

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

**SUMMARY OF
THESIS**

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

relationship between physical/chemical and environmental factor, optimized with multiples variables of these factor need for the development of algal productivity. The macro and micro nutrient present in wastewater are suitable for algal growth and their development. This types of low cost integrated approach contains: reduction of wastewater cost treatment, biomass production on annually, reduction of freshwater burden, reduction of media cost. Therefore, keeping in views, the designed objectives have been taken to prove the concepts which is based on experimental validations.

Chapter 1: Introduction

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

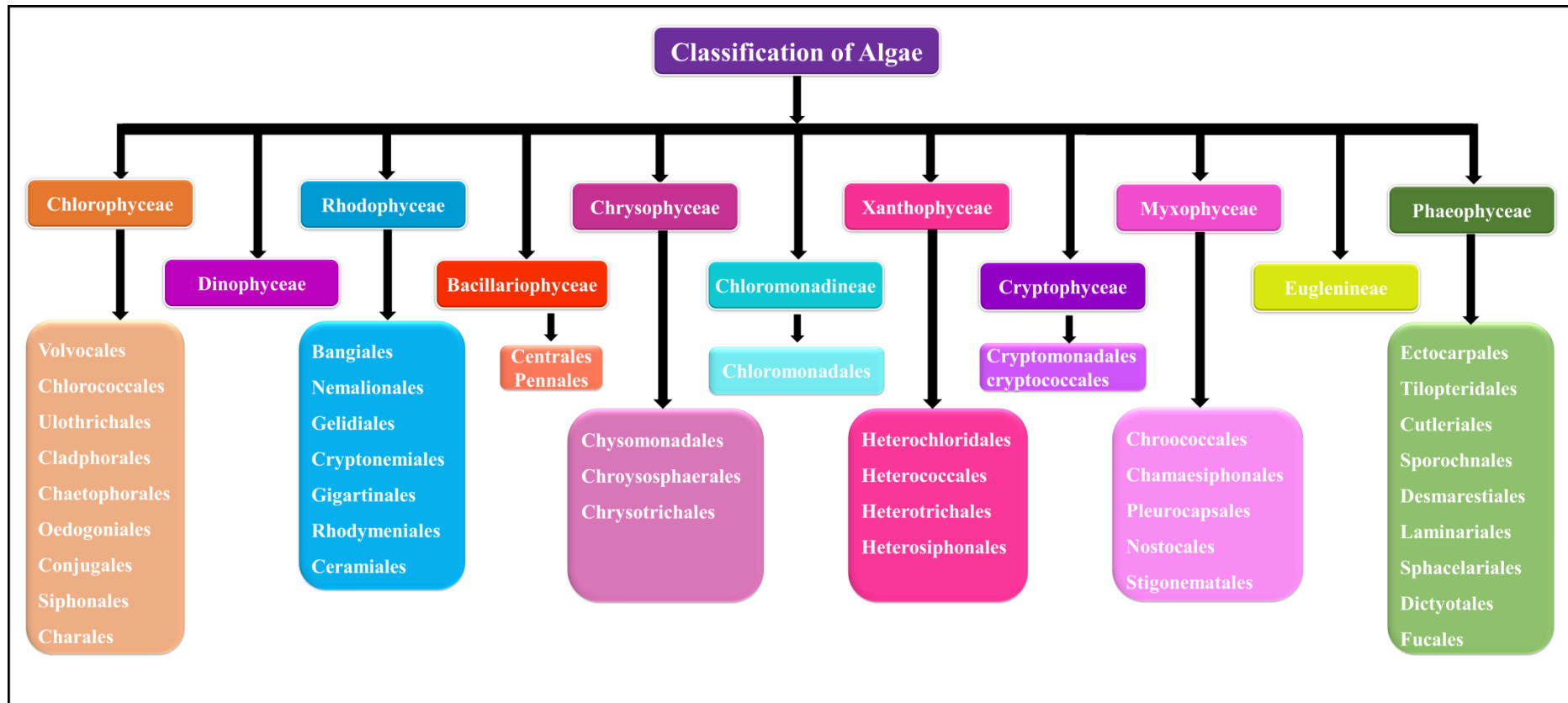


Figure 1. Classification of algae (Fritsch 1935)

