Studies of CO_2 sequestration by *Chlorella vulgaris* in dairy waste water in stirred tank batch type photobioreactor

Dissertation

submitted in partial fulfillment of the requirement for the award of degree of

Master of Technology
in
Biotechnology

Submitted

by

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Declaration

I, the undersigned, hereby declare that the research work presented in the M. Tech dissertation entitled **"Studies of CO_2 sequestration by Chlorella vulgaris in dairy waste water in stirred tank batch type photobioreactor"** has been carried out by me under the supervision and guidance of Dr. Haripada Bhunia, Department of Chemical engineering, Dr. Niranjan Das, Professor, Department of Biotechnology, and Thapar University, Patiala. Further, I declare that no part of this dissertation has been submitted for a degree or any other qualification of any other university or examining body in India/elsewhere.

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CERTIFICATE

This is to certify that dissertation entitled "Studies of CO_2 sequestration by Chlorella vulgaris in dairy waste water in stirred tank batch type photobioreactor" submitted by Ms. Anjali chauhann partial fulfillment of the requirements for the Masters in technology Degree in Biotechnology at Thapar University, Patiala is an authentic work carried by her under our guidance and supervision.

To the best of our knowledge, no part of this dissertation has been submitted for a degree or any other qualification of any other university or examining body in India/elsewhere.

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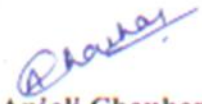

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Abstract

Carbon dioxide, a Greenhouse Gas (GHG), is the one of the principle pollutant, warming earth. Enough carbon dioxide have pumped into the earth atmosphere raising its level to 400 ppm in the past 150 years because of anthropogenic activities, higher than they have been for hundreds of thousands of years. A number of CO_2 capture technologies and storage technologies are being considered to tackle this climate change. The techniques to reduce the effects of Carbon di oxide are classified as chemical reaction based strategy (abiotic) and biological mitigation method (biotic). Abiotic method captures CO_2 by reaction with chemical compounds before it is released into the atmosphere. But, the disadvantages of this method are that the chemical reactions are highly energy intensive and costly; and the problematic disposal of the wasted chemical compounds. On the other hand, biological mitigation is more favorable as it directly converts CO_2 into biomass. Because of photosynthetic ability of microalgae, the potential microalgae such as Scenedesmus sp., Chlorella vulgaris, Chlamydomonas sp. And Chroococcus sp. have been actively used globally in open and closed photobioreactor for CO_2 mitigation. Algae are also used as feed stocks for bio-energy diverse that include microalgae, macroalgae (seaweed) and cyanobacteria (blue-green algae). Algae occur in a variety of terrestrial habitats and natural aqueous in environment. Some microalgae also have the potential to store lipids under certain conditions. This dissertation describes the effectiveness of *Chlorella vulgaris*, used in a photobioreactor at different concentrations (0.04%, 5%, 10%, 15% and 20%) of CO_2 in Fogg's media with 10% (of total volume) inoculum. 10% is the CO_2 concentration at which most of the kinetic parameters as well as total CO_2 fixed and fixation rate is found to be maximum. The pH and CO_2 concentration for this culture were also optimized.

Contents

List of tables

List of Figures

Abbreviation

AMOP	Aquatic Microbial Oxygenic Phototrophs
ASU	Air Separation Unit
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
ECE	Energy Conversion Efficiency
GHG	Green House Gases
IGCC	Integrated Coal Gasification Combined Cycles
IPCC	International Panel On Climate Control
NADP	Nicotinamide Dinucleotide Phosphate
PBR	Photobioreactor
TAG	Triacylglycerol
TDS	Total dissolved solids
TSS	Total suspended solids
TS	Total solids

CHAPTER 1

INTRODUCTION

Global warming due to increased carbon dioxide concentration in the atmosphere is receiving a great deal of attention **[1]**. Global warming is rise in temperature because of increased emission of global greenhouse gases, particularly carbon dioxide (CO_2), methane, tropospheric ozone, and chlorofluorocarbons. However, Carbon di oxide is considered to be the most anthropogenic greenhouse gas. **[2].**

Takeuchi (1988) reported that the amount of fossil fuels being burned is emphatically correlated with the atmospheric increase in carbon dioxide. **[1]**. Fossil fuels utilization for the purpose of meeting our energy requirements has resulted in unpropitious effects on the climate, over rely on foreign oil and economic requirements . The increasing surface temperature because of global warming, causing catastrophic effects, necessitate the scientists to tag it as an issue of prior concern .To tackle climate change effects, CO_2 level should not be allowed to elevate much higher than 550ppm and the current level is 380 ppm **[3]**

Since the pre industrial era, the ongoing economic and population growth has led to an enormous increase in the emission of anthropogenic greenhouse gases. This has resulted to atmospheric concentration of carbon di oxide, methane and nitrous oxide unparalleled in at least the last 800,000 years. This ongoing climate change is the result of these anthropogenic drivers that have been detected throughout the climate system and are likely to be one of the dominant reason of the observed global warming since the mid-20^{th} century **[4]**

Three different approaches may be followed in order to reduce the rising CO_2 concentrations **[5]**

1. Increase efficiency in electricity generation and its optimum use

2. Expand use of renewable energy sources such as wind, solar, biomass, geothermal and other alternate energy sources, viz. nuclear power

3. Capture carbon dioxide emissions at fossil-fuelled electricity generating plants and permanently sequester the carbon.

According to fourth IPCC Assessment Report, (Fig. 1) is showing the current atmospheric CO_2 concentration at 388 ppm **[4].**

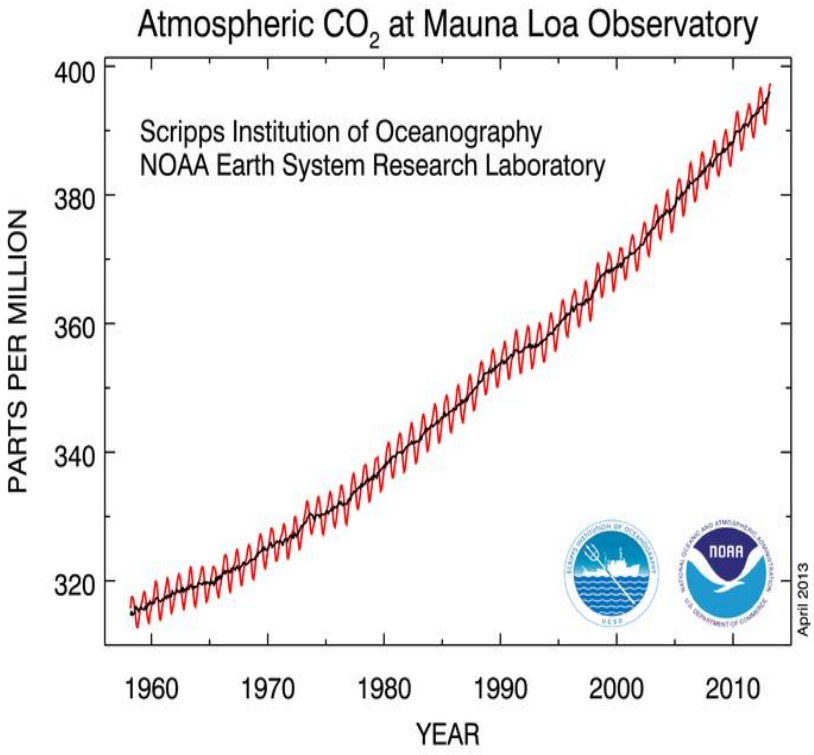

Figure 1.1: CO_2 concentration rise over the years (4)

According to the IPCC a significant fall in CO_2 emissions below current levels to decline the impact of climate change is required for the stabilization of the atmospheric CO_2 concentration **[6]**.The scientific community is currently examining potential approaches in order to reduce the anthropogenic contributions to global warming **[7]**. Physio-chemical methods and conversion of CO_2 to microalgae are the sequestration options in the research and development panorama **[6].** The extent of use will depend on factors such as cost, capacity, environmental impact, the rate at which the technology is being introduced, and social factors such as public acceptance.

Research objectives

The overall objective of the research is to capture CO_2 by micro algal sequestration that would help mitigate global warming. The specific objectives are:

- Optimization of pH and CO_2 concentration for maximizing growth, productivity, protein content and CO_2 fixation rate
- Kinetic study of CO_2 sequestration at different CO_2 concentrations.

- Growth studies of microalga in dairy waste water.

Dissertation overview

This dissertation consists of five chapters. Chapter 1 contains a brief introduction to the research topic. Chapter 2 contains a more detailed literature review relevant to the research such as basic information of microalgae, their place and importance in the modern world, the factor effecting process of CO_2 sequestration, photo-bioreactor technology that is currently used for their proper cultivation, listed including descriptions of the different photo-bioreactor configurations and parameters monitored and their pros and cons. In chapter 3, materials and methods briefly discussed about the instrumentation and methodology involved. In chapter 4, results obtained are shown, analysed and discussed. The final chapter (chapter 5) includes the conclusions that can be made from the results obtained as well as recommendations for future work.

CHAPTER 2

LITERATURE REVIEW

2.1 CO_2 CAPTURE AND SEQUESTERATION (CCS)

2.1.1 CO_2 CAPTURE

CO_2 capture and storage or CO_2 sequestration is the term used to describe a set of technologies aimed at capturing carbon dioxide emitted from industries and other energy-related sources before it enters the atmosphere, compressing it, and administering it deep underground in secure geological formations, and ensuring it remains stored there indefinitely **[8]**. CO_2 capture is applicable to all sources which release large volumes of CO_2. These include - fossil fuel or biomass energy facilities, major CO_2-emitting industries, natural gas production, synthetic fuel plants, oil refineries etc. Capturing CO_2 from small sources such as from transportation industries, is difficult and economically not feasible **[9]**. Therefore, the development of an efficient and cost-effective CO_2capture technique is considered to be one of the highest priorities in the field of Carbon capture and sequestration (CCS) **[10]**. Sequestration strategies adopted can be broadly categorised into physio-chemical and biological means .There are 3 basic approaches to CO_2 capture from fossil fuels**[11]** –

i) Pre-combustion capture
ii) Post combustion capture
iii) Oxy- combustion capture

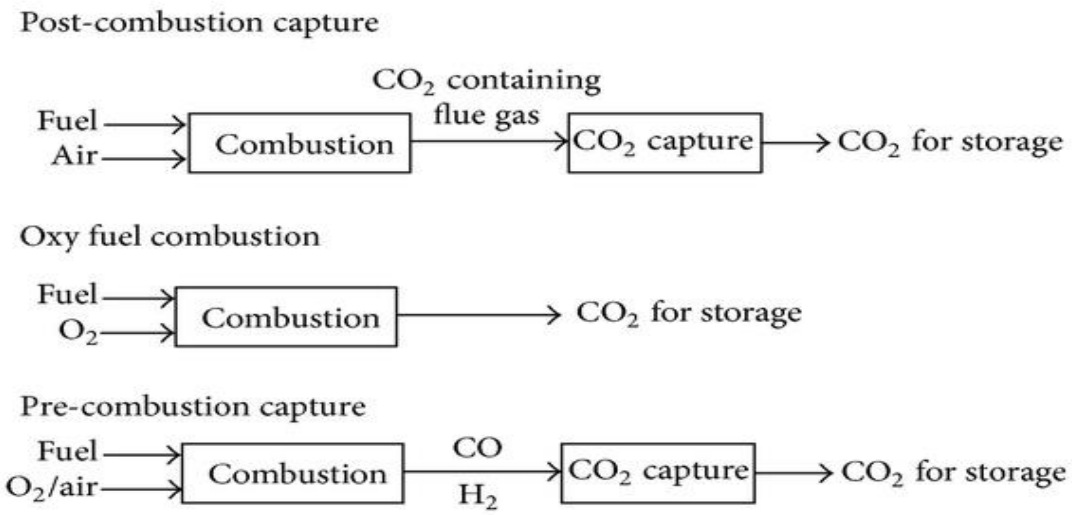

Figure 2.1 Different approaches to CO_2 capture – schematic diagram **[10]**

i) **Oxy-fuel combustion**- In this nearly pure oxygen is used for combustion instead of ambient air, resulting in a flue gas, mainly CO_2 and H_2O, which are separated by condensing water. High CO_2 concentration in output stream (above 80% v/v), high flame temperature, and easy separation of exhaust gases are three major advantages of this method. The major disadvantages are large electric power requirement to separate oxygen from air and high capital cost **[12]**.

ii) **Pre combustion carbon capture** - As the name suggests is prior to combustion. In this method the fuel reacts with air or oxygen in a shift converter and in some cases with steam, a catalytic reactor, to give CO_2 and more hydrogen. Cryogenic distillation and chemical absorption process are used for separating CO_2, resulting in a fuel that is hydrogen rich that can further be used in many applications, such as, gas turbines, furnaces, fuel cells and engines **[10]**.

iii) **Post combustion capture-** its principles involves separation of CO_2 from flue gas after combustion. Generally, the CO_2 in flue gas is diluted (8–15% CO_2) with inert gases such as nitrogen, argon, and water in addition to oxygen. Post-combustion capture does not require expensive technologies such as syngas separation, hydrogen turbine, and fuel cell. The reason why post-combustion capture is more important because it offers flexibility and does not need to change combustion cycle. Shutting down of capture plant will not affect the power plant. Unfavourable condition of flue gases is the major dis advantage of this method **[10]**.

