CO_2 CAPTURE AND GROWTH KINETICS STUDY OF *CHLORELLA VULGARIS* AND *NANNOCHLOROPSIS OCULATA* IN BATCH AND SERIES PHOTO-BIOREACTORS

BY

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To my parents, family and friends

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"In the name of Allah, The Most Merciful and The Most Gracious"

All praise and thanks to Almighty, Allah, the Creator of all worlds for giving me the courage to accomplish this work sincerely and successfully. May there be every peace and blessings upon the holy prophet Muhammad (PBUH), his family and his companions.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

MBR	:	Membrane Bioreactor
SWW	:	Synthetic wastewater
C.V	:	Chlorella Vulgaris
N.O	:	*Nannochloropsis Oculata*
LED	:	Light Emitting Diode
L-D-C	:	Light Dark Cycles
CCS	:	Carbon Capture and Storage
GHG	:	Green House Gases
PBRs	:	Photobioreactors
OCD	:	Optimal Cell Density
PPP	:	Pentose Phosphate Path
EMP	:	Embdenn Meyerhoff Path
BOD	:	Biological Oxygen Demand
BBM	:	Bolds Bessel Media
COD	:	Chemical Oxygen Demand
OD	:	Optical Density
X_m	:	Biomass Produced
F/2	:	Microalgae Cultivation Media

ABSTRACT

Full Name : [Muhammad Ilyas]

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CO_2 capture by microalgae cultivation is a promising technology to address the greenhouse gas emission. The microalgae can also contribute to wastewater treatment as they consume various minerals present in the wastewater. The cultivated biomass is one of the promising sources of the renewable fuels/biofuels. Thus, the microalgae culture can contribute to CO_2 fixation, wastewater treatment and as an energy resource.

In this work *Chlorella vulgaris* and *Nannochloropsis Oculata* have been investigated as promising microalgae species for CO_2 capture and wastewater treatment using laboratory scale photobioreactors. The experimental results show that pH (CO_2/air ratio) is the main controlling parameter for CO_2 capture and microalgae growth. In an F/2 media the maximum CO_2 fixation rates are found to be 0.087 g L^{-1} d^{-1} and 0.086 g L^{-1} d^{-1} while in synthetic wasterwater, CO_2 fixation rates are 0.11 $gL^{-1}d^{-1}$ and 0.098 $gL^{-1}d^{-1}$ for *Chlorella vulgaris and Nannochloropsis Oculata*, respectively. In synthetic wastewater the maximum biomass produced is 1.66 gL^{-1} which is almost the double as compared to the commercial F/2 media. These results indicates that the mixtrophic cultivation of microalgae is promising approach for CO_2 capture integrated with with wastewater treatment.

ملخص الرسالة

الاسم الكامل: محمد الياس

عنوان الرسالة: التقاط CO_2 والنمو حركية دراسة *Chlorella vulgaris* و*Nannochloropsis Oculata* في دفعة وسلسلة صور-المفاعلات الحيوية

التخصص: الهندسة الكيميائية

تاريخ الدرجة العلمية: أبريل 2014

يعتبر احتجاز غاز ثاني أكسيد الكربون بزراعة الطحالب الدقيقة تقنية واعدة لمعالجة انبعاث الغازات الدفيئة. الطحالب الدقيقة ايضا يمكنها ان تساهم في معالجة مياه الصرف الصحي حيث انها قادره على استهلاك الاملاح المختلفه الموجوده في مياه الصرف الصحي.و كذلك الكتلة الحيوية الناتجة هي مصادر واعده لانتاج الوقود/الوقود الحيوي المتجدد. اذا فإن زراعة الطحالب الدقيقة تساهم في احتجاز غاز ثاني أكسيد الكربون, معالجة مياه الصرف الصحي وكذلك كمصدر للطاقه.

في هذا البحث نوع الطحالب الدقيقة *Chlorella vulgaris* و *Nannochloropsis Oculata* تم التحقيق في كونها فعاله في احتجاز غاز ثاني أكسيد الكربون و معالجة مياه الصرف الصحي وذلك باستخدام مفاعلات ضوئية. النتائج المتحصل عليها تفيد بان الوسط الهايدروجيني (نسبة ثاني أكسيد الكربون \ الهواء) هي العامل الاساسي المتحكم في احتجاز غاز ثاني أكسيد الكربون ونمو الطحالب الدقيقة. في بيئة النمو F/2 اعلى نسبة لاحتجاز غاز ثاني أكسيد الكربون هي 0.087 و 0.086 جرام\ لتر\يوم اما في بيئة مياه صرف صحي مصطنعة فهي 0.11 و 0.098 جرام\ لتر\يوم لنوع الطحالب الدقيقة *Chlorella vulgaris* و *Nannochloropsis Oculata* على التوالي. في بيئة مياه صرف صحي المصطنعة اعلى انتاجية للكتلة الحيوية هي 1.66 جرام\ لتر وهي ضعف الكمية المتحصل عليها عند استخدام في بيئة النمو F/2. هذه النتائج تشير بان الزراعة مختلطة التغذية للطحالب الدقيقة هي طريقه واعدة لاحتجاز غاز ثاني أكسيد الكربون مع معالجة مياه الصرف الصحي.

CHAPTER 1

INTRODUCTION

1.1 Motivation

The aim of present research is to address two important global environmental issues, i: capture and fixation of anthropogenic CO_2 mostly produced by burning process of fossil fuels which is major part of greenhouse gases causing global warming, ii: production of sustainable environmental friendly biofuels. Particularly this research study focuses on the biological CO_2 capture and growth kinetics study of microalgae cultivation to utilize maximum CO_2 to produce biomass. The proposed technology uses the concept of photosynthesis for consuming CO_2, nutrients and water in the presence of sunlight to produce biomass using photobioreactors. This technology has potential to integrate CO_2 capture with secondary and tertiary wastewater treatment – source of nutrients– to produce biomass which is further converted to fuel and nonfuel application. The objective of this chapter is to introduce with this potential technology from environmental, renewable and economic prospective to present the motivation.

1.2 Background

As CO_2 is a major environmental concern worldwide lot of research is being done for the mitigation purpose with different techniques and technologies. The fossil fuel rich countries of Middle East especially Saudi Arabia are using this fuel for power plants and transportation purposes. In these days there is major concern to reduce CO_2 produced by power plants and other fossil fuel sources in Saudi Arabia. Biological CO_2 capture is the potential technology being explored worldwide which comprise plant photosynthesis process and conversion of the produced biomass to fuels, with results of no net CO_2 production as a whole.

Management of even a small fraction of CO_2 by biological cycle would make a major contribution to fixation of greenhouse gas. A novel approach for mitigating CO_2 by direct biological process could be made practical by using flue gases from point source, nutrient rich wastewater and sunlight could be used for cultivation of photosynthetic organisms. The biomass produced can be transformed to biofuels, food products and biochemical which can provide revenue for mitigation process. In this research different types of microalgae species are studied with varying CO_2 ratios for CO_2 fixation and growth kinetics studies in details with respect to Saudi Arabian environment and conditions.

Since the usage of fossil fuels has become essential part of our daily life needs. Specifically, the burning of fossil fuel for the production of energy and electrical generation purpose, which has played important role in the improvement and development of standards of living and quality of life . However, fossil fuels are the sources of energy which are limited and will end up one day. In addition, burning of

fossil fuels has caused many problems to environment including the emissions of greenhouse gases especially CO_2 which is major cause of global warming (Lam & Lee, 2012).

As due to the increase of global warming issue, which can be mainly due to the increased CO_2 level in the atmosphere, the United Nations signed the Kyoto Protocol (1997) with the objective to reduce greenhouse gases emission by 5.2% on the basis of year 1990, and more than 170 countries of the world have accepted the protocol. Various CO_2 mitigation techniques have been investigated, which can be normally categorized into two major groups as (i) chemical reaction-based approaches and (ii) biological CO_2 mitigation method (Stephens et al., 2010).

The chemical reaction-based fixation of CO_2 typically consists of three techniques of separation, transportation and finally sequestration of CO_2. The separation cost of CO_2 and compressing to 110 bars (for the transportation purpose) is estimated to be $30 to 50 per ton of CO_2, and transportation and sequestration are expected to cost about $1to 3 per ton per 100 kg and $1–3 per ton of CO_2 respectively (Gupta & Fan, 2002). As these techniques are comparatively costly and more energy-consuming so it is so essential to develop the cost-effective and appropriate alternatives method.

Biological CO_2 capture is more smart as an alternate because it leads to manufacture of biomass energy as derivative in the process of fixation of CO_2 through photosynthesis (Huang et al., 2010). The research being done on CO_2 removal process by microalgae mainly focus in two major areas of the flue gas (with 10–20% CO_2) and air in a atmosphere (generally around 1.0% CO_2) However, much consideration is required on

the following two main factors relating the maximizing the efficiency of CO_2 removal in the bio-regenerative systems.

Now a days conventional type of fossil fuels are facing many global challenge which have lead scientists to discover alternative renewable fuel production from biological sources (Demirbas et al., 2009). The microalgae based renewable fuels are gaining quick attention as it has great potential to replace the petroleum-based fuels (Bhola et al., 2011).

The main attention has been focused on the lowering the cost of production of biofuel, emissions of GHG, land requirements and lowering of water resource needs, with the improvement in compatibility of fuel delivery systems and vehicle engines as well (Demirbas, 2011; Balat and Balat, 2010). In last few years much attention has been focused on the investigation of potential biomass production sources. Based on these results biomass has been recognized as viable feedstock based on input required and GHG emissions in their production (Singh and Gu, 2010). In recent years the potentials of using microalgae as a source of biodiesel and biogas for different energy applications has been explored (Singh et al, 2011)

Microalgae are eukaryotic photosynthetic microorganisms, which are used to produce highly valuable compounds (Spolaore et al, 2006). Marine microalgae are the fastest growing organism, that are less than 2mm in diameter floating in the upper 200 M of the ocean where sunlight is available for photosynthesis. Microalgae are cell factories driven by sunlight that convert CO_2 into renewable potential biofuels, foods additives, feeds and large number of high value bioactive metabolites (Ramanathan, 2011). In recent times, production of fuel from microalgae has been getting substantial attention because of the

world growing energy prices, emissions of the GHG and continuing reduction of fossil fuels (Xiong et al, 2010; Johnson and Wen, 2010). Microalgae are considered as ideal source of biofuel production due to their fast biomass production capability, higher photosynthetic efficiency and their ability to accumulate a large amount of lipid in biomass (Rasoul et al, 2011).

Microalgae are the rapidly growing photosynthetic unicellular organisms which converts solar energy source into chemical energy through the process of carbon dioxide fixation (Mata et al, 2010). Microalgae have greater photon of light conversion efficiency compared to other terrestrial plants. Photoautotrophic mode of microalgae culturing is the most common way of cultivation, which uses light source as the main source of energy and CO_2 as inorganic carbon source(Mohsenpour and Willoughby, 2013) and (Huang et al., 2010).

Microalgae are the microorganisms that may convert solar into chemical energy with the efficiency of 10–50 times higher than any other terrestrial plant (Khan et al., 2009). During the microalgae photosynthesis process, microalgae consumed CO_2 from the air as carbon source to grow cells and replicate. Microalgae cells comprise of approximately 50% carbon of their weight, in which around 1.8 kg of CO_2 are fixed by production of 1 kg of microalgae biomass(Chisti, 2007a).

Generally, microalgae have three culturing modes phototrophic, heterotrophic and Mixotrophic. Phototrophic mechanism uses light as source of energy and CO_2 as inorganic source of carbon, while heterotrophic process is independent of light source and utilizes only organic carbon substrate (e.g. glucose, acetate) as the energy and carbon

source (Mata et al., 2010). For mixotrophic mode of culture, microalgae are able to grow by phototrophic or heterotrophic or both mechanism, depending on the application source of organic carbon and light intensity (Lam and Lee, 2012).

Up to now phototrophic mode was technically and economically more viable to culture microalgae in the commercial scale process, usually at outdoor culturing where sunlight is abundant and free of cost (Borowitzka, 1999). In addition to that, phototrophic microalgae are capable to capture CO_2 from industrial flue gases and acts as a larger carbon sink which is added advantage to the current culture system.

