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

SUMMARY

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SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

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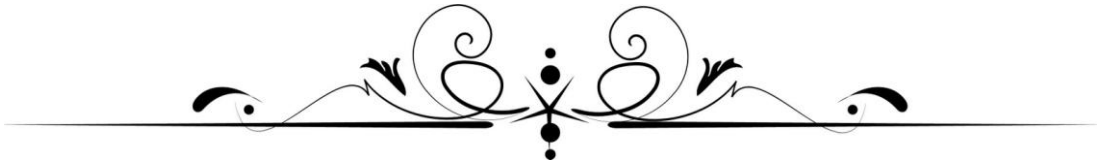
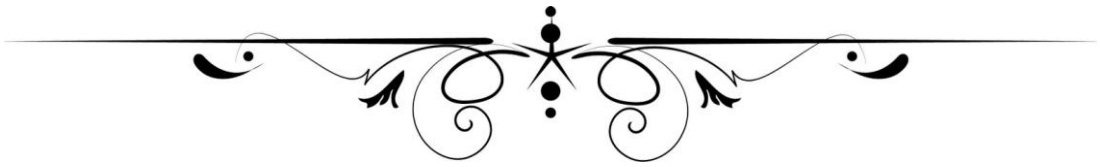


Dedicated to

My Beloved Parents



Declaration

This is to certify that the material embodies in the present work entitled "**Spectral conversion of light with different induced stress conditions to improve algal growth in wastewater for 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.

Shamshad Ahmad

Name and Signature

(Candidate)

Rishi Kothari

Countersigned

(Supervisor)

CERTIFICATE

This is to certify that the thesis titled **"Spectral conversion of light with different induced stress conditions to improve algal growth in wastewater for biofuel production"** submitted by **Mr. Shamshad Ahmad** 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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Date:

Place: Lucknow

Shamshad Ahmad

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ABBREVIATIONS

GIP	Green Industrial Policy
MW	Mega watt
GW	Giga Watt
MNRE	Ministry of New and Renewable Energy
AD	Anaerobic Digestion
LCFA	Long Chain Fatty Acids
TAG	Triglyceride
BBM	Bold Basal Medium
ATP	Adenosine Triphosphate
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PAR	Photosynthetic Active Radiation
PSII	Photosystem II
PPFD	Photosynthetic Photon Flux Density
RSM	Response Surface Methodology
NCIM	National Collection of Industrial Microorganisms
OD	Optical density
Arc GIS	Arc-Geographic Information System
CCD	Central Composite Design
LED	Light-Emitting Diode
FQI	Fuel Quality Index
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
PPA	Point Prediction Analysis
FTIR	Fourier-Transform Infrared Spectroscopy
SEM-	Scanning Electron Microscope
EDX	Energy Dispersive X-Ray spectroscopy
GC	Gas Chromatography
ABA	Absisic Acid
DIWW	Dairy industry wastewater
COD	Chemical Oxygen Demand
TN	Total Nitrogen
TP	Total Phosphate
RSQM	Response Surface Quadric Model
FFA	Free Fatty Acid
N.A	Not available
TIWW	Textile industry wastewater
NER	North-Eastern Region
BOO	Build Own Operate
MJ kg^{-1}	Mega Joule Per Kilogram
GC-FID	Gas Chromatography With Flame Ionized Detector
USFA	Unsaturated Fatty Acid
MUFA	Monosaturated Fatty Acid
PUFA	Polysaturated Fatty Acid
FAME	Fatty Acid Methyl Ester

Chapter 1

Introduction and Review of Literature

1. Introduction

The increased consumption of fossil fuels at present as well as its persistent demand that will continue in the future and reduction in available energy resources are the main problems needed to be solved for a sustainable world. If this situation is not resolved in time, it will be a threat for modern human existence. So, to fulfill the global energy demand of the world, the energy sector requires some bountiful alternative resources of energy.

This chapter is delineating the current energy scenario at global as well as in Indian context with respect to present resources and alternate resources explicitly for biomass based energy resources. Various generations of biofuels, specifically algal based biofuel generation and its influencing parameters which affect the upstream and downstream processing in general and the recent researches on this area are also included as a segment of this chapter. Due to the anthropogenic disturbances, there are imbalances in the parameters during real algal growth incidence which directly creates a stress on cell's physiology as an individual parameter as well as multivariable interaction. Few recent workers focused their work on such type of interactions, which is also included in this chapter as a part of review of literature with their findings. Recent reviews and experimental studies available on algal biomass harvesting and their use for biofuel production are also summarized with specific remarks which are helpful in framing the other chapters of this work.

1.1 Energy scenario

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 an environmental constituent. Every nation's economic development is very much dependent upon its energy capitals. Highly developed nation have a high rate of energy utilization in

comparison to 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 every individual in the world to live comfortably within their 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. Greenhouse gasses (GHGs) in the environment have already passed dangerously high levels (>450ppm CO_2) and are still rising due to global use of fossil fuels for transport and power generation. Such an enormous increase in CO_2 in the atmosphere will have disastrous environmental consequences like increase in the earth's temperature, rise in the sea level, acidification of ocean, melting of glaciers, extreme weather conditions, change in ecosystems, coral reef bleaching *etc.* (Geetanjali *et al.*,2014). If the current trend of increase in CO_2 levels will continue at this rate, it has been predicted that by the year 2050, the carbon dioxide concentration will reach 550 ppm in the atmosphere (Twine *et al.,* 2013). The major emitters of CO_2 are not only power, steel and cement plants but also transportation sectors like automobiles, ships and aeroplanes which primarily run on fossil fuels. Therefore, CO_2 sequestration has been found as one of the effective ways to reduce the carbon foot print in the atmosphere (Geetanjali *et al.,* 2014). Establishment of adequate energy is a necessary (but not satisfactory) mean to meet the overall energy challenge.

However, there is a proliferating peril posed by pollution as a consequence of fossil fuel burning. So, there arises an exigent requirement to elude the gratuitous use of pollution emancipating conventional energy sources and shift towards alternatives

energy resources which are environment friendly.

1.2. Energy crisis

Recently, with increasing oil prices, researchers, academicians and partners of commercial sector are focusing on energy alternatives to decrease the energy crisis as a whole. Depletion of natural energy resources at an alarming rate also aids in focusing on agreements among scientific communities and governments for sustainable economic security at global level. The population expansion and their demands are indirectly responsible for variations in the existent climatic conditions due to immense use of traditional fuel resources which lead to energy crisis. According to Coyle *et al.,* (2014) total global primary energy supply in 2010 was estimated as 12700 Mtoe (Million tonnes of oil equivalent), which will increase in year 2035 upto one hundred percent. Fig.1.1 clearly delineates the situation of conventional resources of fuel and their utilization as resources as per the present scenario.

The quantum of conventional energy resource utilization is consummate in advanced countries like China, Australia and other European states in spite of austere environmental coercions that cause global warming via greenhouse gas (GHGs) effect. The trend at which the global environment is changing rapidly is prodigious in the entire geological history. As a consequence of this change, the hostile conditions aroused like temperature elevation and ocean acidification due to GHGs making the existence of humans on earth challenging (Beardall *et al.,* 2009). The main components of the Green House Gases are CO_2, CH_4, and N_2O, *etc.* out of which CO_2 is the highest contributor at present (Chung *et al.,* 2011). The atmospheric carbon dioxide magnitude has amplified from 280ppm in 1750 during the pre-industrial period to 380ppm at present (Kierzkowska et *al.,* 2013).Sustainable growth of future

depends on the long-term availability of different source of energy which is affordable, accessible, and environmentally friendly (Oyedepo O, 2013).

In the starting decades of 21st century, the world faced lots of problem of growing energy consumption and diminishing supplies of fossil fuels (Foster *et al.*, 2017). In addition, the estimated reserves of tar sands and proven reserves of coal, oil and gas depleted at an exponential rate. The year 2082 mark the point that we will run out of fossil fuels as shown in (Fig.1.1) .There are about 5,570 billion tonnes of coal equivalent and 1330 million of oil reserve present in India.

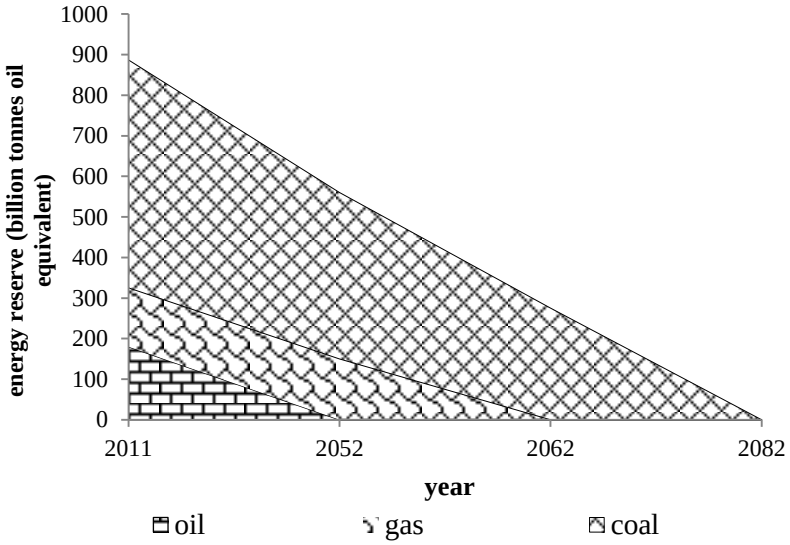


Fig 1.1: Future energy demand of conventional fuels (Sharmina *et al.*,2017)

1.3. Indian energy scenario

India, a country having the world's second largest population of 1.28 billion reckoning for more than 17.5% of the global population. From this 1.28 billion, 32% of the population is vested in urban region and the rest in rural (Khot, 2015). This developing country encounters an ominous challenge in furnishing the passable energy to its citizens at a judicious cost. The challenge of energy production and supply in India is of paramount importance (Garg, 2012).

1.3.1. Nonrenewable energy resources

The energy producing natural resources in India are disseminated haphazardly and arbitrarily focused in a few places only. The hydro energy resources are sited in two places i.e. the foothills of Himalayas and the north-eastern region (NER). States like Jharkhand, Orissa, West Bengal, Chhattisgarh and parts of Madhya Pradesh are intensified with the natural reserves of coal while Tamil Nadu and Gujarat have lignite natural reserves. An unexploited enormous hydro potential estimating about 35,000 MW in North Eastern Region, near about 15,000 MW in Bhutan and about 8,000 MW in Sikkim is located in these places (Sen and Srivastava,2015; Kumar *et al.*,2014). India's energy deployment and installed electricity capacity has amplified 16 and 84 times respectively from the last few decades. (Jain, 2015; Agarwal *et al.*, 2014; Khoiyangbam *et al.*, 2011). An increase from 967.150 BU during 2013-14 to 1048.673 BU during the year 2014-15 has been discerned in the complete energy generation of the country. The energy consumption of India was the 5^{th} highest in the world (Priyadarshani *et al.*, 2015). As a result of population explosion and economic advancement, the energy consumption by India has augmented alarmingly in the recent years even however the base ratio is rather truncated. The energy demand is expected to increase expressively because of the hasty urbanization and the upgraded living principles of millions of households in India and a projected economic growth of 8-9% per annum (Sahoo, 2016). The extensive apparition behind India's integrated energy policy is to meet the energy services demand of all the various sectors steadfastly. As a part of this integrated policy, a safe, clean and expedient energy at a very nominal cost will be provided to the impuissant households in every part of the country for meeting their energy needs. The goals of this policy would be achieved in ways that are viable economically, efficient technically and environmentally

sustainable by making use of both the conventional and non-conventional forms of energy and also by impending and incipient energy sources to corroborate energy disbursement at all time with approved assurance.

1.3.2. Renewable energy resources

India is facing acute energy scarceness which is obstructing its industrial evolution as well as economic development. Establishment of several new power plants is unavoidably highly dependent on import of fossil fuels which creates huge pollution. Therefore, it is necessary to mitigate this prevailing energy plight sensibly using the copious resources of renewable energy like wind, geothermal, solar and biomass energy (Mohtasham, 2015).

Apart from supplementing the energy, non-conventional resources will relief India in mitigating climate change. India is earnestly dependent on conventional energy resources for energy desires. Most of the power generation is carried out by fossil fuel based power plants which produces huge amount of GHGs. In India, per capita energy utilization is approximately 0.55 kW, which is lesser than that of developed nations $1/13^{th}$ of USA,$1/11^{th}$ Saudi,$1/9^{th}$ of Japan *etc*. Nevertheless, this amount is predicted to sharply upsurge due to exorbitant economic development and expeditious industrialization. In this viewpoint, renewable energy resources potentially reduce the dependency on fossil fuels. Keeping with global trend, Indian government is also pursuing Green Industrial Policy (GIP). In this policy, use of clean energy based power generation has rightly been prioritized for attaining the green growth goals for sustainable development (Alves *et al.,* 2017; Dhillon and Wuehlisch, 2013). Renewable energy resources have been recognized as potential alternatives to replace conventional energy sources, which include forms such as energy from sun, wind, biomass *etc*. Currently, India holds 6^{th} rank in the world with renewable based energy

generation for extensive economic efforts to achieve renewable based power generation targets in 12th five year plan (Owusu & Asumadu-Sarkodie,2016; Luthra *et al.*,2015) as given in Table.1.1. The total grid-interactive power (MW) achieved in the financial year of 2014-15 was 2104 MW against the target of 3770 MW (Yenneti, 2016). It is clearly depicted that wind is the largest sources of energy in the total share of energy in grid interactive power. Small hydro power comes next to wind power in terms of power generation. Waste to power development still needs momentum to grow, however it has huge potential for power generation in India (Mishra *et al.,* 2015).

The bioenergy sector of India was among the top markets at global level in 2013 with capacity addition of 0.4 GW of power (Salvo *et al.,* 2015). The total off grid power capacity of India is about 1123.32 MW in which biomass based energy dominates over the other energy sources with 30% share in total primary energy consumption (Chauhan and Saini, 2016). Gasifier based energy generation has successfully achieved a capacity of 18.23 MW in rural areas while 153.4 MW capacity in industrial sector (Kothari *et al.,* 2015). Thus, there is an enormous potential in bioenergy sector of India in comparison to other renewable and non-renewable sectors.

1.3.2.1. Biomass based energy: The need of the Hour

Amongst the several sources of energy like solar, hydroelectric, geothermal, wind and biofuels which are being used as potential energy sources, biomass based bioenergy are realized as authentic modes for attaining the aim of substituting fossil fuels (Khan *et al.*,2017).Biomass which is a carbon neutral source and the chief component responsible for greenhouse effect. Being the fourth largest energy source in the world after coal, petroleum and natural gas, it contributes >14% consumption (Yan, 2015).

Table.1.1: Renewable energy options for India: Available potential and future aspects.

Types of Renewable/ Sustainable energy forms in India	Available potential (GW)	Utilized (%)	Future prospects
Solar energy	700-2100	2.208	By the year 2022, 20 GW of solar power production has to be accomplished.
Wind energy	100	21.13	20,000 MW power installation has been anticipated by the Ministry of New and Renewable Energy (MNRE) by 2022
Hydro energy	150	39.78	By the end of the 12th Five Year Plan in 2017, the ministry had intended to expand its small hydro power capacity to 7 GW
Geothermal energy	10.6	0.009	Country's first geothermal power plant would be inaugurated in the Balrampur district of Chhattisgarh by its government.
Biomass energy	23	1.28	Though biodiesel is not being currently vended in India, however the country is proposing to fulfill 20% of diesel supplies of the country through biodiesel by 2020
Tidal energy	8	0.001	A 3.75 MW tidal power plant project has been endorsed by the ministry of MNRE.
Wave energy	40	40	On the basis of BOO (Build Own Operate), policies have been declared by the Government of Maharashtra as well as Government of India to draw attention of the private stockholders

Globally, biomass is being appraised as an imperative renewable energy resource. A wide variety of energy necessities are abated through biomass energy like electricity generation, vehicular fuel, process heat for industries etc. (Saxena *et al.,* 2009). Biomass gets its distinctiveness amongst all the renewable energy forms as it commendably reserves solar energy and is the sole exclusive carbon renewable

resource which could be easily transformed into solid, liquid and gaseous forms of fuel by disparate transformation procedures. The biomass based energy generated by the biochemical conversion includes biohydrogen, biodiesel, biogas and bioethanol, which are the possible biofuels of future generation as given in Fig.1.2 (Subramanian and Babu, 2013).

1.3.2.1.1 Biohydrogen

Biohydrogen is a sustainable substitute of hydrogen (H_2) gas. Bio-hydrogen designated as "Green Energy" has accrued appreciable contemplation in the recent years because of its sustainability and waste curtailing aspects. Extensive use of biohydrogen as a source of conventional energy could mitigate global climate change, enhance energy efficiency, and improve quality of air (Kothari *et al.,*2017) Production of Bio-H_2 can be understood by anaerobic as well as photosynthetic microbes using rich organic compound materials. Through the transmutation of organic substrate, Bio-H_2 is formed as the resulting by-product (Azbar, 2015). But in the photosynthetic processes, algae are also included which uses CO_2 and H_2O as substrate for production of hydrogen (Holladay *et al.,* 2009). The ascendancies of the previous procedures are higher production of H_2 as well as utilization of waste materials (Mehmeti *et al.,* 2018) for the bio-hydrogen production is given in Table1.2. However, the technological advancement of this particular process is required as the H_2 production rate is low. As the demand for cleaner energy resources has increased these days, the deployment of waste biomass for the generation of Bio-H_2 seems to be a unique and propitious strategy for meeting the rising energy demands and as safer alternatives to the conventional sources.

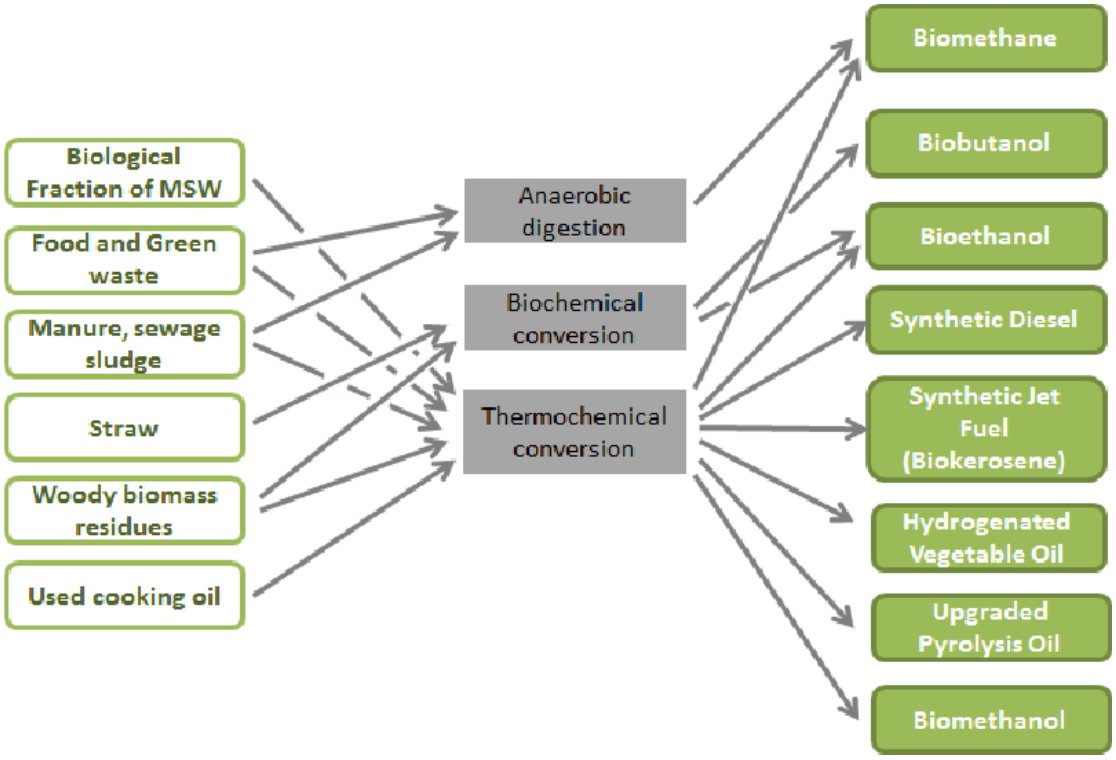


Fig 1.2: Biochemical conversion routes for the conversion of biomass into valuable products

1.3.2.1.2. Biogas

Biogas typically contains 50-75% methane (CH_4), 25-50% carbon dioxide (CO_2) minor amounts of other gases like nitrogen (N_2) and water vapor (Chen *et al.*, 2018). Biogas is formed through the disintegration of complex organic matter by making use of microorganisms in the anaerobic digestion (AD) process (Weiland *et al.*, 2010).This ability of biomass has been utilized by man-made systems (anaerobic digester) for a long time (Caroline,2017).

Table.1.2: Various routes for the conversion of biomass into Bio-hydrogen

Methods	Types of Algae	General reactions and comprehensive categorization of the used microorganisms	Advantages	Disadvantages	References
Direct biophotolysis	*Green algae* *Chlamydomonas* MGA 161 *Platymonas subcordiformis* *Chlamydomonas reinhardtii* CC-124	$2H_2O+Light=2H_2+O_2$	Harvest H_2 from water and sunlight directly. Require high intensity of light Solar conversion energy increased by ten-folds as compared to trees, crops	Require high intensity of light O_2 can be dangerous for the system	Azwar *et al.,* 2014 Guan *et al.,* 2004 Laurinavichene *et al.,* 2006 Chader *et al.,* 2009
Indirect biophotolysis	*Cyanobacteria* *Chlamydomonas* MGA 161 *Clostridium butyricum* GS2 *Escherichia coli,* DJT135 *Clostridium sp.* R1 *Bacillus coagulans* IIT-BT S1 *Clostridium butyricum* CWBI1009 *Citrobacter freundii* *Clostridium pasteurianum*	$6H_2O+6CO_2+light=C_2H_{12}O_6$ $C_2H_{12}O_6+2H_2O=4H_2+2CH_3COOH+2CO_2$ $2CH_3COOH+4H_2O+light=8H_2+4CO_2$	• Can produce H_2 from water • Has the ability to fix N_2 from the atmosphere	Lower photochemical efficiency uptake. Hydrogenase enzymes are to be removed to stop degradation of H_2. About 30% of O_2 is present in gas mixture. O_2 has an inhibitory effect on nitrogenase	Tamburic *et al.,*2010 Gadhamshetty *et al.,*2009 Ho *et al.,*2010 Kotay and Das,2010 Masset *et al.,*2010 Makinen *et al.,*2012 Cui *et al.,*2012 Lo *et al.,*2013
Dark fermentation	*Klebsielle oxytoca* HP1 *E. cloacae* IIT-BT 08	$C_2H_{12}O_6+6H_2O=12H_2+6CO_2$	It can produce H_2 all day long without light	Relatively lower achievable	Minnan *et al.,*2005 Kumar and Das,2000

	E. coli *Thermoanaerobacterium* *Clostridium sp.*		(ii) A diverse range of sources of carbon could be deployed as substrates (iii) It produces valuable metabolites such as lactic acid and butyric acid as reaction's by products (iv) It is an aerobic method; hence O_2 constraint is not an issue.	yields of H_2 CO_2 in gas mixture has to be separated Light conversion efficiency is very low, only 1 to 5 % O_2 is a strong inhibitor of hydrogenase	Podesta *et al.*,1997 Liu *et al.*,2003 Lay ,2001
Photo fermentation	*Rhodopseudomonas* *R. sphaeroides* RV *R. capsulata* *R. sphaeroides*	$2CH_2COOH+2H_2O+Light=4H_2+2CO_2$			Barbosa *et al.*,2001 Fascetti and Todini, 1995 Fang *et al.*,2005 Ero˘glu *et al.*,1999 Yokai *et al.*,2001
Hybrid reactor system (combined dark and photo-fermentation)	*C. buytricum, E. aerogenes, Rhodobacter* sp. M-19 *C. buytricum, E. aerogenes, Rhodobacter* sp. M-19 *Lactobacilus amylovorus, R. marinum* A-501 *C. buytricum, Rhodobacter* sp. M-19	**Stage1** $C_6H_{12}O_6+2H_2O=4H2+2CH_3COOH+2CO_2$ **Stage2** $2CH_3COOH+2H_2O+Light=4H_2+2CO_2$	Two stage fermentation can improve the overall yield of hydrogen		Khanal *et al.*,2004 Yakoi *et al.*,1998 Akkerman *et al.*,2002

It plays significant role in future sustainable energy scenario and it can be used as renewable source of electricity when needed and can be stored. Moist herbaceous plants (sugar cane, vegetables, corn, sugar beet, cotton, sorghum); some non-food crops, marine crops (macroalgae and microalgae) and waste manure are most suitable for biogas production (Baltrėnas & Misevičius, 2015). Chanakya *et al.* (2007) has explained that regions where biomass is preponderantly available, the plant derived biomass proves to be a persuasive cleaner energy source in India meant for cooking and other activities. Biogas is considered as an integral component of the several renewable energy programmes run by numerous developing nations (Azeem *et al.,* 2016).

1.3.2.1.3. Biodiesel

Biodiesel which comprise of long chain fatty acids (LCFA) is identified as non-conventional, non-petroleum based diesel. Biodiesel is procured from vegetable oil, animal fat or waste cooking oils by the process of transesterification. The edible oils essentially olive, soyabean, palm, sunflower, mustard and peanuts have been used as fuels for about 100 years. Depending on the preparatory complex organic material converted into simple form, biofuel could be divided into three generations as given in Fig.1.3.The agricultural crops used to produce simple sugars are subsequently converted into ethanol and used as 1st generation biofuels (Ullah *et al.,* 2014). Different nations search diverse categories of plant oils as alternatives of liquid fuels. For instance, palm oil in Southeast Asia and Indonesia, soya-bean oil in U.S, sunflower and rapeseed oil in Europe, and coconut oil in Philippines (Chaurasia *et al.,* 2016). The 2nd generation biofuels use endemic, perennially growing plants that requires no farming or whole grass flora for biofuel production (Sang and Zhu, 2011). The practice of photosynthetic algae biomass to produce biodiesel has often been

referred to as the 3rd generation biofuels.

1.4. Generation of biofuel

Biofuels are a renewable energy resource because they are rapidly replenished. On the other hand, fossil fuels are non-renewable since, they require millions of years to replenish. There are three types of biofuels: 1st, 2nd and 3rd generation biofuels. They are characterized by their sources of biomass, their limitations as a renewable energy source and their technological progress.

1.4.1. First generation biofuels

Edible crops are the main source of first generation biofuels. The biofuel is obtained from the various components of edible crops like the starch, sugar, and edible oil. Corn, wheat, and sugarcane are the most commonly used biofuel feed stocks in 1st generation. It is noteworthy that, as far as the generation is concerned, there are no modifications in the structural configuration of the biofuels, nonetheless, change in the biofuel genesis is observed between generations.

Biofuels obtained by transesterification and fermentation of crops is currently an established energy industry in countries like Argentina, Brazil, USA and the European Union (EU) (Timilsina & Zilberman, 2015). Biofuels of the first generation can be classified into bioethanol and biodiesel. The 1st generation biofuels poses both direct and indirect competition between crops for food or fuel. It also causes an indirect competition for food crops production as it is responsible for the transfiguration of forests into agricultural land (Gaudreau, 2015). Milner *et al.,* (2016) points out the issue of the competition of land use with explicit reference to USA and Brazil, the two leading biofuel producing countries. A slight influence is done on the prices of food through these agro-energy techniques.

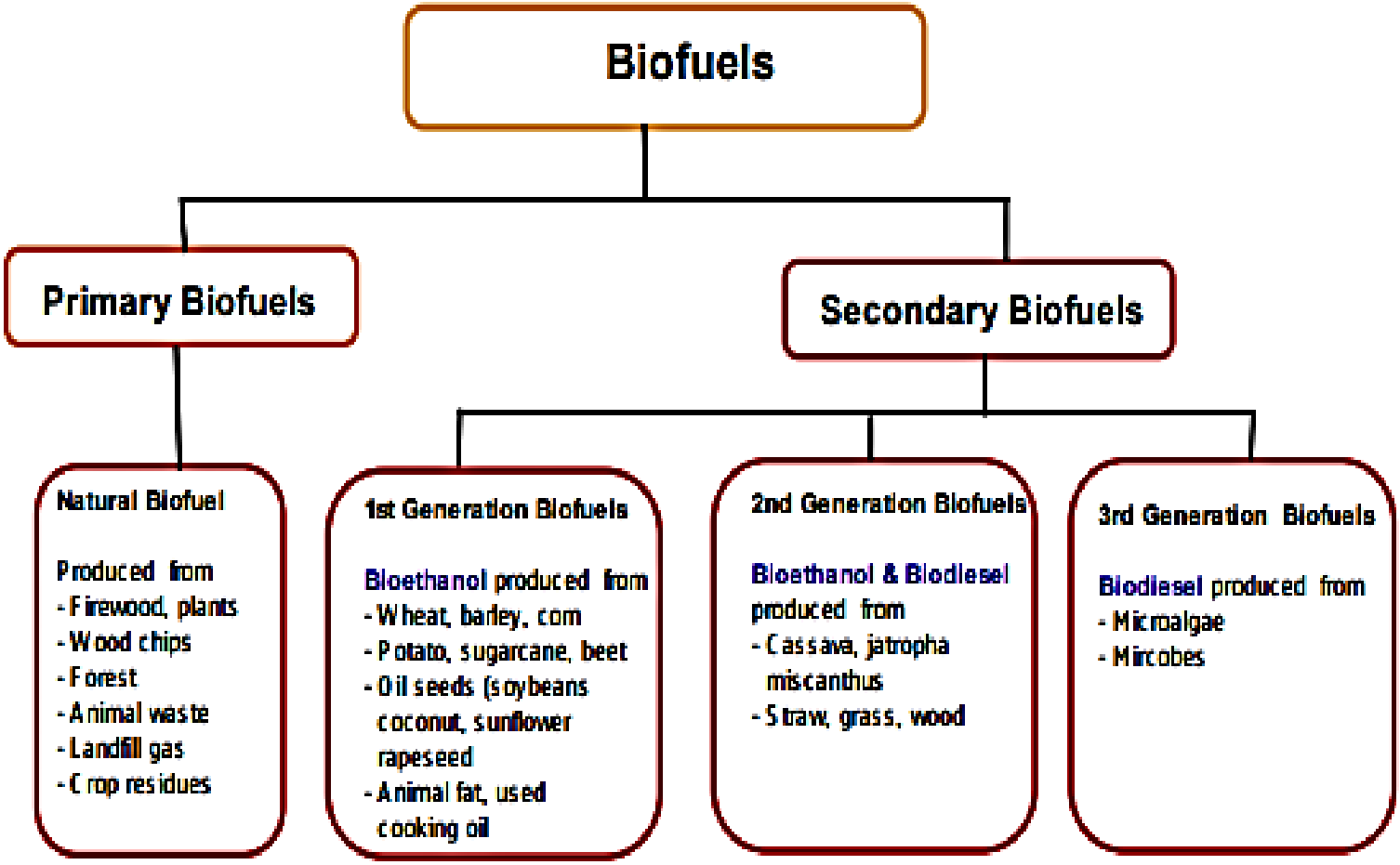


Fig.1.3: Generations of biofuel

No competition is observed with food when we take the instance of Brazil where bioethanol is produced from sugarcane (Milner *et al.,* 2016).Nevertheless, the environmental, economic and social complications caused by the land-use changes invigorated more arduous research in the area of biofuel. Facing a global challenge, crops requirement augmented in the fuel industry, particularly cereals reaching their top in 30 years (Sassi, 2013). The price hiking is egregious for the sub-Saharan African countries because 80% of their nutritive energy is emanated from the imported cereals (Konandreas, 2012). Rigorous researches to find the substitute of the contemporary sources and technologies has been done due this major quandary of price hiking.

1.4.2. Second generation biofuels

Research is in progress and efforts are being made to unfold techniques for utilizing the discarded non-food components like leaves, husks and stems. Moreover, cultivation of non-food crops like jatropha, euphorbia seed, mahua, and miscanthus is being emphasized (Kerckhoffs & Renquist, 2013).The regulatory policies and accomplishments are misleadingly using the terms such as sustainability and sustainable which have extensive meanings (Terano *et al.,* 2015). The economic, environmental and social sustainability objectives are very well achieved via the production process, whereas traditional sustainability relied solely on economic sustainability (Rinaldi, 2017). Sustainable biofuel should fulfill the following properties: (1) Cheaper than prevailing fossil fuels; (2) Causes a reduction in GHG emissions; (3) Doesn't cause environmental degradation; (4) provides employment opportunities thereby enhancing economic development; (5) strife with food crops is evaded. Though a challenging task, yet for the approximation of sustainability of the prevailing and the forthcoming generations of biofuel, a concrete and arduous

valuation device is a prerequisite. Second generation biofuels are said to be sustainable as they are derived from non-food biomass and crops and these don't compete with arable land (Chisti, 2007). They are environment friendly because zero fertilizer, less water and negligible pesticides input is entailed (Devarapalli & Atiyeh, 2015). Nevertheless, the sustainability of these crops is being questioned because of the land use changes ensued for the growth of such crops (Bryan *et al.,* 2016). Commercial production of the 2nd generation non-food crops (jatropha *etc.*) is done on fertile land which gives a direct competition to the land required for food production (Achten *et al.,* 2013).

There are no proper controls set for the estimation of these changes and the calculations are restricted to certain innumerable whimsical suppositions (Mathews and Tan, 2014). 2nd generation biofuels have low ability to satisfy the energy demand as they have lower conversion rates to fuel. To satisfy the ever increasing fuel need through second generation biofuel, vast areas should be brought under cultivation as specified in Table.1.3. The discrepancy in net energy balance (the total amount of output of biofuel energy versus input of fossil fuel energy) is prominently dependent upon the species of the plant, method of energy production and type of fuel *etc*. There is still a dilemma on the favorable influence of the biodiesel. There is a great dispute among arguments regarding the sustainability and suitability of these biofuels for a healthy ecosystem where Pimentel and Haq *et al.,* (2016) indicated that for corn production, an extra 29% fossil energy than the ethanol is compulsory. Solomon (2010) publicized that 25% additional energy could be harvested than the energy endowed for producing it. This slow conversion rate is because of the difficulty in converting cellulose to biofuel. The corn starch is effortlessly converted to ethanol by direct fermentation process (Devarapalli & Atiyeh, 2015).The problem with biofuel is

economic production limitation as the raw materials are distributed on marginal land as well as on farms leading to a dispersed collection.

1.4.3. Third generation biofuels

After extensive research works on first and second generation biofuels, various limitations and challenges are still persistent that limits to make it commercial like increased pressure on arable land use and water use by first generation biofuels whereas second generation biofuels very much limited to pretreatment of woody biomass, so these are not economically viable as specified in Table.1.3. Hence, a search of viable alternative energy resources at economic, environment and social scale needs to be resolved. Furthermore, algal biomass is considered to be the best alternative for biofuel option that has the potential to produce 15-300 times more oil for biodiesel production than traditional crops on an area basis, short harvesting cycles (~1-10 days for continuous mode and 10-15 days for batch mode).Use of fresh water can also be substituted with wastewater to be used as a nutrient media for growth of algal biomass. Microalgal biomass could be produced from huge open ponds or through contrived closed compartments, termed photobioreactors (PBR) and subsequently subjected to drying and processing for the production of bioethanol or biodiesel (Nguyen & Hoang, 2016). Species selection and conditions for microalgal growth (e.g. amount of sunlight, water, and nutrients) should be given cautiously, so that it helps to accelerate growth rates.

Table.1.3: An overview of existing and proposed liquid fuels from sustainability perspective

	Environmental	Social	Economic
1st generation	• Possible carbon neutrality Reforestation potential on marginal lands • Stripping of land and increased carbon arrears—outstanding 17yrs for Brazilian sugarcane biofuel while worst 423 yrs for palm oil from Indonesia and Malaysia • Monocultural farming leading to soil degradation	• Fulfilled the energy requirements besides triggering the economic development of the rural area • Increase employment, particularly in rural areas Dispute arises as foreign shareholders are greatly fascinated by weak land tenure security • Problems of poverty and hunger arises due to the conflict amid crops to be used as a fuel or food	• High biofuel blends can require engine modifications • Raised prices of food crops as growers switch to supplying bioethanol market. • Artificially inflates Prices for corn in US, reducing the need for government price support or export subsidies
2nd generation	• Waste/by-product stream eliminates the requirement for advanced inputs/resources e.g. bagasse and molasses from sugar production • Reduce waste production since recycling by-products • Carbon release is greatly cutailed (relying on land-use) • Refrain changes in land use practices and deforestation • Proliferation into forest zones have been reported	• Food versus fuel perplexity should be circumvented (in case of non-arable land usage). • Since waste streams are widely available especially for rural areas, hence dispersed energy systems are feasible • Energy requirements of the rural areas are fulfilled as well as rural economic growth are encouraged • Over-selling of yields creates frustration and suspicion e.g. Kenyan jatropha	• Rates of infrastructure are quite high for e.g. biogas capture/distribution, but can be viable at small scale • Dispersed nature of resource avoids monopoly domestic source of fuel can avoid expensive imports
3rd generation	• Ratio between energy and available land area id high, so avoid deforestation impacts and land tenure conflicts • Potential escape of invasive algae impacting waterways	• Doesn't obligates productive land hence no dispute with food-crops • Rural areas could be developed as there is a vast capacity to dispense a span of high-skill and low-skill occupations	A rigorous R&D should be done of the organisms as these reduce the amount and processing large-scale production necessary to be economical high plant costs and supporting infrastructure

Simple cellular structure of microalgae makes them an auspicious candidate for increased yields through emetic modification (Nguyen & Hoang, 2016).Oil palm produces 5950 L ha^{-1} yr^{-1} of oil while Jatropha produces 1892 L ha^{-1} yr^{-1} but, at the same time, algae yields 12,000 L ha^{-1} yr^{-1} having 30 wt % oil comparatively and volumetric productivity of algae is also high as given by some researchers in literature. It is expected that that 20.5% of arable land would be needed for algae production for meeting the oil demand worldwide. This estimate could be reduced to zero when non-arable land is used for positioning the huge open ponds and closed photobioreactors (Narala *et al.,* 2016). The global energy demand could be met, when for algal cultivation 1.5-2.7% of the entire non-arable land is used (Trentacoste *et al.*, 2015).

1.5. Algae: Sustainable alternative biomass

Algae are an assemblage of eukaryotic microorganisms whose adherents have a unique attribute of oxygenic photosynthesis. Algal species come in various sizes; they could be of a micron (µ) sized single cell, multicellular colonies or could be paradoxically of intricate spiny leafy structures. Surprisingly, they could have a length of less than 2 mm (picoplankton) or 50–60 m (giant kelp).In some cases, they could exhibit an unexpected fast growth reaching 60m in length (Rajkumar *et al.*, 2013).Fritsch (1935) has developed the algae categorization on the basis of farm and texture and recently, Bold and Wynne (1978) have given the auto-ecological classification. Among them, microalgae are the ones that have attracted the attention and interest of the researchers for bio-fuel industry and sustainable environment. The prokaryotic members of this group are the Cyanophyta and Prochlorophyta while the eukaryotic microalgae has been classified into nine divisions or sections, viz., (1) Chlorarachniophyta (2) Cryptophyta (3) Chlorophyta (4) Dinophyta (5) Euglenophyta

(6) Glaucophyta (7) Heterokontophyta (8) Haptophyta (9) Rhodophyta (Bajpai *et al.*,2013) Pigmentation, storage product structures, cell wall composition, life cycle (for eukaryotes) and the elementary cellular configuration are the basis of classification of algae by the phycologists. Considering pigmentation, algae are categorized into three major classes: (1) brown seaweed (*Phaeophyceae*) (2) green seaweed (*Chlorophyceae*) (3) Red seaweed (*Rhodophyceae*). Microalgae are primitive organisms having a simple unicellular structure as depicted in Fig.1.4.

The photosynthetic mechanism of microalgae is similar to land-based plants, but, because of their simple cellular structure, they have ample contact with water, CO_2 and other nutrients. The terrestrial plants and the microalgae exhibit analogous mechanism of photosynthesis, but the microalgae have more ample contact with CO_2, water and the nutrients as they remain inundated in aqueous environment because of their rustic cellular structure. Hence, the solar energy is proficiently converted into biomass by the microalgae than the plants (Ort *et al.,* 2011).Algal cell requires nutrients like as phosphorus, iron, nitrogen and occasionally silicon for its growth.So, the growth medium should have abundant amount of these inorganic elements. Many microalgae predominantly produces neutral lipids exclusively, triacylglycerols (TAG) and hydrocarbons (Maeda *et al.*, 2017), the raw matter for biofuels, under impelled environmental conditions. Currently, microalgae are being used commercially for the manufacturing of many products like nutrient supplements, nutraceuticals and moreover for the drug-screening and wastewater treatment.

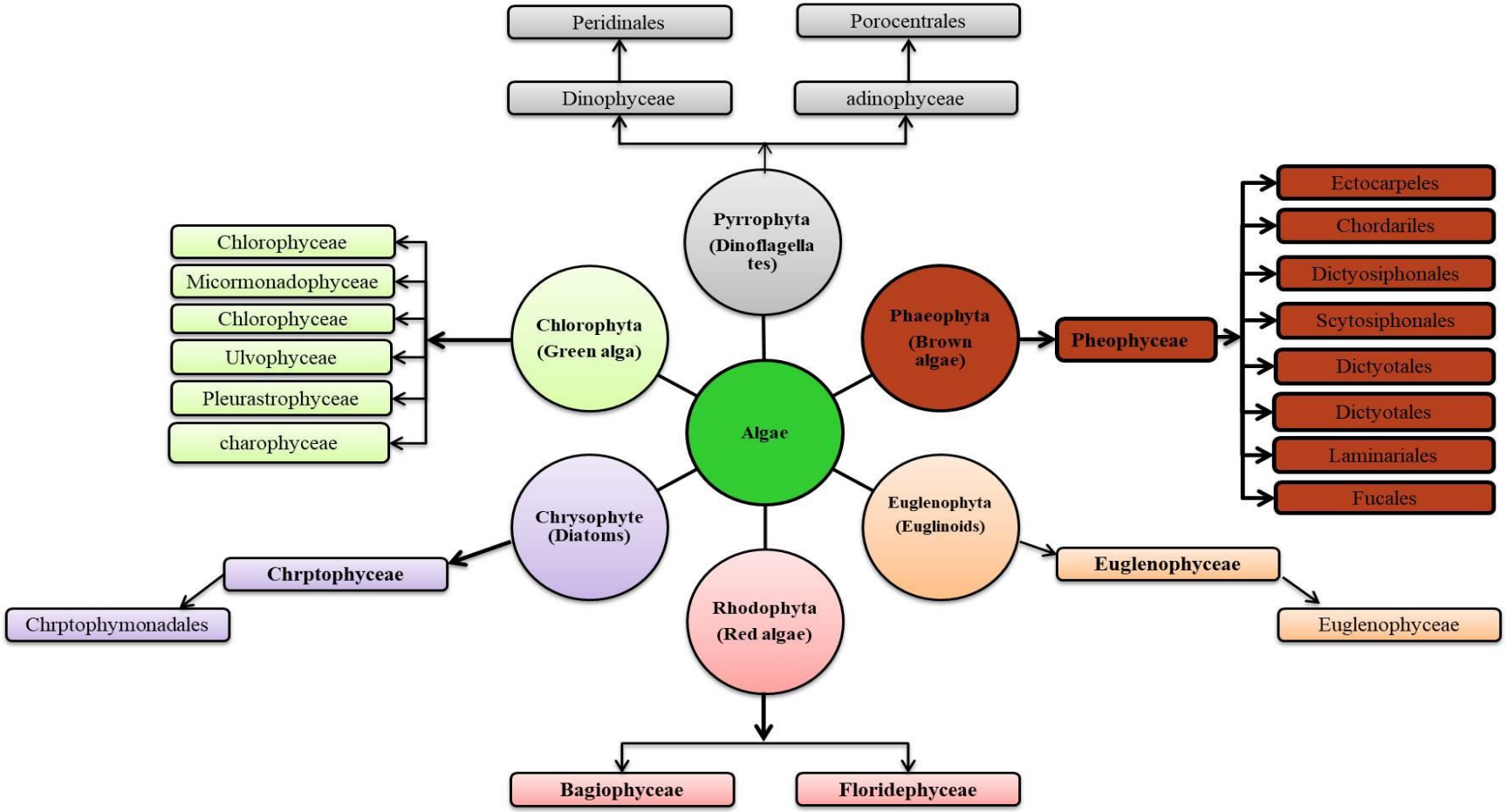


Fig1.4: Classification of algae (Fritsch 1935)

A variety of applications of microalgae prevail such as biofuel production (biodiesel, biomethane, biohydrogen), CO_2 mitigation and also for wastewater treatment. Compared to the terrestrial plants, microalgae, the indubitable diminutive biochemical factories, are more proficient in photosynthesizing (Randrianarison and Ashraf, 2017) and fixing CO_2 effectively (Cuellar-Bermudez *et al.*, 2015).

For screening purposes, an algal strain is firstly isolated, then purified and subsequently grown under sterilized environments in the laboratory. An ideal strain should exhibit certain remarkable characteristics like lipid content and productivity should be high,a hasty growth rate, excess TAG fatty acid profile, could endure an extensive range of pH, temperatures and salinities, could efficiently extract CO_2 from effluent sources and contrive sustainable byproducts (Gong and Jiang, 2011).

1.5.1. Growth parameters

The most influencing parameters regulating algal growth for higher yield of biomass are nutrient quality and quantity, light, pH, turbulence, salinity and temperature. Nutrient quality and quantity depends upon the culture medium, optimal parameter ranges are species specific and they all are interdependent to each other (Fig.1.5). Optimal range requirements for each parameter with their specific remarks, in general, are documented in the upcoming sections (1.5.1.1-1.5.1.6) of this chapter as;

1.5.1.1. Nutrients

Biodiesel production from microalgae is lucid as well as intricate.The basic requirement for survival is only water (H_2O), carbon dioxide (CO_2) and sunlight. For biodiesel production, consideracble amount of microalgae is required which can be obtained by providing sufficient amount of nutrients. Carbon dioxide (CO_2) needed for it's growth is provided by the air.Chemical media is also required for enhancing the growth of algal culture. Large scale algal concentrations are not possible without

any media or proper nutritional support. The macronutrients like nitrogen (N) and phosphorus (P) are the prerequisites for the growth of algae. Besides this, they also require some other nutrients like potassium (K), magnesium (Mg), calcium (Ca), Copper (Cu), Manganese (Mn), Sulfur (S) and Zinc (Zn). Nitrogen plays a vital role for an effective and efficient protein, carbohydrate and lipid metabolism and in its shortage, the division of cell is slackened and a product diversion from protein to lipids is observed. Iron (Fe) is an obligatory element for a pertinent photosynthesis. Cells lose chlorophyll in case of Mg deficit. In the absence of S, cells become incompetent to divide (Gikonyo, 2013). For media preparation of marine algae, salt like sodium chloride is (NaCl) is also an imperative aspect. Certain prevalent media compositions are enlisted in Table.1.4.

However, the environmental circumstances and algal nutrient composition could chiefly affect the N content in the algae (Wells *et al.,* 2016). For a good and maximal algal growth, the elements (silicon for diatoms) should be provided in abundance for an uninterrupted growth. Medium should be appended with some salts like potassium, in case of usage of saline groundwater for growth of algae. Maximum wastewater types have been tested for the culture of algae.The microalgal cells consume N and P for its growth and this is how the N and P are removed (Su *et al.,* 2011).The degradation of nutrients is also performed by the microorganisms on the condition, that they are present in the microalgal culture Through the process of nitrification and denitrification, the degradation of ammonia, nitrate and nitrite could result with the aid of few distinct bacteria (Zhu *et al.,* 2011).

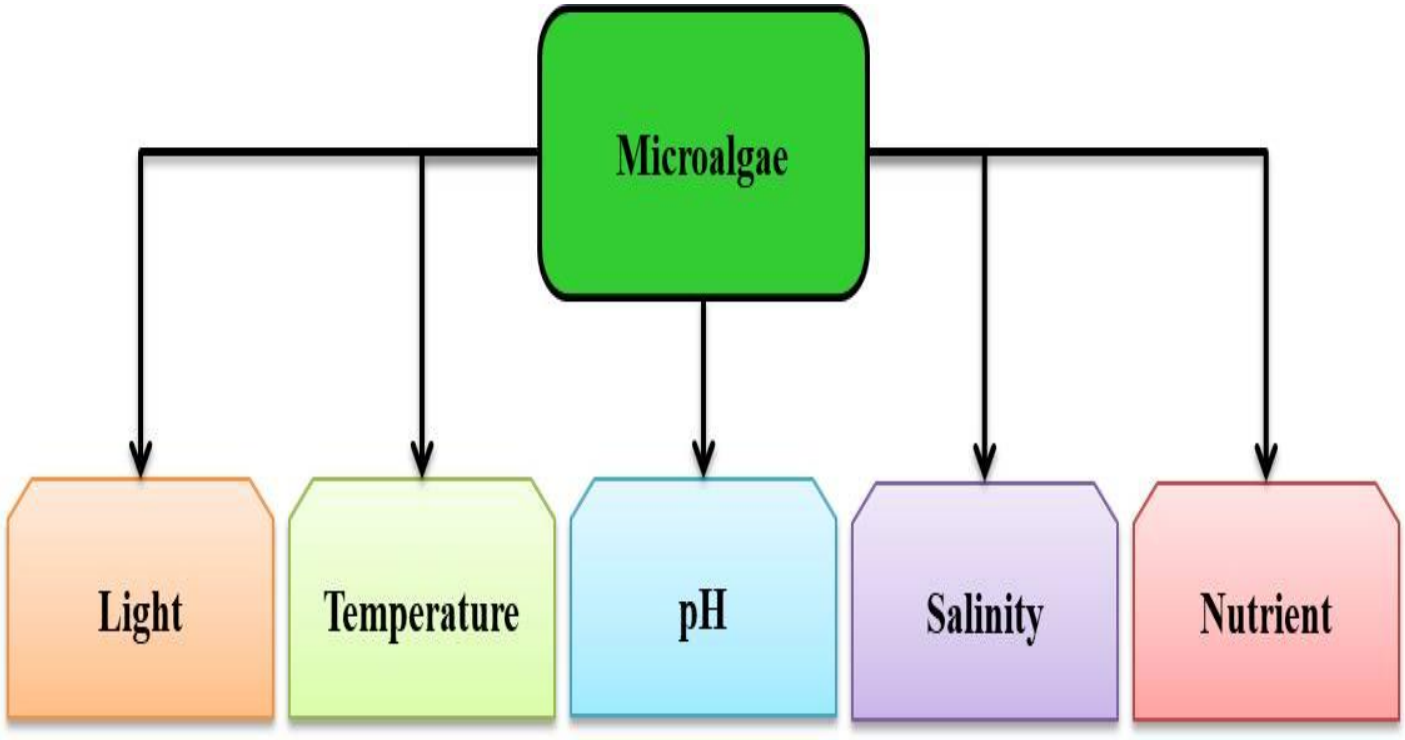


Fig.1.5: Factors affecting algal growth

Table 1.4:Compostion of media used for algae (gL^{-1})

Composition	BBM	BG11	Modified chu1	Basal
KNO_3	-	-	200	100
$NaNO_3$	250	1.5	-	-
K_2HPO_4	74	4	40	-
KH_2PO_4	17.4	-	-	-
$CACl_2.2H_2O$	24	3.6	80	-
$MgSO_4.7H_2O$	73	7.5	100	40
Na_2CO_3	-	2.0	-	-
NaCl	25	-	-	-
$FeSO_4$	5	-	-	-
EDTA	45	-	-	-
Citric Acid	-	0.6	100	-
Ammonium Ferric Citrate	-	0.6	-	-
$Ca(NO_3)_2.4H_2O$	-	-	10	150
BETA-Na_2.Glycerophosphate	-	-	-	50
EDTA-Na_2	-	1.0	-	2.71
Vitamin B_{12}	-	-	-	0.0001
Biotin	-	-	-	0.0001
Thymine-HCl	-	-	-	0.01
H_3BO_3	-	2.86	-	-
$MnCl_2.4H_2O$	-	1.81	-	0.108
$ZnSO_4.7H_2O$	-	0.22	-	0.066
$Na2MoO_4.2H_2O$	-	0.39	-	0.0075
$CuSO_4.5H_2O$	-	0.08	-	-
$Co(NO_3)_2.6H_2O$	-	0.05	-	-
$FeCl_3.6H_2O$	-	-	-	5.888
$CoCl_2.6H_2O$	-	-	-	0.012
Tris-aminomethane	-	-	-	500

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 (Kabir *et al.,* 2017). Through microbial actions too, phosphate could be despoiled to a great extent (Zhu, 2014). Moreover, P could be removed through precipitation once the pH of microalgal culture upsurges.

The concentration of lipids increases as a result of nitrogen deficit. But, at the same time, biomass production is hampered as a result of lipid accumulation. In case of Nitrogen deficiency, sunlight, a limiting factor, is to be augmented so that the biomass could be retained (Juneja *et al.,* 2013). Algal cells develop a deficiency of protein but a boosting of carbohydrate is seen as nitrogen is required for protein rather than carbohydrates. In case of macronutrient deficiency, photosynthesis occurs at a slower rate as stated in Table 1.5. Commercial grade fertilizers envisioned for traditional crops could be used as a source of nutrient supply instead of using media. The discarded biomass may be used as a fertilizer after oil extraction (Benemann *et al.,* 2012).Cassava is a media source for algal culture. The addition of nutrient is considerably reduced while using wastewater (high in N and P) to feed the algae (Neves *et al.,* 2016).

1.5.1.2. Water

For the production of algal biomass, huge amount of water is mandatory for growth as well as for the conversion of biomass into biofuel (Efroymson & Dale, 2015). Notwithstanding climatic conditions, algal species grown in raceway ponds and closed photobioreactors has enormous water availability (Rastegary *et al.,* 2013).

Table.1.5:Role of nutrients (organic and inorganic) for biochemistry of algal growth

Nutrients	Effect on algal growth	References
Carbon (C)	Indispensible constituent for the growth of microalgae. Besides the growth, respiration rates are also stimulated due to glucose present in heterotrophic environments. Nevertheless, growth rate is reduced as concentration of C is enhanced and obstructed if specific limit is crossed.	Hsia *et al.*,2015
Nitrogen (N)	The chief nutrient vital for microalgae growth. Many enzymes have been activated for oxygen generation due to the presence of some vital nitrogenous compounds like ammonia, nitrates and nitrites.	Peccia *et al.*,2013
Phosphorus (P)	Crucial feature for 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.	Singh *et al.*,2018
Magnesium (Mg)	Employed for activation of enzymes. Central atom in chlorophyll. The genetic material like DNA and RNA are created with its aid.	Hanifzadeh *et al.*,2018
Potassium (K)	The osmoregulation, synthesis of proteins as well as ion exchange in cell growth membranes are achieved by this.	Iyer *et al.*,2015
Zinc (Zn)	Carboxylases are stimulated for the production of fatty acids.	Djearamane *et al.*,2018
Iron (Fe)	Chief feature for the synthesis of proteins like ferredoxin and cytochrome. Microalgal cultivation is curtailed by its high concentration.	Rizwan *et al.*,2017
Molybdenum (Mo)	Integral part of the Nitrogenase as well as nitrate reductase enzymes. Absorption of nitrogen is curbed by its deficit.	Juneja *et al.*,2013
Chromium (Cr)	Inhibitor of Photosynthesis i.e., Cell machinery accountable for liberation of oxygen is hampered by high concentration.	Ouyang *et al.*, 2012
Copper (Cu)	Besides the biomass harvest, oil content is also enriched.	Juneja *et al.*,2013

Subsequently, freshwater sources are restricted in regions where productivity of algae is maximum (i.e. expanses of intense solar radiation throughout the year). Saline water is also essential for growth of algal culture. Culture in freshwater source is not possible in almost all situations as the supply of freshwater is limited. Algal growth would be affected temporally when the fatalities through evaporation would be substituted by the open pond salinity. A fractional ('blow-down') or comprehensive emancipation of the open pond water should be done when the endurance range of algae is narrow; same as many marine algal forms. Fortunately, algae have the potential to growth both in fresh water as well as in wastewater including seawater. Similarly, water and nutrients are the most critical variables for algal biomass production. Various researchers are focusing on this and trying to make it sustainable and environmental friendly, with a synergistic solution and integrated approach for algal production by the treatment of nutrient rich wastewater (Kothari et al.,2018 & 2017; Pathak *et al.*,2015).

1.5.1.3. Light

Light is the source of energy, like other plants, for algal photosynthesis. Light intensity, spectral quality and photoperiod are the important variables for algal growth. Light intensity (high/low) requirements are very specific to the type of culture/strain used. This holds virtuous as the light energy could be made easily accessible through sunlight. However, it becomes rather challenging at times for exploiting the energy from biomass fabrication as there is an exertion of exposing the algal culture to a sufficient amount of the light energy as depicted in Fig.1.6. For microalgal growth, sun is the quintessential 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. The photosystems receives a good amount of the captivated light energy by pigment–protein antenna complexes. There is a separation of charge by the red photon's energy and consequently, quinones become the receiver of the liberated electrons in the Photosystem II (PSII).Like all terrestrial plants, algae photosynthesize and transform gaseous CO_2 into organic substance, specifically carbohydrate by procuring energy from sunlight (Fig.1.7). Because of the primary pigments, chlorophyll a and b, algal growth is enhanced in blue and red lights as these pigments are subtle to such wavelengths and used for light harvesting (Wu *et al.,* 2016). The cells of *Chlorella vulgaris* showed a light absorption in the range of 400-500 nm (blue) besides 625-675 nm (red) however the remaining solar light was dissipated in between the algal cells (Wang *et al.,*2015). Similarly, it was found that the volume of *Scenedesmus obliquus* cells enlarged and the nuclear splitting eventuated expeditiously in red light (625-675 nm) (Přibyl, 2013). The autotrophic microalgae in the photosynthetic process, transforms the carbon dioxide of the air into organic compounds by harnessing the visible light which is the leading energy source (Carvalho *et al.,* 2011). With the aid of various microalgal pigments like phycobilins, carotenoids, in addition, to the chlorophylls could be engrossed in the array of visible light which is depicted in Table.1.6.Artificial light in photoautotrophic and mixotrophic microalgae is also explained. . In the fabrication of microalgae, solar light is regarded as a lucrative source of energy. Nonetheless, use of biomass as feedstock for prominent value yields like food or feed supplements (e.g., carotenoids and n-3 polyunsaturated fatty acids) or nutraceuticals.

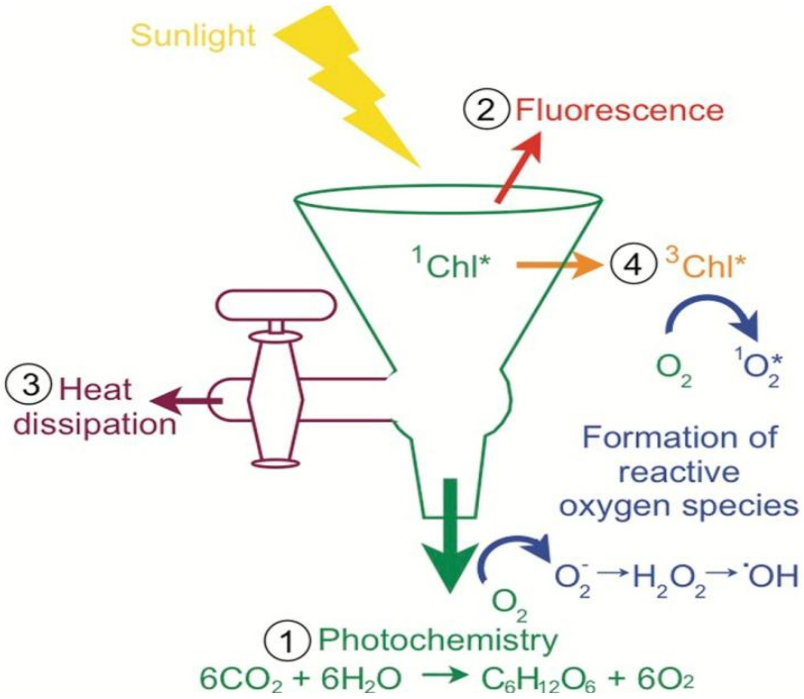


Fig.1.6: Light and wavelength distribution in chlorophyll for product formation

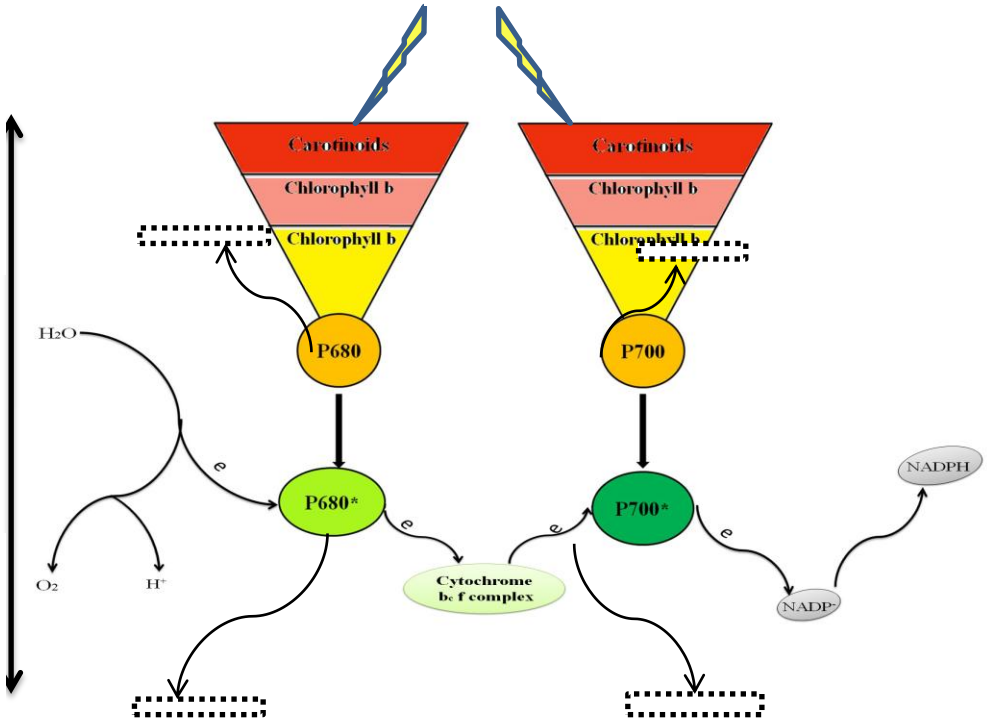


Fig.1.7: Photosynthetic funneling of light-induced excitations from the antenna complex to the reaction center

The yield and superiority of the biomass are the two regulatory features when the achievement of any industrial or agricultural merchandise is to be determined. The

photosynthetic photon flux density (PPFD), photoperiod, and light spectral regulation could be very well synchronized comparatively for the microalgal production by the artificial light and this would help in achieving the aforementioned features. A restricted proportion of sunlight is available during the daytime and the sunlight aggregate is quite capricious (cloud cover). It is such that the available sunlight for photosynthesis is present only in adequate concentrations for a limited duration in the day as well as year and at times energy is not present in the accurate wavelength. In the course of winter months, certain world regions receive scanty sunlight thereby necessitating for the instigation of an alternative source of light, that could be manipulated for uninterrupted algal production. Table 1.7 summarized the various sources of light according to availability, naturally as well as artificially, and their specificity in terms of algal growth.

