted Biodiesel production (70 % conversion)
Biomass and biofuel at favourable conditions naturally (with optimum required temperature)								
50%	1.4 gL^{-1}	27	2660 kg/cycle	53200 kg	718	14364	502	≈10054.8
100%	0.3 gL^{-1}	12	570 kg/cycle	11400	68	1368	47.6	≈957.6
Biomass and biofuel production with TES based PBR (PBR-II)								
**50%	4.8 gL^{-1}	35	9120 kg/cycle	91200	3192	31920	2234	22340
Biomass and biofuel production with TES based PBR (PBR-I)								
**50%	0.85 gL^{-1}	35	1615 kg/cycle	16150	565	5650	395	3950

4(* 1.9 MLD discharge of CETP wastewater; ** Annual discharge calculated with maximum working days of 300 **Annual production with respect to TES based PBR, 1 cycle = 12 days, total number of cycle per year 10, 4 months have been considered with temperature fluctuations *etc.* winter season)

Although it’s not experimentally done with CETP wastewater due to limitation of dried biomass of *Chlorella pyrenoidosa*, but dairy industry wastewater has been used for biogas production with unidentified algal biomass collected from nearby pond just to check the viability of co-digestion with few other waste materials as a source of nutrient in anaerobic digestion (Kothari et al., 2017). This experimental study is not the part of our objective but it can be part of future research in recommendation. Hence, it is clear from the above discussion that algal biomass and biofuel productivity can be enhanced by the application of phase change materials to maintain the temperature of PBR even in adverse climatic conditions. The applied method for this study has potential to minimise the chemical based fuel.

6.3. Photobioreactor

Temperature control through thermal energy storage materials is a new idea in designing of energy efficient buildings and passive heating devices though; its application in photobioreactor is novel approach and not reported by researchers. So, many heating and cooling systems are being incorporated in typical conventional type of bioreactors (hot water buffer, thermostat *etc*.), but all these systems are not economically feasible. Subsequently, to maintain the temperatures of BRs with phase change material are recommended but it is not widely used therefore, innovative approach should be concern in this vicinity. This type of energy storage system reduces the mismatch between supply and demand and provides an optimized temperature range for better algal biomass productivity. Thermal energy can be stored as a change in internal energy of a material as sensible heat, latent heat and thermo-chemical or combination of these which can be used later for the growth of algal biomass even in the absence of temperature.

In order to maintain the temperature of the algal suspension in photobioreactor, thermal energy storage based phase change material has been used in this research work. For thermal energy storage, PCM materials are idyllic as they are economically viable and cost effective, stable and environment friendly. It is advantageous in terms of maintaining desired temperature without the need of external source of electricity at the period of operation in photobioreactor. It is useful to provide as a backup source of energy to the system. It limits the surplus temperature in day time by storing excess heat and releases during night when sunlight not present yet the system required maintained temperature. PCMs consent to large amount of energy stored in small volume thus, it supports lowest storage media to maintain the temperature of photobioreactor.

6.3.1. Cost analysis and energy requirement calculation of the PBR

As per literature survey, it was found that 25-30°C temperature range is required to increase the life cycle of algae. Thus to maintain this temperature range either electric heating or gas heating was adopted. In this paper novel PCM based system is designed and implemented to get desired temperature range. In this section authors did techno-economic analysis of electric coil based PBR (EC-PBR), LPG gas based PBR (GS-PBR) and TES based PBR (TES-PBR). Basis on the Indian market survey, Table 6.3 enlist the comparison of features of EC-PBR, GS-PBR and TES-PBR.

Table 6.3: Comparison of different water heating system

S.No.	Feature	EC-PBR	GS-PBR	TES-PBR
1	Initial capital cost	3000 INR	2500 INR	1500
2	Safety	Safe	Completely unsafe: Strictly not recommended on the basis of safety	Very safe
4	Life span range	7-10 Years	6-8 Years	9-10 Years
5	Installation	Easy	Difficult as you need to connect the LPG and should arrange outlet for fumes	Easy
6	Technology	Electricity	Gas burning	Solar energy
7	Pollution	Pollution due to the production by coal	May releases carbon monoxide which needs to vented out carefully	No pollution

6.3.1.1. Annual cost method (ACM)

Annual cost method (ACM) is implemented due to the fact that systems under our consideration for evaluation did not have same life times. The system annual cost (A_C) by considering time value of money concept may be evaluated as:

$$A_C = A_{FC} - S_{FF} * SV + I_C * C_{RF} + A_{MC} \tag{6.1}$$

WhereA_{FC}, annual cost of fuel, [INR]; SV, salvage value of system; I_C, system initial cost, [INR]; A_{MC}, annual cost for maintenance, [INR/year].

The termsS_{FF}, C_{RF} are the sinking fund factor and capital recovery factor respectively. These two terms can be written as:

$$S_{FF} = \frac{i}{[(i+1)^j - 1]} \tag{6.2}$$

$$C_{RF} = \frac{(i+1)^j * i}{[(i+1)^j - 1]} \tag{6.3}$$

Where$i$, interest rate, [INR]; j, operational life of system.

The annual fuel cost of TES-PBR is the total cost of electrical energy consumption for heating of water during cloudy or rainy days. Where, the annual cost of fuel for EC-PBR and GS-PBR is the total cost of electricity and LPG gas used to meet hot water requirement for desired temperature range (Kablan 2004). For evaluation of annual cost of fuel, daily hot water need for growth of algae has to be calculated. The energy required to meet daily hot water requirement may be evaluated as:

$$E_{DHR} = m_w * C_{pw} * \left(T_{wf} - T_{wi}\right) \tag{6.4}$$

Where, m_w, mass of hot water requirement per day, [Litre/day];C_{pw}, specific heat of water [kJ/kg. K];T_{wf}andT_{wi} are the initial and final temperature of water respectively. Thus annual cost of fuel of TES-PBR and EC-PBR could be calculated as per according to following equation:

$$A_{FC} = \frac{N * E_{DHR} * E_C}{\eta_{el}} \tag{6.5}$$

Where,N, number of days electricity used to fulfill the need of hot water requirement [days] ;E_C,electricity cost [INR/kWh];η_{el}, electrical coil efficiency.

The annual cost of fuel of GS-PBR can be calculated as:

$$A_{FC} = \frac{P_{LPG} * E_{DHR} * 365}{CV_{LPG} * \eta_{GB}} \tag{6.6}$$

Where,P_{LPG}, price of LPG [INR./kg];CV_{LPG}, calorific value of LPG [kWh/kg];η_{GB}, efficiency of gas burner.

The salvage value of system is varying with operational time (j) of the system. Therefore, by considering that system depreciation with respect to time is linear in nature, hence salvage value can be calculated as:

$$SV(j) = j * I_C\left[\frac{1}{j} - \frac{1}{j_{max}}\right] \tag{6.7}$$

Wherej_{max}, is the maximum time after that system is totally discarded [Year]

For making the analysis simpler, from market survey it is noted that annual cost for maintenance for EC-PBR and GS-PBR is 5% of annual capital cost whereas in case of TES-PBE, no maintenance is required.