2.1.2 Physico-chemical methods for CCS include the following –

i) Absorption (solvent scrubbing)

A well-established carbon di oxide capture system primarily used in the oil and chemical industries. Being pressure and temperature dependent the physical absorption occurs at high pressures and low temperatures. Chemical absorption of CO_2 from flue-gases relies on acid–base neutralization reactions using basic solvents. For removal of CO_2 some of the preferred solvents are amines. The released CO_2 is compressed and the regenerated absorbent solution is recycled to the stripper column **[10,13].**

ii) Cryogenic distillation

Cryogenic distillation, an air separation process, where gaseous components of a mixture are separated by condensation. Cryogenic method can capture CO_2 in a liquid form, being easier to transport and storage or send for enhanced oil recovery fields. CO_2 and H_2O are separated from flue gas on the basis of differences in dew and sublimation points **[13**].

iii) Membrane Separation

The membrane operation relies on the variations in physical and chemical interactions between gases and therefore the material of the membrane is changed thus permitting one element to labour under membrane quicker than the opposite. The modules of membranes can be used both as typical membrane separation units and as a gas absorption column **[10]**. within the former case, greenhouse gas removal is achieved as a result of the intrinsic property of the membrane between greenhouse gas and alternative gases concerned, whereas within the latter case, greenhouse gas removal is accomplished by gas absorption wherever the membranes, sometimes microporous, hydrophobic and non-selective, square measure utilized as a hard and fast interface for greenhouse gas transfer **[14**].

iv) Adsorption

Solid adsorbents like mesoporous silicates, zeolites and activated carbons, metal oxides, alumina are extensively used for gas separation. These carbonaceous adsorbents have molecular sieving ability which can be can be controlled during their fabrication process making them preferentially adsorb a specific gas (e.g. CO_2) according to the adsorbing molecule's shape and size **[10].**

v) Electrochemical pumps for separation of CO_2 from flue gas

The aqueous alkaline fuel cells and molten carbonate have been studied for use in separating CO_2 from flue gases and air. Carbonate ions get transported across the membrane when an external emf is applied while operating molten carbonate fuel cell in a closed circuit. As oxidizing conditions are required for the formation of carbonate from carbon di oxide, the molten carbonate electro-chemical separator is less applicable for separating CO_2 from flue gases directly **[15].**

vi) Chemical looping

Chemical looping, is an innovative method used for facilitating CO_2 separation from the flue gases. A regenerable metal oxide catalyst provides O_2 for the purpose of combustion of the fuel. A flue gas along with the nitrogen is formed when the lattice oxygen from a metal oxide catalyst is used as the oxidizing agent and the obtained flue gas is not diluted by the nitrogen. This flue gas is enriched with carbon di oxide. In the combustion of the fuel air is not used as a result of which NO emissions are generally low **[15].**

vii) Pressure swing adsorption

In pressure swing adsorption the high pressure of the IGCC is employed to enhance the extent of CO_2 adsorption. When CO_2 passes over the adsorption tower, it gets adsorbed on the adsorbent to

much greater extent than it should in the normal conditions. When compared to adsorption and absorption methods, the thermal energy losses are much smaller. However, the optimum size and actual operating conditions are still unknown **[16]**.

vii) Biological method

There are several disadvantages associated with physical methods such as, having high cost associated with it there by need to develop the suitable technologies. Biological method is an alternative to physical methods of carbon di oxide sequestration.

Biological carbon dioxide fixation is presently achieved through the photosynthesis of all terrestrial plants and an incredible range of photosynthetic microorganisms. Reduction in 3-6% world greenhouse gas emissions is expected to be solely due to plants. In comparison to terrestrial plants the CO_2 fixation potency of cyanobacteria and microalgae is very high and can grow a lot quicker than them. Microalgal- carbon dioxide fixation is environmentally sustainable when combined with various environmental protecting processes, like effluent treatment or significant metal removal. A mixture of carbon dioxide fixation, biofuel production, and effluent treatment might therefore provide a really promising alternative to current carbon dioxide mitigation methods **[17].**

The idea of photosynthetic conversion to fix carbon dioxide using bacteria or micro-algae is sort of promising because the by-product it may result is going to be oxygen that lay the very foundation of human existence. Blue-green algae or micro-algae are steered to perform the role of photosynthesis. In order to push uniform growth of the organisms, the distribution of photosynthetic photon flux light within the wavelength range of 400–700 nm has to be delivered to the bioreactor **[18].**

2.2 MICROALGA FOR CO_2 SEQUERSTRATION

Microalgae are microscopic photosynthetic organisms. They habitat includes both marine and freshwater environments.

The most frequently cited microalgae a for economical and efficient CO_2 biofixation, the lipid synthesis and waste water treatment toward biofuel production are **[19]:**

1. Bacillariophycea: The diatoms. These are also known as diatoms. Majority of them are found in the oceans, though some are also found in brackish and fresh water. Their cell walls consist of polymerized silica (si) and store carbon in different forms. The carbon is stored in the form of polymer of carbohydrates known as Chrysolaminarin or natural oils.
2. Chlorophyceae: The green algae. Mostly present in freshwater, occur in the form of single cells or colonies. These are said to be the modern plant's evolutionary progenitors. Starch is

the main storage compound for green algae, though, under certain conditions natural oils can also be produced.

3. Cyanophyceae: The blue green algae. Cyanophyceae plays a key role in fixing atmospheric nitrogen. Structurally they are much closer to bacteria. Approximately two thousand known species of these algae lives in a variety of habitats.
4. Chrysophyceae. The golden algae. Possessing similarity to the bacillariophycea in biochemical composition and pigmentation, their pigmentation system is more complex i.e.., brown, yellow or orange in colour. These algae produce carbohydrates and natural oils as storage compounds.

2.2.1 Commonly cultivated microalgae species

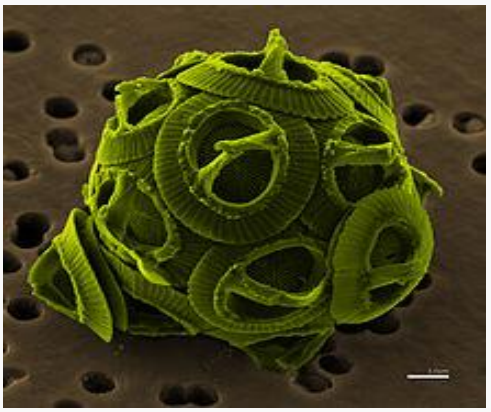

Figure 2.2 Microalgae are microscopic forms of algae, like this coccolithophore which are between 5 and 100 micrometres across **[14]**

Species	Application
Chaetoceros sp.	Aquaculture

Chlorella vulgaris	Source of natural antioxidants
Dunaliella salina	Produce carotenoids (β-carotene)
Haematococcussp.	Produce carotenoids (β-carotene), astaxanthin,canthaxanthin
Phaeodactylum tricornutum	Source of antioxidants
Porphyridium cruentum	Source of antioxidants
Rhodella sp.	Colourant for cosmetics
Skeletonema sp	Aquaculture
Arthrospiramaxima	High protein content – Nutritional supplement
Arthrospiraplatensis	High protein content – Nutritional supplement

2.2.2 Microalgal biofuel system

Lately, the development of microalgae for biofuels production is a subject of substantial interest and investment. Some of the advantages of microalgae biofuel system are larger production yields and accessible land area when compared with terrestrial crops; algae has ability to capture carbon dioxide as bicarbonate in ponds, reducing atmospherical carbon dioxide emissions; and less competition for land, significantly cultivable land used for food production . When compared to the most effective terrestrial systems the additional biomass per unit area increases two to multiple folds in case of algae. There are many reasons for the larger biomass yields of microalgae than the terrestrial plants. Because of higher skills to capture light and converting it into usable chemical energy the photosynthetic efficiency of algae are higher than land plant. Under suitable growth

conditions most of the algae energy is directed into cell division (6- to 12-hour cycle), resulting a faster biomass accumulation. Also, the large amounts of biomass produced in unicellular algae is not partitioned into supportive structures like stems, roots that are not only energetically expensive to provide but also tough to harvest for biofuel production. Additionally, in algae photorespiration is supressed by the carbon-concentrating mechanisms. With algae, the harvesting of biomass can be done at any time of the year, instead of seasonally. Algae fully grown under nerve-wracking conditions (e.g., low nitrogen) or in the presence of supplemental reductants (sugar, glycerol), have their metabolism shifted toward the assembly and accumulation of energy-dense storage compounds like lipids. Several unicellular algae are among the foremost economical biofuel production systems known because of their ability to manufacture up to 60 minutes of neutral lipids (triacylglycerol [TAG]) per gram of dry weight **[20].**

2.2.3 Photosynthesis

Photosynthesis is the physico-chemical method by which plants, algae and photosynthetic microorganism use light energy to perform the synthesis of organic compounds. In plants, algae and certain kinds of microorganism, the process ends up in the release of molecular oxygen and also the removal of carbon dioxide from the atmosphere that's used to produce carbohydrates (oxygenic photosynthesis). Other kinds of bacteria use light energy to make organic compounds however don't produce oxygen (anoxygenic photosynthesis).

Photosynthesis provides the energy and reduced carbon needed for the survival of nearly all life on our planet, as well as the molecular oxygen required for the survival of oxygen intense organisms. Additionally, the fossil fuels presently being burned to supply energy for human activity were produced by ancient photosynthetic organisms. Although, photosynthesis happens in cells or organelles that are usually only a few microns across, the method incorporates a profound impact on the earth's atmosphere and climate.

