Spectral conversion of light with different induced stress conditions to improve algal growth in wastewater for biofuel production

SUMMARY
of
THESIS

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Rapid consumption of fossil fuels and their limited availability has caused the energy crisis at global level. Energy is measured to be a significant factor in the inception of bringing prosperity, a good health and wealth and an imperative economic as well as environment constituent. Every nation's economic development is very much dependent upon its energy capitals. Highly developed nation have a high rate of energy utilization associated with other developing nations. It has been appraised that the complete primary energy utilization of India is approximately 1/1.6th time of Japan, 1/7th of USA and 1/29th of that of the world (Garg, 2012).

The major challenge of the 21st century is to provide adequate water, food, and energy for everyone in the world to live comfortably with surroundings, in the expression of growing population, the warning of climate alteration, and (sooner or later) deteriorating fossil fuels and increasing level of CO_2. In 2014, fossil fuels accounted for 86% of global energy consumption.

Hence, these conditions attracting the attention of researchers and stakeholders for development of alternative fuels. Biomass based energy generation has been found as potential resource for replacement of fossil fuels and termed as biofuel. Various generation of biofuel has been developed based on variety of feedstocks such as first generation, second generation and third generation biofuel. First generation biofuel was associated with food versus fuel dilemma while the second generation biofuel was associated with challenges like land availability and irrigation cost. On the other hand, third generation biofuel was developed to resolve the issues associated with first and second generation biofuels and was based on algal based biomass feedstock. Algal based biomass feedstock not only provides useful resource for bioenergy generation but also reduce carbon dioxide from the surroundings due to its higher photosynthetic efficiency than the higher terrestrial plants. Apart from these

advantages, alga can be cultivated in to wastewater released from various sources (industrial and municipal). The other advantages with algal biomass involve its application as a nutraceuticals, antioxidants and anti-ageing agent etc.

After extensive literature survey on biomass based value added products including conversion route of biomass to biofuel, influencing parameters and further uses of residual biomass for secondary bioenergy option, providing a new insight for bio economy. The bio economy concept is significant for further challenges like energy crisis environmental imbalances and unsustainability on planet earth So, algal biomass has a potential to solve all the associated problem in an integrated way from wastewater treatment to fuel conversion. Keeping all associated challenges with algal growth biomass and their utilization for biofuel as well as other value-added end products different objectives are formulated to complete the task in sustainable manner. Hence this research work trying to provide an illustration for all the challenges like material and methodology adopted to carry out the experimental plans discussed in Chapter-2. Chapter-3 is providing the experimental plans related to steps for upstream processing for algal biomass growth using wastewater in general and use of response surface methodology (RSM) with their impacts on process variables for optimization and with designed bioreactor in particular. Similarly, Chapter-4 is delineating the downstream processing steps like harvesting of *Chlorella pyrenoidosa*using low cost flocculants and comparing their harvesting efficiency with commercially available chemical based catalyst. Reuse of low-cost catalyst for transestrification of harvested algal biomass also studied with optimization of dose using RSM. Development of fuel quality index (FQI) is explaining the qualitative assessment of biofuel in comparison with commercial biodiesel on the basis of fatty acid methyl ester (FAME) content and its application as a fuel for commercial engines using experimental data base generated in Chapter-3 and Chapter-4,part of Chapter-5. Techno-economic feasibility of “selected approach” for conversion of selected algal biomass to biofuel with various value added end products (Algal biorefinary concept) also overviewed in Chapter-5. Chapter-6 providing a concluding remark for selected research problem and future recommendations for research (from lab to land) in concern area.Brief discussions of each chapter with their specific concluding remarks are provided below:

Chapter 1: Introduction and Review of Literature

Microalgal cells consume N and P for its growth and synthesize protein and nucleic acid. Algal cells could enthrall the inorganic nitrogen after it has been converted to NO_3^- form through nitrification process or it could be degraded into gas nitrogen continually. Besides, the physical reactions, chemical reactions like absorption, ion exchange, sedimentation as well as precipitation perform an essential part in order to reduce P. Through microbial actions too, phosphate could be despoiled to a great extent, moreover, P could be removed through precipitation once the pH of microalgal culture upsurges. Phosphorus plays crucial role in the energy metabolism of microalgae. Creation of nucleic acid, lipids and adenosine-tri-phosphate (ATP) is also sustained by this. Via phosphorylation reactions, cell growth as well as metabolism of microalgae is supported by the inorganic form of this essential nutrient. For microalgal growth, sun is the essential source of energy as a reasonable amount of light intensity reaches the ground. The microalgae could harness the wavelength range of 400-700 nm from an expansive realm of solar radiation. This particular solar radiation is termed as photosynthetic active radiation (PAR), 43% of the incoming solar radiation lies in PAR expanse on the basis of energy. Under closedcultivation condition variety of light sources have been used to explore their impact on algal growth such as Incandescent light (bulbs), Fluorescent lamps, Halogen light etc. Temperature proves to be a strong influencing parameter.

Temperature alone determines nitrogen uptake, CO_2 fixation, organization of the cells thereby persuading the rate of growth of every algal species. It‘s a universal fact that an increase in temperature registers an increase in the growth rate of algae till it reaches the optimum range.

After cultivation of algal biomass its cultivation is another bottleneck for algae based biofuel production process. Algal harvesting cost could be in excess of 50% (Greenwell *et al.*2010).Harvesting and dewatering together constitute the 90% equipment cost in open organization for manufacturing of algal biomass. Extraction of algal oil and its conversion in to biodiesel is a part of downstream processing and involves various physical and chemical based conversion processes. Oils from algal cell can be extracted by using the organic solvents such as methanol, n-hexane, ether etc. Pressurized high solvent extraction using ionic liquid is applied to replace the toxic organic solvents and to achieve high oil yields. Recent investigators have also explored the use of supercritical CO_2 and found more efficient, simple and cheap method for oil extraction. After extraction of lipid which is used to convert into fatty acid by the process of transestrification. Transesterification refers to the chemical way to convert the fatty acid in to the fatty acid methyl esters (FAME), which is termed as biodiesel. This process reduces the viscosity of FAME. Initially transestrification process was carried out by employing the base or acid catalysis, but now researchers have explored the other potential ways such as direct methanolysis, enzymatic transesterification and microwave assisted transesterification. The present study involves the investigation on spectral variation under different stress condition (temperature nutrient, carbon dioxide, bicarbonate) for growth of selected algal strain, design and development of suitable photobioreactor for growth of algae at different visible spectrum condition with wastewater, optimization of biomass cultivation condition and the feasibility of present approach with the conventional process.

Chapter 2: Materials and methods

The microalgae *Chlorella pyrenoidosa* was obtained from National Collection of Industrial Microorganism (NCIM), Pune and maintained in recommended Fog's growth medium (Kothari *et al*., 2012). The initial pH of the medium was maintained to 7.2.The mother culture of alga was grown in one liter Erlenmeyer flask provided with white fluorescent cool light (12:12 h) and optimum room temperature 25±2°C. All cultures were manually shaken to avoid sticking of microalgal cell on wall of culture flask. Growth of microalgae was optimized within the manipulated culture conditions and measured in terms of biomass concentration, biomass productivity.

This chapter describes the experimental methodology used in present study overall. The materials and methods used for study are described in detail, explaining the purpose of each experiment and the analytical technique used. The analytical techniques used in present study are explained under the sections of upstream and downstream processing including microalgal culture requirements and its growth optimization under different wastewater concentrations with harvesting techniques adopted to scale-up the algal biomass. It also outlines the algal culture requirement and multifactor optimization of biomass, lipid and Fatty acid methyl ester (FAME) using Response surface methodology (RSM).