Chapter 2: Review of literature

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

Chapter 3: Materials and methods

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

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

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

Therefore, the objective of this chapter is focused on a novel approach for bioremediation. Presently, the algal biomass production at the commercial level uses the existing freshwater resources which are not sustainable due to their demand for drinking and other purposes and is available at a high cost. It is also an alarming condition for the environmental sustainability and contributes as one of the factors in capital production cost. Thus, the utilization of wastewater as an alternative source for freshwater and to reduce the cost in a dual way *i.e.* as a medium for biomass and providing low-cost source nutrients from those instead of high-cost chemicals, decrease the production cost and break the limitations with its wide applications. Hence, the present study explores to assess the impact of variation in the concentration of SHWW for the growth of *C. pyrenoidosa* and *C. vulgaris* for high biomass production. Keeping in view, *C. vulgaris* was taken in this experiment study for the assessment of algal growth and lipid production potential using SHWW with

an aim for treatment and one of the important value-added products *i.e.* bio-oil. The main important part of this work is based on the growth of both strains of *C. pyrenoidosa* and *C. vulgaris* was done by using different kinetic models by nutrient availability in SHWW via phycoremediation and conceptualization of freshwater footprinting is also investigated on the part of reusability of treated water and freshwater crisis. The SHWW was treated with two *Chlorella* sp. (*C. vulgaris* and *C. pyrenoidosa*). The growth and algal biomass production were optimized with various concentrations of wastewater, and this experiment was conducted for 15 days. During the growth period, the biochemical parameters were assessed at every 5 days interval, in terms of optical density (680 nm) with organic load (COD, BOD), nutrient load (nitrate and phosphate), reduction of pollution load, and nutrient load. Besides, the treated wastewater was analyzed thoroughly before it releases to the surroundings. Co-relational analysis between the physicochemical and biochemical profile of SHWW of algal biomass is also studied in both *Chlorella* sp. In the present study, it is concluded that algal-based wastewater treatment is an effective and low-cost method without any harm to the environment. Therefore, different concentration (25%, 50%, 75% and 100%) of wastewater have been taken for this experimental investigation. In comparison to the modified Gompertz model, the logistic model produced better results. SHWW treatment is an effective and best alternative nutrient source to enhance the biochemical profile of algal biomass for their products. Only 50% concentration of wastewater was selected for the changes in chlorophyll, protein, lipid and carbohydrate along with growth dynamics monitored during the uptake of nutrients from wastewater. Based on growth, 50% concentration is the best for the production of biomass (430 mg/l on dry weight basis) in SHWW. Therefore, the pH of wastewater during the phycoremediation process needs to be within the optimal pH

range. The present experimental investigation results clearly show as the potential for algal biomass production with favorable biochemical profile with SHWW at 50% concentration. the correlation between two variables indicates the high differences among them. The highest positive correlation was observed in 25% and 50% concentration of SHWW of TS, BOD, COD, nitrate and phosphate between the chlorophyll, protein, and carbohydrate are highly correlated which is also present. The best positive correlation was found between the 25% and 50% concentrations of wastewater. In *C. vulgaris* the correlation analysis was comprised between the physicochemical parameters and biological parameters of SHWW with 50% concentration. Furthermore, using the concept of construction of on-site pits in combination with algal biomass near slaughterhouses may be able to solve the problem of wastewater discharges without treatment for healthy, clean and commercial solutions in favor of bio-economy. The SHWW has a high potential to treat algae for the production of algal biomass and lipid extraction. Therefore, the phycoremediation based process of SHWW indicates that is used for value-added products and its main aim is to reduce pollutants from waste such as sewage. Therefore, it represents a very good resource for the production of biomass.

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

The present chapter deals with the current global environmental issues raise unavoidable challenges for the utilization of natural resources. Increasing the human population is a challenge that affects the clean water body becomes a global problem. The biomass production from algae using industrial wastewater offers the possibility of recycling industrial residues to create new sources of raw materials for energy and

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

But in addition, there is a need of information on the kinetic assessment of lipid production for algae cultivated in nutrient-limited wastewater.