The overall equation for photosynthesis is deceptively simple:

$$CO_2 + H_2O \longrightarrow (CH_2O)_n + O_2$$

Generally, a complex set of physical and chemical reactions should occur in a well-coordinated manner, for the production of carbohydrates. To synthesise a sugar molecule such as sucrose, plants need nearly thirty distinct proteins that work inside a complicated membrane structure. Further analysis of the mechanism of photosynthesis focuses on understanding the structure of the

photosynthetic parts and the molecular processes that requires radiant energy to run carbohydrate synthesis **[21].**

The process of photosynthesis is divided into two phase, first is light dependent and the second one is calvin cycle **[17].**

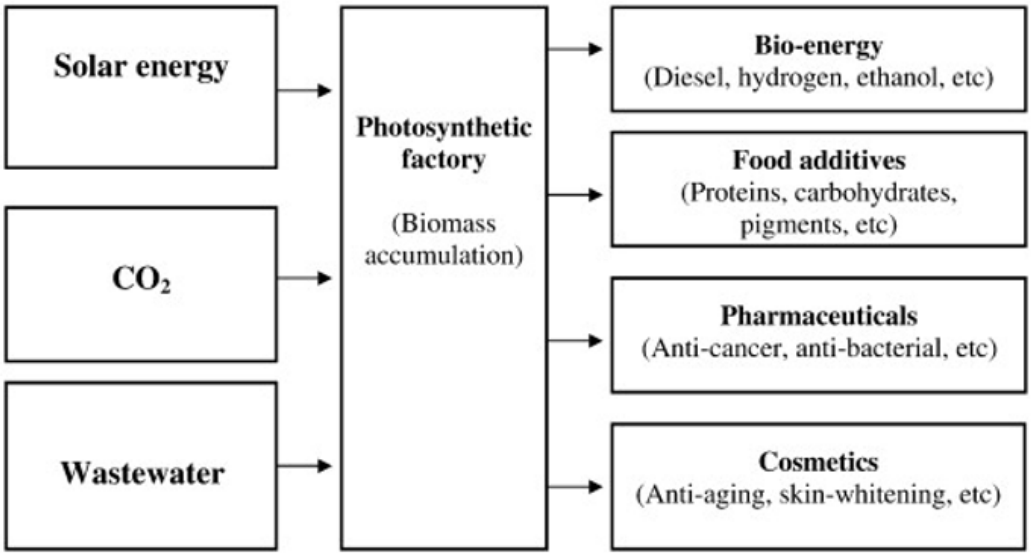

Figure 2.3 Schematic description of photosynthetic conversion of CO_2, solar energy, and wastewater into a variety of valuable end products (e.g., bioenergy, food additives, pharmaceuticals, and cosmetics) by using microalgae **[17]**

light cycle

In the light reactions, chlorophyll's one molecule absorbs one photon along with the loss of one electron. This electron is transported to Pheophytin, a modified form of chlorophyll which transports the electron to a Quinone molecule, allowing the initiation of a flow of electrons down an ETP chain (electron transport chain), leading to the ultimate reduction of NADP to NADPH. In addition, a proton gradient is created across the chloroplast membrane; its dissipation is used by ATP synthase for the concomitant synthesis of ATP **[22].** The lost electron is regained by the chlorophyll molecule from a water molecule by a process called photolysis, which releases a Dioxygen (O_2) molecule. Under the conditions of non-cyclic electron flow the overall equation for the light dependent reactions in green plants is :

$$2\ H_2O + 2\ NADP^+ + 3\ ADP + 3\ P_i + light \rightarrow 2\ NADPH + 2\ H^+ + 3\ ATP + O_2$$

Calvin cycle

In Calvin cycle, CO_2 is captured by the enzyme RuBisCO from the atmosphere and in a process that requires the newly formed NADPH, called the Calvin-Benson cycle, releases three-carbon sugars, which are later combined to form sucrose and starch. The overall equation for the light-independent reactions in green plants is **[23]**

$$3CO_2 + 9\ ATP + 6\ NADPH + 6\ H^+ \rightarrow C_3H_6O_3\text{-phosphate} + 9\ ADP + 8\ P_i + 6\ NADP^+ + 3\ H_2O$$

The carbon dioxide fixation or reduction is a process in which carbon dioxide combines with, Ribulose 1,5-bisphosphate (RuBP), a five carbon sugar, to yield two molecules of Glycerate 3-phosphate (GP), a three carbon compound, also known as 3-phosphoglycerate (PGA). Glycerate3-phosphate is reduced to glyceraldehyde 3-phosphate (G3P), in the presence of ATP and NADPH from the light dependent stages. This product is also named as 3-Phosphoglyceraldehyde or even as Triose phosphate. Triose is a 3-carbon sugar. Most of the G3P is used to regenerate RuBP for the process to continue. The 1 out of 6 molecules of the triose phosphates not "recycled" often condense to form hexose phosphates, which finally yield sucrose, starch and cellulose. The carbon skeletons that are produced during carbon metabolism can be used for other metabolic reactions like the production of amino acids and lipids **[24]**

2.2.4 Growth requirements for microalgae

Reduction in the cost of separating CO_2 gas is one of the advantage of utilizing flue gas directly. Due to higher concentration of CO_2 in power plant flue gas, high CO_2 tolerant species is important. Although CO_2 concentrations differs on the basis of the flue gas source, 15- 20% v/v is typically assumed. While selecting micro-algae for sequestering purpose different parameters need to be considered. These are **[25]**

(a) CO_2 tolerance: CO_2 tolerance should be high. Almost all species of green micro-algae can tolerate CO_2 up to an extent. Some strains has the ability to grow under 80% CO_2 concentration but the maximum growth was observed in 10-20% CO_2 concentrations, e.g strains like Scenedesmus sp.. Chlorella sp. can grow under different concentrations of Carbon di oxide. Other than high CO_2 tolerance, several other factors also need to be taken into consideration for better growth or CO_2 uptake.

(b) Temperature tolerance: Temperature tolerance should be high. Thermophiles are usually considered because the temperature of waste gas emitted from thermal power station is around 120^{o} c .Thermophiles are high temperature tolerant species which can grow in range of temperature from 42-100^{0}C. An obvious advantage of the use of thermophiles for CO_2 sequestration is. Reduced cooling costs, unique secondary metabolites, are obvious which may reduce overall costs for CO_2 sequestration.

Table 2.1 illustrates the temperature and CO_2 tolerance of multiple algal species.

(c) CO_2 assimilation / CO_2 uptake: CO_2 assimilation ability is of crucial importance in choosing algae species. But the comparison is not straightforward because culture conditions differs from experiment to experiment.

(d) Light conditions: light energy being one of the crucial component of photsynthesis makes it an important factor to be considered. Microalgae has lower light intensity requirements as compared to high plants. Microalgae often exhibit photoinhibition under excess light conditions, the major reason behind reducing algal productivity. Photoinhibiton is a reduction of the photosynthetic activities caused by the exposition to high photon photosynthetic flux density (PPFD). The concentration of high-energy electrons in the cell get so high that they cannot be completely consumed through the calvin process and this happens when the chloroplasts absorbs a high flux of photons. These electrons damages the cell structure by forming H_2O_2

Attenuation of light intensity depends on following:

a) wavelength
b) cell concentration
c) penetration distance of light
d) The geometry of photo-bioreactor.

Microalgae mostly consume blue and red light, however, it penetrates little in microalgae suspension than green light. In the dense culture this effect is more pronounced. In the engineering point of view, geometry of reactor can reduce the attenuation of light in micro algal suspension. The light utilization efficiency and overall photosynthetic efficiency is determined by the saturation light intensity (Is). Saturation light intensity roughly varies from 30 to 45 W/m2 with a good estimation. Photo-inhibition or photo-oxidative death is caused by reactive oxygen species, which is produced when the pigments present in the photo system interrupt the alteration of light harvesting complex synthesis and degradation due to overloaded pigment system with the incoming light **[26].**

2.2.5 Factors affecting microalgal growth

An optimum environment is required for proper microalgal growth, which depend on the below described factors. However, different outcomes may be observed if we change these factors.

i. Light provision

In microalgal bioreactors the utilization efficiency and intensity of the light supplied are of crucial importance because for phototrophic organism light is the basic energy source. Normally the wavelength of the he photosynthetically active radiance is assumed to be in range of 400–700 nm **[19]**. Experimental investigations reveal that for microalgal productivity the ratio of light to dark periods in a cycle is of great importance. Photoinhibition can occur due to formation of free radicals leading to toxic H_2O_2 formation if exposure to high intensity of light is provided. Generally 4000-6000 lux is the ideal range of light intensity for high algal growth.

ii. Gas transfer

In microalgal cultivation the gases are introduced to serve a number of purposes, such as **[19]**:

a) Supply of CO_2 as sources of carbon for biomass primary and secondary metabolism
b) Provision of internal mixing,
c) Promotion of exposure of all cells to light, while minimizing self-shading and phototoxicity.

Among the various alternatives, the most frequently used approach is bubbling CO_2 into the bottom of the bioreactor with bubble diffusers. Moderate overall transfer efficiencies (13–20%) can be achieved by this mode of gas delivery is used to achieve moderate overall transfer efficiencies i.e. 13-20% **[27].**

iii. Mixing rates

Mixing is a crucial parameter for achieving acceptable performance of microalgal bioreactors. The gaseous mass transfer is hindered by the low mixing rates and can even cause biomass settling. In either case, stagnant zones will be formed because of poor mixing. This will lead to insufficient availability of light and nutrients in the media because of which /anaerobic conditions will prevail, resulting in decrease of productivity. On the other side, high mixing rates causes shear damage to the cells and requires a large energy input. Pumping, mechanical stirring and gas injection are some of the most common methods adopted for mixing in microalgal bioreactors **[19].**

iv. Temperature effects

Temperature is one of the utmost factors that regulate the morphological, physiological and cellular responses of microalgae: The metabolic rates of microalgae are generally accelerated by the higher

temperatures, whereas at low temperatures inhibition of microalgal growth occurs. The optimal temperature varies from microalgal species to species; however, other environmental parameters also influences the optimal temperature, such as light intensity. The temperature range at which optimal growth have been reported for some species is 15–26 °C, though the maximum is obtained at 23 °C **[19]**.

vi. pH effects

Some microalgal species are tolerant to higher pH (e.g. *Spirulinaplatensis* at pH 9) and some to lower pH (e.g. *Chlorococcumlittorale* at pH 4) whereas most are favoured by neutral pH. CO_2 concentration and pH in microalgal bioreactor systems has a complex relationship. Increased CO_2 concentrations leads to higher biomass productivity, but will also results in decrease in pH, which can effect microalgal physiology negatively. By contrast, CO_2 uptake by microalgae in open ponds cause a rise in pH to 10-11. This increase in pH can be both beneficial and harmful. Beneficial as it causes inactivation of pathogens in microalgal wastewater treatment, harmful because this can also inhibit its growth **[19]**.

vi. Nutrient requirements

Other than carbon, another most important element that is required for microalgal nutrition is nitrogen. And, being the constituent of both proteins and nucleic acids, it is directly linked with the primary metabolism of microalgae. The third most important nutrient for microalgal growth is phosphorus. Because not all phosphorus compounds are bioavailable the phosphorus shoul be supplied in significant excess. The energy stored in lipids are twice the energy stored per C atom of carbohydrates, which directly leads to twofold increase in fuel energy content **[19]**. The relative amounts of lipids or starch increases twofold to threefold in many AMOPs with the depletion of nitrate, silicate, or phosphate from the growth medium. When grown under nutrient deplete conditions most AMOPs become rich source of proteins **[28]**.

Different species of cyanobacteria and microalgae are listed in **Table 2.1**

S.no	Species	Requirements	Advantages
1	*Chlorococcum*	Temp 15-27^{o}C, pH 4-9	Doubling time 8 hrs, densely culturable, CO_2 uptake 70%
2	*Chlorella*	Temp 15-45^{0}c, pH 3-7	Doubling time 2.5-8 hrs, high temp. tolerance, high growth ability,dispersible,CO_2uptake 60%
3	*Euglena gracilis*	Temp 27^{o}c, pH 3-5	Grows well under acidic conditions, high amino acid content, good digestibility (effective fodder), not easily contaminated
4	*Viridiella*	Temp 15-42^{o}C, pH 2-6	Accumulates lipid granules inside the cell, high CO_2 and high temp tolerance.
5	*Anabena*	Temp 25-35^{0}C, pH 7-7.5	N_2 fixation, temp resistant
6	*Enteromorpha clathrata*	Temp 24-33^{o}C, pH 7.5-8.0	Very little agitation is required
7	*Spirulina platensis*	Temp 35-37^{o}C, pH 8.3-11	Very tolerable to pH changes

Table 2.1 Notable species of cyanobacteria and microalgae

2.3 Algae culture systems

For macroalgae (seaweed) and microalgae, culture systems are very different. Different cultivation systems (placed on land or floating on water) are being designed for different microalgae because of their small (μm) size. There are certain conditions that have to be met for algae to grow: water, light, nutrients, light, carbon source and a suitably controlled temperature. Many different culture systems that meet these requirements have been developed over the years, many different culture systems have been developed that meet these requirements **[29]**. However, the difficulty arises when

the system needs to be scaled up as meeting these requirements gets difficult then. A large-scale systems is prerequisite to grow algae commercially for purposes like energy production. These algae systems ranges from very simple open air systems to highly controllable, optimized but more expensive closed systems. The technology necessary for developing profitable algae-based fuel generation is still in progress and the final configuration is yet to be determined and demonstrated at the industrial scale. **[30]**.