6.3.1.2. Saving per day

The total saving/day for TES-PBR may be calculated by evaluation of savings by production of hot water by EC-PBR, GS-PBR, and TES-PBR at desired temperature per litre and then this saving multiply by total hot water requirement in a day.

$$S_{hw/l} = C_{cs/l} - C_{PCM-PBR/l} \tag{6.8}$$

Where, $S_{hw/l}$ savings in hot water production per liter [INR];$C_{cs/l}$ cost of production of hot water per liter by conventional systems (EC-PBR and GS-PBR) [INR];$C_{PCM-PBR/l}$ cost of production of hot water per litre by TES-PBR [INR]

Where,

$$C_{cs/l} = \frac{C_{CS/A}}{P_{cs/A}} \tag{6.9}$$

$$C_{PCMWHS/l} = \frac{C_{PCM-PBR/A}}{P_{PCM-\mathrm{PBR}/A}} \tag{6.10}$$

$$S_{day} = S_{hw/l} m_w \tag{6.11}$$

Where,$C_{cs/A}$ and $C_{PCM-\mathrm{PBR}/A}$cost of production of hot water [INR] by conventional system and TES-PBR per annum respectively; $P_{CS/A}$ and $P_{PCM-PBR/A}$ hot water production per year by conventional and TES-PBR respectively [Litre]; S_{day}savings in hot water production per day [INR].

6.3.1.3. Payback period

Payback period is the period of time required for an investment to recover its initial investment in terms of savings (Sreekumar 2010). In order to calculate the economic feasibility of TES-PBR payback period is evaluated by following equation:

$$PP_N = \frac{ln\left[1-\frac{C_I}{S_1}(i-f)\right]}{ln\left(\frac{1+f}{1+i}\right)} \tag{6.12}$$

WherePP_N, payback period for N years;S_1 benefit during first year (INR)

6.3.2. Outcomes

6.3.2.1. Techno-economic analysis of TES-PBR

The economical parameters that are to be considered for Techno-economic analysis of EC-PBR, GS-PBR and TES-PBR are based on situation of India. As given in previous section two methods are there for Techno-economic analysis of systems which are given below.

6.3.2.2. Annualized cost method

In this section annual cost of TES-PBR, EC-PBR and GS-PBR is evaluated. The economical parameters of Indian market, based on which the Techno-economic analysis of the considered systems is carried out provided in Table 6.4.

Table 6.4: Economical parameters of Indian market

Parameters	Value
Interest rate	9%
Inflation rate	3%
Average daily solar radiation	5.20 kWh/m^2/day
Cost of Electricity per kWh	Rs. 5.25
Cost of LPG	Rs. 60/kg
Calorific value of LPG	12.87 kWh/kg

The economic analysis of TES-PBR is carried out by supposing that system can easily work in 300 days in a year and annual cost of fuel to produced hot water for 65 days is done by electric coil/gas burner. The Fig.6.1 shows the comparative annual cost and fuel cost for TES-PBR with auxiliary systems (EC-PBR and GS-PBR).

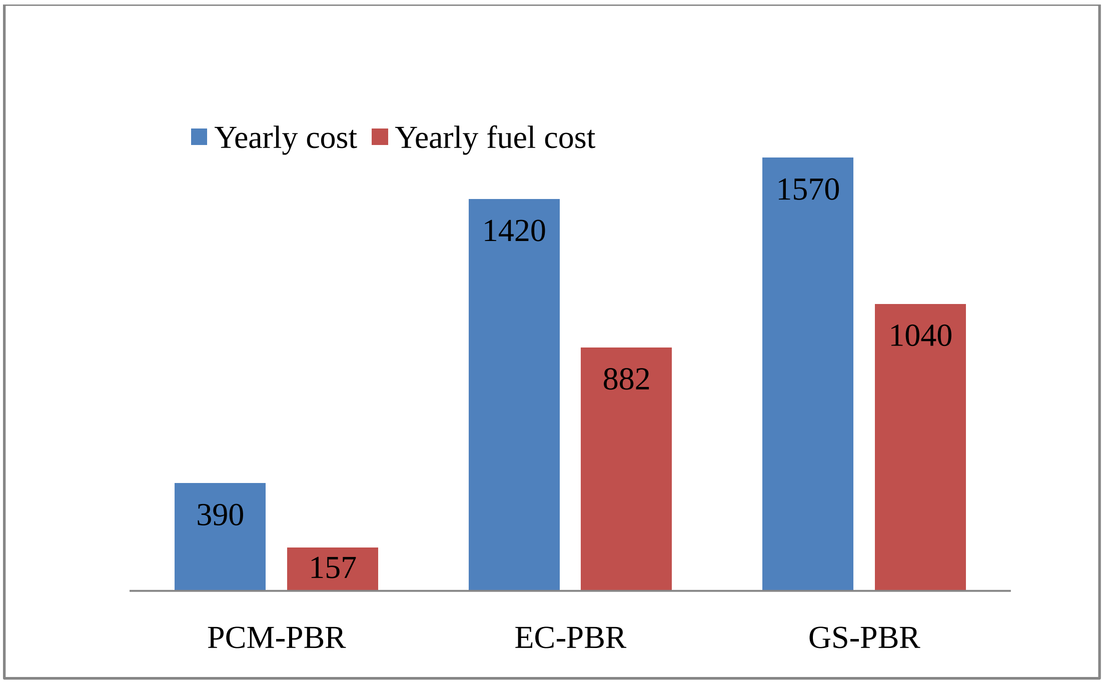


Fig. 6.1: Comparison of yearly cost and yearly fuel cost of different PBRs

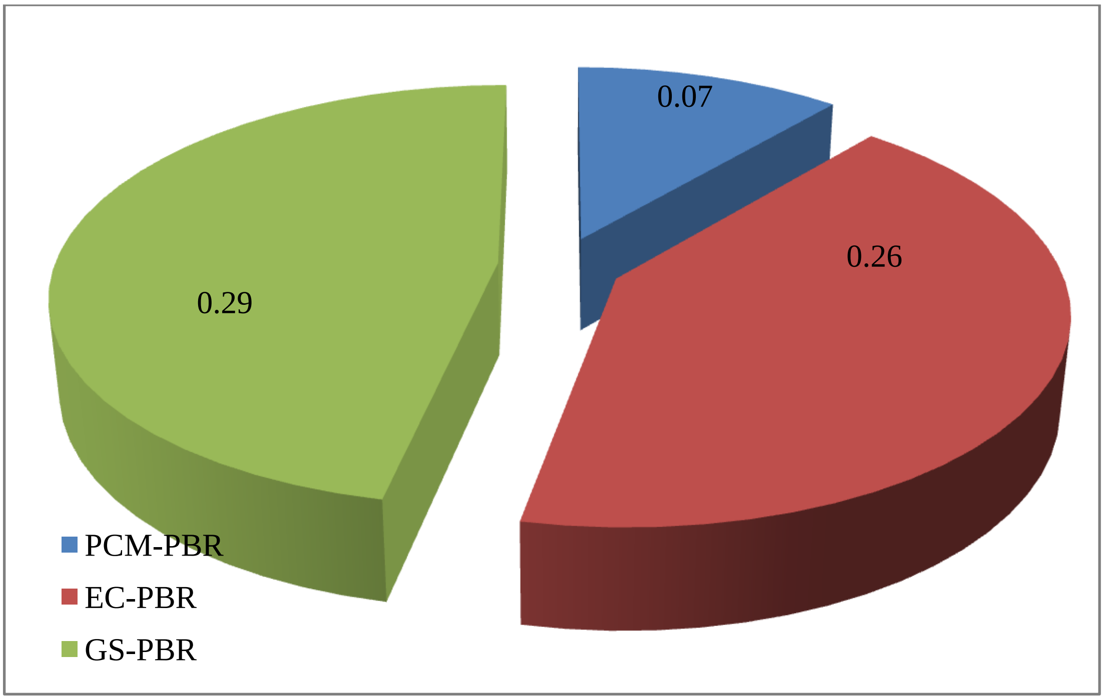


Fig. 6.2: Comparison of production cost of hot water/litre by considered systems

Basis on the basic specifications of considered systems and economical parameters of Indian market the greatest value of annual capital cost (1420 INR) and annual cost of fuel (882 INR) is evaluated for EC-PBR. The values of annual capital cost (390 INR) and annual cost of fuel (157INR) becomes lowermost for TES-PBR with auxiliary system. The price in INR for the production of hot water/litre by considered systems is also calculated. It was found from Fig. 6.2 that the price of hot water/litre by TES-PBR with auxiliary system, EC-PBR and GS-PBR are INR 0.07, 0.26 and 0.29 respectively.