1.5.1.4. Temperature

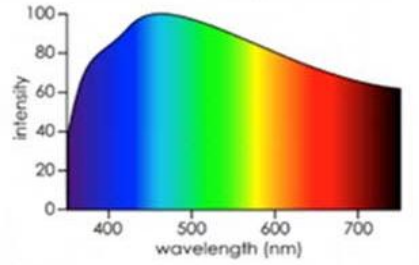
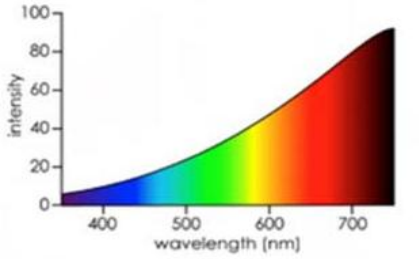
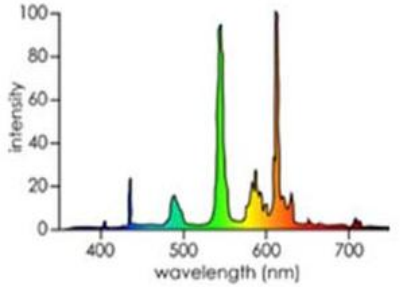
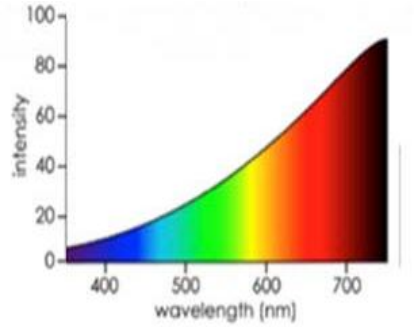
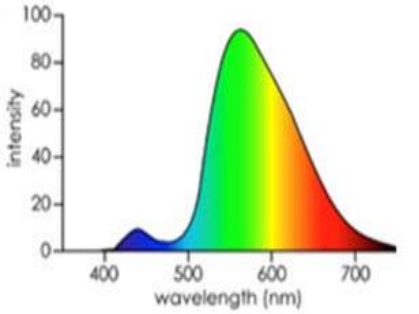
For algal growth, temperature proves to be a strong influencing parameter. Temperature alone determines nitrogen uptake, CO_2 fixation, organization of the cells thereby persuading the rate of growth of every algal species. It's a universal fact that, an increase in temperature registers an increase in the growth rate of algae till it reaches the optimum range. After reaching the optimal value, the growth rate shows a brisk decline as a result of increasing temperature as observed and specified in Table 1.8.

A positive correlation is seen between temperature augmentation and photosynthesis as well as cell division beneath optimum growth temperatures.

Table.1.6:Photonic features of major spectrums with detailed spectral pattern of visible light

Pigment group	Color	Range of absorption bands(nm)	Pigments
Chlorophylls	Green	450-675	Chlorophyll a,b,c_1,c_2,d
Phycobilins	Blue Red	500-650	Phycocyanin, Phycoerythrin,
Carotenoids	Yellow, orange	400-550	β-carotene, α-carotene, Violaxanthin, Fucoxanthin

Table.1.7:Comparison between natural and artificial sources of light and their specific properties

Light source	Spectral pattern of light	Specific Properties	References
Day light		• Sun is the only source of light accessible in adequate amounts to carry out photosynthesis for the period of certain times of the year • Required amount of energy is not in the veracious wavelength	Arnon, (1971).
Incandescent light		• Incandescent light include bulbs, as per data available artificial light from bulbs, specially use for algal growth have very challenging due to heat energy production and very difficult to concentrate at one point as required	Yeh, & Chung,2009
Cool white florescent lights		• Fluorescent lamps exacerbate temporally thereby producing less powerful light. Normally, the lifespan of 10000 hours has been shown in this Fluorescent (roughly an year).	McConnell et al.,2002
Halogens		• Halogen light as well as the incandescent lamps share the analogous spectrum therefore, has a truncated competence for the purpose of photosynthesis	Hogewoning, 2010
Light emitting diodes (LED)		• Most of the radiated light would be concentrated into the PBR as LEDs are very directional. • The lifespan of LEDs is longer than the traditional light sources. It curtails the cost comparative with the traditional light sources for prolonged time period. • Entire red LEDs have been used in this particular experiment so there is diminutive light produced in the wavelength range of 400-500nm.	Ouzounis *et al.,* 2013

The tendency is elucidated by the augmentation of enzymatic accomplishments associated to the Calvin cycle (Michelet *et al.,* 2013).A comprehensive study as well as modeling has been done for the interconnection amongst the rate of growth and below optimum temperature (Ras *et al.,* 2013). By making use of the Arrhenius function, temperature coefficient Q_{10} (growth rate escalation by a with a 10ºC upsurge in the temperature, rate of growth escalates) is frequently used as a factor and is anticipated that it gives a value nearby 2. Till the increment in temperature becomes hostile for algal growth, photosynthetic activity, division of cells and growth duple with every 10ºC rise in temperature. Microalgal growth rate shows an abrupt fall when temperature required for growth surpasses the optimal temperature. This abrupt decline in microalgal growth is due to the heat stress that distresses the enzymatic functions (inactivation, denaturation) and proteins are amended that are entailed in the processes of photosynthesis thus deterring growth (Mohamad *et al.*,2017). When a graph is plotted for growth of microalgae alongside temperature, a bell-shaped curve is obtained. The optimal growth temperature for microalgal cultivation generally ranges between 20-24°C, although this may vary with the composition of the culture medium and culture. The effect of temperature on the phosphorous amount of the wastewater was also studied using algae and it was found that, at elevated temperatures say 25 °C, the phosphorous amount was comparatively advanced than at lower temperatures.(Pasaribu *et al.*,2016). The temperature (0-22°C) influence on the acceptance of metals like As, Cd, Cu, La, W and Zn was also studied and it was revealed that arsenic, tungsten, zinc and cadmium uptake amplified along with the increment of temperature as studied experimentally (Cassidy, 2011).

Table.1.8:Effect of temperature on the growth of algae

Strain	Temp (ºC)	Growth rate (gL^{-1})	Specific growth rate ($g\ L^{-1}d^{-1}$)	References
Enteromorpha intesinalis	20±1	0.273		Sousa *et al.*,2007
Botryococcus strain SK	25±1	-	0.135	Yeesang *et al.*,2011
Botryococcus strain TRG	25±1	-	0.182	
Botryococcus strain PSU	25±1	-	0.061	
Botryococcus strain KB	25±1	-	0.0223	
B. braunii IPE 001 B	25	-	0.15	Yoshimura *et al.*,2013
B. braunii Yayoi B	25	-	0.2	
B. braunii 765	25	-	0.13	
B. braunii KMITL 2	25	-	0.1	
B. braunii UC 58	25	-	0.42	
N. oleoabundans	25	-	1.74	Loera-Quezada *et al.*,2011
H. pluvialis UTEX 2505	27	-	-	Evens Terence *et al.*,2008
P. donghaiense	27	-	0.77	Yamaguchi *et al.*,2010
C. ovata CO2	30	1.21	-	
C. ovata CO3	25	1.11	-	
C. ovata CO8	30	1.47	-	
N. thermalis	22±1	0.5	-	Mercado *et al.*,2004
N. incerta	22±1	0.3	-	
P. reticulatum	22±1	0.11	-	Roder *et al.*,2012
S. minutum	30	-	-	Bouterfas *et al.*,2012
C. microporum	30	-	1.55	
C. subprotumidum	30	-	1.59	
P. globosa	18±1	1.17	0.88	Shuxia *et al.*,2011

1.5.1.5. pH

For the growth of microalgae along with the production of biofuel, pH is considered as an imperative environmental factor. The optimal pH lies in the range of 7 and 9, for maximum cultivated species of microalgae (Ho *et al.*, 2011).The features of microalgal biochemical reaction often determines the pH of the cultivated medium. The whole algal culture may subside, if the optimum conditions of pH are not given because all the cellular processes may get disturbed by much raised pH. As per the findings of Liu *et al.*, (2007), *C. marina* recorded a normal growth in the pH range of 7.5-8.5, however, when pH reached further to pH 9, microalgal growth was substantially decremented. For microalgal and cyobacterial growth, the optimum pH ranges are prominently dependent on species type.

1.5.1.6. Bioreactor

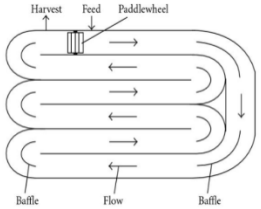
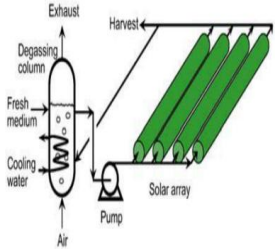
The cultivation system can vary depending on the product and strain. Various biomass-based products used in energy recovery include biodiesel, bioethanol, biobutanol, biohydrogen and biogas. As in conventional heterotrophic cultivations, for microalgal Biotechnology, high volumetric productivities are required to reduce the size of cultivation systems and consequently reduce production and downstream processing costs. This entailed a high efficiency of light utilization besides high biomass concentrations because light energy is the growth limiting substrate. The basic idea of using sunlight to produce high-value compounds brings along several limitations, which are related to light regime inside the cultivation system and have to be considered in the design and scaling up of photobioreactor. 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. Hence, to achieve the maximum viability of

CPBRs at the commercial scale, several researchers 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.A tubular PBR may be the best algal culture system in cooler climatic settings where there are no worries of overheating (Kimberly *et al.,* 2013). In a tropical environment, raceways or open ponds may be more suitable so that large amount of the water requirement is met. A hybrid reactor is made by combining a PBR and a raceway (Narala *et al.,* 2016). In the morning hours, culturing of algae could be done in the PBR and by afternoon, they could be moved to the raceway. Yet again, the culture could come back to the PBR in the evening hours. For diverse segments of the life cycle, a PBR as well as an open pond could be used simultaneously (Shamshad et al., 2017). Various types of bioreactors are available for culturing of algal biomass. According to review of literatures and according to working conditions.open and closed type bioreactors are used for most of the experimental studies. Table.1.9 recapitulates the benefits and shortcoming besides a few good features of all photobioreactors varieties.

1.5.2. Factors affecting lipid productivity for value added products

Generally, algal biomass processing for various value added products is divided into upstream and downstream sections. Upstream section totally depends on the type of selected algal strain with parameters, whereas, downstream processing involves harvesting through various techniques like centrifugation, filtration, flocculation etc. This process totally depends upon the type of the end product we are planning for value added products and end products. If we are moving towards bioenergy option, using algal biomass as a substrate, then extraction of lipids and their conversion to fuel also becomes the part of downstream processing

Table 1.9:Comparison of various types of reactors used to culture microalgae on a large scale

System	Reactor design	Algae	Max growth	Advantages	Limitation	References
Raceway pond		*Phormidium valderia*	30 mg L^{-1} d^{-1}	Relatively cheap Easy to clean	Poor biomass productivity	Dinesh *et al.*,2017
		Spriulina platensis	0.18 g L^{-1}	Utilization of non-arable land	Large area of land required	Brennanand,2010
		Scenedesmus sp.	17 g m^{-2} d^{-1}			De-Godos *et al.*,2014
		Chlorella vulgaris	0.35 g m^{-2} d^{-1}	Low energy inputs	Limited to a few strain of algae	Li *et al.*, 2013
		Tetraselmis MUR 233	29.6 g m^{-2} d^{-1}	Easy to maintain Good for mass cultivation	Difficulty in growing algal cultures for long ponds	Sing *et al.*,2014
		Botryococcus sp.	0.1 g m^{-2} d^{-1}			Rao *et al.*,2012
		Scenedesmus sp.	0.16 g m^{-2} d^{-1}			Ketheesan, 2011
		Dictyosphaerium sp	N.A			Gressel *et al.*,2013
Tubular photobioreactor		*Arthospira plantesis SPGG*	7.18gL^{-1}	Large illuminating surface area	Some degree of wall growth	Watanabe & Hall, 1995
		Arthospira sp.	47.718 mg $m^{-2}d^{-1}$	Suitable for outdoor cultures	Fouling	Watanabe & hall, 1995
		Arthospira platensis	30. mg $m^{-2}d^{-1}$		Require large space	Watanabe & hall, 1995
		Spirulina sp.	0.22 gL^{-1}	comparatively cheap	Gradiants up pH ,dissolved oxygen (DO_2) and CO_2 along with the tube	Aslam & Mughal,2016
		Chlorella sorokiniana	0.6 g m^{-2} d^{-1}	excellent biomass productivities		Solovchenko *et al.*,2014

Flat plate collector		*Arthospira platensis*	55-60tonn/hec/y	High biomass productivities	Improvement require in many compartments and supporting materials	Provost *et al.*,2012
		Anabaena azollae	4-7 gL^{-1}			Tredici *et al.*,1991
		C. pyrenoidosa FACB-9	0.63 g m^{-2} d^{-1}	Low oxygen build up		Tan *et al.*,2014
				Good light path		
		Chlorella kessleri	N.A	Relatively cheap	Difficult temperature control	Caporgno *et al.*,2015
		Chlorella sp	34.6 g m^{-2} d^{-1}	Large illumination surface area		Hu *et al.*,2013
		Chlorella vulgaris FC-16	2.97 g m^{-2} d^{-1}		Small degree of hydrodynamic stress	Choi *et al.*,2015
				Suitable for outdoor culture		
		Oocystis sp.,	0.111 g m^{-2} d^{-1}		Wall growth	Riano *et al.*,2011
Column photobioreactor		*Limnothrix redekei*	0.5gL^{-1}	High biomass transfer	Small illumination area	Mundt *et al.*,2001
		Synechococcus elongata	212 mg L^{-1} d^{-1}	Require Less energy consumption	Shear stress	Gao *et al.*,2012
		Arthospira sp.	0.22 g L^{-1} d^{-1}	Good mixing with low shear stress	Construction is sophisticated	De-Morais & costa 2007
		Phormidium sp.	N.A	Easy to disinfect	Decrese of illumination surface area upon scale-up	Cheng *et al.*,2017
		Sprirulina sp.	0.45 gL^{-1}	Reduced photoinhibition and photo oxidation		Ruiz-Martinez *et al.*,2012
		Chlorococcales	0.234 g L^{-1} d^{-1}			

These all are discussed in detail in subsequent sections (1.5.2.1 and 1.5.2.2) of this chapter.

Furthermore, this harvested biomass is used as an animal feed. Similarly, residual biomass, after processing of bioenergy products, also have a potential for some value added products like as biofertilizer, substrate for biogas (Kothari *et al*., 2017).

1.5.2.1. Harvesting

Collection of algal biomass is another bottleneck for algae based biofuel production process. This process is considered as one of the main steps of downstream processing to achieve the solid content from >1.0% to up to 20% of solid and it costs about 20 to 30% of the total cost in biofuel production. An important aspect that limits the use of micro-algae commercially is, its cost-effective harvesting for biofuel generation from microalgae (Greenwell *et al.,* 2010). A 20-30% of the price of the biomass of microalgae is needed in the harvesting phase (Mata *et al.*, 2010; Verma *et al.,* 2010). Algal harvesting cost could be in excess of 50% (Greenwell *et al.*,2010).Harvesting and dewatering together constitutes the 90% equipment cost in open organization for manufacturing of algal biomass (Amer *et al.,* 2011). The dilute suspension requires constant harvesting which makes it more expensive than the harvesting of terrestrial flora. The energy required for harvesting through settlement and centrifugation on dry biomass basis is 1 MJ kg^{-1} for microalgae whilst that of wood is 0.7–0.9 MJ kg^{-1} (Sawayama *et al.* 1999). Instead of concentrating on recovery, works related to micro-algal biodiesel production had pivoted on the yield and configuration (Brennan and Owende 2010). For harvesting the microalgae, numerous ways and means are made use of; enlisting a few are sedimentation, flocculation, flotation, centrifugation and filtration or an amalgamation of all these as specified in Table 1.10.

1.5.2.2. Extraction of lipid and its conversion to fuel

The lipid extraction method from algal cell comes next to the cell harvesting. The algal cell wall must be shattered to extract lipids. Intended for biodiesel generation expertise through microalgae, techniques like bio-prospecting of high-lipid-containing strains and also stimulation for inflated lipid generation through strain enhancement approaches both physiologically and genetically is being conceded. Thus, lipid extraction is an exceptionally imperative process for microalgal biodiesel production. Algal biorefinery is a process to yield numerous algal products and bio-crude is produced by thermochemical technology. Lipid extraction is feasible in both technical and economical ways in joint concepts. Even the algal strains that have lipid content as low as 10%, lipid extraction is possible for biodiesel production. One could not produce lipid from microalgae in a successful and efficient way from algal cell biomass. Every time solvent extraction doesn't prove to be an apt technique for oil recuperation through the microalgal biomass; hence a concern arises for oil extraction in an environmentally sustainable manner. Some of the procedures like solvent extraction, supercritical fluid extraction, mechanical pressing, milling, homogenization, osmotic shock, ultrasonic-assisted extraction and enzymatic extractions are presented extensively in literature part for oil extraction from microalgae (Ranjith *et al.,* 2015).These entire processes have limitations also along with advantages, which all are summarized in Table 1.11 according to the availability of literatures till date.

Table.1.10:Advantages and disadvantages of various techniques with their efficiencies for harvesting the algal biomass

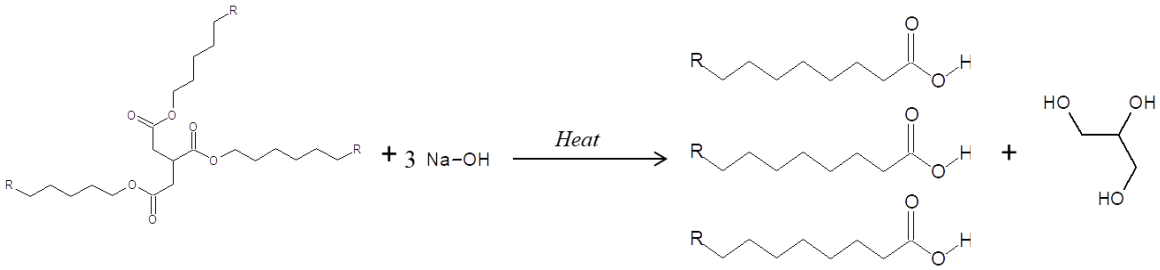
Harvesting Technique	Efficiency	Advantage	Disadvantage	References
Centrifugation	80-90	Steadfast, rapid as well as proficient since 80% of biomass is acquired within 2–3 minutes.	Energy intensive Damage of cells.	Mata *et al.*,2009
Sedimentation	10-50	Simple and recognized technique, regulated effortlessly, residual stream could be reprocessed to growth chamber.	When no additional flocculants are present, becomes time consuming for small constant suspended culture (0.1–2.6 cm h^{-1})	Milledge and Heaven, 2013
Filtration	70–95	Greatly reliant on size of the particle. Greatly resourceful for big size microalgae, enhanced fluid velocity reduces the fouling	Time intense, Filters fouling, high operational charge increases because of backwashing, effectiveness reduced	Barros *et al.*,2015
Flotation	80–90	Excellent interrelation of the air bubbles with zeta potential of the cells of algae, Inexpensive at huge volume scale	Though the bubble size is small operating cost is costly expensive for ozone floatation, High area to volume ratio is needed, relies on flow rate of air	Pragya *et al.*,2013
Flocculation or Coagulation	70–90	Increased effectiveness, less energy intensive and economical No biomass contamination by polymers as compared with chemical flocculants	Flocculent chemicals increase the operational charge biomass slurry contains good amount of water content	Pittman *et al.*,2010
Bio-flocculation	99	Extremely efficacious	A complex is formed amid bacteria and algae/ creation of precipitation	Ndikubwimana *et al.*,2016)
Methods of Electrolysis	80–95	Use of coagulants / flocculants is not done, speedy harvesting, functions at natural pH, requisites a reduced amount of time	Because energy consumption is elevated, it requires an increased price, scaling of electrodes. Reduced competence for marine microalgae.	Kligerman and Bouwer , 2015)

Table.1.11. Different types of extraction processes and their efficiencies for algal lipid yield: Advantages and disadvantages

Method	Efficiency rating	algae	% yield	Advantage	Disadvantage	References
Organic solvents like chloro-form/methanol, hexane, ether are used	Moderate	*Shizochytrium limacinum*	45	Economical as well as lucid. Apt for small scale production, Maximum proficiency	Extraction time is long; Large volume of solvent required, toxic and highly flammable Solvent recovery is energy intensive. High process cost associated with its infrastructure and operation.	Liau *et al.*,2010
		Nannochloropsis oculata	9			Liau *et al.*,2010
		Nannochloropsis oculata	5.79			Liau *et al.*,2010
		Nannochloropsis oculata	40.9			Liau *et al.*,2010
		Pavlova sp	45.2			Liau *et al.*,2010
		Nannochloropsis oculata	8.2			
Pressurized solvent extraction	High	*Nannochloropsis oculata*	6.1	User- friendly, solvent is not obligatory	Cumbersome process, sample desired in surplus	Pieber et al.,2012
Ionic liquid	Moderate-high	*Chlorella vulgaris*	11.1	Replace toxic organic solvents with ionic liquids, the so-called "green" designer solvent.	Anion and cation arrangement is discrete Solvent's specific polarity, hydrophobicity, conductivity and solubility could be designed as per necessities	Young *et al.*,2012
		Chlorella sorokiniana	23			Pan *et al.*,2016
		Nannochloropsis oculata	12.8			Olkiewicz *et al.*,2015
		Scenedesmus obliquus	10.4			Chiappe et al.,2016
		Scenedesmus obliquus	8.1			Chiappe et al.,2016
		C. vulgaris	10.6			Kim et al.,2012
		Dunaliella	8.6			Young et al.,2010
Supercritical CO_2	High	*Shizochytrium limacinum*	33.6	Favorable mass transfer equilibrium due to transitional dispersion/ features of the fluid viscosity, the extract is free from any solvent	Environmental and safety issues	Tang *et al.*,2007
		*Pavlova s*p.	34			Cheng *et al.*,2012
		Spirulina platensis	9.6			Sajilata *et al.,* 2008
		Nannochloropsis sp	25			Andrich *et al*., 2005
Expeller press	Low-	-	-	Very simple and	Production of heat.	-

	moderate			cheap Good for small scale	Compounds are mutilated	
Bead beating	Moderate	*Chlorella sp.*	30	Easy to use	Hefty biomass quantity is a prerequisite •	Prabakaran and Ravindran,2011
		Scenesdesmus sp.	32		Sluggish/Moderate procedure	Prabakaran and Ravindran,2011
		Neochlorsis sp.	34		Dissipation of high power, escalation problematic	Prabakaran and Ravindran,2011
Microwave	Very high	*Botryococcus sp*	28.1	More economical	Efficiency of microwaves can	Lee *et al.*,2010
		Chlorella vulgaris	18.1	Environmental	be very poor when either the	Zheng *et al.*,2011
		Chlorella PY-ZU1 GM	18.7	friendly,	target compounds or the	Cheng *et al.*,2013
		Nannochloropsis sp	53	Reduced extraction time, Reduced	solvents are non-polar, or volatile.	Iqbal and Theegala,2013
		Chlorella vulgaris SAG 211-12	9.59	solvent usage		Šoštarič *et al.*,2012
		Scenedesmus dimorphus	17.2	Improved extraction yield.		Axelsson and Gentili,2014
Sonication method	High	*Scenedesmus* sp.	6	Solvents used are	Most organic solvents are	Ranjan *et al.*,2010
		Dunaliella sp	30.1	relatively	highly	Converti *et al.*, 2009
		N. oculata	20	inexpensive;	flammable and/or toxic;	Araujo *et al*., 2011
		Botryococcus sp.	9	results are	solvent recovery is expensive	Koberg *et al*., 2011
		Chlorella sp.	52.5	reproducible	large volume of solvent is required	Raujo *et al.*, 2013
Osmotic shock method	Moderate-high	*Schizochytrium* sp.	48.7		Requires longer treatment time (not	Avinesh *et al*., 2015
		Thraustochytrium sp.	29.1			Avinesh *et al*., 2015
Electroporation	Very high	*Chlorella vulgaris*	22		Appears promising but detailed	Flisar *et al.*,2014
		Spirulina	-		pilot-scale studies have to be	Qin *et al.*,2014
		Nannochloropsis sp	-		carried out	Grimi *et al.*,2014
		Dunaliella salina	-			Foltz, 2012
		Isocrysis sp	-			Kempkes *et al.*,2011

After extraction of lipid, it is used to convert into fatty acid by the process of transesterification. Transesterification refers to the chemical way that converts the fatty acid into the fatty acid methyl esters (FAME), which is termed as biodiesel. This process reduces the viscosity of FAME. Initially, transesterification 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.



Triglyceride **Sodium Hydroxide** **Fatty Acid Methyl Ester**

Alcohol and triglycerols (TAG) is mixed in a proportion of 3:1 M for generating biodiesel. The distinct properties of the Glycerol and FAME is demarcated as the former gets down in the base due to substantial density whereas the latter stays at the apex (Chew *et al.*,2017).Approximately 80% of the extricated algal oil volume could yield biodiesel (Rawat *et al.*,2012).

During Transesterification, usually chemical catalysts (acids, bases) or biological catalysts (enzymes) are applied (Oncel, 2013).Numerous chemical catalysts chiefly bases like NaOH, KOH, Al_2O_3, BaO, SrO, Cao, Zeoloites, HCO_3, Na and K alkoxides like sodium methoxide (Na-OCH_3), sodium ethoxide (Na-OC_2H_5), sodium propoxide (Na-OC_3H_7) and sodium butoxide (Na-OC_4H_9) are employed for biodiesel production (Ogunkunle *et al.*,2017;Leung *et al.*,2017;Salam *et al.*,2016; Dall'Oglio *et al.*,2014). Some chemical catalysts mainly acids like sulfonic acid, sulfuric acid and hydrochloric acid are used for biodiesel generation. For the action of enzymatic

biocatalysts, lipases are deployed (Teo *et al.*, 2014).Transesterification catalyzed by alkali catalyst is expeditious (4000 times) than the acid catalyzed. Though alkali catalyzed reaction is hasty, however it could result in saponification because of the amount of free fatty acids (FFA) (>2%) (Mandal *et al.*,2009).The water formed during the alkaline transesterification could usher the saponification reaction therefore; triglycerides and alcohol used in the process should be extensively anhydrous (Cheah *et al.,* 2016). When exorbitant amount of FFA (>2%) is to be transformed into esters, acid catalyzed transesterification proves to be a pragmatic approach (Makareviciene *et al.,*2013).The problem that ensues from acid catalyzed transesterification is that besides being a cumbersome method, it is attained at high temperature (>100°C) and pressure, hence it is quite expensive (Razzak *et al.,*2013).Energy intensiveness, saponification menace, segregation problem of catalyst and products, obstacle in glycerol retrieval are some of the challenges while using chemical catalysis (Oncel *et al.,*2013).For transesterification, the enzymes could be very well employed as they demonstrate notable endurance to lipid's FFA content. At a temperature range of 35–45 °C, lipase transesterification is performed guaranteeing no catalytic activity of saponification (Razzak *et al.,* 2013.The actions of the enzymes are curtailed in the transesterification reaction as the restrained surface of the lipase coheres with glycerol. The reason behind why this method is not being used commercially is because the lipase enzyme is quite expensive and there arises a difficulty in salvaging the glycerol from restrained lipase surface (Huang *et al.,* 2009).

Table.1.12:Effect of different types of catalysts with their conversion efficiencies

Transesterification method	Algal	Catalyst	Efficiency %	Advantages	Disadvantages	References
Homogeneous Acid catalyzed	*Schizochytrium limacimun*	H_2SO_4	52-66	• Gives relatively high yield • Insensitive to FFA content in feedstock, thus preferred method if low-grade feedstock is used • Esterification and transesterification occur simultaneously	• Corrosiveness of acids damage equipment • More amount of free glycerol in the biodiesel • Relatively difficult to separation of catalyst from product. • Has slower rate of production (relatively takes longer time)	Johnson and Wen 2009
	Chlorella protothecoides	H_2SO_4	80			Xu *et al.*, 2006
	Chlorella protothecoides	H_2SO_4	>70			Miao and Wu 2006
	Tetraselmis suecica	H_2SO_4	78			Wahlen *et al.*, 2011
	Schizochytrium limacimun	H_2SO_4	63.47			Johnson and Wen 2009
	Chaetoceros gracilis	H_2SO_4	32.9			Wahlen *et al.* 2011
	Nannochloropsis sp.	H_2SO_4	2.9			Koberg *et al.* 2011
Homogeneous Base catalyzed	*Oedigonium* sp	NaOH	>90	• Faster reaction rate than acid catalyzed transesterification • Reaction can occur at mild reaction condition and less energy intensive • Popular catalysts such as NaOH as well as KOH are economical as well as extensively accessible	• Sensitive to FFA content in the oil • Saponification of oil is the main problem due to quality of feedstock • Recovery of glycerol is difficult, • Alkaline wastewater • generated requires treatment	Hossain *et al.* 2008
	Nannochloropsis sp.	KOH	>77			Patil *et al.* 2011
	Nannochloropsis sp	SrO	37.1			Koberg *et al.* 2011
	C. vulgaris	NaOH	46.8			Farooq *et al.*,2013
	R. hieroglyphicum	NaOH	44.8			Farooq *et al.*,2013
	C. vulgaris	KOH	46			Farooq *et al.*,2013
	R. hieroglyphicum	KOH	45.7			Farooq *et al.*,2013
	Oedigonium sp.	NaOH	>90			Hossain *et al.* 2008
Heterogeneous Base Catalysis	*Nannochloropsis oculata*	Al_2O_3/CaO	80	• Improved selectivity • Easy to separate catalyst from reaction mixture • Reduced process stages and wastes • Enable to regenerate	• Catalyst might be poisoned when exposed to ambient air • Sensitive to FFA content in the oil so selective to feedstock type • Soap will be formed if there is	Umdu *et al.*,2009
	Chlorella vulgaris	Al_2O_3/KOH	>89			Ma *et al.*,2015
	Nannochloropsis sp.	Mg-Zr	23			Sistiafi *et al.*,2018
	N. oculata	NaOH/Zeolite	83.5			Sistiafi *et al.*,2018
	Chlorella vulgaris	NaOH/Zeolite	98			Cercado *et al.*,2018

	Chlorella vulgaris	$Ca_{12}Al_{14}O_{33}$/CaO	84	and reuse the catalyst	high FFA content	Cercado *et al.*,2018
	Chlorella vulgaris	$NaAlO_2$	80	• Moderate reaction conditions ensues the reaction and requires less energy	• Soap formation associated with reduced biodiesel yield besides problem in product purification	Cercado *et al.*,2018
	Chlorella vulgaris	K_2CO_3/α-Al_2O_3	54			Cercado *et al.*,2018
Heterogeneous Acid Catalysis	Algae oil	Zirconia, Titania	90.2	• Has reduced process stages and wastes • Insensitive to feedstocks' FFA content. • Preferred-method if lowgrade oil is used • Esterification and transesterification occur simultaneously • Solid acid catalyst can be easily removed recycled	• Complicated catalyst synthesis procedures lead to higher cost • Requires high reaction temperature, high alcohol to oil molar ratio and long reaction time. • Relatively energy intensive	McNeff *et al.*,2008
	Algae oil	NiO-MoO_3/ alumina	99			Sani *et al.*,2013
	Algae oil	Pt-SAPO-11	83			Sani *et al.*,2013
	Algae oil	NiO, MoO3/HZSM-5	98			(Sani *et al.*,2013
	Algae oil	Microporous Titania	94.7			Sani *et al.*,2013
	Algae oil	(HY-340) niobium oxide	94.2			Sani *et al.*,2013
	Algae oil	Hierarchical H-Beta zeolites	99.5			Sani *et al.*,2013
	Algae oil	Amberlyst-15	>98			Dong *et al.*,2013
Lipase catalyzed transesterification	*Chlorella protothecoides*	Candidiasis sp.	98	• Insensitive to FFA and water content in the oil, thus preferred when low grade feedstock is used • It is carried out at low reaction temperature • Purification requires simple step, by enabling easy separation from the byproduct, glycerol • Gives high purity product (esters) • Enables to reuse immobilized enzyme	• The cost of enzyme is usually very high • Gives relatively low yield • It takes high reaction time • The problem of lipases inactivation caused by methanol and glycerol	Li *et al.* 2007
	Chlorella vulgaris ESP-31	-	95.			Tran *et al.,* 2013
	Scenedesmus incrassatulus CLHE-Si01	Novozyme 435	71.7			Arias-Penaranda *et al.* 2013
	Chlorella sp. KR-1	Novozyme 435	75.5			Lee *et al.* 2013
	Oedogonium sp.	Novozyme	92			Haq *et al.* (2014
	Chlorella pyrenoidosa	Penicillium expansum	90.7			Guldhe, 2015
	Chlorella vulgaris ESP-31	Burkholderia lipase	72.12			Tran *et al.*,2012

Nano catalyzed transesterification	*Algal oil*	TBD-Fe_3O_4@silica	99.15	• Relatively with shorter reaction time • Less amount of catalyst can be enough since has high specific surface area • Catalyst can be reused many times • Wide range of catalyst	• Requires relatively more alcohol for effective yield • In some cases preparation of appropriate catalysts costs more	Chiang *et al.*,2015
	Algal oil	Nano-CaO	86.14			Pandita and Fulekar, 2017
	Nannochloropsis sp.	Nano $Ca(OCH_3)_2$	99.0			Teo *et al.*,2016
	Synechocystis sp.	Nano-TiO_2	36.5			Jawaharraj *et al.*,2017
Ionic liquid catalyzed transesterification	*Scenedesmus obliquus*	[HDBU][$MeOCO_2$/HCO_3]	88	• Easy to separate final products due to formation of biphasic. • Efficient and time saving	•	Chiappe *et al.*,2016
	Nannochloropsis sp.	[EMIM][$MeSO_4$]	36.79	• suit a particular need • Catalyst can be easily separated and reused many times • High catalytic activity, excellent stability	• High cost of ionic liquid production • Requires relatively more alcohol for effective yield	(Wahidin *et al.*,2016
	C. vulgaris	IL([Bmim]CF_3SO_3)	19			Ana *et al.*,2013
	C. vulgaris	IL([Bmim]$MeSO_4$)	17.4			Ana *et al.*,2013
Supercritical transesterification	*Crypthecodinium cohnii*	Carbon dioxide (SC-CO_2)	72	• It takes very less time to complete • Insensitive to greater water content of the feedstocks • Produces more than a kilo of fuel per kilo of feedstock • No need of washing the product as there is no catalyst used	• Requires higher temperature and pressure • It is not an economic alternative due to its high operating cost, due to high pressures and high temperatures • Relatively there is high methanol consumption (e.g.,high methanol/crude-oil molar ratio of 40/1)	Couto *et al.*,2010
	Nannochloropsis (CCMP1776)	SC-CH_3OH	84.15			Patil *et al.*,2012
	Chlorella sp.	SC-CH_3OH	45.62			Jazzar *et al.*,2015
	Nannochloris sp	SC-CH_3OH	21.79			Jazzar *et al.*,2015
	Chlorella CG12	SC-CH_3OH	98.12			Srivastava *et al.*,2018
	Microalgae	SC-C_2H_5OH	89			Levine *et al.*,2013
	nannochloropsis salina	SC-CH_3OH	67			Reddy *et al.,*2014

The enzymatic transesterification would be a preferred method in the future only if the restriction of the enzyme and multiple enzymes is performed in an opposite way. A concoction of glycerol, alcohol, esters, catalyst as well as tri-, di- and monoglycerides are the resultants of the transesterification. When there are contaminations found in the esters like di- and monoglycerides, then there would be a difficulty in attaining pure esters. By the centrifugation and gravitational settling methods, glycerol (a by-product) could be recuperated. For the biodiesel production, the abstraction and transesterification procedures are united into one single phase in the in-situ (direct) transesterification process. The step involving abstraction of oil is eradicated by adding alcohol as well as catalyst and consequently, the harvested cells of microalgae are transesterified into fatty acid esters instantly (Perez *et al.,* 2016). In Table.1.12, various processes of transesterification are compared on the basis of catalyst used in the process available in literatures till date.

1.5.2.3. Other value added products

The potential use of algal biomass is not restricted only to biodiesel and biohydrogen production but also have multiple applications such as biogas, bioethanol, bio-methanol, bio-plastics, bio-fertilizer, medicinal value and animal food *etc.* (Tong *et al.,* 2014; Gebreslassie *et al.,* 2013; Gallezot *et al.,* 2012). Microalgal cultivation is significant to achieve high algal biomass, having a wide range of applications in different fields as enlisted in Table 1.13.

1.5.3. Future scopes and challenges

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 options, providing a new insight for bio-economy.

Table 1.13:Algal derived bioproducts concerning bioreactor and current status

Algal sp.	Products	Uses
Many	Isotopic compounds	Medicine
Red	Phycobiliproteins	Research, Food colour
Other	Pharmaceutical	Antibiotics
Dunaliella	Beta-Carotene	Food suppl.
Diatoms	Xanthophyll's	Chicken feed
Greens	Vitamins C&E	Vitamins
Chlorella, Spirulina	Health foods	Supplements
Porphyridium,	Polysaccharide	Viscosities, gums
Diatoms	Bivalves feeds	Seed raising
Chlamydomonas	Soil inoculum	Fertilisers
Chlorella	Amino acids	Proline
Green algae,	Single-cell protein	Animal feeds
Greens	Vegetable oils	Food, feed

The bio-economy concept is significant for further challenges like energy crisis, environmental imbalances and unsustainability on planet earth. Henceforth, algal biomass has a potential to solve all the associated problems in an integrated way from wastewater treatment to fuel conversion.

The aforementioned bioenergy technologies are very efficient in terms of economic, environmental and sustainable aspects as specified in some sections (1.3.2.1.1-1.3.2.1.1) Among them, biofuel production has started a new arena of wastewater treatment (industrial wastewater) with simultaneous bioenergy production even under stress conditions due to natural as well as anthropogenic activities. All the influencing parameters are discussed in the previous sections (1.5.1.1-1.5.1.6) of this Chapter.

Keeping all these issues and associated challenges with this concept, following objectives are formulated to complete the task in a sustainable manner. The significant objectives are as follows:

1. To study the spectral variation under different stress conditions (temperature, nutrient, carbon dioxide, bicarbonates) for the growth of selected algal strain.

2. To study the design and development of suitable photobioreactor for the growth of algae at different visible spectrum conditions with wastewater.
3. To study the potentially optimized conditions of biomass production after stress condition for biodiesel production using transesterification with designed reactor system.
4. To study the feasibility of "selected approach" in comparison to the conventional.

From the above literature, effect of every parameter with production mechanism at individual level as well as in combination with optimization levels were discussed with various algal strains but studies explicit to *Chlorella pyrenoidosa* is not the part of literature till date. Hence, this research work is trying to provide an illustration for all the challenges like material and methodology adopted to carry out the experimental plans as 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 transesterification of harvested algal biomass is 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 and is detailed in Chapter-5. Techno-economic feasibility of "selected

approach" for conversion of selected algal biomass to biofuel with various value added end products (Algal Biorefinery concept) is also overviewed in Chapter-5. Chapter-6 provides a concluding remark for the selected research problem and the future recommendations associated with the research (from lab to land) in the pertinent area.

Chapter 2

Materials and Methods

2.1. Introduction

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

2.2. Reagents, chemicals and glasswares

In order to minimize the contamination problem, all reagents were prepared with distilled/deionized water. All glasswares and plastic containers were used only after proper washing and rinsing with Milli-Q water. All chemicals used in the present study were of analytical reagent grade. All the aqueous solutions were prepared in mili-Q water.

2.3. Algal species

Due to escalation of prices, competition in demands between food and biofuel sources, algaculture (farming of algae) has gained profound attention. Algae have enormous potential as a food, feed, fodder and fuel (4Fs). It also has the potential of being the fastest growing and photosynthesizing organism capable to compete their growth life cycle in few days (5 to 15 days).

According to literature, algal systems are well reported with experimental studies, for;

- Using waste stream from municipalities as well as industrial plants as water sources;
- Processing algal biomass for 4Fs;

- Carbon capture from smoke stack to increase algal growth rates;

There are over 20,000 different species of algae that have been identified at laboratory as well as commercial scale for various bio-products. Keeping this in mind, in the present work, algal biomass of *Chlorella* sp. is considered as an experimental tool for our objectives and optimizing its processing parameters for growth during stress conditions.

Among the various species of algae, microalgal species *Chlorella pyrenoidosa* is a freshwater green algae as depicted in Fig.2.1, this is one of the oldest life forms on the earth. The species name *pyrenoidosa* refers to the presence of a prominent pyrenoid within the *Chlorella's* chloroplast and scientific classification is given in Table 2.1.

Its chlorophyll concentration from which it owes its name is remarkably higher than that of the other food stuffs including green vegetables and fruits. Unlike *Spirulina* microalga, *Chlorella* contains a 5 -10 times higher concentration of chlorophyll. With the help of chlorophyll, algae catch the sunlight and convert it in energy forms which are used by human beings. *Chlorella* species also have the ability to treat the wastewater of different compositions.

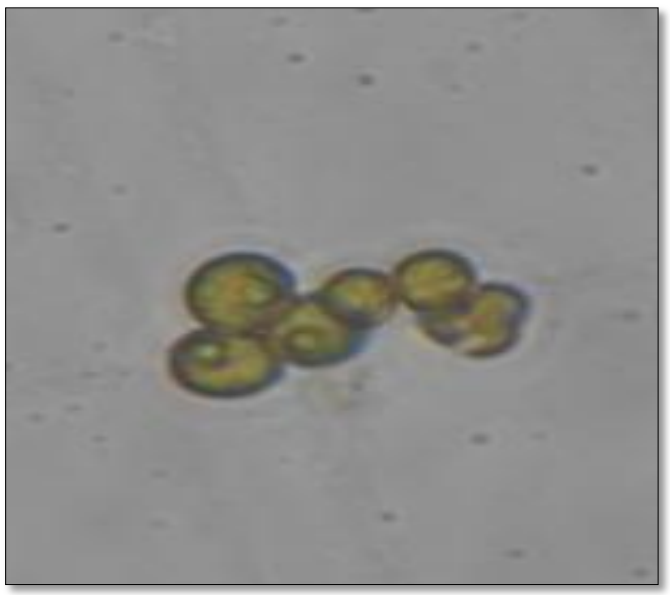


Fig.2.1: Microscopic structure of *Chlorella pyrenoidosa*

2.3.1. Medium and culture conditions

Chlorella pyrenoidosa (NCIM 2738) was obtained from the National Collection of Industrial Microorganisms (NCIM, Pune) and cultured in BG-11 medium (in gL^{-1}): $NaNO_3$, 1.5;K_2HPO_4, 0.04; $MgSO_47H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; EDTA (disodium salt), 0.001; Na_2CO_3, 0.02; 1 mL trace elements solution (in g L^{-1}: H_3BO_3, 2.86; $MnCl_2.4H_2O$,1.81; $ZnSO_47H_2O$, 0.222; $NaMoO_4$ $2H_2O$ 0.39; $CuSO_4$ $5H_2O$,0.079; $Co(NO_3)_2.6H_2O$,0.0494),with a pH of 7.0±1. Media and flasks were sterilized by autoclaving for 20 min at 15 Psi and 121°C before use. Similarly glasswares were cleaned and sterilized at 120°C for 6–7 hours before use. The medium was then inoculated with culture density equivalent to 0.1 at 660 nm and incubated at 25±1ºC in a 12h light (10 Wm^{-2})/dark cycle.

Cultivation of microalgae *Chlorella pyrenoidosa* was carried out under the ambient conditions (mentioned in BG 11 medium). Growth of algal cell was measured at 680 nm by UV-Visible Spectrophotometer (Schimadzu model). All experimental flasks were inoculated with an initial OD of about 0.05 cells. The cells were harvested by centrifugation, dried (60°C) and its dry weight was determined. The calibration curve obtained between biomass OD and dry biomass weight yields a linear equation which is used for analysis of dry weight of alga. The OD corresponding to 1 was found to be equal to 0.61 gL^{-1} of dry weight of biomass as shown in Fig 2.2.

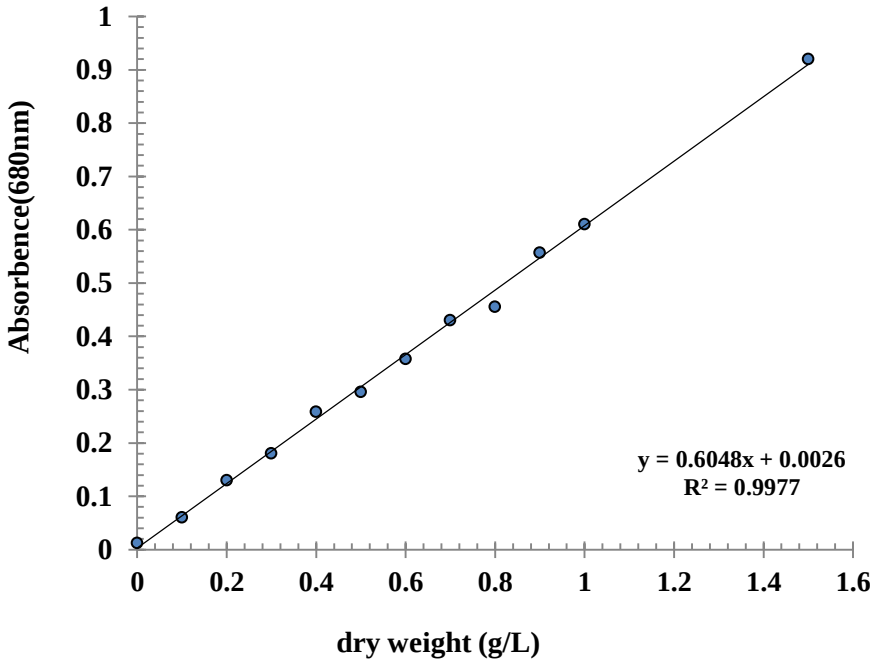


Fig.2.2: Standard graph of algal biomass on dry basis

2.3.2. Biochemical composition of *Chlorella pyrenoidosa*

Algal growth characterizations were observed by analysis of its biochemical composition such as proteins, carbohydrates and pigments. These parameters were analyzed on every alternate day to access its growth on regular basis as specified in Table 2.2.

2.4. Industrial wastewater

Wastewater as a substrate for algal cultivation was selected to establish cost effective algal cultivation technology. After extensive survey of literatures, wastewater from dairy and textile industry was selected as substrate. Fig.2.3 shows the geographical location of sampling sites which is plotted through Arc GIS software.

Table.2.1. Scientific classification of *Chlorella pyrenoidosa*

Division	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellales
Family	Chlorellaceae
Genus	*Chlorella*
Species	*pyrenoidosa*

Table. 2.2.Biochemical properties of *Chlorella pyrenoidosa*

	Method	**Unit**	**Amount**
Protien	Phenol sulphuric acid	%	56
Carbohydrate	Lowry method	%	24.5
Lipid	Sulpho-phosphovalin	%	10.3
Total chlorophyll	90% acetone	μgmL^{-1}	0.45

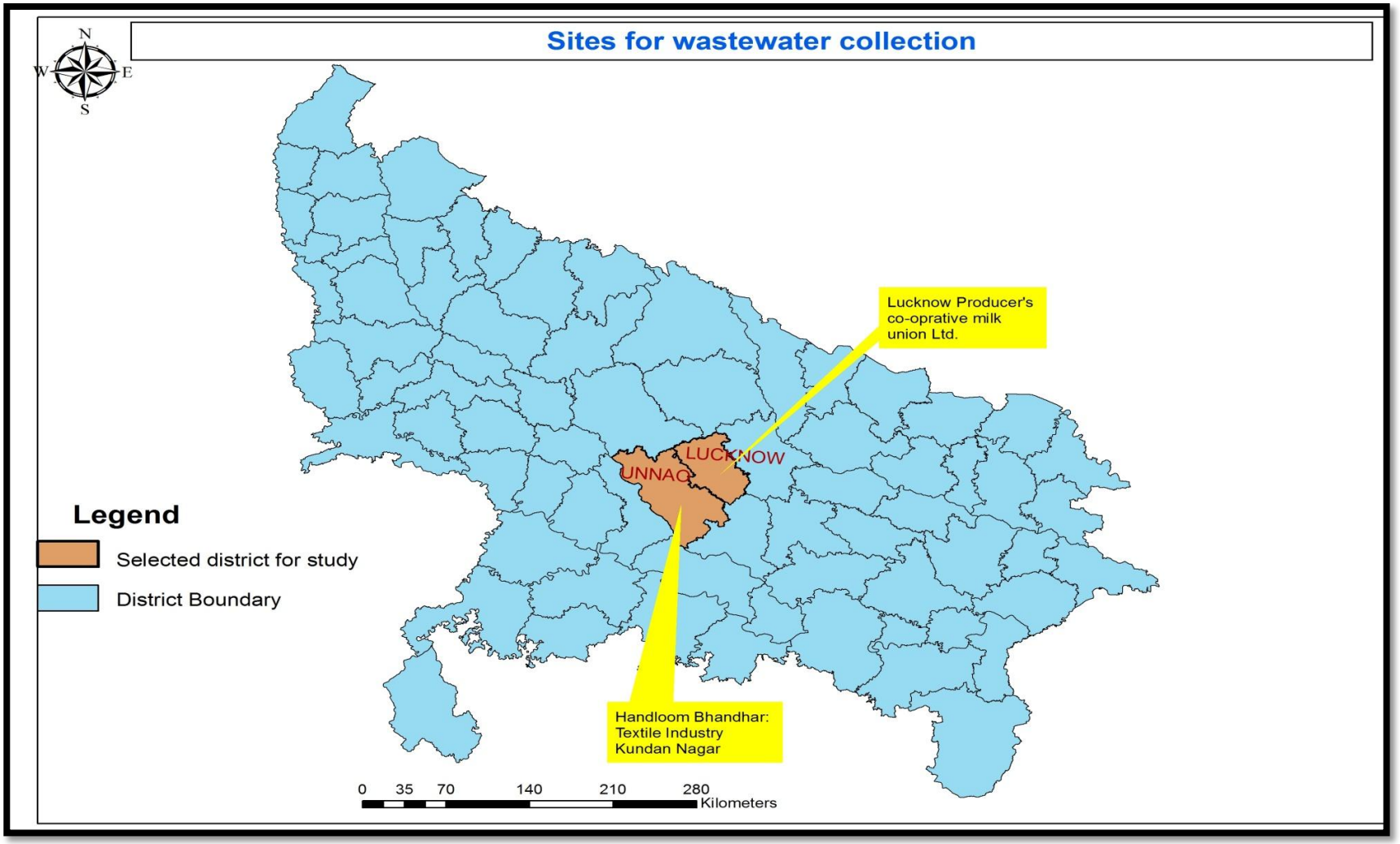


Fig.2.3. Sites for collection of wastewater

2.4.1. Dairy industry wastewater (DIWW)

The dairy industry effluent is the second most important single source of pollution in streams (Fig.2.4). It is one of the major agriculture industries and the effluent discharged by raw milk quality control laboratories are more complex than the ones commonly generated by dairy factories because of the presence of certain chemicals. The environmental impact of these factories can be very high, especially due to the discharge of very large wastewater with high content of organic matter and nutrients (nitrogen and phosphate). High organic load with significant nutrient concentration makes it feasible to use as a substrate for algal cultivation. Wastewater treatment plant of dairy industry named as "Producers Cooperative Milk Union Limited", Lucknow (U.P.) was selected as the source (Fig.2.3).The wastewater samples were collected after primary and secondary treatment processes and termed as effluent from treatment plant.

2.4.2. Textile industry wastewater (TIWW)

Textile industry in India is a prominent contributor in national economy which accounts for 14% of industrial production closely linked with rural sector (Annual Report, 2010-11, Ministry of Textiles). Approximately more than 100,000 commercially available dyes and more than 7×10^5 tonnes of dye stuffs are produced worldwide annually (Carmen *et al.*,2012) (Fig.2.3). Textile industry wastewater sample was collected from handloom Bandar, near Kundannaga, Unnao (U.P), India. Final discharge (effluent) of textile industry treatment plants was collected for the study purpose. Textile industry wastewater contains significant concentration of dyes and pigments which are highly toxic in nature. Fig.2.5 is depicting the generalized process scheme for industry**.**

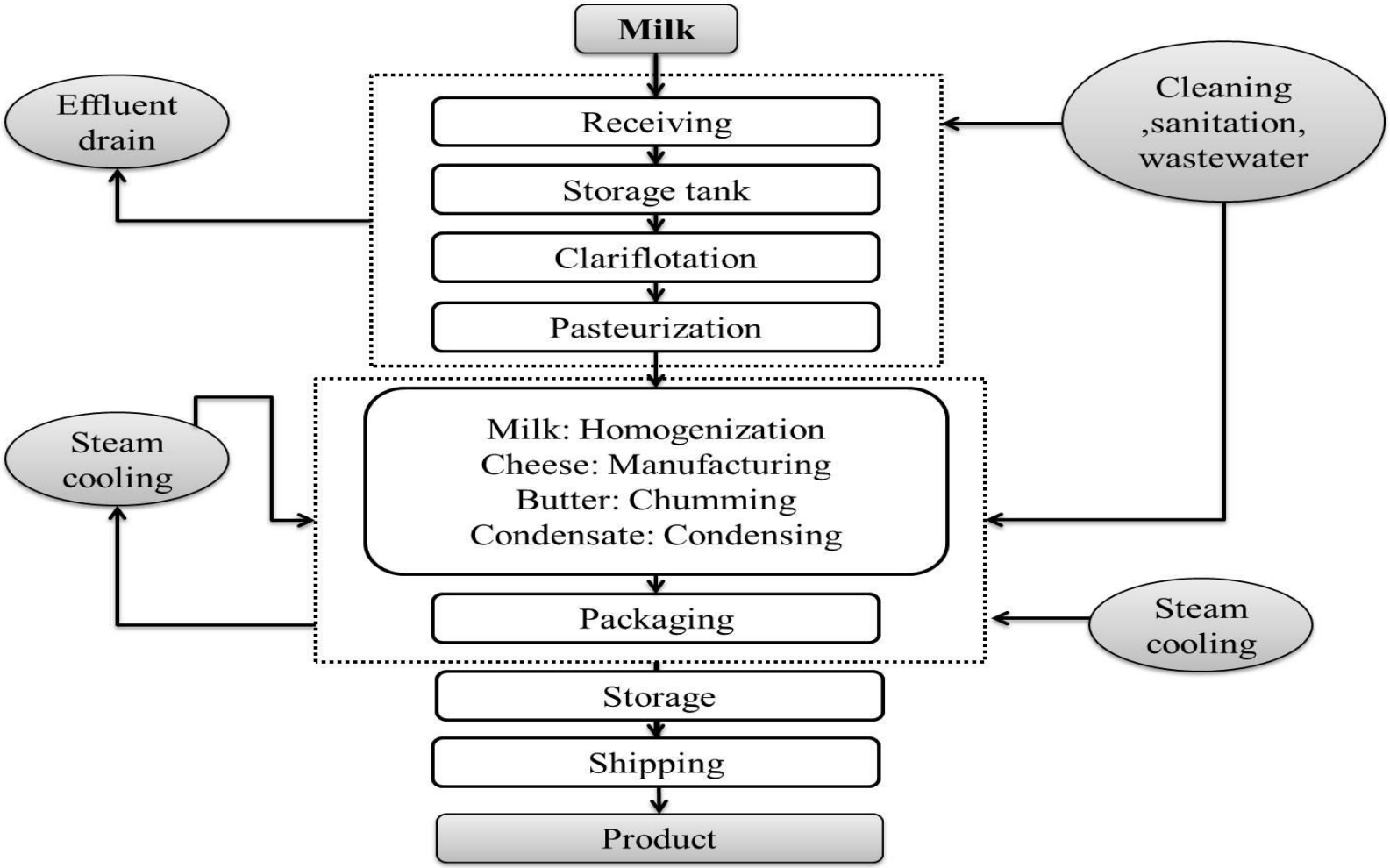


Fig.2.4: Wastewater generation sources in dairy industry (FAO, 2009)

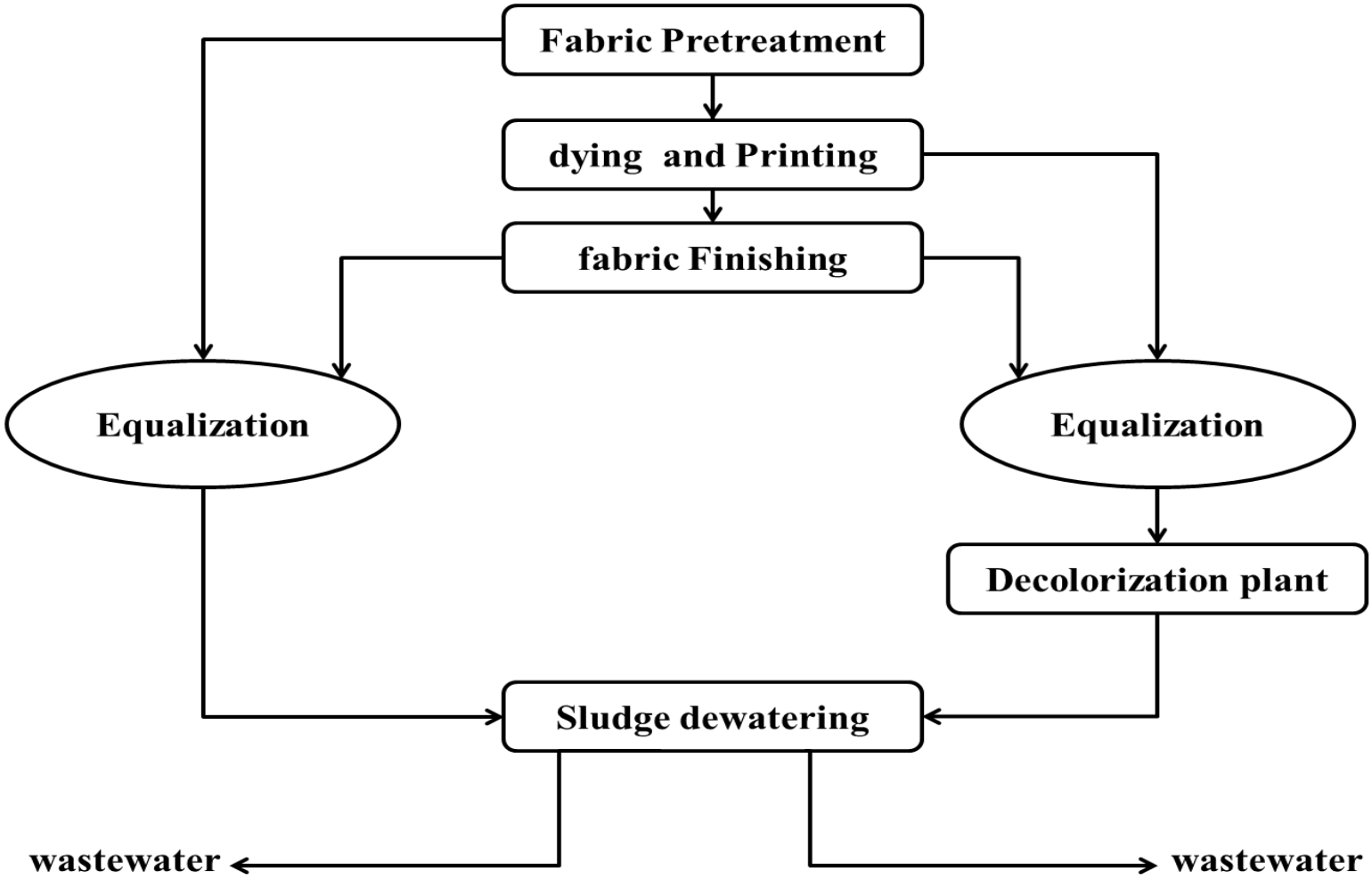


Fig.2.5: Wastewater generation sources in textile industry

2.5. Designing of Photobioreactor

A cylindrical transparent reactor was used as closed photo-bioreactor, specifications of the bioreactor are provided in Table 2.3. The bioreactor consists of a sample chamber to hold a sample of 10 liters of wastewater, however, in the present study, working volume of PBR was set up to 5 Litres in each set up given in Fig.2.6.

During upstream processing, after optimization of process parameters coupling with response surface methodology (RSM), central composite design (CCD) analysis, final findings were done in designed photobioreactor to validate the experimental results at laboratory scale in Chapter-3.

2.6. Experimental Plans

Experimental plan consists of growth optimization of microalgae at variable growth factors. Experimental data was used for calculation of parameters of growth kinetics to indicate the suitability of growth factors for rate of biomass production as displayed in Fig.2.7. The combined influence of growth factors on biomass production was also analyzed using Response Surface Methodology (RSM) as given in Table.2.4.

2.6.1 Experimental Set-up

Experimental setup was divided into three phases of study *i.e.* Phase-I, Phase-II and Phase-III. Phase-I of the study comprises characterization of wastewater sample obtained from selected industries (dairy and textile) and optimization of wastewater concentration for algal biomass cultivation and lipid productivity using multifactor analysis with the help of response surface methodology and experimental validation of the findings with PBR. Phase-II involves the harvesting of algal biomass and extraction of oil from algal biomass cultivated on industrial wastewater by using response surface methodology and

Table 2.3:Specifications of closed photo-bioreactor

S.No.	Specifications	Description
1.	Material	Polycarbonate Transparent plastic polymer
2.	Volume	10 litre
3.	Diameter	26 cm
4.	Aeration	Aqua air pump
5.	Tubing's	Plastic tubing's (2 meter)
6.	Light source	LED 12 volt
7.	Temperature sensor	K type thermocouple (Envirometer)
8.	Radiation measurement	Solar power meter
9.	pH measure	Handy pH meter

Table 2.4: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.