2.3.1 Open ponds

In the past few years the use of open ponds for the cultivation of algae has been extensively studied. Open ponds can be categorized into artificial ponds or containers, natural waters (lakes, lagoons, ponds). Shallow big ponds, circular ponds, tanks and raceway ponds are the most commonly used systems. In comparison to closed systems the open ponds are easier to construct and operate, the major advantage of open ponds **[31]**. However, poor light utilization by the cells, evaporative losses, diffusion of CO_2 to the atmosphere and requirement of large areas of land are major limitations in open ponds. Furthermore, the commercial production of algae is restricted by other fast growing heterotrophs and contamination by predators. Also, mass transfer rates in open cultivation systems are very poor due to inefficient stirring mechanism resulting to low biomass productivity **[32]**.

2.3.2 Closed system

As per Several studies use of microalgae in Photobioreactors (PBRs) is a promising and effective method. Closed PBRs offers more advantages than open system such as: high CO_2 fixation efficiency, with the extra benefits of low contamination risk, regulated and well- controlled cultivation, controllable hydrodynamics and high metabolic flexibility. Such system prevents the microalgal inhibition in open systems by reducing the shadow effect by providing a larger surface area exposed to the light. However, the scaling of most closed PBRs having a high tube length has some serious limitations related to biomass circulation, utilization of light, the control of growth parameters and the mass transfer. A good microalgal CO_2 fixation PBR system has certain criteria that needs to be meet such as gas transfer, good mixing and light distribution. Different closed photobioreactors that are currently used for microalgae cultivation are [17]:

(1) Vertical tubular systems

(2) Plate-type systems

(3) Column systems,

The above listed have various advantages and disadvantages, as shown in **Table 2.2**. Normally, for large-scale outdoor cultivation the most attractive systems are tubular PBR and column PBR system are comparatively low cost. Some previous studies claim that the most CO_2 fixation efficiency can be achieved by air-lift PBRs, the vertical tubular type, as they provide better circulation and mass transfer. In addition, because of their circulation times and well-defined flow patterns, bubble column PBRs are assumed to be superior. However, in airlift the cell density and CO_2 fixation ability is affected negatively because of slow circulation times and photo-inhibitory effects. In addition it is seen that small bubbles reflected the most light thus adversely influencing the photosynthetic efficiency, but while using air-lift PBRs found that airlift PBRs are similar to refined bubble column PBRs because of their designed principle. Although, the most suitable system for effective microalgal CO_2 fixation are tubular PBR systems. Their length and reactor sizes are limited by excess oxygen removal, parameter control, CO_2 depletion, and elevated cost. Thus, closed tubular PBR systems are difficult to scale up indefinitely, and depends on the multiplication of reactor units. **[33]**

Table 2.2 Comparison of open and closed systems for microalgae cultivation [17]

Characteristics	**Open systems**	**Closed systems**
CO_2 fixation ability	Low	High
Biomass productivity	Low	High
Specific growth rate	Low	High
Contamination risk	Extremely high	Low
Evaporation losses	High	Low
Photosynthetic efficiency	Low	High
Surface area	Low	Extremely high
Process control	Difficult	Easy
Operation cost	Low	High
Scale-up	Easy	Difficult

2.4 Photobioreactors

Although the term 'photobioreactor' has been designated to open algal ponds and channels, it is best suited for devices that allow monoseptic culture which is fully isolated from a potentially

contaminating environment **[34]**. Algal culture systems can be illuminated by solar light, artificial light or by both. Open ponds, inclined tubular photobioreactors, flat-plate, and horizontal/serpentine have large illumination surface areas and are naturally illuminated algal culture system. Generally, fluorescent lamps and other light distributors are used for artificially illuminating laboratory-scale photobioreactors. Some of these photobioreactors include airlift column, bubble column, stirred-tank, conical, helical tubular, torus and seaweed-type photobioreactors. Furthermore, tempering of some photobioreactors can be easily performed, it can be easily tempered by placing it in a constant temperature room. This approach is limited to compact photobioreactors. High technical efforts are required for large scale outdoor systems like tubular photobioreactors . However, bioreactors such as BIOSTAT photobioreactors (commercially available photobioreactor) can be readily tempered. For designing temperature controlled photobioreactors like double-walled internally illuminated bioreactor with a heaing and cooling water circuit efforts are being made. **[32]** Irrespective of the specific reactor configuration that is used, several essential issues need addressing; (i) supply of carbon dioxide while minimizing losses (ii) efficient and effective provision of light (iii) removal of photosynthetically generated oxygen that could inhibit metabolism or otherwise may damage the culture accumulates in the reactor (iv) sensible scalability of the photobioreactor technology **[34]**.

Flat-plate photobioreactors

Because of large illumination surface area a much attention is given to flat-plate photobioreactor for cultivation of photosynthetic microorganisms. Generally, transparent materials are used for construction of flat-plate photobioreactors for maximum utilization of solar light energy **[32]**. When compared to horizontal tubular photobioreactors, in flate photobioreactors the accumulation of dissolved oxygen is very low **[35]**. It has been reported that, high photosynthetic efficiencies can be achieved with flat plate photobioreactor. For mass cultures of algae flat plate photobioreactors are very suitable. But, they also have some limitations as shown in Table 1. A schematic representation of flat Plate bioreactor is given in Figure 2.4 **[36]**. Provision of an open gas transfer which overcome the problem of oxygen buildup is one of the greatest benefits of this system; however, this can also restricts the effectiveness of contamination control, when compared with completely closed reactors **[37]**.

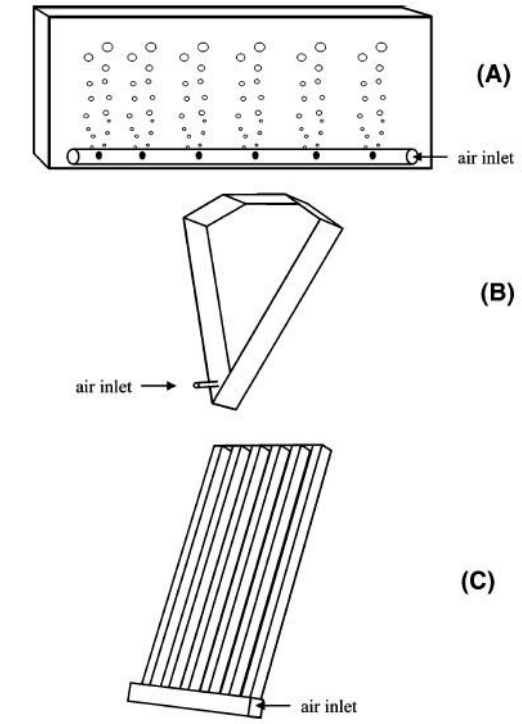

Figure 2.4 Schematic representation of flat panel reactors: flat panel bubbled in the bottom(A), V-shaped panel (B) and alveolar panel (C)

Tubular photobioreactors

For outdoor mass cultures tubular photobioreactor is said to be the one of the most suitable types. Either plastic or glass tube are mostly used for construction of outdoor tubular photobioreactors and their cultures are re-circulated either with pump or preferably with airlift system **[38]**. They can be in form of vertical, horizontal/serpentine, near horizontal, inclined and conical photobioreactor.

Air-pump or airlift systems are used for mixing and aeration of the cultures in tubular photobioreactor system. Advantages and disadvantages of tubular photobioreactors are shown in Table 1. These are found to be much efficient for outdoor mass cultures of algae because of their large illumination surface area. Poor mass transfer is one of the major limitations of tubular photobioreactor. When tubular photobioreactors are scaled up mass transfer (oxygen build up) becomes a problem.

Also, photoinhibition is very common problem in outdoor tubular photobioreactors. When it is scaled up by increasing the diameter of tubes, there is a decrease in illumination surface to volume ratio **[32]**.

A tubular photobioreactor can be scaled up by increasing the diameter of the tubes and the length of the tube can be kept as short as possible. In this case, the growth of cell present at the lower part will slow down as that part will noe receive enough light (due to light shading effect) unless there

is a good mixing system. In any case, by improving the mixing system in the tubes an efficient light distribution system can be achieved.

Also, it is difficult to controling culture temperatures tubular photobioreactors is very difficult, though, they can be equipped with a temperature controller, thermostat to maintain the desired culture temperature, but this could be difficult and very expensive to implement. Also the adherence of the cells to the walls of the tubes is common in tubular photobioreactors.

Furthermore, gradients of oxygen and transfer of CO_2 along the tubes are used for characterization of photobioreactor .The increase in pH of the cultures would also lead to frequent re-carbonation of the cultures can also occur due to increase in pH , which would results in an increase in the cost of algal production.

Tubular photobioreactors are of three types: 1) simple airlift and bubble column, composed of vertical tubing in the form of a vertical tubular reactor that is transparent thus allowing light penetration and CO_2 is supplied via bubbling; 2) horizontal tubular reactor, composed of horizontal transparent tubing, mostly bearing gas transfer systems attached to the connections; and **[32]**; 3) helical tubular reactor, composed of a flexible plastic tube coiled in a circular framework. Air lift and bubble column reactors have been sketched in Figure 2.2.

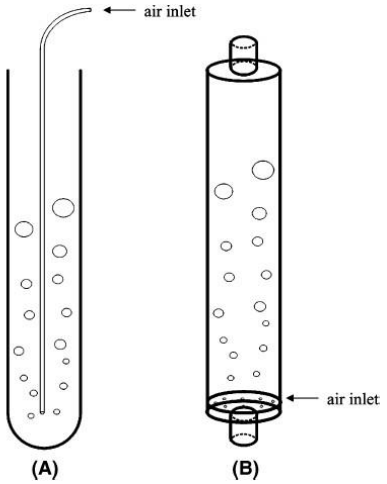

Figure 2.5 Schematic representation of (A) airlift (B) bubble column reactors

The airlift and bubble column reactors comes under the category of vertical tubular reactors (VTR), generally composed of polyethylene or glass tubes, which allow good light penetration as they are sufficiently transparent and are non-expensive because manufactured with sufficiently common materials. To provide good overall mixing air is bubbled at the bottom and CO_2 is provided sufficiently with efficient removal of O_2. Because of advantage like low cost, high transparency and good sterility polyethylene bags are frequently used **[37]**.

Horizontal tubular reactors (HTR) have been on the rise; a gas-exchange unit or a tube connection is used for the purpose of gas transfer, efficient light harvesting is there as the angle toward sunlight is particularly adequate, less susceptible to contamination thus can handle large working volumes. Also, a considerable amounts of heat is generated which may attain amplitudes of 20°C within a single day if (costly) temperature control systems are not provided; thus, it will likely to cause a problem for regular operation. A basic outline sketch of horizontal tubular reactors is given in Figure 2.3.

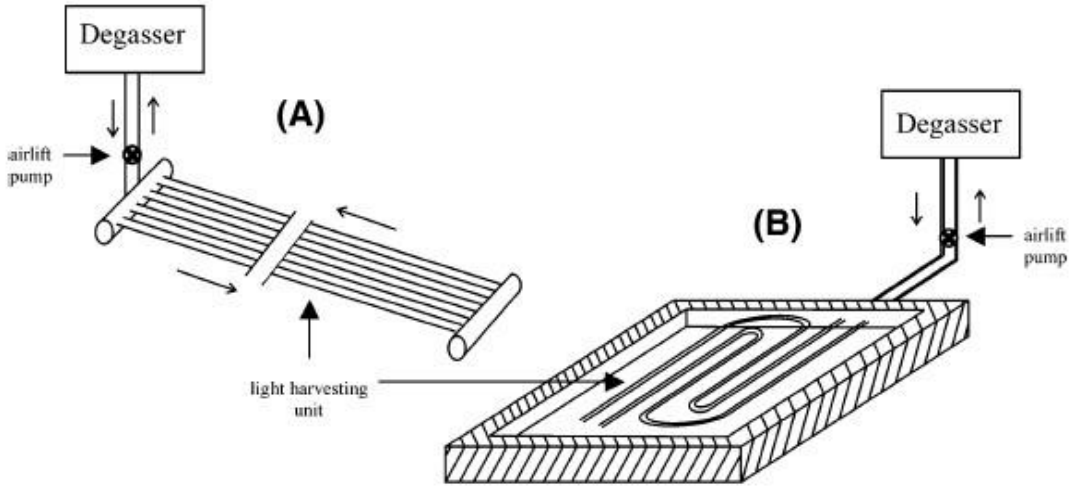

Figure 2.6 Schematic representation of horizontal tubular reactor with a degassing unit and a light harvesting unit, composed of parallel sets of tubes (A) or a loop tube (B) **[37]**

Table 2.3 Advantages and disadvantages of different closed photobioreactor systems for algal cultivation.