6.3.2.2. Payback period

The Payback period is calculated from Eq (6.12). The initial capital investment on TES-PBR is recovered in 1.99 years of operation. Therefore, TES-PBR delivered hot water to desired temperature for 18 hours free of cost after 1.99 years.

6.4. Conclusion

The maximum annual biomass productivity with 50% concentration of CETP wastewater estimated 16150 kg but with TES based PBR (PBR-II) it increases five times (91200) even in adverse climatic condition (temperature fluctuation). Lipid content of *Chlorella pyrenoidosa* is also enhanced 27% to 35% with PBR-II, as culture system. Therefore, techno-economic assessment of any system should be a part of research and development for sustainable and economically feasible way to produce algal biomass based biofuel and other (nutritional supplement, cosmetic products, medicines *etc.*) value-added products.

Chapter 7

Conclusion and Future Recommendations

7.1. Conclusion

In view point of objectives decided for this thesis, all work has been successfully completed by its experimental validation. The experimental results were significantly analysed by using algal growth kinetics and Pearson correlation coefficient between process parameters (pH, light photoperiod, nutrients *i.e.* nitrate, phosphate, carbon in salt and gaseous forms) and algal biochemical compounds (protein, carbohydrate, pigments, and lipid) to analyse positive or negative correlation between these variables. Optimization of CETP wastewater to obtain maximum algal growth with efficient wastewater treatment (in terms of physico-chemical and metals removal) through selected algal species *i.e. Chlorella pyrenoidosa* is a significant part of this research work. Average removal rate kinetic was applied to investigate the affinity of algal biomass for metal uptake. Impact of different concentrations of CO_2 with optimised concentration of CETP wastewater has also been studied in Phase-I of experiment. Designing of thermal energy storage based photobioreactor for enhanced cultivation of algal biomass and biofuel production coupled with optimised parameters obtained from Phase-I of experimental study was carried out in Phase-II. Phase-III of experimental study emphasised on advanced algal biomass harvesting techniques with optimization of various parameters *i.e.* dose concentration, temperature, and pH. The experimental data has been supported by Pseudo-second order model, thermodynamic function, and zeta potential analysis. Though, conclusion of each chapter has been given at the end of chapters, however, the major conclusion drawn from all the chapters of this thesis is summarized here and given in upcoming pages of this chapter.

- Application of algal biomass for wastewater treatment with biofuel production is an integrated approach to promote green economy in terms of phycoremediation of wastewater and low-cost and clean bioenergy generation. But a wide range of practical challenges have been faced during execution of above said concept. Various practical challenges are faced at developing stage of experiments *i.e.* change in physical (pH, light photoperiod) and chemical (nitrate, phosphate, carbon, CO_2) process parameters for enhanced algal biomass production at commercial scale. In order to reduce the burden over fresh water sources, wastewater from common effluent treatment plant has been selected as nutrient substrate for algal biomass (*Chlorella pyrenoidosa*) growth and development. Effect of various influencing parameters independently with BG-11 media as well as with CETP wastewater have been taken into account to enhanced algal biomass and biochemical productivity. Pearson correlation coefficient analysis has been applied to discuss and conclude the positive and negative correlation among various process parameters and algal biochemical compounds.

- Experimental studies carried out for this research work on optimization of various process parameters to enhance algal biomass production are directly affected by different ranges of process parameters. At neutral or slight alkaline pH algal biomass shows maximum biomass productivity whereas, low pH (acidic) supports maximum lipid productivity. Similarly, long duration of light and short duration of dark cycle supports maximum algal biomass and lipid productivity. Higher concentrations of nutrients (nitrate, phosphate, and carbon) support maximum algal biomass productivity whereas, low concentration/stressed conditions favourable for maximum lipid productivity with selected media (BG-11 and CETP wastewater). Pearson correlation coefficient analysis has been applied to discuss and conclude the positive and negative correlation among various process parameters and algal biochemical compounds.

- Among different types of closed photobioreactor, two types (tubular photobioreactor and thermal energy storage based photobioreactor) are executed in this research study. It has been found that closed type of reactor is significant to minimise chances of contamination and reduces water loss. Similarly, thermal energy storage based photobioreactor (PBR-II) is a novel approach for cultivation of algal biomass (*Chlorella pyrenoidosa*), that supports biomass productivity approximate five times more in comparison to uncontrolled temperature system (photobioreactor) at adverse environmental conditions (extreme hot/cold). Use of CETP wastewater as culture media for algal cultivation also supports phycoremediation approach (heavy metal removal) in PBR-II system.
- In order to harvest algal biomass, advanced flocculant material based harvesting techniques has been examined *i.e.* bioflocculant (waste egg shell) and chemical flocculant (laboratory available calcium carbonate) to know the harvesting efficiency of flocculants. A significant result has been obtained with bioflocculant without any algal cell surface structural deformities used as harvesting agent. A significant effect of alteration in dose concentration, temperature and pH has also been investigated on harvesting efficiency of flocculants. Hence, it is very important to select an appropriate harvesting technique to make algal based value-added products feasible to support green product based economy.
- Techno-economic analysis of the proposed and selected approach also evaluated and discussed in this research work. An innovative approach consist

of algal biomass cultivation on wastewater collected from Common Effluent Treatment Plant, its harvesting with waste material based bioflocculant, extraction of bio-oil from harvested biomass in coupling with Thermal Energy Storage based Photobioreactor was analyzed and shown a feasible way for resource utilization with positive results/findings in comparison to others on economic as well as environmental scale of sustainability. The selected approach of TES-PBR would have better overall benefits with internal rate of return as discussed in detail in Chapter-6. Both types of macro/micro nutrients in wastewaters are suitable for algal growth and simultaneously treating the wastewater also including heavy metal removal. Benefits of this type integrated system includes: reducing the cost of wastewater treatment and biomass production in bulk on annual scale, significantly lowering the use of high grade electricity (room heaters) for temperature maintenance during low ambient temperature range for desired range of algal growth, less burden on freshwater resources and chemical media. Furthermore, additional revenues by biofuel (bio-diesel/biogas) production justify the economic soundness of this integrated system. From the experimental studies, examined and evaluated, the finding are clearly conclude that research efforts, particularly with low capital cost, operating cost, and lifespan of reactor is the demand for large-scale cultivation system. Furthermore, use of wastewater for algal growth as a nutrient media may also be potential alternative for treatment option which may further be used for bioenergy options (biodiesel, biogas, biohydrogen *etc*.) although whole processing system efficiency depends on selection of algal strain and its compatibility with composition of wastewater selected. Similarly, role of bioreactor in coupling with algal strain and wastewater is an important physical structure to support for large cultivation of biomass. Simultaneously, algal biomass used for treatment of wastewater can further processed for bio-diesel (bio-oil) and deoiled biomass can also be used for biogas production again in co-digestion with nutrient rich wastewater as a raw material.

7.2. Future recommendations

Although, this novel thermal energy storage based system has been experimentally investigated but it needs further research on the basis of factors like, light distribution, mass transfer, heat transfer from phase change material to media to maintain the temperature of photobioreactor and enhance algal growth rate at very low/high ambient temperature. Use of other phase change materials suitable for algal biomass growth, should also be investigated in future research. Similarly, use of different nutrient rich wastewater with other selected algal strains should also be the part of future research studies for treatment as well as biofuel and other value added end products. Lipid content also has a large variability with different types of algal species, if grow with different media sources (chemical media/wastewater resources as a media) may also be recommended for future work. Furthermore, techno-economic feasibility of each adopted technology should also be assessed to make it commercially accessible for sustainable bio-economy in long-term.