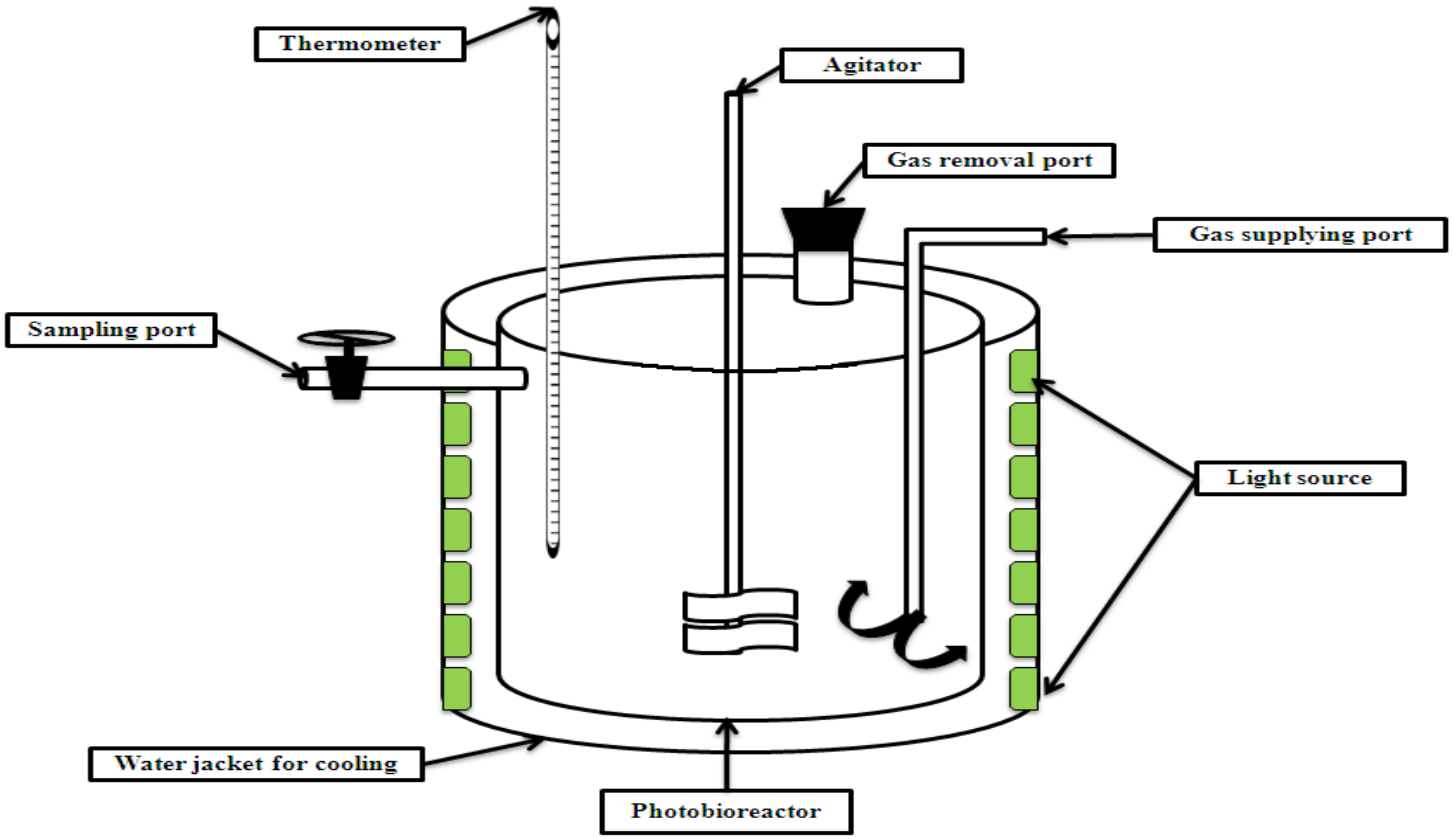


Fig. 2.6: Schematic diagram of photo-bioreactor used for present study

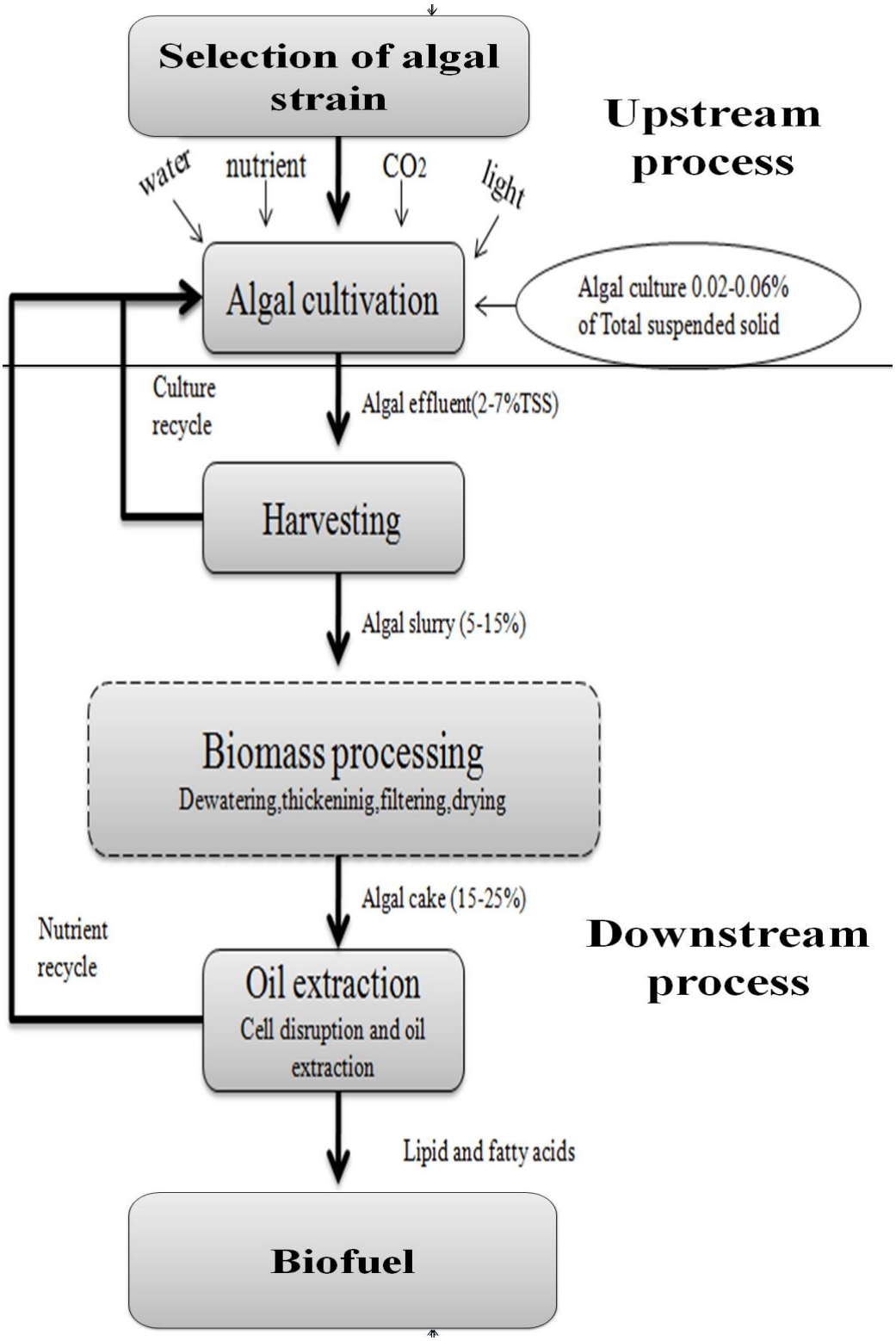


Figure.2.7: Block diagram for producing biofuel from algae with Upstream and Downstream processing steps

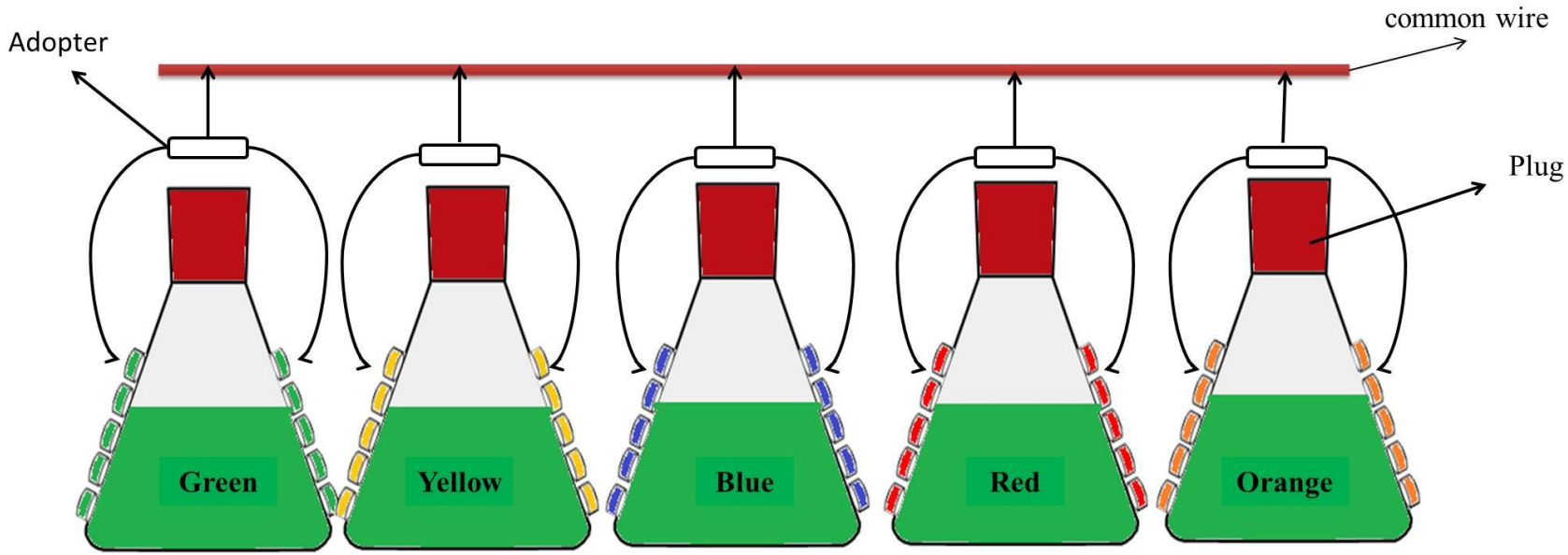
Phase-III involves the development of fuel quality index (FQI) for identification of quality of lipid and fatty acid at commercial scale and providing the techno-economic feasibility of selected approach in favor of sustainable bio-economy.

2.6.1.1. Phase-I: Optimization of upstream process for algal growth and lipid productivity using response surface methodology

Culturing of algal biomass is the prime important step for upstream processing. It can be cultured by different methods under different conditions. Interest on microalgae as clean and sustainable feedstock of bio-products, has increased now a days. Similarly, growth enhancement may be used to scale up their biomass growth rate for the future .Here, in this study, light is used by algal species to convert absorbed water and CO_2 into biomass through photosynthesis. This spectral light factor in combination with other important parameters like N and P (major nutrients), CO_2 uptake, temperature and concentration of DIWW with selected algal biomass is studied here in the Phase I^{st} of study. RSM is used to study the multifactor analysis for algal biomass and lipid productivity as well. Similarly, the optimized findings of the experimental results is validated through designed photobioreactor (Fig.2.6) to make it more viable for further studies from laboratory scale to pilot scale.

2.6.1.1. Light source

Photons of light are the important energy source for the algal cell growth. According to literature, growth rate of algal species is very much influenced by the light source during culture conditions at laboratory scale (Chen *et al.,* 2010). In comparison to conventional tubular lamps and light bulbs (Table.1.6 of Chapter-1) LEDs with low power consumption and narrow band wavelength are considered here as a light source for cultivating the algal biomass.



(a)

(b)

Fig.2.8. Experimental setup plan with LEDs as light source (a) Diagrammatic (b) Working setup plan

Five different colour LEDs (LEXCO LED strip) Red (680±5nm); yellow (620±5nm); orange (580±5nm); Green (480±5nm); and Blue (420±5nm) were used for the experimental Phase-I. All the LEDs were driven by a 6 Volt power supply. The light intensity was measured via a light meter..The cultivation was carried out in 250 ml Erykmen flask containing 200 ml BG-11 medium with manual shaking on every hour for 5 minutes on regular basis Fig.2.8 (a) diagrammatic and (b)showing the experimental setup used for this phase with LEDs. All the parameters are discussed in detail individually and their importance for scaling up the biomass and lipid productivity. This phase is divided into two main sections to study and optimize the coupling behavior of multiple factors specific to spectral (light) conditions in detail with the findings (Chapter-3).

2.6.1.2. Temperature source

Temperature is other major factor that regulates cell morphology as well as the by-products of the microbial biomass. In Phase-I, growth experiments were done at different temperature ranges in 250 ml erykmen flask. The medium and flask were sterilized in an autoclave for 20 min at 121ºC in order to prevent any contamination. Temperature in the medium was selected as an independent variable in Phase-I plan based on the literatures; temperature ranges selected for the experimental plan are 20ºC, 30ºC, 40ºC and 50ºC that is shown in the Fig.2.9. Temperature was controlled for the system with the help of water bath with thermostat (80±5ºC). The ambient temperature of the room was in the range of (27±3ºC).

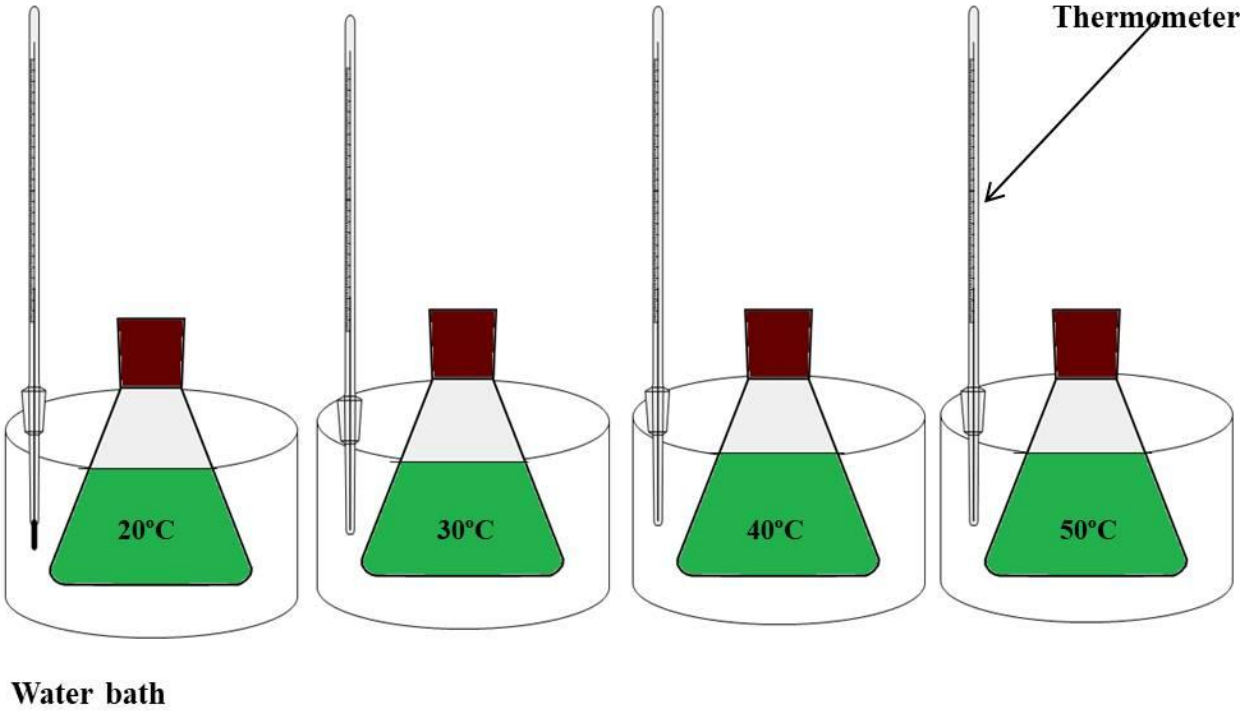


Fig.2.9: Experimental setup plan at different temperatures

2.6.1.3. Nutrient

Growth of photoautotrophic biomass like algae is dependent on nutrients such as carbon, nitrogen, phosphorus and micronutrients. As per the demand of our experimental setup, nitrogen, phosphorus and carbon are taken as major nutrient source for algal growth and their stresses on algal growth are also studied at independent as well as dependent level, which are discussed in detail in the upcoming section of this chapter and the findings related to them in the next chapter (Chapter-3 of this research work).

2.6.1.3.1. Nitrogen

Nitrogen is the most growth limiting factor for eukaryotic microalgae and would be one of the first nutirents to be depleted during algal cultivation. It is relatively easy to apply on controlled N stress for microalga by substracting the nitorgen sources in the growth media (Minhas *et al.*,2016). Lipid acculmulation in microalgae usually occurred when micoralgae are cultivated under stress conditions (eg.,Nitrogen starvation, Nutirent dificiency, pH variation *etc*.,) (Paes *et al.*,2017).Among these stress conditions, nitrogen limitation is the most effectively used strategy for stimulating lipid accumulation in microalgae. Recent reports demostrated that cultivation under nitrogen starved conditions leads to a marked increse in the lipid content. Different concentrsation of nitrate $0.05gL^{-1}$, $0.1gL^{-1}$, $0.15\ gL^{-1}$ and $0.2\ gL^{-1}$in the form of sodium nitrate ($NaNO_3^-$) were used during Phase-I of the study as dependent and independent variables.

2.6.1.3.2. Phosphorus

Phosphorus (P) availability is often a limiting factor in aquatic ecosystems. In some microalgae, nutrient starvation limits cell division and leads to the accumulation of organic carbon (e.g. in the form of triacylglycerols TAGs)(Yang *et al.*,2017). To cope

up with P limitation, there is an interplay of P recycling and changes in lipid classes in green algae. It is required in biomolecules such as DNA, long-chain polyphosphates (polyPs), and phosphoproteins, all of which have long half-lives. Phosphate is used within the cell in the form of adenosine phosphates or NADPH. Thus, P limitation will affect a variety of energy-requiring processes, such as RNA transcription, the carbon cycle and protein synthesis. Inorganic orthophosphate (Pi) is supplied and translocated into the cell by specific transporters, followed by incorporation into organic compounds or polyP storage through a plethora of biochemical reactions. Di-potassium dihydrogen phosohpate (K_2HPO_4) for phosphorus source in the present study with different concentrations $0.025gL^{-1}$, $0.05gL^{-1}$, $0.075gL^{-1}$ and $0.1gL^{-1}$ was used for the present study.

2.6.1.3.3. Carbon

Carbon sources are usually the most critical factors for the growth of microalgae.In genral, microalgae can be grown under photoautotrophic, heterotrophic and mixotrophic conditions using diversified carbon sources such as CO_2, methanol, acetate glucose or other organic compounds. Photoautotrophic cultivation means that micoralgae use inorganic corbaon (CO_2 and HCO_3).Some microalgal species can directly use organic carbon as source in the presence or absence of a light supply (Abinandan & Shanthakumar,2016). In present study, CO_2 supply is used with the help of cylinder and with different concentrations of CO_2 (0.04%, 5%, 10%, 15%, 20% and 100%). The schematic sketch for plan is shown in Fig.2.10.

To carry out the Phase-I study with CO_2 as one of the nutrients; different concentrations of CO_2 were used for aeration of algal culture. Pure air containing 0.03% CO_2 along with ambient air were used for aeration. Various CO_2 concentrations were used for gas flow rate as given in Table.2.5.

Table.2.5:Flow rate of CO_2

CO_2%	Air flow (ml/min)	CO_2 flow (ml/min)
0%	100	0
5%	95	5
10%	90	10
15%	85	15
20%	80	20
100%	0	100

2.6.1.4. Growth kinetics of algae

Generally, algal growth in exponential phase is considered to calculate the growth rate. The duration of exponential phase depends on the size of the inoculum and the capacity of culture conditions to support the algal growth. A growth model was applied to measure the kinetics of microalgal growth. Specific growth rate represents a measure of the ability of the organism to grow under a given set of environmental conditions, doubling times are more easily understood or meaningful Specific growth rate was calculated by using Eq.(2.1.),

$$\textbf{Specific growth rate}(\mu) = \ln(N_o\text{-}N_t)/(t_1\text{-}t_0) \qquad \textbf{(2.1)}$$

Here, μ is the specific growth rate of *C. pyrenoidosa* (mgL^{-1} d^{-1}), Nt is the biomass concentration at time t and No is the initial concentration (Duong *et al.*, 2015) until all treatments had approximately reached,their maximum densities were used for calculation.

The doubling time is simply the time required for the cells to divide. A large doubling time value means slow growth, while a small doubling time value means rapid growth. Once the value of specific growth rate was determined, the other parameters such as doubling time and division per day can be calculated by following Eq (2.2)

$$\textbf{Doubling time } T = \ln(2)/\mu \qquad (2.2)$$

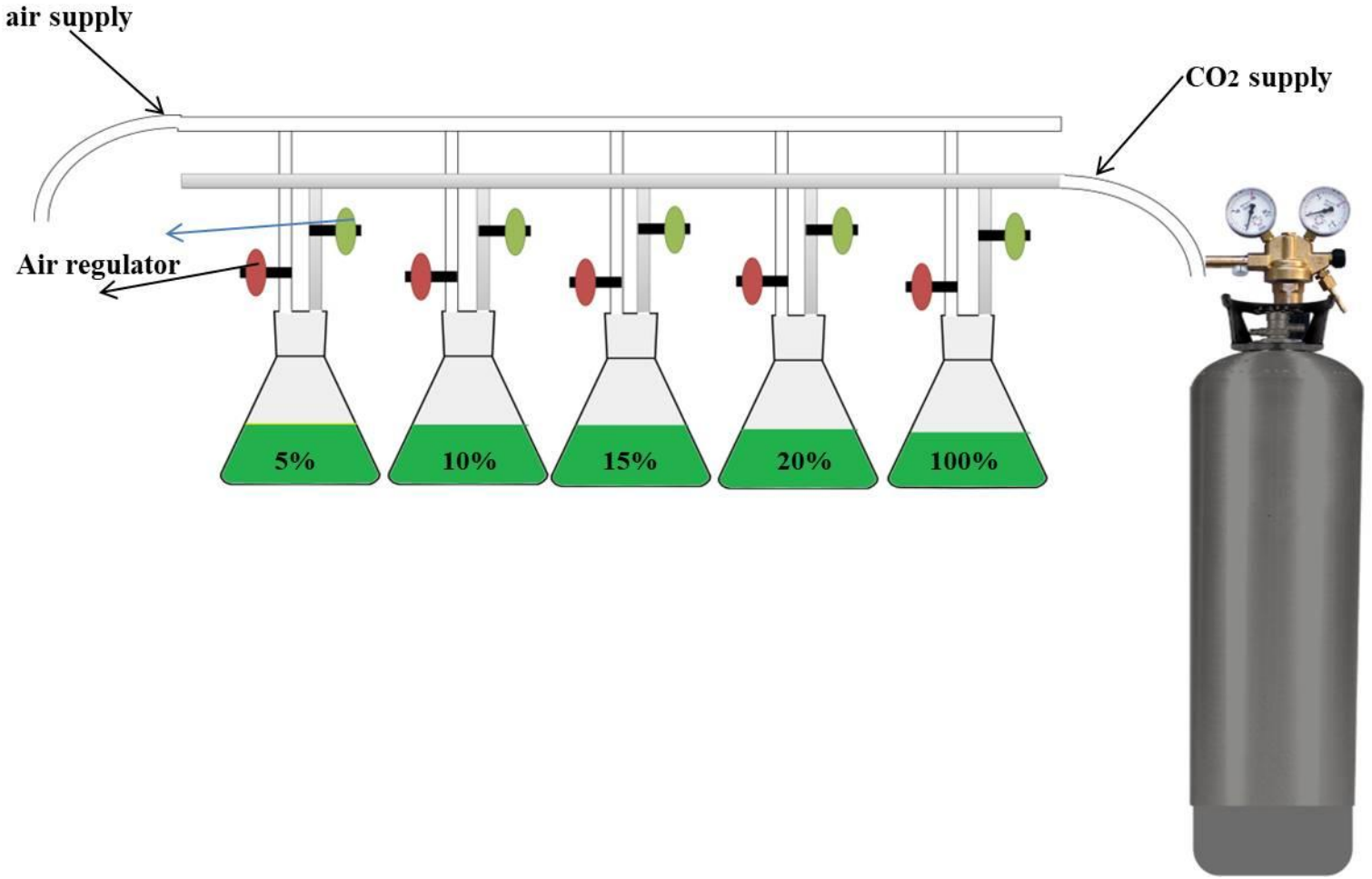


Fig.2.10: Experimental setup with differet flow rates of CO_2

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 occur when either a specific requirement for cell division is limiting or any factor inhibits 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 the present study, kinetic parameters were 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 sections.

2.6.1.5. Statistical analysis

All experiments are designed by using by RSM Design Expert software v11 (Student version).

2.6.1.5.1. Combined influence of multiple variables

Statistical modeling such as response surface methodology (RSM) has been widely used in researches to study effects of several independent factors on response. Usually, when it comes to investigating different variables, one single factor would be studied in several levels while all others remain constant. Laboratory method is a single dimensional search involving changing one variable while fixing the others at a certain level that is laborious and time consuming, especially when the number of variables is enormous. Therefore, an alternative and potential method in microbial system is the function of statistical methods. Statistical inference techniques can be used to evaluate the importance of individual factors, as well as the appropriateness of functional form and sensitivity of the response to each factor (Mason, *et al.,* 2003).Three different levels such as low concentration level (-1), middle level (0) and

High level (+1) and three independent variables represented as X_1, X_2, X_3 and dependent variable of design. Then, the rest variables are studied in the same way, one at a time. Such approach is simple and straightforward, yet it would neglect the possible interactions between different variables.

Recently, many statistical experimental design methods have been employed in bioprocess optimization. Among them, response surface methodology (RSM) is one of the suitable methods deployed for identifying the effect of individual variables and optimizing the conditions for a multivariable system efficiently Multiple regression and correlation analysis are used as tools to assess the effects of two or more independent factors on the dependent variables. Another advantage of RSM is that, it requires less experimental trials to be performed, thus saving both time and resources. However, this would also lead to loss of certain information, compared to a full factorial design. Therefore, a combination of the two might generate more accurate models, in which a full factorial design is used for the experiment, while the response surface method could be used later to model the data. Such combination could guarantee the completeness of the information in the data, while being able to investigate the possible interactions between different factors.

2.6.1.5.2. Similarity of regression analysis with RSM

The regression analysis assists the assessment of variable quantities which is affected by the effect of the factors on the behavior of the system. From the set of experimental data, a mathematical model can be established depicting the relationship between the response variables and the factors that influence the process. It is one of the effective tool used for investigating effect of relationships having applications of physical, chemical, engineering and biotechnology, as well as in social sciences. Response surface methods are additional techniques that are employed before and

after a regression analysis performed on the data. The experiment must be designed using regression analysis that selects input variables and their values during the actual experimentation assigned. After the regression analysis is performed, certain model testing procedures and optimization techniques were applied. Thus, the subject RSM includes the application of regression as well as other techniques in an attempt to gain a better understanding of the characteristics of the response system under study.

2.6.1.5.3. Central Composite Design (CCD) for upstream process and downstream process

The central composite design (CCD) is an alternative class of 3^k factorial design and it was introduced by Box and Wilson (1992). A central composite design consists of a complete (or a fraction of a) 2^k factorial design, where the factor levels are coded to the usual -1, 0, +1 values. This is called factorial portion of the design. Two axial points on the axis of each design variable at a distance of α from the design center. Thus, the total number of design points in central composite design (CCD), consisting of k variables is given in Eq (2.3):

$$N = 2^k + 2k + n_0 \qquad (\mathbf{2.3})$$

Where N is the total number of experiments, k is the number of factors and m is the number of replicates. The test variables were coded according to the following Eq.(2.4).

$$x_i = \frac{X_i - X_I^0}{\Delta X} \qquad (\mathbf{2.4})$$

Where x_i is coded variables and X_i is the natural value of independent variables, $X_I^0$is the value of the variable at the center point and ΔX is the step change value.

The lower and upper bounds of each of N design variables in the optimization problem needs to be defined. The allowable range is then discretized at different

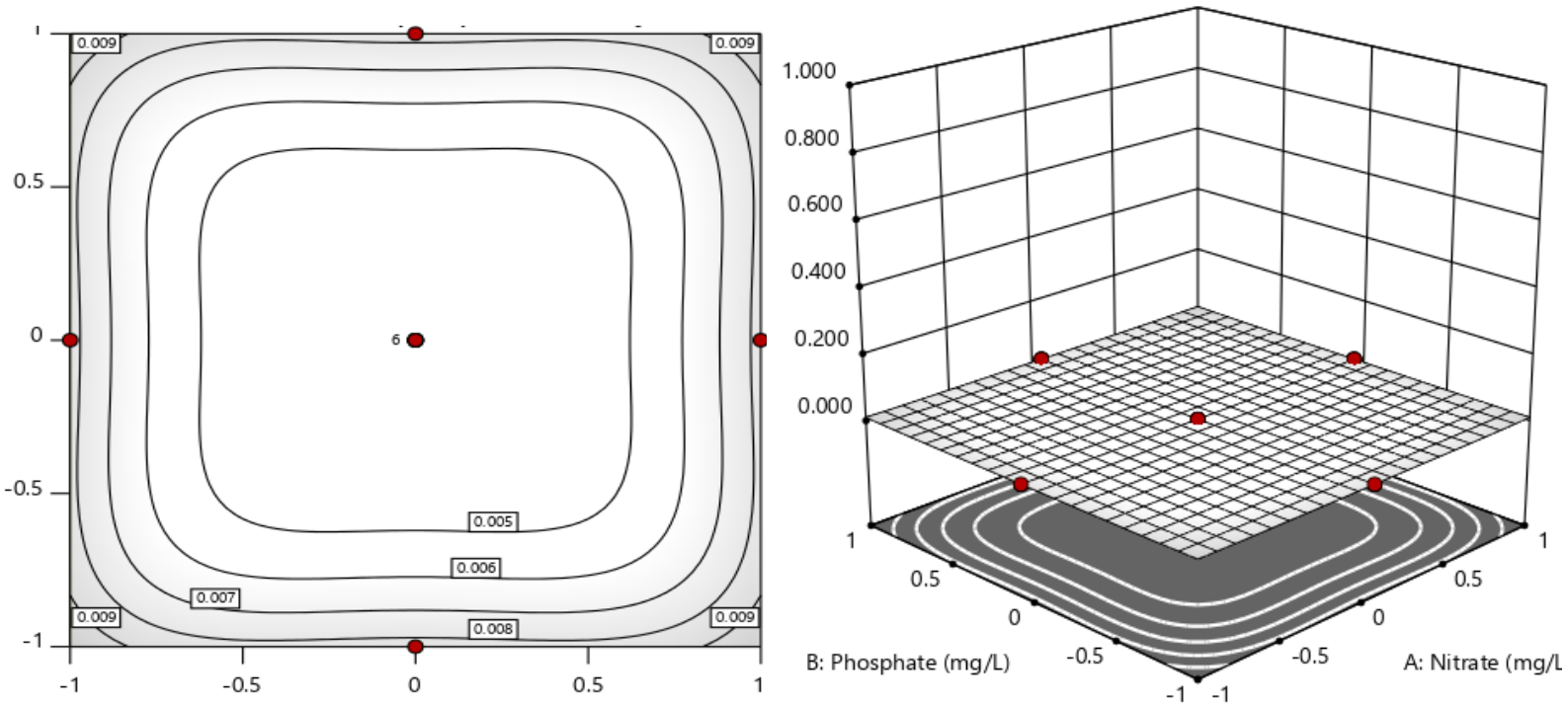
levels. If each of the variables is defined at only the lower and upper bounds (two levels), the experimental design is called 2^k full factorial. Similarly, if the midpoints are included, the design is called 3^k full factorial To make the design rotatable, the variance of the predicted response remains constant at all points which are equidistant from the design center 2D and 3D as could be inferred from the Fig.2.11 (a and b). To make central composite design (CCD) rotatable, it is chosen the value of α that satisfies the condition.

Factorial designs can be used for fitting second-order models. A second-order model can significantly improve the optimization process when a first-order model suffers lack of fit due to interaction between variables and surface curvature. A general second-order model is defined as Eq.2.5.

$$Y = \beta_0 + \sum_{i=1}^{k} B_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + e_{ij} \qquad \mathbf{2.5}$$

Where, Y is the response of prediction and$x_i, x_{j,\ldots\ldots\ldots,} x_k$ are the input variables, which affect the response Y;,$x_i^2, x_j^2 \ldots\ldots\ldots, x_k^2$ are the square effect of variables;$x_i x_j$, $x_i x_k$…….,$x_j x_k$ are the interaction effect of variables;,B_0 is the intercept;B_i (i=1,2……) is the linear effect;β_{ii} (i=1,2……) is the effect of squared;,β_{ij} (i=1,2……k, j=1,2……) is the effect of interaction; and random error is denoted by e.

Therefore, to study the multifactorial analysis for algal biomass growth using RSM, in this study, coupling of influencing parameters without LEDs and with LEDs following specification in references of chemicals and other experimental conditions were designed as:



(a) **(b)**

Fig.2.11: Graphical representation of RSM (a) 2D graph (b) 3D graph

2.6.1.5.3.1. The experimental design consisted of three nutrient stress variables: $NaNO_3$ (nitrogen source), K_2HPO_4 (phosphorus source) and DIWW (pollutant stress and aquatic medium). All experiments were performed in 500 mL conical flasks with light intensity of 10 W/m^2 using cool fluorescent tubes in a culture room at temperature of 25±1°C. A total of 20 experiments in triplicates containing varying nutrient concentrations were performed. The table with codes are defined in section. 3.4.3 of Chapter-3.

2.6.1.5.3.2. The experimental design consisted of three nutrient stress variables: $NaNO_3$ (nitrogen source), K_2HPO_4 (phosphorus source) and multicolor LEDs (All experiments were performed in 500 mL conical flask in a culture room at temperature of 25±1°C.

2.6.1.5.3.3. The experimental design consisted of three stress variables under various manipulated concentrations of CO_2 rate (0, 5,10,15 and 20%), different wavelength light emitting diodes LEDs (420, 460, 520, 580 and 620nm) and DIWW (pollutant stress and aquatic medium) on biomass productivity (gL^{-1}), lipid content (%) and percentage variables.

2.6.1.5.3.4. The experimental design consisted of three stress variables under various manipulated concentrations of CO_2 rate (0, 5,10,15 and 20%), temperature rate (20, 25,35, 45 and 50°C) and different wavelength light emitting diodes LEDs (420, 460, 520, 580 and 620nm) on biomass productivity (gL^{-1}), lipid content (%) and percentage variables.

2.6.1.6. Bioreactor and point prediction analysis (PPA)

Designing of bioreactor for growth of selected algal biomass is explained in section 2.5 of this chapter with all specifications. Point prediction analysis was performed to

analyze the experimental data base and its viability with optimized process parameters to scale up and enhance algal growth with selected concentrations for all parameters.

2.6.1.6.1. Point prediction analysis (PPA)

PPA is the data driven approach, helpful in planning to generate the data base with existing findings. It is also helpful is focusing the challenges associated with practical situations during experiments. It is also helpful during unfavorable experimental conditions without setting experimental studies and when less number of existing data is available.

2.6.1.2. Phase-II: Optimization of downstream processes (harvesting and transesterification) using response surface methodology to scale up algal biomass

Algal biomass is considered as the most assuring raw material to counterbalance the unremitting global demand for food, feed, biofuel and biomolecule. Microalgae have appreciable growth rate, high lipids and carbohydrate yield and other biochemicals such as proteins and vitamins. Microalgae cultivation can be incorporated in different environmental bioremediation schemes. However, regardless of these advantages, the major challenge lies in the dewatering of the microalgal cultures due to their high dilution rate, minor cell dimensions and electronegative cell surface charge .Significant advances have also been made in upstream processing to generate cellular biomass and oil. However, the process of extracting and purifying oil from algae continues to be a significant challenge in producing both microalgal bioproducts and biofuel, as the oil extraction from algae is relatively energy-intensive and expensive. Therefore, to optimize the downstream process steps, this Phase-II has been divided into two sections. Section 1 of this phases explores the low-cost approaches for harvesting of *C. pyrenoidosa* and scale up the quantity of algal biomass for biofuel production, whereas, Section 2 focused on the transesterification of algal oil from the

biomass extracted from Section 1 with notable findings. Here, incorporation of nano-particles based catalyst has been introduced to make the system of low-cost and less-energy intensive using industrial wastewater. RSM is also used to optimize the conditions and is explained in detail in upcoming sections of this chapter. The current methods used for harvesting and concentrating microalgal biomass and perform a comparative analysis in order to determine the most efficient economical methods for large scale processing of microalgal biomass for production of biodiesel and other value added products is discussed in sections of Chapter.1.

2.6.1.2.1. Preparation of catalysts

The most common harvesting processes are flocculation, micro screening and centrifugation. These must be energy-efficient and relatively inexpensive. Hence, selecting easy-to harvest strains is important. Macroalgal harvesting employs manpower whereas, microalgae can be harvested using micro screens, centrifugation, flocculation or by froth flotation. In comparison with other methods, chemical flocculation is considered to be one of the best methods for cell harvesting because it can handle large amounts of microalgae, can be used with a wide range of species, reliable and cost-effective.

2.6.1.2.1.1. Impregnated Tungustate

The 5 % of tungustun oxide (WO_3) (by weight Tungustun) on calcium oxide was prepared by the method as given in Fig.2.12(a), was impregnated at room temperature for 12 h, and then dried at 110ºC for 2 h and calcined at 550ºC for 4 h.

2.6.1.2.1.2. Impregnated Zirconium

The 5% of Zirconium acetate ($Zr(CH_3COO)_2$) (by weight zirconium) on calcium oxide was prepared by method as given in Fig.2.12(b), was impregnated at room

temperature for 12 h, and then dried at 105ºC for impregnation with the solution, dried at about 125°C and calcined at 900°C.

2.6.1.2.1.3. Synthesis of Ca-Nanoparticles

The waste egg shell remained after peeling of boiled/unboiled egg was collected from the local market due to ease of availability. Egg shells were washed with distilled water and dried at 40ºC in an oven. Dried egg shell was grind to obtain the fine powder and sieved manually using micro sieve (Preeti and Fulekar,2017). The Ca-nanocatalyst was prepared using calcination hydration dehydration method of egg shells as specified in Fig.2.13 (a).

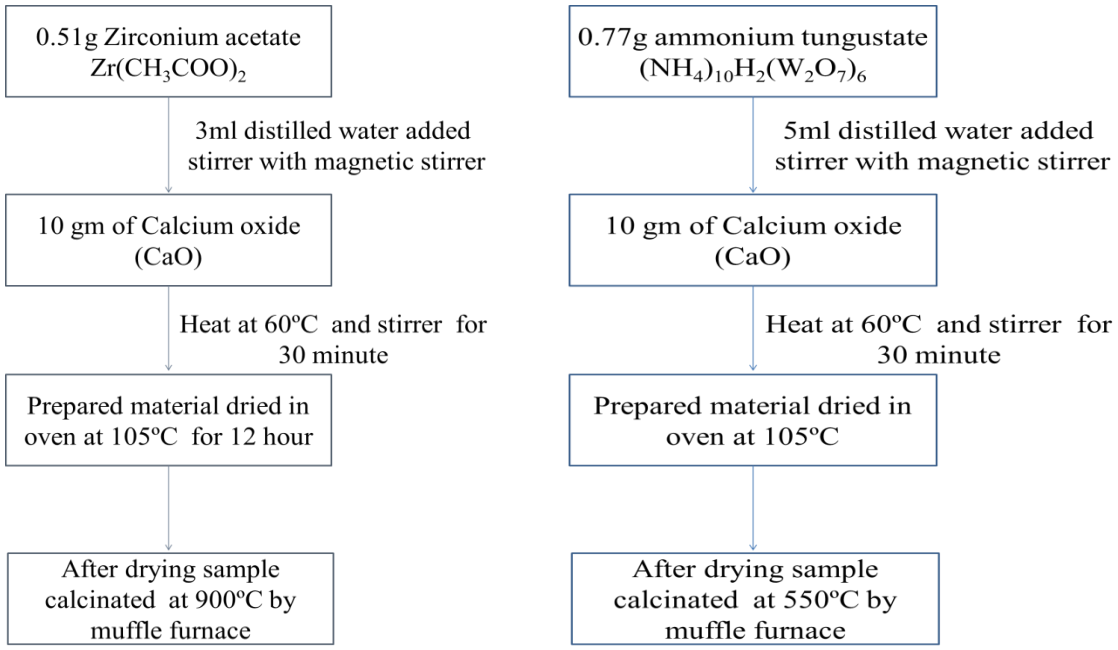
2.6.1.2.1.4. Synthesis of Mg-Nanoparticles

The synthesis of Mg-nanoparticles is divided into various steps, such as mixing, stirring, filtering, drying and calcination as described in Fig.2.13 (b). Finally, by calcinating the powder at 400^{o}C for 3h, the Mg was obtained in the form of nanoparticles (Wahab *et al.*, 2007).

2.6.1.2.2. Variables for the Phase-II study

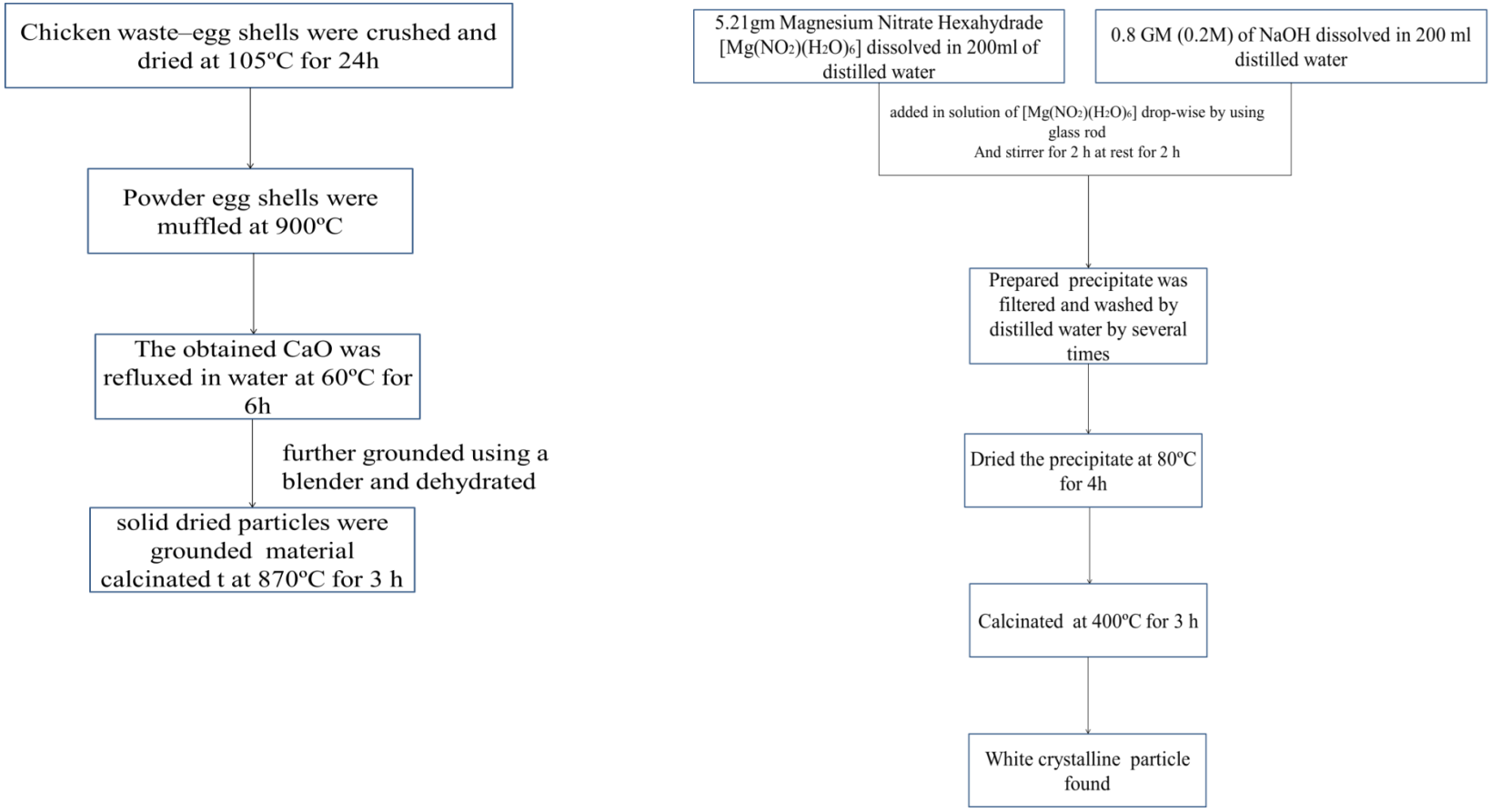
2.6.1.2.2.1. Dose

Dose which is responsible for flocculation of microalgae should not only be effective in terms of flocculation efficiency but also in terms of settling rate and concentration of the biomass.Harvesting efficiency and transesterification efficiency are also affected by the amount of dose. In the present study, the doses 0 mg, 25 mg,50 mg, 75 mg and 100 mg were used in harvesting as well as in transesterification process.



(a) **(b)**

Figure.2.12:Method of preparation of impregnated catalyst (a) Tungustate (b) Zirconium



(a) **(b)**

Fig.2.13:Methods for preparation of Nano-Catalyst for Phase-2;(a) Ca-Nanocatalyst (b) Mg-Nanocatalyst

2.6.1.2.2.2. pH

pH is the main factor which helps in coagulation of algae and binds the algal cell due to electrostatic attraction by increisng and decresing pH. In the present study, pH ranges of 5- 9 was used for the harvesting of algal biomass.

2.6.1.2.2.3. Temperature

Temperature values for the downstream process (harvesting and transesterification) reaction varies depending on the literature source. It is a well known fact that higher temperatures speed up the reaction and shorten the reaction time. Apart from that, higher temperatures usually mean obtaining higher ester yields. However, it should also be noted that if the reaction temperature is higher than the boiling point of the alcohol, it will evaporate, resulting in a lower yield. The reaction temperature at the time of harveting is maintained by a water bath and during transesterification process, temperature is maintained by hotplate. The temeprature ranges of 20ºC, 30ºC, 40ºC and 50ºC were used in harvesting process and 0-100ºC range was used during thre transesterification process.

2.6.1.2.2.3.Statistical analysis

All experimets were designed by RSM, Design Expert Software v11 (student version). All the theoretical aspects are already explained in section 2.5.1.5.(2.6.1.5.1, 2.6.1.5.2, 2.6.1.5.3) of this Chapter. Hence to studuy the transerstification of algal oil from *Chlorella pyrenoidosa* under the downstream processing following specifications were designed as;

2.6.1.2.2.3.1. In the present study, *C. pyrenoidosa* was grown with three independent variables viz. dose, pH and temperature as shown in Table.4.9. 4 types of flocculents (Imp-Zr, Imp-W, Nano-Ca and Nano-M) as depicted in Fig.4.15 are used for

comprative analysis requiring 19 sets of experimental runs comprising 5 replicates of center points, 6 axial (star points) and 8 factorial (cubic points).

2.6.1.2.2.3.2.1 gram of dried algal biomass of *C. pyrenoidosa* was mixed with Nano-Ca in powder form and suspended in required methanol (6:1) ratio. The reaction was allowed at constant continuous speed (200 rpm) with variable temperature, time, dose of Nano-Ca catalyst and (40-70) for a period of (30-180) at different dose of Nano-Ca catalyst. After completion, the transesterification reaction mixture was cooled down and centrifuged to separate layer. Filtered biodiesel was evaporated at 80ºC to remove excess methanol by using rotatory evaporator and mass of biodiesel was determined gravimetrically.

2.6.1.3. Phase-III: Development of fuel quality index (FQI) for qualitative assessment of FAME

Fuel quality index (FQI), estimated for the first time, depends on the basis of water quality index (WQI) measured by Brown and Forsy (1979). Like WQI, different fuel quality parameters were mathematically summarized into a single number to demonstrate the quality of fuel compatible with the commercially available fuels. The fuel quality index (FQI) includes different parameters like density, kinematic viscosity, acid value, peroxide value, iodine value, flash point, pour point etc. This FQI is calculated using the following equation:

$$\text{Fuel quality index(FQI)} = \sum_{i=1}^{n} \mathbf{q_i^{fi}} \quad \textbf{(2.6)}$$

$$\sum_{i=1}^{fi} q_i^{fi} \quad \textbf{(2.7)}$$

Where, FQI is a number ranging between 0 and 100 to indicate the quality of fuel; and qi is fuel quality parameter, ranging between 0 and 100, obtained from the respective "mean quality" variation, as a function of measurement concentration; n denotes the

number of parameters used to calculate FQI and, *wi*, the weighting factor of parameter i, a number between 0 and 1, assigned as a function of its importance for the FQI as described in Eq. (2) and n is the number of parameters (Shamshad *et al.*,2018).

2.7. Instrumental analysis

2.7.1. Spectroscopic method

Biomass lipid fatty acid methyl ester (FAME) was analyzed by using UV visible spectrophotometer (HALO-DB 20, Thermo-Scientific).

2.7.2. Fourier Transform Infrared Spectroscopy (FTIR)

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 create problems in interpretation of functional groups. Spectral adsorption bands were identified in relation to the published information. Supporting information on bands was also obtained by analyzing a range of pure biochemical standards (protein, nucleic acid, fatty acid and soluble carbohydrate) as detailed in Sigee *et al*., (2002).

2.7.3. Scanning Electron Microscope-X ray spectroscopy (SEM-EDX)

Surface characteristics of algal cells were analyzed by using SEM facility at University Science Instrumentation Centre (USIC) of Babasaheb Bhimrao Ambedkar University. 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 (OsO_4). 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. The samples were washed about 3-4 times to remove the salt. The samples were dehydrated by passing through a series of alcohols in increasing concentrations (25%, 50%, 75%, 95%, 100% V/V). The dried material was processed for critical point drying (CPD), in which ethanol is replaced by liquid carbon dioxide under controlled conditions of pressure and temperature. Pressure is reduced to evaporate the carbon dioxide without causing surface tension on algal cells. Then samples were dried under the atmospheric conditions. Prior to the SEM analysis, samples were coated with metal coating.

2.7.4. Gas Chromatography

Gas chromatography equipped with Flame Ionization Detector (FID) (Perkin Elmer) was performed to analyze the composition of produced methyl ester from algal oil. The FAME analysis was carried out with a split injection on to analytical column with a polar stationary phase and an FID detector. The details of instrumental parameters are given in Table.2.6.

2.7.5. XRD analysis

X-Ray Powder Diffractometry is one of the most powerful and established techniques for material structural analysis, capable of providing information about the structure of a material at the atomic level. Low and high temperature measurement facilities are available. The temperature attachment enables analysis from -170ºC to +450ºC under vacuum. Change in unit cell dimensions, structural changes at phase transitions etc., as a function of temperature can be determined given in Table.2.7.

Table 2.6. Instrumental parameters of GC

Gas Chromatograph	**PerkinElmer Clarus GC**
Inlet Temperature	250 ºC
Column flow	1 mL min^{-1}
Split flow	50 mL min^{-1}
Injection volume	0.5 µL
Oven program initial temperature	210 ºC
Oven program final temperature	230 ºC
Equilibrium time	0.0 minute
Column	Elite-Famewax- 30 m x 320 µm x 0.25 µm film
Carrier Gas	Helium
FID temperature	250 ºC
H_2 Flow	45 mL min^{-1}
Air Flow	450 mL min^{-1}
Range	1
Attenuation	-5

Table 2.7. Instrumental parameters of XRD

Make/Model	Bruker AXS D8 Advance
Configuration	Vertical, Theta/2 Theta geometry
Measuring circle diameter	435, 500, and 600 mm predefined
Angle range	360°
Max. Usable angular range:	3° to 135°
X-ray source	Cu, Wavelength 1.5406 A°
Detector	Si(Li) PSD
Temperature attachment	
Make /Model	Anton Paar, TTK 450
Temperature Range	-170 °C to +450 °C

2.8. Analytical method

Analysis based protocol adopted for the study of Phase-I and Phases-II are given in annexure section. Wastewater analysis, lipid analysis, Modified Bligh and Dyer method are given in Annexure 1

2.9. Conclusion

This chapter summarized the basis requirement needed to execute the experimental as well as model (RSM) objectives in general and analytical instrumental requirements to investigate the findings of Phase-I, Phase-II and Phase-III in particular.

Result and Discussion

Chapter 3

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

3.1.Introduction

Microalgae can be cultured by different methods and under different conditions. They need light as an energy source to convert the absorbed water and CO_2 into biomass through photosynthesis. Photosynthetic products accumulate in various forms, such as cell components or storage materials and vary from 20 to 50% of total biomass. Algae also need nitrogen and phosphorus as major nutrients, which account for 10–20% of algal biomass. Other requirements for growth are the macronutrients Na, Mg, Ca, and K; micronutrients, such as Mo, Mn, B, Co, Fe, and Zn and other trace elements (Khan *et al.*,2018;Mondal *et al.*,2017). Wastewater is a good source of the required nutrients for microalgae cultivation. Thus, application of organic effluents from the food and agriculture industries can nourish microalgae. During growth, the algae cells pass through different phases (e.g., lag, exponential, stationary, death). Different species of microalgae may vary in their need for growth media. However, the major requirements are the same for almost all species and include essential nutrients, an organic or inorganic carbon source, nitrogen, phosphorus, and iron.

Other than nutrients, bioreactor is also important in algal culturing. One of the most important parameters in algae culturing is the type of bioreactor used. This should be designed according to the species and the purpose of culture. On a large scale, algae can be cultured in open ponds (high-rate ponds) (Mondal *et al.*,2017). Open culture systems are comparatively inexpensive, but they become easily contaminated. Other bioreactors have continuous or batch culture facilities. Some species of algae grow very well in heterotrophic culture. For commercial cultivation, it is feasible to grow microalgae in waste water.

Optimization of growth parameters play the most thriving criterion to achieve the maximum production of biomass for value added end products. The optimization tool

used in this study was design expert, having several methods such as RSM *etc.* Response surface methodology (RSM) is ideally suited for most of the optimization studies as it requires only a fewer amount of data to identify the optimum response of the process as already discussed in section 2.6.1.5.1 of Chapter-2. The response surface methodology (RSM) is an effective and convenient method for screening key factors rapidly from multiple factors and optimizing culture conditions, which can avoid the defects brought by single-factor optimization. Only a few reports related the application of RSM in the optimizing of autotrophic microalgal medium for lipid production, where RSM had been shown to enhance lipid production by a two-step strategy with initial optimization of microalgal growth and final optimization of lipid accumulation. To our knowledge, the research using RSM directly for improving the value of lipid production by one-stage culture in the autotrophic microalgae has been scarcely reported by very few researchers. Therefore, for each individual microalga, systematic studies are needed to optimize the medium in order to obtain its maximum biomass and lipid production. Hence, this chapter emphasizes on the effect of various parameters on algal biomass of *Chlorella pyrenoidosa* and their lipid productivity with (LEDs) and without spectral variation. Furthermore, experimental validation is also confirmed by designed bioreactor.So, this chapter is divided into three main sections as:

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

3.1.(b). Spectral variations and their impacts on biomass and lipid productivity coupling with other different variables using RSM

3.1.(b).i. Nutrient (NO_3^-, PO_4^{-3}) with variation in DIWW concentrations without LEDs.

3.1.(b).ii. Different light emitting diodes (LEDs) with nutrients (NO_3^- and PO_4^{-3})

3.1.(b).iii. Different light emitting diodes (LEDs) with the use of CO_2 and DIWW.

3.1.(b).iv. Different light emitting diodes (LEDs) with variable temperature and CO_2 concentration.

3.1.(c).Bioreactor: Experimental validation of bioreactor with point prediction analysis.

3.2. Materials and methods

3.2.1. Algal species *Chlorella pyrenoidosa* was selected for the study and all relevant informations regarding the collection, media used is given in section.2.4.1. of Chapter-2.

3.2.2 Growth parameters

Various parameters are responsible for growth of algal biomass. Among them, the temperature, nutrients (N, P and CO_2) and wastewater are studied here. Concentration used for Phase-I for all these are discussed in Chapter-2 of section 2.3.1.All the chemicals used were of analytical grade.

3.2.3 Experimental setup

Experiments were done in Phase-I according to batch setup plans as shown in Chapter-2 (Table 2.4).As discussed in section 3.1 of this chapter, whole chapter is divided into 3 main sections, among these 3 sub-sections of the chapter, Section.3.2 have been carried out with RSM studies. Therefore, variables selected for RSM studies are given in Tables-3.1;3.2;3.3.3 and 3.4,indicative of the 5 coded levels for influencing parameters without LEDs (Section. 3.5.2.1) and with LEDs coupling with other influencing parameters (Section. 3.5.2.2, 3.5.2.3, and 3.5.2.4) respectively.

Table 3.1:Code limit for variables used in the experimental design (NO_3^-, PO_4^{-3} and DIWW)

Factor	Unit	Symbol	Coded level				
			-α	**-1**	**0**	**+1**	**+α**
DIWW	**%**	X_1	0	25	50	75	100
Nitrate	**mg/L**	X_2	0	50	100	150	200
Phosphate	**mg/L**	X_3	0	25	50	75	100

Table 3.2:Code limit for variables used in the experimental design (NO_3^-, PO_4^{-3} and LED)

Factor	Unit	Symbol	Coded level				
			-α	**-1**	**0**	**+1**	**+α**
Light	**nm**	X_1	420	460	580	620	660
Nitrate	**mg/L**	X_2	0	50	100	150	200
Phosphate	**mg/L**	X_3	0	25	50	75	100

Table 3.3:Code limit for variables used in the experimental design (LED, DIWW and CO_2)

Factor	Unit	Symbol	Coded level				
			-α	**-1**	**0**	**+1**	**+α**
DIWW	**nm**	X_1	420	465	555	640	660
CO_2	**mg/L**	X_2	0	50	100	150	200
LED	**mg/L**	X_3	0	25	50	75	100

Table 3.4:Code limit for variables used in the experimental design (LED, CO_2 and Temperature)

Factor	Unit	Symbol	Coded level				
			-α	**-1**	**0**	**+1**	**+α**
CO_2	**%**	X_1	0	5	10	15	20
Temperature	**ºC**	X_2	20	25	35	45	50
LED	**Nm**	X_3	420	460	520	580	620

3.2.4. Analytical methods

The analytical method required for pollution removal was estimated by standard methods. The detailed procedure of each method is given in Annexure.1. Biomass productivity and lipid content is also estimated by standard methods and given in Annexure.1.

3.3. Results and discussion

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

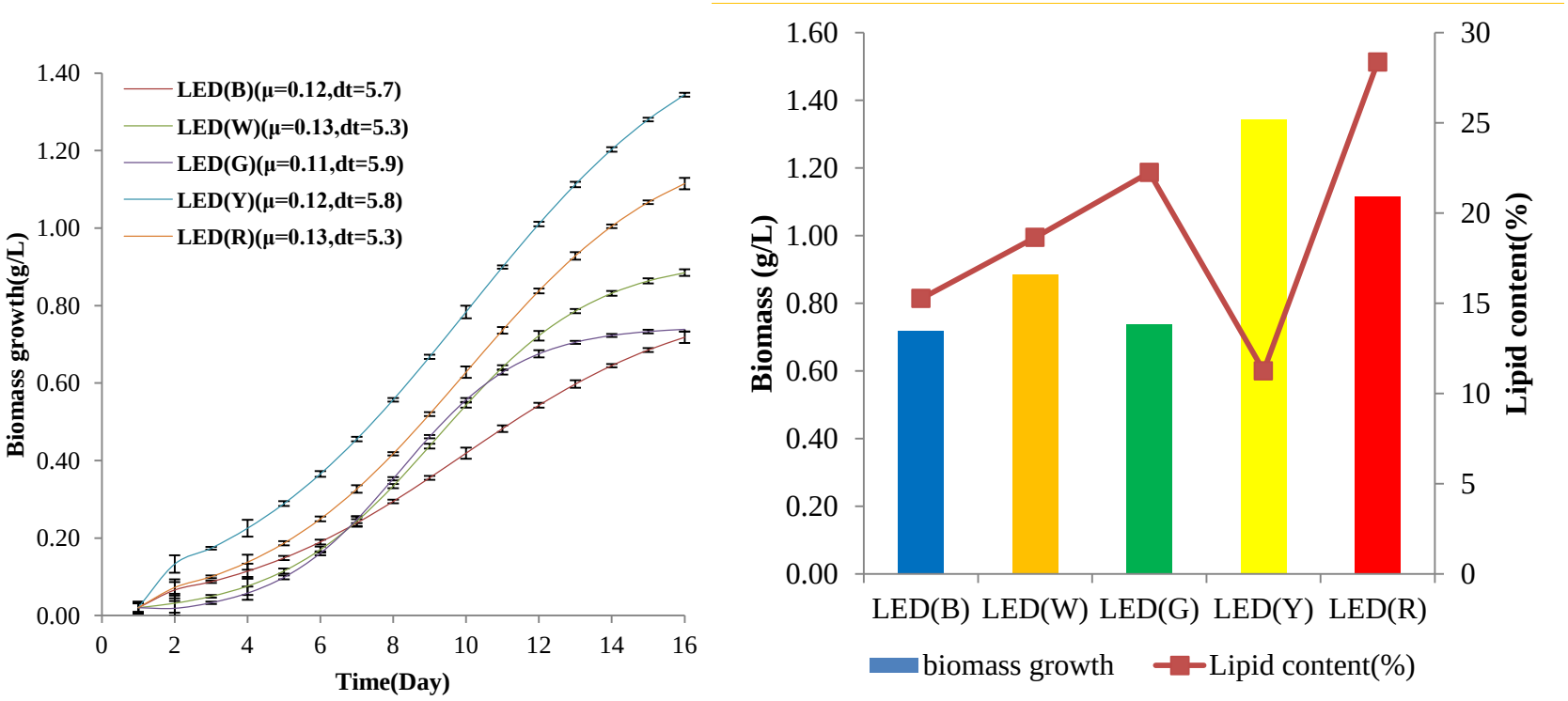
3.3.1.1.Light

Specific growth rate, biomass productivity and lipid content are highly influenced by the light source. LEDs with specific wavelength for microalgal culture have advantages of long life expectancy, low heat generation and efficient light conversion when used as a light source. Yan et al., (2013) and Xu *et al.,* (2013) also support that red light promote the algal biomass productivity for *Chlorella vulgaris*. Similar trend with increase in biomass productivity 0.91 g/L was also showed by Zonathsan, (2015) with red LED light source. Furthermore, experiments conducted with photoautotrophic cultivation of *Spirulina platensis* also exhibit the highest specific growth ($0.4d^{-1}$) with red LEDs (Chih *et al.,* 2007). Whereas blue LEDs show the least efficiency for *Spirulina platensis* in the same experiment. Similar trends were also observed in our study with red LED. The microalgal productivity of *Chlorella* with LEDs was found maximum (1.57±0.042) at 700 nm and minimum (0.746±0.054) at 550 nm (Fig.3.1b). Light is also responsible for lipid content and photosynthesis is done by green pigment chlorphyll.(Chih *et al.,* 2007) whereas, the light from other colour LEDs can only be observed partially for photosynthesis. Biomass productivity

as well content of microalgae was maximum (28.36%) at red and minimum (11.25%) at yellow LED light (Fig.3.1.b).

3.3.1.2.Temperature

Temperature is one of the most influencing factors for algal growth rate, size distribution for cell and compositional behavior in biochemical content which varies with increase and decrese in temperature ranges. Generally, 20-35ºC is the optimal range for temperature in Indian environmental conditions. It has also been founnd that this range sometimes vary according to the type of strain, geographical regions and seasonal variation (Juneja *et al.*,2013). It has been noted that photoinhibition process is also affected by the temperature variation, which has a direct impact on algal biomass. In the present study, *Chlorella pyrenoidosa* shows an adaptability for temperature ranges from 20-50ºC for specific growth rates. Similarly, biomass productivity for selected algal strain was found maximum at 30ºC(1.12±0.04g/L) whereas minimum biomass productivity was noticed at 50 ºC (0.423±0.03g/L). Similar trend of biomass productivity of *Chlorella vulgaris* was also observed by Chinnasamy *et al.,* (2009) and Converti *et al.,* (2009). They also suggested that increase in temperature from 30ºC leads to an abrupt halt in algal growth and cell dies. Whereas with *Chlorella pyrenoidosa*, growth rate was observed till 50ºC but deformation was noticed at higher temperature ranges.



(a) **(b)**

Fig.3.1:(a) Influence of LEDs on *Chlorella pyrenoidosa* for (a) Red (R),Yellow (Y),Orange(O), Green (G) and Blue(B)

(b) Biomass productivity (g/L) and Lipid content (%)

Graphical representation for biomass productivity and lipid content clearly depicts the data obtained from experimental findings in Phase-I setup as given in Fig.3.2. a and b.

Botrycoccus bruni, a green algae is observed with the difference in lipid consumption during different growth temperatures. A decrese in lipid content leads to an accumulation of polysaccharides. Furthermore, similar effects were also observed with other algal strains like *Nannochloropsis oculata* and *Chlorella vulgaris*. Bajguz, (2009) discussed the role of heat stress and heat shock for algal protien content with decrese/increase in temperature and produce absisic acid (ABA) as a stress hormone. Hence, temperature is the important stress factor which influences the lipid content ranges. This statement is supported by the finding of our results like maximum lipid content was observed at 20ºC (33.26%) and minimum lipid content (20.43%) at 20ºC.