Closed photobioreactor systems	Advantages	Disadvantages
Tubular PBRs	• Large illumination surface area • Fairly high biomass productivity • Relatively cheap • Suitable for outdoors	• Fouling • Large area of land needed • Poor mass transfer • High O_2 accumulation • Photoinhibition risk • Hard to control temperature
Flat-plate PBRs	• Huge illumination surface area • Good light path • High biomass productivity • Easy to scale up • Suitable for outdoors • Relatively low O_2 accumulation • High photosynthetic efficiency	• High hydrodynamic stress • Hard to control temperature
Column PBRs	• Low shear stress • Low energy consumption • Relatively cheap • Easy to operate	• Small illumination surface area

CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

3.1.1 Microorganism and culture medium

The strain of *Chlorella vulgaris,* the photoroph which was used, a fast growing microalgae was obtained from Science Technology and Entrepreneur's Park (STEP), Thapar University, Patiala.

The media which was used for the maintenance as well as subculturing of the stock was Fogg's media. The composition of the media is given below:

Table 3.1 Fogg's medium composition

Ingredients	**Concentration**
Potassium nitrate	0.5 g/l
di-potassium hydrogen phosphate	0.2g/l
Magnesium sulphate	0.2g/l
Calcium chloride	0.1g/l
A5 micronutrient solution	1 ml/l
Fe-EDTA stock solution	1 ml/l

For the preparation of Fe-EDTA stock solution, add 26.1 g 0f EDTA in 286 ml water that has 19 g KOH. Dissolve 24.9g of $FeSO_4.7H_2O$ in 500 ml water. Aeration and stirring is required while adding iron sulphate solution to the potassium EDTA solution. The solution become wine red and very little precipitation occurred. The pH of the solution rises to 7.1. Final volume is made up to 1 L and the bottle is stored in dark.

Algae also requires certain ions and salts in minute amounts. These ions and salts acts as co-factors by enzymes for growth of algae. This solution of salts and ions is known as A5 micronutrients solution. The composition of A5 micronutrient solution is given in **Table 3.2**

Table 3.2 Composition of A-5 micronutrient solution

Ingredients	**Concentration**
Boric acid	2.86
Manganese chloride	1.81
Zinc sulphate	0.22
Sodium molybdate	0.018
Copper sulphate	0.08

3.1.2 Characterization of dairy waste water sample

Dairy waste water sample used for cultivation of algae is obtained from verka dairy plant, Sangrur, Patiala on 12 December 2015. Characterization of the sample is performed which includes turbidity, colour, pH, Biological oxygen demand (BOD), Chemical oxygen demand (COD), Total dissolved solids (TDS), Total suspended solids (TSS), Total solids (TS), nitrite. The chemicals and materials used for different tests are given below in **Table 3.3**

Table 3.3 Reagents and materials required for characterization

Characteristics	Reagents and materials
BOD	Sodium hydroxide, oxitop respirometer system, magnetic beads
COD	Potassium di chromate, sulfuric acid reagent, thermodigester, conical flask, ferroin indicator
TDS	Filter paper, funnel, evaporating dish
TSS	Filter paper, funnel, crucibles
Turbidity	Turbidometer
Nitrite	Stock nitrite solution, working nitrite solution, colouring agent (NEDA), sodium acetate
pH	pH meter

All chemical were of AR grade and obtained from Himedia.

3.2 Instruments / Equipment

3.2.1 Set-up for sub-culturing

The set-up for sub-culturing included a 85 watts cool white fluorescent light of CFL as light source and a temperature controlled orbital shaker. Flasks containing the cultures were placed in the orbital shaker, maintained at 27^{o}C and 110 rpm. The luminous intensity was kept around 5000lx. A diagram of the setup is provided **in Figure 3.1.**

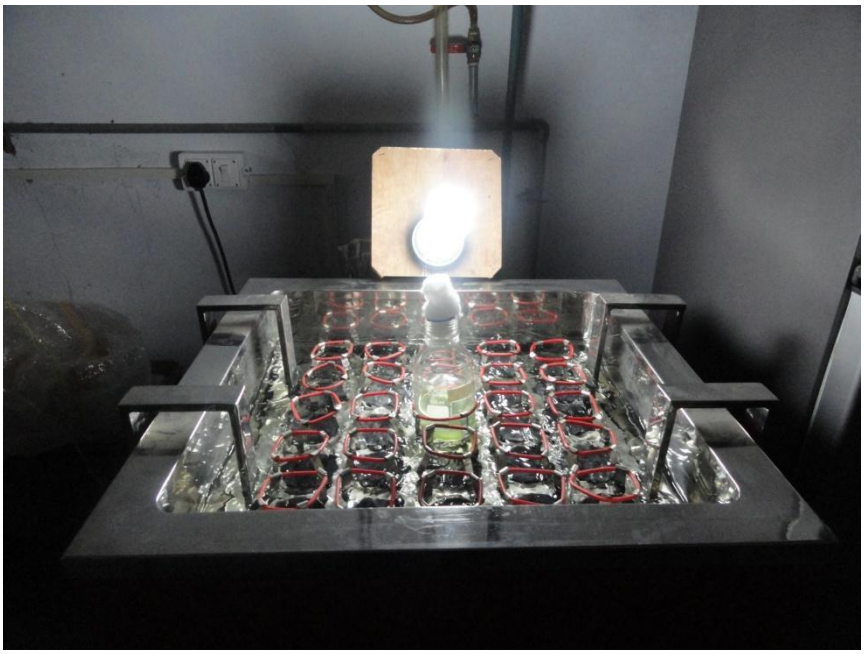

Figure 3.1 set up for sub-culturing

3.2.2 Set-up for CO_2 fixation

Set up for CO_2 fixation consisted of a Bioflo® / celligen 115 Bioreactor (New Brunswick sci., USA) of 7.5 L capacity with working volume of 5.5 l, two light sources, inoculum, CO_2, Air gas supply. Compressed CO_2 gas cylinder required for supplying CO_2 gas is obtained from Lalit gases, Patiala. An oil free compressor of Apcon, India is used for supplying air.

Schematic diagram of the experimental set up is given in Figure 3.1(a)

The total process flow diagram is shown in Figure 3.1 (b)

3.2.3 Centrifuge

Remi, R- 8C, BL centrifuge was used for phase separation of micro-algal cells and growth media.

3.2.4 pH meter

pH meter (Thermo Scientific, Orion 5-Star model) is used for determining pH of the media. 1N NaOH and 1 N HCL is used for adjusting pH, buffers of pH 7 and pH 9 is used for calibrating the pH probes.

3.2.5 Microscope

Images of live algal cells, were captured using a Nikon eclipse 50 i microscope at 40X and 100X magnifications.

3.2.6 UV-Vis spectrophotometer

UV-Vis spectrophotometer is used foe checking the absorbance of algal cells suspension. The absorbance is taken at regular intervals, at 680 nm by a Lambda 35 UV-Vis spectrophotometer (Perkin Elmer, USA).

Figure 3.2 UV-Visible spectrophotometer

3.2.7 Autoclave

Autoclave obtained from Equitron, India was used for the purpose of sterilizing reactor and media.

Figure 3.3 Autoclave

3.2.8 Lux meter

Light affects the biomass yield of the *C. vulgaris*. To monitor the light intensity provided to the algal culture**,** Lux meter was obtained from Mastech, India, model MS 6610 to monitor the light intensity provided to the algal culture.

3.2.9 BOD incubator and Thermo reactor

For the analysis of BOD and COD of samples, BOD incubator and Thermo reactor are used respectively. To incubate oxitop respirometer system (OxiTop IS 6, IS 12) for 5 days to determine BOD, BOD incubator (Aar Kay Enterprises, Ambala cant..) was used . For sample digestion for COD analysis, Thermos reactor (Model 2025 D) was used.

3.2.10 Laminar air flow chamber

For transferring the culture in sterile conditions, Laminar air flow chamber from Thermodyne Pvt. Ltd., Faridabad was used.

Figure 3.4 Laminar flow chamber

3.3 Procedure

3.3.1 Subculturing

10% inoculum was used for subculturing in 200 ml of fogg's media in shake flasks in temperature controlled orbital shaker at temperature 27^{O}C and 110 rpm. Stirring for 5 days. Cotton balls are used for plugging and the only carbon source is self-dissolved carbon from air, this is to avoid other heterotrophic growth. Continuous illumination of 5000 lx is provided with the help of 85 watts CFL. pH of the media was set at 7.2±0.2.to maintain fresh stock the culture was subcultured after every two weeks.

3.3.2 Autoclaving

Sterilization of the photobioreactor is the most important step before starting a batch. Autoclave is used for sterilizing because moist heat kills not only microorgansims but their spores too. Before autoclaving, photobioreactor is unassembled and washed with chromic acid and then rinsed with soap solution and water. Photobioreactor was air dried and reassembled. Probes were cleaned up using ethanol. Afterwards media was filled in the photobioreactor, pH was set before autoclaving at 7.2±0.2. Stoppers are used to tighten up the pipes to prevent any escape of media from them during autoclaving. Each pipe's opening is further closed using cotton balls and aluminium foil. Water level of autoclave is checked and pH probes, DO probes and impeller shaft were covered with their respective caps. The bioreactor was autoclaved at 121^{o}C and 15 psi for half an hour. Before autoclaving it is ensured that air is removed completely.

3.3.3 Inoculum

As cells divides actively during mid exponential phase, culture was always taken during this period. 10% inoculum was used and the head plate of photobioreactor was always sterilized before inoculation using Bunsen flame and ethanol. Funnels used were also sterilized.

3.3.4 CO_2 fixation experiment

Bioflo/ Celligen® 115 Bioreactor (New Brunswick Sci., USA) of capacity 7.5 L with 5.5 L working volume was used. A sensor named Resistance Temperature Detector (RTD) was used for measuring temperature of 27±1^{o}C and is maintained with the help of chiller and heating jacket. pH probe is used for measuring pH. Two 85 WATTs CFL was used for providing illumination of 5000 lx. Rotameter

was used to maintain the gas mixture flow at 2.75 l/min. The photobioractor was equipped with a Rushton disk type impellor which performed the function of proper distribution of nutrients alongwith improving the mass transfer of CO_2 by breaking the bubbles. The CO_2 and air mixture was passed through micropore filter with 0.2-µm pore size prir to injection in photobioreactor. Filters were also employed on the otlets if air to prevent any algae from leaving the photobioreactor. To prevent the medium from evaporating an exhaust condenser is installed at the outlet. Turbulence was created for the purpose of proper mixing by the helps of baffles that were installed in the photobioreactor.

3.3.5 Gas mixture

Pure CO_2 from a CO_2 cylenders with a preheater and air and zero air through an oil free compressor were mixed. The aeration rate was fixed at 0.05 vvm with varying CO_2 concentration in each batch.

3.3.6 Estimation of dry weight of biomass

Optical density of the chlorella culture was measured using UV-VIS spectrophotometer. The OD was converted to dry weight using a calibration curve. The equation for calculating dry cell weight by using OD is **[37]**:

$$\text{Dry cell weight} = 0.28\ OD_{682}\ \text{nm}$$

3.3.7 Determination of kinetic parameters

Biomass vs time graph is plotted to construct the growth curves. To calculate specific growth rate µ (1/d) from the exponential phase following equation was used.

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} ----(3.1)$$

X_2 and X_1 are the dry biomass weight (g/l) at t_2 and t_1 are time respectively. The maximum specific growth rate μ_{max} (1/d) was determined from the different µ values. The cell doubling time was estimated by the following equation **[39]**:

$$t_d(d) = \frac{\ln 2}{\mu_{max}}$$

X_{max} (g/L) is used to designate the maximum biomass obtained. The biomass concentration ΔX (g/L) cultivation time Δt was calculated as $\Delta X = X_t - X_0$. To calculate the biomass productivity $P_{overall}$ (g/L/d) following Equation was used (3.3) **[40]**

$$P_{overall} = \frac{\Delta X}{\Delta t} - - - -(3.3)$$

Where, X_o is the initial biomass concentration at inoculation time (t_o) and X_t is the biomass concentration at time (t). The maximum productivity is designated by P_{max} (g/l/d).