Keeping in view this Chapter aims on the the production of algal biomass and lipid extraction for value-added products. The *Chlorella* sp. is the main objective of this chapter by using OSCCW to evaluate the physico-chemical properties, for the production of biomass and lipid. Algae is a rich source of crude oil and high value-added products that are not only applied to food, feed but diverse new fields such as photovoltaic. Algae produced various photosynthetic pigments, secondary metabolites such as chlorophylls, carotenoids and phycobiliproteins are a good source of sensitizers. The result shows that the growth of algae in absence of OSCCW did not give the best result due to lack of nutrients. But in controlled conditions with BG-11 medium the growth of algae was observed with both *Chlorella* sp. and the best result was found in *C. pyrenoidosa* based on growth curve as compared to *C. vulgaris*. However, the growth of *C. vulgaris* was much noticed between on 13th – 15th days of the experiment with 50% concentration of OSCCW may be due to the level of nutrients being high as compared to *C. pyrenoidosa*. The highest biomass content was analyzed at 0.902 g/l in *C. pyrenoidosa* whereas 0.848 g/l biomass content was analyzed in *C. vulgaris* at 15th day of experiment. In this study, they were used three models (Logistic, Gompertz and Richards model), but the Logistic and Gompertz are best after the assessment for the analysis of algal growth. Therefore, *C. pyrenoidosa* has high potential for algal biomass production as compared to *C. vulgaris*. But based on the profile of chlorophyll, protein and carbohydrate in *C. pyrenoidosa* were found best result due to the increased level of biochemical profile from 0th to 15th day of the analysis. In *C. pyrenoidosa* the highest positive correlation was observed with the 0th to 5th day of TDS, COD, BOD, nitrate and phosphate as compared with carbohydrate,

chlorophyll and protein, was observed with 10th to 15th day was observed, that is highly correlated with each other. In *C. vulgaris* correlation TDS, BOD, COD, nitrate and phosphate between two variables are not positively correlated with chlorophyll, protein, and carbohydrate was not found highly correlated. Thus, the analysis and observation on the basis of correlation with positive results indicate it is a very effective method for wastewater treatment and their management. The significant correlation between the algal biomass and the nutrients that indicate the importance of microalgal communities can be used as an important tool for the various phases of wastewater treatment. The algal-based OSCCW has high potential for the removal of nutrients from wastewater. In this study, the *C. pyrenoidosa* achieved the high efficient amount of nutrient removal rate and high lipid production. The algal-based OSCCW treatment is a very low and environmental friendly method to treat the *Chlorella* sp. with wastewater for the extraction of lipid. The remediation of wastewater using *Chlorella* sp. provides an environmentally acceptable and effective option for wastewater remediation, which not only recycles valuable nutrients but also improves the quality of water. Therefore it may be regarded as an efficient nutrient removal process for the removal of nutrients from wastewater.

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

The present chapter deals with the investigation of culturing of algal biomass under various parameters for the production of maximum biomass. Algae are cultured by the application of various conditions. Due to the large scale of cultivation of algae increase the green technology economically. In the natural or artificial process, it is exposed to a number of environmental factors like pH, nutrient sources as well as light. The bio-chemical compounds (carbohydrates, protein, lipid, carotenoids and

pigments) are the main compounds which help to produce maximum biomass for value-added products. Despite all of this, the biogenic accessible elements concentrations are the main parameters that affect the growth of algae. In this work, the bio-chemical compounds are derived from algae by treating with different wastewater (SHWW, OSCCW) for the estimation of valuable results, which is based on value-added products. Algae play an important role in the daily diet and future life. It is considered as a rich source of proteins, carbohydrates and lipids for value-added products and it is an essential food for human beings as well as animal feed all over the world. However, regardless of these challenges is associated with the completely harvesting of algal biomass. Whereas, harvesting of algal biomass is an important part of this experimental research for value-added products. Therefore, various conventional treatment processes (flocculation, centrifugation, sedimentation, electrocoagulation and gravity) are used for the cultivation and dewatering for the harvesting of algal biomass. Numerous compounds extracted from algae are widely used as cosmetic industry as water-binding agents, thickening agents, and antioxidants in skin and facial care products. It is well known that algae contain valuable products that are proteins, carbohydrate, lutein, β -carotene, phenolic and flavonoids compounds that are useful for several industrial applications. Despite of these excellent and advanced benefits, there are several limitations that should be focused as well. Therefore, to overcome these issues some of these bio-chemical active compounds are presently articulated in proper vectors to relieve for purification and hence for the reduction of purification as well as production costs. These novel approaches provide a innovative approach by using the algae to obtain the valuable bio-chemical compounds for the treatment of various disease. Furthermore, in genetic engineering the algae have high metabolites towards natural organism for drug