Saudi Arabia has a climate that is well suited for growing algae, with high levels of sunlight, favorable temperatures and large areas that can be used for algae cultivation without negative impacts on biodiversity (Campbell and Batten, 2010). This combined with the fact that there are plentiful sources of CO_2 emissions, based on the amount of energy produced through natural gas and others means that water access and nutrition are the remaining factors required for large scale algae production for carbon sequestration. Using wastewater as a growth medium provides multiple advantages, such as the removal of nitrates, phosphates and even some heavy metals from the wastewater whilst providing nutrients for the algae (Mata et al., 2010) and (de-Bashan and Bashan, 2010).

1.3 Research objectives

This research is focused on the development of an algae based CO_2 capture technology combined with wastewater treatment. The cultivated microalgae (biomass) can be further processed to produce biofuels and/or syngas. Towards the end, *Chlorella vulgaris* and *Nannochloropsis Oculata*, two promising microalgae species, have been cultivated in laboratory scale photobioreactors to demonstrate their CO_2 capture proficiency and growth kinetics. The following are the specific objective of this research:

i. To study the effects of pH at different CO_2/Air ratio on the growth of the microalgae species (*Chlorella vulgaris* and *Nannochloropsis Oculata*).
ii. To investigate the growth kinetics of *Chlorella vulgaris* & *Nannochloropsis Oculata* in different culture media at various compositions.
iii. To study the bio-fixation rate of CO_2 at different CO_2/Air ratios and the culture media.
iv. To determine the nutrients uptake from the culture media, i.e., the destruction/consumption of nitrate, ammonia, and phosphate that is present in wastewater.

CHAPTER 2

LITERATURE REVIEW

2.1 Carbon dioxide capture and storage

A transition into sustainable and CO_2 neutral systems requires new technology both to put an end to our dependency on fossil fuels, and to manage and mitigate current CO_2 emissions. Although cleaner production opportunities should always be a first hand choice, energy supply will continue to be heavily fossil loaded, and there are at present technologies in use, and in development, that can mitigate and remediate CO_2 emissions. In order to avoid further irreparable environmental damage, sequestration of carbon from the atmosphere and from industries is essential.

Facing the problem of global warming, people all over the world are making efforts to find the proper solutions. To prevent the further huge amount of carbon dioxide generated from the combustion of fossil fuel emitting into the atmosphere, people are trying to take actions to capture carbon dioxide from the large point sources. These large CO_2 point sources could be the fossil fuel, natural gas burning, others synthetic fuel plants and hydrogen production plants base on fossil fuel.

Research focused on effective, efficient and cleaner ways of CO_2 reduction is needed along with cleaner fuel production. Figure 1 gives the brief summary of current technologies being explored for CO_2 mitigation purpose, which can be divided in to three groups, each group is reviewed in detail in the following section briefly.

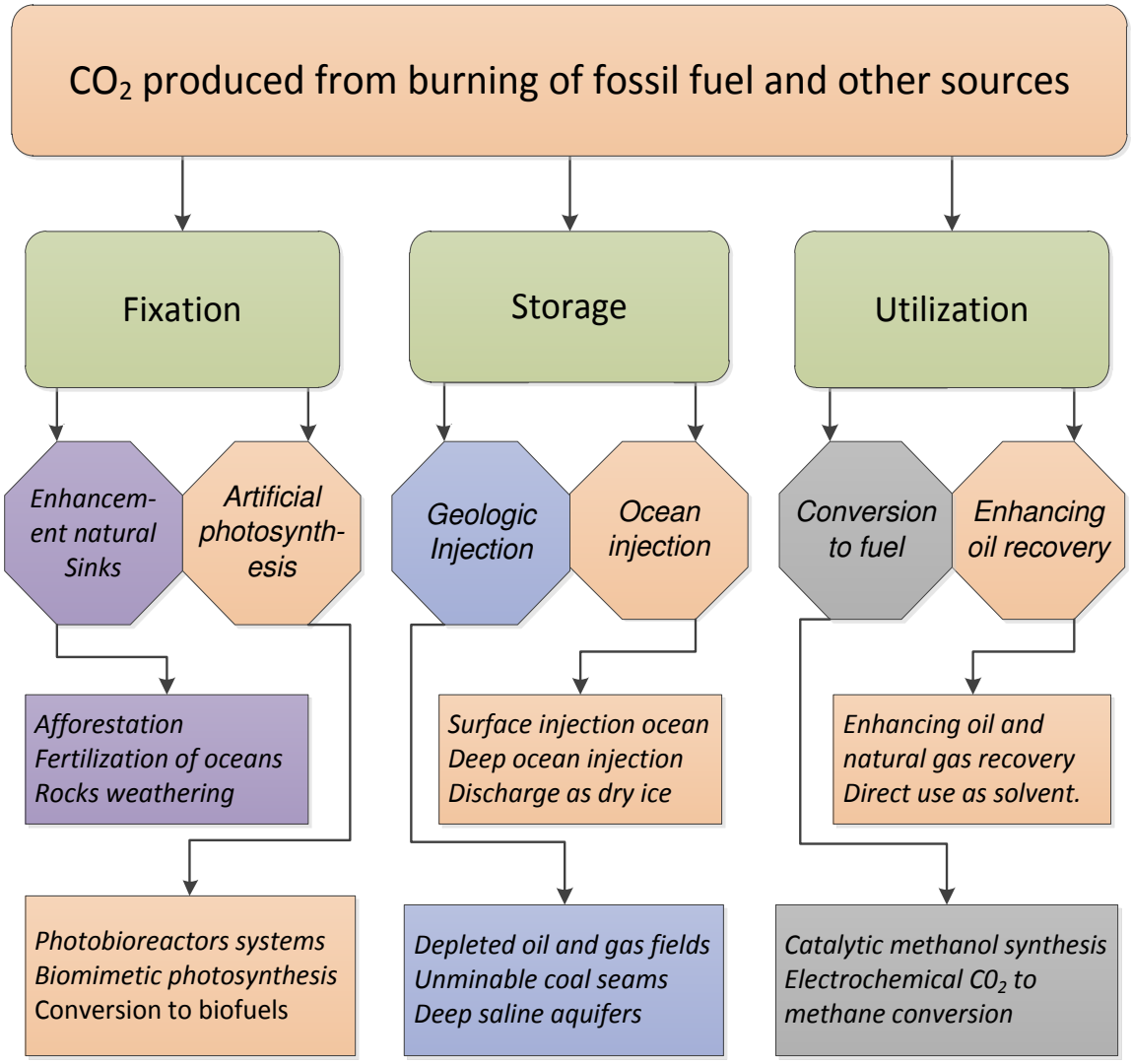


Figure 1: CO_2 managing existing technologies worldwide.

For the carbon capture and storage CCS process, it mainly contains three ways to capture the CO_2:

Post combustion capture: the CO_2 capture will happen after the fossil fuel combustion, it mainly capture the CO_2 from the flue gas. It is can be applied for the fired power plant and waste incineration plant.

Pre-combustion capture process: this method often be used in the gasification and fermentation process, the CO_2 emitted from the gasifier and fermentor can be captured. It can be applied in the H_2, CH_4 and fertilizer production process.

Oxy-fuel combustion capture: in the process, the fuel is burned in the combustion chamber with oxygen instead of air. As a result, the flus gas generated from this process mainly contain CO_2 and water steam, with the water condensing in the latter process, we can get nearly pure CO_2 stream.

Fixation of CO_2 is a biochemical process, where CO_2 through photosynthesis process is stored in a stable organic form. For this research project from the review we have selected the microalgae based CO_2 capture process based on the following potential key advantages.

2.2 Key potentials of microalgae based process

With the arising of global warming and GHG emission issue, algae are also studied to capture the CO_2. The potential ability of microalgae is positive, through the related theoretic calculations; the result is that per kilogram microalgae could capture nearly 1.83

kg CO_2. (Brennan and Owende, 2010a) The reasons for using microalgae to capture carbon dioxide are demonstrated as below:

2.2.1 Masters of photosynthesis

The microalgae have much higher growth rate than the most land-based plant due their higher photosynthesis efficiency. Algae have much shorter growth cycle, the weight double time is about three to five days (Chang et al., 2011), and some species can have two harvest seasons in one day. The algae yield weight per year is nearly several times or even hundred times of food crop yield. In conclusion, the algae absorb more CO_2 in a shorter time.

2.2.2 High biomass productivity

Microalgae have higher photosynthetic efficiency and growth rates. Many microalgae exist in single cell form or cluster a few cells. Their simple cellular structures usually facilitate microalgae to grow faster and store more lipid or starch at excessive energy. Microalgae have several metabolic activity pathways and they are exchangeable under different conditions of nutrient and light source situations.

Microalgae photobioreactors could be building up in different horizontal and vertical directions to utilize maximum CO_2 and nutrients and even some of fast growing microalgae species could be harvested on daily basis. All these factors contribute to higher biomass production per area per unit time (Pandey, 2011).

2.2.3 Higher CO_2 uptake

In comparison to other conventional agricultural crops, microalgae utilize much higher doses of CO_2 present in the industrial flue gases, which is indication that they have the higher potential to reduce the emission of greenhouse gases. It is great interest of researchers to explore if the effect of higher-dose CO_2 alteration could further improve photosynthesis efficiency and thus biomass as well (Pandey, 2011).

2.2.4 No occupation of agricultural land

The algae can be cultivated in the water condition; there will be no land competition between agricultural crops and algae. In Saudi Arabia, the natural sea can be good cultivation base for the algae. It is especially important, the fresh water resource is very limited in Saudi Arabia(Wenying et al., 2009)

2.2.5 On site solution

Most of the fire power plants in Saudi Arabia are established along the coast line, these algae cultivation sites can be built near these power plants to reduce the cost of flue gas transportation. And this system does not need to make big technical changes for the power plants.

2.2.6 Additional revenue generation

The harvest algal biomass can be made into different byproducts. The most important product from the algae is the biodiesel, and also the biomass can be processed into healthy food, animal feedstock, biogas and fertilizer. By selling these products can give

extra profit to support this system, and this contributes to attract investments and participation to impulse this process.

2.2.7 Higher safety of process

The whole process is much more like a natural process, no toxic substances, no radiation, no pollutants are supposed to be released during the whole process (Sydney et al., 2010) and the whole system can work under the normal pressure; there is no potential risk for the workers.

2.3 Microalgae wastewater treatment potential

It has been suggested that CO_2 capture, biofuel production coupled with wastewater treatment is the area with most viable commercial application which could be achieved in short term. This process provides the mechanism for wastewater treatment in term of removal of toxic chemicals, heavy metals, pathogens from wastewater and organic contaminants from wastewater during the process of CO_2 capture and biomass production (Brennan and Owende, 2009).

Microalgae have the capability to grow in environments rich of nutrients and collect different nutrients and heavy metals from wastewater (Bashan et al., 2010) and (Mallick, 2002) . This makes heterotrophic cultivation of microalgae one of the viable options for lipid biosynthesis. Algae-based process of biodiesel production is considered both economically as well as environmentally viable when wastewater is used as substrate (Brune et al., 2009 ; Chisti, 2007b; Stephens et al., 2010; Prathima and Venkata, 2012).