3.3.1.3.DIWW

Wastewater can be a sustainable growth medium for algal feedstock (Kothari *et al.*,2012; 2013, Shamshad *et al.*,2018). Certain marine algal species like *Scenedesmus, Chlorella* sp. *Spirulina etc.*, can be an effective tool for wastewater treatment since, they provide an integrated approach like wastewater treatment with algal biomass growth and different value added products can also be explored. In this present study, initial value of DIWW was recorded on the frist day of collection and higher ranges of parameters (N and P) were noticed. Higher ranges of N and P in wastewater supports the algal growth deliberated by various researchers in literature. So, selected wastewater DIWW supports the algal growth of *Chlorella pyrenoidosa* species selected by us. Similarly, algal growth was monitored in term of increase in the optical density at 680nm with different concentrations of (0% 25% 50%,75% and 100%) industrial diary wastewater. The results show that algal growth increased with increasing wastewater fraction (10-75v/v %) (Kothari *et al.*,2013). However, algal

growth at 100% concentration of wastewater was as such uninhibited and 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 of dairy industry waste water, when compared with the growth obtained at 100% concentration of wastewater. However, lower concentrations of wastewater (10–50%) did not support better algal growth as compared to control, perhaps due to low level of nutrients at lower concentrations of waste water. Based on these observations, the concentration of dairy industry wastewater selected for further experiments was 75% (v/v). Similar results are obtained on dairy industry waste water treatment by using the alga *Botrycoccu*s, which presented a good example of phycoremediation by consuming the nitrogen and phosphorus components of the wastewater (Kothari *et al.,* 2013).

Similarly, Ding *et al.,*(2014) observed the growth of unicellular algal strain. Gurviaiah *et al.,* (2012) also provide that *Scenedesmus* and *Chlorella* sp. have the potential to grow in DIWW with concentration (10-80 v/v%) and best finding was observed with 80% wastewater. DIWW concentration supports the biomass growth and lipid productivity at optimal level maximum biomass growth (0.58g/L) at 75% and lipid content (21.27%) at 100% DIWW as shown in Fig.3.3(a and b).

3.3.1.4.Nitrogen

Nitrogen is responsible for cell growth as well as biochemical composition in algal biomass which makeup the lipid and carbohydrate content for biofuel and other value added products (Juneja *et al.,*2013). N stress is an important concept which is rarely considered.It seems probable that the occurrence of physiologically induced stress from the basis for the several studies have shown that lipid tends to be accumulated in nitrogen different conditions (Phaniendra *et al.,*2015). In general, there is an inverse

relationship between lipid content and nitrate concentration. Algal biomass utilize the various forms of nitrogen NO_3^- NO_2^- and NH_4^+ organic N sources such as urea.Each N_2 source is frist reduced to the amino acid through avariety of pathways. Several researches focused their studies on nitrogen stress with different strains of algae (Minhas *et al.*,2015).It has been reported that *Chlorella* sp. prefer NO_3^- rather that NH_4^+ for growth and they also effectively utilize Organic-N sources such as urea, glycine and yeast extract (Kim *et al.*,2016).

Similarly, N sources are also helpful in gaining the end product as demanded eg. Protien content of *Dunella* is 2 times higher with NH_4^+ (Hannon *et al.*,2010) but lipid content of *Chlorella sorokiniana* in comparison to urea or NO_3^- as a supplement. Similarly Daliry *et al.,* 2017 observed that an increase in algal biomass growth was noticed with increasing nitrate concentration from 0.2 to 5 mM and lipid content was found highest in lower concentration of NO_3^-.Similarly biomass productivity for selected algal strain *Chlorella pyrenoidosa* was found maximum (0.86±0.04 g/L) at 0.2 g/L and minimum (0.21±0.03 g/L) at 0.05 g/L of NO_3^- concentration as shown in Fig.3.4(a and b).

3.3.1.5.Phosphorus

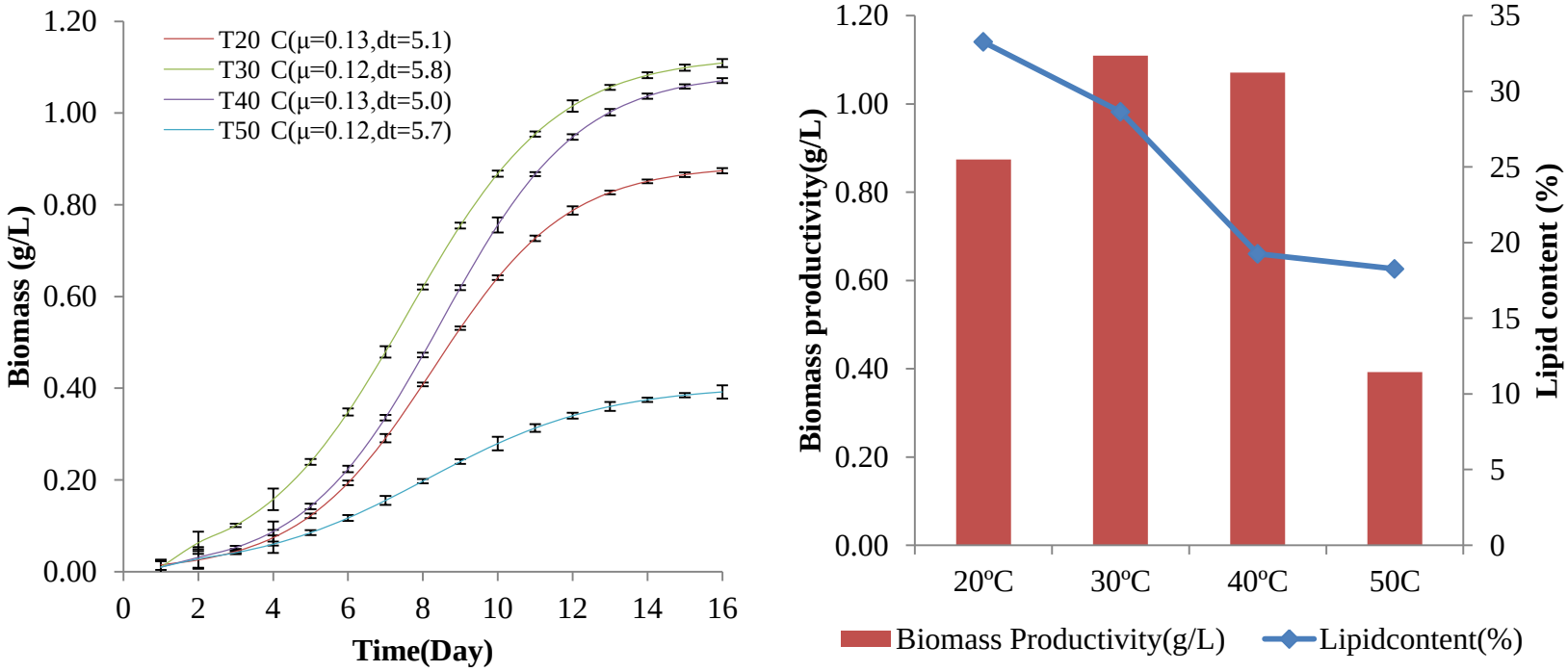
Phosphorus in the form of orthophosphate is generally considered the main limiting nutrient in fresh water aquatic systems that are if all phosphorus limited system, excess phosphorus will trigger eutrophication (Correll, 1999). The higher doses of phosphate reduced growth and chlorophyll, but an increase in soluble sugar and amino nitrogen was noticed. Li-Yeguang *et al*.,(2006) found that growth rate increased linearly with the increase of P concentration when it was below 0.05 mg/L. and was almost unchanged when the P concentration exceeded 0.2mg/L. According to Patel *et al*.,(2012) Phosphorus uptake was dependent on species, duration of exposure

and treatment, with significant interaction effects. Growth was dependent on species and treatment. Not all species showed increased P removal with increasing P addition, and no species demonstrated higher growth. Kalla & Khan (2016) stated that decreasing phosphorus concentration in medium had an effect on biomass and lipid content of *C. vulgaris*. Significant decrease in growth and biomass was observed with the decrease of concentration in the medium from (1.5g/l to 0.0g/l) and (0.04g/l to 0.0g/l) respectively. Whereas the lipid accumulation showed reverse trend of increase when the concentration of both phosphorus and nitrogen decreased in medium. Biomass productivity of *Chlorella* at different concentrations of PO_4^{-3} found maximum (0.65 g/L) at 0.75 g/L and minimum (0.5 g/L) at 0.050 g/L. Lipid content and productivity was also affected by the concentration of phosphate of *Chlorella pyrenoidosa*, maximum (18.3%) at 0.050 g/L and minimum (16.5%) at 0.75 g/L respectively as given in Fig.3.5(a and b).

3.3.1.6.Carbon

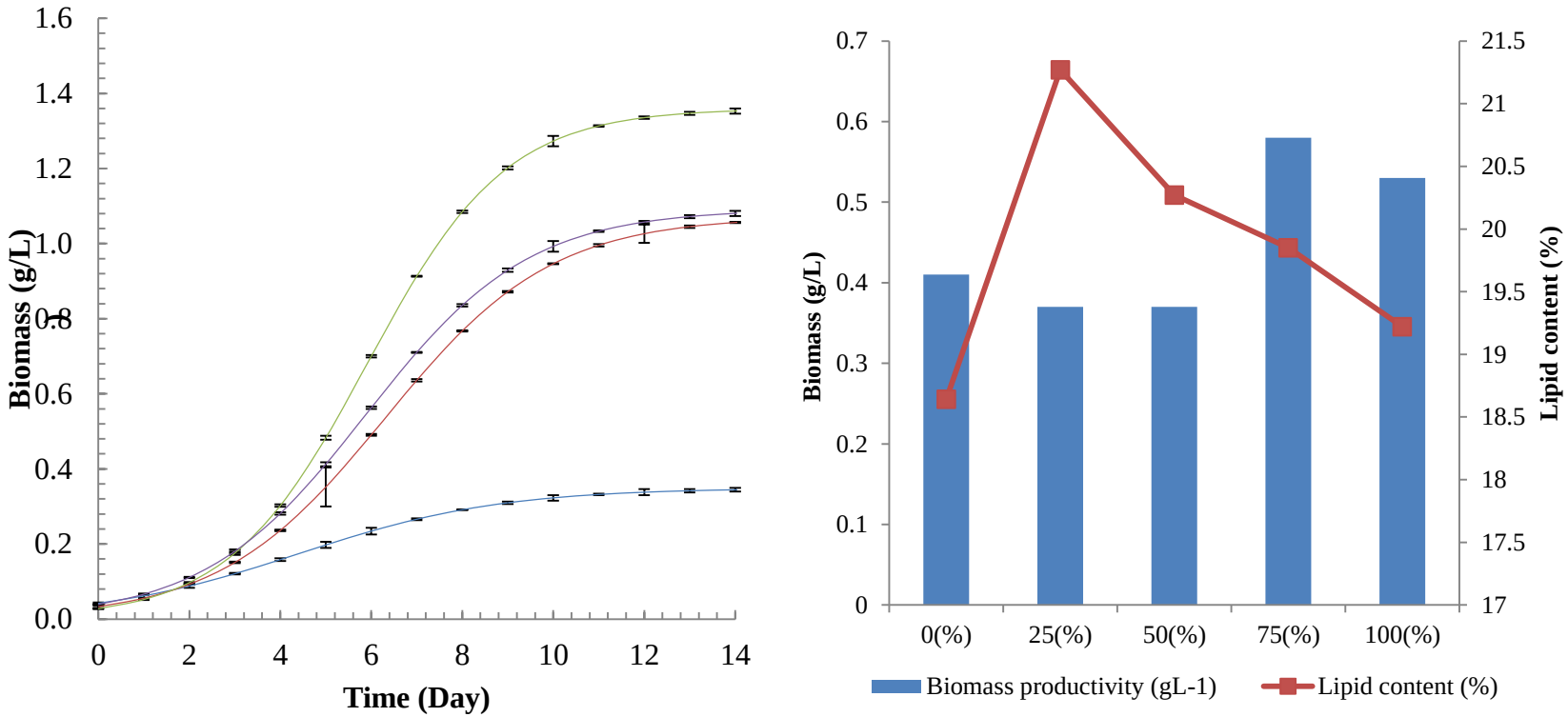
Natural changes in climate due to internal as well as external factors, like anthropogenic emissions, fossil fuel combustion, transportation and heating which cause CO_2 emissions is one of the major issues which causes global warming (increasing concentrations of greenhouse gases). The production of algae is identified as one of the solutions of carbon sequestration along with the production of renewable fuel solving the problem of food crisis to a certain extent. Microalgae are photosynthetic microorganisms that can help in CO_2 mitigation and at the same time produce value added compounds. Carbon capture and sequestration is a safer technology to reduce the environmental carbon dioxide. The robust interest in CO_2 to algae results from its implicated photosynthesis conversion efficiency of as much as 12%.

Most current research on oil extraction is focused on microalgae to produce biodiesel from algal oil. Optimizing the cultivation conditions and increasing CO_2 tolerance are indispensable to the efficient application of CO_2. According to Adamczyk *et al.,* (2016), species of the genera *Chlorella, Scenedesmus, Spirulina, Nannochloropsis*, and *Chlorococcum* are characterized by rapid growth, tolerance to stress factors, and tolerance against high concentrations of CO_2, which indicates its effective accumulation and utilization. Rendeon *et al.,* (2013) found that *Chlorella* sp can grow relatively rapidly under the ambient air concentrations of CO_2 (0.037%) but maximizing growth with increasing the CO_2 concentration upto maximum level 20%to 50%. *S. obliquus* show highest growth and biomass productivity at 10% of CO_2 but lipid content maximum at 5% CO_2 (Adamczyk *et al*., 2017). In present study, selected range of CO_2 concentration support the growth upto optimum level, the highest biomass growth (1.13 g/L) at 15% and minimum (0.43 g/L) at 100% CO_2 and maximum CO_2 fixation efficiency was noticed at 10% CO_2 as given in Fig.3.6 (a and b).



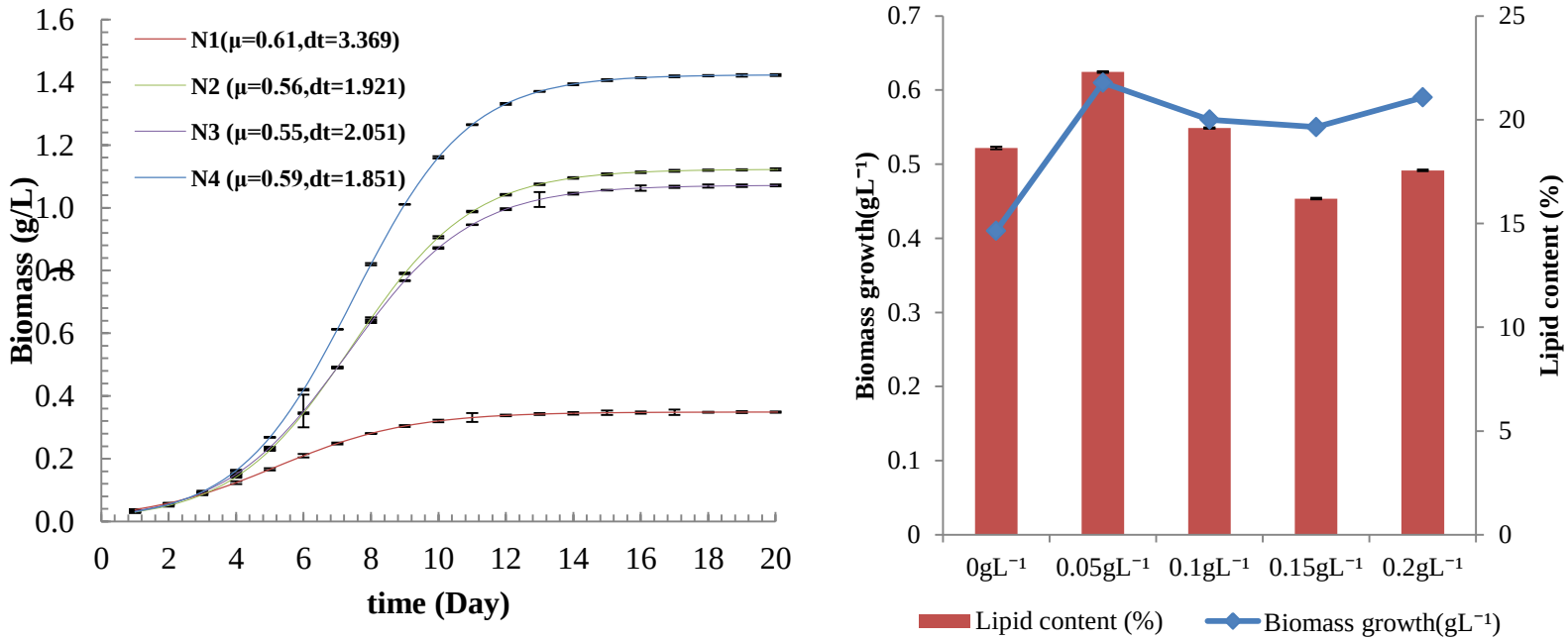
(a) **(b)**

Fig.3.2:(a) Growth kinetics of *Chlorella pyrenoidosa* at different temperature (b) Biomass productivity and lipid content at differenert temperature



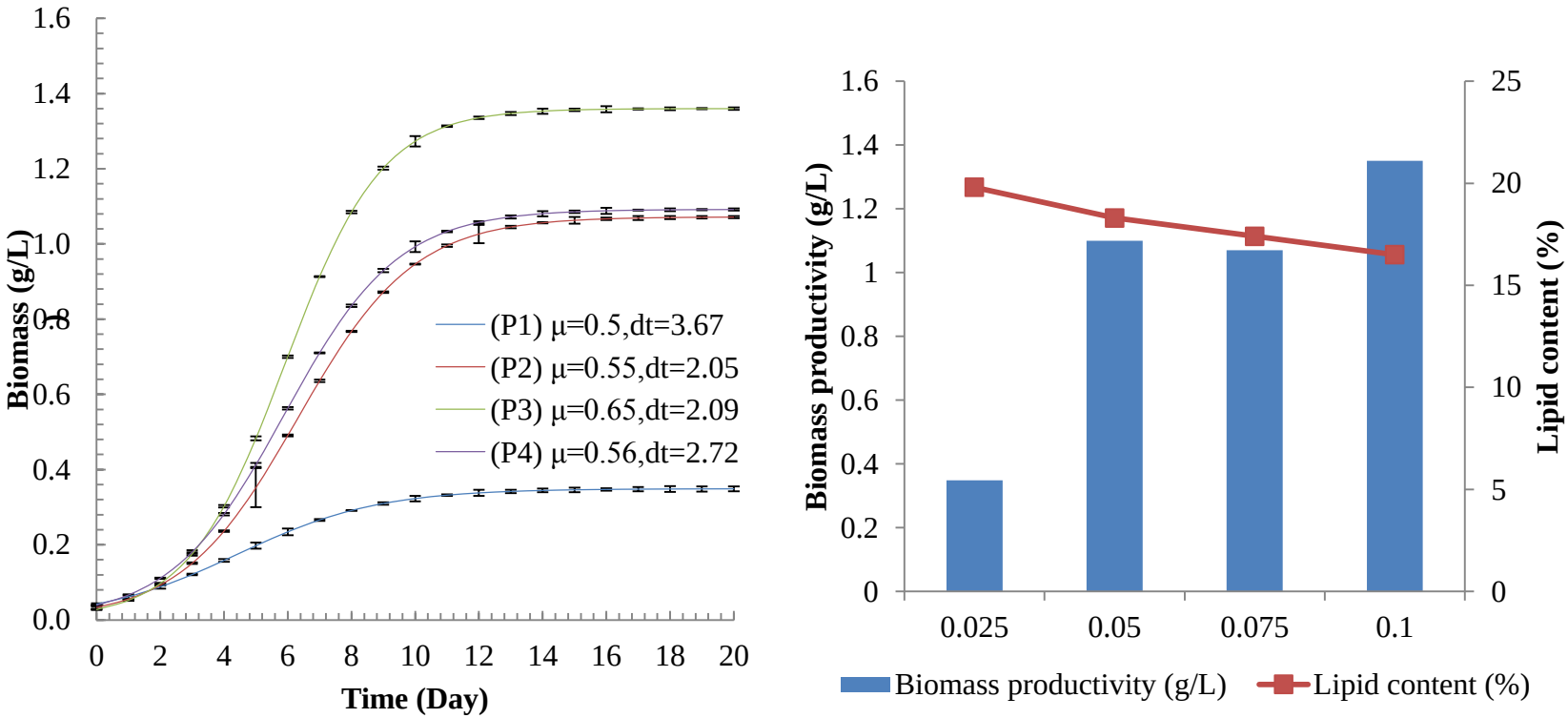
(a) **(b)**

Fig.3.3: (a) Growth kinetics of *chlorella pyrenoidosa* at different concentration of DIWW (b) Biomass productivity and lipid content at differenert concentration of DIWW



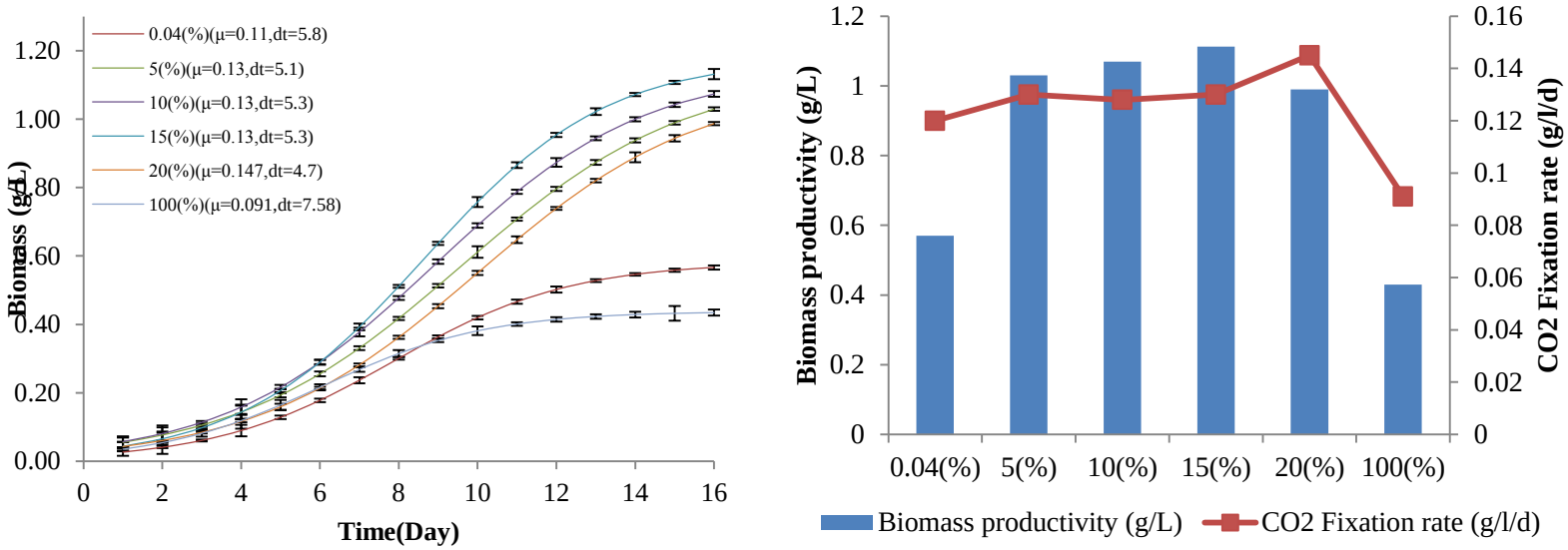
(a) **(b)**

Fig.3.4: (a) Growth kinetics of *Chlorella pyrenoidosa* at NO_3^- concentration (b) biomass productivity and lipid content at differenert NO_3^- concentration



(a) **(b)**

Fig.3.5: (a) Growth kinetics of *Chlorella pyrenoidosa* at different PO_4^{-3} concentration (b) biomass productivity and lipid content at differenert PO_4^{-3} concentration



(a) **(b)**

Fig.3.6: (a) Growth kinetics of *Chlorella pyrenoidosa* at different % of CO_2 concentration (b) biomass productivity and CO_2 uptake differenert CO_2 concentration

3.3.2. Spectral variations and their impacts on biomass and lipid productivity in coupling with other different variables using response surface methodology (RSM)

In conventional optimization processes, an individual factor is varied while keeping the remaining factors constant, which represents a single factor time operation. Conventional experimental and statistical methods are therefore unable to explain the effects of the interaction of variables during real time situation. Thus, RSM provides a systematic statistical design approach to explore the combined influence of two or more variables used in the experiment. To get the more appropriate results and findings, various combinations of influencing parameter were taken as variables and all experiments were designed accordingly, predicting these situations as probabilities in real life condition for higher yields of biomass and lipid productivities. The combination of data from RSM and experiment helps in development of framework for growth and oil from algal strain for cost efficiently and scale up value added end products.

3.3.2.1. Effect of Nutrients (NO_3^-, PO_4^{-3}) with variable in DIWW concentration without LEDs

In the present study, *C. pyrenoidosa* was grown under various manipulated concentrations of nitrate (0, 50, 75, 100, 200 mg/L), phosphate (0, 25, 50, 75, 100 mg/L) and DIWW (0, 25, 50, 75, 100%). Biomass productivity (mg/L/d), lipid content (%) and percentage removal of pollutant load (COD, TN and TP) were considered as response variables. In present study, *Chlorella pyrenoidosa* was grown under various manipulated conditions done to influencing parameter and results observed for the experimental basis is divided into three main subsections to study the impacts extensively with RSM as;

3.3.2.1.1. Growth Kinetics with NO_3^-, PO_4^{-3} and DIWW concentration

Data shown in Fig.3.7 revealed algal growth pattern influenced by culture medium conditions. Data for variables of growth kinetics such as O.D. (a), rate constant (K) and doubling time (μ) are provided in Table 3.5. In a total of 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 mg/L NO_3^- and 50 mg/L 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 4^{th} run, which was designed with a combination of 75% DIWW, 150 mg/L NO_3^- and 25 mg/L PO_4^{-3} concentration. Thus, it is clear that phosphorus acts as a limiting factor in growth of algal biomass in comparison to nitrate and DIWW content. The increase in DIWW content was found to negatively affect algal growth, which is possibly due to excessive nutrient inhibition of algal growth. Similar findings were also observed on limited increment in growth of *Chlorella* sp. with increasing concentrations of industrial wastewater (Benavente-Valdés *et al.,* 2016; Pathak *et al.,* 2015).

The results suggest that the model used was highly suitable with a minimal error at different concentrations of DIWW with normal and depleted nutrient values. The stress conditions assessed a decrease in nutrient content appeared to accelerate neutral lipid accumulation in algal biomass (Duong *et al.,* 2015). Based on the results of previous studies, this increased lipid accumulation leads to a decrease in growth rate but increased production of oil (James and Boriah, 2010).

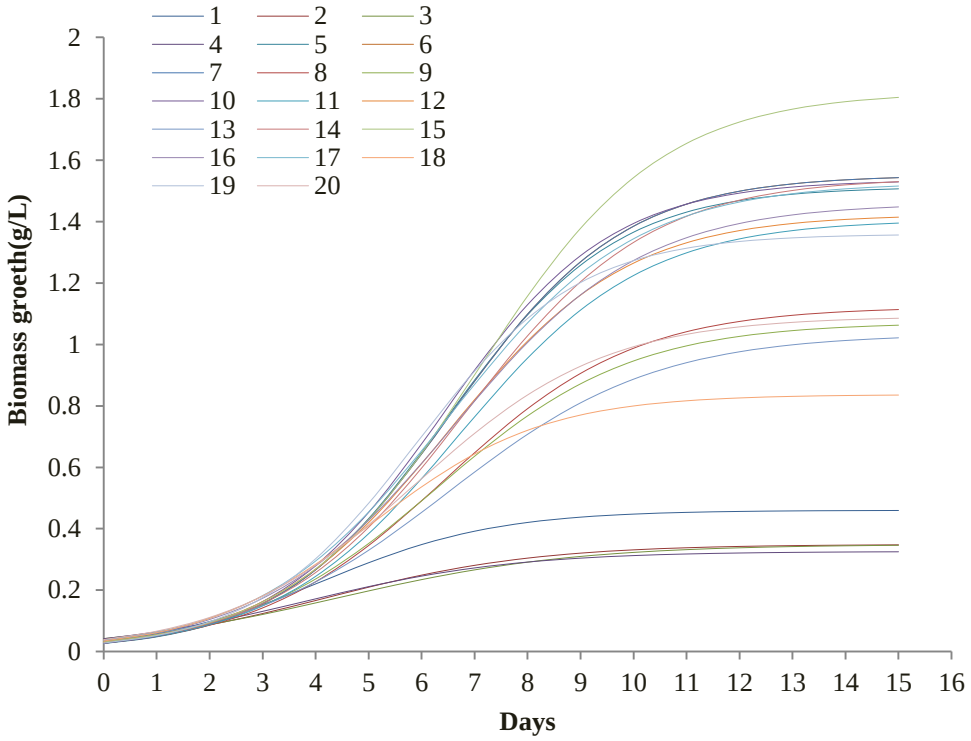


Fig.3.7:Growth kinetics of *C. pyrenoidosa* at different variable conditions

Table 3.5:Summary of growth kinetics for *C. pyrenoidosa* grown at different concentrations of DIWW, $NO_3^-$and PO_4^{-3}

S.N	DIWW	NO_3^- (mg/L)	PO_4^{-3} (mg/L)	μ (day^{-1})	dt (d^{-1})
1	0	0	0	0.62	2.80
2	100	0	0	0.61	3.37
3	0	200	0	0.5	3.67
4	100	200	0	0.45	3.84
5	0	0	100	0.5	4.07
6	100	0	100	0.63	1.78
7	0	200	100	0.61	1.77
8	100	200	100	0.56	1.92
9[a]	50	100	50	0.55	2.05
10[a]	50	100	50	0.63	1.77
11[a]	50	100	50	0.58	1.74
12[a]	50	100	50	0.59	1.87
13	-34.08	100	50	0.51	2.35
14	134.08	100	50	0.57	1.70
15	50	-68.17	50	0.58	1.64
16	50	268.17	50	0.56	1.92
17	50	100	-34.08	0.57	1.89
18	50	100	134.08	0.62	2.78
19[a]	50	100	50	0.65	2.10
20[a]	50	100	50	0.56	2.72

[a] central point

3.3.2.2. RSM for optimization of process variables

The Central composite design (CCD) of the RSM was proposed for identifying the optimum levels for selected variables such as DIWW (0-100%), NO_3^- (0-200mg/L), and PO_4^{-3} (0-100mg/L) and results of observed and predicted responses after statistical and matmatical analysis is given in Table .3.6. The second order polynomial was applied to determine the relationship between variables and responses and regression coefficients were also calculated. A flat surface on the three-dimensional (3D) response graphs indicates an optimum condition for a given response as shown in Analysis of variance (ANOVA) which also ensures the goodness of fit model for biomass productivity and lipid content. A prob >F value of <0.5 and >0.5, indicated the model terms which were significant and non-significant, respectively. In this assumption, the residual graph was applied to check the homogeneous effrontery by plotting the studentized residuals against the predicted value of probability. Homogenously spreading of COD removal, TN (%), TP (%), biomass productivity and lipid content data on either side of the zero line indicated the suitability of the model in this study as depicted in Fig.3.8 (a-e).

The coefficient of variance and standard deviation (SD) indicated very high degree of accuracy and excellent reliability of the experimental values of biomass productivity (g/L) and lipid content (%) as shown in Table 3.7. and COD removal (%), TN (%), TP (%) in Table 3.8.

3.3.2.2.1 Effect of optimized process variables on pollutants removal (COD and nutrient removal)

In order to achive high removal efficiency of pollutants (COD, TN and TP), the major factors NO_3^- and PO_4^{-3} by using CCD model by using 20 run discussed below:

Table .3.6:The minimum central and maximum variable levels in the central composite design(CCD) for various responses (observed and predicted values) for study on *C. pyrenoidosa* grown at different concentrations of DIWW, $NO_3^-$and PO_4^{-3}

	Variables			Observed value					Predicted value				
S.N	DIWW	NO_3^- (mg/L)	PO_4^{-3} (mg/L)	Biomass productivity (g/L)	Lipid (%)	COD (%)	TP (%)	TN (%)	Biomass productivity (g/L)	Lipid content (%)	COD (%)	TP (%)	TN (%)
1	0	0	0	0.86	29.65	42.28	99.5	98.24	0.99	30.04	41.71	99.44	95.76
2	100	0	0	1.12	27.56	42.33	99	95.32	1.16	27.51	42.05	98.47	91.56
3	0	200	0	1.07	26.54	41.26	98.25	91.37	1.32	25.6	40.89	97.18	93.12
4	100	200	0	0.85	25.31	42.25	98.64	69.23	1.28	25.06	41.77	99.17	70.6
5	0	0	100	0.85	32.54	41.68	>99	92.34	0.68	31.71	41.22	99.07	88.02
6	100	0	100	1.15	28.31	43.32	97.32	97.34	1.06	28.17	42.75	98	92.66
7	0	200	100	1.49	24.76	41.68	97.25	85.67	1.43	23.73	41.03	97.37	86.5
8	100	200	100	1.36	23.65	43.47	99.6	73.25	1.61	22.18	43.1	99.26	72.82
9[a]	50	100	50	1.24	28.65	42.88	>99	96.33	0.85	28.5	42.75	99.52	95.93
10[a]	50	100	50	1.54	28.65	42.88	99	96.33	0.85	28.5	42.75	99.52	95.93
11[a]	50	100	50	0.85	28.65	42.88	99.5	96.33	0.85	28.5	42.75	99.52	95.93
12[a]	50	100	50	0.62	28.65	42.88	99.4	96.33	0.85	28.5	42.75	99.52	95.93
13	0	100	50	0.85	25.32	39.8	96.25	86.34	1.25	26.05	40.43	97.15	86.985
14	100	100	50	1.13	22.19	42.05	98.36	69.36	1.54	22.62	42.45	97.93	71.9658
15	50	50	50	1.26	34.41	42.34	99.8	93.64	0.62	34.08	42.85	>99	>99
16	50	200	50	1.52	23.82	41.94	99	85.92	1.35	25.31	42.46	99.21	81.9443
17	50	100	-0	1.37	27.21	40.75	98.2	92.34	1.13	27.02	41.16	98.62	92.2931
18	50	100	100	0.85	24.65	41.25	98.35	84.42	1.14	26.013	41.86	98.38	87.6563
19[a]	50	100	50	0.58	28.65	42.88	99.9	96.33	0.85	28.5	42.75	99.52	95.93
20[a]	50	100	50	1.12	28.65	42.88	99.6	96.33	0.85	28.5	42.75	99.52	95.93

a central point

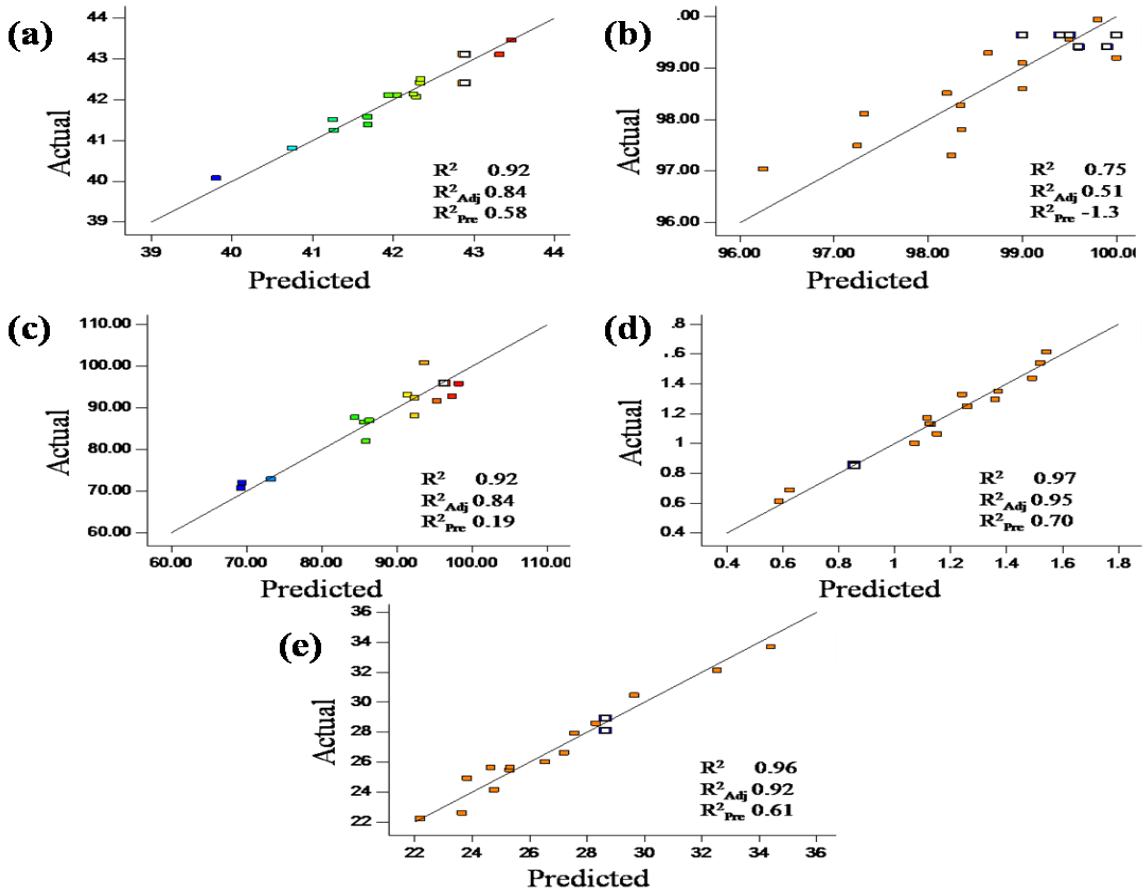


Fig.3.8:Normal plot of actual versus predicted probability for [(a) COD $_{(removal\ \%)}$;(b) phosphate(%) removal; (c)nitrate(%) removal; (d)biomass productivity (g/L); (e) lipid accumulation (%) associated with for *C. pyrenoidosa* grown at different concentrations of DIWW, NO^{3-} and PO_4^{-3})

Table 3.7:Analysis of variance (ANOVA) for nutrient removal, including COD, for *C. pyrenoidosa* grown at different concentrations of DIWW, NO_3^- and $PO4^{-3}$

		Response (3) for total phosphorus (%) ANOVA for Response Surface Quadratic Model(RSQM)				Response (3) for total nitrogen (%) ANOVA for Response Surface Quadratic Model(RSQM)				Response (3) for $COD_{(removal)}$ (mg/L) ANOVA for Response Surface Quadratic Model(RSQM)			
Source	**df**	**Sum of Squares**	**Mean Square**	**F Value**	**Prob > F**	**Sum of Squares**	**Mean Square**	**F Value**	**Prob > F**	**Sum of Squares**	**Mean Square**	**F Value**	**Prob > F**
Block	1	0.36	0.36			32.16	32.16			2.69	2.69		
Model	9	14.77	1.64	3.05	0.0560	1471.43	163.49	11.47	0.0006	12.19	1.35	11.22	0.0007
X_1	1	0.71	0.71	1.32	0.2810	272.79	272.79	19.14	0.0018	4.97	4.97	41.15	0.0001
X_2	1	0.86	0.86	1.60	0.2380	430.80	430.80	30.23	0.0004	0.19	0.19	1.58	0.2401
X_3	1	0.069	0.069	0.13	0.7293	26.10	26.10	1.83	0.2090	0.60	0.60	4.96	0.0530
X_1X_2	1	4.38	4.38	8.14	0.0190	167.81	167.81	11.78	0.0075	0.15	0.15	1.22	0.2973
X_1X_3	1	$6.05x10^{-3}$	$6.05x10^{-3}$	0.01	0.9179	38.90	38.90	2.73	0.1329	0.71	0.71	5.85	0.0387
X_2X_3	1	0.16	0.16	0.30	0.5960	0.61	0.61	0.042	0.8413	0.20	0.20	1.65	0.2314
X_1^2	1	7.10	7.10	13.2	0.0055	489.12	489.12	34.32	0.0002	3.11	3.11	25.73	0.0007
X_2^2	1	0.021	0.021	0.04	0.8469	37.33	37.33	2.62	0.1400	0.018	0.018	0.15	0.7113
X_3^2	1	1.86	1.86	3.46	0.0959	63.82	63.82	4.48	0.0634	2.78	2.78	23.01	0.0010

***Values of 'Prob > F' less than 0.05indicate model terms; NA implies that the co-efficient are not applicable for linear model.**

Table 3.8:Analysis of variance (ANOVA) for biomass and lipid productivity (mg/L) for *C. pyrenoidosa* grown at different concentrations of DIWW, $NO_3^-$and PO_4^{-3}

Source		Response (1): Biomass Productivity (g/L); ANOVA for Response Surface Quadratic Model (RSQM)				Response (2): Lipid Productivity (mg/L); ANOVA for Response Surface Quadratic Model (RSQM)			
		Sum of Squares	Mean Square	F Value	p-value Prob > F	Sum of Squares	Mean Square	F Value	p-value Prob > F
Block	**df**	$3.1x10^{-5}$	$3.1x10^{-5}$			3.72	3.72		
Model	1	1.52	0.17	37.45	< 0.0001*	158.35	17.59	23.28	< 0.0001*
X_1	9	0.10	0.10	22.57	0.0010*	14.20	14.20	18.79	0.0019*
X_2	1	0.66	0.66	145.09	< 0.0001	92.85	92.85	122.88	< 0.0001*
X_3	1	$6.67x10^{-6}$	$6.67x10^{-6}$	$1.47x10^{-3}$	0.9702	1.23	1.23	1.63	0.2332
X_1X_2	1	0.020	0.020	4.45	0.0641	1.98	1.98	2.62	0.1400
X_1X_3	1	0.022	0.022	4.81	0.0559	0.51	0.51	0.67	0.4325
X_2X_3	1	0.089	0.089	19.76	0.0016*	6.27	6.27	8.29	0.0182*
X_1^2	1	0.53	0.53	118.21	< 0.0001*	31.16	31.16	41.24	0.0001*
X_2^2	1	0.031	0.031	6.96	0.0270*	2.59	2.59	3.42	0.0972
X_3^2	1	0.14	0.14	31.72	0.0003*	7.10	7.10	9.39	0.0135*

***Values of 'Prob > F' less than 0.05indicate model terms; NA implies that the co-efficient are not applicable for linear model.**

3.3.2.2.1.1.COD (removal %)

Statistical analysis of the positive linear coefficient indicated that time was the most important factor affecting all responses in Eq.3.1. Therefore, the relationship of COD (removal %) with variables as DIWW, TN and TP concentration using *C. pyrenoidosa* can be inferred from Eq.(3.1). The significant 3D plot define sensitivity of all the responses with two interacting variables by accommodating the other variables at the central values. The positive value for nitrate and phosphate variable indicated that among the selected variables, these two parameters had the most pronounced effect on COD removal.

The high value of 0.60 for the linear coefficient of DIWW concentration and linear (X_1), interactive (X_1X_3) and Quadratic (X_1^2, & X_3^2) in Eq.(3.1), illustrate the significant positive effect of these variables on COD (removal%).The negative quadratic effect on the coefficient of the independent variables (X_2^2) on COD (removal %) was observed as a function of these variables by keeping all other variables at a fixed level. These results suggested that a higher level of both these factors yielded a smaller response. The non-significant value (>0.05) for all the quadratic variables indicated that there was no interaction among all the variables, i.e. a change in one variable did not affect another. The COD (removal %) properties of *C. pyrenoidosa* under different initial NO_3^- and PO_4^{-3} levels are shown in Fig.3.9a.

The F-value of 13.35 of the model was significant (p=0.0001), which further substantiates the suitability of the model for the given data. From the analysis by the model (R^2_{adj} and R^2_{pred}), the R^2_{pred} of 0.59 was not in agreement with the R^2_{adj} of 0.83.

$$Y_{COD\ removal\ (\%)} = 42.76+0.6X_1-0.12X_2+0.21X_3+0.14X_1X_2+0.3X_1X_3+0.16X_2X_3-0.46X_1^2-0.035X_2^2-0.44X_3^2 \quad \textbf{(3.1)}$$

3.3.2.2.1.2.TN and TP removal

Coupling of the microalgal growth and lipid production with DIWW treatment implies that utilization of nutrients (TN&TP) should be examined in addition to algal growth with lipid accumulation. The potential of nutrient removal (TN and TP) after 15 days of culture are shown in Table 3.6. The removal efficiency of phosphorus was >99%, however, the efficient removal of nitrogen was under the influence of the initial concentration of phosphorus. The PO_4^{-3} removal potential of *C. pyrenoidosa* under different NO_3^- and DIWW levels are shown in Fig.3.9b which indicates the interactive (X_1X_2) and quadratic (X_1^2) model are significant using Eq.(3.2) (F-value of 6.21).

$$Y_{(TP\%)}=99.52+0.23X_1-0.25X_2-0.07_1X_3+0.74X_1X_2-0.027X_1X_3+0.14X_2X_3-0.70X_1^2 +0.038\ X_2^2-0.36X_2^3 \quad \textbf{(3.2)}$$

Nitrogen removal efficiency data suggest that linear (X_1 & X_2), interactive (X_1X_2) and quadratic (X_1^2) model terms were significant (F-value of 11.47; Fig.3.9c.When the added P was greater than or equal to 4 mg/L, nitrogen removal efficiency reached >99% which authenticates the findings of other studies. The efficiency of nitrogen removal decreased the limitation of phosphorus which influences the microalgal growth. TN and TP could be analyzed and optimized by Eq.(3.3). For TN during the growth of *C. pyrenoidosa*, uptake of nutrients (N/P) was not necessarily the same as the constituent of algae.

$$Y_{(TN\%)} = 95.93-4.47X_1-5.62X_2-1.38X_3-4.58X_1X_2+2.21X_1X_3+0.28X_2X_3-5.83X_1^2 +1.61X_2^2-2.11X_2^3 \quad \textbf{(3.3)}$$

3.3.2.2.2. Effect of optimized process variables on algal growth productivity (biomass productivity and lipid content)

3.3.2.2.2.1. Biomass productivity

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} is well presented by the 3D plot (Fig.3.10a). The prob>F values for the linear (X_1& X_2), quadratic (X_1^2, X_2^2, and X_3^2) and interactive (X_2X_3) effects of DIWW, NO_3^- and PO_4^{-3} on growth of *C. pyrenoidosa* were found significant in Table 3.9. This observation also suggests that DIWW concentration (trace X_1) had the least effect on algal biomass weight. Alternatively, *C. pyrenoidosa* dry weight was greatly influenced by NO_3^- (increasing throughout the range; trace X_2) and quite sensitive to PO_4^{-3} (trace X_3). It should be noted though that the optimal range of nutrients (NO_3^- and PO_4^{-3}) lies outside the range of experiment values. The lack of fit test with p-value of 0.05 showed that the model is satisfactorily fitted with the experimental design data (Abinandan & Shanthakumar, 2016).

When R^2_{pred} values were compared to the actual values of biomass yield for *C. pyrenoidosa* R^2_{adj} was 0.948, indicating 94.8% model variability. The predicted and experimental values suggest that data fitted well with the RSM model and gives an accurately estimated response for the system using Eq.(3.4). Predicted and actual response of lipid content is given in Fig.3.8 d. On the other hand, the residual responses versus predicted responses showed a structureless plot suggesting that the model is acceptable and does not show any violation.

$$Y_{Biomass(mg/L)}=0.85+0.086X_1+0.22X_2+6.99x10^{-4}X_3+0.05X_1X_2+0.052X_2X_3+0.19X_1^2+\ 0.047X_2^2 +0.1X_3^2 \quad \textbf{(3.4)}$$

3.3.2.2.2.2. Lipid content (%)

Lipid productivity of *C. pyrenoidosa* was greatly influenced by different DIWW combinations with nitrate and phosphate, which is shown in Fig.3.10b. The observations revealed higher lipid accumulation with lower concentrations 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 values 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 from Table 3.9. These results indicate that DIWW, NO_3^- and PO_4^{-3} ratio have a significant effect on lipid content in *C. pyrenoidosa*. The predicted values of lipid content calculated using the quadratic Eq.(3.5) ranged between 11.16–56.62% (dry basis). The experimental design model observed that the effects of the linear terms (X_1&X_2), quadratic terms (X_1^2 and X_3^2) and interactive terms (X_2X_3) were highly significant (P<0.05). However, linear term (X3), quadratic term (X_2^2) and interactive term (X_1X_2 &X_1X_3) did not affect lipid content significantly (P>0.05). The combined influence of DIWW, nitrate and phosphate can be represented in terms of second order polynomial Eq.(3.5), which is represented as:

$$Y_{lipid(\%)}=28.5-1.02X_1-2.61X_2-0.3X_3+0.5X_1X_2-0.25X_1X_3-0.88X_2X_3-1.47X_1^2+0.42X_2^2-0.7X32 \quad \textbf{(3.5)}$$

The significant prob>F values for the linear, quadratic and interactive effects of DIWW, NO_3^- and PO_4^{-3}, were evaluated. Moreover, the F-value of 23.28 demonstrated that the model was meaningful, as revealed by a p-value <0.0001, which supported the data for model validation. From the analysis of data, the R^2_{pred} of 0.61was in good agreement with the R^2_{adj} of 0.92 in Fig.(3.8e) and the data was well explained by this model. It is well known that deprivation of NO_3^- increases lipid

content in microalgae by 2 folds (Simionato et al., 2013). Some researchers observed that maximum lipid content and biomass production occur at different values of the same parametric response. Hence, it is unlikely that maximum values for both responses can be found within the same experimental set-up. The microalgal biochemical system is similar to that of photosynthetic plants, in that it depends on macro- and micro-nutrient supply (Karemore et al. 2013). Advantages of coupling algal growth with wastewater treatment are that the wastewater provides nutrients for algal growth while at the same time saves freshwater and promotes lipid accumulation for biofuel production. Whilst some of the nutrients found in wastewater are growth limiting, others are involved in several enzymatic reactions for metabolite biosynthesis (Karemore *et al.,* 2013).

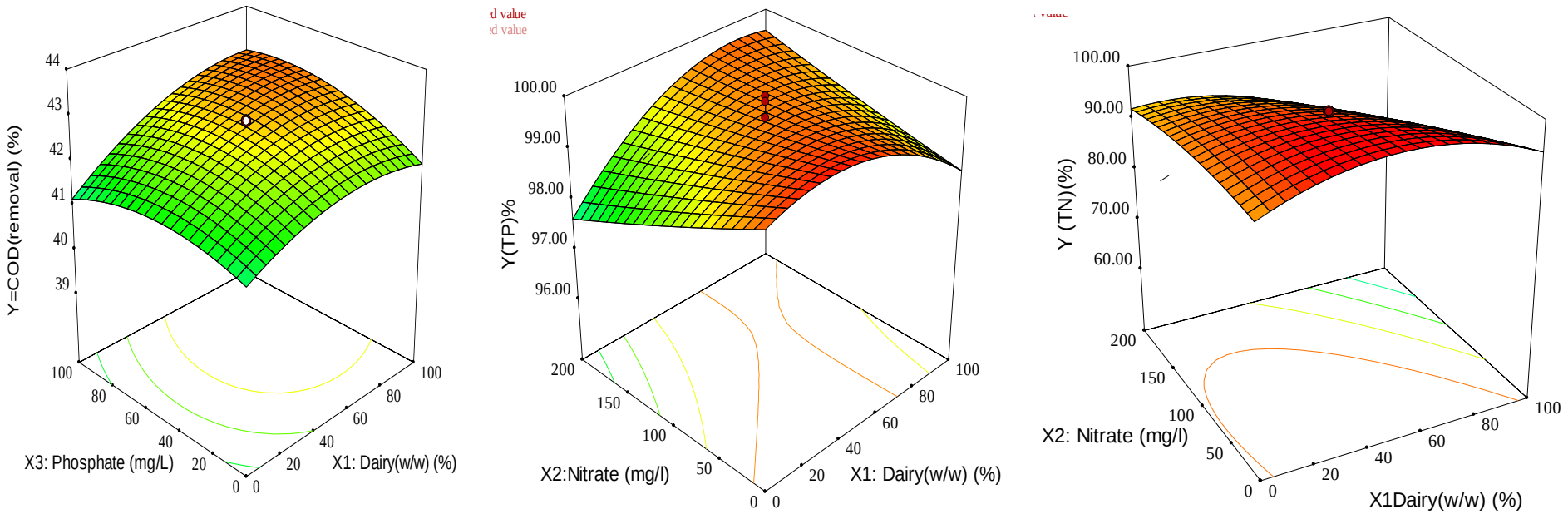


Fig.3.9: Effect of NO_3^- ,PO_4^{-3} and DIWW variables for removal by *C. pyrenoidosa* (a)COD $_{(Removal\%)}$ (b) Total Nitrogen (TN) (c) Total Phosphorus (TP)

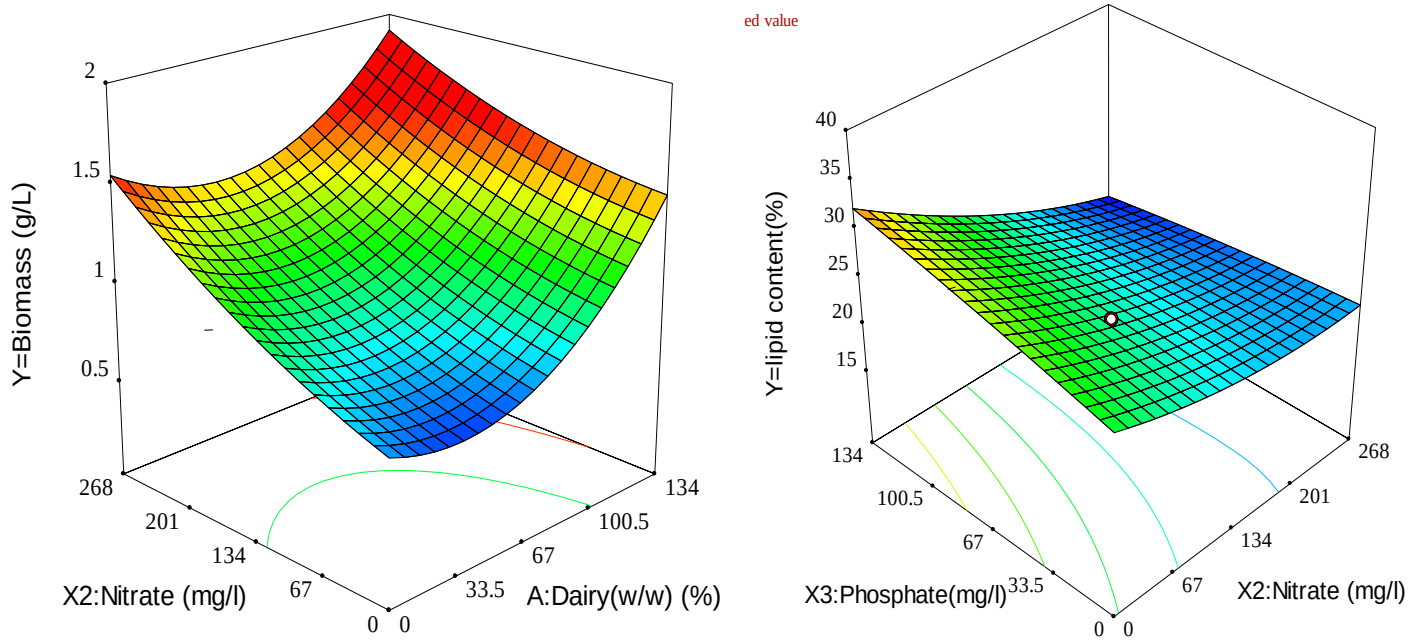


Fig.3.10:.Effect of NO_3^-, PO_4^{-3} and DIWW variables (a) Biomass productivity (b) Lipid Content

3.3.2.2. Effect of different light emitting diode (LEDs) with nutrient (NO_3^- and PO_4^{-3})

In order to cultivate microalgae commercially, a durable, reliable, low cost and high efficiency light source is required. Nowadays, among the variety of light sources, light-emitting diodes (LEDs) are the only ones that can achieve this criteria. LEDs are light and small enough to fit into almost any type of photobioreactor and their advantages such as lower heat generation, longer life-expectancy, higher conversion efficiency and others has made them commercially viable. For the growth of different kinds of algae, different nutrients in the growth medium and different wavelengths may be necessary. In this study, all environmental conditions, except for the wavelength of light and light intensity, were kept constant when growing *Chlorella pyrenoidosa* in internally illuminated bubble column photobioreactor. In the experiments, red, blue, green and white LED lights were used as illumination sources. The results after a period of ten days of growth showed the effect of the four illumination sources relative to algal biomass.

3.3.2.2.1. Growth kinetics with LEDs and NO_3^-, PO_4^{-3}

Data for variables of growth kinetics such as O.D in the terms of absorbance, rate constant (K) and doubling time (dt) of *C. pyrenoidosa* growth studies is given in Fig.3.11a and b. Experiments were carried out using LED light as per the design with variations in most influencing parameters i.e. nitrate (NO_3^-) and phosphate (PO_4^{-3}). The experiment was repeated in triplicate to determine the reproducibility of determined biomass concentration and lipid accumulation.

The average density corresponding to the design of experiments, is reported in columns X_1 and X_2 respectively. The variation of biomass concentration with growth time for all the designs of experiments provide in Fig.3.11(a). The variation of

biomass concentration with any one factor was set at the highest level (+α), and one factor was set at the lowest level (-α). Biomass growth with the intermediate factor is depicted in Fig.3.11(b). It was observed that the maximum biomass concentration was obtained from15 run. Ong *et al.,* (2010) obtained specific growth rates of 0.40-0.85 d^{-1} for *Chlorella* sp. grown in f/2 medium, which are higher than that reported in Obata et al. (2009) for *C. vulgaris* in enriched C medium under semi-continuous conditions (0.40 d^{-1}). Similarly, 23.5 mM, 0.72 mM, and 0.028 mM concentration of $NaNO_3$, KH_2PO_4, and FeC_{l3} support the maximum specific growth rate for some species.

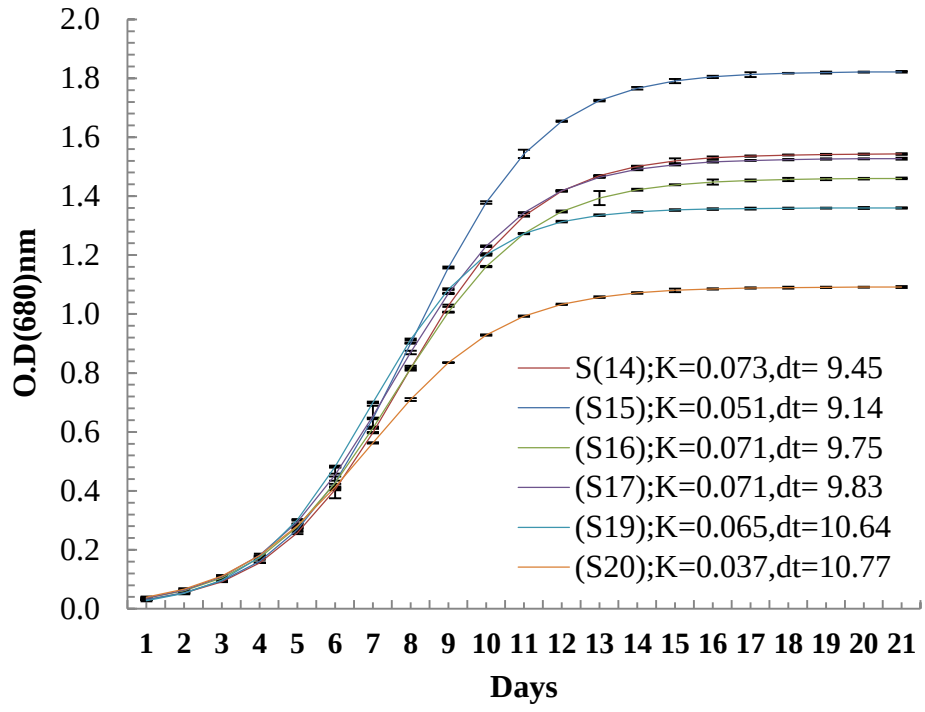
3.3.2.2.2.Development of regression model

ANOVA was carried out using experimental design model and actual responses as given in Table 3.10. to select suitable model to predict the response values (*i.e.* $Y_{BM,}$ Y_{Lipid}, Y_{FAME}) and found accurate with these responses respectively. Chronologically F-test was performed for each model and conforming probability was calculated for significance of the model in Fig.3.12.