3.3.8 Determination of CO_2 utilization efficiency

X_0 and X_t (g/L) respectively were used to designate the initial biomass concentration of inoculum and max. biomass concentration achieved in the bioreactor.

Thus, the CO_2 fixation rate F_C (g/L/d) was calculated according to Equation (3.4)

$$F_c = \frac{P_{overall} * 0.5 * 44}{12} - - - -(3.4)$$

Where, 0.5 is taken as carbon content of dried biomass which is calculated with the help of CHNS analyzer, 12 (g/mol) and 44 (g/mol) presents the molecular weight of carbon and $_{CO2}$, respectively **[41]** .

3.3.9 Biomass analysis

CHNS analysis

To determine organic carbon, carbonate and the composition of the organic matter, depending on total organic carbon/total nitrogen [C/N] ratios, elemental analysis of total carbon, nitrogen and sulfur was done.

5-10 mg freeze dried and crushed samples were weighed and mixed with vanadium pentoxide [V_2O_5], an oxidizer in a tin capsule, then combusted at 1000°C in a reactor. A flash combustion is promoted in oxygen enriched atmosphere by the tin along with the melting of sample and container. A constant flow of carrier gas (helium) carries combustion products NO_2, CO_2 and CO_2 and a copper reducer, kept at 1000°C while passing through a glass column packed with an oxidation catalyst of tungsten trioxide (WO_3). The NO_2 was reduced to N_2, at this temperature. The CO_2, SO_2, and N_2 were then transported

to a 2-m-long packed column (Poropak Q/S 50/80 mesh) by the helium and quantified with a Thermal Conductivity Detector (set at 290°C).

3.3.10 Determination of growth kinetic using logistic equation

To determine the growth kinetics of algae the logistic equation was used. As it does not use substrate for explaining the entire growth profile of the algae it is a good choice for explaining the growth profile. A sigmoid variation of X as a function of t was obtained by X v/s t and the entire growth profile (lag, exponential, and stationary phase) of the culture can be explained by this satisfactory **[42]**.

Equation 3.5 is the logistic equation.

$$\frac{\mathrm{dx}}{\mathrm{dt}} = K_c X\left(1 - \frac{X}{X_{\max}}\right)$$

Here, in the above equation X denotes the dry cell weight (g/l) max X is maximum dry cell weight (g/l) and K denotes apparent specific growth rate (1/d) for this strain. When integrated with the boundary conditions $X(0) = X_0$ and rearranged the above equation 3.5 can be written as equation 3.6, where X_o, the initial biomass concentration is in g/l.

$$X = \frac{X_{\max}}{1 + \left(\frac{X_{\max}}{X_0} - 1\right)e^{-K_c t}}$$

This can also be written in the form of equation given below:

$$y = \frac{a}{1 + be^{-kt}}$$

Where X_{max} is '*a* 'is X_{max} and is '*b*' is $\left(\frac{X_{\max}}{X_0} - 1\right)$.

By fitting the experimental data in equation 3.7 in origiPro 8.0 using curve fitting tool these constants were determined. To find the fit a confidence bound of 95% was taken.

$$y = \frac{a}{1 + be^{-K_c x}}$$

3.3.11 Characterization of dairy waste water

Dairy waste water sample used for cultivation of algae is obtained from verka dairy plant, Sangrur, Patiala on 12 December 2015. Characterization of the sample is performed which includes turbidity,

colour, pH, Biological oxygen demand (BOD), Chemical oxygen demand (COD), Total dissolved solids (TDS), Total suspended solids (TSS), Total solids (TS), nitrite.

pH: The pH was measured using pH meter.

Turbidity: it was measured by using turbidometer.

BOD: Both filtered and unfiltered sample were taken for BOD measurement in oxitop respirometer systems having pressure sensors for BOD testing and measurements in a closed system after 5 days of experiments.

COD: Add 5ml diluted sample waste water to the culture tubes, the sample was kept at $1/10^{th}$ dilution and add 3 ml standard dichromate solution, 7 ml sulfuric acid reagent along the walls of the tube . Close the tube and shake the content carefully. Digest the sample in thermoreactor at 120°C for 2 hrs. Finally, the glass tube was cooled and sample was taken in cuvette for its spectrophotometric analysis.

TDS: Wash filter paper. Dry evaporating dish & weigh it, stir the sample. Pipette 50 ml while stirring filter it using filter papers. Transfer filtrate to evaporating dish & dry. Cool & weigh the filterate, Calculate in mg/ L **[43]**

$$\text{mg Dissolved Solids/L} = \frac{A-B\ X\ 1000}{ml\ sample},$$

where,

A = weight of dried residue + dish(mg), B = weight of dish(mg)

TSS: weigh filter paper and assemble filtration apparatus. Stir sample and pipette 50ml while stirring .Filter and transfer filter to evaporating dish & dry. Cool & weigh. Calculate in mg/ L.

$$\text{mg Suspended Solids / L} = \frac{A-B\ X\ 1000}{ml\ sample}$$

Nitrite: Take 50 ml of diluted sample and add 2 ml of colour reagent (NEDA). Incubate sample for 10 minutes. Take OD at 543 after 10 minutes and before 2 hours. Prepare suitable series of standards by taking 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 to 2.0 ml oof working stock and make the volume 50 ml with distilled water. They corresponds to 2, 4, 6, 7,10, 12 to 20 µg/l respectively. Shake them well and add 2ml of colouring agent and after 10 minutes takes the OD. Prepare a std. curve by plotting absorbance of standards against NO_2N^- concentration, compute sample concentration.

3.3.12 Microalgae culturing in dairy wastewater

The dairy waste used for algae culturing was obtained from Verka plant, Sangrur, Patiala. *Chlorella vulgaris* was cultured in it for 5 days at 27°C in orbital shaker in shake flask for studying the effect

of wastewater on growth of microalga. Prior use the dairy waste sample was filtered and autoclaved. Algal sample was centrifuged and supernatant was taken for BOD measurement in oxitop respirometer systems having pressure sensors for BOD testing and measurements in a closed system after 5 days of experiments. CO_2 is formed by the microorganisms by consuming the oxygen and this CO_2 was absorbed by NaOH which creates a vaccum which can be read directly as a measured value in mg/l of BOD.

The OxiTop® BOD instrumentation heads have an AutoTemp function. The start of BOD measurement gets automatically delayed (by at least 1 hour) when the sample temperature is too cold, till a constant temperature has been reached.

The measured BOD values are automatically stored (1 value per day) and can be read at all times during or after the period of 5 days and check values or measurements can also be tracked.

COD

Chlorella vulgaris sample was centrifuged at 10,000 rpm for 10 minutes and the obtained supernatant was diluted 20 times in distilled water and mixed thoroughly. 15 mL of distilled water was taken in a glass tube and 5ml of sample was added. After that, 20 mL of concentrated H_2SO_4 and 10 ml of 0.25N $K_2Cr_2O_7$ solution were also added in the glass tube. Then it (glass tube) was kept in the thermoreactor at 150°C for 2 hours. Finally, the glass tube was cooled and sample was taken in cuvette for its spectrophotometric analysis.

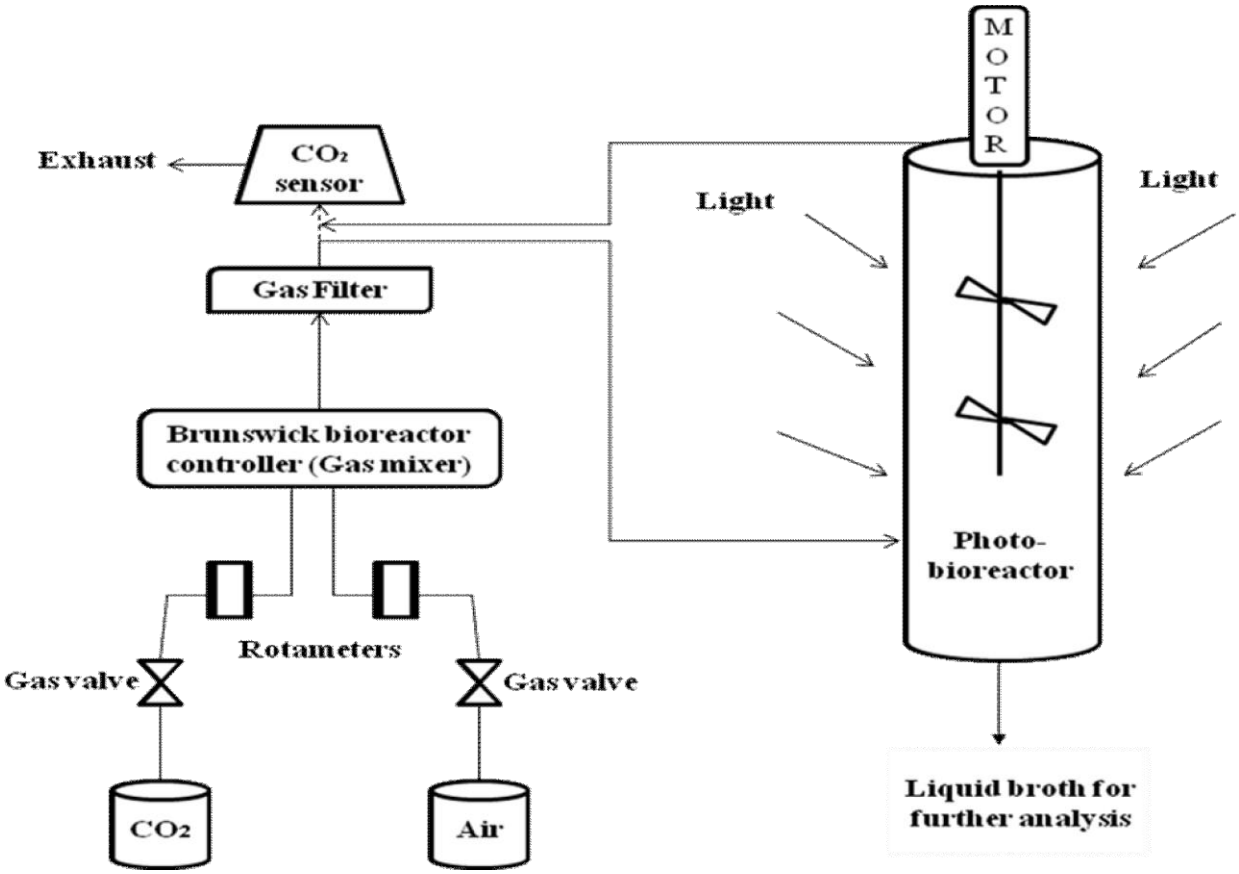

Figure 3.1(a) Schematic diagram for CO_2 fixation experimental set up

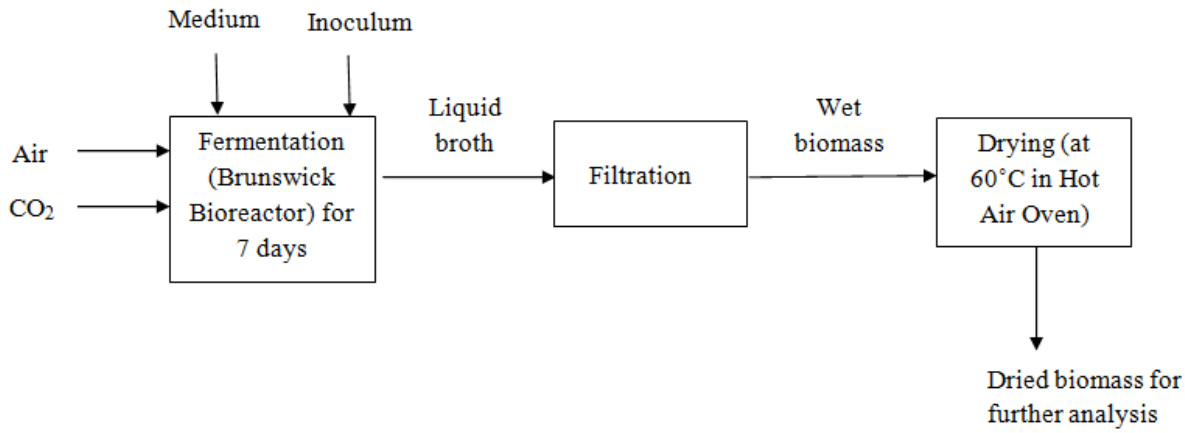

Figure 3.1(b) Process flow diagram of CO_2 fixation experiment

CHAPTER 4

RESULTS AND DISCUSSION

4.1 pH optimization

Any variation in pH significantly affects the growth of the algae. It affects the solubility and availability of nutrients, and transport of substrates across plasma membrane, enzyme activity and electron transport in respiration and photosynthesis. Thus, pH was optimized for the microalga *C. vulgaris.*