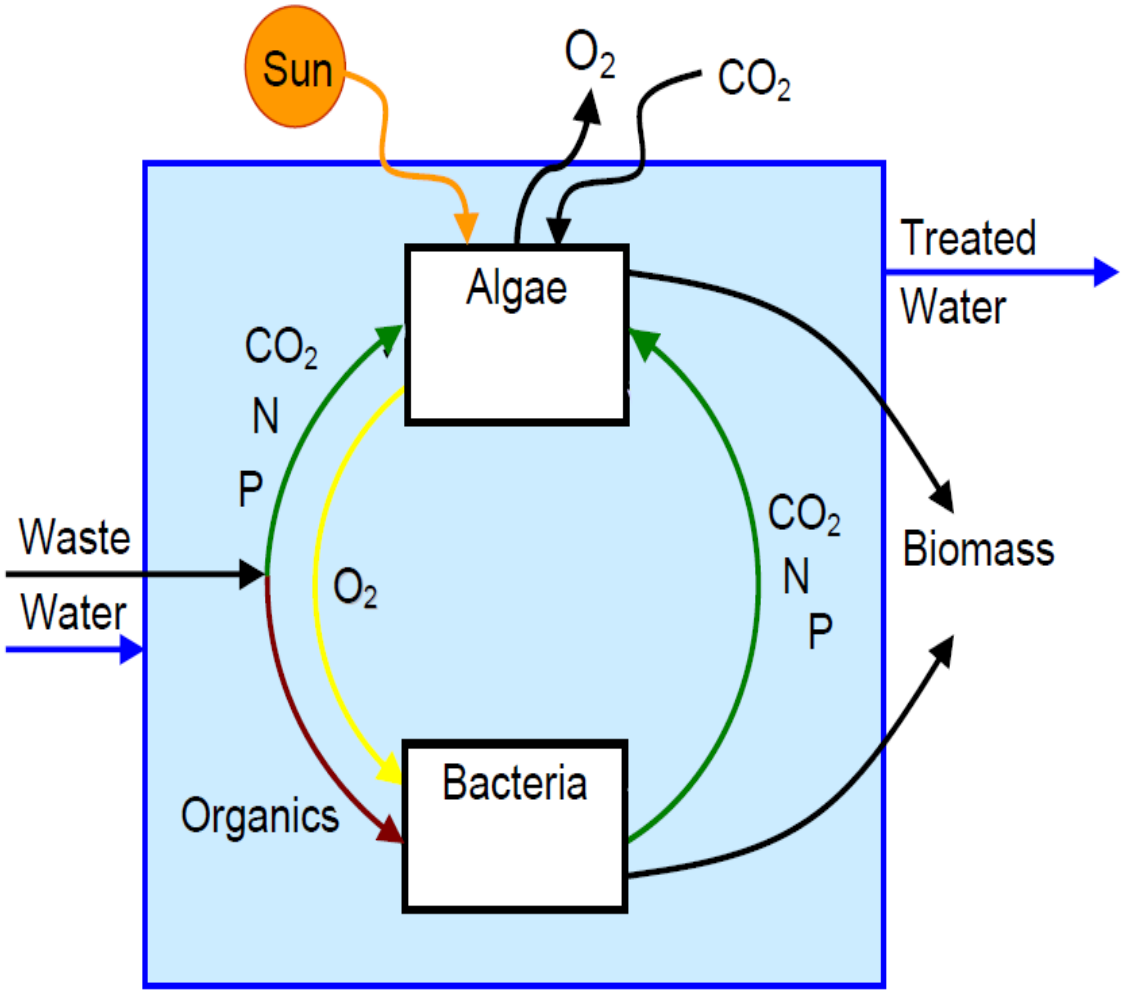


Figure 2: Algae-bacteria combined symbiosis in wastewater treatment (Lundquist et al., 2007)

Use of algae as a biocatalyst was generally documented for wastewater treatment in conventional oxidation ponds, raceway ponds, and suspended algal ponds to remove high concentrations of nutrients, especially for polishing purposes.

Microalgae are the microscopic photosynthetic organisms found in aquatic environment. The idea for the use of microalgae for the purpose of wastewater treatment formerly established in the 1950s in California by William Oswald (Oswald, 1957). In this ideal the microalgae role was to consume nutrients in wastewater by providing oxygen to bacteria. Mainly bacteria were involved the degradation process of organic contaminants. The complete process description is shown in Figure 2

Algae-based treatment systems are efficient in removing nutrients from wastewater compared to chemical-based treatments (Bux, 2013; Pandey et al., 2014; Bux, 2013) and are environmentally amenable and provide efficient recycling of nutrients (Wilkie and Mulbry, 2002). Usually Chlorella sp. and Scenedesmus sp. are predominantly observed in the oxidation ponds (Bhatnagar et al., 2010). Especially for industrial wastewater treatment, the algae-based remediation process was used as a tertiary unit operation for the removal process of heavy metal and organic toxins rather than nutrients (Ahluwalia and Goyal, 2007). Microalgae culturing process with wastewater treatment is a potential field for environmental sustainability and carbon neutrality.

2.4 Microalgae cultivation systems

The purpose of the culture system is to provide an environment for the algae culture to grow in. There are a number of different ways to do this. In general, culture systems can be divided into two types open and closed systems depending on whether the culture

grown is open to the surrounding environment, such as in lakes, or enclosed in a photobioreactors (Borowitzka, 1999a). Microalgae can grow well in open ponds systems or closed reactors. The production process in open ponds systems depends primarily on the local climatic conditions due to the trouble of control of conditions in this type of bioreactors is not preferable.

The contamination is a big drawback of this algae cultivation system (Silva et al., 2013). So high biomass productivity in open ponds are achieved with those microalgae strains which resist to harsh culture environment conditions (Harun et al., 2010; Lee, 2001). Besides the technology is simple but the production in open ponds systems is not economical due to the higher cost of downstream processing. While choosing the microalgae cultivation system following parameters should be considered:

i. Biological nature of microalgae species
ii. The cost of land and energy
iii. Nutrients and water for cultivation
iv. Local environment and climate conditions
v. Desired purpose of cultivation

Table 1 gives the comparison between open pond system and closed photo-bioreactors regarding the production of microalgae (Carvalho et al., 2006).

Table 1: Comparison of microalgae production in open and closed reactor system (Carvalho et al., 2006)

Factor	Open reactor system	Closed reactor system
Space requirement	High	Low
Evaporation rate	High	No evaporation
Water losses	Very high	Low
CO_2-losses	Higher	Lower
Temperature	Variable	Cooling is required
Weather dependency	High	Low
Process-control	Difficult	Easy
Shear stresses	Lower	Higher
Cleaning	No need	Required
Contamination	Higher	None
Quality of biomass	Variable	Reproducible
Harvesting efficiency	Lower	Higher
Harvesting cost	Higher	Lower
Light-efficiency	Poor	Good
Expensive parameters	Mixing	temperature control
Capital investment	Low	High

2.4.1 Open ponds

According to (Borowitzka, 1999) four most commonly used commercial cultivation techniques which are described as: large scale open ponds systems, circular ponds systems with mixing elements, raceway ponds culturing and large bags culture systems and closed photo bioreactor.

Open ponds systems are mostly designed in similar way to raceway ponds by providing proper mixing tools in the form of paddle wheels and baffles. Figure 3 illustrates examples of raceway ponds system in schematic way. Raceway ponds are constructed in such a way that they are shallow (between 10 to 50 cm deep, to keep the proper illumination of light and economically low energy utilizing paddlewheels for circulation and mixing purpose. The culture is exposed to open atmosphere which helps in regulation of temperature by evaporation process and these systems are normally used for production of microalgae biomass and cyanobacteria on commercial scale(Jorquera et al., 2010). Pound depth is optimized by keeping in mind the provision of enough light and deep enough to provide proper mixing and prevention of evaporation (Kunjapur and Eldridge, 2010a).

As for open ponds is concerned they are cheapest system for microalgae cultivation than closed system (Chisti, 2008) but they have some serious technical issues in addition to economic drawbacks. One of the major issue of open ponds systems is the presence of contaminants and difficulty to maintain monoculture of desired species (Silva et al., 2013). According to research evaluation done by (Lee, 2001) and (Borowitzka, 1999), the best commercially effective species that grow in open ponds are resistant in harsh environment which adverse the competition . As example Dunaliella, Spirulina and

Chlorella are those species which grow in high saline water, more alkaline and nutrient rich water respectively. Another major issue towards success of open pond system is the evaporative loss of water (Brennan and Owende, 2010b). However, at the major drawback of open pond systems is in the insufficient control mechanism of necessary design factors for optimum growth of microalgae culture (Kunjapur and Eldridge, 2010a).

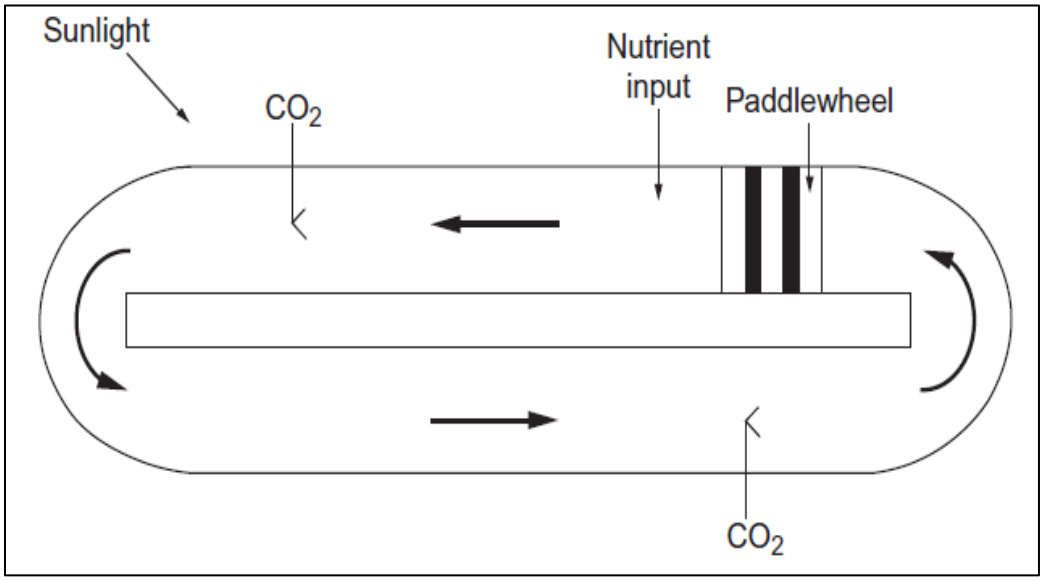


Figure 3: Schematic diagram of a raceway pond (Kunjapur and Eldridge, 2010a)

According to (Xu et al., 2009) open-air farming systems mainly consist of natural and synthetic ponds, raceway type ponds systems and inclined outward systems which are driven through paddle wheels. These types of systems symbolize the old microalgae biomass production processes. As different types of open culturing reactors have been used but most commonly used are open shallow ponds, raceway systems and circular ponds. Open type of culture system are directly exposed to sunlight, easier to design and operation than close systems. Over the past fifty year study of open systems a number of challenges and drawbacks have been found (Xu et al., 2009; Pushparaj et al., 1997) which are:

i. Limited to few species of microalgae growth successfully
ii. Presence of predators species is a major drawback
iii. Evaporation loss cause water supply issue
iv. Efficient CO_2 utilization is also major issue
v. Large area requirement can only make use of waste land
vi. Lower biomass productivity compared to closed process
vii. Harvesting cost of microalgae is very high

Even though different struggles to develop open ponds systems with better control of temperature, improved nutrients supply, optimizing pond depth, better CO_2 sparging systems etc. But its productivity is still remains very low in comparison to closed system (Borowitzka, 1999). Due to these drawbacks and limitations the main focus has now shifted towards development of more effective closed photobioreactors which are discussed below in detail.

2.4.2 Closed reactor system

From the literature it's found that open pound cultivation systems has reached its maximum productivity which could not be enhanced economically so many authors suggested the need of close reactor system for large scale future production of biomass.as the cost will be reduced with time and closed reactors will become most popular microalgae culturing systems.

Many designs of closed photo-bioreactors systems have already been done in order to achieve better growth control and operating parameters. The three core classifications most commonly appropriate for larger scale cultivation systems includes horizontal tubular reactors, vertical column reactors and flat plate or panel (FP) type photo-bioreactors (Kunjapur and Eldridge, 2010b).

One of the most substantial issues related with the production of microalgae biomass through photobioreactors is the requirement of power to run system in maintaining culturing conditions within the required parameters of mixing, nutrients supply, oxygen removal and CO_2 supply (Hulatt and Thomas, 2011). However making comparison to open pond systems, closed photobioreactors have higher photosynthesis efficiency, biomass production capability and biomass purity as well shown in Table.1. In the last three decades large number of different photobioreactors has been investigated for culturing process. In this literature review we will briefly describe their advancements in this field.