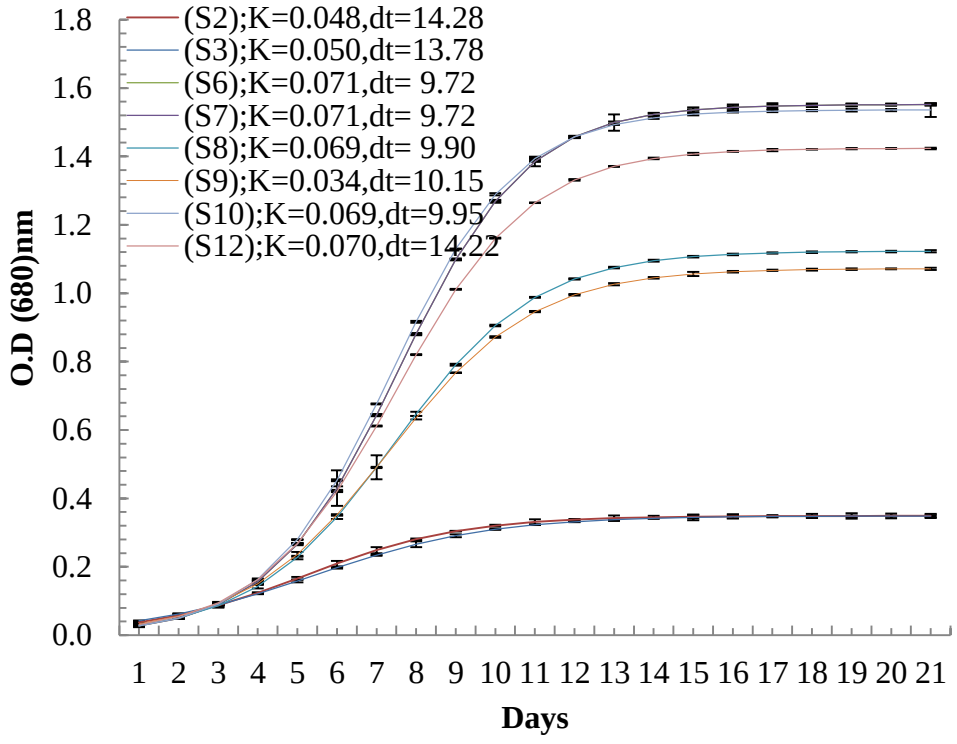
For different responses, quadratic model was selected with the prob>F<0.0001 and detailed analysis of variance is given in Table 3.10.The predicted response value of the Y_{BM}(g/L), Y_{Lipid}(%),and Y_{FAME}(%) is given in quadratic Eq.(3.6-3.8) that were obtained after substituting the coded operating variables X_1,X_2,X_3 given in Table (3.10).

$$Y_{BM}(gL^1)=1.05+0.13*X_1+0.069*X_2+0.28*X_3+0.03*X_1X_2+0.0227*X_1X_3+0.0143*X_2X_3+0.022*X_1^2+-0.133*X_2^2+-0.23*X_3^2 \quad \textbf{(3.6)}$$

$$Y_{(lipid)}(\%)=31.5+-5.09*X_1+-1.79*X_2+-1.39*X_3+-0.83*X_1X_2+-1.51*X_1X_3+-0.32*X_2X_3+3.03*X_1^2+1.27*X_2^2+1.15*X_3^2 \quad \textbf{(3.7)}$$



(a)



(b)

Fig.3.11:Time variant logistic growth model for the design of experiment with (a) anyone of the factor at ranges (-α to +α)(experiment no.14,15,16,17,19,20); (b) Factor at the intermediate level (-1 to +1)(Experimental no.2,3,6,7,8,9,10,12)

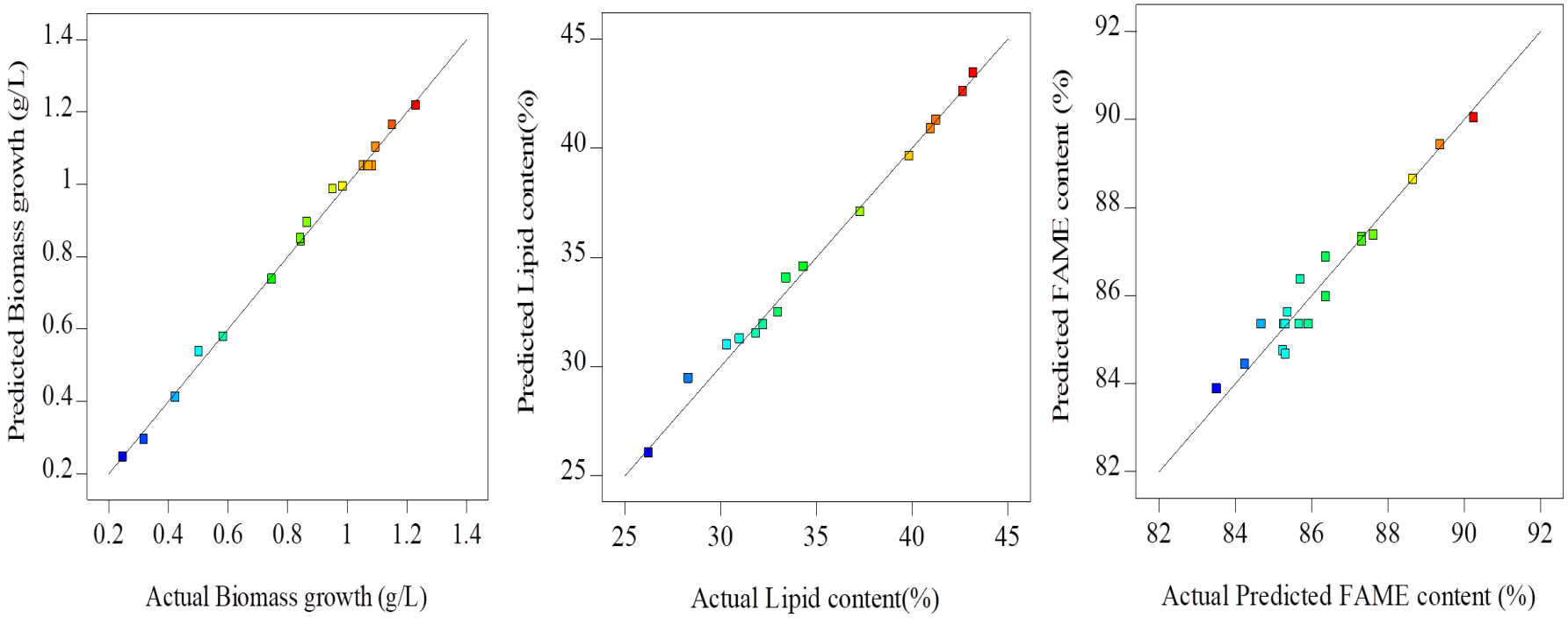
$Y_{FAME}(\%)=85.3+-0.88*X_1+1.09*X_2+0.85*X_3+-0.68*X_1X_2+-0.49*X_1X_3+-1.04*$

$X_2X_3+ 1.145*X_1^2 +0.46*X_2^2+0.165*X_3^2$ **(3.8)**

The goodness of fit of regression equation was tested for the determination of coefficient R^2_{Adj} value for Eq.(3.6), (3.7) and (3.8) and they were found to be 0.95 ,0.73 and 0.93 indicating the high degree of agreement between the actual and predicted values of Y_{BM}(g/L), Y_{Lipid}(%) and Y_{FAME}(%) concentration using the proposed quadratic models. The prob>F (probability of error) is also used to check the significance of each regression coefficient, which also indicates the effect of interaction on each cross product. The lesser prob>F value indicating the higher significance of the corresponding coefficient and the detail of prob>F value of all intercepts, linear, quadratic and interactive coefficient for Y_{BM}(g/L),Y_{Lipid}(%),and Y_{FAME}(%) are given in the Table 3.10 respectively. 'F value' and 'Prob>F for Y_{BM}(g/L) 37.45 and 29.01 and <0.05, whereas these values 6.34 and <0.05 for the Y_{Lipid}(%). This implies that selected model for Y_{BM}(g/L), Y_{Lipid}(%),and Y_{FAME}(%) is significant. Moreover, the coefficients of determination (R^2) 0.97, 0.86 respectively for Y_{BM}(g/L), Y_{Lipid}(%), and 0.96 for Y_{FAME}(%) this models were very close to the unity which indicate the proposed model Eq.(3.6-3.8) were very much accurate to predict biomass concentration, lipid and FAME content. According to Yang *et al., (*2014) the combination $NaHCO_3$, 15.49 mgL^{-1} $NaH_2PO_4{\cdot}2H_2O$ and 803.21 mgL^{-1} $NaNO_3$. Maximum biomass growth 3.07 gL^{-1}, and the increased 54.64% lipid. Miranda *et al.,* (2016) with 0.04 $g{\cdot}L^{-1}$ NO_3^-, 0.01 $g{\cdot}L^{-1}$,PO_4^{-3} and 5.0 $g{\cdot}L^{-1}$ sodium chloride resulted to be the most effective condition to growth and fatty acids accumulation. Using this optimal condition, *Ankistrodesmus* sp. and *Chlamydomonas* sp. increased in 2.1 and 2.4 folds their fatty acids yield, respectively.

Table 3.9:The minimum central and maximum variable levels in the central composite design(CCD) for various responses (observed and predicted values) for study on *C.pyrenoidosa* grown at different variable (NO□□ ,PO□□³ and LED light)

	Variables			Observed value			Predicted value		
S. N	**NO□□ (mg/L)**	**PO□□³ (mg/L)**	**Light (nm)**	**biomass productivity (g/L)**	**Lipid content (%)**	**FAME Productivity (g/L)**	**biomass Productivity (g/L)**	**Lipid content (%)**	**FAME Productivity (g/L)**
1	0	0	420	0.247	42.65	83.51	0.247	42.61	83.89
2	200	0	460	0.423	37.28	84.25	0.412	37.10	84.45
3	0	100	460	0.318	41.25	89.36	0.296	41.31	89.43
4	200	100	420	0.583	32.98	87.32	0.579	32.49	87.32
5	0	0	660	0.746	43.21	88.65	0.738	43.46	88.65
6	200	0	660	0.984	32.21	87.32	0.995	31.92	87.25
7	0	100	660	0.845	40.96	90.25	0.845	40.90	90.05
8	200	100	660	1.23	26.24	86.36	1.219	26.05	85.98
9	0	50	580	0.865	39.85	87.62	0.895	39.64	87.38
10	200	50	620	1.15	28.31	85.37	1.165	29.46	85.62
11	100	0	580	0.843	34.32	85.25	0.851	34.58	84.75
12	100	100	520	0.951	30.32	86.37	0.988	31.00	86.88
13	100	50	420	0.502	33.41	85.32	0.538	34.06	84.67
14	100	50	660	1.095	30.98	85.71	1.104	31.27	86.37
15	100	50	620	1.083	31.83	85.32	1.052	31.52	85.36
16	100	50	580	1.067	31.83	84.68	1.052	31.52	85.36
17	100	50	580	1.062	31.83	85.26	1.052	31.52	85.36
18	100	50	580	1.053	31.83	85.92	1.052	31.52	85.36
19	100	50	580	1.067	31.83	85.67	1.052	31.52	85.36
20	100	50	580	1.071	31.83	85.31	1.052	31.52	85.36



(a) **(b)** **(c)**

Fig.3.12:Relation between predicted and actual responses: (a) Biomass, Y_{BM}(g/L) (b)Lipid, Y_{Lipid}(%),and (c) FAME, Y_{FAME}(%)

Table 3.10. ANOVA of different variables on biomass productivity, lipid content and FAME content

		Response (1): Biomass productivity (g/L) ANOVA for Response surface quadratic model (RSQM)				**Response (1): Lipid content (%) ANOVA for Response surface quadratic model (RSQM)**				**Response (1): FAME content (%) ANOVA for Response surface quadratic model (RSQM)**			
		Y_{BM}(g/L),				**Y_{Lipid}(%),**				**Y_{FAME}(%)**			
Source	**df**	**Sum Squares**	**Mean Square**	**F-Value**	**Prob > F**	**Sum Square**	**Mean Square**	**F-Value**	**Prob > F**	**Sum of Squares**	**Mean Square**	**F-Value**	**Prob > F**
Model	9	1.63	0.1811	269.86	< 0.0001	449.14	49.90	143.75	< 0.0001	52.93	5.88	21.20	< 0.0001
X_1	1	0.1820	0.1820	271.14	< 0.0001	259.08	259.08	746.28	< 0.0001	7.69	7.69	27.73	0.0004
X_2	1	0.0468	0.0468	69.71	< 0.0001	32.11	32.11	92.50	< 0.0001	11.41	11.41	41.12	< 0.0001
X_3	1	0.7992	0.7992	1190.7	< 0.0001	19.52	19.52	56.22	< 0.0001	7.28	7.28	26.23	0.0004
X_1X_2	1	0.0070	0.0070	10.37	0.0092	5.48	5.48	15.78	0.0026	3.56	3.56	12.85	0.0050
X_1X_3	1	0.0041	0.0041	6.17	0.0323	18.24	18.24	52.54	< 0.0001	1.92	1.92	6.92	0.0251
X_2X_3	1	0.0016	0.0016	2.42	0.1508	0.7938	0.7938	2.29	0.1614	8.57	8.57	30.89	0.0002
X_1^2	1	0.0013	0.0013	2.00	0.1877	25.35	25.35	73.03	< 0.0001	3.61	3.61	13.00	0.0048
X_2^2	1	0.0483	0.0483	72.03	< 0.0001	4.48	4.48	12.90	0.0049	0.5819	0.5819	2.10	0.1781
X_3^2	1	0.1469	0.1469	218.81	< 0.0001	3.65	3.65	10.50	0.0089	0.0749	0.0749	0.2699	0.6147
Residual	9	0.0067	0.0007			3.47	0.3472			2.77	0.2774		
Lack of Fit	5	0.0062	0.0012	12.62	0.0073	3.47	0.6943			1.89	0.3775	2.13	0.2131
Pure Error	4	0.0005	0.0001			0.0000	0.0000			0.8862	0.1772		
		Mean=0.85		**Std.Dev=0.589**		**Mean =34.25**		**Std=0.589**		**Mean=86.24**		**R^2_{Adj}=0.95**	
		R^2=0.99		**R^2_{Adj} =0.99**		**R^2=0.99**		**R^2Adj=0.98**		**Std=0.526**		**R^2=0.90**	
		CV%=3.02				**CV%=1.72**				**CV%=0.601**			

Statistical analysis of biomass concentration (g/L), lipid and FAME content was shown in 3.12 (a-c) which reveals the variation of predicted and actual observation of Y_{BM} (g/L), Y_{Lipid}(%), and Y_{FAME}(%). Fig.4.16(a & c) shows the variation of predicted vs actual values are distributed normally in straight line and error is insignificant within the range of operating variables but little fluctuation was found in predicted vs actual values of lipid content. So, this suggests that the model is satisfactory and does not show any violation of independence or constant variance assumption as shown in Table 3.10. A low coefficient of the variation in Y_{BM} (g/L), Y_{Lipid} (%) and Y_{FAME} (%) assured high degree of accuracy and good authencity of the experimental data.

3.3.2.2.2.1.Effect of factors on biomass concentration, lipid content and FAME content

It was depicted from Table 3.10, these responses in terms of biomass concentration, Lipid and FAME content varied between 0.247-1.23g/L, 26.24-43.21% and 83.21-90.2% lipid respectively for different combinations of operating variables. The best-fitted response for Y_{BM}(g/L), Y_{Lipid}(%), and Y_{FAME}(%) are given in Eq.4.6-4.8 which are composed of several intercepts, linear, quadratic and interaction coefficient and among them some coefficients (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. Details of statistically significant and extremely significant coefficient for biomass concentration are given in the Table 3.10.

3.3.2.2.2.2. Individual factors contribution of operating variables for Y_{BM} (g/L), Y_{Lipid}(%),and Y_{FAME}(%)

The contributions of individual operating variables toward Y_{BM}(g/L), Y_{Lipid}(%), and Y_{FAME}(%) are shown in the Fig.3.13(a-c) respectively. 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 is influenced by PO_4^{-3}>NO_3^->light. The variation in biomass productivity, lipid and FAME for individual factor is shown in Fig.3.13 (a-c), keeping the remaining variables fixed at '0' level. It was observed that biomass growth increased by increasing the concentration of NO_3^- whereas, the lipid productivity was inhibited by the excess of NO_3^- concentration and PO_4^{-3} after reaching the maximum level and FAME content affected by concentration of NO_3^- and PO_4^{-3}.

NO_3^- and $PO_4^{-3}$are the two most important nutrient sources mainly altering growth and metabolism of a species depending on its concentration in the medium. The biomass productivity is inhibited by using PO_4^{-3} in excess mainly due to the complex feedback mechanism. When these concentrations are limited, it leads to a shifting in the metabolic pathway of cells from membrane lipids to synthesis of neutral lipids in the form of triacylglycerides (TAGs). On the other hand, PO_4^{-3}, is another nutrient known to induce lipid accumulation in the cells. It has a key role in the transportation of metabolic energy and is a vital structural element of phospholipid molecules and nucleotides in living cells (Saumya *et al.*, 2017).

3.3.2.2.2.3.Interactive contribution of operating variables for Y_{BM} (g/L), Y_{Lipid} (%), and Y_{FAME}(%)

The percent contribution of interacting variables determined after the statistical analysis from Table 3.10 as shown in Fig.4.14(a-c) for Y_{BM} (g/L), Y_{Lipid}(%),and Y_{FAME}(%).It is seen from figure 3.14(a)>55%, contribution of NO_3^-:PO_4^{-3} interactive variables for biomass concentration of *Chlorella pyrenoidosa*. Similarly, the most influencing interaction variables for lipid content ~75% of NO_3^-:light, as shown in Fig

4.14b. But, in case of FAME content~55% of light:PO_4^{-3} interacting effect was observed as shown in the Fig.3.14c, which helps in formation of FAME content.

Therefore, three quadratic interaction variables were considered to generate 3D response surfaces and 2D contour plots. These plots are the graphical representation of the regression equation containing the information of the interacting effect between pair variables and optimal condition could be identified by analyzing their effect. These 3D graphs were generated for pairwise combination of any two significant factors. Among the three LED, $NO_3^-$and PO_4^{-3} variables combination are responsible for biomass concentration, lipid and FAME content. Considering the most influential interaction $NO_3^{-+}PO_4^{-3}$, of 3D response surface plots for biomass concentration as shown in Fig 3.15(a-d). Similarly, Fig.3.15(e-h) demonstrates that the NO_3^-+PO_4^{-3}, is the most significant operating variables for lipid content.Considering the significant interaction between PO_4^{-3} + NO_3^- details of 3D response surface plots are shown in Fig.3.15(i-l)for FAME content.

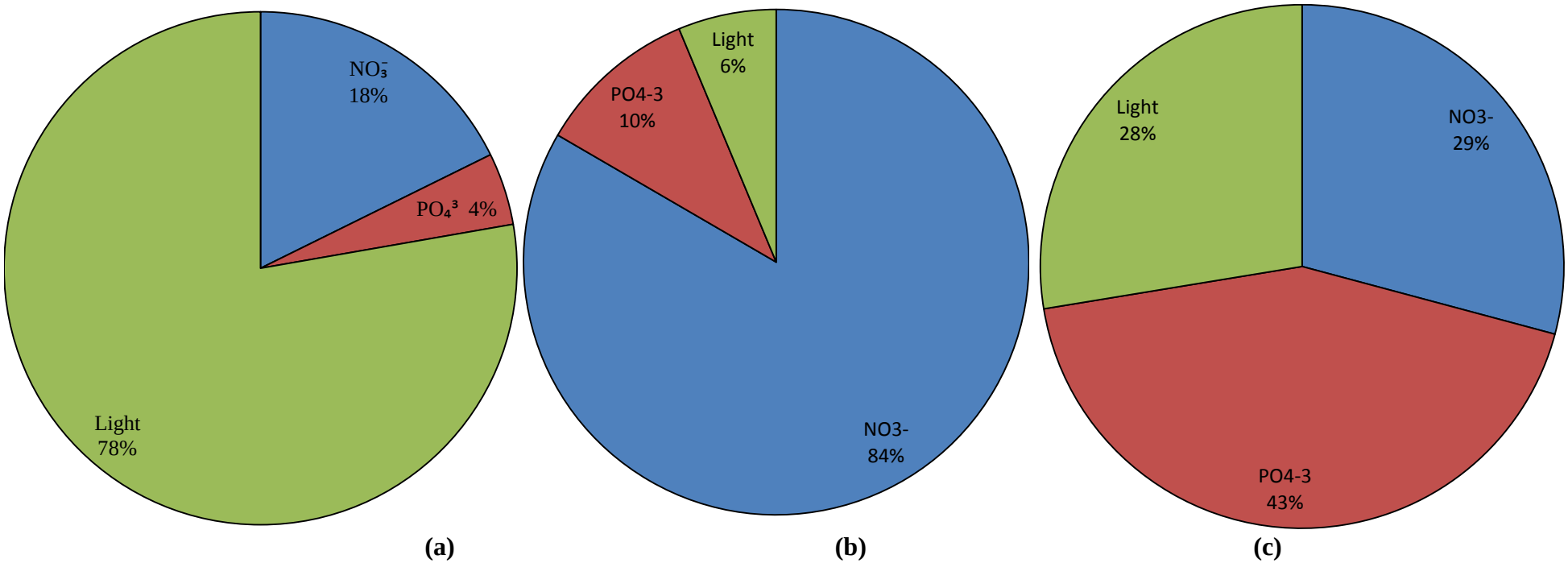


Fig.3.13:Perent contribution of individual factors:(a) Biomass, Y_{BM}(g/L) (b)Lipid, Y_{Lipid}(%),and (c) FAME,Y_{FAME}(%)

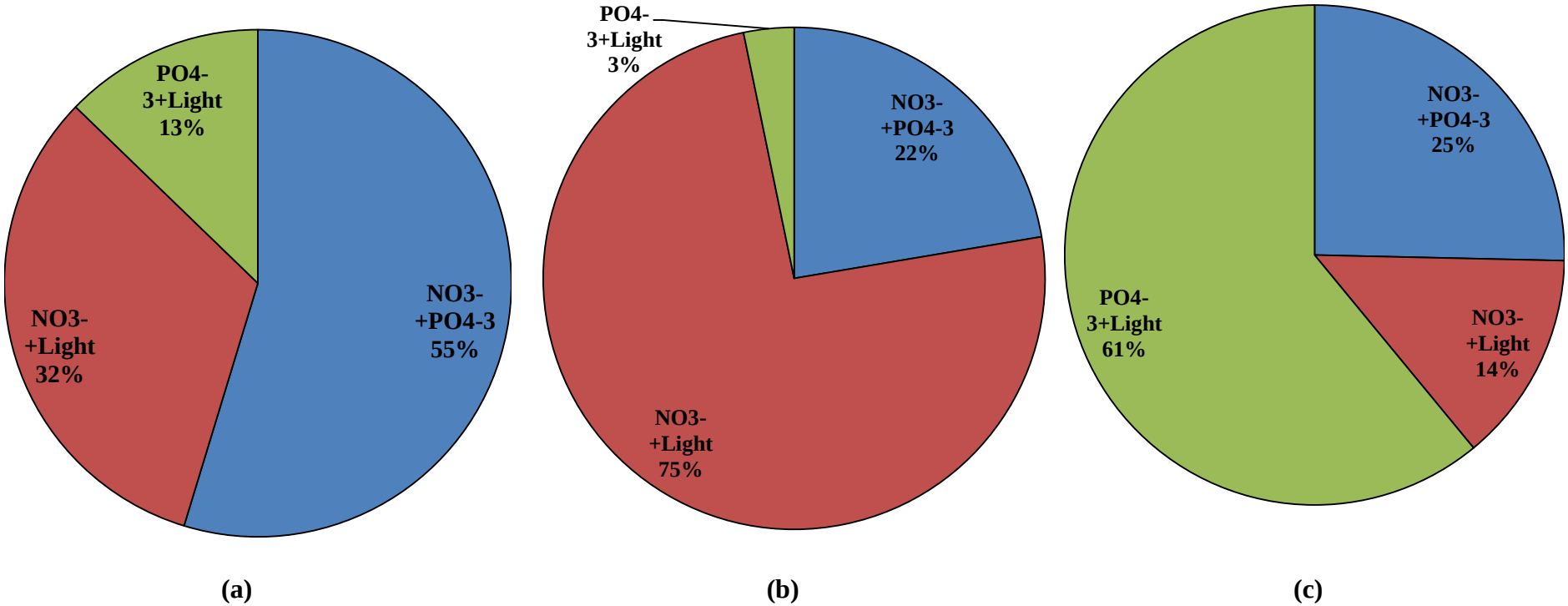
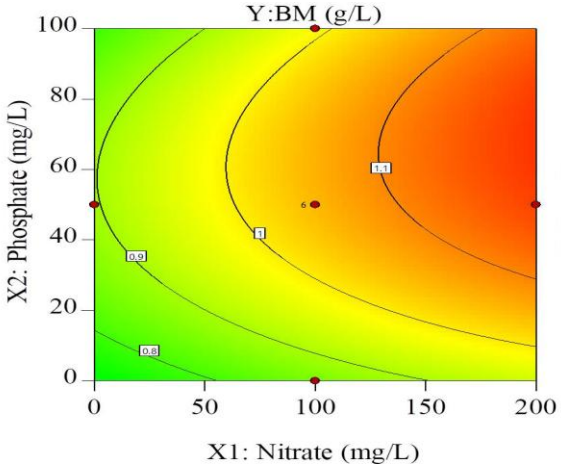
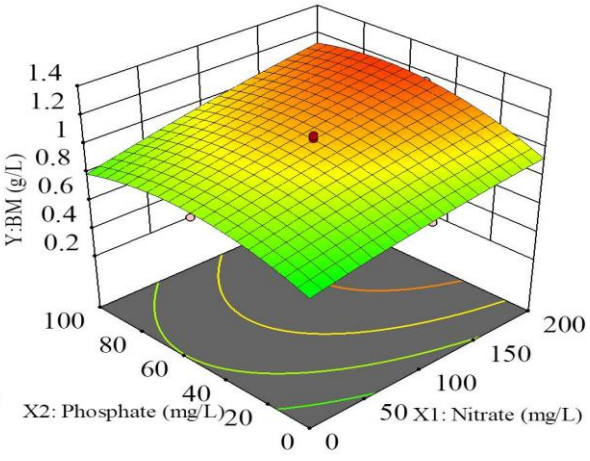


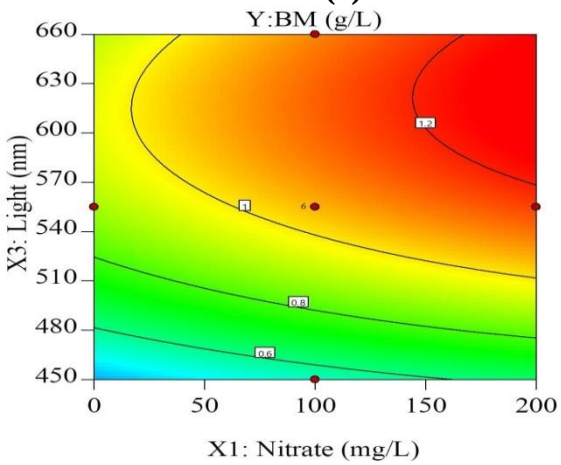
Fig.3.14:Contribution of interactive factors (a) Biomass (b)Lipid content and (c)FAME content


Y:BM (g/L)
X2: Phosphate (mg/L)
X1: Nitrate (mg/L)
100
80
60
40
20
0
0
50
100
150
200
0.9
1
1.1
0.8
6

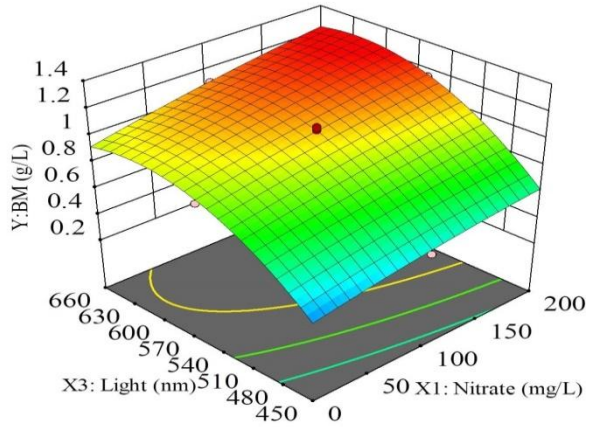
(a)


Y:BM (g/L)
1.4
1.2
1
0.8
0.6
0.4
0.2
X2: Phosphate (mg/L)
X1: Nitrate (mg/L)
100
80
60
40
20
0
0
50
100
150
200

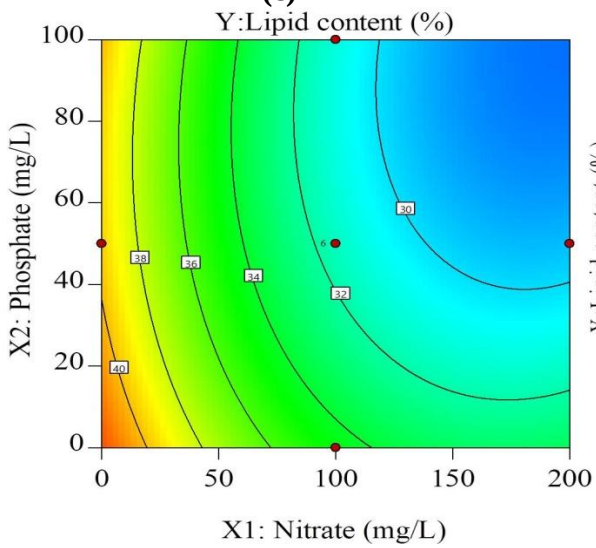
(b)


Y:BM (g/L)
X3: Light (nm)
X1: Nitrate (mg/L)
660
630
600
570
540
510
480
450
0
50
100
150
200
1
1.2
0.8
0.6
6

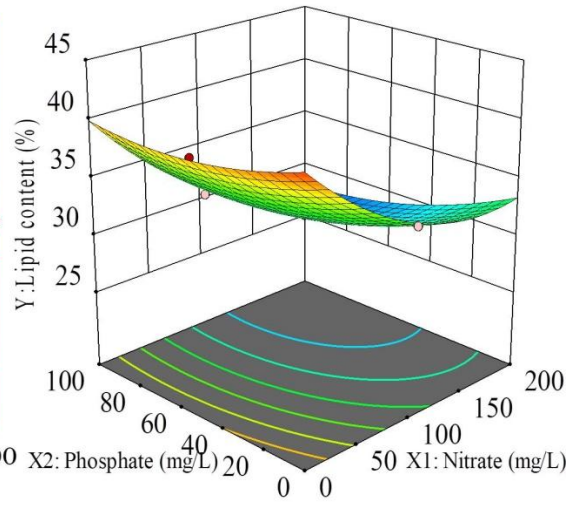
(c)


Y:BM (g/L)
1.4
1.2
1
0.8
0.6
0.4
0.2
X3: Light (nm)
X1: Nitrate (mg/L)
660
630
600
570
540
510
480
450
0
50
100
150
200

(d)


Y:Lipid content (%)
X2: Phosphate (mg/L)
X1: Nitrate (mg/L)
100
80
60
40
20
0
0
50
100
150
200
40
38
36
34
32
30
6

(e)


Y:Lipid content (%)
45
40
35
30
25
X2: Phosphate (mg/L)
X1: Nitrate (mg/L)
100
80
60
40
20
0
0
50
100
150
200

(f)

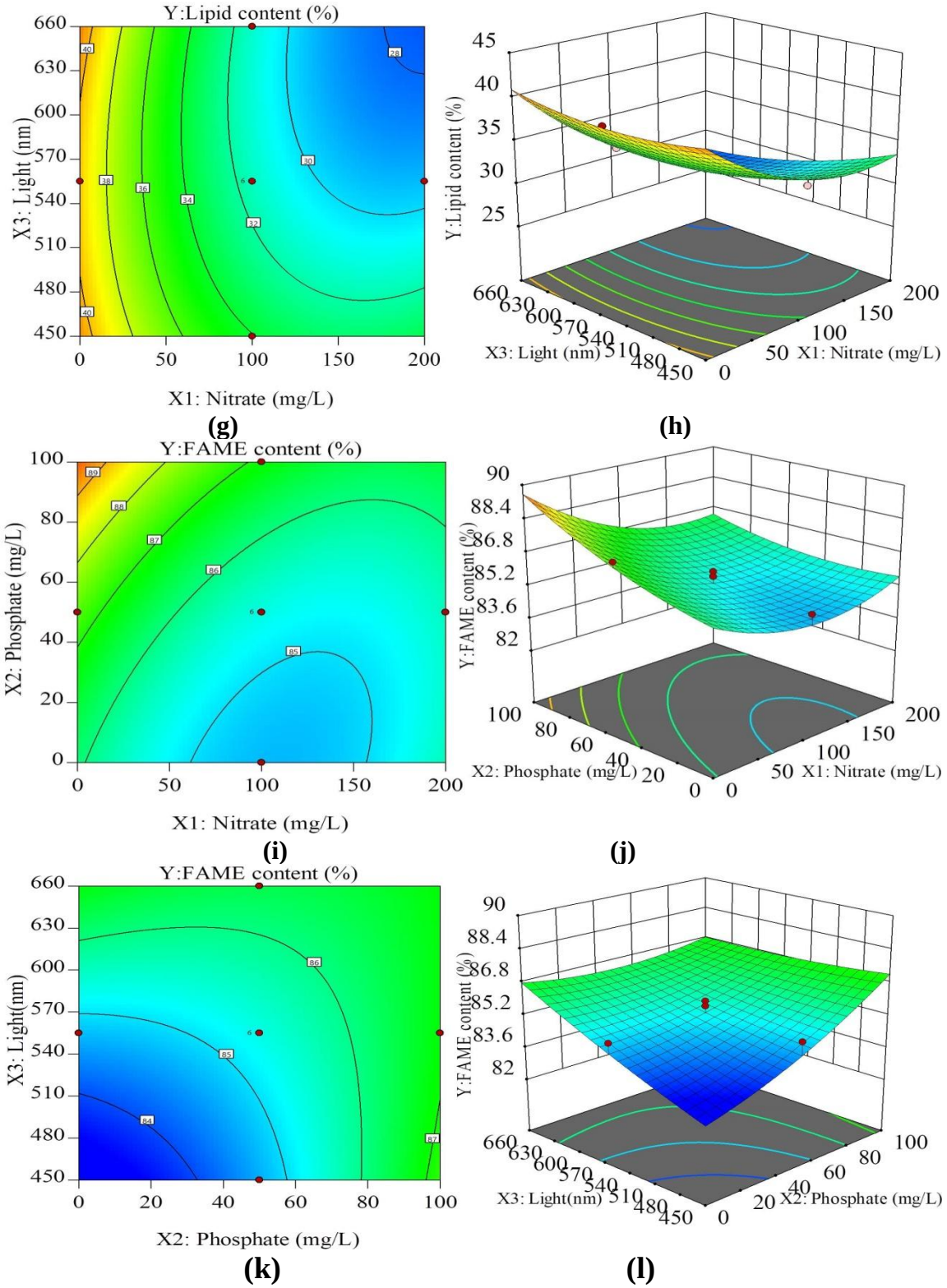


Fig.3.15:2D and 3D representation of significant factor and responses: (a-b) Effect of NO_3^- and $PO4^{-3}$ (c-d) light and NO_3^- biomass productivity(e-f) Effect of NO_3^- and $PO4^{-3}$ (g-h) Light and NO_3^- on Lipid productivity (i-j) Effect of NO_3^- and PO_4^{-3} (k-l) Light and PO_4^{-3} on FAME productivity

3.3.2.2.3. FT-IR analysis of transestrification of lipid at different variables (NO_3^-, PO_4^{-3} and Light)

The importance of the IR spectroscopy for empathy of the arrangement of molecules originates the possibility to assign some certain absorption bands related to the functional group. In all oils, most of the peaks and bands are recognized for the functional group. Since, triglycerides (TGAs) are the main component in algal oil and all types of oil consists of fatty acid therefore, the IR spectra show only characteristic absorption bands. At different variable (DIWW,NO_3^- and PO_4^{-3}) concentration, IR spectra show notable difference at 3009 cm^{-1} due to NO_3^- and PO_4^{-3} limitation as shown in Fig.6 which assign to the C-H stretching vibration of double bond (=CH-). The exact position band is the function of oil and shifts with the change in fatty acid composition in algal oil as shown in Fig.3.16. Absorption band found at around 2920 cm^{-1} to 2855cm^{-1} due to the symmetric stretch in vibration of the aliphatic group. Peak height for algal oil of NO_3^- deficiency show great difference than PO_4^{-3} deficiency. Spectral band arises at approximately 1743cm^{-1} due to the ester carbonyl group of triglycerides. It is noted that carbonyl band stretching shifts in according to change of concentration of NO_3^- & PO_4^{-3}. Besides, this band appeared at approximately 1743 cm^{-1} region due to the ester carbonyl (-COO-) functional group of triglycerides. But due to variable concentrations of NO_3^- & PO_4^{-3} band of ester carbonyl (-COO-),functional group shifted left to right at 1743cm^{-1}. There is no band absorbed at 1700 cm^{-1} because of no free fatty acids (FFA) which affects the quality of algal oil or very low proportion is present that is not detectable in IR spectrum. The C=C stretching found at the region of 1657cm^{-1}, the intensity of band is not affected by any variable.

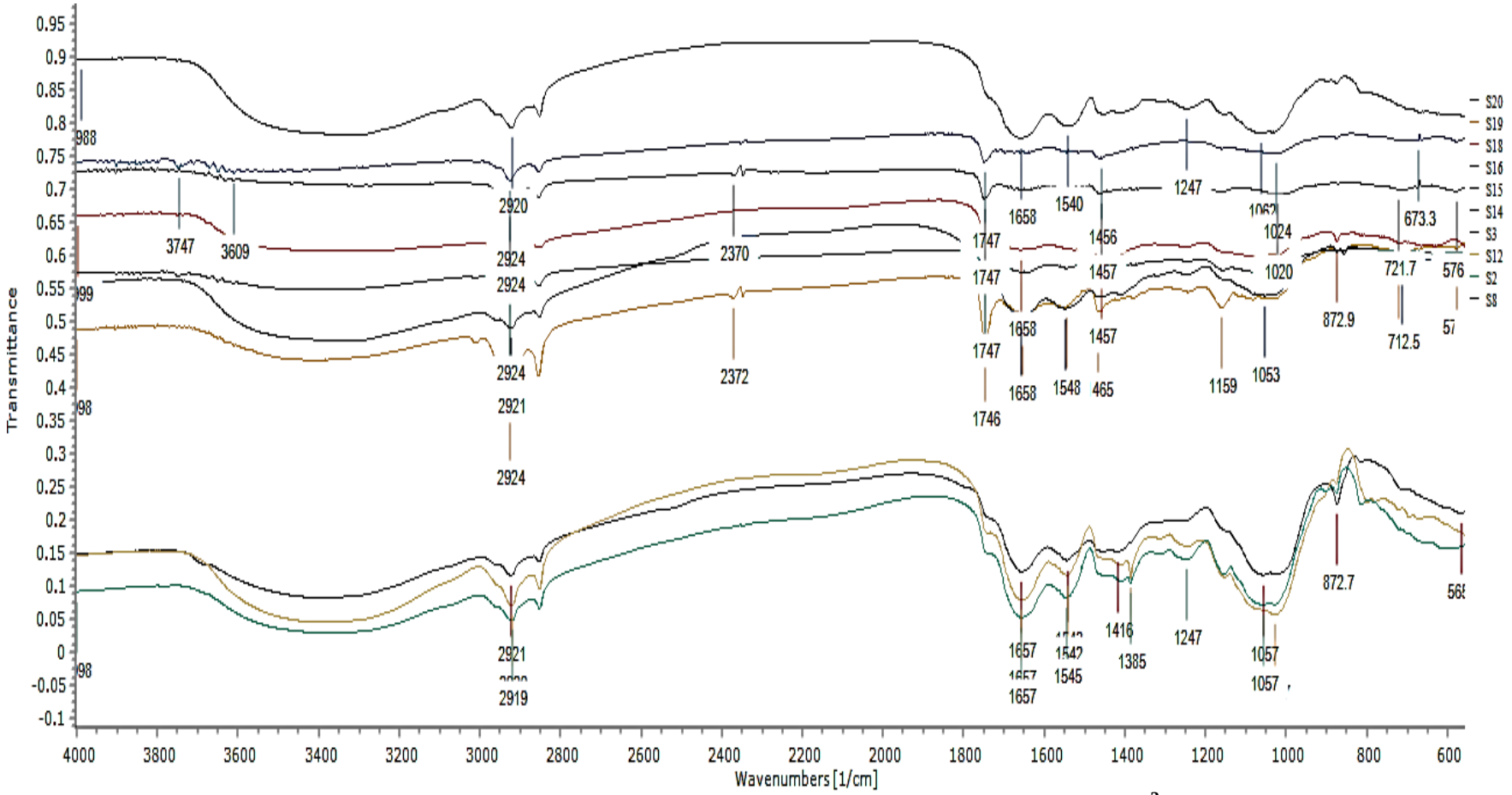
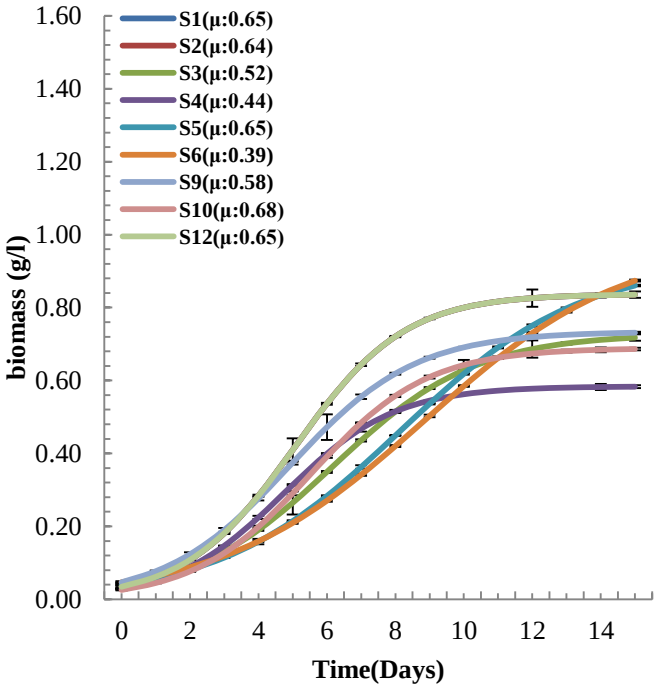


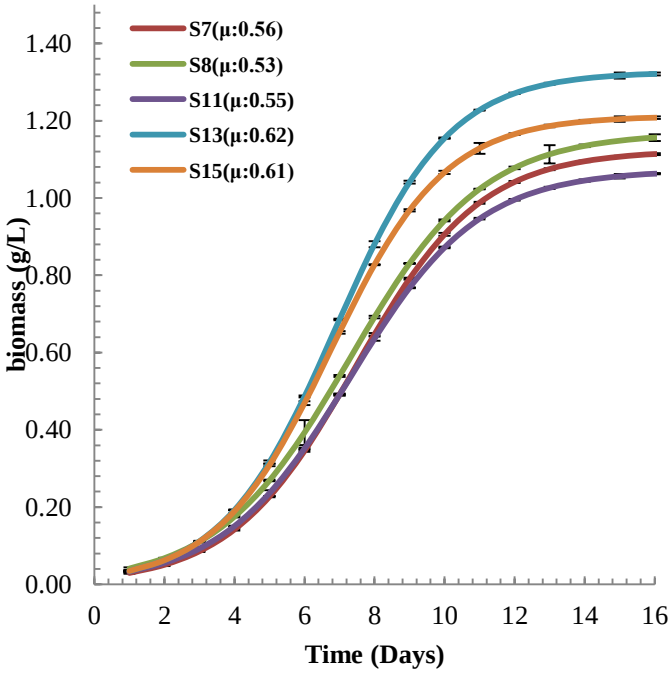
Fig.3.16:FT-IR analysis of different variables (Light, NO_3^- and PO_4^{-3}) for FAME

3.3.2.3. Effect of different light emitting diodes (LEDs) with CO_2 and different concentration of DIWW

DIWW, wavelength of light emitting diode (LED) and supply of CO_2 affect the algal growth. These multivariable studies explains that growth kinetics is affected by the variation in concentration as shown in Fig.3.17(a-b). In central composite design, the experimental run with the highest value (1.32g/L) for algal growth was obtained with 19 run which was designed in combination of the experiment and was repeated in triplicate to determine the reproducibility of experimentally determined biomass concentration. The variation in biomass concentration was set at the highest and one factor was set at lowest for biomass growth as shown in Fig.3.17b. Pahija *et al.*, (2017) found the specific growth rate at 0.28 day^{-1} and 0.32 day^{-1} for the 22°C and 27°C. Whereas, Huesemann *et al*.,(2016) also found maximum specific growth rate was 5.9 day^{-1} for *Chlorella* sp. at 36.1°C, and only 1.1 day^{-1} for *N. salina* at 26.3 °C and 1.8 day^{-1} for *Picochlorum* sp. at 30 °C respectively. The maximum specific growth rates decreased with light intensity, resulting in complete or almost complete growth inhibition of the 14 °C and 18 °C cultures at 280 μmol/m^2s^{-1} and 1280 μmol/$m^2 \cdot s^{-1}$, respectively. While all species failed to grow below 13°C. Kasiri, (2015) found 22% CO_2 concentration, 29 mM phosphate concentration and 70 μmol photons.$m^{-2}s^{-1}$; light intensity maximized specific growth rate to 0.31day^{-1}.



(a)



(b)

Fig.3.16:Time variant logistic growth model for the design of experiment with (a) anyone of the factor at ranges (-α to α)(experiment no.7,8,11,13and 15(b) Factor at the intermediate level (-1 to +1)(Experimental no.2,3,6,7,8,9,10,12)

3.3.2.3.1. Optimization of algal biomass, lipid productivity and FAME content

The growth studies of *Chlorella pyrenoidosa* were carried out as per the design of experiments (DOEs) with varying concentration of DIWW, CO_2 loading and LED light with different wavelengths (Table 3.11). Among the 19 experiments, 6 experiments were repeated. CCD-RSM technique was used to obtain best relationship between the responses (Y_{BM}, Y_{lipid} Y_{FAME} and Y_{CO_2}) and factor. The quadratic models were used in this study and the ANOVA criterion was employed. ANOVA tables were formulated by calculating the (i) sum of squares, (ii) degree of freedom, (iii) mean square, (iv) F-value and, (v) p-value, and the details are given in Table 3.12 and 3.13. Among the models considered, the quadratic model was found to be the best-suited model with p-value <0.0001 and maximum F-value to predict Y_{BM}, $Y_{lipid,}$ Y_{FAME} and Y_{CO2} responses accurately for the coded factors and the details are given by:

$$Y_{BM(g/L)} = -4.35+1.53x10^{-2}X_1-0.104X_2+0.0195X_3+11x10^{-4}X_1X_2+8.95x10^{-6}X_1X_3+3.0x10^{-4}X_2X_3-1.96x10^{-4}X_1^2-2.54x10^{-3}X_2^2x10^{-5}X_3^2 \quad \textbf{(3.9)}$$

$$Y_{Lipid}=-126.24+0.031X_1-2.10X_2+0.59X_3-2.44x10^{-3}X_1X_2-5.6x10^{-5}*X_1X_3+3.8x10^{-3}X_2X_3-1.63x10^{-4}X_1^2+6.58x10^{-3}X_2^2-5.62x10^{-4}X_3^2 \quad \textbf{(3.10)}$$

$$Y_{FAME}=-73.09-0.28X_1+1.73X_2+0.55X_3-9.3x10^{-3}X_1X_2+5.83x10^{-4}X_1X_3-2.27x10^{-3}X_2X_3+9.2x10^{-4}X_1^2-1.94x10^{-3}X_2^2-5.04x10^{-4}X_3^2 \quad \textbf{(3.11)}$$

$$Y_{CO2}=-0.91+4.92x10^{-3}X_1-7.54x10^{-3}X_2+3.44x10^{-3}X_3-4.1x10^{-5}X_1X_2-3.75x10^{-7}X_1X_3+5.5x10^{-5}X_2X_3-4.5x10^{-5}X_1^2-8.84x10^{-4}X_2^2-3.56x10^{-6}X_3^2 \quad \textbf{(3.12)}$$

3.3.2.3.2. Effect of factors on biomass concentration, lipid content and FAME content

The Design Expert Software developed a 2^3 factorial CCD design resulting 19 run with 6 axial point and 6 replicates using second order polynomial equation predicted

by the model for maximum biomass concentration, lipid content and FAME content and CO_2 sequestration. It was seen from Table 3.11, the responses of biomass concentration, Lipid content, FAME content and CO_2 sequestration varied between 0.615-1.32g/L, 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-fitted response for Y_{BM}(g/L) Y_{Lipid}(%), Y_{FAME}(%) $Y_{CO_2\ sequestration}$ is given in Eq.3.9 to 3.12 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}(g/L) 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}(g/L) 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}(g/L) and Y_{Lipid}(%).

Positive coefficients of Eq.3.9 to 3.12 indicate the synergistic effect, whereas negative coefficients shows the antagonistic effect of response variables due to multiple factors. The detailed analysis of variance (ANNOVA) for Y_{BM}, Y_{lipid} Y_{FAME} and Y_{CO2} responses are given in Table 3.12 and Table 3.13, respectively. Coefficient of determination (R^2: goodness of fit with respect to all factors), adjusted (R^2_{Adj}: accuracy of fit with important factors) and predicted R^2_{pred} (R^2_{pred}; predictive quality the model for new set of data) were determined for YBM, Y_{lipid} and Y_{CO_2}, and R^2, R^2_{Adj} and R^2_{pred} values for Y_{BM}, Y_{lipid} Y_{FAME} and Y_{CO2} responses were found to be (0.98, 0.97 and 0.89), (0.98,0.96 and 0.84) and (0.97,0.94 and 0.77) and (0.99, 0.99 and 0.95) respectively. All R^2 values suggested that the regression models were very accurate within the limits of the operating variables. Wu *et al.*, (2014) found that *Synechococcus* strain under 33˚C, 300 μE/m^2 /s with 10% and 20% CO_2, respectively. The result for 10% CO_2 culture was very promising, achieved an Xmax of 3.1 g/L and μmax of 0.0180 hr^{-1}. Above regression models also satisfied the statistically non-

significant lack of fit test, where p-values for Y_{BM}, Y_{lipid} and Y_{CO2} respectively, indicating that the proposed models fitted satisfactorily with the experimental data. Statistical analyses of Y_{BM}, Y_{lipid}, Y_{FAME} and Y_{CO2} variations of predicted responses versus actual responses are shown in Fig.3.18(a-d). The difference between observed and predicted responses (called residuals) was determined to verify the model accuracy, which is shown by plotting (i) normal percent probability vs. residuals.

3.3.2.3.1. Effect of individual operating variable on algal biomass growth, lipid content, FAME content and CO_2 sequestration

The percentage contribution of DIWW, CO_2 and Light (X_1, X_2 and X_3) towards the responses variable ($Y_{Biomass}$, Y_{lipid}, $Y_{FAME\ content}$ and $Y_{CO2\ sequestration}$) calculated on the basis of Prob>F value of coefficient as given in Table 3.12 and Table 3.13 and predicted in Fig.3.18(a-d) respectively. The order of influence for the operating variables on biomass productivity was found to be Light> CO_2 >DIWW (Fig.3.18a). Similarly, the order of influence for operating variables for lipid content, FAME content were: Light>DIWW>CO_2 (Fig.3.18b) and for CO_2 sequestration were: Light>CO_2>DIWW (Fig.3.18c-d) respectively. Parameters, like CO_2 concentration, nutrients ratio, light intensity, temperature and type of algae are responsible for CO_2 uptake rate as well as biomass productivity(Cheng *et al.,*2013) In the present study, it was observed that light is the most influencing variable for $Y_{Biomass}$, Y_{lipid}, $Y_{FAME\ content}$ and $Y_{CO2\ sequestration}$. The above analysis clearly revealed that multicolor LED,CO_2 and DIWW had significant impact in improving the microalgal metabolism to enhance the algal growth, lipid content, FAME content and CO_2 sequestration. Similarly, direct and indirect relationship is also observed for biomass productivity with increase and decrease in wavelength from LED light radiations.

Table 3.11:Design of experiment and predictive responses (Algal growth (g/L) ; Lipid productivity (%); FAME content(%); and CO_2 sequestration (g/L/d)

		Factor			Observed				Predicted			
Std	**Run**	**X_1:DIWW**	**X_2:CO_2**	**X_3:Light**	**Biomass**	**Lipid**	**FAME**	**CO_2 fixation rate**	**biomass**	**lipid**	**FAME**	**CO_2 fixation rate**
		%	%	nm	g/L	%	%	**(g/L/d)**	%			**(g/L/d)**
1	2	20	5	460	0.87	25.37	76.96	0.07	0.82	25.76	76.47	0.07
2	3	80	5	460	0.857	23.92	78.36	0.07	0.78	24.36	78.28	0.07
3	6	20	15	460	0.736	22.62	81.24	0.06	0.63	23.11	81.01	0.06
4	13	80	15	460	0.615	20.91	77.64	0.04	0.52	20.24	77.20	0.04
5	10	20	5	580	0.952	28.21	80.12	0.06	0.87	28.92	79.79	0.07
6	8	80	5	580	0.962	27.56	86.34	0.06	0.89	27.12	85.80	0.07
7	7	20	15	580	1.135	31.25	82.29	0.13	1.04	30.85	81.60	0.13
8	19	80	15	580	1.12	27.93	82.26	0.10	0.99	27.59	81.98	0.10
9	12	0	10	520	0.785	30.05	81.84	0.05	0.68	29.53	82.70	0.05
10	17	100	10	520	0.682	24.82	83.86	0.03	0.60	25.65	84.53	0.03
11	9	50	0	520	1.05	30.12	80.24	0.06	0.92	29.75	80.74	0.05
12	16	50	20	520	0.886	26.93	80.85	0.08	0.83	27.57	81.46	0.08
13	20	50	10	420	0.735	18.25	72.36	0.09	0.71	18.00	72.88	0.09
14	11	50	10	620	1.32	26.19	78.62	0.15	1.15	26.75	79.64	0.14
15	4	50	10	520	1.22	27.92	81.26	0.15	1.13	28.00	81.30	0.15
16	5	50	10	520	1.22	27.92	81.26	0.15	1.13	28.00	81.30	0.15
17	1	50	10	520	1.22	27.92	81.26	0.15	1.13	28.00	81.30	0.15
18	15	50	10	520	1.22	27.92	80.85	0.15	1.13	28.00	81.30	0.15
19	18	50	10	520	1.22	27.92	81.26	0.15	1.13	28.00	81.30	0.15
20	14	50	10	520	1.22	27.92	81.26	0.15	1.13	28.00	81.30	0.15

Table 3.12:Analysis of variance (ANOVA) for Biomass productivity, Lipid content, and FAME content

		ANOVA of Response surface quadratic model for Biomass productivity (Y_{BM})				ANOVA of Response surface quadratic model for Lipid content (Y_{lipid})				ANOVA of Response surface quadratic model for FAME content (Y_{FAME})			
Source	**df**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	**Sum of Square**	**Mean Square**	**F-value**	**p-value**	**Sum of Square**	**Mean Square**	**F-value**	**p-value**
Model	9	0.9055	0.1006	83.83	< 0.0001	187.94	20.88	55.14	< 0.0001	147.87	16.43	35.70	< 0.0001
X_1-DIWW	1	0.0071	0.0071	5.93	0.0351	18.53	18.53	48.91	< 0.0001	3.99	3.99	8.67	0.0147
X_2-CO_2	1	0.0082	0.0082	6.86	0.0256	4.76	4.76	12.58	0.0053	0.5148	0.5148	1.12	0.3151
X_3-Light	1	0.3149	0.3149	262.36	< 0.0001	92.25	92.25	243.5	< 0.0001	54.75	54.75	118.9	< 0.0001
X_1X_2	1	0.0022	0.0022	1.84	0.2045	1.07	1.07	2.83	0.1232	15.82	15.82	34.37	0.0002
X_1X_3	1	0.0021	0.0021	1.73	0.2174	0.0820	0.0820	0.216	0.6517	8.80	8.80	19.12	0.0014
X_2X_3	1	0.0643	0.0643	53.54	< 0.0001	10.51	10.51	27.75	0.0004	3.74	3.74	8.13	0.0172
X_1^2	1	0.4301	0.4301	358.37	< 0.0001	0.2968	0.2968	0.783	0.3968	9.60	9.60	20.85	0.0010
X_2^2	1	0.1053	0.1053	87.78	< 0.0001	0.7028	0.7028	1.86	0.2030	0.0616	0.0616	0.133	0.7221
X_3^2	1	0.0691	0.0691	57.55	< 0.0001	56.49	56.49	149.1	< 0.0001	45.44	45.44	98.73	< 0.0001
Residual	10	0.0120	0.0012			3.79	0.3787			4.60	0.4603		
Lack of Fit	5	0.0120	0.0024			3.79	0.7575			4.46	0.8925	31.86	0.0008
Pure Error	5	0.0000	0.0000			0.0000	0.0000			0.1401	0.0280		
Cor Total	19	0.9175				191.73				152.47			
		Std. Dev=0.035		**R^2=0.98**		**Std. Dev.=0.61**		**R^2=0.98**		**Std. Dev.0.68**		**R^2=0.97**	
		Mean=1.0		**R^2_{Adj}=0.97**		**Mean=26.5825**		**R^2_{Adj}=0.96**		**Mean=80.5**		**R^2_{Adj}=0.94**	
		C.V. %=3.46		**R^2_{Pred}=0.89**		**C.V. %=2.31**		**R^2_{Pred}=0.84**		**C.V. %=0.84**		**R^2_{Pred}=0.77**	

Table 3.13:ANOVA of Response surface quadratic model for CO_2 sequestration (g/L/d)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.0387	9	0.0043	202.67	< 0.0001
X1-DIWW	0.0005	1	0.0005	21.61	0.0009
X2-CO_2	0.0008	1	0.0008	39.78	< 0.0001
X3-Light	0.0037	1	0.0037	175.40	< 0.0001
X_1X_2	0.0003	1	0.0003	14.38	0.0035
X_1X_3	3.645E-06	1	3.645E-06	0.1718	0.6873
X_2X_3	0.0022	1	0.0022	103.59	< 0.0001
X_1^2	0.0228	1	0.0228	1072.53	< 0.0001
X_2^2	0.0127	1	0.0127	597.23	< 0.0001
X_3^2	0.0023	1	0.0023	106.70	< 0.0001
Residual	0.0002	10	0.0000		
Lack of Fit	0.0002	5	0.0000		
Pure Error	0.0000	5	0.0000		
Cor Total	0.0389	19			
			Std. Dev.=0.0046		**R^2=0.99**
			Mean=0.09802		**R^2_{Adj}=0.99**
			C.V. % =4.67		**R^2_{Pred}=0.95**

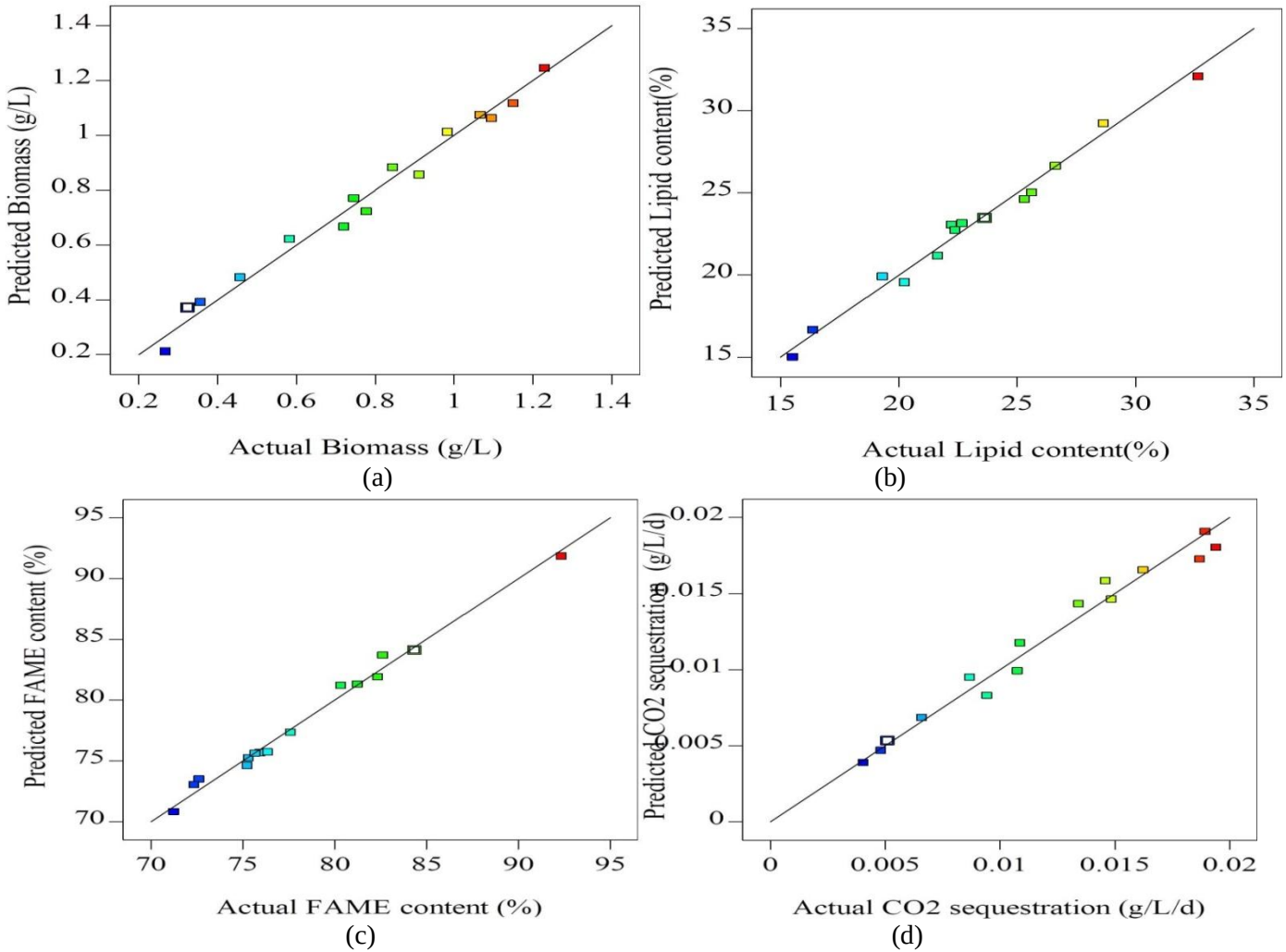


Fig.3.18:Relation between predicted and experimental responses (a) Biomass productivity (b) Lipid content (c) FAME content (d) CO_2 sequestration

3.3.2.3.2. Interaction of operating variables; $Y_{Biomass}$, Y_{lipid}, $Y_{FAME\ content}$ and $Y_{CO2\ sequestration}$

The percent contribution of interacting variables determined after the statistical analysis is given in Table 3.12 and Table 3.13.The pairwise (DIWW+CO_2; DIWW+Light and CO_2+Light) interactive contributions between the factors toward Y_{BM}, Y_{lipid} Y_{FAME} and Y_{CO_2} are shown in Fig.3.19(a–d), respectively. The order of binary interaction among the variables was found CO_2+Light> DIWW+CO_2> DIWW+Light for *Chlorella pyrenoidosa* as shown in Fig.3.19a. Similarly, the most influencing binary interactive variable for lipid and FAME content were found CO_2+Light> DIWW+CO_2> DIWW+Light given in Fig.3.19b and DIWW+CO_2 >DIWW+Light >CO_2+Light given in Fig.3.19c. In case of CO_2, binary interactive variable of Y_{CO2} is CO_2+Light> DIWW+CO_2 >Light+DIWW (Fig.3.19d). It clearly shows that multicolor LED interaction with CO_2 supply and DIWW (Light-CO_2 and DIWW-CO_2) significatly impact microalgal biomass, responsible for enhancement of lipid content and CO_2 uptake rate.

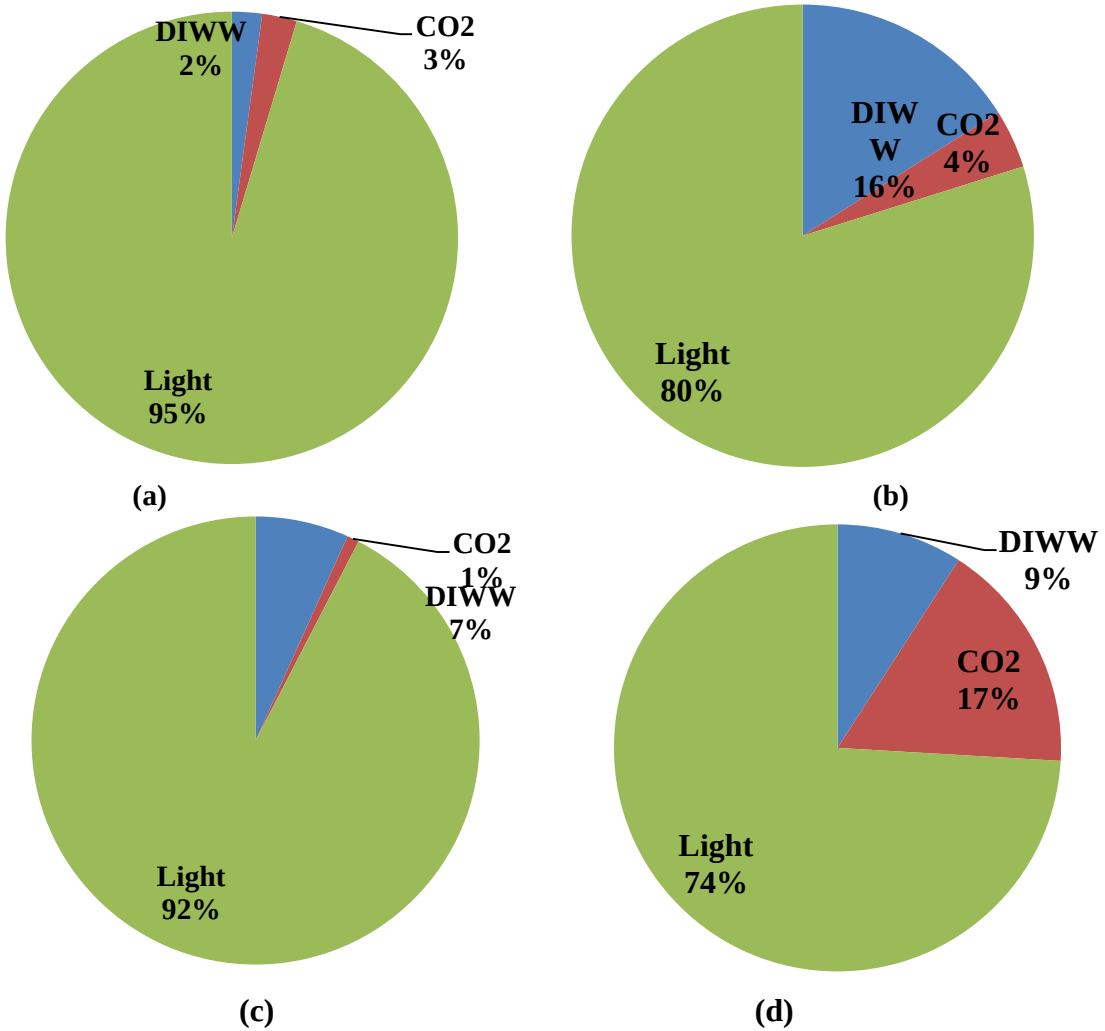


Fig.3.19:Percentage contribution of individual factors (X_1: DIWW, X_2:CO_2, and X_3: Light) on (a) Biomass production (b) Lipid content (c) FAME content and (d) CO_2 sequestration

According to Ho *et al.,* (2012), the use of RSM to maximize the fixation of CO_2 and growth rate by CO_2 manipulation, the CO_2 concentration and light intensity by using algal strain *Scenedesmus obliquus*. Similarly, the interaction of supply of CO_2 with DIWW and Light i.e (DIWW-CO_2 and DIWW-light) help for the quality improvement of FAME content of microalgal lipid after transestrification. Li *et al.,(*2012) suggests that the light intensity and CO_2 concentration had a significant effect on biomass accumulation for both *Chlorella kessleri* and *Chlorella protothecoides.* Therefore, three quadratic interaction variables were considered to generate 3D (Response Surface Plot) and 2D (Contour Plot). These plots are graphical representations of regression equation containing the information of the interacting effects between the most significant pairwise variables and optimal conditions could be identified by analyzing the effect. Kasiri, (2015) uses RSM-CCD model and validated the model and was found in good agreement between predicted and experimental data with 35% CO_2 concentration, 29 mM phosphate concentration and 70 µmol photons.$m^{-2}s^{-1}$ Light intensity maximized the CO_2 uptake rate upto 65.03 mg/L/day. Most significant interactive model for Y_{BM} (CO_2-Light) and 2D and 3D are shown in Fig3.21(a-b) whereas 3D and 2D plots for Y_{lipid} are shown in Fig.4.25(c-d).similarly most suitable interactive model for (CO_2-Light) and (DIWW-CO_2 ,DIWW-Light and CO_2-Light) for Y_{FAME} represented in Fig.3.21(e-j) Similarly, for $Y_{CO_2\ uptake}$ most significant interactive model is (DIWW-CO_2 and CO_2-Light) and represented in 3D and 2D as shown in Fig.3.21(k-n).