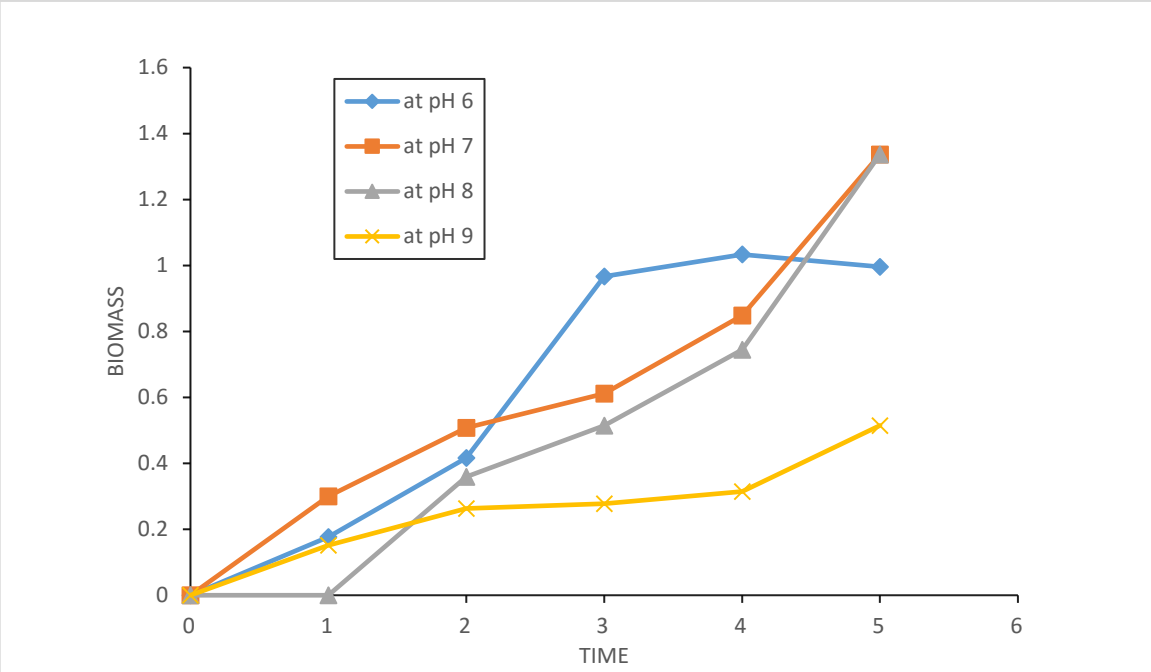

Figure 4.1 Algae growth pattern at different pH

When *chlorella vulgaris* was grown at different pH regimes, from 6-9 different growth patterns was observed. At pH 7 the best growth was found, at this the lag phase was found to be of minimum span and biomass found maximum. The organism was found to grow well between 7 and 8. So, a pH shift above 6 was not found to affect the growth negatively. Though when pH was below than 6 or greater than 8, the growth was noticeably reduced. When the culture was grown at pH 9, the growth starts ceasing after 5, whereas at 7 and 8 the growth was still increasing.

Maximum growth was found in between 6-8 pH but at 6 pH lag phase is found to be longer and at 8 growth was found to be longer and at 8 growth was found slow in comparison to 7. So, the best pH is found to be 7. Photobioreactor when the experiment was conducted the pH of media was observed around 8 and adjusted to 7 by using 1N HCL before inoculation. However. On the first day pH was

found to decrease sharply because of effect of CO_2. In photobioreactor decrease in pH was found proportional to increase in CO_2 concentration; though the pH varies in window of 5 to 7.

4.2 Dry weight calibration curve of *Chlorella vulgaris*

Biomass dry weight (g/l) is used as a measure of growth concentration. Absorbance is used in place of cell dry weight for calculating the biomass ae it is easy as compared to methods adopted for calculating dry weight. Linear regression was used for establishing relationship between optical density taken at 680 nm and cell dry weight. It was found that optical density correctly predicted the dry weight that is (R^2= 0.9916). Thus the biomass was calculated by using OD

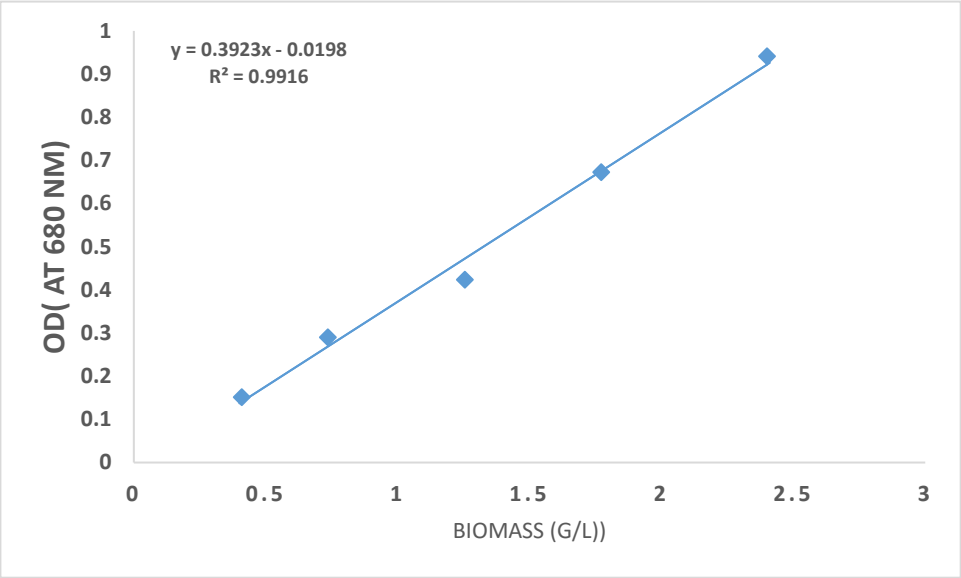

Figure 4.2 Calibration curve of optical density (OD) v/s algal biomass concentration

The value of x is biomass concentration and the value of y is optical density of the sample measured vu UV-VIS spectrophotometer.

4.3 *Chlorella vulgaris* growth kinetics

Biomass dry weight was used as the parameter for measuring growth of *Chlorella vulgaris.* It showed the maximum growth of 8.3 at the input of 10% CO_2 concentration which corresponds to 14.3g/l. of CO_2 sequestered. The graph as shown in **Figure 4.3** shows an upward trajectory till 10% CO_2

concentration and then starts falling down. So, it can be esily concluded that at 10% concentration the growth is higher,. The maximum biomass concentrations corresponds to different CO_2 concentrations o.o5%, 5%, 10%, 15%, and 20% are 0.70 g/l, 5.46g/l, 8.01 g/l, 7.30 g/l and 6.992 g/l respectively.

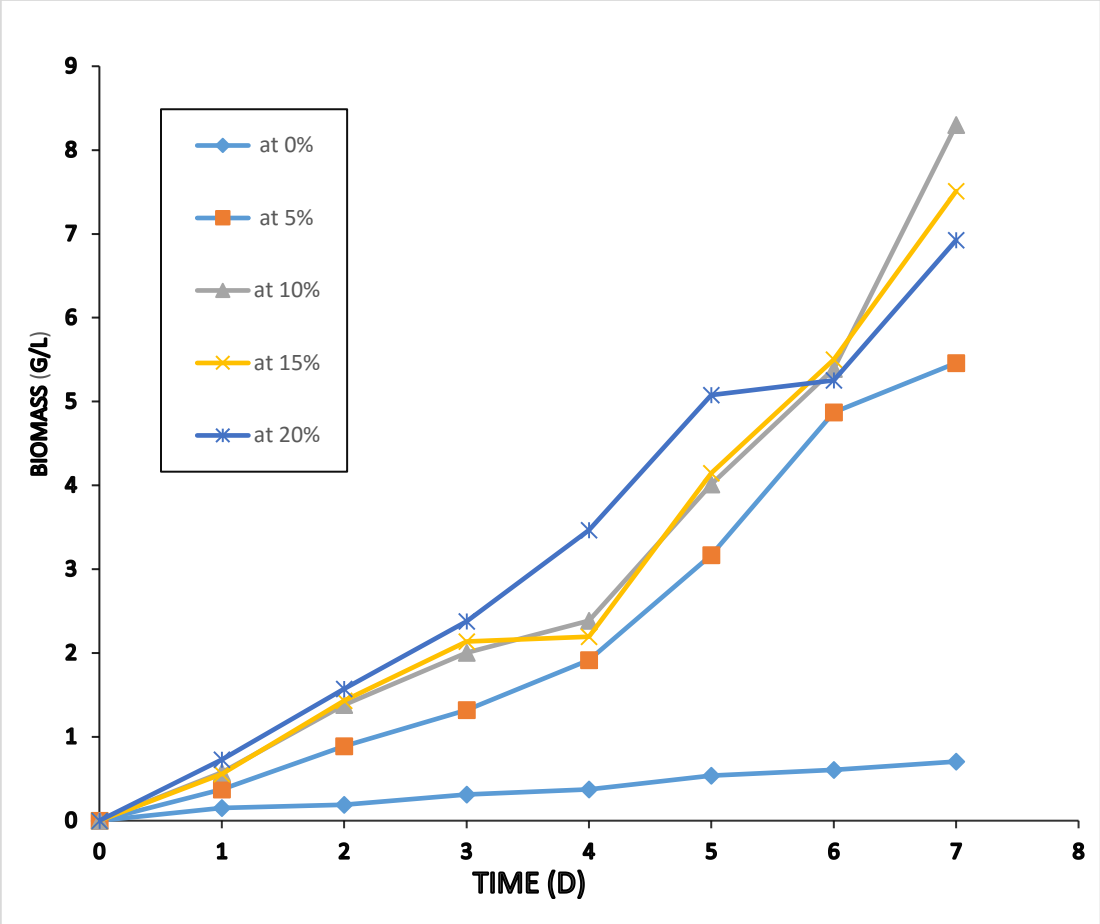

Figure 4.3 Biomass v/s time curve for microalga at different CO_2 concentrations

After 7 days the X_{max} value for chlorella was found to be 8.3g/l at 10% CO_2 concentration and the values of μ_{max}, productivity and doubling time (t_d) were calculated as 0.32, 1.101 g/l/d and 2.16 respectively. In case of autotrophs the growth is affected by depletion of growth limiting factors and the stationary phase is very unlikely to see.

4.4 Kinetic parameter studies

Kinetic parameters of *chlorella vulgaris* were calculated for different CO_2 concentrations. Specific growth rate (μ_{max}) was calculated by using biomass values at different times and exponential regression

during the logarithmic phase. The increment in CO_2 fixation rates and captured CO_2 indicates that algal cells are still functioning at an optimum level. The CO_2 concentration at which all the kinetic parameters were found to be maximum was 10%. Approximately 9-15% carbon di oxide is present in flue gas and can be utilized by the microalgae for their growth and subsequently removing CO_2 from the environment making it evident that flue gas can be directly used for the growth of microalgae.