Table 2: Some of prospects and limits of culture systems for microalgae (Ugwu et al., 2008)

Culture systems	Prospects	Limitations
Open ponds systems	i. relatively more economical ii. easier to clean before cultivation iii. good for farming of algae species having tolerance to harsh environment	i. less control of culture environments ii. hard in growing long times & poorer productivity iii. occupy large space iv. limited to only few strains &easily polluted
Vertical-column photo bioreactors	i. greater mass transfer ii. better mixing and lower shear iii. lower energy intake iv. higher capacities scalability v. good for immobilization vi. lower photo inhibition and photo-oxidation	i. small illuminated area ii. design involve the refined materials iii. produce shear stress to cells iv. further decrease in lighting area upon scale-up process
Flat-plate photo bioreactors	i. large illuminated area ii. suitable for outside culturing iii. suitable for immobilization iv. better light path & high biomass productivity v. comparatively cheaper & easier to clean vi. lower oxygen build-up	i. scale-up requires supporting materials ii. difficult temperature control iii. wall growth occurs iv. possibility of offering shear stress to some microalgae strains
Tubular photo bioreactors	i. larger lighting surface area ii. suitable for open-air cultures systems iii. better biomass productivities iv. comparatively cheaper	v. pH, dissolved oxygen and CO_2 gradients exists vi. fouling factor and wall growth is involved vii. requires higher land area

2.4.3 Closed photobioreactors

A photo bioreactor is close culturing illuminated vessel, specially designed for the purpose of organized biomass production system. Photo bioreactor is a system which is enclosed to the environment and cannot directly exchange contaminants and gases with environment. Photo-bioreactors have majors advantages over open culture systems (Richmond, 2004) which includes:

i. They minimize contamination by growing only one selected species..
ii. Offer good control over bio-cultural conditions such as pH, intensity of light, carbon dioxide, and temperature of culture.
iii. They can prevent better the water evaporation loss which is main issue with open ponds.
iv. They can much reduce CO_2 losses due to at exhaust.
v. Concentrations of cells in growth are higher in these reactors.
vi. They allow the producing complexes like biopharmaceuticals in the knockout mosses in the GMP conditions and as a biotechnology known as molecular farming.

The efficiency of a photo-bioreactor depends on the integration of capture, transport, distribution, and use of light by the microalgae through photosynthesis (Zijffers et al., 2008). Closed photo-bioreactors are highly efficient at bio-fixation of CO_2, mainly due to enhanced homogeneity of the medium and the mass transfer. However, these reactors are limited by the excess O_2 produced (Chang et al., 2011).The main feature of the photo-bioreactor that influences the exposure of microalgae to light is the surface to volume

ratio. Some of materials used for construction of reactors are glass, Plexiglas, polyvinyl chloride, acrylic-PVC, fiberglass and polyethylene (Wang et al, 2012). Each type of material should be analyzed prior to be used for specific application.

The overall process mainly focuses on uses of microalgae process for industrial waste CO_2 capture in photo bioreactors, coupled with treatment of the nutrient rich wastewater. Mainly CO_2 is transformed into algae biomass by the method of photosynthesis in the incidence of light source in photo bioreactor. After processing one of the biomass treatment (physical, biological or thermochemical) we transform biomass into green fuels like methane, biodiesel, food products and others by products (Kumar et al., 2010a).

Based on these advantages of closed reactor system especially with respect to environmental condition of Saudi Arabia closed photobioreactors system is more suitable for cultivation. In this research we will use closed photobioreactors for our proposed experiments.

2.5 Commonly used photobioreactors types

There are many types of photobioreactors used for microalgae cultivation process. Here with the objective of CO_2 capture we will discuss the most commonly used reactors types in reference to efficient CO_2 fixation, biomass productivity, wastewater treatment potential and efficient utilization of nutrients and light source.

2.5.1 Membrane photobioreactors

Membrane photo-bioreactor is a term that is used to describe a membrane contactor that is integrated with a photo-bioreactor to enhance CO_2 mitigation by microalgae (Li-Hai et al., 2008). Different ranges of biomass production and CO_2 fixation are originate in the literature showing the many factors that may affect the biomass production and CO_2 reduction by culture. The extent of CO_2 fixation and biomass production are noted to be higher and easier in bubble column reactors.

Still, quite a lot of issues needs to be discussed for membrane type bioreactors for micro algal production such as membrane strength and efficiency and bio-fouling factors during prolonged operation of the reactor (Kumar et al., 2010a). Gas to liquid transfer of mass is the key factor of photo-bioreactors, and the major challenge is the design of reactor higher productivity of biomass.

The poor transfer of mass may increase the risk of CO_2 stripping in the photo-bioreactor which results in lower growth of culture. Which is due to the fact that higher photosynthetic rate will produce more dissolved oxygen. The dissolved oxygen accumulate in enough amount to obstruct the growth of microalgae (Hoekema et al., 2002).

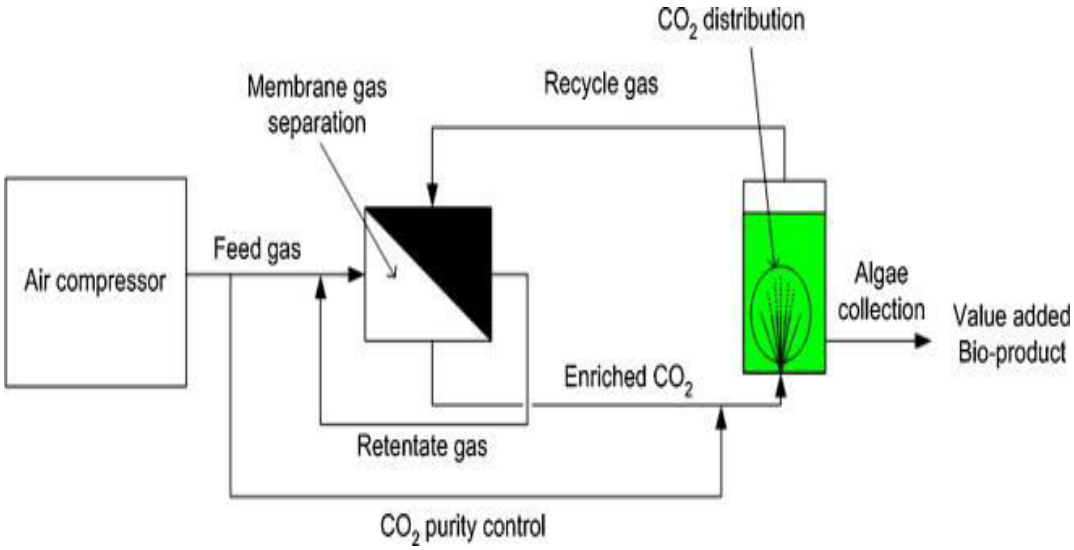


Figure 4: Schematic of the gas membrane type separation combined photo-bioreactor on CO_2 enrichment process (Cheng et al., 2011)

The basic feature of a membrane-integrated photo-bioreactor for CO_2 utilization by microalgae culture consists of CO_2 supply set, a membrane set, and photo-bioreactor system. The CO_2 supply set consists of an air compressor, gas mixer, gas filter (optional), and a CO_2 tank. The membrane set consists of a peristaltic pump and one or more membrane contactor modules. The photo-bioreactor system consists of an illumination source and a closed culture vessel (bioreactor) to culture microalgae (L.-Hai et al., 2007).The basic idea of membrane reactor for CO_2 mitigation is graphically explained as in Figure 5.

The membrane-integrated photo-bioreactor serves two major roles in biofuel production. The first role is to increase the mass transfer of exchange CO_2 and O_2 gases in the photo-bioreactor and the other is to enhance the photosynthetic rate of microalgae, thereby increasing microalgae productivity. Although it has been proven that a membrane contactor can increase the mass transfer rate in the gas exchange process in a photo-bioreactor, the issue involving pressure drop due to fouling of the pores of the membrane has become a major challenge to the use of the membrane photo-bioreactor.

2.5.2 Tubular photobioreactors

Among the projected photo-bioreactors, tubular photo-bioreactor is one of the most appropriate outdoor type mass cultures reactors. Most outdoor type tubular photo-bioreactors constructed with glass or plastic material having re-circulation system either driven by motor or airlifts systems (Ugwu et al., 2008; Tredici and Zittelli, 1998). Fully enclosed type tubular photo-bioreactors are possibly more suitable for large scale production outdoor culturing of microalgae (Molina et al., 2001).

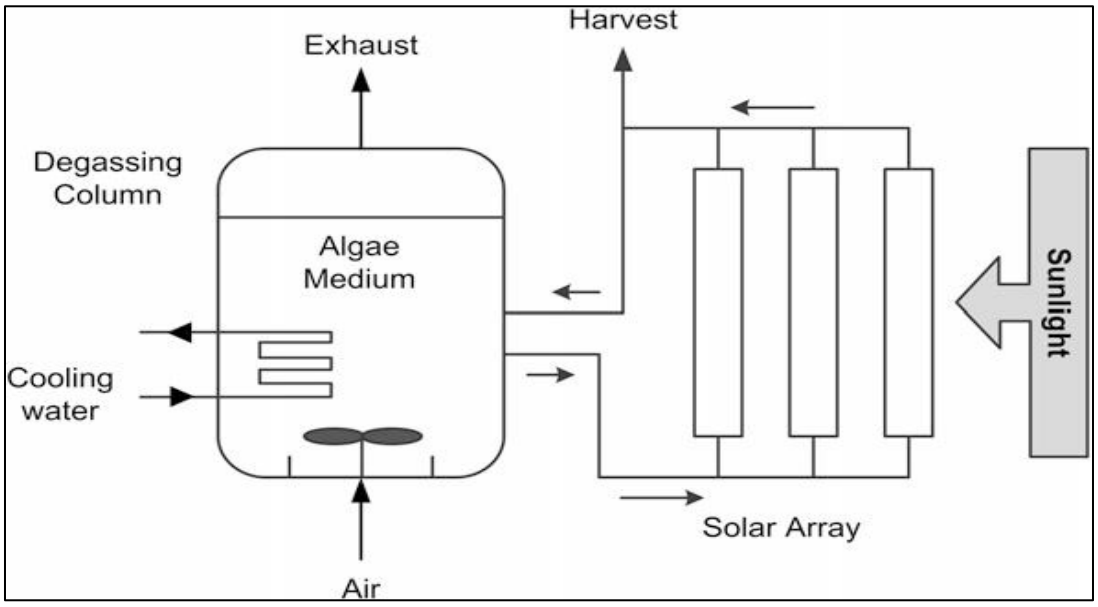


Figure 5: A tubular photo-bioreactor with parallel-run horizontal tubes (Wilcox, 2012)

Tubular photo-bioreactors comprise of straight, long coiled or looped translucent tubing prepared in several ways to make best use of sunlight utilization. Phototrophic modes of cultures are circulated using the tubes by different methods; airlift used for circulation is particularly common. Well-designed tubular photo-bioreactors fully separate the culture from possibly contaminating outdoor environments, by allowing prolonged duration mono-algae culture (Garc and Rubio, 2000). photo-inhibition is very common issue in outdoor tubular photo-bioreactors (Vonshak and Torzillo, 2004).

Bubble-column PBRs have been widely used in the bioprocessing reactions, wastewater treatment purpose, and in chemical processes industry (Xu et al., 2009). Generally, bubble-column reactors are vertical and compact – requiring less land, relatively cheap, and easy to operate. The high mass transfer coefficient and extremely low physical stress obtained in these reactors make them suitable for microalgae culture, especially for aquaculture (Mirón, 2000; Xu et al., 2009).

Productivity of microalgae biomass in tubular photo-bioreactors mainly depends on type of algal species, location of reactor, biomass concentration, diameter and distance of tubes and the number of horizontal tubes per unit stack in vertically stacked type systems (Slegers et al. 2013). Microalgae are distributed through pump, tubes or airlift system to reactor system with continuous agitation.

Use of the airlift scheme deals some advantages, as CO_2 and O_2 exchange between the rising gas phase and liquid, possible cell damage related with power-driven pumping system may be decreased and rotation is accomplished without any mechanically moving system (Xu et al., 2009).

In some cases the temperature of the tube-shaped photo bioreactor is better organized by fluctuating or immersing the tubes in the pool of water (Pulz et al., 1998).

Moreover, elongated tubular photo-bioreactors are characterized mass transfer of CO_2 and dissolved oxygen gradients (Fernandez et al., 1999; Ugwu et al., 2003). These reactor systems are categorized into two classes of vertical and horizontal system.

2.5.3 Vertical tubular photobioreactors

The two major categories of vertical column type photo-bioreactors air-lift type and bubble-column they have wide range of applications in bio-processes, gas-liquid contacting , chemical industry and wastewater treatment due to their better gas liquid hydrodynamics and mass transfer applications (Mirón, 2000).

This type of reactors is made by vertical transparent tubes to allow the illumination. Sparger of reactor is close to the bottom which transforms the sparged gas into small bubbles phase. Sparging gas combination provides complete mixing, mass transference of CO_2 and also do away with O_2 produced during the process of photosynthesis.