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Appendix

Methodology for Wastewater Characterization

The industrial wastewater were analyzed on each 5th day time interval for various physicochemical parameters such as pH, TDS, TSS, BOD, COD, Nitrate, Phosphate, 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:

$$\text{TDS (mg/l)} = \frac{\text{A-B}}{\text{V}} \times 10^6$$

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:

$$\text{TSS (mg/l)} = \text{TS} - \text{TDS}$$

(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.

$$\text{pH} = -\log_{10}(\text{H}^+)$$

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 $Na_2HPO_4.7H_2O$ and 0.42gm 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 prepare 100 ml of solution.

e). Calcium chloride: 2.75gm of anhydrous $CaCl_2$ was dissolved in distilled water to prepare 100 ml of solution.

f). Ferric chloride: 0.25gm $FeCl_3.6H_2O$ was dissolved in distilled water to prepare 1 liter of solution.

g). Sodium sulfite solution: 1.57gm Na_2SO_4 was dissolved in 100 ml distilled water and 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 sulfate was 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:

$$\text{COD (mg/L)} = \frac{\text{(b-a) X N of FAS X 1000X 8}}{\text{mL of sample}}$$

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 volume to 100 ml. The pink colour develops slowly, does not affect the sensitivity.

C). Sulphuric acid solution: 500 ml conce.H_2SO_4 was added in 125 ml distilled water and then cooled.

D). Sodium chloride solution: 300gm NaCl was dissolved in distilled water and made its 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 to 1 liter. The solution contains 100mgN/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, exactly for 20 minutes.
- Cooled the contents again in cold water bath and the readings were taken at 410nm on spectrophotometer. The concentration of NO_3-N from the standard curve was found out.

(4) Phosphate (mg/L)

Phosphate in extract is measured by the reaction of phosphate with ammonical molybedate in an acid medium to form molybdohpsphoric acid .the molybdohpsphoric acid is then reduced to a pink coloured complex and these blue coloured compound detected through at absorbance 640 nm using spectrophotometer.

$$\mathbf{H_3PO_4+12H_2MoO_4 \xrightarrow{\qquad\qquad} H_3P(Mo_3O_{10})_4+12H_2O}$$

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 molybedate $(NH4)_2MoO_4$

2) $SnCl_2$

Procedure:

- Take 10 ml sample in a test tube.
- Add 0.4 ml ammonium molybedate $(NH4)_2MoO_4$ 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 the 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 distilled water. Added 50 ml 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 100mg/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 on a Spectrophotometer at 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 and pigments. These parameters were analyzed on every alternate day of growth optimization experiment.

(1) Carbohydrate

Phenol sulphuric acid test was originally described as a nonspecific quantitative test for carbohydrate (Dubois et al. 1956). The interaction of phenol solution with carbohydrate produces a finite absorbance, which is measured at 490 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 490 nm followed by cooling at room temperature.

Calculation:

The carbohydrate concentration can be calculated from the calibration curve of known concentration.

(2) Protein (gmL^{-1})

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 used with protein concentrations of 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 100C 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 OD at 650 nm in 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 Whatman 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 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:

$$\textbf{Chlorophyll 'a'} = (12.7\times Abs_{663}) - (2.69 \times Abs_{645})$$

$$\textbf{Chlorophyll 'b'} = (22.9\times Abs645)-(4.7\times Abs663)$$

$$\textbf{Chlorophyll 'a+b'} = (20.2\times Abs645)+(8.0\times Abs663)$$

(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. 3 ml of extraction 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 extraction 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 weighted (White et al., 1979).

(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 residual 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 rotator 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.

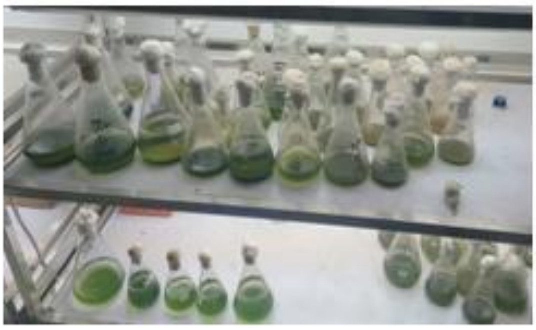
(E) Scanning electron microscope

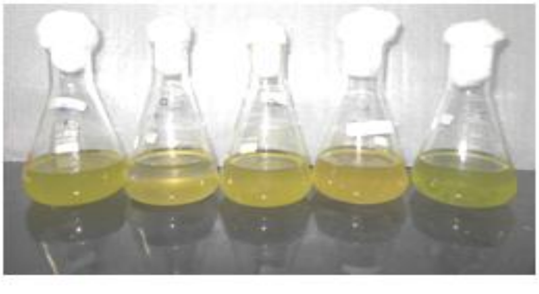
Surface characteristics of algal cell were analyzed by using SEM facility. The algal samples used for SEM analysis were fixed with osmium tetroxide (OsO_4). A 10% working solution of osmium tetroxide in distilled water was used. Samples were fixed for 10-30 minutes with a final concentration of 1-2% of osmium tetroxide. Following steps were followed for sample preparation:

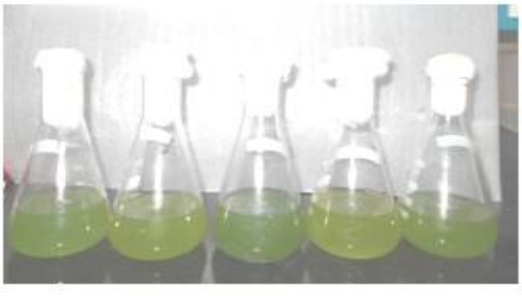
1. 1. A volume of 200-500 µL of culture was filtered by applying the light pressure on the plunger of the syringe to avoid the damage of sample.
2. Wash the samples about 3 times to remove the salt.
3. Dehydrate the samples by passing through a series of alcohols in increasing concentrations (25%, 50%, 75%, 95%, 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.

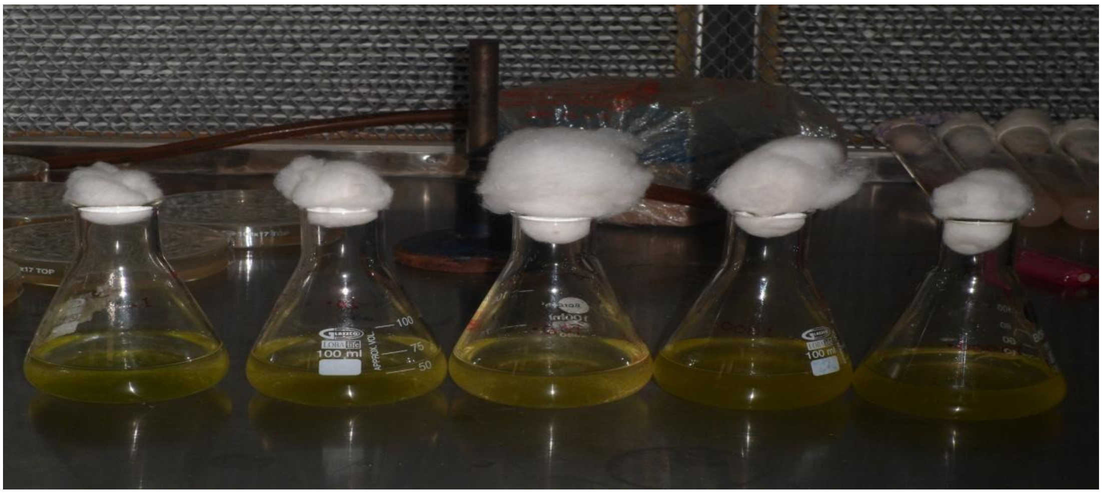
(F) Fourier Transform Infrared Spectroscopy