The present study was accomplished within three Phases and shown in Table.2.1**Phases:Phase-I**involve the optimization of upstream process parameters for algal growth and lipid productivity using response surface methodology. In this phase biomass, lipid productivity and pollutant removal study of microalgae (*Chlorella pyrenoidosa*) were performed using Response surface methodology (RSM). In **Phase-II** optimization of downstream processes (harvesting and tranestrification) using Response surface methodology to scale up algal biomass was observed which involves harvesting of algal biomass and extraction of oil from wastewater grown algal biomass, while the **Phase-III** was set-up for development of fuel quality index (FQI) for qualitative assessment of FAME with the help of instrumental analysis such as UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy, Gas chromatography performed in various steps of the study.

Chapter 3: Optimization of upstream process parameters for algal growth and lipid productivity using response surface methodology

This chapter is divided into three main sections as:

3.1. Impact of single factor variables (Light, Temperature, Nutrient and industry wastewater) on algal growth:

Conventional optimization of light, temperature, nutrient and industry wastewater was found to have significant impact on the algal growth. Among various wavelength of light, red was found to have more pronounced impact on the lipid productivity (28.36%) while algal cells exposed under yellow showed minimum lipid synthesis (11.25%).The temperature optimization was performed within a range of 20-50ºC to study specific growth rates. Maximum algal growth was obtained at 30ºC (1.12±0.04gL^{-1}), whereas minimum biomass productivity was noticed at 50 ºC (0.423±0.03gL^{-1}). The impact of dairy industry wastewater was found effective for scaling up the biomass. The increasing wastewater fraction (10-75v/v%) showed positive influence on the algal growth. However, algal growth at 100% concentration wastewater was as such uninhibitedand it was comparable to the rate of algal growth obtained in control set (medium supported growth). However, the growth of alga was stimulated by about 25% at 75% concentration ofdairy industry waste water, when compared with the growth obtained at 100% concentration ofwastewater. However, lower concentrations of wastewater (10–50%) did not support better algal growth as compared to control, perhaps due to low level nutrient at lower concentrations of waste water. The impact of Nitrogen and Phosphorus variation in culture medium was also found significant for biomass growth and lipid synthesis. The influence of CO_2 on algal growth was also observed and it was found that selected range of CO_2 concentration support the growth upto optimum level the highest biomass growth

(1.13 gL^{-1}) at 15% and minimum (0.43 gL^{-1}) at 100% CO_2 and maximum CO_2 fixation efficiency notices in 10% CO_2.

3.2. Spectral variations and their impacts on biomass and lipid productivity in coupling with other different variables using RSM

3.2.1. Nutrient (NO_3^-, PO_4^{-3}) with variable in DIWW concentration without LEDs:

Response surface methodology (RSM) based optimization studies provided the combined influence of nutrient, light source, dairy industry wastewater on the lipid content and

biomass growth of *C. pyrenoidosa*. Initially the combined influence of NO_3^-, PO_4^{-3} and DIWW concentration was studied and it was found that among 20 experimental runs, the highest rate constant value (0.65 day^{-1}) for algal growth was obtained with 19^{th} run, which was designed with combination of 50% DIWW, 100 mgL^{-1} NO_3^- and 50mgL^{-1} PO_4^{-3} concentrations. Similarly, maximum biomass concentration in terms of O.D was also obtained in the 19^{th} run. The minimum algal growth rate and biomass concentration occurred in the 4th run, which was designed with a combination of 75% DIWW, 150 mgL^{-1} NO_3^- and 25 mgL^{-1} PO_{4-}^{3} concentration. Significant positive effect of these variables was found with removal of chemical oxygen demand from dairy industry wastewater. The results suggested that a higher level of both these factors yielded a smaller response. On the other hand efficiency of nitrogen removal decreased the limitation of phosphorus which influences the microalgal growth.