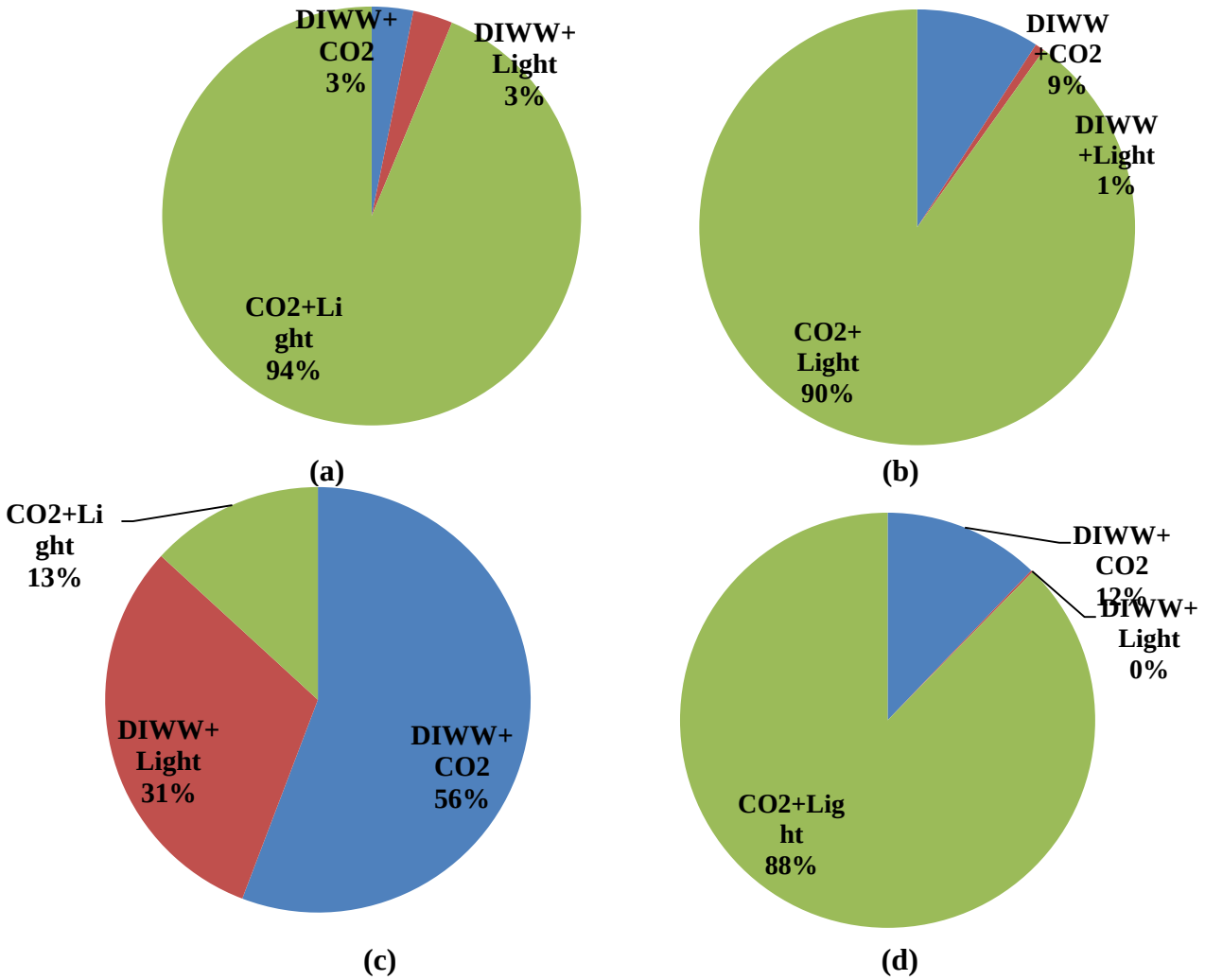
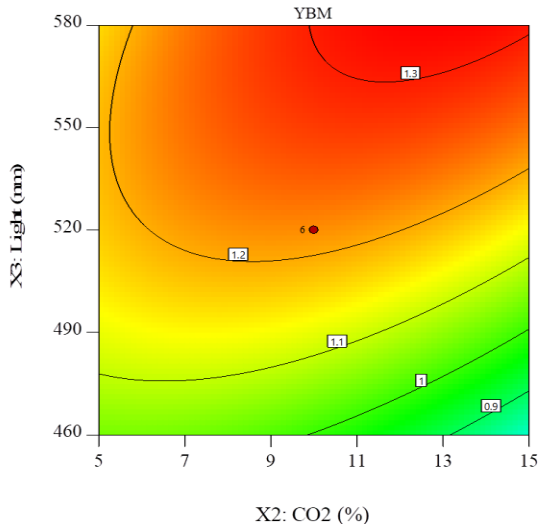
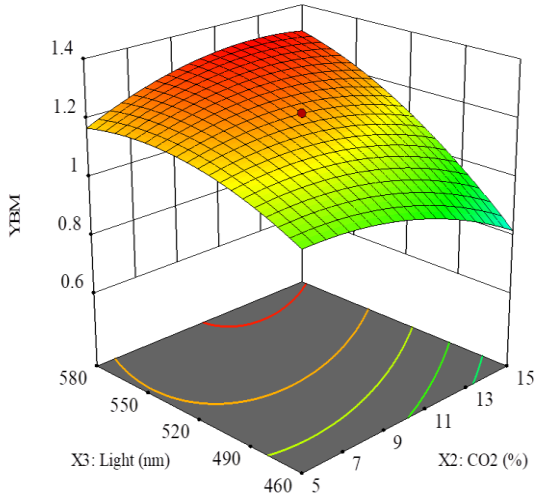
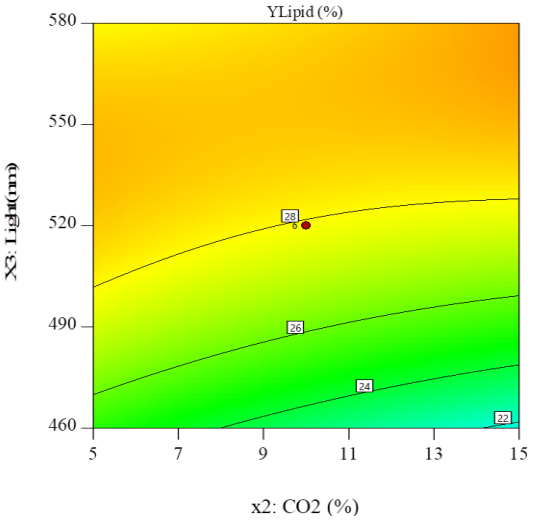


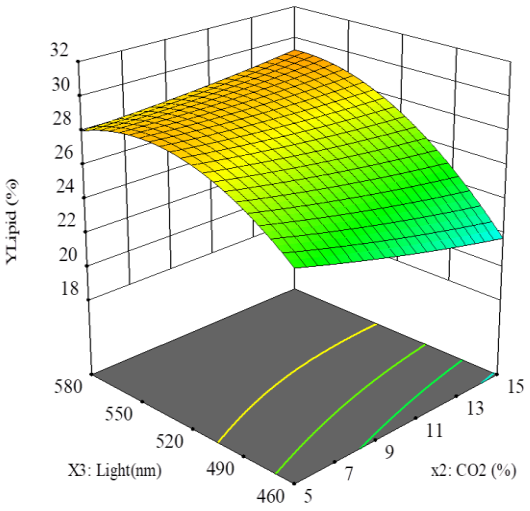
Fig.3.20.Percentage contribution of interactive factors (DIWW-CO_2, DIWW-light and CO_2-Light) (a) Biomass production (b) Lipid content (c) FAME content and (d) CO_2 sequestration


YBM
X3: Light (nm)
X2: CO2 (%)
580
550
520
490
460
5
7
9
11
13
15
1.3
1.2
1.1
1
0.9
6

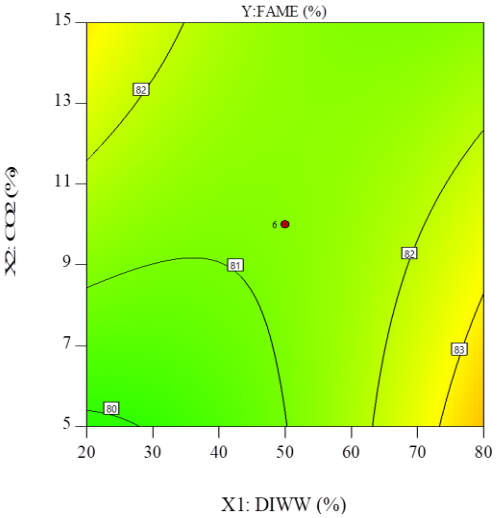

YBM
1.4
1.2
1
0.8
0.6
X3: Light (nm)
X2: CO2 (%)
580
550
520
490
460
5
7
9
11
13
15

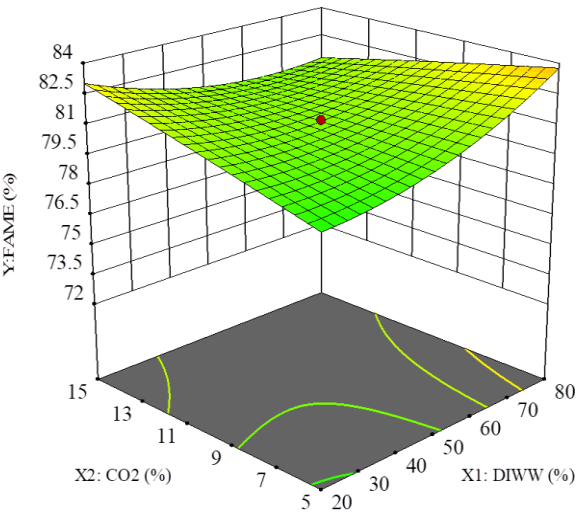
(a) **(b)**


YLipid (%)
X3: Light(nm)
x2: CO2 (%)
580
550
520
490
460
5
7
9
11
13
15
28
26
24
22
6

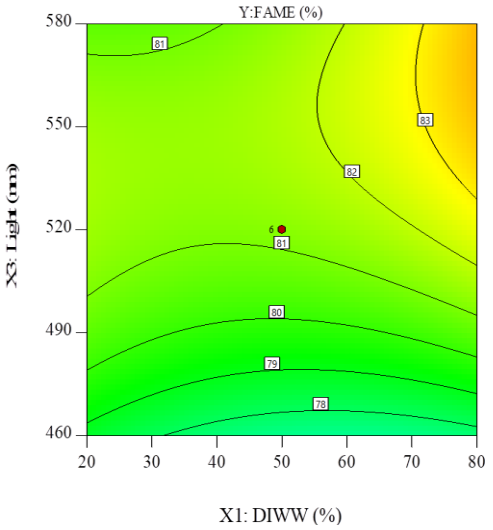

YLipid (%)
32
30
28
26
24
22
20
18
X3: Light(nm)
x2: CO2 (%)
580
550
520
490
460
5
7
9
11
13
15

(c) **(d)**

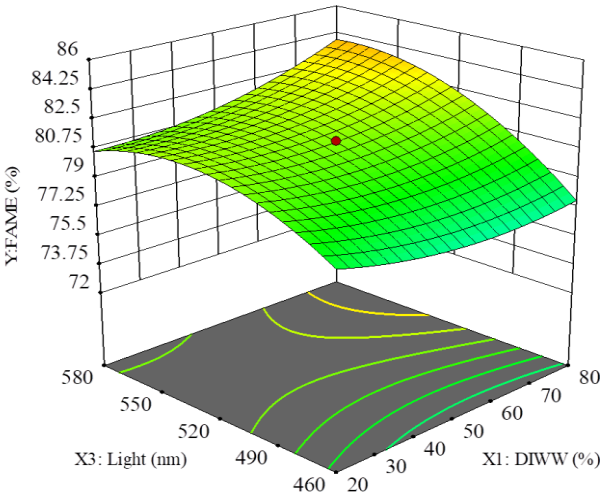

Y:FAME (%)
X2: CO2 (%)
X1: DIWW (%)
15
13
11
9
7
5
20
30
40
50
60
70
80
82
81
83
6


Y:FAME (%)
84
82.5
81
79.5
78
76.5
75
73.5
72
X2: CO2 (%)
X1: DIWW (%)
15
13
11
9
7
5
20
30
40
50
60
70
80

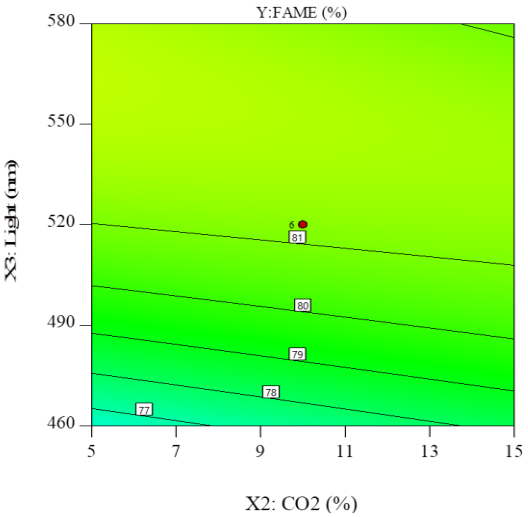
(e) **(f)**


Y:FAME (%)
X3: Light (nm)
X1: DIWW (%)
580
550
520
490
460
20
30
40
50
60
70
80
81
82
83
80
79
78

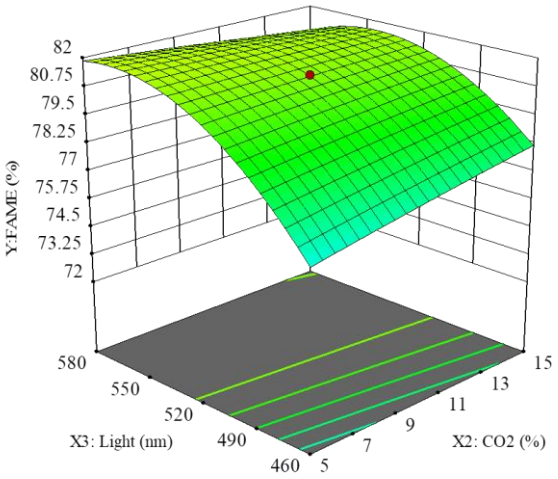
(g)


Y:FAME (%)
X3: Light (nm)
X1: DIWW (%)
86
84.25
82.5
80.75
79
77.25
75.5
73.75
72
580
550
520
490
460
20
30
40
50
60
70
80

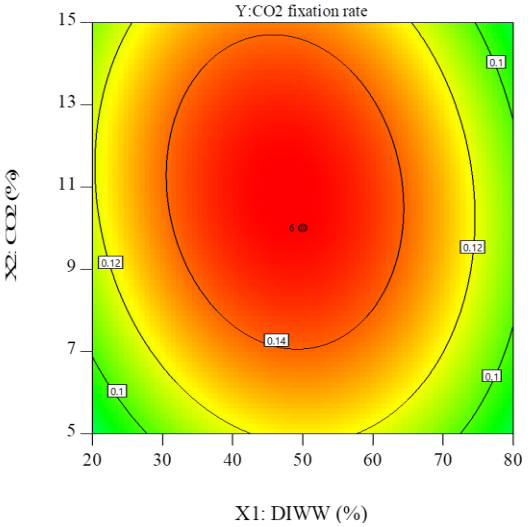
(h)


Y:FAME (%)
X3: Light (nm)
X2: CO2 (%)
580
550
520
490
460
5
7
9
11
13
15
81
80
79
78
77

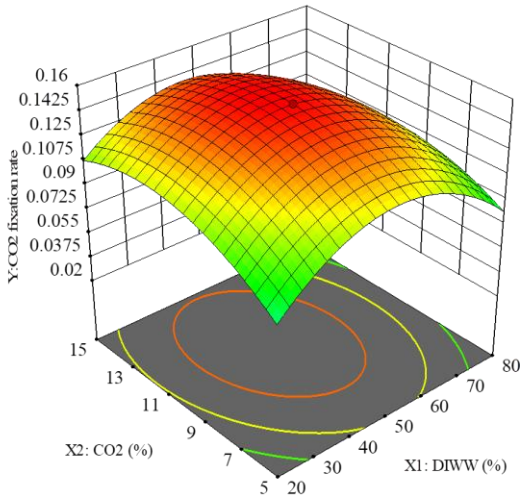
(i)


Y:FAME (%)
X3: Light (nm)
X2: CO2 (%)
82
80.75
79.5
78.25
77
75.75
74.5
73.25
72
580
550
520
490
460
5
7
9
11
13
15

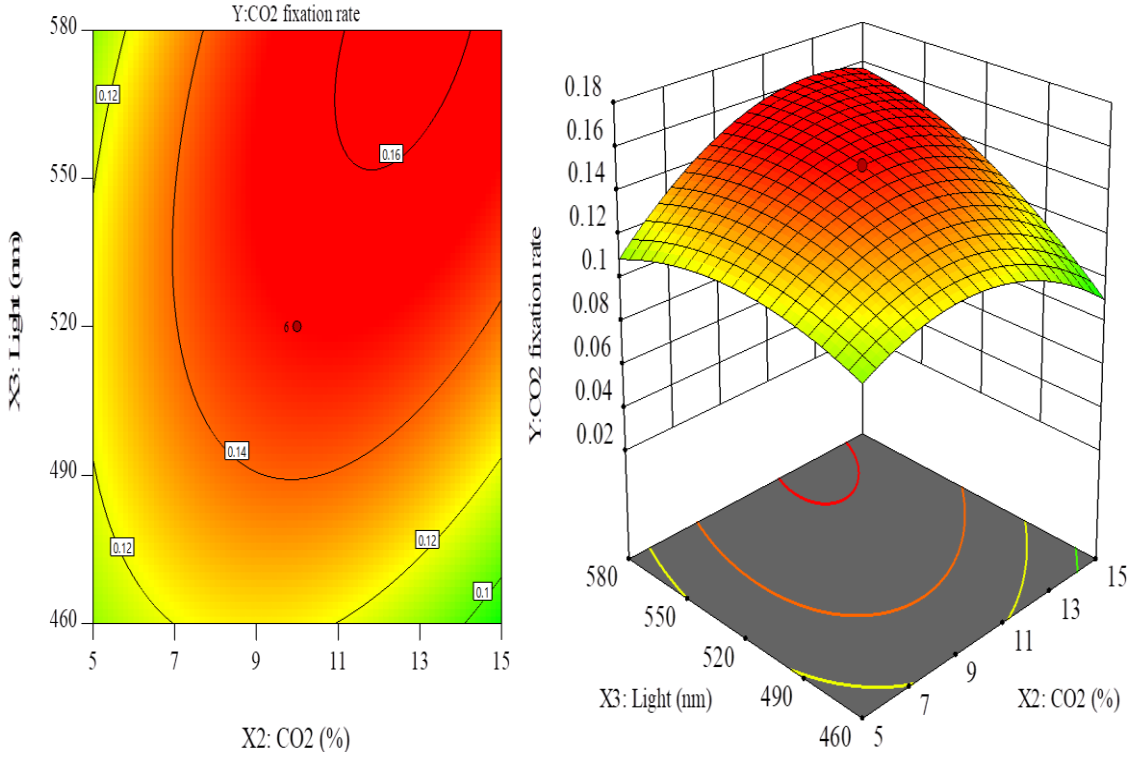
(j)


Y:CO2 fixation rate
X2: CO2 (%)
X1: DIWW (%)
15
13
11
9
7
5
20
30
40
50
60
70
80
0.1
0.12
0.14

(k)


Y:CO2 fixation rate
X2: CO2 (%)
X1: DIWW (%)
0.16
0.1425
0.125
0.1075
0.09
0.0725
0.055
0.0375
0.02
15
13
11
9
7
5
20
30
40
50
60
70
80

(l)



(m) **(n)**

Fig.3.21:2D and 3D representation of significant factor and responses: (a-b) Biomass productivity (c-d)lipid content (e-j) Fame content (k-n)CO_2 sequestration

3.3.2.3.3.FTIR analysis of transesterification of lipid by different variables (DIWW, CO_2 and LED light)

The importance of the spectroscopy for empathy of the arrangement of molecules originates for the possibility to assign certain absorption bands related to the functional group. In all oils, most of the peaks and bands are recognized for the functional group. Since, triglycerides (TGAs) are the main component in algal oil and all type of oil consists of fatty acids therefore, the IR spectra showing only characteristic of absorption bands at different variables (DIWW concentration, CO_2 flow and Effect of LED) concentrations IR spectra show notable difference at 3009 cm^{-1} due to CO_2 and LED light limitations which assign to the C-H stretching

vibration of double bond (=CH-). The exact position band is the function of oil and shifts with the change in composition of fatty acid of algal oil (Fig.3.22).

Absorption band found at around 2920-2855cm^{-1} due to the symmetric stretching vibration of the aliphatic group. Peak height for algal oil of DIWW deficiency show great difference than CO_2 deficiency. Spectral band arise at approximately 1743cm^{-1} due to the ester carbonyl group of triglycerides, it is noted that carbonyl band stretching shifting with according to concentration change of DIWW and LED light. Besides this band appeared at approximately 1743 cm^{-1} region sue to the ester carbonyl (-COO-) functional group of triglycerides. But due to variable concentration of NO_3^- and PO_4^{-3}, band of ester carbonyl (-COO-) functional group shifted to right at 1743cm^{-1}.There is no band absorbed at 1700 cm^{-1} because there is no free fatty acid (FFA) which affects the quality of algal oil or very low proportion is not detectable in IR spectrum. The intensity of C=C stretching found at the region of 1657cm^{-1} is not affected by any variable.

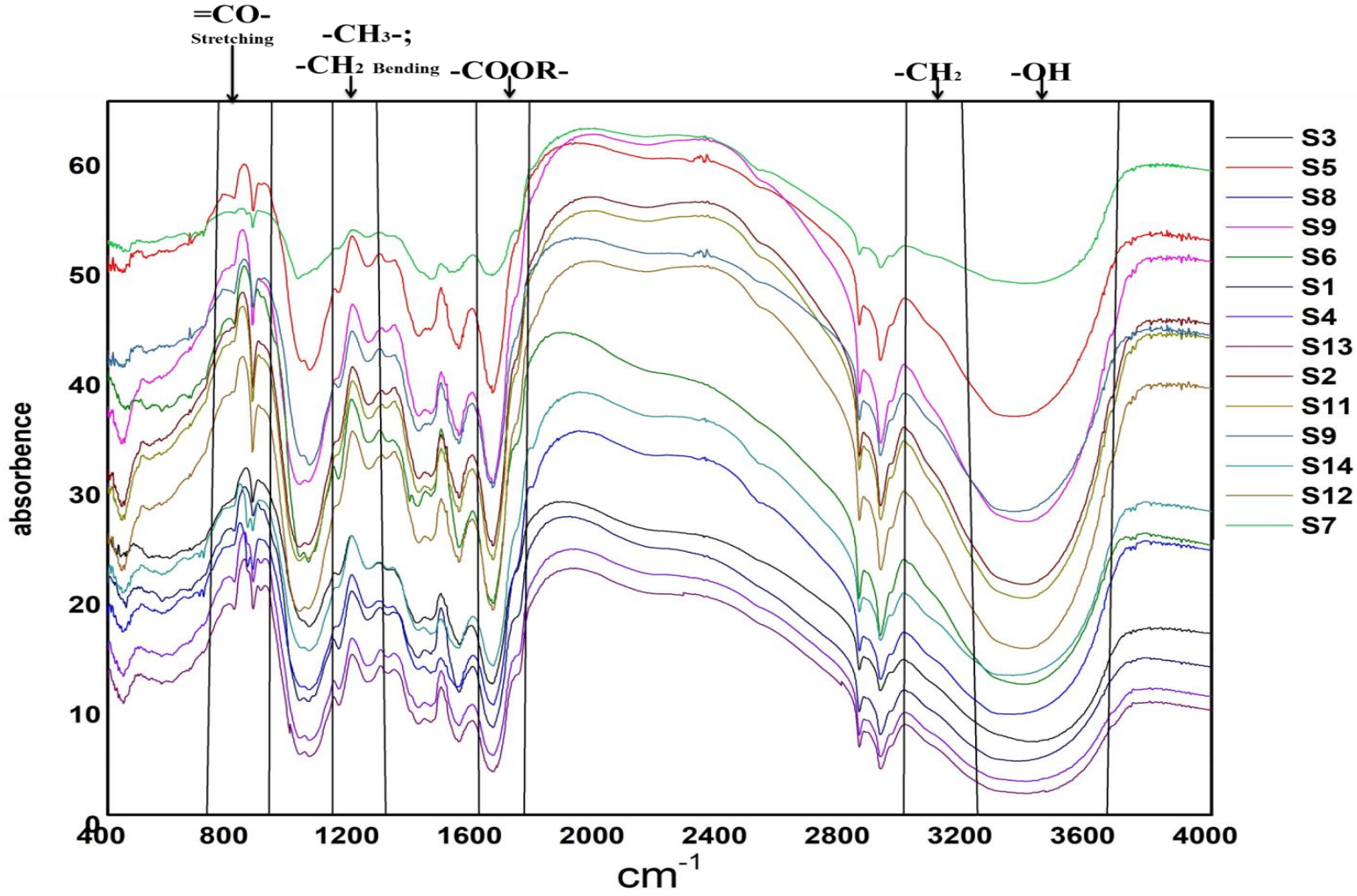


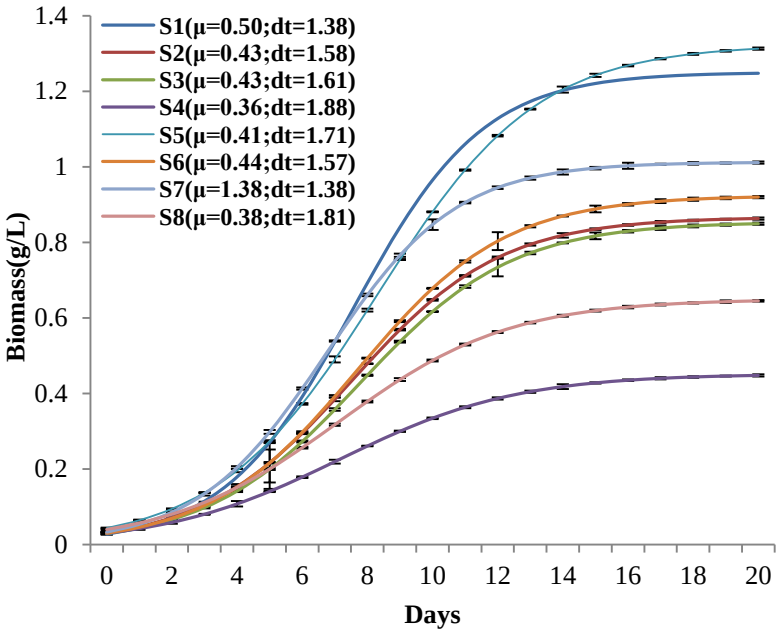
Fig.3.22:FTIR analysis of Transesterification of lipid by different variable (DIWW, CO_2 and LED light)

3.3.2.4. Effect of different light emitting diodes (LEDs) with temperature and CO_2

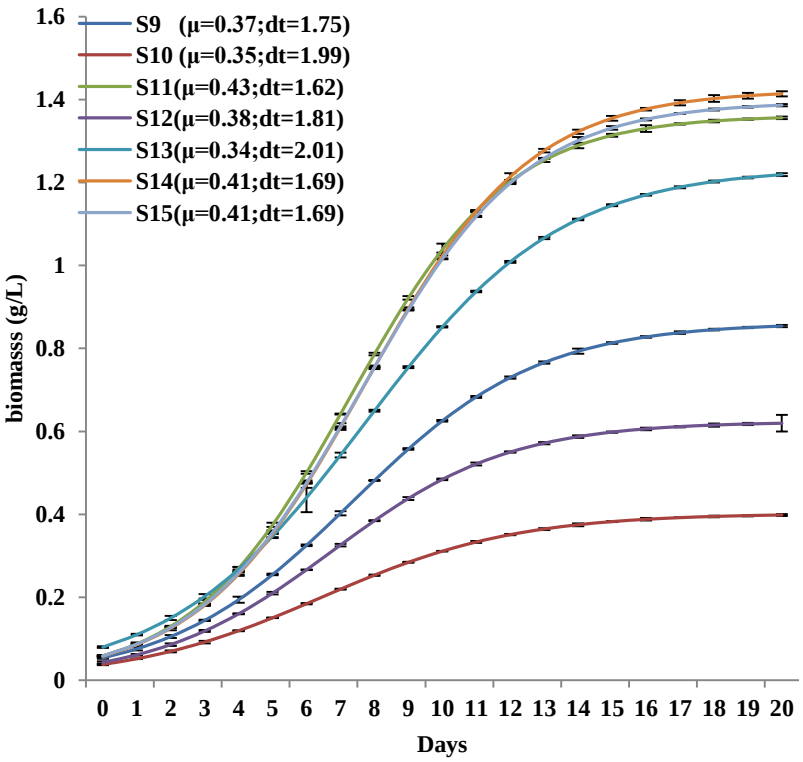
The algal growth and rate kinetics (μ) and doubling time (dt) of *C.pyrenoidosa* are depicted in Fig.3.23 a & b. This was carried out using different concentrations of CO_2 with different LEDs and temperatures as variables. The experiment was designed by using Central Composite Design (CCD) and 20 run in combination with different variables. The variation in biomass concentration, specific growth and doubling time was affected by combination of variables. The variation of biomass concentration with any concentration, where one factor is set high (+2) and other factor set (-2) as shown in Fig.3.23(a) and on the other hand, all factors were set (+1) to (-1) as given in Fig.3.23(b).

3.5.2.4.1.Optimization of algal biomass, lipid productivity and lipid content

The design expert developed a 2^3 factorial design resulting 20 run with 6 axial, 8 facial and 6 replicate using second order polynomial equation to predict by model for maximum concentration of algal biomass, lipid content, lipid productivity, FAME content and CO_2 sequestration 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 as shown in Table 3.14. Second order polynomial model were applied to determine the relationship between the different variables and responses. Analysis of variance (ANOVA) ensures the goodness of fit of model and is calculated by degree of freedom, sum and mean square of F and p values as given in Table 3.14. 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) is given in Eq.3.13 to Eq.3.17 that are composed of linear (X_1,X_2 and X_3), quadratic (X_1^2,X_2^2 and X_3^2) and interactive coefficient.



(a)



(b)

Fig.3.23:Time variant logistic growth model for the design of experiment with (a) anyone of the factor at ranges (-α to +α)(experiment no. 9,11,12,16,17,and 20 (b) Factor at the intermediate level (-1 to +1) (Experimental no. 2,3,6,7,8,10,13 and 18)

(X_1X_2,X_1X_3 and X_2X_3) among them linear coefficient (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 $X1$ for $Y_{Lipid\ content}$ (%) interactive coefficient (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 coefficient (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} (%).

$$Y_{BM}(g/L)=-1.86+0.12X_1+0.11X_2+3.4x10^{-3}X_3+8.4x10^{-5}X_1X_2*+1.6x10^{-5}X_1X_3+5x10^{-5}X_2X_3-8.7x10^{-3}X_1^2-2.2x10^{-3}X_2^2-4.2x10^{-6}X_3^2 \quad \textbf{(3.13)}$$

$$Y_{Lipid\ content}(\%)=-15.08+2.01X_1+2.62X_2-0.033X_3+0.011X_1X_3-7.5x10^{-4}X_1X_3+7.7x10^{-5}X_2X_3-0.11X_1^2-0.039X_2^2+3.8x10^{-5}X_3^2 \quad \textbf{(3.14)}$$

$$Y_{FAME(\%)}=-199.46+1.75X_1+1.46X_2+0.96X_3-0.032X_1X_2^{-3}x10-3X_1X_3+6.9x10^{-4}X_2X_3+0.037\ X_1^2-0.028X_2^2-8.99x10^{-4}X_3^2 \quad \textbf{(3.15)}$$

$$Y_{Lipid\ productivity}\ (g/L/d) =0.11+4x10^{-3}X_1+4.4x10^{-3}X_2+1.7x10^{-4}X_3+1.2x10-5X_1X_2-1.04x10^{-6}X_1X_3+5.2x10^{-7}X_2X_3-2.32x10^{-4}X_1^2-7.3X10^{-5}X_2^2-1.5x10^{-7}X_3^2 \quad \textbf{(3.16)}$$

$$Y_{CO2seq}\ (g/l/d) =-1.32+2.3x10^{-2}X_1+1.33x10^{-3}X_2+4.8x10^{-3}X_3-1.33x10^{-4}X_1X_2+2.08x10^{-6}X_1X_3+2.8x10^{-5}X_2X_3-1.07x10^{-3}X_1^2-1.9x10^{-4}X_2^2-5.26x10^{-6}X_3^2 \quad \textbf{(3.17)}$$

Synergistic effect caused by positive coefficient and antagonistic effect caused by negative coefficient of different variables due to the effect of interaction is given in Table 3.15 and Table 3.16. Coefficient of determination (R^2: goodness of fit with respect to all factors), R^2_{adj} accuracy of important factor and R^2_{pred} quality were determined for Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L) and responses were given in Table 3.15 and Table 3.16 respectively. All R^2 values suggested that regression model was accurate within the limits of operating variables. Regression model satisfied

Table 3.14:Design of experiment and responses (Algal biomass growth; Lipid productivity; FAME content; and CO_2 sequestration)

	Factor			Observed					Predicted				
Std	**X_1:CO_2**	**X_2:Tem**	**X_3:LED**	**Y_{BM} (g/L^{-1})**	**Y_{Lipid} (%)**	**Y_{FAME} (%)**	**$Y_{Lipid\ prod}$ ($g\ L^{-1}d^{-1}$)**	**Y_{CO2seq} ($g\ L^{-1}d^{-1}$)**	**Y_{BM} (g/L^{-1})**	**Y_{Lipid} (%)**	**Y_{FAME} (%)**	**$Y_{Lipid\ prod}$ ($g\ L^{-1}d^{-1}$)**	**Y_{CO2seq} ($g\ L^{-1}d^{-1}$)**
1	5	25	460	1.3	26.4	79.6	0.022	0.08	1.26	26.57	79.76	0.022	0.075
2	15	25	460	0.9	22.3	82.3	0.013	0.08	0.87	23.15	82.99	0.014	0.080
3	5	45	460	0.9	25.4	71.4	0.015	0.08	0.85	25.10	72.48	0.014	0.079
4	15	45	460	0.5	23.4	69.5	0.007	0.05	0.47	23.91	69.33	0.009	0.057
5	5	25	580	1.3	27.4	83.3	0.025	0.08	1.31	27.23	83.60	0.025	0.078
6	15	25	580	0.9	22.2	84.3	0.014	0.09	0.93	22.91	83.24	0.015	0.085
7	5	45	580	1.0	26.3	78.6	0.018	0.15	1.01	25.95	77.99	0.018	0.149
8	15	45	580	0.7	23.7	71.3	0.010	0.13	0.65	23.85	71.24	0.011	0.130
9	0	35	520	0.9	22.4	88.6	0.013	0.06	0.88	22.86	88.22	0.014	0.061
10	20	35	520	0.1	18.3	84.4	0.0012	0.05	0.13	17.34	84.70	-0.0008	0.046
11	10	20	520	1.1	23.6	83.6	0.019	0.09	1.13	22.77	83.56	0.018	0.094
12	10	50	520	0.6	22.3	69.3	0.009	0.13	0.61	22.38	69.11	0.009	0.130
13	10	35	420	1.2	32.3	72.4	0.027	0.08	1.23	31.67	71.33	0.026	0.071
14	10	35	620	1.4	32.2	75.3	0.031	0.13	1.43	32.18	76.12	0.030	0.134
15	10	35	520	1.4	31.2	82.4	0.030	0.16	1.37	31.54	82.72	0.030	0.155
16	10	35	520	1.4	31.3	82.6	0.029	0.15	1.37	31.54	82.72	0.030	0.155
17	10	35	520	1.4	31.6	83.0	0.029	0.15	1.37	31.54	82.72	0.030	0.155
18	10	35	520	1.4	32.0	83.1	0.031	0.16	1.37	31.54	82.72	0.030	0.155
19	10	35	520	1.4	31.5	82.6	0.030	0.16	1.37	31.54	82.72	0.030	0.155
20	10	35	520	1.4	31.6	82.6	0.029	0.15	1.37	31.54	82.72	0.030	0.155

Table 3.15.Statistical and Mathematical analysis of variance for Y_{BM} (g/L^{-1}) Y_{Lipid} (%), $Y_{Lipid\ prod}$ ($g\ L^{-1}d^{-1}$)

		ANOVA of Response surface quadratic model for Y_{BM} (g/L^{-1})				ANOVA of Response surface quadratic model for Y_{Lipid} (%),				ANOVA of Response surface quadratic model for $Y_{Lipid\ prod}$ ($g\ L^{-1}d^{-1}$)			
Source	**df**	**Sum of square**	**Mean square**	**F-value**	**p-value**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**
Model	9	2.57601	0.28622	1175.24311	0.00000	377.86	41.98	96.47	< 0.0001	0.0016	0.0002	100.26	< 0.0001
X_1-CO_2	1	0.56565	0.56565	2322.59903	0.00000	30.44	30.44	69.95	< 0.0001	0.0002	0.0002	118.38	< 0.0001
X_2-Temperature	1	0.37225	0.37225	1528.45834	0.00000	0.2087	0.2087	0.4794	0.5044	0.0001	0.0001	65.69	< 0.0001
X_3-LED	1	0.04535	0.04535	186.21373	0.00000	0.3141	0.3141	0.7216	0.4155	0	0	11.95	0.0062
X_1X_2	1	0.00014	0.00014	0.57944	0.46410	2.48	2.48	5.69	0.0383	3.00E-06	3.00E-06	1.65	0.2279
X_1X_3	1	0.00018	0.00018	0.72562	0.41424	0.4095	0.4095	0.9409	0.3549	7.81E-07	7.81E-07	0.4295	0.527
X_2X_3	1	0.00722	0.00722	29.66211	0.00028	0.0171	0.0171	0.0393	0.8468	7.81E-07	7.81E-07	0.4295	0.527
X_1^2	1	1.24117	1.24117	5096.27417	0.00000	214.48	214.48	492.82	< 0.0001	0.0009	0.0009	482.64	< 0.0001
X_2^2	1	0.50018	0.50018	2053.76770	0.00000	161.1	161.1	370.15	< 0.0001	0.0005	0.0005	300.56	< 0.0001
X_3^2	1	0.00320	0.00320	13.15722	0.00463	0.266	0.266	0.6111	0.4525	4.02E-06	4.02E-06	2.21	0.168
Residual	10	0.00244	0.00024			4.35	0.4352			0	1.82E-06		
Lack of Fit	5	0.00144	0.00029	1.44317	0.34855	3.98	0.7957	10.65	0.0107	0	2.97E-06	4.46	0.0633
Pure Error	5	0.00100	0.00020			0.3735	0.0747			3.33E-06	6.67E-07		
Cor Total	19	2.57844				382.22				0.0017			
S.D		0.0156				0.65				0.0013			
Mean		1.05				26.86				0.0201			
C.V%		1.49				2.46				6.7			
R		0.99				0.98				0.98			
R^2 adj		0.99				0.97				0.97			
R^2pre		0.99				0.91				0.91			

Table3.16:Statistical and Mathematical analysis of variance for Y_{FAME} (%) and Y_{CO2seq} ($g\ L^{-1}d^{-1}$)

		ANOVA of Response surface quadratic model for Y_{FAME} (%)				ANOVA of Response surface quadratic model for Y_{CO2seq} ($g\ L^{-1}d^{-1}$)			
Source	**df**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**
Model	9	633.9	70.43	119.35	< 0.0001	0.0308	0.0034	340.17	< 0.0001
X_1-CO_2	1	12.37	12.37	20.97	0.001	0.0002	0.0002	20.17	0.0012
X_2-Temperature	1	289.97	289.97	491.37	< 0.0001	0.0018	0.0018	181.23	< 0.0001
X_3-LED	1	28.05	28.05	47.53	< 0.0001	0.0047	0.0047	467.9	< 0.0001
X_1X_2	1	20.38	20.38	34.54	0.0002	0.0004	0.0004	34.89	0.0001
X_1X_3	1	6.46	6.46	10.95	0.0079	3.13E-06	3.13E-06	0.3105	0.5896
X_2X_3	1	1.39	1.39	2.35	0.1564	0.0023	0.0023	226.34	< 0.0001
X_1^2	1	22.91	22.91	38.83	< 0.0001	0.0169	0.0169	1681.68	< 0.0001
X_2^2	1	81.54	81.54	138.17	< 0.0001	0.0037	0.0037	370.27	< 0.0001
X_3^2	1	145.74	145.74	246.96	< 0.0001	0.005	0.005	496.26	< 0.0001
Residual	10	5.9	0.5901			0.0001	0		
Lack of Fit	5	5.53	1.11	14.75	0.0051	0.0001	0	1.89	0.2509
Pure Error	5	0.3748	0.075			0	$6.9x10^{-6}$		
Cor Total	19	639.8				0.0309			
S.D		0.77				0.0032			
Mean		79.5				0.11			
C.V%		0.96				2.88			
R^2		0.99				0.99			
R^2 adj		0.98				0.99			
R^2pre		0.92				0.98			

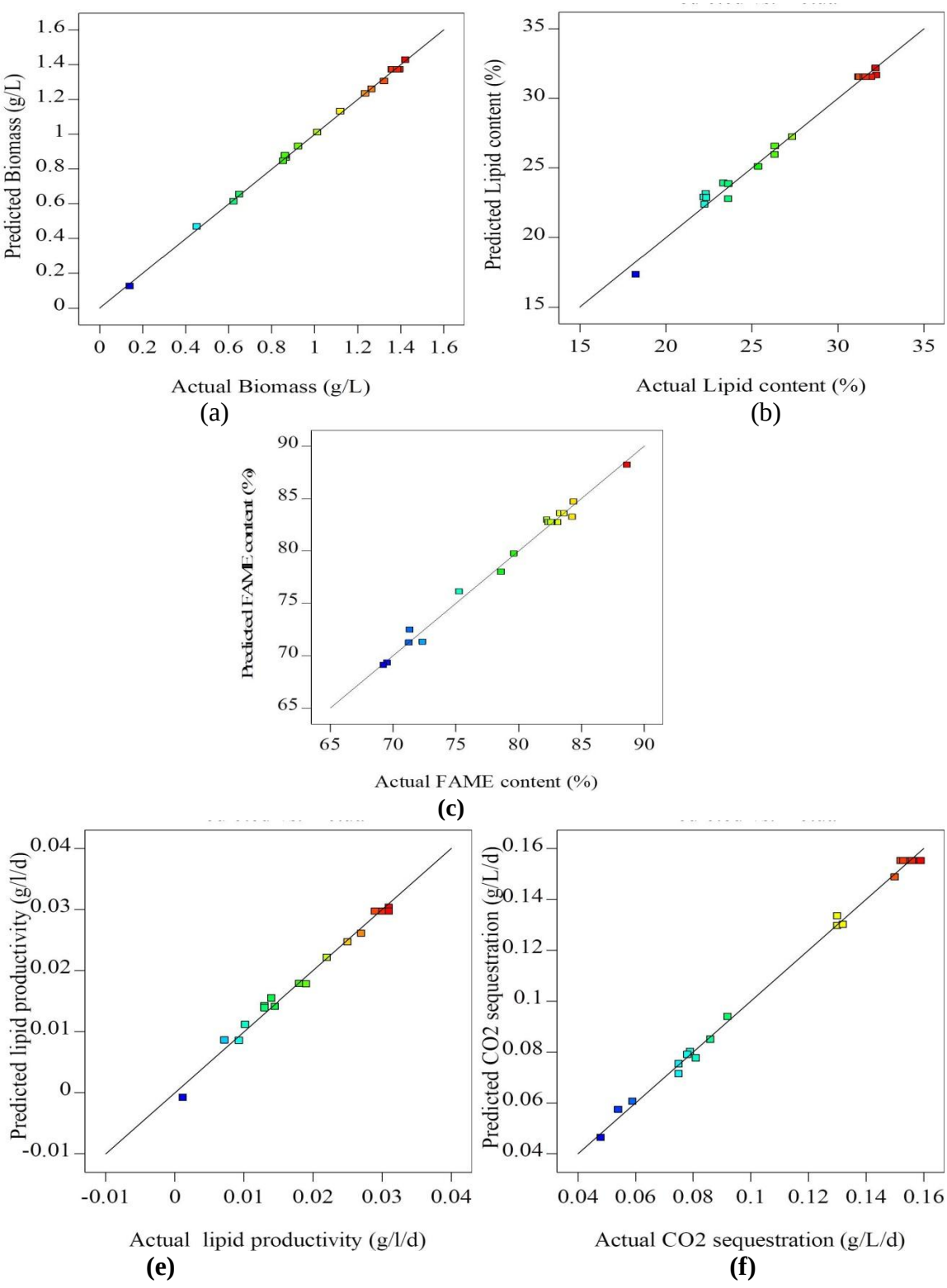


Fig.3.24:Relation between predicted versus actual Responses Probability for (A) Biomass (Y_{BM} (g/L) (b)Lipid content (Y_{Lipid} (%) (c)Lipid productivity ($Y_{Lipid\ prod}$ (g/L d^{-1}) (d) CO_2 sequestration $Y_{CO2\ seq}$(g/L d^{-1}) (e) FAME content (Y_{FAME} (%)

the statistically non-significant lack of fit where p value of Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L) responses indicated the proposed model fitted satisfactory between experimental data and observed data as shown in Fig.24(a-e). So, this model is satisfactory and does not show any or slight violation of independence or constant as shown in Table 3.15 and 3.16; low coefficient of variance in Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L) assumed high degree of accuracy and authencity between the experimental and observed data. The difference between observed and predicted responses called residue was determined to verify the accuracy of model, which is shown by plotting % probability vs residuals.

3.3.2.4.1.1.Individual contribution of CO_2, Temperature and LED

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 values in Table 3.15 and Table 3.17 and change in % contribution of variables is given in Fig.3.25(a-e) variation in (Y_{BM} (g/L) and $Y_{Lipid\ productivity}$ (g/L/d), influencing variables is CO_2>Temp>LED in Fig.25a, $Y_{Lipid\ content}$ (%) influenced by CO_2>LED>Temp as shown in Fig.3.25b. Similarly, the order of influencing variables for $Y_{Lipid\ content}$ (%) Y_{CO2seq} (g/L/d), and Y_{FAME} (g/L) was CO_2>LED>Temp, Temp>LED>CO_2 and LED>Temp>CO_2 as shown in Fig.3.25a to 3.25e.The above analysis revealed that CO_2 and temperature have significant impacts on the metabolism of microalgae which impacts biomass growh lipid content and lipid productivity. Increase in CO_2 concentration upto 2-6% enhances upto 45% growth of *Chlorella vulgaris* (Zheng *et al.,* 2012). Tipawan *et al.,* (2016) suggested that 20% of CO_2, decreased biomass growth as well as lipid production. Several studies reported that biofixation rate of CO_2 is below 1 g $L^{-1}d^{-1}$

when high concentration of CO_2 used in the algal culture (Ho *et al*., 2010; de Morais and Costa, 2007; Huntley and Redalje, 2006). From the Eq.3.13 to 3.17, CO_2 and temperature positively effects the Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L) but only LED light negatively affects the lipid content. Toledo-Cervantes *et al.,* (2013) reported that CO_2 fixation rate was affected by light intensity and CO_2 feeding rate during algal growth.

3.4.2.4.1.2.Interaction of operating variable:Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L):

The percent contribution of interacting variables(CO_2+temperaure,CO_2+LED and temperaure+LED) determined after statistical and mathematical analysis of variables (Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L)) and was calculated prob>F b using Table 3.15 and Table 3.16 value and change in % contribution of variables is given in Fig.3.25(a- e). The most influencing binary interactive variables for biomass production and CO_2 sequestration are LED+Temp>CO_2+Temp>CO_2+LED as shown in Fig. 3.26 (a) and 3.25(d). From the above result, multicolor LED and effect of temperature enhances the growh by utilization of CO_2. The lipid content, lipid productivity and FAME content behavior depends on the interaction of ((CO_2+Temp)>(CO_2+LED)>(LED+Temp)). Lipid content and FAME content is directly affected by the supply of CO_2 and temperature. For effective fixation, light becomes the limiting factor for biomass production for microalgal cultivation. Kumar *et al*.,(2011) suggested that temperature affects the solubility of CO_2. High temperatures affect the ratio of O_2 to CO_2 concentration. This leads to O_2 fixation by enzymatic activity of RuBisCO. High temperature also leads to decrese in RuBisCO affinity which decreses the CO_2.

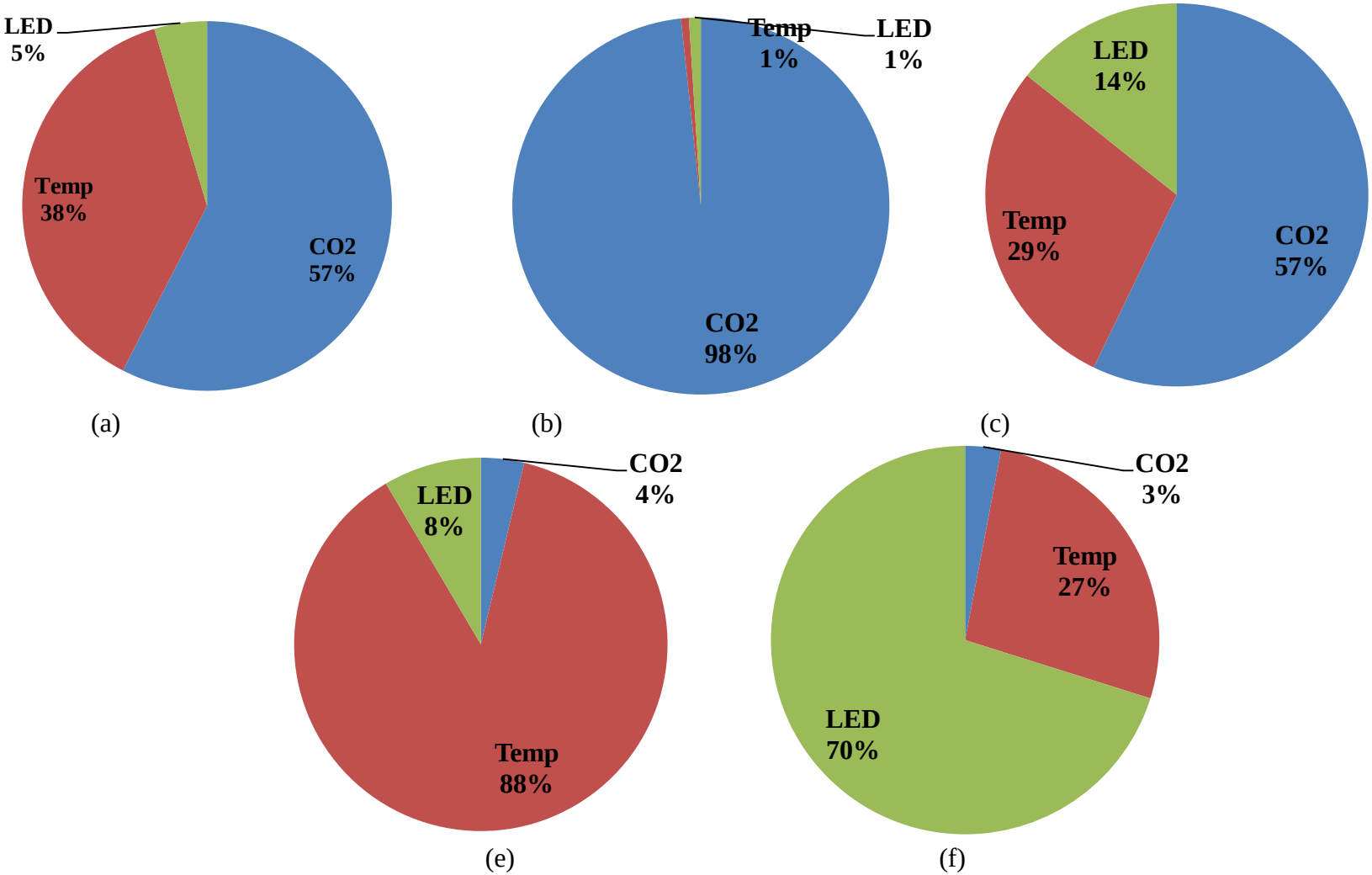


Fig.3.25: Percent contribution of individual factors (X_1:CO_2, X_2: Temperature and X_3: LED) on (a) Biomass production (Y_{BM} (g/L) (b) Lipid content (Y_{Lipid} (%) (c) Lipid productivity $Y_{CO2\ seq}$(g/L d^{-1}) (d) CO_2 sequestration $Y_{CO2\ seq}$(g/L d^{-1}) (e) FAME content (Y_{FAME} (%)

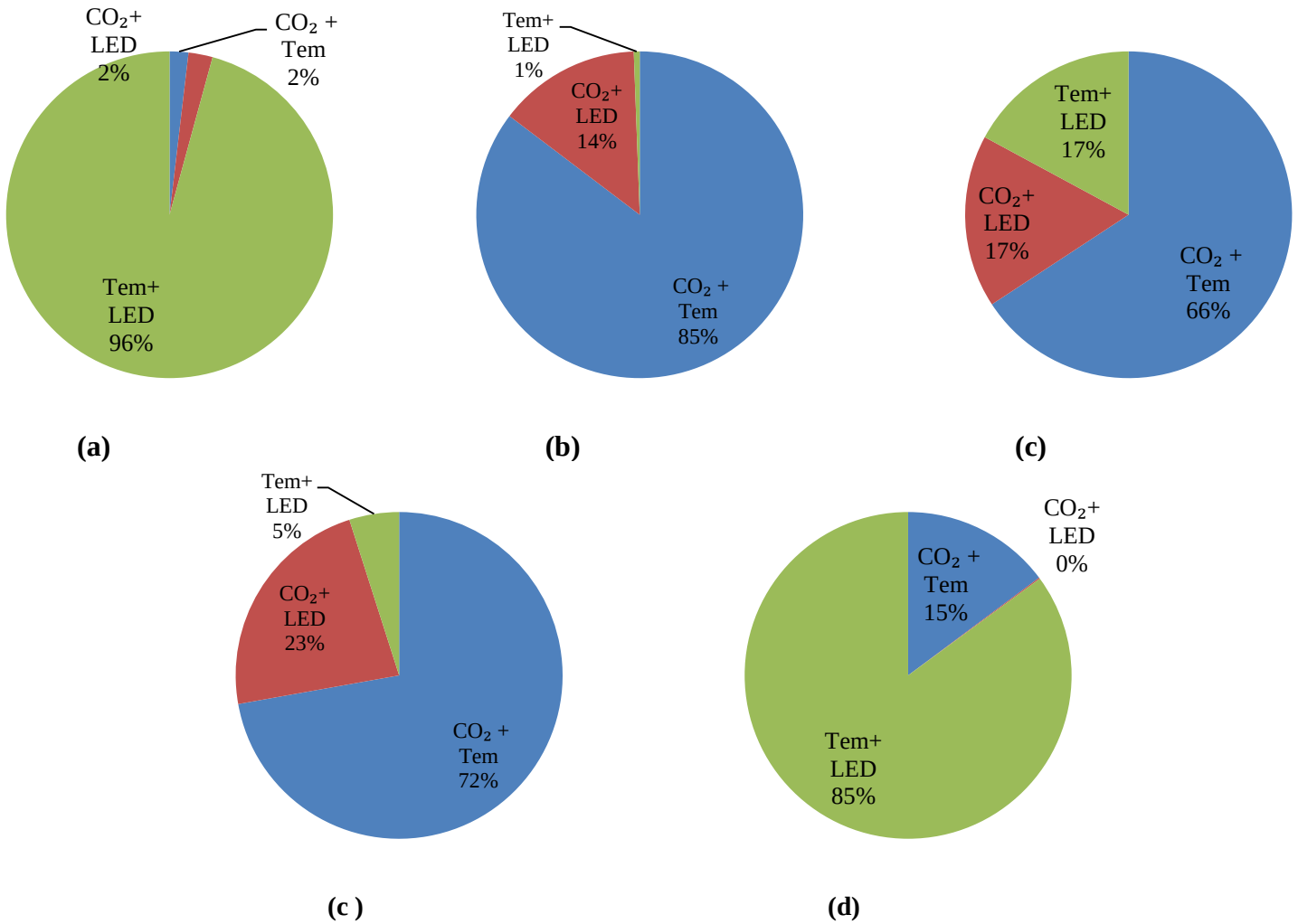
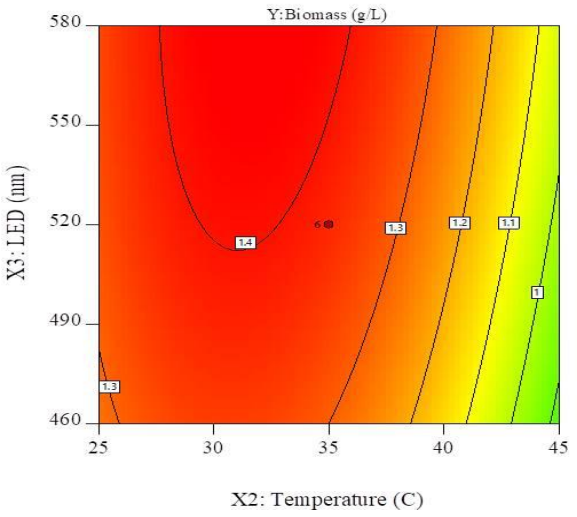
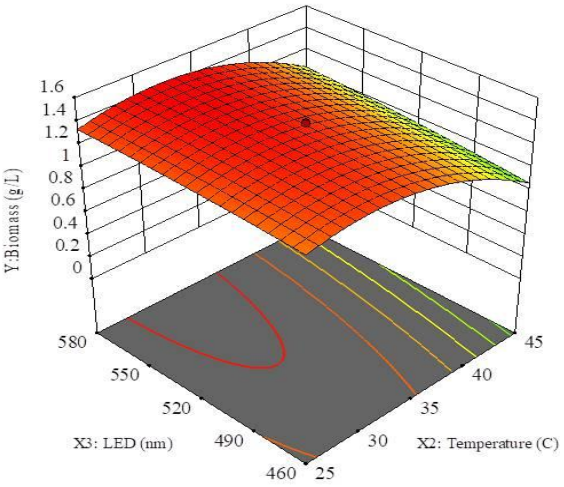


Figure.3.26:Percent contribution of interective factors (X_1:CO_2, X_2: Temperature and X_3: LED) on (a) Biomass production (Y_{BM} (g/L) (b) Lipid content (Y_{Lipid} (%) (c) Lipid productivity $Y_{CO2\ seq}$(g/L d^{-1}) (d) CO_2 sequestration $Y_{CO2\ seq}$(g/L d^{-1}) (e) FAME content (Y_{FAME} (%)

From the equation, interaction of (CO_2+LED) negatively affects the content of lipid and FAME after transestrification. Therefore, three quadratic interaction variables, after statistical analysis, generated 2D (contour plot) and 3D (surface response plot) interaction surface response graph with representation of regression equation containing the effect between the most significant pairwise variables and optimal condition could be identified by analyzing the effect. Michał *et al*., (2016) found utilization rate of CO_2 for *Chlorella* sp.0.71g L^{-1} d^{-1} in 4% CO_2 and 1.77 g L^{-1} d^{-1} for *Nannochloropsis* species in 8% CO_2 concentration. Higher productivity means higher rates of biomass growth which also favours the CO_2 sequestration. The significant interactive quadratic model (Y_{BM} (g/L) is (Temp+LED) as shown in Fig.3.27(a-b) and from the Eq.3.13, it is revealed that all interactive factors positively contribute to biomass growh. Besides microalgae growth, the most significant and most attractive interactive model for lipid content (CO_2+Temp) and lipid productivity (CO_2+Temp and CO_2+LED) is shown in Fig.3.27(c-d) and interaction of (CO_2+LED) negatively effects lipid content and (CO_2+Temp) negatively effects the lipid productivity of algae, calculated from the Eq.3.14. Similarly, most interactive model for FAME content as shown in Fig.3.27(f-j) is (Temp+LED) and (CO_2+Temp and Temp+LED) for CO_2 sequestration. Eq.3.15 describes that (CO_2+LED) and (CO_2+Temp) negatively affect FAME content and uptake of CO_2 as given in Fig3.27(k-n).