Vertical type tubular photo bioreactors may be further divided into two main types of bubble column reactors and airlift reactor system based on the liquid flow systems (Kumar et a, 2011a). Microalgae is harvested through immobilized technology where it makes small beads which settle downs and are separated (Moreno, 2008).

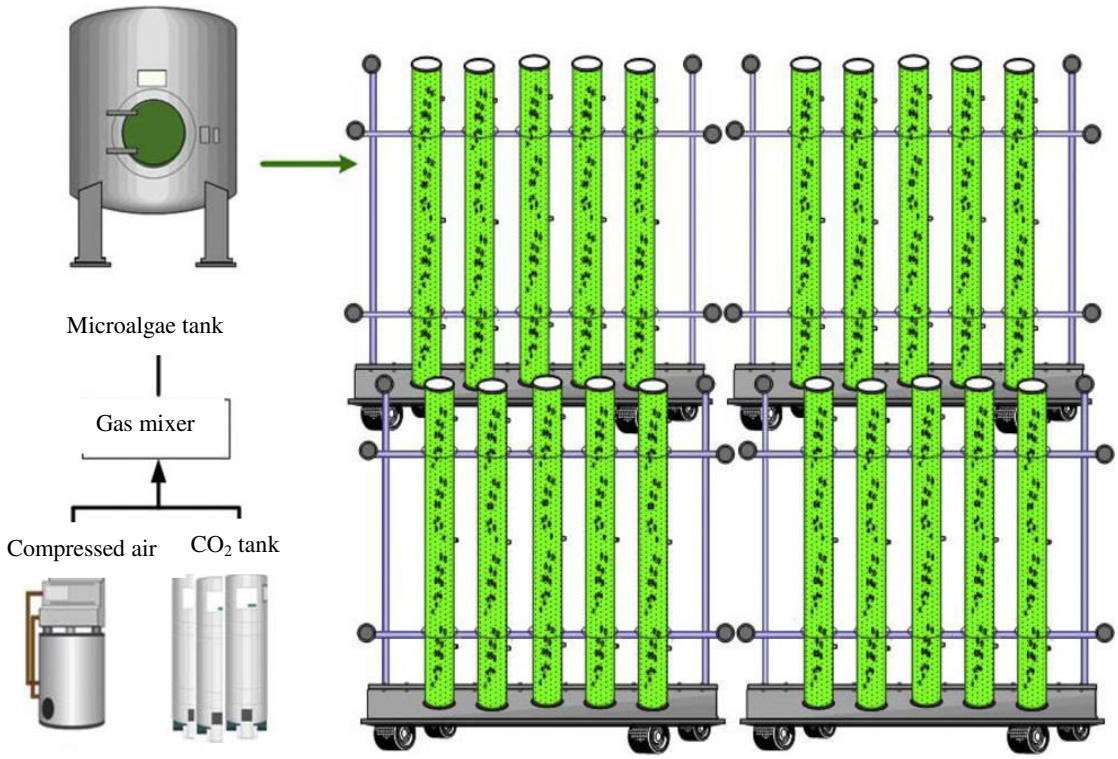


Figure 6: Vertical-column photo-bioreactors for microalgae cultivation (Pandey et al., 2014)

2.5.4 Horizontal tubular photobioreactors

Horizontal type tubular reactors are positioned horizontally giving the enterprise of parallel tubes bundles their shape ,inclination or horizontal shape as shown in Figure 7. Its shape gives benefit in outdoor culture for their alignment to sunlight bring about in high light exchange efficiency of the reactor (Singh and Sharma, 2012).

The most significant characteristic of this tubular system that is different from the vertical column bioreactor is the improvement of air-residence time inside the tubular bioreactor, which can provide more dissolved CO_2. These arrangements could use artificial light, but they are also designed based on natural light (sunlight) provided from outside of the tube (Briassoulis et al., 2010). Tubular photo-bioreactors that have air lift system for circulation of culture is becoming popular due to many reasons: culture circulation is done without using any moving device which promote a concentrated possible for contamination (Molina et al., 2001).

The operational difficulties are similar to other systems, including growth of microalgae on the wall of the tubes, thus blocking the light penetration; high concentration of oxygen that may hinder photosynthesis; and may affect the limits on length of the reactor tube in a single run (Briassoulis et al., 2010). The scaling up of these reactors is relatively easy compared with other photo-bioreactor designs. The increase of tubular photo-bioreactor working volume can easily be achieved by simply extending the tube length to the designed volume if the air pump can affordably provide enough power to pump in air bubbles (Chisti et al., 1999).

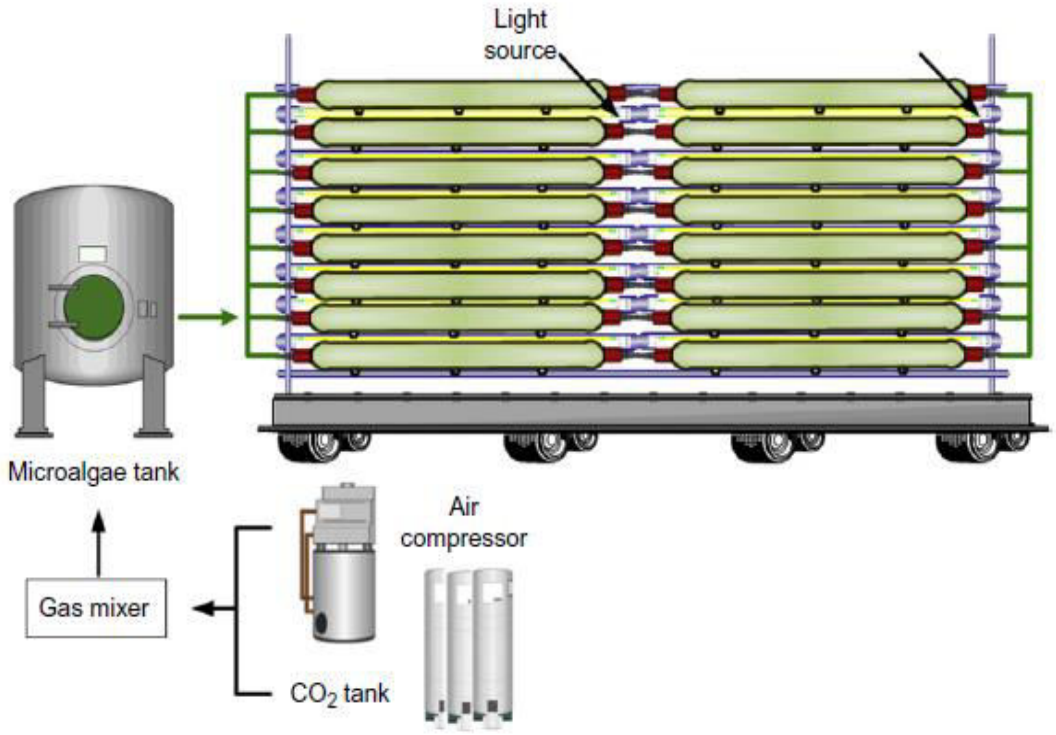


Figure 7: Horizontal tubular photo-bioreactor adapted from (Singh et al., 2012)

2.5.5 Stirred tank photobioreactors

Stirred tank type reactor is conventional where mixing is provide using impellers and vertex are reduced using baffles (Figure 8) (Muñoz et al., 2004) that produces the turbulence for mixing (O., 2001). For the culture process of microalgae, CO_2 mixed with air is bubbled at the bottom of the reactor using sparging system (Ogbonna et al., 1996).

These kind of photobioreactors has been modified into better form of photo bioreactor by providing external illumination in the form of lamps or optical fibers, still they have major problem of small area to volume ratio (Franco and Havel, 2006). Large disengagement space is required for the separation process of unused gases and oxygen produced during process which increases the cost and space requirement (Singh & Sharma, 2012)

Based on these advantages of closed reactor system especially with respect to environmental condition of Saudi Arabia closed photobioreactors system is more suitable for cultivation. In this research we will use closed photobioreactors for our proposed experiments. More specifically based on multiple advantages as described in tubular section we have chosen vertical tubular photobioreactors for our process.

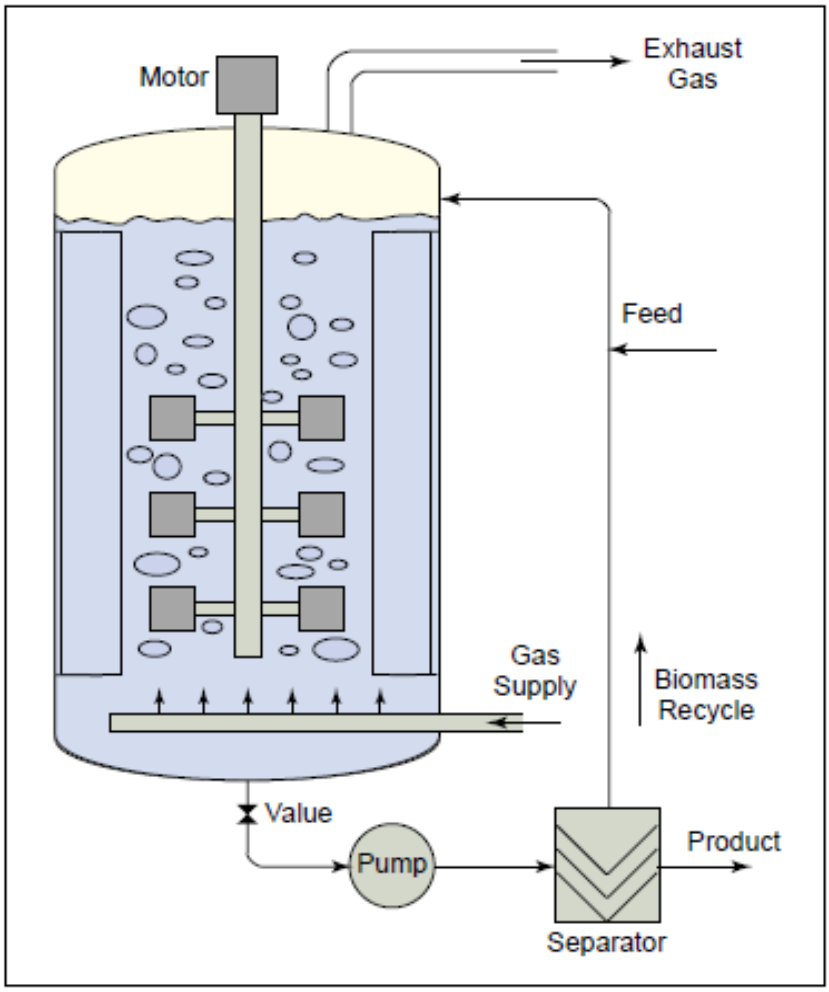


Figure 8: Stirred tank photo-bioreactors uses agitator and baffles system for proper mixing and recycling of biomass (Williams, 2002)

CHAPTER 3

CO_2 CAPTURE PROCESS VARIABLS

3.1 Factors affecting the growth and CO_2 fixation process

Photo bioreactor advancement is possibly, one of the most important steps which could be efficient way of microalgae cultivation. For maximizing the production of large capacity there are major challenges in design to be faced, which are maximum solar light utilization, less land occupancy, high surface area exposed to light and efficient mass transfer for high productivity of biomass (Singh & Sharma, 2012). For CO_2 sequestration higher mass transfer is the major requirement of bioreactor design. CO_2 mass transfer resistance is increased in mass transfer from gas phase to cells in liquid phase (Kumar et al., 2011a). An economically feasible photo bioreactor may be designed by considering the subsequent features: (Vasumathi et al., 2012).

i. Provision of higher surface area to volume ratio.

ii. Stabilizing the absorbed concentration of CO_2.

iii. Sufficient time provision for light photon absorption.

iv. Sustaining optimal concentration and highest growth rate.

v. Keeping up the required level of nutrients.

Despite the large number of photobioreactors developments, only few of which are practically used for mass cultivation of microalgae utilizing solar light efficiently. For the development of efficient culture system photobioreactors design is the major step to be considered. Photobioreactors are used to maximize the solar energy utilization having large surface to volume ratio to achieve higher biomass productivity (Vasumathi et al., 2012). For the efficient design of photobioreactors following points to be considered (Wang et al., 2012):

i. Cultivation of different species in same reactor universally

ii. Fast mass transfer should exist between CO_2 and O_2 and efficient utilization of light

iii. Adhesion of microalgae of microalgae cells on the surface of reactor walls is very common which inhibit the necessary light transfer to cells, should be considered.

iv. Prevention the fouling of reactor materials to a minimum value by efficient design

v. Without damage of produced cells higher mass transfer achievements and biomass production

vi. Reactor should be design in such a way that it should work under foaming conditions which is frequent in bioreactors.

vii. Design should be in such a way that minimum parts of reactors be non-illuminating for higher light utilization.