deliveries have been explored. As per the experimental observation light and temperature are the main factor for the improvement of biochemical profile, biomass growth and their development. There are various applications of algae, which are used as a feed supplements. The obtained biomass from algae has multifunctions which are used as a feedstock for fish towards farm animals. So for the best of our knowledge the result obtained in our study by using SHWW and OSCCW by treating with both strains (*C. pyrenoidosa* and *C. vulgaris*) compare to previous study is best and it can be utilized as fish meal as well as animal feed. The main application of carbohydrates are antioxidants, anticoagulant, antiproliferative and immunostimulatory. In spite, of all these more efforts required to develop the multifunctional ranges of products, that are affordable and also inter-related with nutritional science that give a more health benefits in current market. The main aim of this chapter is completely focused on algal based biomass for value-added products. Major finding can be concluded that the value-added products production is an integrated approach for commercialized application, but the main challenges cannot be ignored at the part of environment. Therefore algal based wastewater treatment is a zero investment on the basis of cost and have high potential on market level.

Chapter 7: Techno-economic assessment: comparative study

Due to the current environmental issues, renewable energy is the best option to solve the environmental issues, to attract increasing attention on both the academic and industrial sectors. The techno-economical assessment system depends on its research, development, commercial aspects and environmental elements. Keeping in view of all the objectives, algal biomass-based biofuels in addition with wastewater, bioreactors analysis and its assessment are evaluated theoretically and experimentally. Therefore, the process, which is done through a laboratory scale, is unable to compile the

requirements on a pilot scale. Hence, the utilization of *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) is the best option for the integrated approach up to final products from biomass. The 50% concentration of SHWW and OSCCW was utilized as nutrient medium that can reduce load on fresh water sources and chemicals. Therefore, algae can be produced high amount of biomass and biomolecule with simultaneous treatment of wastewater at large scale that can be clean and cost cutting technique for cotemporary world. The main finding in this experimental study by using two *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) cultivated with SHWW and OSCCW with bioreactor for the production of biomass in pilot scale for further commercial application.

The biomass growth of *C. pyrenoidosa* and *C. vulgaris* was analyzed in SHWW for 15 days of the cultivation time period and best concentration was optimized at 50%, of both strains. The 50% concentration of OSCCW was also utilized for biomass growth of *C. pyrenoidosa*, and *C. vulgaris* of both strains. The algal biomass growth was optimized in the 500 ml conical flask with 300 ml working volume of SHWW and OSCCW. After optimization of algal growth, both algae were grown in a vertical flat plate bioreactor with 5 L working volume of both wastewaters to estimate the large scale cultivation of both *Chlorella* sp. *C. pyrenoidosa* and *C. vulgaris* produced 1290.75 mg biomass in 300 ml, whereas 21512.5 mg biomass was recorded in vertical flat plate bioreactor which has working volume 5 L (5000 ml). *C. vulgaris* generated 1170 mg biomass in 300 ml culture medium of SHWW and 19500 mg biomass was observed 5 L culture medium in vertical flat plate bioreactor. The bio-chemical compounds (chlorophyll, protein, carbohydrates and lipid) of *C. vulgaris* were analyzed in SHWW and OSCCW. Only 50% concentration of wastewater were taken for the analysis of this experimental approach where the chlorophyll, protein,