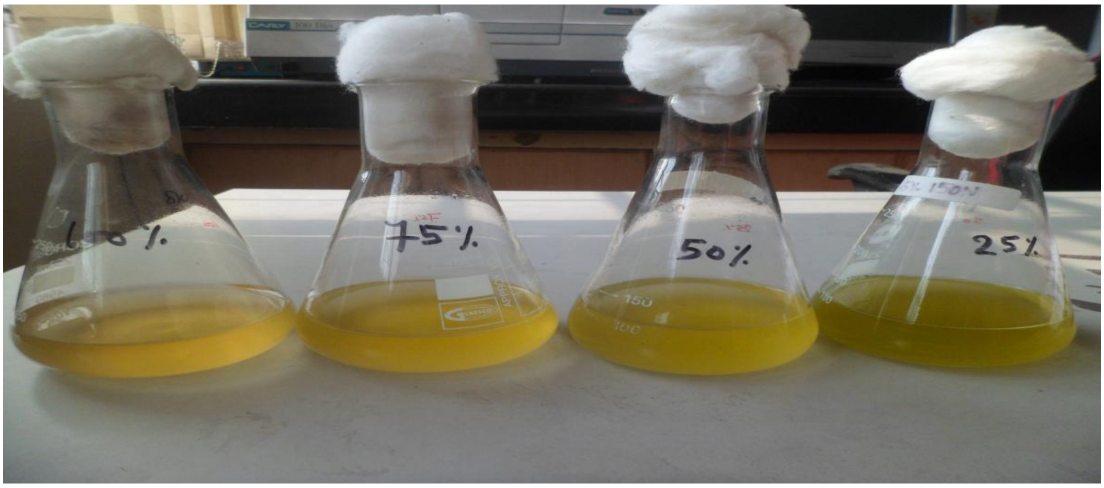
FTIR analysis was performed to characterize the functional groups of algal oil samples and algal based biosorbents (dried and wet algal biomass). A Perkin Elmer spectrum RX/FTIR system was used to obtain IR spectrum within a range of 4000 cm-1 to 500 cm-1 using a KBr disk for reference. Prior to the 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).







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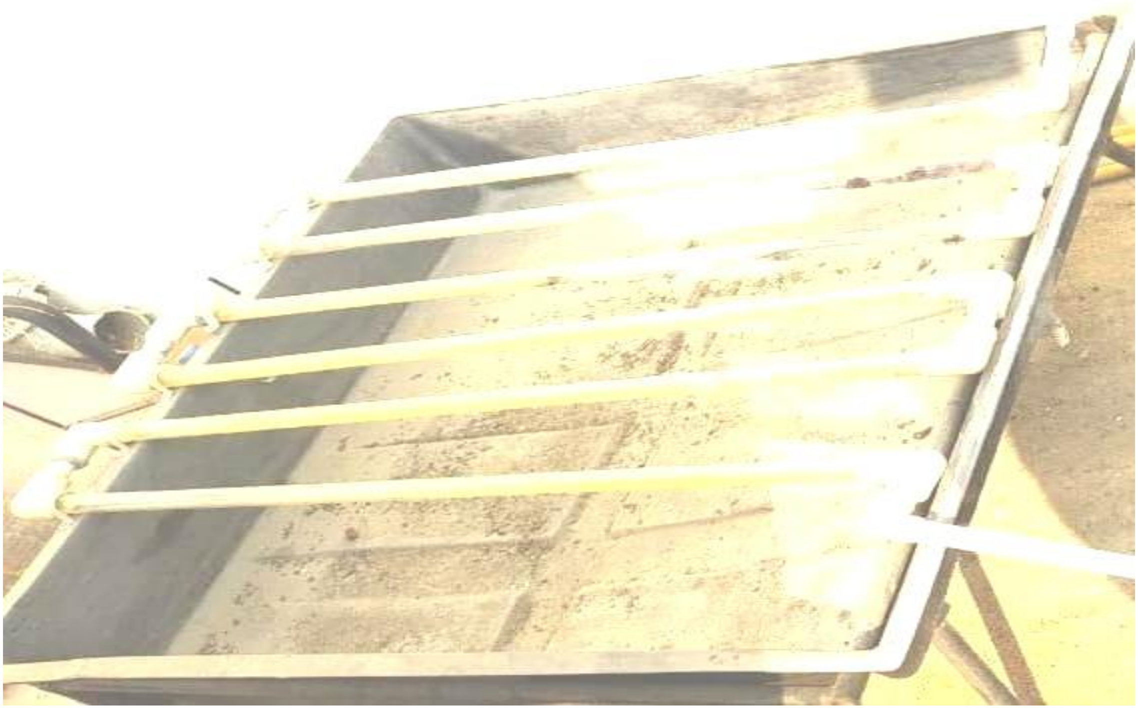


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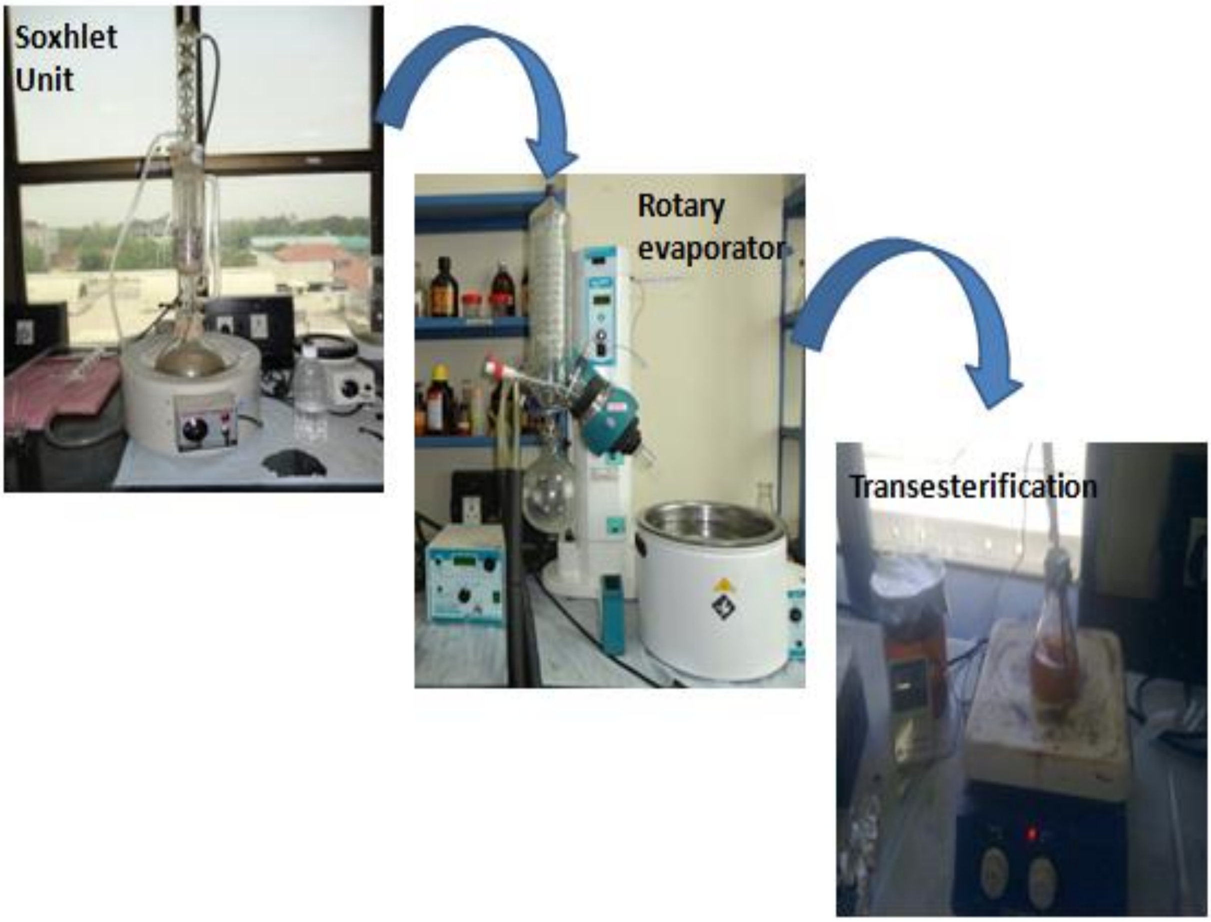
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Publication