Table 2.1:Experimental plan used for present study

Experimental plan		
Phase-I: Optimization of upstream process parameters for algal growth and lipid productivity using response surface methodology	**Phase-II: Optimization of downstream processes (Harvesting and tranestrification) using Response surface methodology to scale up algal biomass**	**Phase-III: Development of fuel quality index (FQI) for qualitative assessment of FAME**
Biomass, lipid productivity and pollutant removal study of microalgae (*Chlorella pyrenoidosa*) using Response surface methodology (RSM): (a) Impact of single factor variables (Light, Temperature, Nutrient and industry wastewater) on algal growth; (b) Spectral variation and their impacts on biomass and lipid productivity in coupling with other different variables using RSM • Nutrient (NO_3^-, PO_4^{-3}) with variable in DIWW concentration without LEDs. • Different light emitting diode (LEDs) with nutrient (NO_3^- and PO_4^{-3}). • Different temperature and light emitting diode (LED) with the use of CO_2 and DIWW. • Different light emitting diode (LED) with variable temperature and CO_2 concentration (c) Designing of bioreactor and its validations using point prediction analysis	Harvesting and extraction of oil from algal biomass cultivated on industrial wastewater by using response surface methodology; (a) Chemically synthesize impregnated catalyst (zirconium, and tungustun) , nanocatalyst (Ca and Mg) for harvesting of algal biomass (b) Optimization of nanocatalyst dose (nano-catalyst-CaO) for tranestrification of bio-oil from harvested biomass (Phase II(a)) and their impact on cell structure.	Development of fuel quality index for identification of quality of lipid and fatty acid at commercial scale Development of fuel quality index for qualitative assessment of biofuel (Phase-I and Phase-II) and its techno economic viability for bio economy in comparison to commercially available biodiesel.

The combined influence of DIWW, NO_3^- and PO_4^{-3} was found synergistic for biomass productivity of *C. pyrenoidosa*. The interrelationship of biomass productivity and variable concentration of DIWW, NO_3^- and PO_4^{-3} revealed that biomass growth of alga was highly influenced by nitrate concentration, while dairy industry wastewater has least impact on the algal biomass growth. Similarly, Lipid productivity of *C. pyrenoidosa* was greatly influenced by different DIWW combinations with nitrate and phosphate. The observation revealed higher lipid accumulation was found with lower concentration of these variables. It was found that higher levels of nitrate and phosphate had an inhibitory effect independently on lipid accumulation. However, the increased and positive value of the interactive effect coefficient for DIWW, NO_3^- and PO_4^{-3}suggests that the accumulation of lipids was enhanced with increasing levels of selected variables.

3.2.2. Different light emitting diode (LEDs) with nutrients (NO_3^- and PO_4^{-3}):

The study on effect of different light emitting diode (LEDs) with nutrient (NO_3^- and PO_4^{-3}) revealed that the biomass concentration, Lipid and FAME content were varied between 0.247-1.23gL^{-1}, 26.24-43.21% 73.5-89.76% lipid respectively for different combinations of operating variables. The best-fitted response for $Y_{BM}(gL^{-1})$, $Y_{Lipid}(\%)$, and$Y_{FAME}(\%)$ are composed of several intercepts, linear, quadratic and interaction coefficient and among them some coefficient (X_1, X_2, X_1X_2, X_1X_3, X_1^2, X_2^2, and X_3^2) for biomass productivity,(X_1, X_2, X_3, X_1X_2, X_1X_3, X_1^2, X_2^2, and X_2^3)for lipid content and (X_1, X_2, X_1X_2, X_1X_3, X_2X_3, and X_1^2) for FAME content. The variation in biomass productivity influencing variables are light>NO_3^->$PO_4^{-3.}$ Similarly, the most influencing variables for lipid content are NO_3^->PO_4^{-3}>light and FAME content influence by PO^{4-}

^{3}>NO_3^->light due to single factor contribution. Similarly, the most influencing interaction variables for lipid content ~75% of NO_3^-: light. But, in case of FAME content~55% of Light:PO_4^{-3} interacting effect was observed, which help in formationof FAMEcontent.