Some of the main parameters influencing the reactor design consideration at most are described along with their effects and merits in Table 3.

Table 3: Key Requirements for microalgae growth in relation to PBR design

Key requirements for algal growth in relation to PBR design (Bux, 2013)			
Key requisite	Concerns if too low	Concerns if too high	Function of following factors
Light	Insufficient for photosynthesis, slow growth	Photo-inhibition, photo and oxidative damage	a. Reactor surface: volume ratio b. Geometry, orientation, and inclination c. Material and thickness of reactor walls d. Culture depth and density e. Mixing
pH	Growth is inhibited	Growth is inhibited	a. Media composition b. Input CO_2 concentration c. Microalgae specie
Temperature	Slow growth, dormancy	Cell death	a. Heat input (ambient temperature, solar radiation, angle to sun, shading) b. Heat dissipation (evaporation, airflow, heating/cooling mechanisms)
Nutrient provision	Growth inhibition	Toxicity	a. Media composition b. CO_2 provision and O_2 removal (mass transfer, sparging and degassing mechanisms, gas holdup volume) c. Mixing
Mixing	Poor mass transfer and biomass	Shear stress high energy use	a. Reactor geometry, b. Mixing technique (e.g., mechanical, air flow, gravity flow)

3.1.1 Mixing

In the growth process of microalgae level of mixing is the major contributing factor. Proper mechanism of mixing provide efficient mixing of culture and equal light distribution to all the cells (Kumar et al., 2011b). mixing effect may increase productivity up to 40% (Ugwu et al., 2002; Bosca et al., 1991).mixing is the key influential factor towards the efficient growth of microalgae culture process (Yr et al., 2003).

Microalgae growth rate is mainly affected by mixing in two ways. Firstly mixing mechanism improves the exposure of sunlight to the cells in efficient way by improving the productivity and mass transfer effects between food source and cells (Qiang Richmond, 1996). Secondly it helps the equal distribution of light in the culture by providing equal opportunities to all the cells (Richmond, 2004). Due to the effects of mixing mass transfer resistance will be reduced by efficient mixing and higher contact area(Vasumathi et al., 2012).

Mixing of culture is necessary for the prevention of sedimentation of cells culture, improving exchange of mass transfer between liquid and cells, uniform distribution of light to all the cells in culture (Wang et al., 2012). Depending on the selection growth method and capacity, mixing could be done by mechanical method, using pumping mechanism and aeration as well or combination of any of these may be used. For provision of mixing mechanism it should be kept in mind that all microalgae species may not tolerate higher mixing (Merchuk et al., 2005).

Higher mixing of culture may cause destruction of cells by inducing shear stress , so mixing level and mechanism should be designed carefully by optimization approach

(Garc et al, 2000). It is found that the destruction is caused mainly by bubble formation and liquid mixing liquid velocity and gas velocity should be optimized. As rising and bursting of bubbles is proven the cause of cell damage (Barbosa et al, 2004). The gas bubbling velocity should be kept minimum than critical values either by increasing the sparging nozzle diameter or by increasing the number of nozzles to reduce the shear stress on cells (Barbosa et al., 2004).

3.1.2 pH effects

The pH values of cultures affect the biochemical processes associated with microalgae, including the bioavailability of CO_2 for photosynthesis and use of the medium nutrients. The optimum pH is determined according to the type of microorganism. Some species have an optimum pH of around 7.0; however, some microalgae are tolerant to high pH (Spirulina, pH 11.0) or low pH (Chlorococcum, pH 4.0) (Kumar et al., 2010b).

The optimum growth of the microorganism in an acidic or basic environment can be maintained if the intracellular pH is 7.5, regardless of the external pH. Living cells have the ability, within certain limits, to maintain internal pH by expelling hydrogen ions. The external pH generally has a drastic change before it affects the cell. The optimum pH of the cultures should be maintained, thereby preventing the collapse of cell cultures by the cellular process of rupture due to high pH. The control of pH must be integrated with the aeration system by the addition of alkaline solution to the culture (Wang et al., 2012).

Some microalgae have high productivity when maintained at an alkaline pH between 10 and 11. The high pH may be beneficial for outdoor cultivation because it inactivates pathogenic microorganisms and other microalgae (Kumar et al., 2010b).

The optimal pH for most cultivated microalgae species is between 7 and 9 (Ho et al., 2011). The pH of the culture medium normally affects the biochemical reaction characteristics of microalgae. Meanwhile, the feeding of CO_2 obviously affects the culture pH as well as microalgae growth. When the CO_2 from the gas phase (molecular CO_2) is transferred in to the culture medium, some of the CO_2 gas will dissolve and become soluble phase (HCO_3) and the conversion of CO_2 to HCO_3 is greatly dependent on the pH value in the culture. The HCO_3 is then utilized by microalgae (Miller et al., 1990).

(Wang et al., 2007) reported that growth of C. marina continued unaffected in the ordinary range of pH value (pH 7.5 to 8.5), whereas a substantial reduction in microalgae progress was experimental when pH was increased outside 9.0 (Liu et al., 2007). Belkin and Boussiba found that a cyanobacteria *Spirulina platensis* exhibited optimal growth at pH 9.0 to 10.0 (Schenk et al., 2008). Apparently the suitable pH range for the growth of microalgae and cyanobacteria is greatly species-dependent.

In the case of cultivation with addition of CO_2, the concentration of this gas may be the dominant factor that will determine the pH of the culture system. In this situation, the CO_2 demand results from the equilibrium between the transference of CO_2 to the liquid and CO_2 intake by the cells (Wang et al., 2012). SO_x and NO_x, present in flue gas from burning coal, can also cause changes in pH, damaging microalgae cultivation. With high concentrations ofCO_2 the pH drops to 5.0, and when exposed to SO_x and NO_x this value is 2.6 (Westerhoff et al., 2010). The pH also influences the removal of ammonia and phosphorus. The high pH may increase the removal of ammonia through its volatilization and phosphorus through its precipitation (Craggs, 2005).

3.1.3 Water consumption

Microalgae can grow in wide range of water resources as compared to other plants. Research has shown that microalgae can grow in fresh, brackish, saline and even in wastewater as well (Yun et al., 1997). Microalgae species are divide into two groups, one growing in fresh water and second one that grows well in saline water (Rao et al, 2007).

Use of wastewater for microalgae cultivation has two major applications. Microalgae could be provided with inexpensive nutrients rich source and further treatment of wastewater could also be done during process (Boonchai et al, 2012). Generally wastewater effluent contain rich source of phosphorous and nitrogen which cause eutrophication in the wastewater receiving bodies. This effect may be overcome by passing wastewater through microalgae cultivation process which reduces the nitrogen and phosphorous concentrations (Aslan and Kapdan, 2006).

Water losses due to evaporation in open ponds system is the major disadvantage of open cultivation (Schenk et al., 2008). With the evaporation of water from culture the concentration of toxic accumulation and salinity increase which cause culture damage is the major problem in open ponds (Kunjapur & Eldridge, 2010a). To overcome this problem closed photo-bioreactors are recommended.

3.1.4 CO_2 requirements

From the analysis of microalgae biomass it shows that more than 50% of biomass is composed of carbon which is main nutrient for microalgae growth, in phototrophic mechanism its provided by inorganic carbon source as CO_2 (Carvalho et al., 2006). Close system photo-bioreactor requires continuous supply of soluble inorganic source of carbon for growing microalgae cells continuously.

CO_2 is the basis of inorganic carbon for photoautotrophic growth process of microalgae. This required CO_2 source is provided by bubbling CO_2 in gas mixture through the rector (Yr et al., 2003). CO_2 is the major carbon source for microalgae in auto phototrophic culture and could be the limiting factor if the CO_2 is in low concentration or there is no proper mixing. However, excess amount of CO_2 may also be inhibiting photosynthesis and detrimental to cells (Wang et al., 2012; Bhola et al., 2011).

Tolerance of microalgae cells towards input concentration of CO_2 is op to certain limits due to two main reasons. Firstly higher concentrations of CO_2 induce environmental stress on the microalgae cells which reduces cells capacity towards biological CO_2 fixation. Secondly pH of culture is decreased due to formation of higher amount of carbonate buffer as result of higher CO_2 inputs concentrations (Mazzuca et al., 2000). With the increase in concentration of CO_2 productivity of biomass is increased up to certain limits above which its value decreases (Kumar et al., 2011a) as this effect is shown in Table 2.

Table 4: CO_2 sequestration capabilities of different algal species (Rahaman et al., 2011)

Microalgae specie cultivated	CO_2 Initial inputs	CO_2 Fixation in percentage	Biomass Production (mg L^{-1} d^{-1})	Lipid productivity (mg L^{-1} d^{-1})
Aphanothece M.Nägeli	15%	~99%	540–770	~40–60
Botryococcus braunii	0.5–10%	–	40–750	40–480
Chlorella kessleri	6–18%	~4%	67–87	–
Chlorella protothecoides	2%	–	2860	417
Chlorella sp.	2–15%	16–58%	760–870	–
Chlorella vulguris	~2%	74%	30–45	15–25
Nannochloropsis Oculata	2–15%	~13–55%	490	80–150
Platymonas subcordiformis	<10%	~3%	44	–
Scenedesmus obliquus	6–18%	14–28%	~60–160	10–80
Spirulina plutensis	4%	61.5%	350	4–9
Spirulina sp.	6%	~5–9%	125–280	5–20

In aqueous environment dissolved CO_2 always exist in equilibrium with H_2CO_3, HCO_3^{-1} and CO_3^{-2} which depends upon pH and temperature. Due to fast inter-convertible reaction among them, consumption of any of inorganic carbon does not affect the equilibrium. Microalgae cells preferentially uptake HCO_3^{-1} over CO_2 despite of the fact that former is a poor source of carbon than later (Carvalho et al., 2006).

CO_2 is supplied through membrane by diffusion in sufficient amount to the microalgae culture to avoid high partial pressure of gas. CO_2 concentrations mostly aerated through culture is from 5% - 15% by volume but maximum growth of culture cells is observed around 1-5% CO_2 (Yr et al., 2003).

Flue gas is one of the main source of CO_2 for microalgae culture growth process because it helps in reduction of global warming effect and cost of biofuel production (Doucha et al, 2005). Flue gas mostly contain around 13% CO_2 emitted from coal fired and natural gas based power plants (Chisti, 2007b). on comparison of performance of growth and production in pure and flue gas CO_2 were found similar , but mass transfer efficiency of CO_2 was lower than that of pure CO_2 however the presence of NOx did not affect the growth of microorganism and (Doucha et al., 2005).

3.1.5 O_2 removal

During photosynthesis water splits which produce oxygen gas which has toxic effects on culture and photosynthetic efficiency (Sa et al., 1999). A well designed degasification system should be present to remove trapped O_2 (Kumar et al., 2011a; Sa et al., 1999). Higher concentration of oxygen and presence of light source cause damage to microalgae cells and inhibits growth(Aiba, 1982).

Generally, trapped concentrations of oxygen must be kept lower than 400% of the saturation value of air. Accumulation of photo-synthetically produced oxygen becomes a main problem in outdoor closed photo-bioreactors with high volume to area ratio (Carvalho et al., 2006). Mostly to date degasser is proposed as solution of oxygen removal constantly. In open ponds oxygen is not accumulated which is the advantage of open pond system (Richmond et al., 1993; Morita et al., 2000; Ferna et al., 2001).

In a tubular reactor microalgae culture is continuously degassed to remove oxygen by passing through airlift zone which removes trapped oxygen (Molina et al., 2001). This process of oxygen removal could also be possible by placing a gas liquid separation unit at the out let of reactor which prevents oxygen accumulation in the tubular reactor (Kunjapur and Eldridge, 2010a).

The oxygen bubbles rise up time must be at least balanced through degasser to avoid any accumulation of oxygen inside culture (Yr et al., 2003). Practically the removal of oxygen as by product may also decrease the cost of CO_2 capture process and biofuel production from microalgae as well.