carbohydrates and lipid content were investigated with SHWW with *C. vulgaris*. Thus, the present experimental study indicates that the *C. pyrenoidosa* and *C. vulgaris* are the prominent strains that produce biomass and bio-chemicals compounds at large scale in bioreactor by utilization of various types of wastewater (SHWW and OSCCW). The algae can be cultivated in these types of wastewater at large scale for commercial application as well as value-added products. OSCCW is an eco-friendly treatment for clean and green environment for future research. The 50% concentration of both wastewater with both strains of *Chlorella* sp. (*C. pyrenoidosa* and *C. vulgaris*) is best as per our investigation on lab-to-land approach. It provides an innovative approach, which is economically viable, proficient as well as expedient green approach for the cultivation of algal biomass for further valuable applications. It is a low-cost technique for the production of algal biomass-based biofuels and other for value-added products that are used in cosmetics, nutrient-supplements, medical and fertilizer, as per techno-economic assessment in between conventional and advanced remediation approaches. Bioreactor based algal cultivation techniques is best for the production of biomass for valuable applications, while as, still there is some technical issue that require to be solved to unfold its potential in up scaling of algal biomass production for biofuels. Thus, the recovery of nutrients by algal treatment of wastewater at a large scale in the bioreactor can be the cost-cutting process that can also enhance the sustainability of wastewater treatment. There are many developed method, which are used for high productivity, biomass quality and its density, to prevent the contamination of algal culture. Algae production has proven to be environmental reimbursement and also reduce potential unpleasant impacts related to the high water and energy demand, emission control, management of wastewater, land use and risk of microbial contamination.

Chapter 8: Conclusions and future recommendations

The experimental results were assessment through algal growth by using growth kinetic models (Logistic and Gompertz) and correlational analysis between the process parameters (TDS, BOD, COD, nitrate and phosphate) with algal biochemical profile (protein, carbohydrates and lipid) for the analysis of positive and negative correlation between two variables. Optimization of different concentration (25%, 50%, 75% and 100%) of wastewater were assessed with two strain of *Chlorella* for the efficient treatment for the production of maximum algal growth for value added products is a important part of this research work. Correlational analysis have been taken as the variables between two process parameters and biochemical profile for the negative and positive correlation of both strains. Hence, utilization of wastewater as a media for the cultivation of algal growth is a good alternative for treatment process, which is further investigated for bioenergy options (biohydrogen, biodiesel and biogas). It also presents a new way forward for future energy options and waste management approach to solve the energy crisis, freshwater crisis and control of environmental pollution from local to national to regional level. The various applications of algal biomass for the treatment of wastewater to produce biofuels is an integrated approach for clean and ecofriendly sustainable environment. Phycoremediation based treatment is low cost process for the generaton of bioenergy. But there are some practical challenges during the implementation of this process. Several practical challenges are faced during the stage of experiment *i.e.* physical and chemical changes at the time of processing for the production of algal biomass in pilot scale for value added products. Inspite of all these challenges, to remove the burden on our freshwater sources, wastewater from different sources have been selected as substrate for algal biomass (*C. pyrenoidosa* and *C. vulgaris*) growth and their

development. Effect of several parameters has been taken into consideration for algal growth, biomass and biochemical productivity. Hence, utilization of wastewater as a media for the cultivation of algal growth is an good alternative for treatment process, which is further investigated for bioenergy options (biohydrogen, biodiesel and biogas). The bioreactor plays an important role for the cultivation of biomass. Simultaneously, algal biomass used for treatment of wastewater can be processed for biodiesel (bio-oil) and its biomass can be used for value-added products. Implication of algal biomass for bioenergy (biofuel) as well as value-added bio-chemical products provide a integral approach for the treatment of wastewater with clean and green environment. This leads to the conclusion that the use of wastewater with (*C. pyrenoidosa* and *C. vulgaris*) have a potential for biofuels production. Whileas, upstream and downstream processing steps require an optimization for “system” working in efficient way with any types of algal strain with wastewater. The work done in this thesis indicates a relevant answer to the problems associated with clean efficient production of biofuels. It also presents a new way forward for future energy options and waste management approach to solve the energy crisis, freshwater crisis and control of environmental pollution from local to national to regional level.