Journal of Environmental Management 231 (2019) 562–569

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Experimental studies on zeta potential of flocculants for harvesting of algae

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ARTICLE INFO

Keywords:
Bio-flocculant
Zeta potential
Algal biomass
Harvesting efficiency
Health risks

ABSTRACT

An experimental study was performed to evaluate the comparative efficiency of bio-flocculant (waste egg shell), laboratory available calcium carbonate (LACC) and alum ($Al_2\,(SO_4)_3$) for harvesting of unicellular microalga, *Chlorella pyrenoidosa*. The influence of pH on zeta potential (ζ) was also studied to explain the chemistry of flocculation process. The maximum harvesting efficiency (99%) was obtained with alum with deformities in algal cell surfaces. Waste egg-shell material is developed as a low-cost bio-flocculant for harvesting of *Chlorella pyrenoidosa* using 100 mg egg-shell bio-flocculant/L and 100 mg LACC/L, zeta potential analysis was completed to further understand the chemistry of harvesting efficiency over the different ranges of pH (2.0, 4.0, 6.0, 8.0, and 10.0). The optimized range for harvesting efficiency (HE) of pH is 4.0–8.0 for both flocculants. Maximal harvesting efficiency was achieved at pH 4.0 (99%) and pH 8.0 (95%) with bio-flocculant and LACC respectively. Hence, bio-flocculant based harvesting method is found as the best way to dewatering the algal biomass from aqueous medium with entire and intact algal cell surface with environment friendly and cost-effective approach.

1. Introduction

Algal biomass has been recognised as potential source for biofuel as well as for animal feedstock (Popp et al., 2016; Abou-Shanab et al., 2013). Large scale algal farming can be established by the adaptation of suitable land use planning and strategies based on optimal culture conditions to enhance algal biomass productivity. Nutritional values of algae have been well documented with the presence of protein, carbohydrate, vitamins, antioxidants and trace elements (Passell et al., 2013). Therefore, algal biomass has potential to replace the synthetic nutrients by natural nutritional supplements for human and animal feed. Consumer awareness for health benefits have been increasing tremendously for natural resources of nutrient supplements (astaxanthin, lutein, beta-carotene, vitamin E, chlorophyll, phycobiliproteins, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, γ-linolenic acid, oleochemical fatty acids, and Beta-1,3-glucan) present in algal biomass (Guldhe et al., 2017; Yaakob et al., 2014). A wide range of algae (*Chlorella* sp., *Haematococcuse*, *Chlorella zofingiensis*, *Chlorococcum* sp., *Tagetespatula, Muriellopsis*sp., *Scenedesmus* sp., *Muriellopsis* sp., *Dunaliellabardawil, Aphanizomenonflos-aquae, Crypthecodinium cohnii, Schizochytrium mangrove, Nannochloropsis* sp., *Laminaria* sp., *Undaria* sp.,*Porphyra* sp., *Spirulina* sp. and *Phaeodactylum tricornutum*) are being cultivated as a source of livestock, poultry, aquaculture, and shrimp feed (Popp et al., 2016; Amparyup et al., 2012; Kiron et al., 2012; Milledge, 2011). *Porphyra* sp. is widely used as livestock feed as it is rich source of vitamin A, B, C, beta-carotene, and essential mineral iodine, and has been established a potential market value of 2.5 billion US$. In order to replace the conventional protein sources in poultry rations, algae can be used up to 10% as partial feedstock (Harun et al., 2010). Hence, algal biomass is a potential source of livestock feed with numerous health benefits. Harvesting process of desired algal biomass is a major step to maximize the algal biomass without any deterioration in structural morphology or degradation in valuable compounds.

At present various harvesting methods are being used including mechanical, electrical, chemical, and biological. Mechanical methods of harvesting of microalgae consist of filtration, sedimentation, centrifugation, dissolved air flotation and ultra filtration methods. The electrical methods of harvesting algal biomass evolve electrophoresis of

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Abbreviations: LACC, Laboratory Available Calcium Carbonate; NCIM, National Collection of Industrial Microorganism; OD, Optical Density; HE, Harvesting Efficiency; SEM, Scanning Electron Microscopy; EDS, Energy Dispersive X-Ray spectroscopy; AOM, Algal Organic Matter

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https://doi.org/10.1016/j.jenvman.2018.09.096
Received 17 February 2018; Received in revised form 11 July 2018; Accepted 27 September 2018

3 Biotech (2017) 7:243
DOI 10.1007/s13205-017-0812-8

REVIEW ARTICLE

Microalgal cultivation for value-added products: a critical enviro-economical assessment

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Received: 20 December 2016 / Accepted: 22 May 2017

Abstract The present review focuses on the cultivation of algal biomass for generating value-added products (VAP) and to assess their economic benefits and harmful environmental impact. Additionally, the impact of bioreactor designs on the yield of microalgal biomass for VAP is also considered. All these factors are discussed in relation to the impact of microalgae production on the bio-economy sector of commercial biotechnology.

Keywords Value-added products (VAPs) · Microalgae · Enviro-economical assessment · Photobioreactor

Introduction

Microalgae, characterized by production of significant amounts of biomass and oil content can be used as feedstock for biodiesel production and has been proposed as a potential source of renewal energy. Additionally, residual microalgal biomass can also be utilized to generate biohydrogen using anaerobic digestion, biogas, bio-ethanol, biomethanol, bio-plastics, bio-fertilizer, medicinal value products, and animal food (Tong et al. 2014; Gebreslassie et al. 2013; Gallezot 2012). However, the most common fuel generated from microalgae is biodiesel which is produced by transesterification of algal lipid (Zhu 2015; Huang et al. 2014; Gonçalves et al. 2013; Zhu and Ketola 2012). The potential benefit of microalgae for biodiesel production is relatively high in compared to crop plants as its' growth requires less land space and can also be easily grown in wastewater (Bhatt et al. 2014; Chisti 2012; Pittman et al. 2011; Wijffels 2008; Brennan and Owende 2010). Previously published works have shown the benefits as well as weakness for the production of microalgal-based VAP, especially in the extraction and purification of VAPs. The improvement of these extraction and purification techniques to produce VAP at the commercial level has not yet been realized due to lack of research, high costs and unavailability of necessary facilities (Oswald et al. 1988). A variety of by-products along with biofuel is being produced in pilot scale by microalgal biomass. To increase biodiesel production, two reactor systems, namely, the open pond system and the close type photobioreactor, have been used to generate a large amount of microalgal biomass (Richardson et al. 2012). Designing and fabrication of a bioreactor (BR) is very important, and BR must be designed with different process options, and in accordance with the desired products such as medicinal, cosmetics, fertilizer, biofuel, bio-plastics, food supplements, etc. (Kumar et al. 2015; Richardson et al. 2012; Dasgupta et al. 2010). Though microalgal-based biofuel generation has advantageous over fossil fuel, it is also important to evaluate its economic feasibility and its environmental impacts prior to mass scale cultivation. Figure 1 shows the range of applications of microalgal biomass which includes biofuels as well as different types of high-value-added products (VAP).