Y:Biomass (g/L)
X3: LED (nm)
X2: Temperature (C)
580
550
520
490
460
25
30
35
40
45
1.4
1.3
1.2
1.1
1
1.3

(a)


Y:Biomass (g/L)
X3: LED (nm)
X2: Temperature (C)
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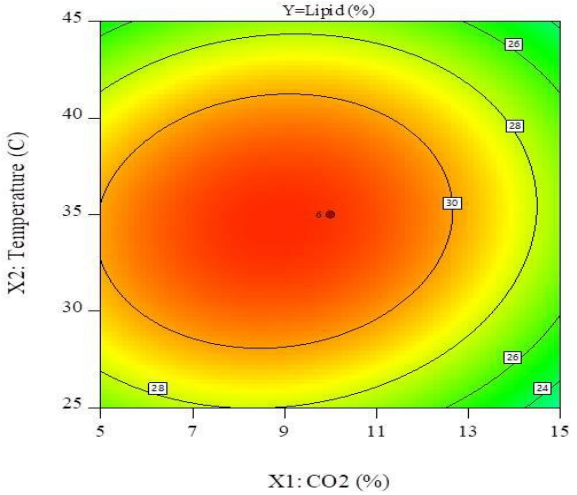
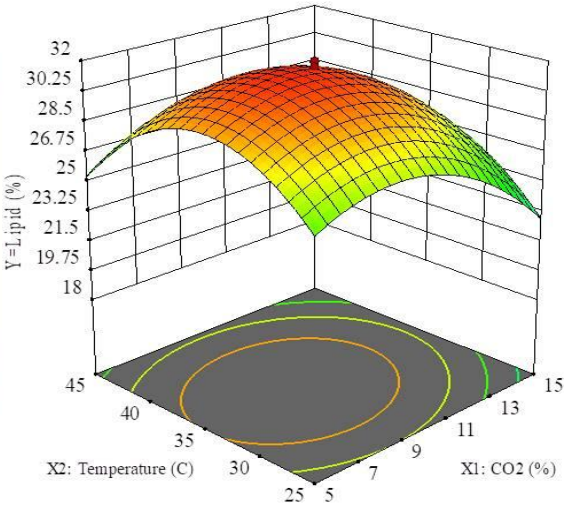
1.4
1.2
1
0.8
0.6
0.4
0.2
0
580
550
520
490
460
25
30
35
40
45

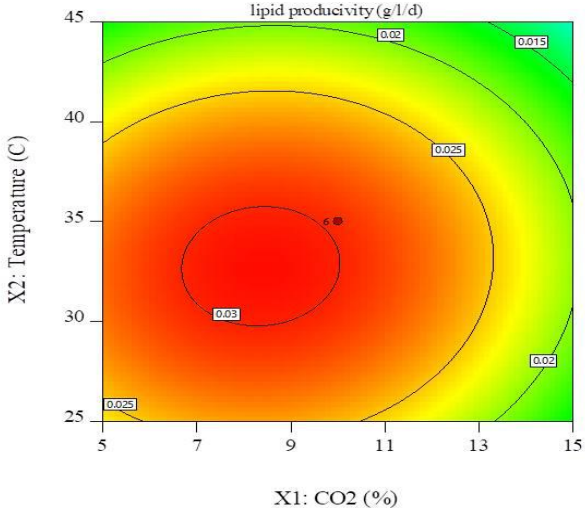
(b)


Y=Lipid (%)
X2: Temperature (C)
X1: CO2 (%)
45
40
35
30
25
5
7
9
11
13
15
26
28
30
24

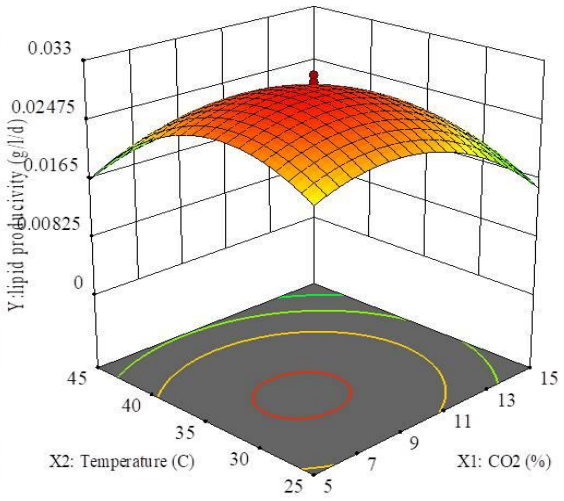
(c)


Y=Lipid (%)
X2: Temperature (C)
X1: CO2 (%)
32
30.25
28.5
26.75
25
23.25
21.5
19.75
18
45
40
35
30
25
5
7
9
11
13
15

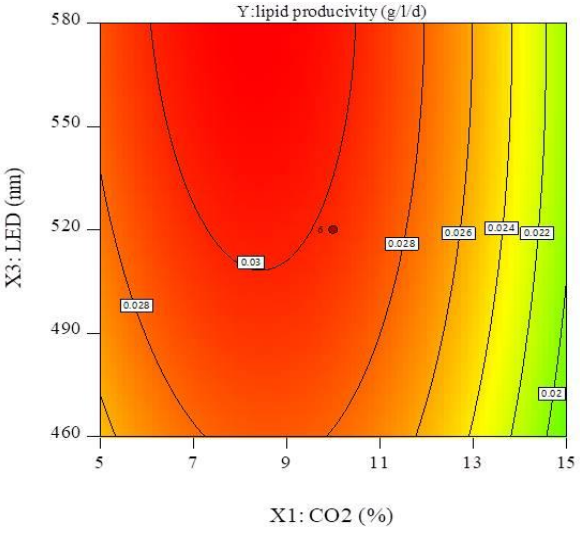
(d)


lipid producivity (g/l/d)
X2: Temperature (C)
X1: CO2 (%)
45
40
35
30
25
5
7
9
11
13
15
0.02
0.015
0.025
0.03
0.02
0.025

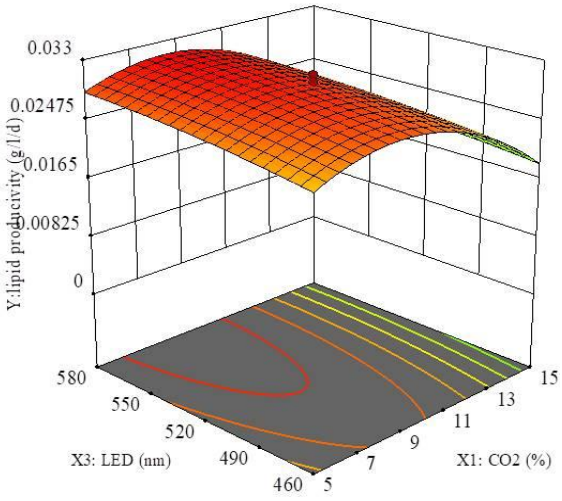
(e)


Y:lipid producivity (g/l/d)
X2: Temperature (C)
X1: CO2 (%)
0.033
0.02475
0.0165
0.00825
0
45
40
35
30
25
5
7
9
11
13
15

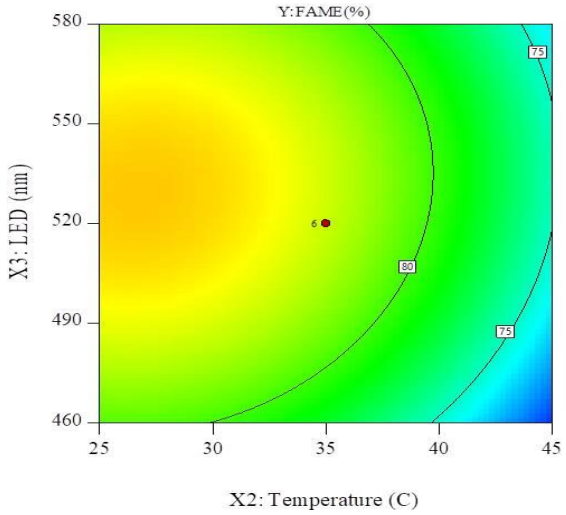
(f)


Y:lipid producivity (g/l/d)
X3: LED (nm)
X1: CO2 (%)
0.03
0.028
0.026
0.024
0.022
0.02

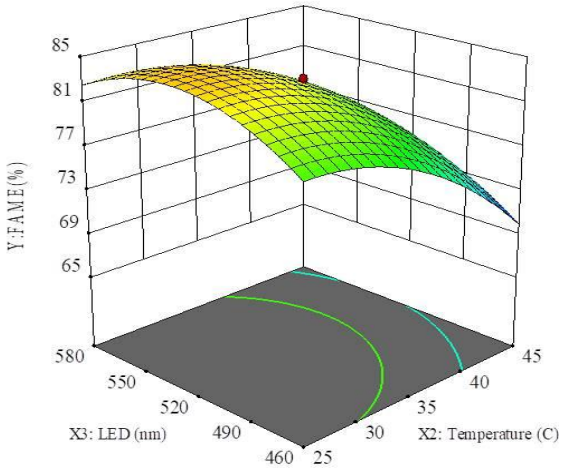
(g)


Y:lipid producivity (g/l/d)
X3: LED (nm)
X1: CO2 (%)

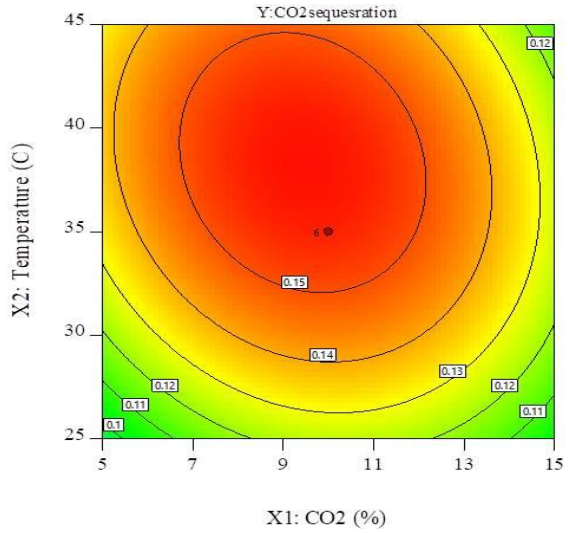
(h)


Y:FAME(%)
X3: LED (nm)
X2: Temperature (C)
80
75

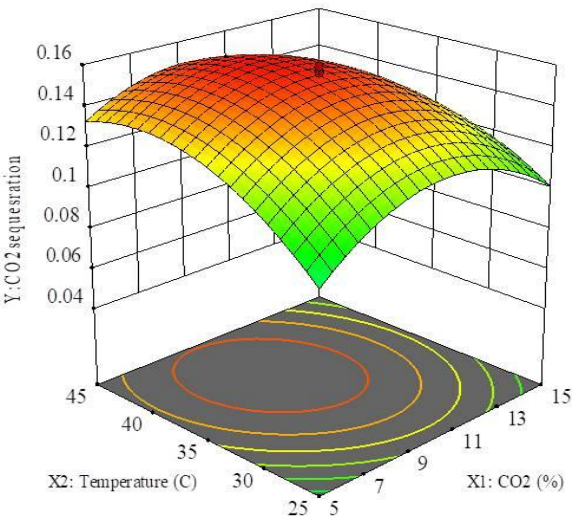
(i)


Y:FAME(%)
X3: LED (nm)
X2: Temperature (C)

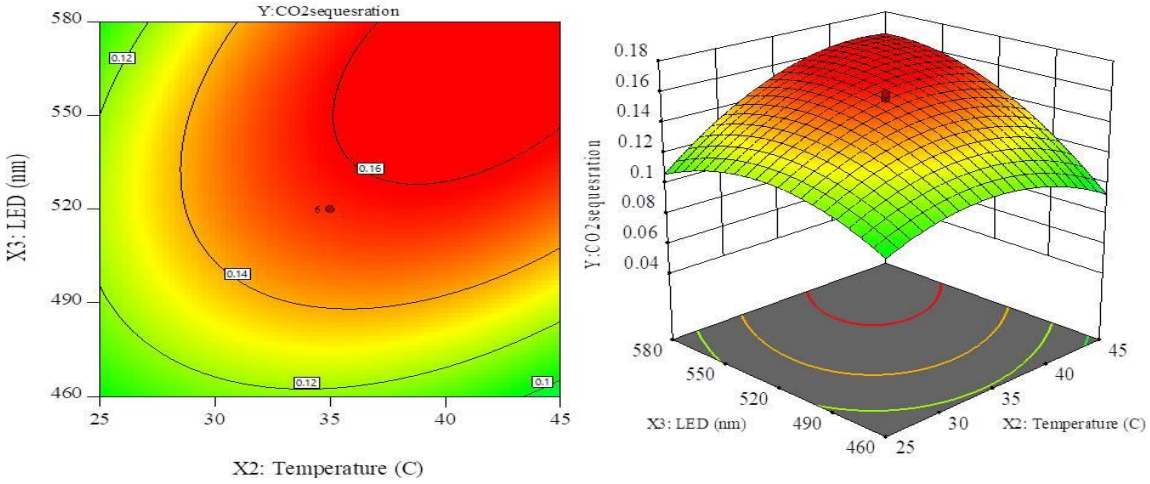
(j)


Y:CO2sequesration
X2: Temperature (C)
X1: CO2 (%)
0.15
0.14
0.13
0.12
0.11
0.1

(k)


Y:CO2sequesration
X2: Temperature (C)
X1: CO2 (%)

(l)



(m) **(n)**

Fig.3.27:Significant interactive quadratic model 2D and 3D (a) 2D;Light+CO_2 for Y_{BM}(g/L) (b) 3D;Light+CO_2 for Y_{BM}(g/L) (c) 2D;Light+CO_2 for $Y_{Lipid\ (\%)}$ (d) 3D;Light+CO_2 for $Y_{Lipid\ (\%)}$ (e) 2D;CO_2+DIWW for Y_{FAME}(%) (f) 3D;CO_2+DIWW for Y_{FAME}(%) (g) 2D;Light+DIWW for Y_{FAME}(%) (h) 2D;Light+DIWW for Y_{FAME}(%) (i) 2D;Light+CO_2 for Y_{FAME}(%) (j) 3D;Light+CO_2 for Y_{FAME}(%)(k) 3D;CO_2+DIWW for $Y_{Lipid\ prod}$ (g/L/d) (l) 2D;CO_2+DIWW for $Y_{Lipid\ prod}$ (g/L/d) (m) 2D;CO_2+Light for $Y_{CO2\ seq}$ (g/L/d) and (n) 3D;CO_2+Light for $Y_{CO2\ seq}$ (g/L/d)

3.4.2.4.2.FTIR analysis of Transesterified Lipid Content of different variables condition

FTIR analysis was conducted for the primary analysis of the functional group desired for biodiesel as shown in Table 3.17. In all oils, most of the peaks and bands are related to the functional group of the oil. The compositionof oil affects the exact position of the FTIR band and yields shift when the proposition of fatty acid with changed variables condition. The spectral band obtained after transestrification, IR band occurring at 3009-3006 cm^{-1} resulted from the =C-H- stretching related to the functional group of alkenes. The peak obtained at 1745-1741cm^{-1} describes Ester carbonyl (-COOR) functional group of the triglycerides. Few short peaks were obtained at 1459-1463 cm^{-1}, 1195-1196 cm^{-1}, 1167-1171 cm^{-1} and 1011-1016 cm^{-1} which describes the functional attribute of (-CH_2-) in aliphatic compound (CH_2 scissors vibration, OH in carboxylic acids (in plane OH- bending and the -C-O ester group.)

Table3.17:.FTIR assignment of functional group at different wavelength (cm^{-})[1] in different variable of sample of lipid after transestrification

S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	Assignment	Functional class
_	_	_	_	3678.5		-	-	-	3673.9	_	_	_	3675.4	3645.6	-OH in alcohol	-
3566.2	3562.9	3528.6	3521.6	_	3566.1	-	-	-		3521.3	_	_			$-NH_2$ in aromatic amines, primary amine	(-O-H) usually free sharp*(str)*
3008.3	3009.1	3006.8	_	3008.8	3008.7	3007.7	3008.5	3008.4	3008.7	3009.7	3007.6	3008.6	3008.2	3009.1	=C-H stretch (aromatic and aliphatic stretch)	Cis-double bond stretching
2934.3	2931.2	2934.6	2934.3	2923.6	2923.6	2923.7	2923.9	2983.4	2923.1	2932.4	2931.6	2931.6	2921.2	2932.1	$(-CH_3)$ and $(–CH_2-)$ in aliphatic compounds antisym and sym stretching	Asymmetric and symmetric stretching vibration of methylene $(-CH_2)$ group
2896.3	2872.2	2825.3	2892.3	2853.7	2853.7	2853.5	2854.7	2853.6	2853.6	2867.2	2854.3	2847.3	2845.1	2851.0	$(-CH_3)$ attached to O or N(-CH stretching modes)	
2678.3	2685.2	2631.2		2678.8	2678.2	-	-	-	2678.2	2632.2	2686.2	2893.2		2892.3	(-CHO) in aldehyde (over tone of CH bending(Fermi resonance)	
1741.3	1742.3	1743.2	1741.2	1741.6	1741.7	1742.8	1741.5	1742.1	1741.9	1746.3	1745.3	1746.3	1742.6	1743.2	(-C=O) in ketoesters (C=O stretch, enol form)	Ester carbonyl(-COOR) functional group of the triglycerides
1462.3	1459.2	1458.3	_	1459.6	1459.5	1460.5	1458.3	1459.1	1459	1460.3	1462.1	1458.2	1457.2	1463.1	$(-CH_2-)$ in aliphatic compound (CH_2 scissors vibration	Bending vibration of the -CH_2 and CH_3 aliphatic group
1438.2	_	_	_	1435.7	1435.7	1435.8	1435.8	1435.8	1435.8	1436.1	1437.2	1438.7	1436.2	1437.2	OH in carboxylic acids (in plane OH- bending)	
1195.2	1194.2	1195.3	_	1195.8	1195.9	1195.9	1195.6	1195.7	1196	_	_	_	1195.3	1196.3	(-C-OH) in alcohols (-C=O Stretch)	(-C-O) stretching
1170.3	1170.9	1168.3	1169.4	1169.9	1170.2	1170.3	1169.6	1169.4	1170.1	1171.3	1170.4	1169.3	1167.2	1168.3	(-C-OH) in alcohols (-C=O Stretch)	
1016.3	1016.3	1017.3	1013.2	1016.3	1016	1013.7	1011.8	1013.9	1015.1	1017.6	1011.2	1012.8	1013.2	1014.2	(-C-OH) in alcohols (-C=O Stretch)	of the -C-O ester group
															(-C-OH) in alcohols (C-O Stretch)	$(-C-O)_{str}$ bending vibration of -CH functional groups of isolated transolefin
722.2	722.3	722.1	722.31	722.0	722.6	722.2	722.1	722.1	722.4	723.3	722.3	722.3	722.3	723.1	$-(CH_2)_n–$ in hydrocarbons (CH_2 rocking in methylene chains, intensity depends on chair length)	Overlapping of the methylene $(-CH_2)$ rocking vibration and to the out of plane vibration of cis-disubstituted olefins

3.3.2.5. Bioreactor and point prediction analysis for algal growth

The regression analysis and optimization of multivariables are taken here as an objective by using design experts for predicting the responses for designed bioreactor using the experimental responses.

3.3.2.5.1. Optimal point prediction analysis for Y_{BM}, Y_{lipid}, and removal of $Y_{TN\%}$, $Y_{TP\%}$ using multifactor (DIWW, NO_3^-, and PO_4^{-3})

In the present study, mathematical and statistical analysis of Central Composite Design (CCD) and possible optimization has been selected for the maximum, minimum range and non-responses to evaluate the optimized output for given conditions. The response surfaces and contour plots for maximum productivity Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), and high removal rate of nitrate and phosphate by the given condition using 73% DIWW, 26 mg/L NO_3^- and 6 mg/L PO_4^{-3}, the given condition after statistical and mathematical analysis of quadratic equation and achieved optimized responses of prediction and experimental data are given in Table 3.18.

3.3.2.2.5.2. Optimal point prediction analysis for Y_{BM}, Y_{lipid}, and Y_{FAME} using multifactor (NO_3^-, PO_4^{-3} and LEDs)

In this study, the input variables (NO_3^-, PO_4^{-3} and LEDs) were used for designing RSM but after statistical and mathematical analysis of data, a correlation established. Using these conditions for casting condition to achieve maximum biomass lipid and FAME content. The maximum achieved biomass 1.23 g/L, 32.19% Lipid content, 89.37% FAME content 560 nm LED and 22 mg/L and 8mg/L are shown in Table 3.19. A comparison between the experimental and predicted results indicates the error was less than 1%. From the results, it was concluded that the developed model could accurately predict the maximum response on given conditions.

Table 3.18:Optimal processing condition for PPA with multifactor (DIWW, NO_3^- and PO_4^{-3})

Factor				Predicted responses				Experimented responses			
S.N	DIWW %	NO_3^- mg/L	PO_4^{-3} mg/L	Y_{BM}, g/L	Y_{lipid} %	TN removal (%)	TN removal (%)	Y_{BM},	Y_{lipid},	TN removal (%)	TP removal (%)
	73	26	6	1.57	32.54	>99	>99	1.412	28.58	>99	>99

Table 3.19. Optimal processing condition for PPA with multifactor (LED, NO_3^- and PO_4^{-3})

Factor			Predicted responses			Experimented responses		
LED (nm)	NO_3^- mg/L	PO_4^{-3} mg/L	Y_{BM} (g/L)	Y_{lipid} (%)	Y_{FAME} (%)	Y_{BM} (g/L)	Y_{lipid} (%)	Y_{FAME} (%)
560	22	8	1.57	32.54	>99	1.23	32.19	89.37

3.3.2.5.3. Optimal point prediction analysis for Y_{BM}, Y_{lipid}, $Y_{FAME\ content}$ and Y_{CO2} sequestration

By using numerical optimization, a desirable value for each input factor and response can be selected. Therein, the possible input optimizations that can be selected includes: the range, maximum, minimum, target, none (for responses) are set, so as to establish an optimized output value for a given set of conditions. After completing the experimental analysis, analysing the data for the development of individual, interactive and quadratic model by a simple point prediction analysis. Optimization of the biomass, Lipid content, FAME content and CO_2 sequestration parameters was carried out in a numerical optimization method as specified in Fig.3.20.

The response surface and contour plot at optimum leaching condition for maximum Y_{BM}, Y_{lipid}, $Y_{FAME\ content}$ and $Y_{CO2\ sequestration}$. In this study, the input variables were given specific ranged values, whereas the response was designed to achieve optimal point for multi responses. Using these conditions, the maximum achieved biomass 1.30 g/L, 29.19% Lipid content, 81.37% FAME content and the sequestration rate is 0.16g/l/d. by using 45% DIWW, 560 nm LED and 13% CO_2 rate. A comparison between the experimental and predicted results indicates that the error was less than 0.08%. From the results, it was concluded that the developed model could accurately satisfy the experimental data for designed bioreactor with multifactor for algal biomass growth. The detail of optimization conditions is shown in Table 3.20

3.3.2.5.4. Point prediction analysis for Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L)

In the present study, mathematical and statistical analysis of Central Composite Design (CCD) and possible optimization and numericalizaion can be selected for the

maximum, minimum range and non-responses to evaluate the optimized output for given conditions. The response surface and contour plots for maximum Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (g/L).Using the given conditions, after statistical and mathematical analysis of quadratic equation and achieved optimized responses of prediction and experimental optimized response as given in Table 3.21.

3.3.2.5.5. Conclusion

This study integrated experimental and theoretical studies to model and validate changes on experimental basis for biomass growth and lipid content. The biomass growth and lipid content estimation is an important tool to predict improvements for bio-oil productivity and their techno-economic feasibility which is studied and presented in Chapter 5.

With rigrous experimental research studies carried out here for optimization of process as well as environmental parameters in integration are concluded according to the findings gathered from different subsections of experimental setup from the single factor impact study to multifactor impact study for steps in upstream processing;

- The highest lipid content (34.41%) and highest biomass productivity (1.54 g L^{-1}) in *Chlorella pyrenoidosa* was obtained under the moderate concentration of dairy industry wastewater (50%).Whereas, in a single factor analysis independent parameter study, findings of algal growth was found more suitable with 75% of concentration of DIWW. Hence, it can be concluded that in coupling with other parameters has a significant impact and increase the lipid content, which is approximately 2 times compared to normal BG-11 nutrient media.

Table 3.20. Optimal processing condition for PPA with multifactor LED, CO_2 and DIWW on (Y_{BM}, Y_{lipid}, $Y_{FAME\ content}$ and Y_{CO2} sequestration

Factor				Predicted responses				Experimented responses			
S.N	DIWW	CO_2	LED light	Y_{BM},	Y_{lipid},	$Y_{FAME\ content}$	Y_{CO2}	Y_{BM},	Y_{lipid},	$Y_{FAME\ content}$	Y_{CO2}
1	45	13	560	1.3	29.19	81.37	0.16	1.312	29.58	82.36	0.162

Table 3.21:Optimized point predictions and model validation for Y_{BM} ,Y_{Lipid} , $Y_{lipid\ prod}$,Y_{FAME} and $Y_{CO2\ seq}$

Factor				Predicted value					Experimental value				
S.N	CO_2	Temp	LED	Y_{BM}	Y_{Lipid}	$Y_{Lip\ pro}$	Y_{FAME}	$Y_{CO2\ seq}$	Y_{BM}	Y_{Lipid}	$Y_{Lip\ pro}$	Y_{FAME}	$Y_{CO2\ seq}$
1	8.5	33.83	540	1.44	31.78	0.0309	83.67	0.155	1.42	32.52	0.032	85.26	0.157

But, taking the spectral variation and coupling with other dependent variables for algal biomass productivity and lipid content, it is concluded along with specific findings of Phase-I, as;

- LED, in combination with NO_3^- and PO_4^{-3} variables are responsible for biomass concentration, lipid and FAME content. Best findings are observed when there are interactive factors of Light>NO3->PO4-3 for biomass .
- LEDs with CO_2 and DIWW showed a significant impact to improve the microalgal metabolism to enhance the algal growth, lipid content, FAME content and CO_2 sequestration. Similarly, direct and indirect relationships are also observed for biomass productivity with increase and decrease in wavelength from LED light radiations.
- Temperatures also had a significant impact on the metabolism of microalgae for biomass growth, lipid content and lipid productivity. CO_2 in integration with temperature positively effects the Y_{BM} (g/L), $Y_{Lipid\ content}$ (%), $Y_{Lipid\ productivity}$ (g/L/d), Y_{CO2seq} (g/l/d), Y_{FAME} (%) but only LED light negatively effects the lipid content.

Point prediction analysis supports the algal growth with process parameters in designed bioreactor. Remarkable algal growth has been noticed on the part of high growth of selected algal biomass of *Chlorella pyrenoidosa*.

Chapter-4

Optimization of downstream processes (harvesting and tranestrification) using Response surface methodology to scale up algal biomass

4.1. Introduction

`A downstream processing step consists of harvesting (i.e., oil extraction) and conversion of algal lipids to advanced bioenergy options i.e. biofuel. It contributes to 60% of total production cost.

Therefore, it is essential to reduce the total combined cost of harvesting, extraction and conversion through a number of technical breakthrough. Additionally, the negatively charged surfaces of the microalgae prevent these organisms from easily settling by gravity. Unfortunately, the best way to harvest various microalgal species has not yet been determined (Uduman *et al.,* 2010). Thus, a proper harvesting method that can improve both the economics and the efficiency of the process according to the desired products and/or the biology of the microalgal species needs to be developed. Although there are technical similarities between microalgal harvest and water purification, it is necessary to develop approaches that can address the technical needs that are unique to microalgal harvest.

- To design efficient as well as ecofriendly approach for harvesting algal biomass strategically is needed at global level to promote bioenergy, like characteristics of selected microalgal species with type of derived end product, hybrid techniques for harvesting also compensate the associated challenges from individual harvesting process; complete cell harvesting should be done by using efficient separators/catalyst flocculants in downstream process and extraction steps needs to minimized by appropriately choosing the harvesting techniques.
- Similarly, research with less energy conversion but high amount of biomass should be gained from culture medium.

- After the extraction of lipids from algal biomass, an appropriate conversion process is needed to produce biodiesel as a fuel, reported by Kothari *et al.*,(2013). Bala, 2005 made use of catalysts and alcohol for production of FAME for biodiesel production. In this way, the reaction becomes fast and helps in formation of products (FAME and Glycerol).
- Other main challenges/drawbacks from the transesterification is the recovery of products from toxic liquid recovery based catalyst, directly or indirectly affects human health and the environment tranestrification is a species dependent reaction, use of co-solvent system where extraction and transesterification occurs at the same time. Free fatty acid content and moisture directly affects the production of high quality biodiesel.

Hence, this chapter emphasizes on the effect of various parameters in harvesting and transesterification process of *Chlorella pyrenoidosa* using RSM. So, this chapter is divided into 2 main sections as:

4.1. (a) To study the effect of flocculants like chemically synthesized impregnated catalyst (zirconium and tungustun) , nanocatalyst (Ca and Mg) on efficiency of *C. pyrenoidosa* using different variables(pH, dose, temperature)

4.1. (b) Optimization of nanocatalyst dose (nanocatalyst-Ca) for transesterification of bio-oil from harvested biomass (Phase 2(a)) and their impact on cell structure.

4.2. Materials and Methods

4.2.1. Types of catalyst, pH and temperature for the harvesting of algal biomass.

4.2.1.1. Impregnated chemically synthesized catalyst: In present research work, two types of impregnated chemically synthesized catalyst, impregnated tungustun (Imp-W) and impregnated zirconium (Imp-Zr). The method of preparation of these

catalysts is given in Section-**2.6.1.2.1**. of Chapter-2 with experimental doses selected for experimental plan Phase-2.

4.2.1.2. Nanocatalyst catalyst: Two types of nanocatalyst was synthesized, Nano-Ca catalyst and Nano-Mg catalyst method of preparation is given in Section-2.6.1.2.1.3 of Chapter-2.

4.2.1.3. pH. In the present study, 5-9 pH ranges was used for the harvesting of algal biomass as given in section-2.6.1.2.2.2 of Chapter-2 with experimental doses selected for experimental plan Phase-2.

4.2.1.4. Temperature. The ranges of temperature for Phase-II given Chapter-2

4.2.2. Statistical analysis

All experiments were designed by RSM, Design Expert Software v11 (student version). All the theoretical aspects are already explained in section 2.5.1.5.(2.6.1.5.1, 2.6.1.5.2, 2.6.1.5.3) of Chapter-2.

4.2.3.Experimetal setup

As we discussed in section 4.1 of this chapter, whole chapter is divided into 2 main sections, which has been carried out with RSM studies. Therefore, variables selected for RSM studies are given in Tables. 4.1 and 4.2 ,indicative of the 5 coded levels for influencing parameters.

4.2.4. Analytical methods

The analytical methods required for harvesting was estimated by standard methods. The detailed procedure of each method is given in Annexure 1.

Table 4.1. Code limit for variables used in the harvesting experimental design

Factor	Unit	Symbol	Coded level				
			-α	**-1**	**0**	**+1**	**+α**
Dose	**mgL^{-1}**	X_1	0	25	50	75	100
pH		X_2	0	5	6.5	7	9
Temperature	**°C**	X_3	30	37.5	40	45	50

Table 4.2.Code limit for variables used in the transesterification experimental design

Factor	Unit	Symbol	Coded level				
			-α	**-1**	**0**	**+1**	**+α**
Nano-Ca	**%**	X_1	0	25	50	75	100
Time	**Min**	X_2	0	50	100	150	200
temperature	**ºC**	X_3	0	25	50	75	100

4.3. Results and discussions

4.3.1. Effects of flocculants on harvesting efficiency of *C. pyrenoidosa with* different variables (pH, dose, temperature) using RSM.

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 conditions for maximum harvest of *C. pyrenoidosa*. According to literatures, a lot of experimental studies based on single-factor analysis have been cited by researchers. Use of very low or high pH ranges directly affects the algal surface and cell size abruptly (Bolton *et al.,* 2016). Similarly, high dose concentration of chemical based catalysts for harvesting of algal trains, also poses a problem in cell deformation, which leads to least use of end-products originated from algal biomass. Furthermore, temperature with high/low ranges creates a stress on algal cells. Therefore, combinations of factors are taken as study part.

A central composite design (CCD) was established for optimizing statistically significant parameters for the harvesting of *C. pyrenoidosa* by different flocculating agents (Imp-Zr, Imp-W, Nano-Ca and Nano-Mg) agent. The responses are noticed with harvesting efficiency (HE %) at different experimental runs under different variables with harvesting dose (mg/L) (X_1), pH (X_2) and Temperature (^oC) as (X_3) shown in Table 4.1. The equation expresses the relationship between the predicted response and independent variables in coded values according to Tables 4.3. The data were analyzed to examine HE (%), Imp-Zr, Imp-W, Nano-Ca and Nano-Mg correlation between the experimental ($Y_{experimental}$) and predicted ($Y_{Predicted}$) responses. It has been observed that the data points were well distributed close to a straight line (R^2>0.98) for different harvesting (Imp-Zr, Imp-W, Nano-Ca and Nano-Mg) agents

which suggested an excellent relationship between the experimental and predicted values of the response and the underlying assumptions of the above analysis were appropriate. The results also indicated that the selected quadratic model was adequate in assuming the response variables for the experimental data. An overall second order polynomial Eq.(4.1-4.4) by multiple regression analysis was obtained for the harvesting efficiency HE(%), Imp-Zr, Imp-W, Nano-Ca and Nano-Mg as represented below:

$$Y_{(Nano\text{-}Ca)}=96.23+38.39*X_1+4.82*X_2+2.23*X_3+1.42*X_1X_2+0.068*X_1X_3+2.73*X_2X_3-33.73*X_1^2-17.63*X_2^2-7.06*X_3^2 \quad \textbf{(4.1)}$$

$$Y_{(Nano\text{-}Mg)}=94.56+36.83*X_1+4.54*X_2+1.66*X_3+1.82*X_1X_2+0.29*X_1X_3+4.36*X_2X_3-34.21*X_1^2-18.4*X_2^2-5.88*X_3^2 \quad \textbf{(4.2)}$$

$$Y_{(Imp\text{-}Zr)}=82.25+29.87*X_1+7.52*X_2+1.43*X_3+7.47*X_1X_2+0.54*X_1X_3-5.6*X_2X_3+-28.66*X_1^2-19.82*X_2^2-6.88*X_3^2 \quad \textbf{(4.3)}$$

$$Y_{(Imp\text{-}W)}=54.86+22.75*X_1+11.16*X_2+2.42*X_3+8.25*X_1X_2+3.3*X_1X_3-3.78*X_2X_3-16.94*X_1^2-7.92*X_2^2-0.35*X_3^2 \quad \textbf{(4.4)}$$

The goodness of fit of regression equation (R^2) developed could be measured by adjusted determination coefficient determines the significance as shown in Table 4.11 of harvesting at different variables and also indicating the high degree of agreement between the actual and predicting harvesting efficiency by using the proposed quadratic model. The R^2 value of 0.98 adjustable R^2_{adj} of 0.95 and predicted R^2_{pred} of 0.92 shows that the quadratic model could be significant for predicting the HE % response and explaining 95% of the variability in the model as given in Fig.4.1 (a-d). The significance of statistical evaluation of the given Eq.4.14 was assessed by ANOVA (Analysis of variance) and F-test which proved that the given model was statistically significant at 95% confidence level ($p<0.05$). ANOVA reported the model F-value of 36.42 which indicated that the model is significant. The p-value of model

helps to understand the interacting behavior among the variables. The most significant ($p<0.05$) value linear factor of this model is X_1, X_2 interacting factor X_1X_2, and quadratic factor X_2^2 and X_3^2 of model. The model also depicted the statistically non-significant lack of fit ($p>0.05$), indicating that the responses are adequate for employing in this model. Three dimensional response surface plots represent regression equations and illustrate the interactions between the response and experimental levels of each variable. These plots let us locate the optimum levels of each variable for the maximum harvesting efficiency to harvest the highest amount of microalgal cells.

Imp-Zr catalyst (%) HE regression value was 0.98, 0.96 for R^2_{adj} and 0.89 for R^2_{pred}. ANOVA and F-test of model was significant statistically at 95% confidence level ($p<0.05$). ANOVA reported the F-value 44.29 of model was significant given in Table 4.4.The most significant ($p<0.05$) value linear factor of this model is X_1, X_2 interactive model is X_1X_2 and quadratic factor X_1^2, X_2^2 and X_3^2 of model.

Imp-W catalyst (%) HE synergistic interactions between X_1 and X_2 was highly significant (>0.05) on checking the value of R^2, the predicted R^2 of 0.97 was reasonable and in agreement with the adjusted R^2 of 0.72. ANOVA reported the F-value 44.29 of the model that was significant as given in Table 4.5.The most significant ($p<0.05$) value, linear factor of this model is X_1, X_2 ,interactive model is X_1X_2 and quadratic factor X_1^2, X_2^2 and X_3^2 of model.

The R^2 value for HE % of algae by **Nano-Ca** was 0.99, 0.98 for R^2_{adj} and predicted 0.89 for R^2_{pred}. ANOVA and F-test of model was significant statistically at 95% confidence level ($p<0.05$). ANOVA reported the F-value of model was 118.86 at significance level Table 4.6.The most significant ($p<0.05$) value linear factor of this model is X_1, X_2 quadratic factor X_2^2 and X_3^2 of model.

Table 4.3:Statistical and mathematical analysis of the variance for harvesting efficiency of algae cell,Imp-Zr, Imp-W, Nano-Ca and Nano-Mg

			Factor	Predicted Value (%)				Observed Value (%)			
S.N	Dose (mg/L)	pH	Temp (ºC)	Y (Imp-Zr)	Y (Imp-W)	Y (Nano-Ca)	Y (Nano-Mg)	Y (Imp-Zr)	Y (Imp-W)	Y (Nano-Ca)	Y (Nano-Mg)
1	0	4	37.5	8.25	8.25	8.25	8.25	3.84	4.34	3.08	2.40
2	100	4	37.5	42.32	33.25	75.65	68.65	48.64	33.34	77.03	72.42
3	0	9	37.5	10.26	10.25	11.25	11.63	3.96	10.16	9.87	7.84
4	100	9	37.5	74.21	68.26	84.32	79.32	78.64	72.16	89.49	85.14
5	0	6.5	25	11.25	12.36	12.65	11.96	15.95	15.70	14.88	16.27
6	100	6.5	25	80.64	55.26	95.85	94.67	74.61	54.60	91.53	89.35
7	0	6.5	50	11.69	13.26	14.89	13.69	17.73	13.94	19.21	19.01
8	100	6.5	50	83.24	69.36	98.36	97.58	78.55	66.04	96.13	93.25
9	50	4	25	41.26	28.67	64.28	66.91	40.99	29.23	67.22	68.44
10	50	9	25	65.63	62.35	72.25	69.31	67.25	59.11	71.40	68.80
11	50	4	50	56.68	38.38	65.37	62.52	55.05	41.63	66.23	63.04
12	50	9	50	58.63	56.95	84.26	82.36	58.91	56.39	81.32	80.84
13	0	9	37.5	10.56	8.98	9.90	8.56	9.76	9.54	8.34	9.23
14	100	6.5	37.5	82.54	84.56	87.65	79.45	83.45	84.32	88.89	76.98
15	50	4	37.5	36.65	39.54	43.23	39.45	38.46	40.34	41.31	38.54
16	50	6.5	37.5	82.25	54.86	96.23	94.56	82.25	54.86	96.23	94.56
17	50	6.5	37.5	82.25	54.86	96.23	94.56	82.25	54.86	96.23	94.56
18	50	6.5	37.5	82.25	54.86	96.23	94.56	82.25	54.86	96.23	94.56
19	50	6.5	37.5	82.25	54.86	96.23	94.56	82.25	54.86	96.23	94.56
20	50	6.5	37.5	82.25	54.86	96.23	94.56	82.25	54.86	96.23	94.56

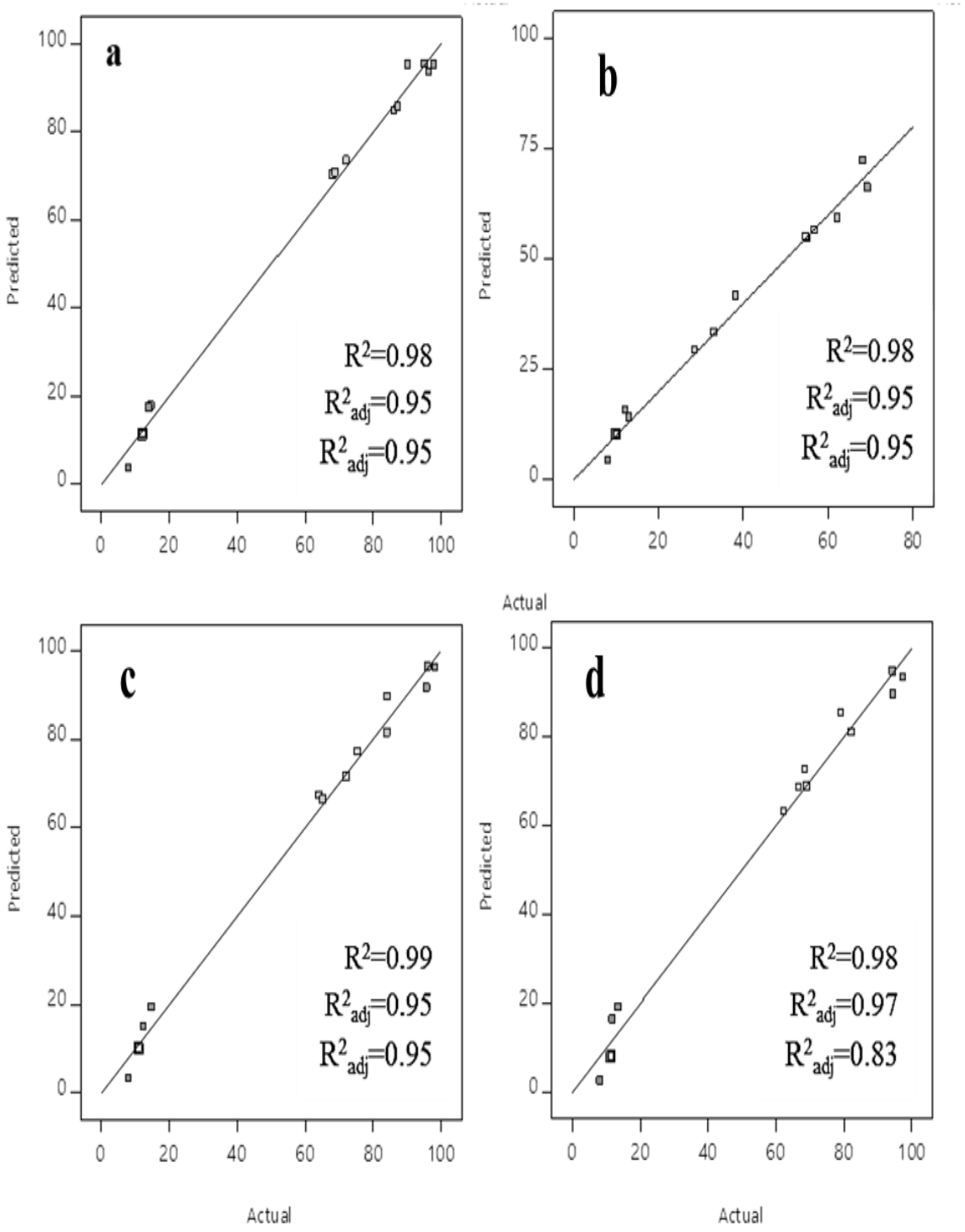


Fig. 4.1: Normal plot of actual versus predicted probability for Harvesting efficiency (%) of algae by [(a) Imp-Zr(b)Imp-W (c)Nano-Ca (d) Nano-Mg(%) associated with different variable (Dose, pH and Temperature)

Table 4.4:.Analysis of variance table for (Imp-Zr)

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Model	13727.03	9	1525.23	44.29	< 0.0001	**significant**
X_1	7137.74	1	7137.74	207.26	< 0.0001	
X_2	453.31	1	453.31	13.16	0.0084	
X_3	16.42	1	16.42	0.4767	0.5122	
X_1X_2	223.20	1	223.20	6.48	0.0383	
X_1X_3	1.17	1	1.17	0.0339	0.8592	
X_2X_3	125.66	1	125.66	3.65	0.0977	
X_1^2	3460.32	1	3460.32	100.48	< 0.0001	
X_2^2	1654.45	1	1654.45	48.04	0.0002	
X_3^2	199.16	1	199.16	5.78	0.0471	
	Std.=5.87	R^2=0.98	R^2pred=0.72			
	C.V%=10.44	R^2_{adj}=0.96				

Table 4.5:Analysis of variance table for (Imp-W)

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Model	7105.43	9	789.49	73.42	< 0.0001	**significant**
X_1	4140.96	1	4140.96	385.07	< 0.0001	
X_2	995.92	1	995.92	92.61	< 0.0001	
X_3	46.61	1	46.61	4.33	0.0759	
X_1X_2	272.42	1	272.42	25.33	0.0015	
X_1X_3	43.56	1	43.56	4.05	0.0840	
X_2X_3	57.08	1	57.08	5.31	0.0547	
X_1^2	1208.62	1	1208.62	112.39	< 0.0001	
X_2^2	263.78	1	263.78	24.53	0.0017	
X_3^2	0.5381	1	0.5381	0.0500	0.8294	
	Std.=3.28	R^2=0.98	R^2pred=0.83			
	C.V%=7.63	R^2_{adj}=0.97				

Table 4.6.Analysis of variance table for Nano-Ca

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Model	18852.41	9	2094.71	118.86	< 0.0001	**significant**
X_1	11791.87	1	11791.87	669.11	< 0.0001	
X_2	185.57	1	185.57	10.53	0.0142	
X_3	39.83	1	39.83	2.26	0.1765	
X_1X_2	8.04	1	8.04	0.4561	0.5211	
X_1X_3	0.0182	1	0.0182	0.0010	0.9752	
X_2X_3	29.81	1	29.81	1.69	0.2346	
X_1^2	4791.08	1	4791.08	271.86	< 0.0001	
X_2^2	1308.70	1	1308.70	74.26	< 0.0001	
X_3^2	209.87	1	209.87	11.91	0.0107	
	Std.=4.2	R^2=0.99	R^2pred=0.89			
	C.V%=6.11	R^2_{adj}=0.98				

Table 4.7:Analysis of variance table for Nano-Mg

Source	Sum of Squares	df	Mean	F	Prob > F	
Model	18107.33	9	2011.93	71.93	< 0.0001	**significant**
X_1	10855.27	1	10855.27	388.12	< 0.0001	
X_2	164.62	1	164.62	5.89	0.0457	
X_3	22.11	1	22.11	0.7906	0.4034	
X_1X_2	13.29	1	13.29	0.4750	0.5129	
X_1X_3	0.3481	1	0.3481	0.0124	0.9143	
X_2X_3	76.04	1	76.04	2.72	0.1432	
X_1^2	4924.44	1	4924.44	176.07	< 0.0001	
X_2^2	1425.32	1	1425.32	50.96	0.0002	
X_3^2	145.89	1	145.89	5.22	0.0563	
	Std.=7.8	R^2 =0.98	R^2_{pred}=0.92			
	C.V%=12.03	R^2_{adj} =0.95				

The R^2 value for harvesting of algae by **Nano-Mg** was 0.98 adjustable R^2_{adj} of 0.97 and predicted R^2_{pred} of 0.82 shows that the model could be significant for predicting the HE %. ANOVA and F-test of model was statistically significant at 95% confidence level ($p<0.05$). ANOVA reported the model F-value of 71.93 as given in Table 4.7.The most significant ($p<0.05$) value linear factor of this model is X_1, X_2 quadratic factor X_2^2 and X_3^2 of model.

4.3.1.1. Determination of condition for optimization of harvesting efficiency by using multivariable

It is depicted from the Tables.4.3 that different responses for harvesting efficiency HE(%) varied for different combination of operating variables. 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) coefficient. 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 show in Fig.4.2 (a-b) Dose: pH for harvesting efficiency are highly significant but Dose: Temp and Temp:pH less significant combination of interactive variable show in Fig.4.2(c-d)

The percent contribution of interactive contribution of factor for harvesting efficiency of algae influencing variables are interactive influencing parameter for nanocatalyst (Nano-Ca and Nano-Mg) and impregnated (Imp-Zr and Imp-W) are (pH+^oC)>(Dose+pH)>(Dose+^oC) show in Fig.4.3(a-d).

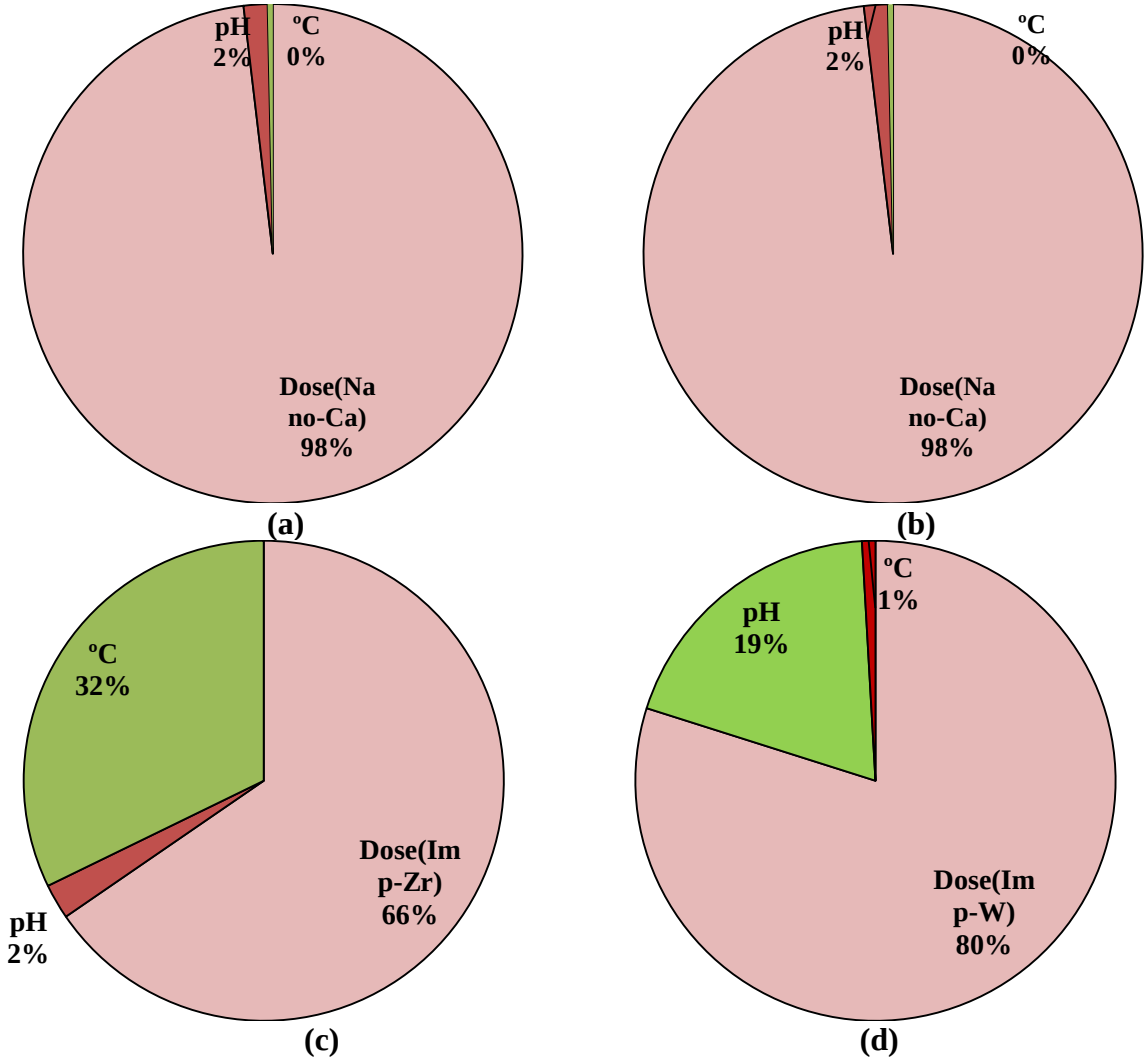


Fig.4.2: Percent contribution effect of intercept variable on HE % by (a) Nano-Ca (b) Nano-Mg (c) Imp-Zr (d)Imp-W,

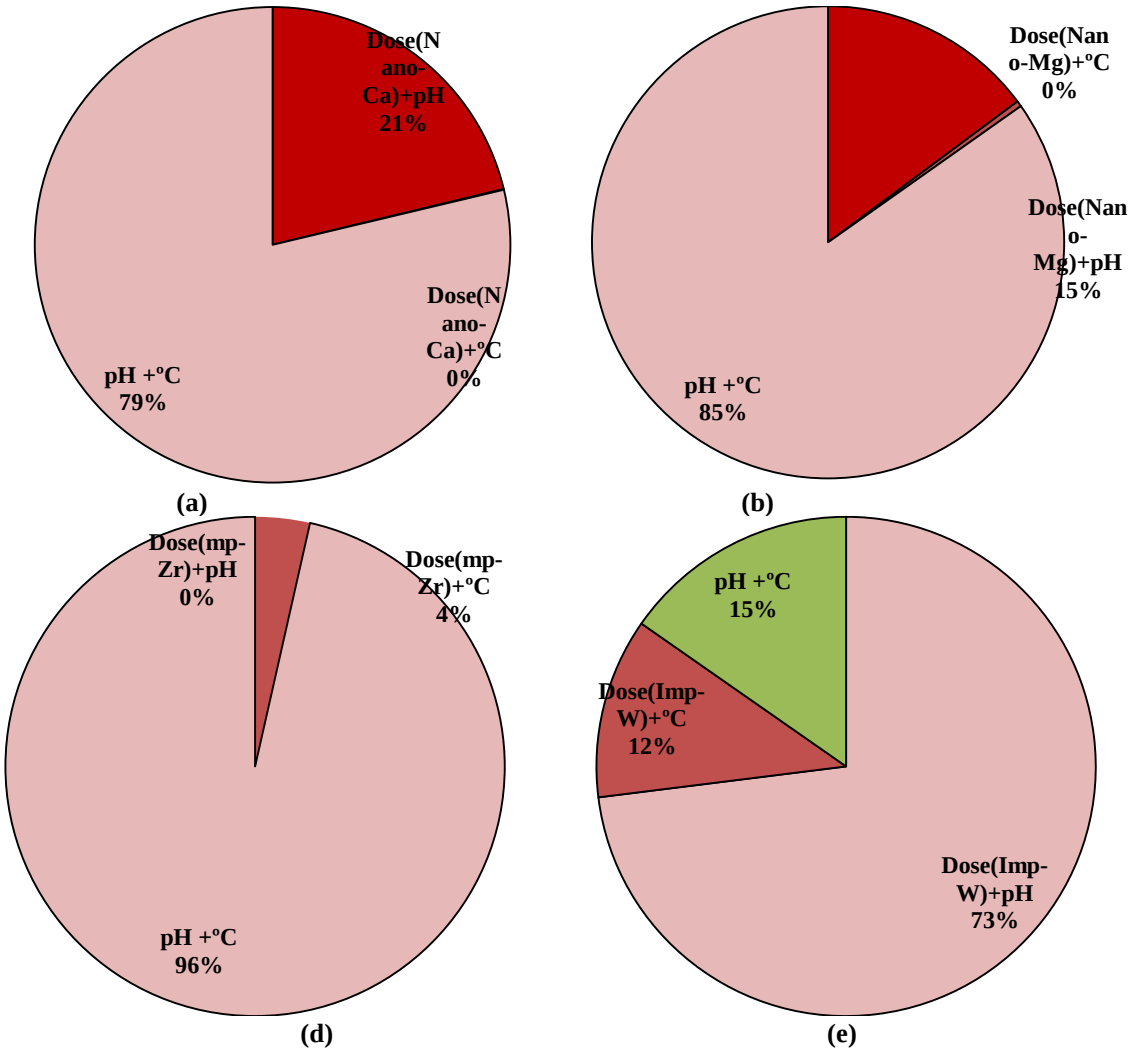


Figure.4.3.Percent interactive contribution effect of intercept variable on HE % by (a) Nano-Ca and(b) Nano-Mg (c) Imp-Zr (d)Imp-W,

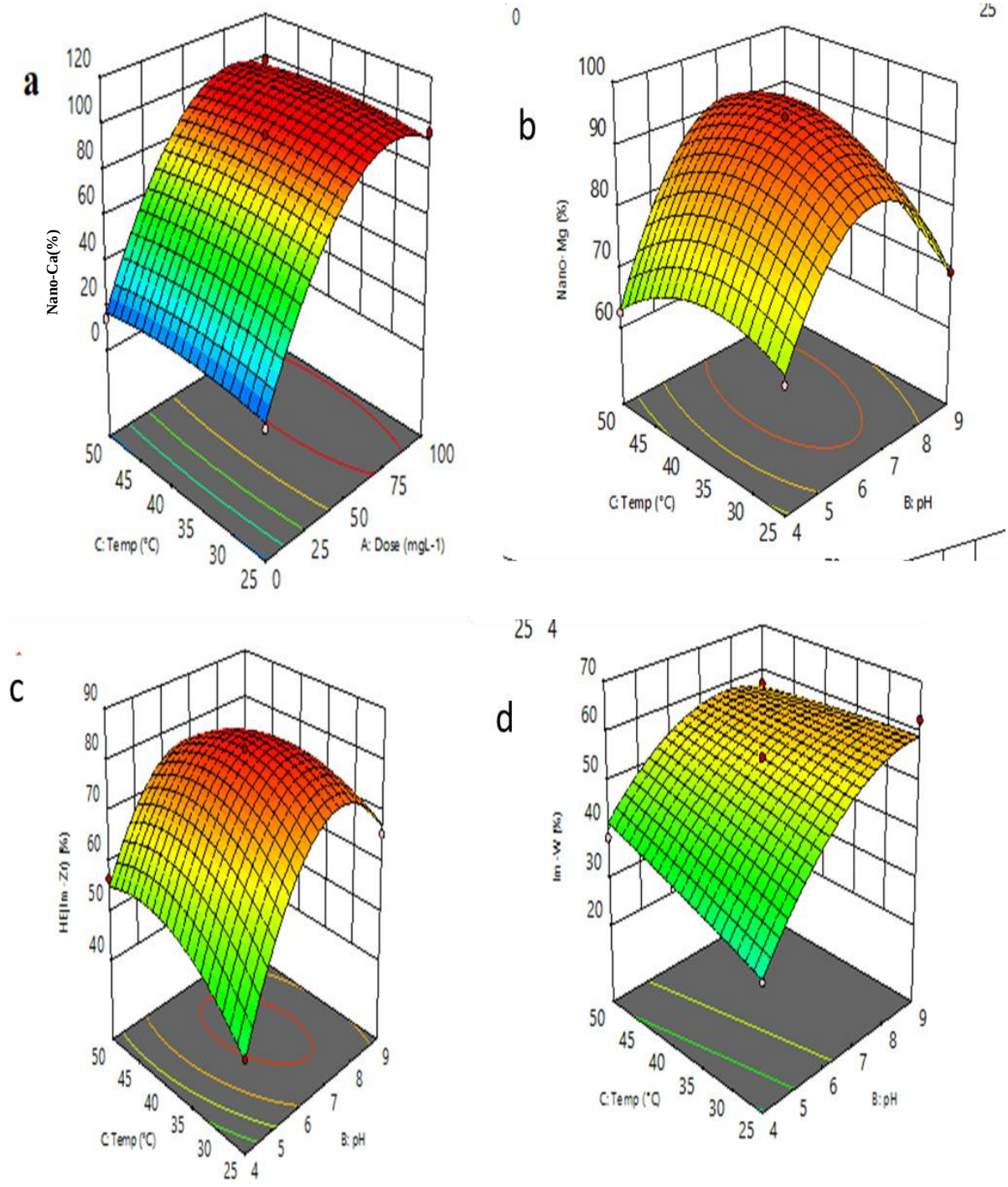


Figure.4.4.3D representation of significant factor and responses on harvesting of algae (a) Nano-Ca: pH+ºC (b) Nano-Mg: pH+ºC (c) Imp-Zr: pH+ºC (d) Imp-W: pH+ºC

Three dimensional (3D) plot are graphical representation of regression equation containing the information of interacting effect between pair variables and optimal condition could be identified by analyzing the effect and locate the optimum levels of each variable for the maximum harvesting efficiency to harvest the highest amount of

microalgal cells by different flocculant , Imp-Zr, Imp-W, Nano-Ca and Nano-Mg. show in Fig.4.4(a-d) Due to increasing the dose of flocculent believed that the zeta potential also shifted to negative to positive and surface charge of algal cell become neutralize and tend to fold tightly and are able to bridge between micro-algal cells to form floc similarly interactive variables Zeta potentials and harvesting efficiencies were both pH dependent and dose of flocculant.

4.3.1.2. Microscopic structure analysis

Microscopic observation of flocculated microalgae shows that, no cell damage was observed in without catalysis as show Fig.4.5a. Imp-Zr, Imp-W, Nano-Ca and Nano-Mg represents no cell damage in microscopic view for microalgae used, as given in Figure 4.5 (a-d).

4.3.1.3. SEM-EDX analysis of algal surface with and without flocculation

Scanning electron microscopy- energy dispersive spectroscopy (SEM-XRD) analysis used to revel that change in morphological and elemental composition with the interaction of mechanism in metal binding. The result of SEM micrograph showed that the algal cell before harvesting had a normal smooth surface and surface and shape with transparent external layer outside cell surface Fig.4.6b and EDX graph show C: 67.87% and O:23.28% and Mg, Fe and Zr in trace show in fig.4.6a SEM-EDX analysis of S. obliquus and C. vulgaris scatter in the absence of control in the suspension (Salama *et al.*,2016). Some of the functional group were deprotonated and provide site for other ions and change in the outer shape may be due to interaction between the metal and provide active site for other microalgal cell. SEM-EDX analysis was performed on algal cell harvested from control and flocculent ($100mgL^{-1}$). The SEM micrograph of algal biomass obtained in controlled shows a smooth cell surface with larger cell size fig.4.6a and without any deposition and having.

Secondary SEM image with EDX analyses showed the carbon almost present over the surface strong signal of Carbon and oxygen >98% show in Fig.4.6b.