3.2.3. Different temperature and light emitting diode (LED) with the use of CO_2 and DIWW:

The combined influence of LEDs, CO_2 and different concentration DIWW revealed responses biomass concentration, Lipid content, FAME content, and CO_2 sequestration were varied between 0.615-1.32gL^{-1}, 18.25-31.25%, 72.36-86.34% and 0.0318-0.152 g/L/d respectively, for different combination of operating variables. The best-fittedresponse for Y_{BM}(gL^{-1}) Y_{Lipid}(%), Y_{FAME}(%) $Y_{CO_2\ sequestration}$ are composed of several intercepts, linear (X_1,X_2 and X_3) quadratic (X_1^2,$X_2^2$and X_3^2) and interaction coefficient (X_1X_2,X_1X_3,and X_2X_3) and among them some linear coefficient for (X_1,$X_2$and X_3) for Y_{BM}(gL^{-1}) Y_{Lipid}(%) and $Y_{CO_2\ sequestration}$ and($X_1$and X_3), significant quadratic responses (X_1^2,X_2^2,and X_3^2) for Y_{BM}(gL^{-1}) and $Y_{CO_2\ sequestration}$ (X_1^2 and X_3^2) for Y_{FAME}(%) and (X_3^2) for Y_{Lipid}(%) and most significant interactive variable (X_1X_2,X_1X_3 and X_2X_3) for Y_{FAME}(%), (X_1X_2 and X_2X_3) for $Y_{CO_2\ sequestration}$ and (X_2X_3) for Y_{BM}(gL^{-1}) and Y_{Lipid}(%).The order of influence for the operating variable on biomass productivity was found to be Light> CO_2>DIWW. Similarly the order of influence for operating variables for lipid content, FAME content were: Light>DIWW>CO_2 and for CO_2 sequestration were: Light>CO_2>DIWW. The order of binary interaction among the variables wasfound CO_2+Light> DIWW+CO_2> DIWW+Light for *Chlorella pyrenoidosa* show *in* biomass. Similarly the most influencing binary interactive variable for lipid and FAME content were found CO_2+Light> DIWW+CO_2> DIWW+Light and DIWW+CO_2 >DIWW+Light >CO_2+Light. In case of CO_2, binary interactive variable of Y_{CO2} is CO_2+Light> DIWW+CO_2 >Light+DIWW. It clearly shows that multicolor LED interaction with CO_2 supply and DIWW (Light-CO_2 and DIWW-CO_2) significantly impact on microalgal biomass, responsible for enhancement of lipid content and CO_2 uptake rate.

3.2.4. Different light emitting diode (LEDs) with variable temperature and CO_2 concentrations:

Effect of multiple variables temperature, CO_2 and different LED on group as well as lipid productivity of *Chlorella pyrenoidosa* found that algal biomass, lipid content, lipid productivity, FAME content and CO_2 sequestration were varied between 0.14-1.423g/L, 18.25-32.25%, 0.0012-0.031g/l/d, 69.25-88.64% and 0.0012-0.031 g/L/d respectively for multiple combination of variables. Best fitted model for Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L) given in (Eq.3.13 to Eq.3.17) Chapter-3 of thesis are composed of linear (X_1,X_2 and X_3) quadratic (X_1^2,X_2^2 and X_3^2) and interactive cofficent (X_1X_2,X_1X_3 and X_2X_3) among them linear cofficent (X_1,X_2 and X_3) for Y_{BM} (g/L),$Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (%) and X_1 for $Y_{Lipid\ content}$ (%) interactive cofficent (X_2X_3) for Y_{BM} (g/L) ,(X_1X_2) $Y_{Lipid\ content}$ (%) and (X_1X_2 and X_2X_3) for $Y_{Lipid\ productivity}$ (g/L/d) and Y_{FAME} (g/L) and quadratic cofficent (X_1^2 and X_2^2) for $Y_{Lipid\ content}$ (%) Y_{CO2seq} (g/l/d) and (X_1^2, X_2^2 and X_3^2) for Y_{BM} (g/L), $Y_{Lipid\ productivity}$ (g/L/d) and Y_{FAME} (%).