3.1.6 Light–dark (L–D) cycles

As microalgae cell density rises, light penetration into liquid culture, decreases exponentially. Which affects two light zones in the photo-bioreactor: illuminated part of reactor in which photosynthesis occurs and dark portion where no light penetration occurs (Richmond, 2004; Grobbelaar, 1994). A significant feature of the optimal cell density (OCD) at this cell concentration is the small light penetration and most of the OCD is exposed at dark (Ferna et al., 2003). For a given path of light, the dark volume of

reactor is function of both light density and cell concentration as well (Ogbonna and Tanaka, 2000).

The length of the light-path greatly affects the frequency of the light/dark cycle which basically originates from the movement of cells in and out of the photic volume, in optimally stirred cultures of cells (Carvalho et al., 2011). The shorter the light path, the higher the frequency of the light/dark cycle accelerates the photosynthetic rate (Sforza et al, 2012).

At optimal cell density, cells are exposed to relatively short flashes of light followed by a relatively longer period of darkness; the higher the frequency of this light/dark cycle therefore, the more efficiently light (particularly high photon flux density) may be utilized for photosynthesis (Al-qasmi et al., 2012). Four factors played a role in determining the compensation light/dark ratio, i.e.: - the duration of the light flash, - the light acclimation state of the algae, - the immediate light history, and - the intrinsic respiratory (maintenance) characteristics of algae (Grobbelaarl et al, 1996).

Indeed, under specific laboratory conditions, reducing the light-path thereby increasing light/dark cycle frequency results in a significant rise in areal productivity (K and Lee, 2001). Since the OPD is inversely related to the light path so using reactors with a very narrow LP (e.g. 1–2 cm) exposed to high PFD maintains cultures of extremely high cell concentrations, e.g. over 100 and up to 1000 mg chlorophyll (Janssen et al., 2001)

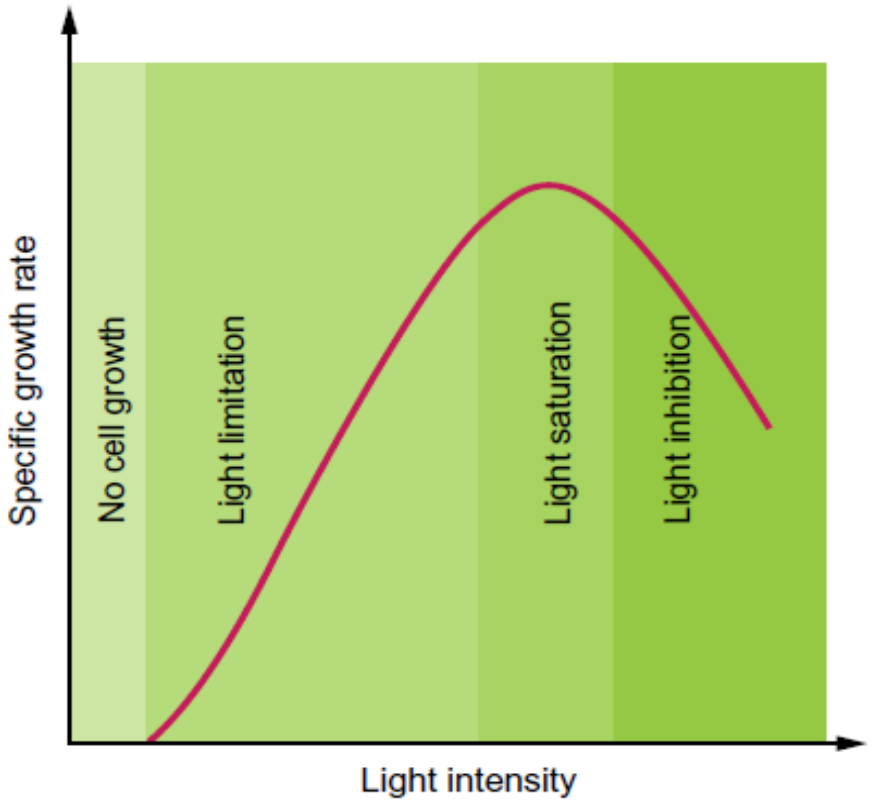


Figure 9: Effect of light intensity on specific growth rate of microalgae under phototrophic cultivation (Ogbonna and Tanaka, 2000)

3.1.7 Other considerations

In order to evaluate a proposed design of reactor, the whole cultivation and production process must be considered by keeping in review the effect on whole process. As an example, the improvement in the oil extraction efficiency and other techniques of improved downstream biomass processing will result in higher content of lipids extraction. Reactor design integration with downstream biomass processing is also a major parameter to be considered for ease of operation (Tredici et al., 1998).

Geographical condition and locations have important role in the assessment of feasibility of microalgae based CO_2 fixation and biofuel production system and reactor selection based on the assumption that certain region of world may be more suitable for microalgae growth than others. Open ponds system provides optimum growth rates and biomass production where cost of land is low, water sources are free of cost and production could be achieved throughout the year based on climate conditions. However, open systems have failed to achieve success in other condition than mentioned above (Kunjapur and Eldridge, 2010a).

3.1.8 Microalgae strain selection

Microalgae are the biological population contains variety of strains. There are nearly more than 50000 species microalgae exist in the earth ecosystems, and around 30000 species have been studied by people. (Gouveia, 2011) Not all these species are suitable to be used to the carbon capture process. It needs to select proper strains according to what the flue gas conditions are and what kinds of products we need. To capture the CO_2

efficiently it needs the microalgae species should possess the following characteristics according to the recent research:

- High CO_2 utilization rate
- High growth rate in shorter duration of time
- High tolerance to the harsh condition(temperature, pH)
- High potential to get useful by-products such as biofuel
- Efficient utilization of light to transform into biomass
- Easy to be collected during harvest process
- Having higher lipid production capability
- No potential risk to human and environment

The CO_2 concentration in the flue gas from the power plant is about from 1% to 20%, otherwise the CO_2 concentration in the atmosphere is around 380 ppm(Oh, 2010) In order to capture the CO_2 from the flue gas, the microalgae species used in the capture process must grow efficiently under the condition of the high CO_2 concentration.

3.1.9 Microalgae biomass treatment

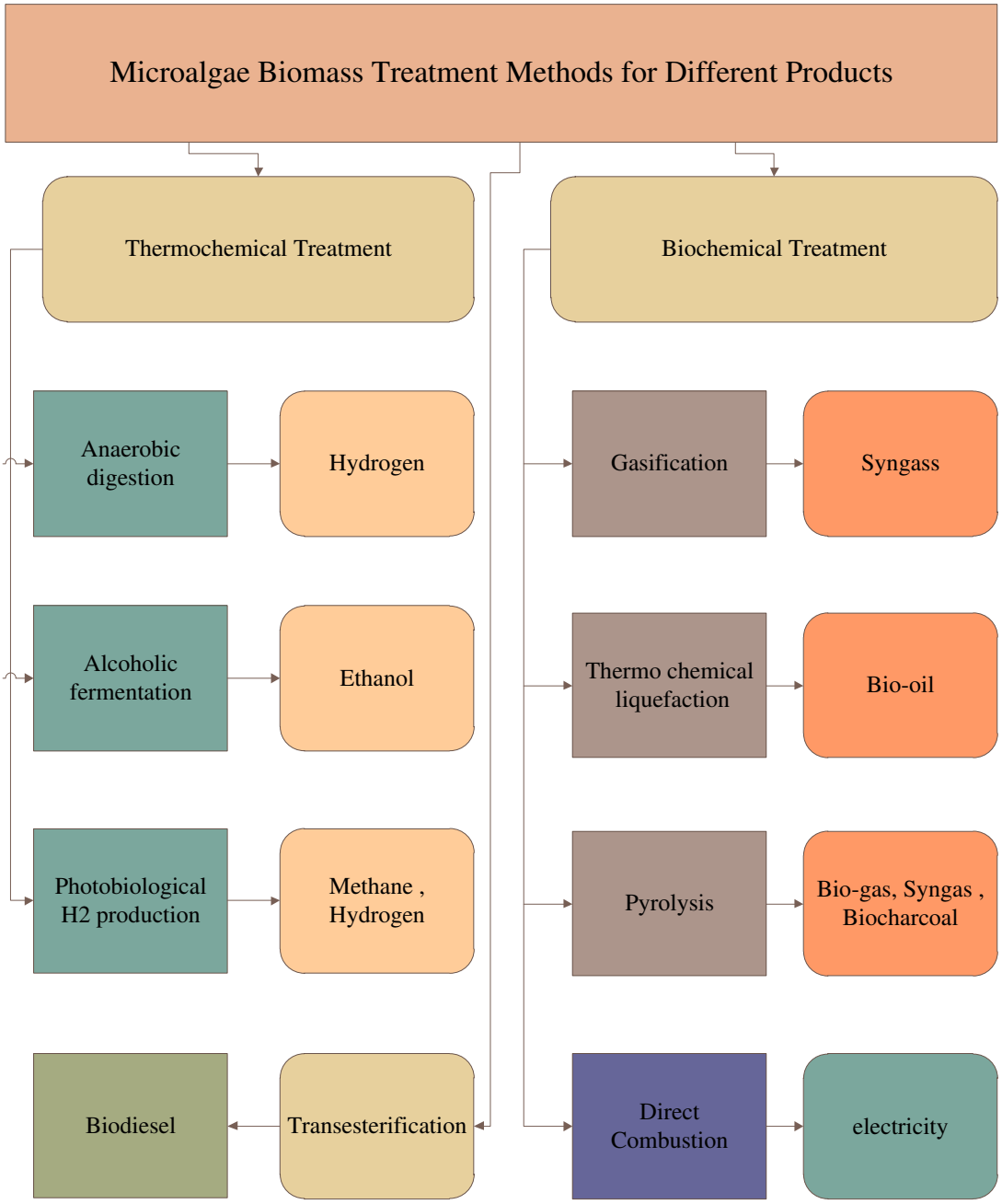


Figure 10: Algal biomass treatment methods to produce different energy products

CHAPTER 4

NUTRITIONAL METHODS OF MICROALGAE

4.1 Nutritional methods of microalgae

According to modes of nutrition microalgae are divided into two main groups, autotrophic and heterotrophic on the basis of their carbon uptake source. Autotrophic microalgae have the ability to convert physical source of energy in light form and chemical source of energy in the form of CO_2 and H_2O sources of energy into carbohydrates, which act as base for other carbon-containing biomolecules (Yoo et al., 2011). Autotrophic microalgae are comparatively self-sufficient and self-sustainable because they obtain their energy from sunlight (Nelson et al., 1994; Eberhard et al., 2008; Nelson and Yocum, 2006; Krause and Weis, 1991).

On the contrary, heterotrophic microalgae consume organic carbon source mostly formed by autotrophic as energy sources for their metabolic purposes because they cannot utilize atmospheric CO_2 as a carbon source. Oxidative integration of carbon initiates by a phosphorylation of glucose/hexose, producing phosphorylated glucose, which is freely present for storage, cell respiration and synthesis.

Nutritional modes significantly affect the carbon assimilation and lipid productivity of the microalgae (Xu et al., 2006). Three types of nutritional modes i) autotrophic ii) heterotrophic iii) mixotrophic are reported to produce algal fuel in the presence of light.

In addition, the dark heterotrophic nutrition mechanism is also found to be capable of lipid biosynthesis by microalgae under specific conditions of cultivation and specie characteristics in natural environment; however productivity may vary in quantity.

4.1.1 Photoautotrophic mechanism

The most commonly used method for culturing of microalgae is the autotrophic mode. Microalgae in photoautotrophic nutrition mode energy source used are sunlight and inorganic CO_2 as the source of carbon to form biochemical energy through the process of photosynthesis (Huang et al., 2010). This is one of the most prevailing environmental conditions for the usual growth of microalgae (Chen et al., 2011).

In photoautotrophic nutritional mode, photo-synthetically fixed CO_2 in the form of glucose serves as a sole energy source for all metabolic activities (Figure: 1).The simpler form of photosynthetic production, such as simpler carbohydrates, serves as sole energy source for carrying out the metabolic activities of the algal cells (Chang et al., 2011). These carbohydrates, under nutrient-limiting and stress conditions, will favor the lipid biosynthesis, which also helps to cope up with the stress (Gouveia and Oliveira, 2009).