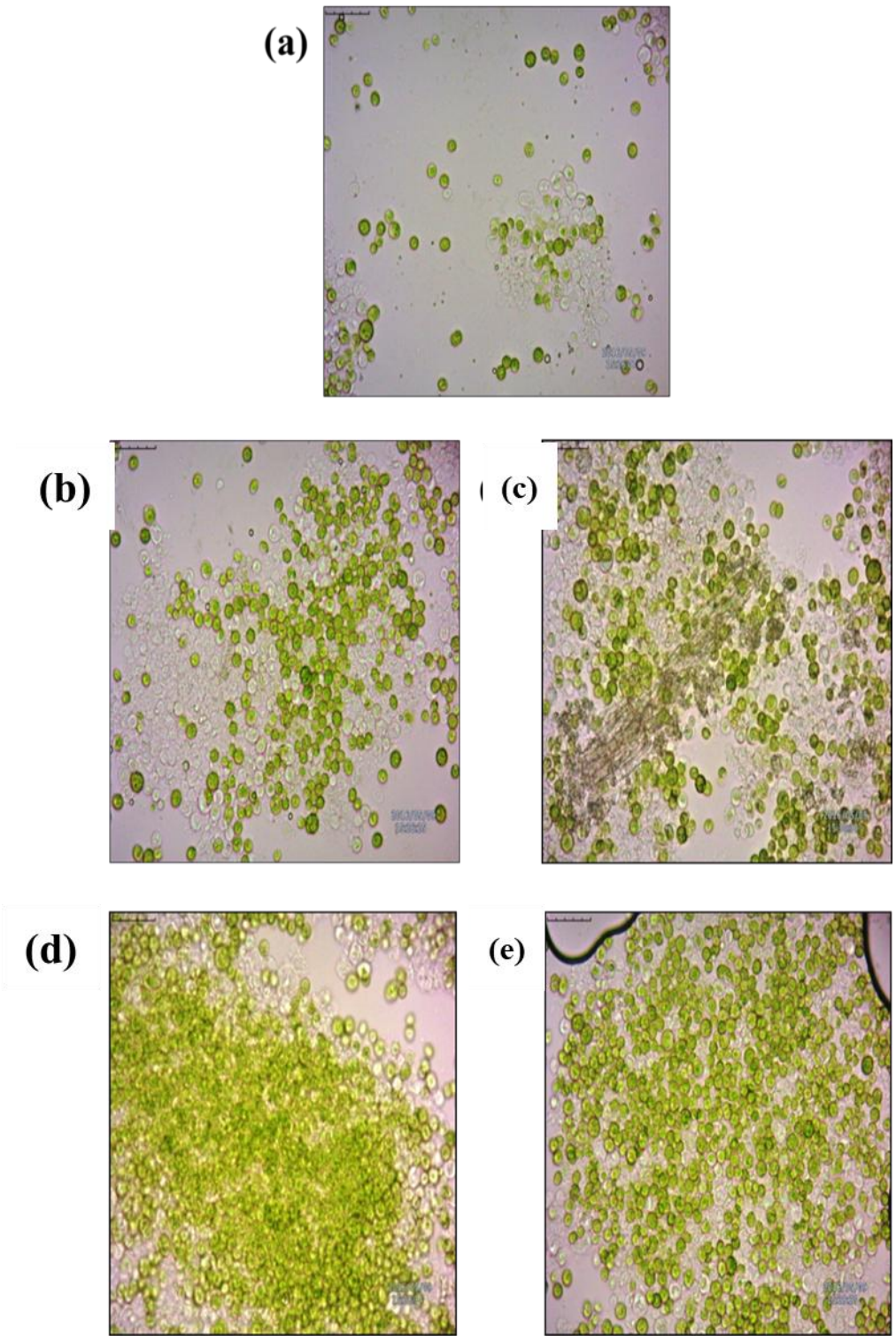


Fig.4.5: Microscopic analysis of different flocculating agents (a) without flocculating agent (b) Imp-Zr (c) Imp-W (d) Nano-Ca and (e) Nano-Mg

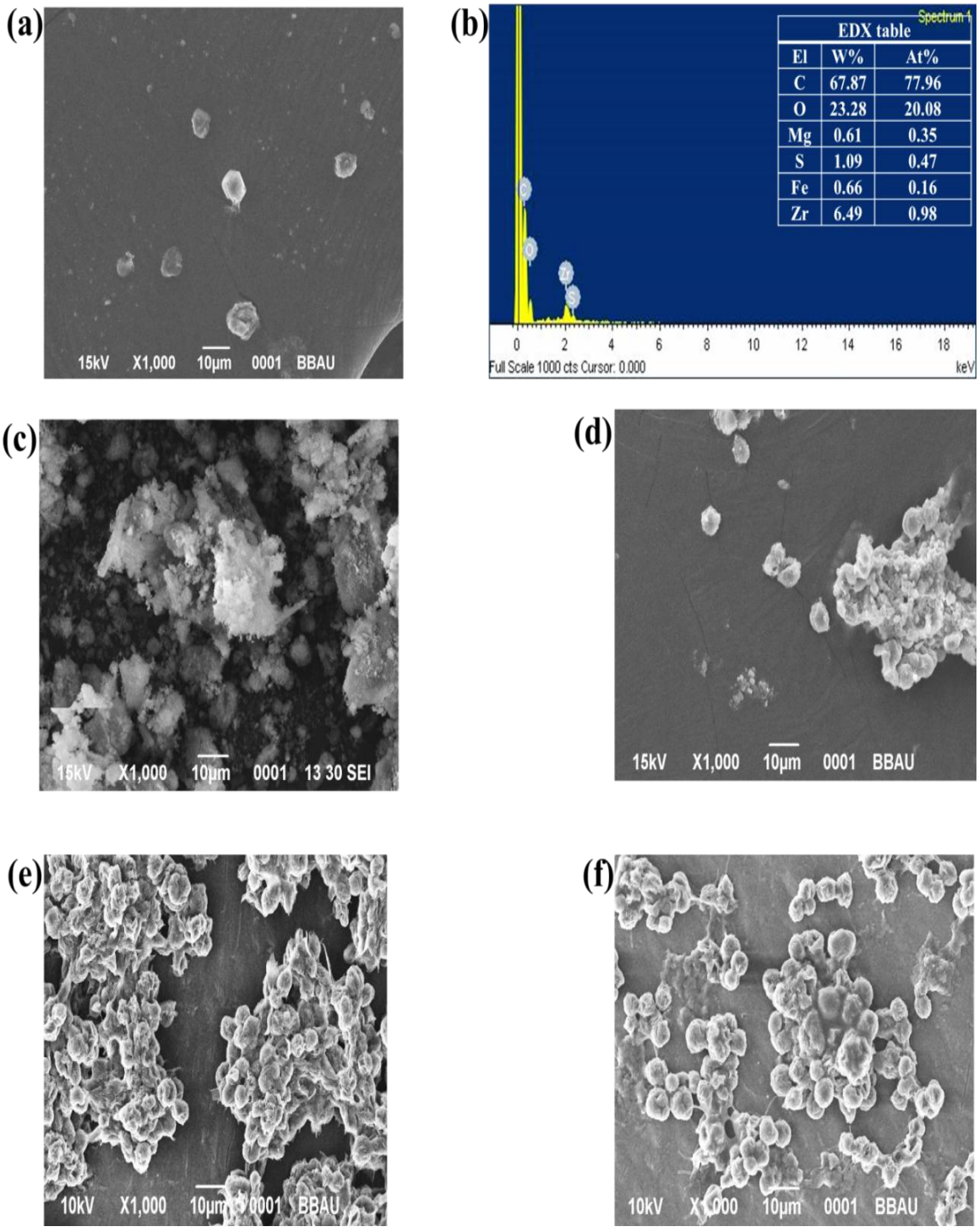


Fig.4.6: SEM analysis of harvesting of algae by different flocculating agent (a) control image (b) EDX of control image (c) Imp-Zr (d)Imp-W (d) Nano-Ca and (e) Nano-Mg

After the process of flocculation, SEM micrographs revealed significant changes in the morphology of algae by the use of flocculant show in Fig.4.6(c-f). The variations in algal cell surface show the several type of deposition on the surface of algal cell

marked effect on the cell surface structure due to its effect on the binding flocculent with the cell surface. Zheng *et al.,* (2012) observed no disintegration in cell surface of *Chlorella vulgaris* flocculated by poly glutamic acid. Another study conducted by Choi *et al.,* (2015) also found that egg shell solution has non-toxic effect on algal cell during the harvesting process. The distinguished difference due to use of bioflocculant on the algal cells have been completely visualized with the help of microscopic images and SEM-EDS analysis in this study.

4.3.2. Optimization of nanocatalyst dose (nano-catalyst-Ca) for tranestrification of bio-oil from harvested biomass (Phase 2(a)) and their impact on cell structure.

4.3.2.1. Biodiesel production using Nano-Ca catalyst

Production of biodiesel from the dry biomass of *chlorella pyrenoidosa* was carried out using the synthesized Nano-Ca nanocatalyst by the process of transesterification. Transesterification reaction perform under 2 neck 100 ml round bottom flask equipped with reflux condenser and magnetic stirrer.1 gram of dried algal biomass of *C. pyrenoidosa* was mixed with Nano-Ca in powder form and suspended in required methanol (6:1) ratio. The reaction was allowed at constant Continious speed (200rpm) with variable temperature time, dose of Nano-Ca catalyst and (40-70) for a period of (30-180) at different dose of Nano-Ca catalyst. After completion the tranestrification reaction mixture was cool and centrifuged to separate layer. Filtered biodiesel was evaporated at 80Cto remove excess methnol by using Rotatory evaporator and mass of biodiesel was determined gravimetrically.

4.3.2.2. Mechanism of transesterification by Nano-Ca catalyst

A large number of researches related to the application of Ca as a catalyst were reported in last decade but few researchers reported the mechanisms for the Nano-Ca catalyzed transesterification given in Fig.4.7.

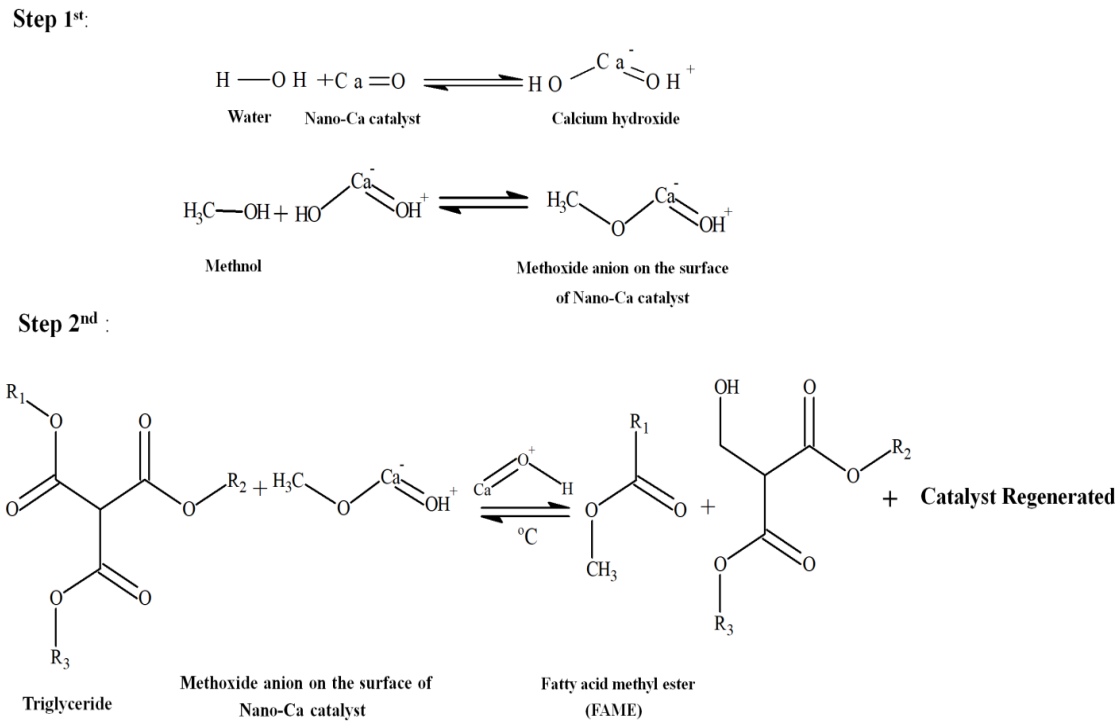


Fig.4.7: Mechanism of transesterification by Nano-Ca

4.3.3.2. Regression model analysis for optimization of biodiesel yield

In order to achive high yield of biodiesel the major factor catalyst dose, temperature and time on process of transesterification by using CCD model using 18 run discussed. Statistical analysis of biodiesel yield (%) show the comparative variation of predicted and actual observation of Y_B(%), show in Fig.4.8. It show the variation of predicted vs actual values are distributed normally straight line and error is insignificant within the range of operating variables but little fluctuation found in predicted vs actual values by the process of transesterification for biodiesel yield So, this regression model suggests that this is satisfactory and does not show any violation of independence or constant variance assumption show in Table4.8 A low coefficient of the variation in Y_B(%), assured high degree of accuracy and good authencity of the experimental data.

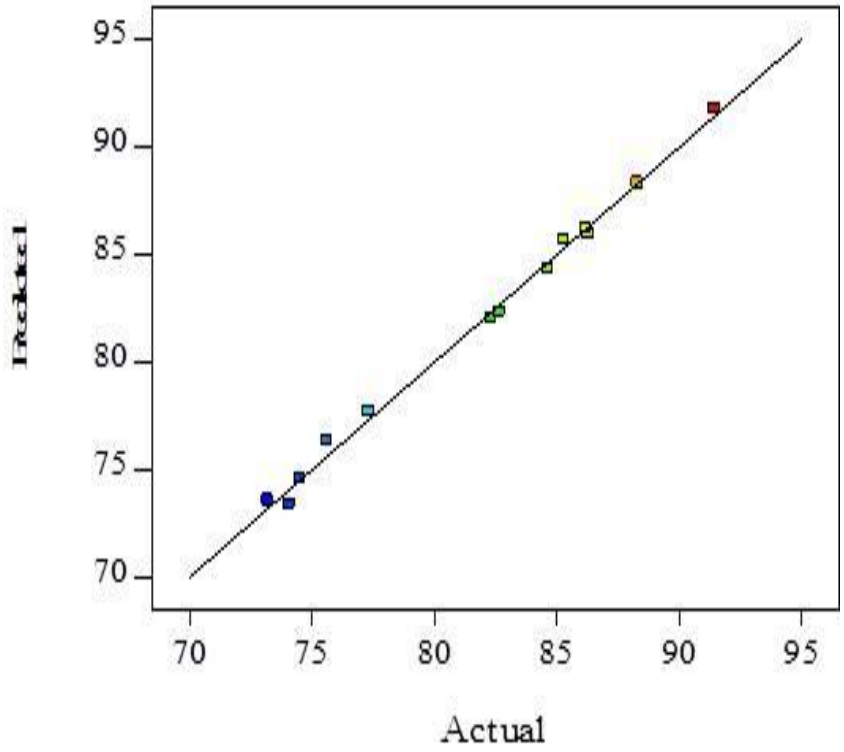


Fig.4.8.Normal plot versus predicted probability for transesterification of algal biodiesel

It was seen from Table 4.8.the biodiesel yield by the process of transesterification was varied between 73-91% respectively for different combination of operating variables. The best-fitted response for Y_B(%) are composed of linear regression coefficient (X_1, X_2,and X_3) Interactive cofficient (X_{12},X_{13} and X_{23}) and quadratic regression coefficient (X_1^2, X_2^2 and X_3^2).The microalgal biodiesel yield was predicted by second–order polynomial equation as:

Biodiesel yield Y_B (%) $=69.94+21.1*X_1-0.253*X_2-0.037*X_3-0.0023*X_1X_2+ 0.0437 *X_1X_3 + 0.00092*X_2X_3-7.13*X_1^2+0.00327*X_2^2 0.000162*X_3^2$ **(4.5)**

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 .All the parameters have positive influence towards the biodiesel yield. Similarly positive effect was projected by the interaction between catalyst concentration and temperature and time and temperature and time.

4.3.2.3.1. Developmental of ANOVA for biodiesel yield

Significantly and suitability of the regression model was proved by analysis of variance ANOVA using experimental design model and actual responses is given in Table 4.9. Chronologically, F-test was performed for each model and conforming probability was calculated for significance of the model. For different responses, quadratic model was selected with the prob>F<0.0001 and detailed analysis of variance is given in Table .4.9

The goodness of fit of the given model for the process of transesterification experiment was predicted by the coefficient value with 99.62% (R^2) and 97.32% as R^2_{adj} value indicating the high degree of agreement between the actual and predicted value of Y_B (%) using the proposed quadratic models. 'F value' and 'Prob>F for Y_B (%) 230.94 . This describes prob>F (probability of error) is also used to check the significance of each regression coefficient, which also indicates the effect of interaction with each cross product. The lesser prob>F value indicating the higher significance of the corresponding coefficient and the detail of prob>F value of all intercepts, linear, quadratic and interactive coefficient for Y_B(%) are given in the Table 4.9. respectively.

4.3.2.3.2. Individual factors contribution of operating variables for biodiesel yield Y_B (%) by process of transesterification

The dry algal biomass to methanol ratio (1:10 wt/vol) and string rate (200 rpm) was kept constant respectively. It was seen from Table 4.9., these responses for biodiesel yield varied between 74.12% to 91.46% respectively for different combinations of operating variables.

Table 4.8: Central composite design (CCD) matrix for reaction condition optimization for microalgal (*C.pyrenoidosa*) biodiesel yield

Std	Run	Nano-Ca (%)	Temp (°C)	Time (Min)	Biodiesel yield (%)	
					Experimental	Predicted
13	1	1.5	45	105	86.21	86.21
11	2	1.5	30	180	85.32	85.67
7	3	0.5	45	180	74.12	73.42
16	4	1.5	45	105	86.21	86.21
17	5	1.5	45	105	86.21	86.21
6	6	2.5	45	30	75.64	76.34
1	7	0.5	30	105	73.23	73.58
3	8	0.5	60	105	77.35	77.70
18	9	1.5	45	105	86.21	86.21
8	10	2.5	45	180	88.32	88.32
9	11	1.5	30	30	82.68	82.33
4	12	2.5	60	105	86.32	85.97
15	13	1.5	45	105	86.21	86.21
2	14	2.5	30	105	82.34	81.99
5	15	0.5	45	30	74.56	74.56
14	16	1.5	45	105	86.21	86.21
12	17	1.5	60	180	91.46	91.81
10	18	1.5	60	30	84.67	84.31

Table 4.9:Analysis of variance for transesterification of algal oil

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	511.07	9	56.79	230.94	< 0.0001	Significant
X_1	139.11	1	139.11	565.74	< 0.0001	
X_2	32.93	1	32.93	133.91	< 0.0001	
X_3	58.70	1	58.70	238.72	< 0.0001	
X_1X_2	0.0049	1	0.0049	0.0199	0.8912	
X_1X_3	43.03	1	43.03	175.01	< 0.0001	
X_2X_3	4.31	1	4.31	17.51	0.0031	
X_1^2	222.22	1	222.22	903.75	< 0.0001	
X_2^2	2.37	1	2.37	9.62	0.0146	
X_3^2	3.64	1	3.64	14.82	0.0049	
Residual	1.97	8	0.2459			
Lack of Fit	1.97	3	0.6557			
			Std.=0.5			R^2=0.99
			Mean=82.96			R^2_{adj}=0.99
			CV%=0.59			R^2_{pre}=0.93

The best-fitted response for Y_B(%) composed of intercepts, linear, quadratic and interaction coefficient as given below. The contribution of individual operating variable towards Y_B(%) is depicted in the Fig.4.9a respectively. It is depicted from Fig. 4.9. that 60% contribution of catalyst dose and 26% time for individual variables for biodiesel yield by the process of transesterification. The deviation in biodiesel yield by influencing variables is Catalyst>Time>Temp. It was described that the biodiesel yield by the process of transesterification was affected by catalyst and time but was inhibited by increasing the temperature.

4.3.2.3.3. Interactive factors contribution of operating variables for biodiesel yield Y_B (%) by process of transesterification

The interactive contribution of the variables (Nano-Ca+Time; Nano-Ca+Temp and Temp+Time) is shown in the Fig.4.9b for optimization of process of transesterification for biodiesel yield. According to statistical analysis, Nano-Ca+Time combination contributes upto 91% role in transesterification process for biodiesel yield. Fig.4.10 a-b show the 2D and surface 3D plot for the interaction between Nano-Ca, Time and Temperature.

Based on surface plot 2D and 3D plots, the combined effect of the increased concentrations of catalyst and reaction time helps to increment the biodiesel yield up to an optimum point. Decrease in concentration of catalyst and time decreases the yield of biodiesel in the process of transesterification as shown in Fig.4.10 (a-d). 3D response surface plot shows the maximum amount of biodiesel yield 93.73 % obtained by using 2% catalyst dose and 180 min reaction time with high doses of catalyst concentration and short time duration decrease the yield of biodiesel. Because the reaction mixture consists of two component system (oil and methnol) which tend to slow down the reaction when dose of catalyst increases or decreases.

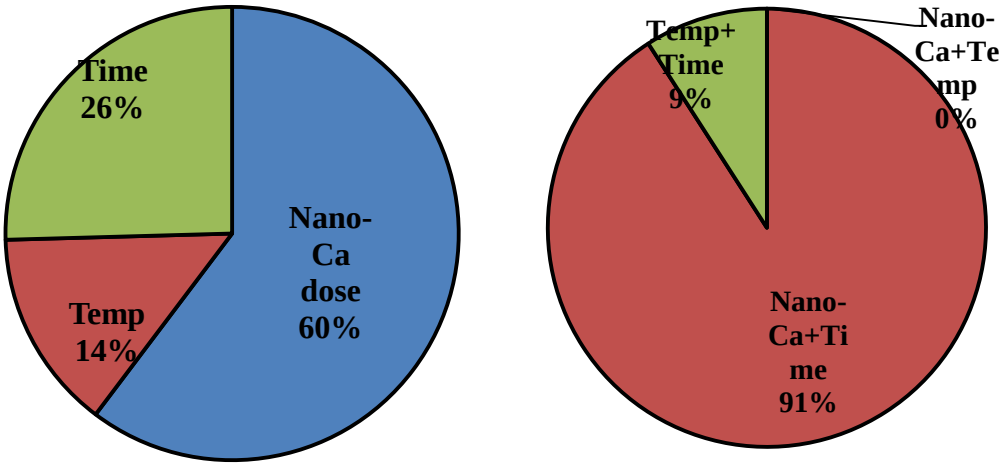


Fig.4.9: Percent contribution of Time, Temperature and Dose for transesterification of algal oil (a) Individual contribution (b) Interactive contribution

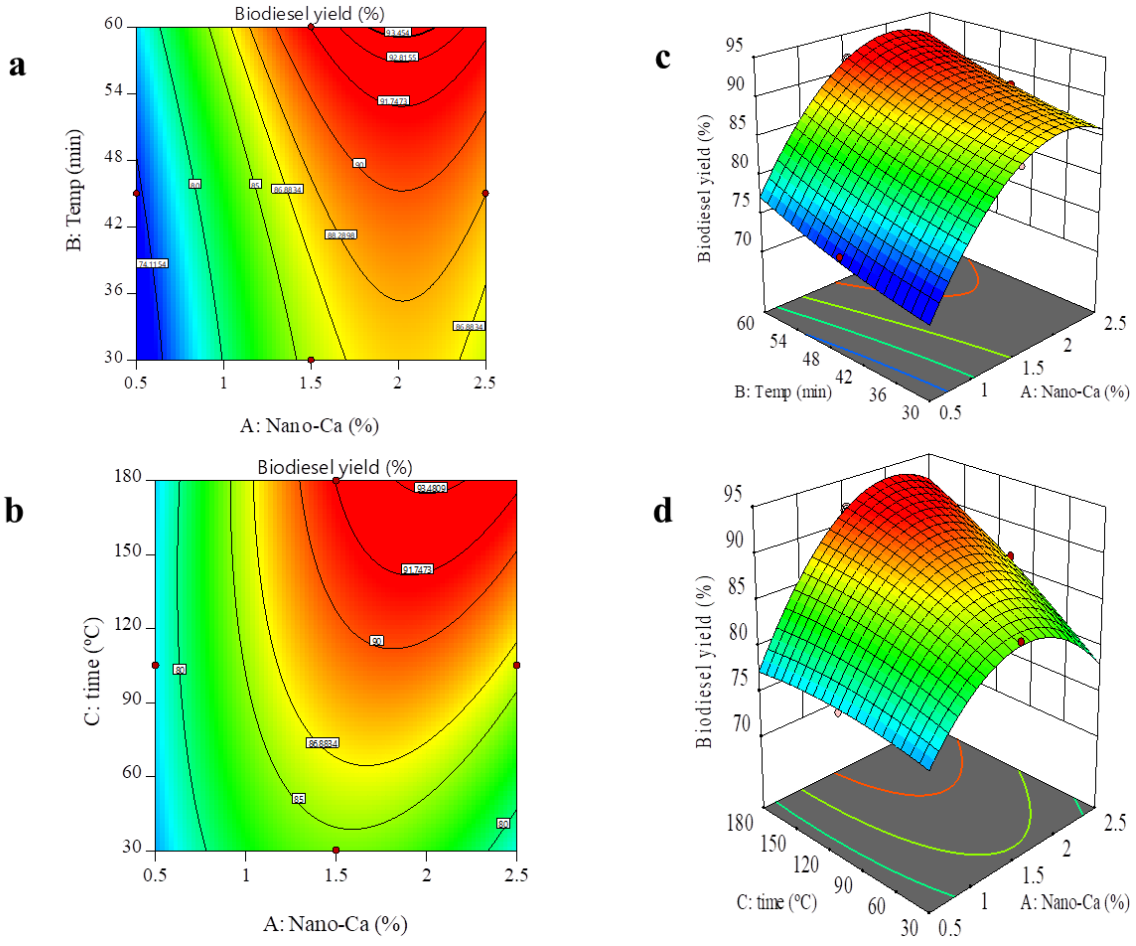


Fig.4.10: 2D and 3D representation of significant factor and response for transestfication of algal oil

Fig 4.10(a-d) illustrates the 2D and 3D plot for the catalyst and temperature interaction on the yield of biodiesel by using 2% catalyst and 60ºC of the reaction temperature. This surface plot indicates that the immiscible phase of methnol and oil and heterogeneous catalyst needs a certain range of temperature. Because at high temperatures, methanol gets evaporated because it is volatile in nature. Higher temperatures should be effective to intensify the activity of catalyst and also accelerate the movement of molecules; so, the frequency of collision among the molecules will be faster. Sabir *et al.*, (2016) reported that 60ºC is the highest temperature for biodiesel yield because there is a limitation in boiling point of methanol.

4.4. Conclusion

In this chapter, significant statistical, regression analysis and response analysis were carried out for harvesting and transesterification by using experimentally obtained values at different variables operating condition to scale up and optimize the algal biomass of *Chlorella pyrenoidosa*. As peer categorization of chapter, concluding remarks are also planned accordingly.

Among all the selected chemical based catalyst, nanocatalyst was found to be the best for harvesting of algal biomass productivity on the basis of present contribution effect. The harvesting efficiency(%) was also found to be significant with multivariables (pH , Dose, temperature).

The selected nano-Ca catalyst used for extraction of oil using transesterification, provides a new concept because it is synthesized from waste-egg shell material. Response model analysis also supported the experimental database for high yield of biodiesel.

Chapter-5

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

5.1. Introduction

To assess the fuel quality of algal oil after upstream and downstream process is very important, if it is to be used on commercial scale. Keeping this view in mind, biofuel (biodiesel) is gaining attention now-a-days, as it is non-toxic, degradable and more feasible for combustion with lesser particulate emissions.

Despite these advantages, feedstock availability and cost effectiveness have always been a serious issue in biodiesel production process. Biofuels are particularly important as an alternative fuel option for transportation. The sustainability of biofuels will depend on the development of viable, sustainable technologies that do not appear to be expensive and are commercially viable. Successful development of algae-based biofuels and co-products industry requires the optimum combination of technical innovations in systems and processes, coupled with economic feasibility in the practical implementation and integrated scale-up for commercial production and marketing. Presently, India produces only 30% of the total petroleum fuels required and the remaining 70% is imported which, costs about 8×10^5 million per year; it is evident that mixing 0.5% biodiesel fuel to the existing diesel fuel can save 4×10^4 million per year. If India plans a 10% mix in the total diesel consumption (46.8 Mton during 2008–2009), ~ 4.7 Mton of biodiesel will be required. Hence, to make the biofuel (biodiesel) comparable at commercial scale at international level, sold biodiesel must meet the American Society for Testing and Materials (ASTM) standard D6751. These specifications cover the biodiesel blend stocks (B100) to be used as a blend component with diesel fuel.

Furthermore, to check the feasibility of biofuel from bio-oil database as a biodiesel, Biodiesel Analyzer (v.1.1) software is used and compared with commercially available biodiesel. Similarly, techno-economical aspects in reference to "suggested

approach" adopted for this work is also validated with conventional/available research outcomes as provided in literatures. So, whole chapter is divided into two main sections to explain the potentiality of experimental database as fuel from Chapter-4 and Chapter 5.

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

5.1(b). Techno economical assessment of the "selected approach"

5.2. Materials and methods

5.2.1. Biodiesel software

It's analytical offline software based on designing fatty acid profile from any feedstock determined by GC. It also helps in prediction of multiple value added products at a glance.

5.2.2. Fuel quality index (FQI) FQI discussed in Phase-III of Chapter-2

5.2.3. Techno-economic analysis

It helps in process designing and economic analysis for the suggested approach selected here for algal biomass towards biofuel as one of the end-products in general and exploration of any other value added end products (Fuel, Feed, Food, and Fodder)

5.3. Results and discussion

5.3.1 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) used in central composite design of Response Surface Methodology with point prediction analysis (PPA) using optimized conditions/induced stress conditions to enhance algal biomass. Similarly, this enhances biomass used for extraction of lipid and convert into fatty acid after the process of transesterification using Nano-Ca catalyst. The Nano-Ca catalyst in the

process of transesterification was used and analyzed by using Gas Chromatography with Flame Ionized Detector (GC-FID) and obtained FAME values are given in Table 5.1. It is also summarized that the chain length of FAME comprising of biodiesel in *C. pyrenoidosa* is between C_{10}–C_{24}. It is composed of saturated fatty acid (SFA), unsaturated fatty acid (USFA) which further categorized into monosaturated fatty acid (MUFA) and polysaturated 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.

From the Table.5.1, the dominated FAME present $C_{22:6}$, $C_{24:0}$ and $C_{24:1}$ more than 60% in Case 1st and 2nd besides this $C_{18:2}$ and $C_{18:3}$ were dominated in Case 3rd and 4th.These fatty acids methyl ester (FAME) have a direct impact on the chemical and physical properties of biofuel to be uses as transportation fuel. According to the data base generated, the selected software also helps in deriving the fuel properties like acid value, kinematic viscosity, cetane number, cloud point *etc* as given in Table 5.2. To be used on commercial scale, Polyunsaturated fatty acid (PUFA), Monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) can be used as transportation fuel, cosmetics and pharmaceutical aspects respectively .The cetane number calculated by ASTM 976 is too low when there is a higher number of saturated acids in the ester mixture and a higher number in the mixture with high levels of poly unsaturated acids whereas according to finding of our results cetane number is on higher side, which support the early ignition rate as a transportation fuel.

Findings of the present study from biodiesel software are used to develop the FQI which is a novel approach in the determination of qualitative assessment for fuel quality. The value near to 100 in FQI, is more suitable to be used as a biodiesel which is determined by the present study.

Table.5.1: Fatty acid composition of different combination of variable after point prediction analysis

Types of Fatty acid	Carbon number	1st case	2nd case	3rd case	4th case
		(NO_3^-, PO_4^{-3} and DIWW)	NO_3^-, PO_4^{-3} and LED Light	DIWW,CO_2, AND LED light	CO_2 temperature and LED Light
Caprylic acid	C_{10}	1.67	1.84	0	0.24
Capric acid	C_{12}	0.89	1.63	1.25	0.74
Lauric acid	C_{13}	0.67	0.23	1.35	0.32
Trideclyic acid	C_{14}	1.28	2.35	0.63	2.32
Mysteric acid	C_{15}	1.34	2.4	0.92	1.42
Pentadecanoic acid	C_{16}	3.13	1.62	0.75	2.34
Palmitic acid	$C_{16:1}$	6.02	4.02	2.34	1.86
Palmitileic acid	C_{18}	0.54	0.26	1.24	2.34
Margaric acid	$C_{17:1}$	0.8	0.4	2.31	1.21
Steraric acid	$C_{18:1}$	3.71	4.23	0.89	0.75
Linoleic acid	$C_{18:2}$	2.5	2.6	20.46	22.32
Linolenic acid	$C_{18:3}$	0.86	0.42	32.63	37.26
Stearidonic acid	$C_{18:4}$	0.26	1.15	0.00	0.00
Archidic acid	$C_{20:0}$	0.4	1.63	0.96	0.94
Eicosenoic acid	$C_{20:1}$	0.96	2.32	4.25	2.34
Archidonic acid	$C_{20:4}$	0.89	1.23	1.21	0.95
Behenic acid	$C_{22:0}$	0.29	1.84	2.64	0.91
Gadolenic acid	$C_{22:2}$	0.29	2.63	0	0.12
Docosahexanoic acid	$C_{22:6}$	32.54	28.36	12.26	10.32
Lignoceric acid	$C_{24:0}$	7.4	4.32	10.2	8.2
Nervonic acid	$C_{24:1}$	32.6	31.26	1.25	1.32
		99.04	96.74	97.54	98.22

Table.5.2:Biodiesel quality parameters after analysis

Properties	**1st case**	**2nd case**	**3rd case**	**4th case**
	(NO_3^-, PO_4^{-3} and DIWW)	**NO_3^-, PO_4^{-3} and LED Light**	**DIWW,CO_2, AND LED light**	**CO_2 temperature and LED Light**
Saturated fatty acid (SFA)	22.14	19.88	22.25	20.98
Monosaturated fatty acid (MUFA)	40.4	41.83	8.73	6.27
Polyunsaturated fatty acid (SUFA)	37.34	36.39	66.56	70.97
Degree of unsaturation	115.08	114.61	141.85	148.21
Saponification value	176.04	175.25	187.14	191.21
Iodine value	192.05	181.13	194.76	198.99
Cetane number	34.09	36.69	31.64	30.02
Long chain saturated factor	16.17	13.39	26.55	19.54
Cold filter plugging point	34.32	25.59	66.94	44.91
Cloud point	-4.29	-4.14	-4.6	-3.76
Alicyclic position equivalent	176.61	160.69	1.72	174.48
Bis alicyclic position equivalent	169.5	150.96	151.36	152.22
Oxidation stability	37.69	41.64	4.81	4.57
High heating value	39.97	39.21	38.58	38.75
Kinematic viscosity	1.47	1.43	1.24	1.2
Density	0.89	0.87	0.86	0.87
FQI	**76.7**	**78.54**	**84.6**	**88.67**

5.3.2. Techno-economical assessment (TEA) for "selected approach"

Techno-economical accomplishment for best production yield of biofuel from algal biomass fully depends on the strategically designed steps of upstream and downstream process. Techno-economic analysis (TEA) for any system totally depends on its research, development, commercial aspects of system including environmental elements .Presently TEA is a part of every R& D projects which covers the lab to land projection and for social growth as fuel cost, eco-friendly fuel from sustainable feedstocks is on emerging stages. In this trend, various generation of fuels (1^{st}, 2nd, and 3^{rd}) are coming with pros and cons, but microalgae based biofuel pathway is providing a potential for sustainable energy solution at global level. Although most of the TEA assessment based on the laboratory scale data which often cannot meet the requirements/ challenges with large scale situations, hence use of integrated system for final products from algal biomass (*C. pyrenoidosa*) should be focused to get higher yield of biofuel. As per concluding remarks from Chapter-3 and 4, it has been justified that through biofuel production, wastewater treatment, carbon dioxide sequestration, nutraceuticals and pharmaceutical provides as an economical soundness with technical route of conversion. Fig.5.1.clearly depicts the endless use of algal biomass but as per our findings optimization of parameters in dependent/coupling manner should not be ignored. Use of regression models with RSM also justified that for practical invention, optimization of influencing/stress parameters saves the time and raw resources (chemical/glassware).

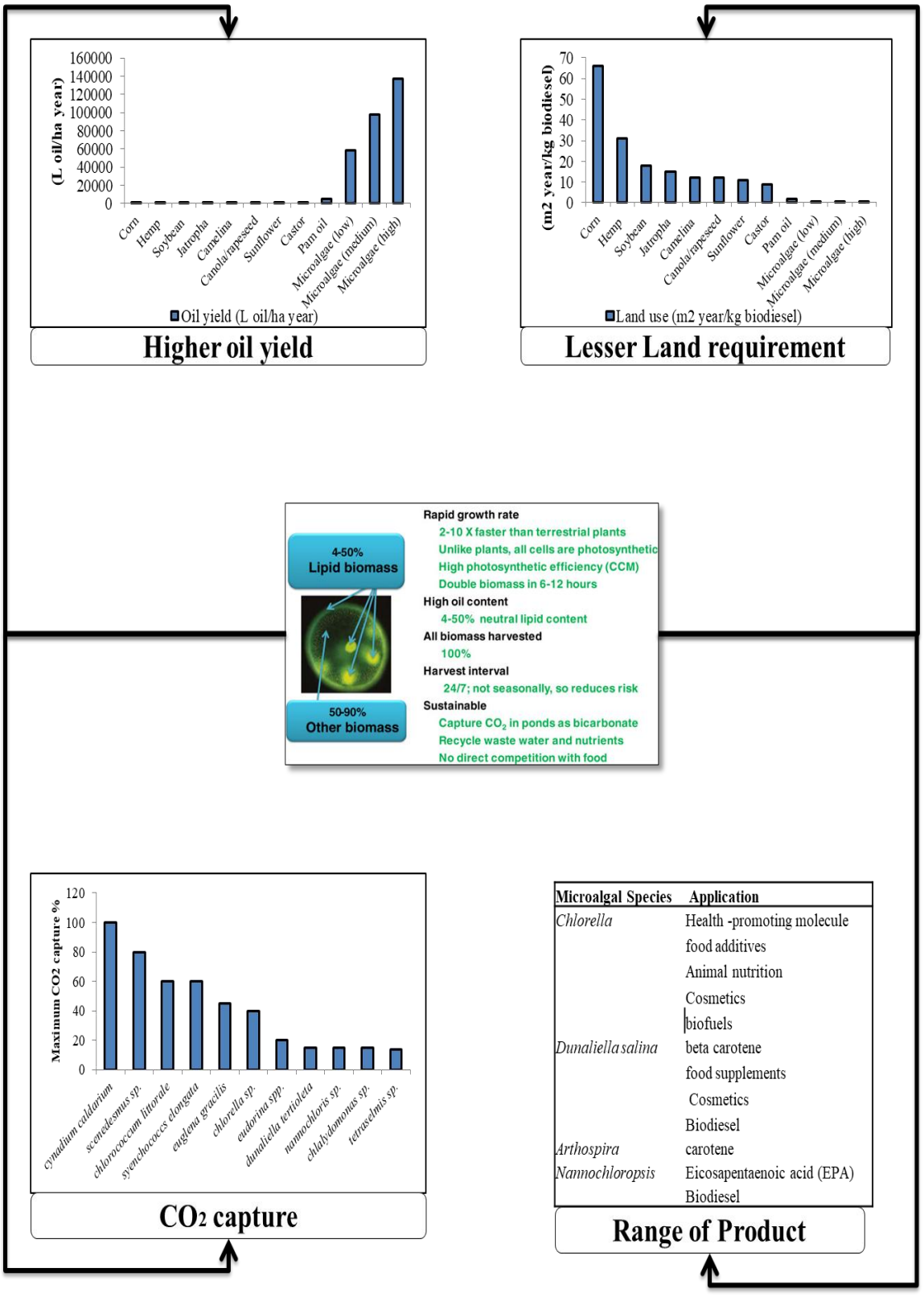


Fig.5.1: Conversion route of algal product in to value added product and its benefit.

Hence, researches on wastewater based algal biofuel production without optimization of multifactor is incomplete to get/enhance algal biomass productivity for higher yield of biofuel. To assess the fuel quality of algal oil after upstream and downstream process is very important, if it is to be used at commercial scale. Keeping this view in mind, biofuel (bio-diesel) is gaining attention now a days, because it is non-toxic, degradable and more feasible for combustion with lesser particulate emissions.

5.4. Conclusion

The influence of different FAME % on biofuel quality using biodiesel software and FQI is explored in this chapter and results can be concluded as;

- Polysaturated fatty acids have the potential to use the algal fuel as transportation fuel with optimized parametric system.
- TEA is an advanced approach to get feasibility with new approaches in sustainable manner.

Chapter-6

Conclusion and Future Recommendations

6. Conclusion and Future recommendations

In view of the objectives decided for the thesis, 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. Where are, 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 was drawn from the thesis can be summarized as given below:

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 developmental technologies like changes in environmental variables (Light, temp, CO_2) and process parameters (Nutrients, pH etc.) to scale the algal biomass productivity at lap/commercial scale. Hence among various industrial wastewater dairy industry wastewater is selected, in computable with algal strain *C. pyrenoidosa* as a growth medium. Effects of influencing parameters independently (BG-11 medium) as well as in coupling with spectral variations (LEDs) are taken into account to improve the biomass productivity ad lipid content. Use of Response surface

methodology (RSM) was used to analyse the feasibility as well as optimization of parameters for best results. 75% concentration of DIWW are found suitable for growth at maximum side with 660 nm (R) light .Temp 30ºC CO_2, 10% nutrient with multifactorial study.

- Experimental and 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. Similarly, limiting concentration of parameters pose a stress (high/low) for algal growth.

- To quantity the effects of varying parameters was subsequently validated against experimental data with optimized conditions using point-prediction analysis in designed bio-reactor, discussed and concluded.

- Effects of chemical-based catalysts and nano-catalysts prepared from waste materials for harvesting of algal biomass from 75% concentration of DIWW with RSM. Furthermore, transesterification of bio-oil from harvested algal biomass from 75% of DIWW with optimization of nano-catalyst (CaO) is also taken as objective to scale-up the algal biomass for further study. According to experimental finding CaO nano-catalyst showed a remarkable harvesting efficiency and performs process of transesterification in comparison to other. This waste-materials based nano-catalyst provides a new dimension for management of environmental pollution. This sustainable approach is also helpful in reducing the cost of systems for "algal biomass to biofuel" in a sustainable manner.

- From the study, it was also concluded that the optimized dose (2%) for waste-materials based nano-catalyst (CaO) with RSM for transesterification of bio-

oil is suitable for higher yield of biofuel, a clean and sustainable product for society as a fuel.

- Trends for qualitative assessment of algal bio-oil developed from harvested algal biomass of *C. pyrenoidosa* are evaluated for fuel quality index (FQI) in comparison with commercial available bio-diesel (*Jatropha*). Techno-economics of the proposed experiment plans (Phase-I, II, and III) has been evaluated and it shows the positive scale at commercial level with concept of sustainable bio-economy.

- FQI concluded with the findings that PUFA (70%) and MUFA (40%) are the responsible for its use as commercial fuel and other value added end-products respectively. C_{18}-C_{22} are the optimized range for biofuel, produced from harvested *C. pyrenoidosa* after Phase-I and Phase-II, similar to other findings reported by researchers, very well observed with own studies.

- In reference to commercial biodiesel, CO_2 temperature and LED Light following parameters from harvested *C. pyrenoidosa* under stress or without stress provides an suitability as a biofuel.

This leads to the conclusion that the use of industrial wastewater with *C. pyrenoidosa* under optimized stressed conditions (with and w/o 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. It also generated a new way forward for the future energy option and waste management approach (DIWW and waste management based catalyst) as to solve the problem of energy crisis and environmental pollution from the local to national, regional to global level.

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Annexure

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: COD (mg/l) =(b-a) X N of FAS X 1000X 8/ ml of sample

Where,

a = ml of titrant with sample

b = ml of titrant with blank

Nitrate (mg/l):

Principle

Nitrate and brucine react to produce a yellow colour, the intensity of which can be measured at 410 nm spectophotometerically.

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.

Phosphate (mg/L)

Phosphate in extract is measured by the reaction of phosphate with ammonical molybdate in an acid medium to form molybdohpsphoric acid .the molybdophosphoric acid is then reduced to a pink coloured complex and these blue

coloured compound detected through at absorbence 640 nm using spectrophotometer.

$$H_3PO_4 + 12H_2MoO_4 \longrightarrow H_3P(Mo_3O_{10})_4 + 12H_2O$$

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

a. **Methodology for growth characterization 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:

A) Spectrophotometer and magnetic stirrer.

B) Phenol Solution (5%): 30 g of phenol dissolved in 1 liter distilled water.

C) 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. **Protein (µg mL^{-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) 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.

Lipid analysis ((µg mL^{-1})

The sulpho-phospho-vanillin (SPV) method has been used for the determination of the total lipid content. Proposed SPV assay for lipid estimation Phosphovanillin reagent was prepared by initially dissolving 0.6 g vanillin in 10 ml absolute ethanol; 90 ml deionized water and stirred continuously. Subsequently 400 ml of concentrated phosphoric acid was added to the mixture, and the resulting reagent was stored in the dark until use.

For SPV reaction of the algal culture for lipid quantification, a known amount of biomass in 100 µL water, which are either suspended in a known volume of liquid culture or harvested via centrifugation at 4000 RPM for 5 min, was used. 2 mL of concentrated (98%) sulfuric acid was added to the sample and was heated for 10 min at 100ºC, and was cooled for 5 min in ice bath. 5 mL of freshly prepared phospho-vanillin reagent was then added, and the sample was incubated for 15 min at 37°C incubator shaker at 200 rpm. Absorbance reading at 530 nm was taken in order to quantify the lipid within the sample.

Modified Bligh and Dyer (MBD) 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 rpmfor 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).

Lipid productivity (% dry weight) = (wt. of lipid/ wt. of alga) ×100 (6)

Transestrification of lipid

Mixture of catalyst (NaOH) and methanol was poured into the flask containing the algal oil. The whole content was then kept for 3 h on continuous rotator shaker (200 rpm) to allow the completion of reaction. After three hours, the biodiesel formed upper layer and the pigment along with glycerine settled down at the bottom. Biodiesel was separated with the help of separating funnel (Hossain Sharif et al., 2008).

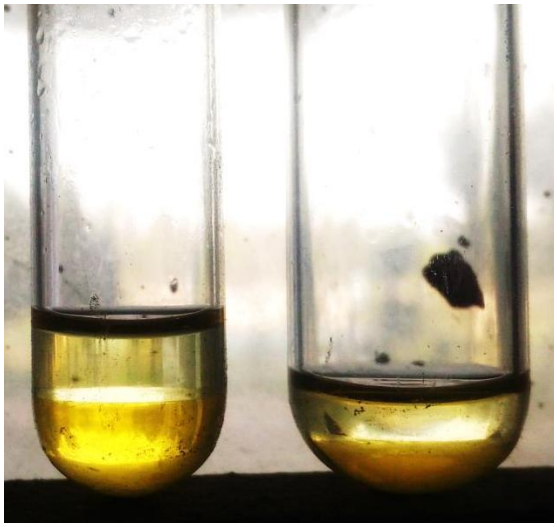
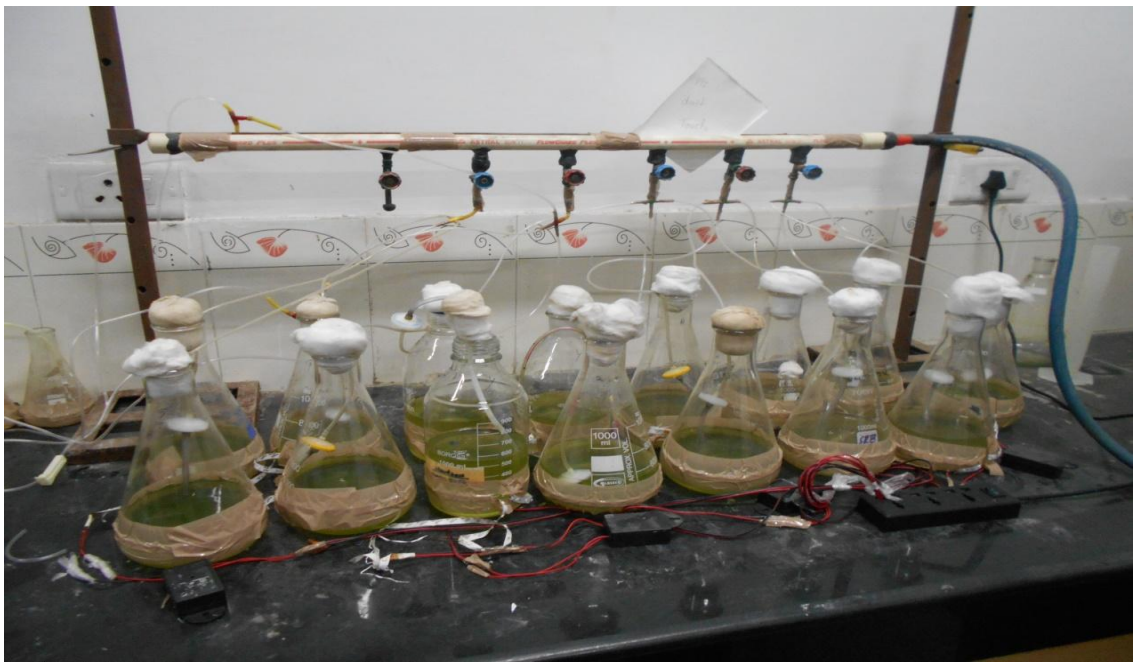


Photo of experiment

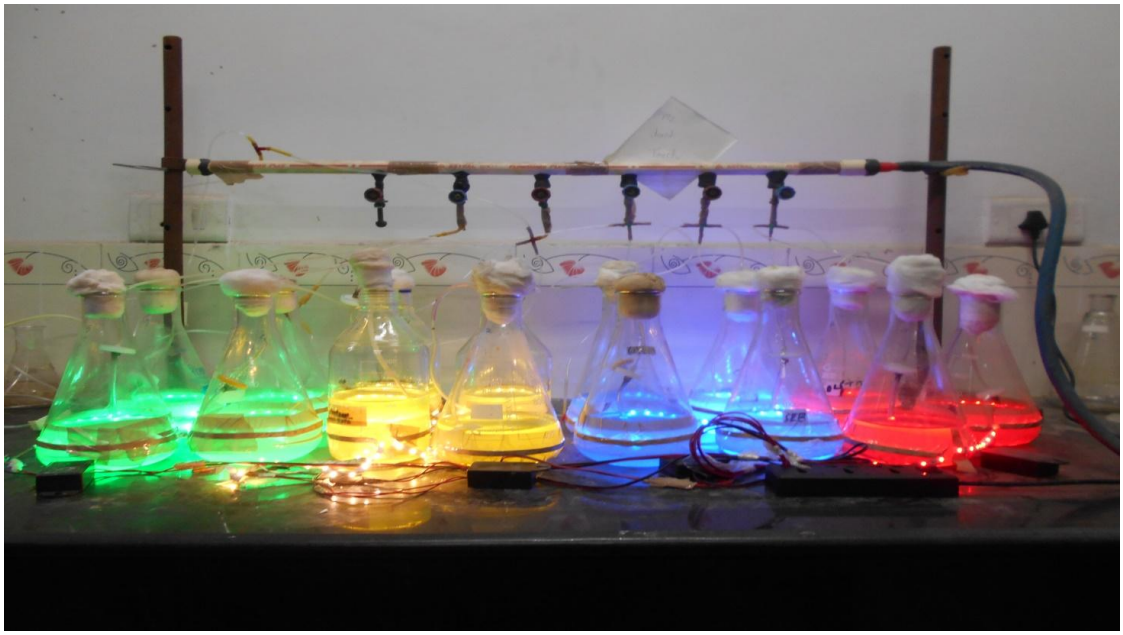
(a)

(b)

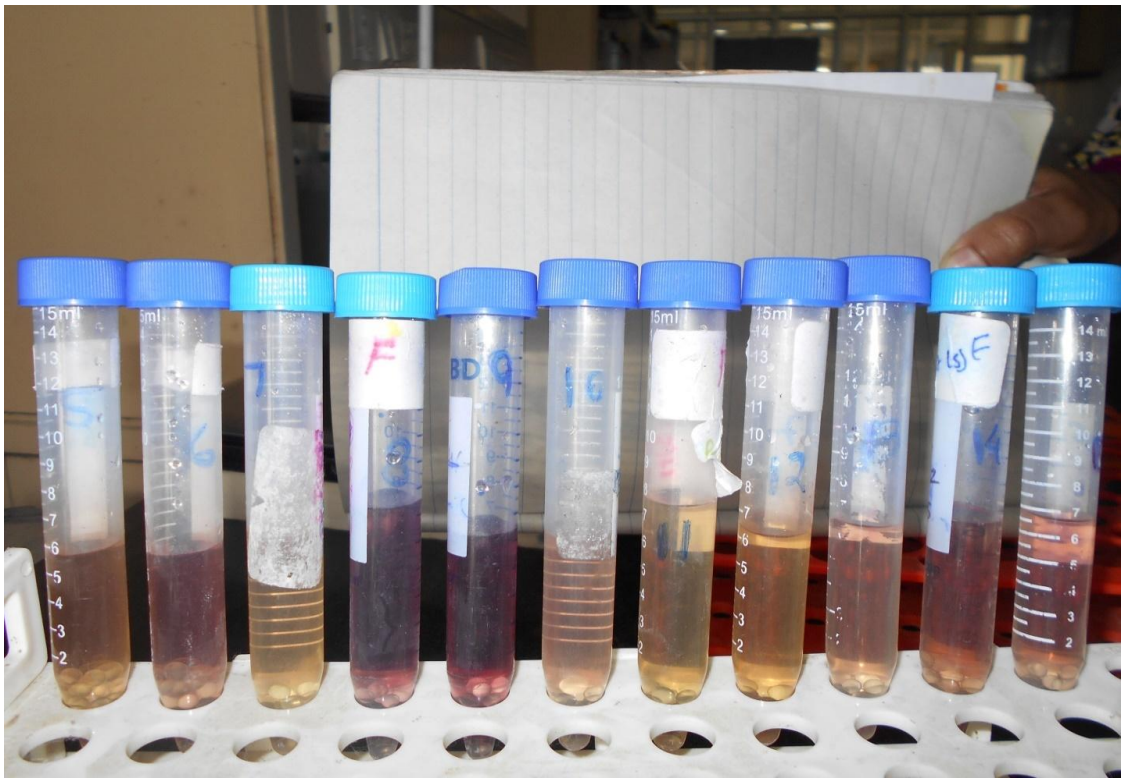
(c)



(d)



(e)



(f)


Tung/mp
Fly Ash
Ca(NaNO)
Fe(NaNO)
Tung/mp
Fly Ash
Ca(NaNO)
Fe(NaNO)
Tung/mp
Fly Ash
Ca(NaNO)
Fe(NaNO)
Tung/mp
Fe(NaNO)
Ca(NaNO)
Fly Ash
Tung/mp
Fe(NaNO)
Ca(NaNO)
Fly Ash

(g)

List of Publications

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ORIGINAL ARTICLE

Optimization of nutrient stress using *C. pyrenoidosa* for lipid and biodiesel production in integration with remediation in dairy industry wastewater using response surface methodology

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Abstract

The present study illustrates optimization and synergetic potential of alga *Chlorella pyrenoidosa* for lipid production and remediation of Dairy industry wastewater (DIWW) through response surface methodology (RSM). Maximum lipid productivity of 34.41% was obtained under 50% DIWW supplemented with 0 mg L^{-1} nitrate (NO_3^-), and 50 mg L^{-1} phosphate (PO_4^{-3}). While maximum biomass productivity (1.54 g L^{-1}) was obtained with 50% DIWW supplemented with 100 mg L^{-1} NO_3^-, and 50 mg L^{-1}, PO_4^{-3}. Maximum removal of COD (43.47%), NO_3^- (99.80%) and PO_4^{-3} (98.24%) was achieved with 8th run (75% DIWW, 150 mg L^{-1} NO_3^-, 75 mg L^{-1} PO_4^{-3}), 15th run (50% DIWW, 0 mg L^{-1} NO_3^-, 50 mg L^{-1}, PO_4^{-3}) followed by 1st run (25% DIWW, 50 mg L^{-1} NO_3^-, and 25 mg L^{-1}, PO_4^{-3}), respectively. Lipid (bio-oil) obtained from 15th run of experiment was converted in biodiesel through base catalyze transesterification process. Fatty acid methyl ester (FAME) analysis of biodiesel confirmed the presence of major fatty acids in *C. pyrenoidosa* grown in DIWW were C11:0, C14:0, C16:0, C16:1, C18:1 and C18:2. Results of study clearly demonstrate enhanced growth and lipid accumulation by *C. pyrenoidosa* in surplus PO_4^{-3} and limitation of NO_3^- sources with DIWW and its suitability as potential alternative for commercial utilization.

Keywords Algal biomass · Wastewater · Nutrients · Lipid · Biodiesel · Response surface methodology

Introduction

Crude oil is the world's largest fossil fuel source to meet the primary energy demand, satisfying 31.3% of the world's total energy demand of 13,699 MT. This may critically explain why the total global emission of CO_2 increased from 15,458 to 32,381 MT during 1973 and 2014 (Lithgow 2017). So, excessive use of fossil fuel for energy generation has proven to be unsustainable from an environmental and economic point of view. Another major environmental challenge is linked to the management of wastewater discharge from industrial sectors such as dairy, textiles and agro-processing (Gothwal et al. 2012). Kushwaha et al. (2011) estimated that Indian dairy industries generate about 275 MT of wastewater annually. In European countries, the average effluent discharge from dairy industry is about 500 m^3 day^{-1} (Kothari et al. 2013). Dairy effluent is characterized by high turbidity and pungent smell, high biochemical oxygen demand (BOD) (40–48,000 mg L^{-1}) and chemical oxygen demand (COD) (80–95,000 mg L^{-1}), and variable pH (6–9) (Demirel et al. 2005). The conventional techniques (physical and chemical)

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ORIGINAL PAPER

Fuel Quality Index: A Novel Experimental Evaluation Tool for Biodiesel Prepared from Waste Cooking Oil

Shamshad Ahmad[1] · Richa Kothari[1,2] · Vinayak V. Pathak[3] · Mritunjay K. Pandey[4]

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Abstract
The potential of biodiesel has been recognized widely, however limited availability of feedstocks have hampered its frequent production. In present study feasibility of waste cooking oil (WCO) for biodiesel production was analyzed and its characterization was performed for development of fuel quality index (FQI). Characteristics of raw oil samples and transesterified oil samples (Soyabean oil and Sunflower oil) after various heating and cooling cycles (1st–10th cycles) were analyzed and compared with American Society for Testing and Materials standards. Fourier Transform Infrared Spectroscopy analysis clearly revealed remarkable changes in the spectralband (1400–1700 cm^{-1}) after transformation of the fatty acid group into ester with selected oil samples obtained at varying heating and cooling cycles. Both oils samples at 10th cycle of heating and cooling showed more pronounced changes in the value of density from 0.9 to 0.93 g ml^{-1} in SFO, 0.91 to 0.87 in SBO and viscosity 41.45 ± 1.52 to 45.65 ± 1.45 mm^2 s^{-1} in SFO and 40.32 ± 1.2 to 44.57 ± 1.68 mm^2 s^{-1}than the control and transesterified samples. The characteristics of transesterified oil samples were used to generate FQI, which is found to be analogous with commercial biodiesel. Thus, the present study provides a competitive alternative feedstock in the form of WCO to produce biodiesel at commercial scale.

Keywords Waste cooking oil · Transesterified oil · Fuel quality index · Biodiesel

Introduction

Conventional sources of energy such as coal and petroleum are diminishing due to rapid industrialization, transportation, and population growth. Combustion of these sources causes potential environmental impacts such as global warming, atmospheric pollution etc. Chemical nature of conventional fuels is also hazardous such as mine wash at coal washery and oil spillage cause serious harm to the surrounding flora and fauna. Thus, in all way fossil fuels have been proven unsustainable but still its demand and consumption are increasing day by day. Keeping all adverse impacts and limitations associated with fossil fuels several efforts have been made to develop new sources of energy such as solar, wind and biomass energy etc. These new sources of energy considered as a clean fuel and its life cycles are found sustainable. Biofuels are biomass derived energy products, which are available in various forms (solid, liquid and gaseous) such as biodiesel, biogas, biohydrogen, bioethanol, butanol etc. [1]. Among all these biofuels, biodiesel has more potential to replace conventional diesel fuel because of its flexibility to be used in conventional diesel engines with no or partial modifications. It has been found that engine run on biodiesel successfully for long duration and the performance emission are quite comparable to that of petroleum diesel fuel [2]. In major oil importer countries like India, indigenous biodiesel production has large potential due to higher demand for diesel fuel in various sectors.

Chemically, biodiesel is non-toxic, degradable and more feasible for combustion with fewer particulate emissions. Despite these advantages, feedstock availability and cost effectiveness has always been a serious issue in biodiesel production process. Biodiesel obtained from edible oil acts

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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.

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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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0960-8524/

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REVIEW ARTICLE

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

Richa Kothari[1] · Arya Pandey[1] · Shamshad Ahmad[1] · Ashwani Kumar[2] · Vinayak V. Pathak[1] · V. V. Tyagi[3]

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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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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]

Published online: 11 October 2016

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,

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Prospects for pretreatment methods of lignocellulosic waste biomass for biogas enhancement: opportunities and challenges

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ABSTRACT

Lignocellulose biomass/lignocellulosic waste biomass (LCB/LCWB) represents the largest renewable pool for potentially fermentable carbohydrates, which provides a good solution for bioenergy production. Although it is assumed to have a lower theoretical yield for biogas than waste material made of sugar or starch, it is free from the problems associated with other generations of biofuels. An inexpensive and efficient pretreatment method of LCB/LCWB is highly desired to achieve an economical biogas production process. This paper reviews the conventional, advanced and infant (i.e. under development) pretreatment methods that have been studied for enhancement of biogas production. In addition to various pretreatment methods, this article also reviews further aspects of the conventional, advanced and infant methods (nanotechnolgy) for pretreatment of LCB/LCWB. Thus, the article provides systematic technological strategies and new pretreatment approaches for sustainable bioprocessing of LCB/LCWB into value-added product.

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Lignocellulosic waste biomass; pretreatment methods; nanomediated pretreatment, biogas

Introduction

At present one of the most important prerequisites for sustainable development is the production of appropriate fuel from biomass which can be utilized as an alternative to fossil fuel. Organic waste material is a promising renewable energy source, which is already being used to meet heat, electricity and transportation fuel requirements in different countries. Biogas production processes can be applied on almost all biological material, but the organic fraction with low degradability limits biogas productivity, thus lowering the efficiency of the process. The potential of lignocellulosic waste has been explored widely due to its frequent availability [1,2]. Lignocellulosic biomass is composed (approximately 90%) of a complex structure of lignin, cellulose and hemicelluloses. Among these constituents, lignin is highly recalcitrant toward chemical and biological (microbial and enzymatic) degradation; hence, digestibility of the organic fraction of lignocellulose biomass/lignocellulosic waste biomass (LCB/LCWB) for biogas production is still a major bottleneck in this technology. However cellulose and hemicelluloses of the organic fraction of LCB/LCWB have been widely used as a feedstock for biogas production [3]. The global production of plant biomass is about 200×10^9 tons/year, of which only 20×10^9 tons/year is accessible as potential lignocellulose biomass [4]. However, due to the recalcitrant constituents of LCB/LCWB, the usable amount of biomass for digestion remains lower than 20%. An essential step in order to increase biomass digestibility is to change the compact structure in order to make cellulose and hemicelluloses more accessible to the enzyme for breakdown and to convert them into fermentable sugar. Several pretreatment methods are available including physical, chemical and biological methods. These methods increase the solubilization of the substrate by breaking down the structure of the lignin and reducing cellulose crystallinity. Hence, the main goal of any pretreatment method is to remove the structural and compositional impediments to subsequent degradation processing steps. In this context, new possibly ecofriendly pretreatment methods are also under experimental investigation, such as nanomaterial-based/nanomediated pretreatment methods to enhance the yield of bioenergy. The development of advanced and promising technologies in pretreatment methods with different types of LCB/LCWB with economic and commercial feasibility on a comparative basis is also extensively discussed in this article. Thus, this article provides a suggestive mode of action for conversion of LCB/LCWB in general and into a value-added bioenergy product, i.e. enhanced yield of biogas, in particular.

Structural components of lignocelluloses

A wide variety of substrate contains lignocellulosic components, such as residues from crops and forests,

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ORIGINAL ARTICLE

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

Richa Kothari[1,2] · Shamshad Ahmad[1] · Vinayak V. Pathak[1,3] · Arya Pandey[1] · Saubhagya Singh[1] · Kapil Kumar[4] · V. V. Tyagi[5]

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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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Effect of Solvent Extraction Methods on Oil Yield and its Parametric Feasibility with C. Pyrenoidosa

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View affiliations

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Abstract

Microalgae are termed as third generation feedstock due to their enormous capacity to synthesize oil, which is a precursor of biodiesel production. The present study aims to analyze the effect of solvents in solvent extraction method to recover algal oil from Chlorella pyrenoidosa biomass grown on dairy industry wastewater. Bligh and Dyer (BD), Modified Bligh and Dyer (MBD), Mixture of n-Hexane and Diethyl ether (M n HDE) and n-Hexane were used to extract algal oil, in which modified Bligh and Dyer (approx 36%) found to be the best. The quality of algal oil was also found to be significant to be used for biodiesel production with specific parameters such as acid value (9.5 mg KOH/g), iodine value (136.24g /100 ml oil), saponification number (192.34 mg KOH/g) and free fatty acid content (0.62%). The FTIR spectrum also shows the presence of larger peaks in the region from 1800-1700cm $^{-1}$, which attributes to the stretching of -C=O, typical of esters. Thus, the present article provides the efficacy for high oil yields with the use of various solvents as a part of solvent extraction methods and obtained oil qualitatively analyzed with parameters on commercial scale, which is an urgent need to scale-up the process for economic sustainability.

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Inspec keywords: sustainable development; product quality; biofuel; mixtures; biotechnology; Fourier transform infrared spectra; wastewater treatment; solvents (industrial)
Subjects: Environmental issues; Biotechnology industry; Testing; Biofuel and biomass resources; Industrial processes; Water (environmental science); Fuel processing industry; Products and commodities; Inspection and quality control

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