The percentage contribution of CO_2(X_1), temperature(X_2) and LED light (X_3) towards responses variables (Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L) was calculated prob>F from the value and changed in % contribution of variables variation in (Y_{BM} (g/L) and $Y_{Lipid\ productivity}$ (g/L/d), influencing variables is

CO_2>Temp>LED, $Y_{Lipid\ content}$ (%) influenced by CO_2>LED>Temp similarly the order of influencing variables for $Y_{Lipid\ content}$ (%) Y_{CO2seq} ($gL^{-1}d^{-1}$), and Y_{FAME} (g/L) was CO_2>LED>Temp, Temp>LED>CO_2 and LED>Temp>CO_2.The interactive variable for biomass production and CO_2 sequestration are in the order LED+Temp>CO_2+Temp>CO_2+LED from the results,multicolor LED and effect of temperature enhance the growth with utilization of CO_2.Lipid content ,lipid productivity and FAME content behaviour depends on the interaction of induced/stress variables(CO_2+Temp)>(CO_2+LED)>(LED+Temp). Lipid content and FAME content directly affected by the supply of CO_2 and temperature for effective fixation of light become the limiting factor for biomass production for microalgal cultivation.

3.3. Bioreactor: Experimental validation of bioreactor point prediction analysis

The regression analysis and optimization of multivariable are taken here as on objective by using design experts for predicting the responses for designed bioreactor using the experiment.Point prediction analysis supports the algal growth with process parameter in designed bioreactor. Remarkable algal growth has been noticed on the part of high growth of selected algal biomass *Chlorella pyrenoidosa*.

Chapter-4:Optimization of downstream processes (harvesting and transesterification) using Response surface methodology to scale up algal biomass

This chapter is divided into two main sections as:

4.1. Chemically synthesize impregnated catalysts (zirconium, and tungustun), nanocatalyst (Ca and Mg) for harvesting of algal biomass:

In this study, three important variables viz. pH, dose and temperature were studied to harvest the selected algal strains in culture media using Imp Zn, Imp W, Nano-Ca and Nano-Mg as a flocculent catalyst in experimental set-up. RSM based CCD was used to get optimized condition for maximum harvest of *C. pyrenoidosa.*

The best fitted responses by the multiple regression are composed of several intercept linear (X_I, X_2 and X_3), interacting(X_1X_2, X_1X_3 and X_2X_3) and quadratic (X_1^2,$X_2^2$and X_3^2) cofficent. The percent contribution of individual factor for harvesting efficiency of algae influencing variables are $X_1>X_2>X_3$ (Dose>pH>Temp) for Nano-Ca, Nano-Mg.The percent contribution of interactive factors for harvesting of efficiency of algae with nanocatalyst (Nano-Ca and Nano-Mg) and impregnated (Imp-Zr and Imp-W) are as (pH+ºC)>(Dose+pH)>(Dose+ºC).

4.2. Optimization of nanocatalyst dose (nano-catalyst-Ca) for tranestrification of bio-oil from harvested biomass (Phase II(a)) and their impact on cell structure

Production of biodiesel from the dry biomass of *Chlorella pyrenoidosa* was carried out using the synthesized Nano-Ca nanocatalyst by the process of transesterification.This Model identified the best fitted model X_1,X_2 and X_3 for linear X_1X_2 and X_2X_3 for interactive and X_1^2 and X_3^2.60% contribution catalyst dose and 26% time for individual variables for biodiesel yield by the process of transestrification. The deviation in biodiesel yield influencing variables is Catalyst>Time>Temp. It was described the biodiesel yield by the process of transestrification affected by catalyst and time but was inhibited by increasing the temperature. But the interactive contribution of the variables (Nano-Ca+Time; Nano-Ca+Temp and Temp+Time) for optimization of process of transestrification for biodiesel yield.According to statistical analysis found Nano-Ca+Time combination contribute upto 91% role in transestrification process for biodiesel yield.