Table 4.1 Effect of CO_2 concentration on various kinetic perimeters of *Chlorella vulgaris*

CO_2concentration (%)	**μ_{avg} (1/d)**	**Productivity (g/l/d)**	**t_d (d)**	**CO2fixation rates (g/l/d)**	**Total CO2 fixed (g/l)**
0.05	0.25	0.100	2.77	0.183	1.3
5	0.30	0.799	2.31	1.46	10.22
10	0.32	1.101	2.16	2.01	14.07
15	0.31	1.105	2.23	1.92	13.44
20	0.39	0.992	1.77	1.81	12.07

From the above figures it is clear that growth of microalgae increases with increase in CO_2 concentration, but up to 10%, after that at 15% and 20% the growth starts declining. As shown in **Table 4.1,** the maximum CO_2 fixation rates was obtained at 10% CO_2 and was calculated to be 2.01 g/l/d by Equation 3.4 and the maximum total CO_2 fixed was 14.07. With further increase in CO_2 concentration the biomass concentration starts decreasing. At 20% CO_2 concentration the μ_{avg} and doubling time were found best. The above mentioned parameters were decided carefully to enhance every positive growth

.

4.5 Biomass analysis

(i) CHNS analysis

Table 4.2 shows that till 15% CO_2 concentration, the percentage of carbon content i.e. 46% remain same but at 20% CO_2 decline in carbon content was observed. This indicates that Chlorella was not able to utilize carbon di oxide properly at higher CO_2 concentrations but capable of doing so in flue gases in which concentration of carbon di oxide is 9-15%. This may be due to non-representative biomass determination which caused lower biomass measurement at 20% CO_2 level. The nitrogen concentration remained approximately same which clearly indicates that protein does not get much affected by high CO_2 concentration.

Table 4.2 Elemental profile of algae at different CO_2 concentration

Sample	CO_2 Concentration (%)	N %	C %	H %	S %
1	5	7.30	46.39	6.912	0.530
2	10	7.01	46.99	6.725	0.500
3	15	6.39	46.40	6.733	0.562

The carbon content obtained from CHNS analysis was used in Equation 3.4 for calculation of CO_2 fixation rate F_c (g/L/d) and studied further. Depending on physiological state and nutrient limitation the nitrogen content of the microalgal cells varies between 4% and 8% **[44]** and we have observed the same from our readings that means the nutrient composition provided was best for growth of microalga. The C/N ratio also increased significantly in cultures exposed to the highest CO_2 concentrations, indicating the cycle focuses more on carbohydrate production than on protein up to 15% CO_2 concentration but C/N ratio significantly decreased at 20% CO_2. S% is found to be less

which is best for growth as it gets converted in to sulphide which is poisonous for microalga as its higher amount decreases pH drastically. Other investigators also established that 1 unit of biomass produced consumes 1.83 units of CO_2 **[41].** We have also used this in our studies and also calculated carbon content fixed in algae by CHNS analysis which supports the fact that it is around 50%.

4.6 Predicting Biomass Productivity Behaviour

Logistic equation as given below was used to determine the growth kinetics of alga which was predicted with the help of OriginPro 8.0 using curve fitting tool.

$$X = \frac{X_{\max}}{1 + \left(\frac{X_{\max}}{X_0} - 1\right)e^{-K_c t}}$$

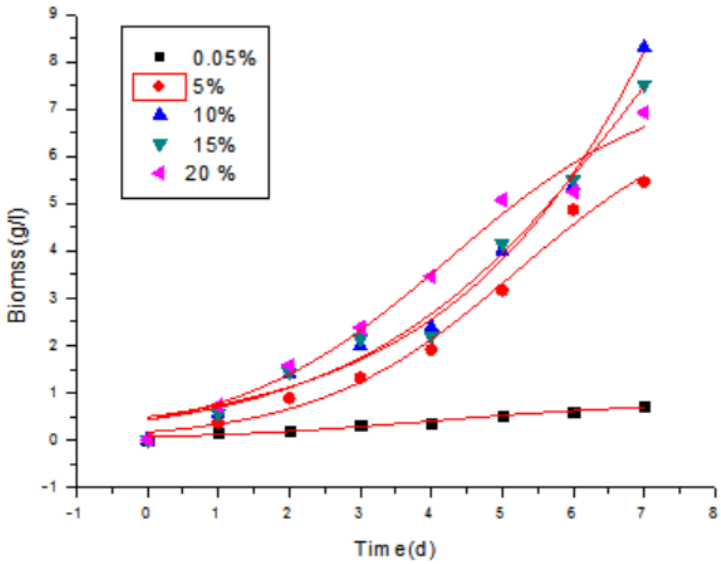

Figure 4.4 Predicted cell growth profile of algal cells at different CO_2 concentrations

Figure 4.4 shows predicted cell growth profile of *Chlorella sp.* in stirred photobioreactor at different percentage of CO_2. Experimental data is fitted with logistic equation. X_{max} and k obtained from predicted logistic model are given in **Table 4.3**. Data points were average of experimental runs, here in **Figure 4.4** line represents the predicted and symbols represent he experimental data. **Table 4.3** shows predicted values of logistic model for maximum biomass and apparent specific growth rate with their R^2 values at different CO_2 concentrations in photobioreactor.

The curve fitting was found to be in good agreement with experimental values as the R^2 values were approximately equal to 0.98 (**Table 4.4**). From the predicted curve, we can also find the values of constant k and X_{max}. The pattern of X_{max} rise and fall was found to be precisely similar to experimental values of X_{max}.

CO_2 % (v/v)	c = k (1/d)	a = X_{max} (g/L)	R^2
0.04	0.5991	0.8114	0.97428
5	0.5601	8.2467	0.98728
10	0.4219	23.5593	0.98552
15	0.4946	15.8757	0.97808
20	0.6701	7.65619	0.97420

Table 4.3 shows predicted values of logistic model

4.7 characterization of dairy waste water

Dairy waste sample was obtained from from Verka dairy plant, Sangrur, Patiala on 12 December 2015. Table 4.3 shows the various characteristics measure of sample.

Table 4.4 characteristics of dairy waste water sample

Parameters	
pH	6.68
Turbidity	995 NTU
BOD	2100mg/l
COD	3150mg/l
TDS	3045 mg/l

TSS	455 mg/l
TS	3500 mg/l
Nitrite	294.4 µg/l

Biochemical oxygen demand (BOD) is used as a measure of the quantity of oxygen required for oxidation of biodegradable organic matter present in water sample by aerobic biological action. It is an important consideration in the design of the treatment facilities . COD is the chemical oxygen demand is used as a means of measuring the organic strength of domestic and industrial wastes. In conjugation with the BOD test, the COD test is helpful in indicating toxic conditions and the presence of biologically resistant organic compounds **[45]**.

According to KSPCB standards the permissible values of B.O.D and C.O.D of untreated effluents ranges between 50-1900 mg/l and 50-6000 mg/l respectively and the BOD and COD values of the sample is 2100mg/l and 3100mg/l. TDS is the total dissolved solids present in a sample, or we can say that the materials that are completely dissolved in water and filterable in nature and sometime can be referred to material which are not dissolved in water and non-filterable. TDS may lead to scaling in boilers, corrosion and degraded quality of the product. As per KSPCB the acceptable range of TSS and TDS is 200-720 mg/l and 275-1120mg/l and the sample has very high TDS but TSS is in normal range. The pH of the sample is 6.68, which is best for microalgae growth as microalgae grows netter in neutral pH. Nitrite are toxic if present in larger concentration in water, however for some aquatic organism even a concentration of 1µg/l is toxic.

4.8 Growth Studies in Dairy Wastewater

As the mode of algal cultivation influences the economy of whole process, the present study is focused more on this decisive step. For this purpose, culture of *Chlorella vulgaris* was grown in dairy wastewater for 5 days at 27°C in orbital shaker in shake flask to study the effect of wastewater on growth of microalga. It can be observed from **Figure 4.7** that alga grew at much higher rate in wastewater than in regular medium.

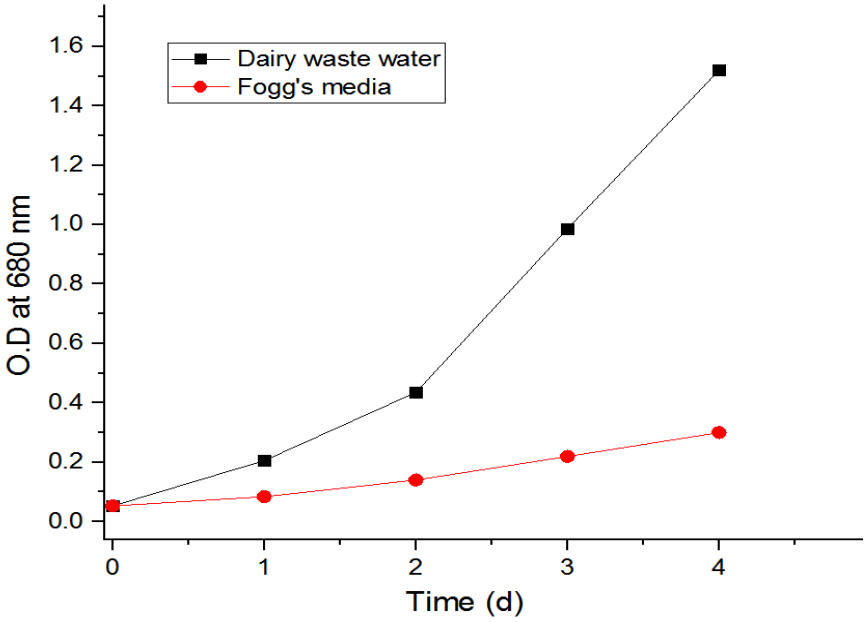

Figure 4.5 Growth profile of microalga in dairy wastewater and synthetic media

From the above graph it can be stated that in comparison to Fogg's media, dairy waste water is better source for growth of algae.

BOD and COD

Table 4.4 shows that the values of BOD and COD of the waste water samples declines after inoculating it with *chlorella vulgaris.* The organic compound present in the waste water sample is utilised by the algae along with atmospheric CO_2. Thus, it can be concluded that using waste water as a medium for algae culturing is not only cost-effective but also beneficial in terms of treatment of waste water

Wastewater sample	BOD(mg/l)	COD (mg/l)
Without filtration	2100	3100
After filtration and autoclaving	1800	2600
After 5 days of inoculation	101	135
Control (Fogg.s media)	39	51

Table 4.5 COD and BOD values after inoculation with *Chlorella vulgaris*

Using it in place of control medium results in lowering the cost of algal production as well as much higher growth.

CHAPTER 5

CONCLUSIONS AND RECCOMENDATIONS

5.1 Conclusions

As the reaction proceeds the CO_2 fixation rates and captured CO_2 amounts continue to rise which indicates that algal cells are still functioning at an optimal level at higher concentrations of CO_2. 10% is the CO_2 concentration at which all the kinetic parameters as well as total CO_2 fixed and fixation rate are found to be maximum. The conclusions obtained from our studies can be summarized as follows

- *Chlorella vulgaris* grows best at pH 7.
- Maximum average specific growth rate is 0.39 (1/d) at 20% CO_2 concentration.
- Maximum carbon fixation rates and CO_2 fixed of *Chlorella vulgaris* are 2.07g /L/day and14.07g/L respectively, at 10% CO_2 concentration.
- The curve fitting was found to be in good agreement with experimental values as the R^2 values of biomass fitted (at all CO_2 concentrations) were approximately equal to 0.98.
- It was observed that dairy wastewater was the most scalable and economic medium for alga cultivation

Recommendations

1. As *Chlorella* was found tolerant to high CO_2 concentrations (15 - 20%), it can be directly used to sequester the CO_2 of flue gases in which CO_2 concentration is 9-15%.
    - Instead of using commercial CO_2 gas cylinder, we can directly use flue gases as a source of CO_2 which will decrease the cost of separation of CO_2.

2. Additionally, instead of using Fogg's media we can use dairy wastewater for growth and maintenance of microalga which will be best for commercial purpose such as
    - Decreasing the cost of media employed for maintenance of microalga.
    - Decreasing the BOD and COD of dairy wastewater and will help in wastewater treatment which we have observed after performing the experiments (replacing media with dairy wastewater).

3. Photobioreactor used had fixed S/V ratio. Bringing change in the dimensions may enhance its performance.

4. In the current research, effect of pH and CO_2 concentrations were studied. However, factors like illumination, media, temperature etc. are also need to be optimized for further improvement of the process.

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