Greater significance should be given to the adaptation of eco friendly and low-cost approaches for the production of

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Bioresource Technology 225 (2017) 84–89

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

A novel method to harvest *Chlorella* sp. via low cost bioflocculant: Influence of temperature with kinetic and thermodynamic functions

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HIGHLIGHTS

- Low-cost bioflocculant based algal biomass harvesting.
- Effect of different concentrations of bioflocculant on algal biomass.
- Effect of temperature with optimized concentration on harvesting efficiency.
- Kinetics and thermodynamic functions to support the experimental data.

ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form 11 November 2016
Accepted 12 November 2016
Available online 15 November 2016

Keywords:
Bioflocculant
Low-cost
Biomass harvesting
Kinetic models
Thermodynamic functions

ABSTRACT

In this study, harvesting efficiency (HE) of bioflocculant (egg shell) was observed with variation in flocculent concentrations (0–100 mg L^{-1}), temperature (30 °C, 35 °C 40 °C, 45 °C and 50 °C) and variable contact time (0–50 min). It was found maximum (≈95.6%) with 100 mg L^{-1} bioflocculant concentration whereas influence of temperature was also observed with optimized concentration of bioflocculant (100 mg L^{-1}) at 40 °C (≈98.1%) and 50 °C (≈99.3%), in 30 min of contact time. Significant changes in algal cell structures were also analyzed after exposure to various temperatures with microscopy, SEM (Scanning electron microscopy) and EDS (Energy dispersive X-ray spectroscopy) images with and without bioflocculant. The experimental data was found to be a good fit with pseudo-second order kinetic model. The thermodynamic functions such as ΔG (Gibbs free energy), ΔH (enthalpy), ΔS (entropy) were also determined. The negative value of ΔG and positive value of ΔH and ΔS shows the spontaneous and endothermic nature of flocculation process.

1. Introduction

Cost effective harvesting technology is a bottleneck in the research and development of algal based bioenergy and value-added compounds production at commercial level (Zhu et al., 2013; Min et al., 2011). Currently, various harvesting methods are in practice for harvesting of algal biomass from their suspension such as centrifugation, gravity sedimentation, natural and pressurized filtration, chemical flocculation, electro-flocculation, and vacuum filtration, *etc.* (Chen et al., 2011; Zhang et al., 2011; Uduman et al., 2010). Among these filtration process with centrifugation, was reported with the highest yield in terms of percentage solid content *i.e.* 22%. However, pressurized filtration process was found with more efficiency (*i.e.* 27%) than centrifugation (Vandamme et al., 2012). The other centrifugation processes range between 1.5 and 18% regarding separation efficiency. The major drawback with centrifugation and pressurized filtration process is high energy usage *i.e.* 8 kw m^{-3} in compare to all above said harvesting process. In general, chemical flocculants have been known for commercial applications (Wu et al., 2012). Flocculation or coagulation, gravity sedimentation, and flotation are quite an inexpensive approach to harvest algal cells. The co-cultivation of some fungal strains along with algal cells promotes the algal biomass flocculation. The main drawback regarding this method is that it requires long culture time and not relevant for harvesting of all microalgal biomass (Knuckey et al., 2006). Therefore, flocculation is an advanced method to harvest algal biomass regarding harvesting efficiency, operation economics, and technological feasibility (Liu et al., 2013; Vandamme et al., 2012; Papazi et al., 2010). There are some inorganic salts like ferric chloride, aluminum sulfate, multivalent metal salts (multivalent aluminium salts) act as a legend to flocculate algal cells and make it settle down (Wan et al., 2015;Udhaya et al., 2014; Letelier-Gordo et al., 2014). These

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http://dx.doi.org/10.1016/j.biortech.2016.11.050
0960-8524/

3 Biotech (2018) 8:34
https://doi.org/10.1007/s13205-017-1023-z

ORIGINAL ARTICLE

Experiment-based thermodynamic feasibility with co-digestion of nutrient-rich biowaste materials for biogas production

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Received: 11 August 2017 / Accepted: 4 December 2017

Abstract

Wild strains of algal biomass, a major contributor for eutrophication in freshwater bodies, can be used as a potential substrate in association with other nutrient-rich biowaste materials like animal excreta and industrial wastewater, for biogas production. This novel concept was experimentally evaluated and analyzed by the modified Gompertz equation for maximum biogas production (μ_m), lag phase (λ), and biogas yield (P). The value of correlation coefficient (R^2) was 0.99 at varying temperature ranges (30, 40, and 50 °C). Thermodynamic functions like enthalpy (ΔH), entropy (ΔS), and Gibb's free energy (ΔG) were evaluated for the chemical oxygen demand removal efficiency. Thermodynamic functions such as ΔG (−), ΔH (+), and ΔS (+) showed the spontaneous and endothermic nature of substrate degradation and biogas production was found to be increased with increasing temperature. So, this novel co-digestion approach using nutrient-rich biowaste materials provides a new insight into biogas production with the aim of waste-to-energy generation.

Keywords Nutrient-rich biowaste materials · Co-digestion · Biogas · Kinetic · Thermodynamic functions

Introduction

Eutrophication can diminish or eradicate the fish population in the concerned pond or lake as a consequence, which can lead to the loss of many enriching services provided by the lake or pond. Due to algal blooms, blue water footprints (BWFP) and green water footprints (GWFP) were reduced into gray water footprints (GrWFP). Algal blooms are not the only cause of river or lake pollution, but industrial sectors also play a crucial role in increasing the gray water (Frank et al. 2017). Food sector is one of the major consumers of water as well as one of the leading producers of effluents per unit of production; in addition to this, it also produces large volume of sludge during biological treatments (Menon et al. 2017). The global water footprint (GWP) for the agricultural sector is 2422 billion cubic meters (BCM) of water, i.e., about 1/4 of the total GWP. Among the various sectors, dairy industry contributes 19% in GWP of agriculture sector (Mekonnen and Hoekstra 2010). India ranks first among the major milk-producing nations and according to an estimate, Indian dairy industries generate about 275 million tons of wastewater annually (Kushwaha et al. 2011). Pollution caused by industrial and dairy effluents is a serious concern throughout the world. Therefore, a green, low-cost, and eco-friendly step is required to minimize the nutrient-rich waste with an alternative use, i.e., to produce biofuel for the society.

As a renewable biomass feedstock, microalgae forfeited of water bodies can be a boon for the sustainable environment such as its capability of mitigating waste CO_2 (Kobayashi et al. 2013) and its potential to grow in organic-rich wastewater. Algae possess a much higher content of water in contrast to other terrestrial energy crops and this makes it more suitable for wet anaerobic digestion processes (Prussi et al. 2014).Water content is known to

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Curr Sustainable Renewable Energy Rep
DOI 10.1007/s40518-016-0052-2

REGIONAL RENEWABLE ENERGY (A SHARMA, SECTION EDITOR)

Deployment of Fermentative Biohydrogen Production for Sustainable Economy in Indian Scenario: Practical and Policy Barriers With Recent Progresses

Vinayak V. Pathak[1,2] · **Shamshad Ahmad**[1] · **Arya Pandey**[1] · **Vineet V. Tyagi**[3] · **D. Buddhi**[4] · **Richa Kothari**[1]

Abstract Depleting fossil fuel reserves and its significant contribution to greenhouse gas emission have created energy crisis and environmental degradation. Therefore, it is necessary to develop alternative fuels with a proper policy framework to support research and development. The potential biomass resource of India such as agricultural products, lignocellulosic waste biomass, industrial waste, and food processing waste has been extensively investigated by Indian researchers via fermentative biohydrogen production. The impact of key factors lowering fermentative biohydrogen yield can be reduced by the intervention of recent advancement in fermentative biohydrogen production such as combined fermentation process, optimized trace metal application, and pH control. A policy dealing with bioenergy promotion should adopt a market pull approach to promote bioenergy as a people-friendly technology. The present review provides recent advances in fermentative biohydrogen production process as well as practical and policy-related barriers in way of biohydrogen energy generation and promotion.