Chapter 5: Development of fuel quality index (FQI) for qualitative assessment of FAME

This chapter is divided into two main sections as:

5.1. Impact of qualitative assessment of FAME using Fuel quality index (FQI) for various end-products:

Different combination of variables (NO_3^-+PO_4^{-3}+DIWW; NO_3^-+PO_4^{-3}+LED; DIWW+CO_2+LED and CO_2+Temperature+LED) of central composite design of response surface methodology with point prediction analysis (PPA) using optimized conditions/induced stress conditions to enhance algal biomass are studied in this Chapter. Similarly, this enhancedbiomass used for extraction of lipid and convert in to fatty acid after the process of transestrification using Nano-Ca catalyst. With use of Nano-Ca catalyst after the process of transestrification analysed by using Gas chromatography with Flame Ionized Detector (GC-FID) and found FAME values are given in Table 5.1 of this Chapter in thesis .It also summarized that the chain length of FAME comprising of biodiesel in *C. pyrenoidosa* between C_{10}–C_{24}.It is composed of saturated fatty acid (SFA) ,unsaturated fatty acid (USFA), which further categorized into Monounsaturated fatty acid (MUFA) and Polyunsaturated fatty acid (PUFA).In case of saturated FAME, the major fatty acids were methyl decanoate and methyl pentadecanoate, while other saturated fatty acids were found below 2% in concentration.

5.2. Techno economical assessment for "selected approach"

Techno-economic study revealed that integrated system based algal cultivation is better than that of its single directed application as the algal cells produces a variety of components of industrial importance. Thus, coupling of biomass production for bioenergy generation with carbon sequestration, wastewater treatment and nutraceuticals production is more cost effective than that of the single application of algal biomass.

Thus the present study revealed that implication of algal biomass for bioenergy option (biofuel) as well as for other value-added end products provides a new arena with integrated approach for waste treatment and clean energy production. But practical challenges are noticed with levels on developmental technologies like changes in environmental variables (Light, temp, CO_2) and process parameters (Nutrients, pH etc.) to scale the algal biomass productivity at lab/commercial scale. Hence among various industrial wastewaters, dairy industry wastewater is selected, which shows compatibility with algal strain *C. pyrenoidosa* as a growth medium. RSM studies carried out for this thesis also supports that both algal growth and lipid production are directly or indirectly affected by two or more nutrients and environmental variable with real time studies as well.

Chapter 6: Conclusions and Future Recommendations

In view of the objectives decided for the, work has been completed successfully by their experimental and model (RSM) validation results. The experimental results were analysed with independent variables (Light, Temperature, DIWW, CO_2, Nutrients (N, P) and integration of each variable (Nutrient with DIWW but w/o LED; LEDs with DIWW, CO_2, Nutrient and Temperature) for algal biomass productivity with lipid contents in Phase-I for upstream process. Similarly, Phase-II was carried to scale-up the downstream process steps size harvesting and transesterification with low-cost materials as a catalyst/nano catalyst and compared their efficiency with chemical based catalysts. Whereas, Phase-III depicts the qualitative assessment of bio-oil derived from algal biomass, harvested from Phase-I and Phase-II on the basis of fuel quality index (FQI) and their techno-economic feasibility also had been studied. Though conclusions are given in the end of each chapter of the thesis, however, the major conclusion drawn from the thesis summarized as;

Uses of industrial wastewater with *C. pyrenoidosa* under optimized conditions (with and without stress) have a potential for biofuel production. But, the quantity of FAME in term of PUFA very much dependent on parameters (Environmental as well as process). Similarly, spectral variation with parameters also imparts a significant impact on FAME quality and quality both. Therefore, upstream and downstream processing steps needs a optimization for "system" working in efficient way with any type of algal strain with wastewater.

The work done in the thesis presents an answer (at least partially) to the problems associated with the clean and efficient production of biofuel from selected influencing parameters and their optimization for higher growth and lipid content. It also generated a new way forward for the future energy option and waste management approach (DIWW and waste material based catalyst) as to solve the problem of energy crisis and environmental pollution from the local to regional, regional to national and from national to global level.

6. Future recommendations

In the foreseeable future for algal based wastewater treatment and biofuel production, must be investigated for better treatment of wastewater and higher yields of biofuel production. Optimization of each parameter i.e. (environmental as well process) should be optimizing with the help of models and experimental setups. The research study should be carried out for investigation of mechanism of lipid accumulations due to climate change or stress conditions. Although, brief discussion on future recommendations are included in this chapter of thesis.