This article is part of the Topical Collection on *Regional Renewable Energy*

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Keywords Biomass · Biohydrogen · Fermentation · Barriers

Introduction

Major energy reforms around the globe have been stimulated due to fluctuation in energy prices, energy scarcity, and environmental pollution due to consumption of conventional fuels. Evidence from several scientific studies clearly reported changes in climatic variables due to global warming. The consequences of this global threat were well recognized; despite this fact, various developing countries such as India largely depend on fossil fuel to run its industrial and transportation sectors. The share of carbon dioxide in total greenhouse gas emissions is projected to increase by double from 14 % in 2000, and total emission would raise to 80 % by 2050 [1]. Thus, there is a need for adaptation of bioenergy under a sustainable economic approach, which is expected to provide a solution to the double challenge of environmental restoration and energy security. Explorations of such energy alternatives are the need in the present, which have the potential to meet the energy demand and supply gap. Hydrogen is the most abundant element in the universe that has a potential to serve as an excellent fuel due to its high heat of combustion (122 kJ/g) with no by-products of pollutant nature (Table 1) [2, 3]. Hence, hydrogen as an option among the other alternatives has emerged as a viable alternative, which has been well explored by recent studies.

Developing countries like India have implemented strategic policies to reduce the carbon emission under the national action plan on climate change. Studies have reported that cumulative emission of greenhouse gasses from developing countries contribute up to 75 %. Thus, significant reduction target could not be achieved without the effort of these fast-growing economies (http://www.cfr.org/climate-change/global-climate-change-regime/p21831). Therefore,

Chapter 20

Solar Photocatalytic Treatments of Wastewater and Factors Affecting Mechanism: A Feasible Low-Cost Approach

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20.1 INTRODUCTION

One of the most significant problems that affect people around the world is scarcity of clean water. Clean water is not only a basic need but also important for the functioning of a civil society. Water crisis is an issue the world over, and the clean water crisis is expected to become worse in the coming decades. Therefore, scientists are developing innovative technologies that can clean, detoxify, and decontaminate wastewater so that it can be reused later (Riaz, Mohamad, Azmi, *et al*. 2014). Waste that contaminates surface and groundwater comes from various sources; it can be industrial waste effluent, agricultural waste like pesticides and fertilizers running off to rivers and ponds, domestic waste, landfill, and so on. Non-degradable organic pollutants, also known as bio-recalcitrant pollutants, are also present in wastewater. Earlier, wastewater was commonly treated through physical, chemical, and biological methods. Conventional methods of wastewater treatment have long been known to remove many contaminants, both chemical and microbial, which are a danger to public health and the environment. However, the effectiveness

Chapter

CLOSED PHOTOBIOREACTORS: CONSTRUCTION MATERIAL AND INFLUENCING PARAMETERS AT THE COMMERCIAL SCALE

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ABSTRACT

In the present era, potential alternatives for energy resources are urgently needed due to continuous depletion of conventional energy resources. Among the various alternative energy sources (solar energy, wind energy, geothermal energy, etc.), biomass-based energy sources (such as algae, organic waste and agriculture biomass) provide a better means of addressing the energy crisis. Various biomass-based products used in energy recovery include biodiesel, bioethanol, biobutanol, biohydrogen and biogas.Furthermore, commercial-scale production of cultured biomass is needed to increase the accessibility of algal biomass, which is directly or indirectly influenced by the type of closed photobioreactors (CPBRs) used. Light availability, construction material, inclination angle are the influencing parameters that mostly affect the growth of algal biomass.Hence, to achieve the maximum viability of CPBRs at the commercial scale, this study investigated CPBRs especially the construction materials used, additionally, other influencing parameters of CPBRs are assessed to evaluate the commercial feasibility of CPBRs for algal biomass production.

Keywords:closed photobioreactor, algal biomass, construction material, parameters

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Algae-Based Biohydrogen: Current Status of Bioprocess Routes, Economical Assessment, and Major Bottlenecks

6

Richa Kothari, Arya Pandey, Virendra Kumar,
and V.V. Tyagi

1 Introduction

Hydrogen is a most efficient fuel and has the highest energy density among known fuels (143 GJ/tonne) in terms of energy values as well as from an environmental point of view. It is a zero emission fuel which does not contain carbon, sulphur, or nitrogen and generates water only as a by-product on combustion. Recently it is being very efficiently used as a vehicle fuel in automobiles and also for electricity generation via fuel cells. Commercially, hydrogen is produced by using fossil fuels such as coal, methane, and other heavy hydrocarbons (Kothari et al. 2008). All these processes of hydrogen production are very expensive and not environmentally friendly. Recently, researchers have sought alternative methods for hydrogen production including photolysis of water and biological methods of hydrogen production (Nayak et al. 2014). Biologically produced hydrogen by using microorganisms such as bacteria and algae by photosynthetic and fermentative routes (Monlau et al. 2013; Julia et al. 2014; Kothari et al. 2011; Venkata et al. 2007; Levin et al. 2004) provides a sustainable approach for society. Biological processes can scale up biohydrogen production by using various microorganisms and making it potentially competitive with chemical processes including thermal gasification, pyrolysis, and reforming among others. Biohydrogen production via a biological route is beneficial because it is neutral regarding CO_2 emission and free from other greenhouse gases such as carbon monoxide and hydrogen sulphide and it does not require any kind of treatment before use in the fuel cell to generate electricity. Yield of biohydrogen production depends on operating cost whereas its rate depends upon its installation cost or reactor cost.

Biophotolysis (direct biophotolysis and indirect biophotolysis), photofermentation, and dark fermentation (Venkata et al. 2009) are the emergent bioprocess routes for the production of biohydrogen. Among these, algae-based bioprocess production routes are projecting more scope in the R&D sector with commercialization. Indeed, algae present several advantages compared to terrestrial plants in virtue of: (1) algae have a higher growth rate than plants and they are more capable in CO_2 fixation; (2) they can be grown easily in water and wastewater (Venkata et al. 2012); (3) they are rich in carbohydrates and have a lack of lignin (Nayak and Das 2013). Besides these, algae is a third-generation biofuel produced from macroalgae, and microalgae are more advantageous than second-generation biofuel produced from nonedible crops because they do not require fertile land for their growth and they have the potential to provide jobs for skilled and unskilled members of society.

There is very modest information available in the literature regarding the journey of lab-scale to large-scale commercial production of biohydrogen with algae. Hence, the present chapter aims to make available considerable research and developmental progress with major bottlenecks through bioprocess routes for algal-biomass–based biohydrogen production with emphasis on the major factors involved.

2 Bioprocess Routes for Biohydrogen Production by Algae

Algae have wide potential for bioenergy generation by their metabolic activity as well as their anaerobic fermentation due to their rapid growth and rich carbohydrate contents. Biohydrogen production through biological process is significant and economically viable by algae because it is less expensive, has an easily available feedstock, and can use

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B. Singh et al. (eds.), *Algae and Environmental Sustainability*, Developments in Applied Phycology 7,
DOI 10.1007/978-81-322-2641-3_6

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Arya Pandey, Vinayak V. Pathak, Richa Kothari, Pradeep K Majhi, Vineet V. Tyagi (2019). Impact of heavy metals on algal biomechanical compounds and their treatment in CETP wastewater. International Journal of Environmental Science and Technology

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