Lipid productivity greatly depends on the photosynthetic activity in terms of atmospheric CO2 fixation and microalgae species natural features dependency. Large variations in lipid productivity, ranging from 5% to 68%, were reported under varying operating conditions during cultivation period and species diversity and characteristics nature of species (Murata and Siegenthaler, 2004; Ohlroggeav and Browseb, 1995; Chen et al., 2011; Mata et al., 2010).

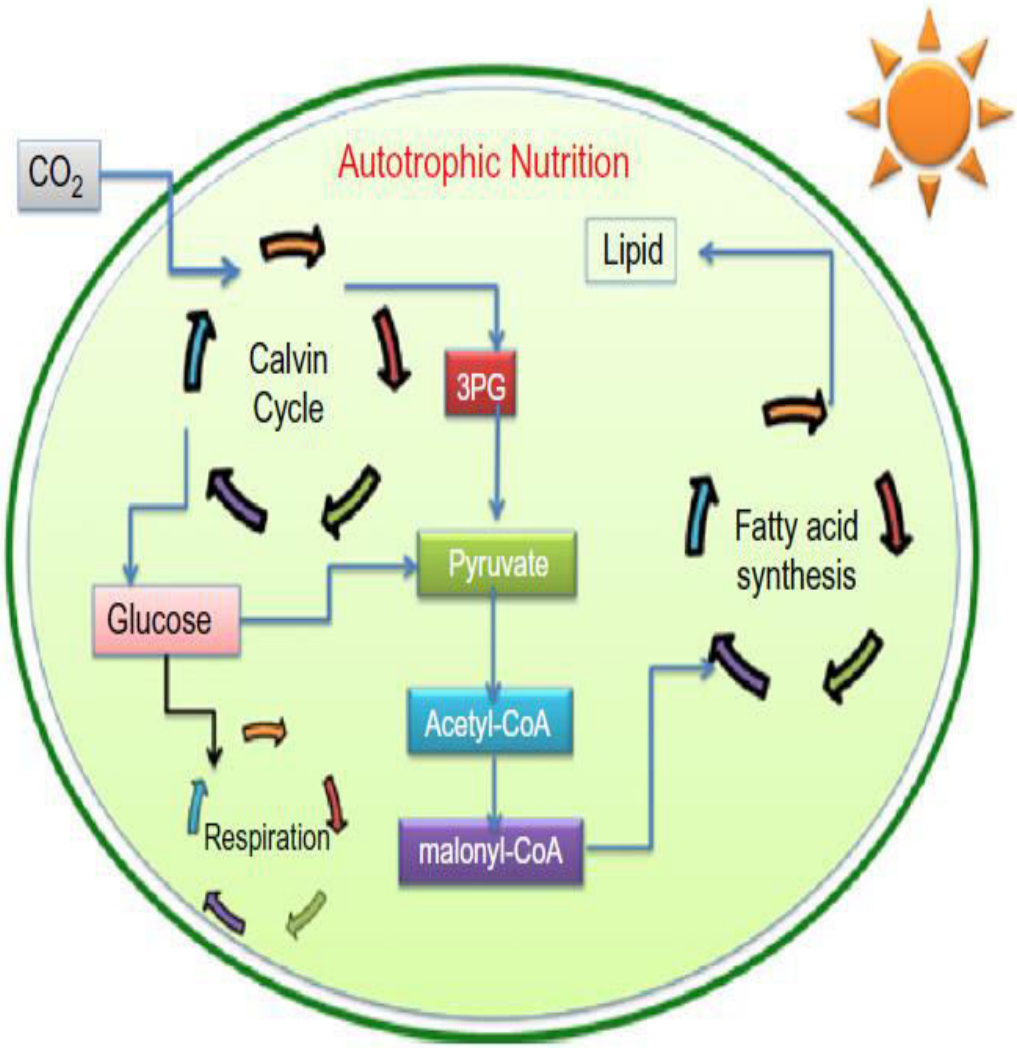


Figure 11: Autotrophic nutrition in microalgae towards CO_2 fixation and lipid biosynthesis.

A major advantage of the autotrophic nutritional mode is the production of algal oil at the expense of atmospheric CO_2. Large measure microalgae cultivation systems are usually run under photoautotrophic environments (Mata et al., 2010). Autotrophic nutritional mode also has fewer contamination problems compared with other nutritional modes. Under autotrophic nutrition, the photosynthetic products also get consumed during respiration associated with the biomass growth, and hence the productivity of lipid represents the collective parameters of algae oil content and capacity of biomass production (Chiu et al., 2008).

4.1.2 Heterotrophic mechanism

Heterotrophic is a mode of nutrition whereby microalgae utilize external substrates as sole carbon sources for their growth and lipid accumulation. The circumstances in which microalgae consume organic molecules as primary energy source and carbon sources is called heterotrophic nutritional mode (Kaplan et al., 1986). In heterotrophic nutrition, the simpler carbohydrates enter the cell and are subsequently converted to lipids and participate in other metabolic pathways such as respiration (Figure 2).

Heterotrophic nutrition takes place both in incidence and absence of light. In photoheterotrophic nutrition, light acts as source of energy, but the source of carbon remains organic only. Heterotrophic growth process in the absence of light condition is sustained by the carbon source substituting the source of light energy. This distinctive capability is shown by different species of microalgae (Perez-Garcia et al., 2011).

Glucose is one of the simplest sources of carbon for heterotrophic microalgae. Higher microalgae growth rates and productivity is obtained by using glucose as sole carbon

source than any other source of alcohols, sugars and organics acids sources. This oxidative assimilation takes place in algae apparently through two pathways; i.e., the Embdenn Meyerhoff path (EMP) and the pentose phosphate path (PPP) (Neilson and Lewin, 1974).

In the heterotrophic growth process of microalgae carbon metabolism occurs via a PPP pathway, whereas the EMP is the main pathway for glycolytic meth in the presence of light (Lloyd, 1974; Neilson and Lewin, 1974; Yang et al., 2000; Hong and Lee, 2007). Both of the pathways are practical in microalgae done in cytosol. Though, the PPP pathway process must have higher flux than other process which depends on light and carbon source (Perez-Garcia et al., 2011).

Light is not required for the transport of glucose inside the cell during dark heterotrophic operation. Glucose transport system in the algal cell become inefficient in the presence of light, because of higher availability of photosynthetic inside the cell due to photosynthesis and down-regulation of hexose transport protein. The carbon is obtained from outside the cell and converted to the acetyl-CoA via pyruvate, which further converts to malonyl-CoA and subsequently enters the lipid biosynthetic pathway (Figure 2) (Perez et al., 2011).

In heterotrophic nutrition mode, because of abundant glucose availability, respiration and other metabolic processes do not compete with the lipid biosynthesis, unlike as the mechanism of autotrophic cultivation mode.

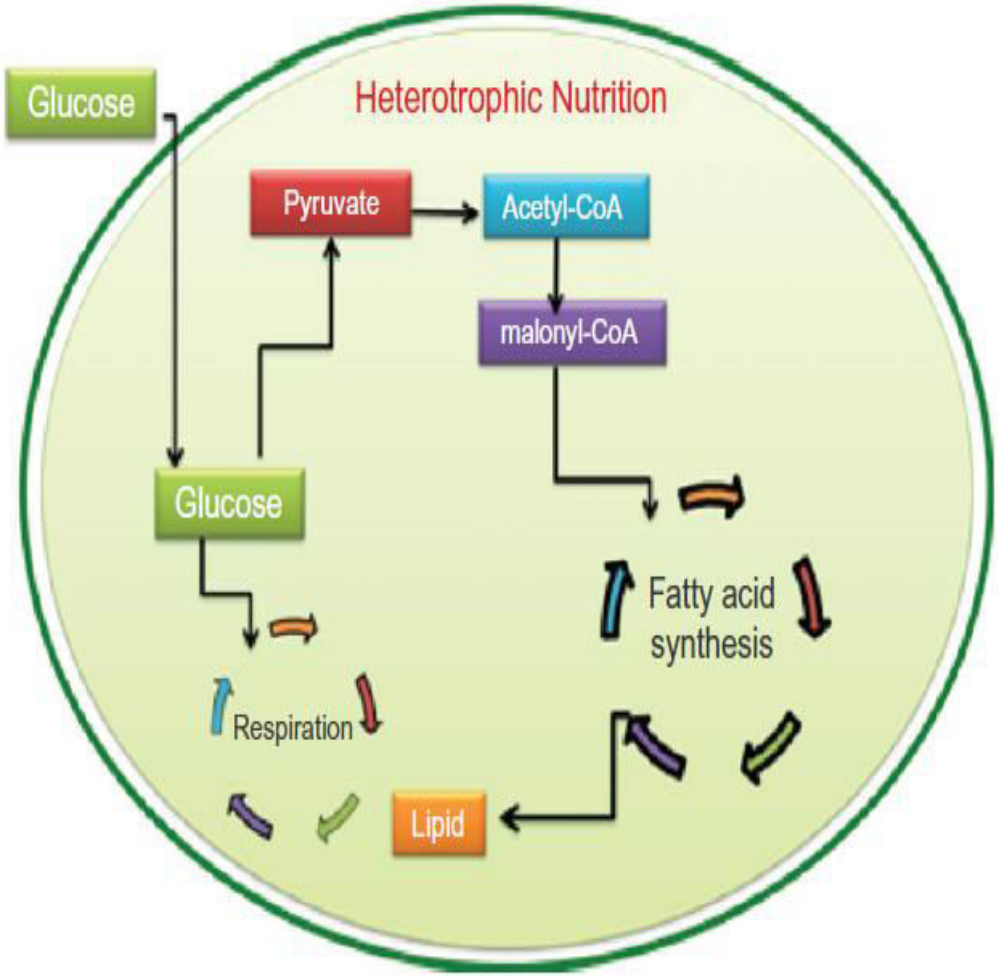


Figure 12: Heterotrophic nutrition in microalgae towards glucose assimilation and lipid biosynthesis

Moreover, microalgae can utilize organic carbon under dark conditions because of the ability of light-independent glucose uptake. Hence, the lipid productivity is high in heterotrophic nutrition mode (Abeliovich and Weisman, 1978).

Heterotrophically higher biomass densities are quite possible that provide an economically more feasible and viable method of microalgae biomass production (Chen, 1996; Chen and Johns, 1996; Lee, 2004; Behrens, 2005; Perez-Garcia et al., 2011). Photoheterotrophic nutritional mode avoids the limitations of light dependency, which is the major obstruction to gaining higher density of cells in large-scale biomass production in photo-bioreactors (Huang et al., 2010).

Chlorella protothecoides showed higher contents of lipids (40%) during the process of heterotrophic growth (Xu et al., 2006). Higher productivity of lipids (3,700 mg/L/d) was also reported by using an upgraded fed-batch type culture strategy in heterotrophic nutritional mode, where the lipid productivity was found 20 times more than that achieved under photoautotrophic cultivation method (Xiong et al., in 2008).

The main advantage of the heterotrophic nutritional method is the acceleration of wastewater treatment as well as lipid productivity, which gives an advantage to its use in the current state of increasing pollution loads. Moreover, cost effective process, relative ease of operation, and maintaining easily are the main advantage of the heterotrophic growth process approach (Perez-Garcia et al., 2011). However, heterotrophic systems suffer from contamination problems (Abeliovich and Weisman, 1978; Olguı´n et al., 2012).

4.1.3 Mixotrophic mechanism

Microalgae can play its role by combining both autotrophic and heterotrophic mechanisms in mixotrophic process. It facilitates fixing atmospheric CO_2 as well as consuming the organic molecules and micronutrients from the growing environment (Figure 13). Microalgae can utilize existing organic carbon source as well as atmospheric source of CO_2 as in mixotrophic cultivation mode. The CO_2 produced by respiration of cells will again be reused in mixotrophic nutritional mode. It differs from photoheterotrophic nutrition mode in terms of CO_2 utilization.

The mixotrophic have the ability to utilize organic carbon; which prevents the light limitation of growth (Chang et al., 2011). The acetyl-CoA pool will be maintained from both carbon sources—that is, by the CO_2 fixation (Calvin cycle) and intake from outside the cell, which can further make malonyl-CoA.

The photosynthetic metabolism utilizes light and CO_2 for growth and organic photosynthate production, whereas respiration uses the organic photosynthates produced during photosynthesis. If an external carbon source is available in the system, there is a less loss of photosynthate during respiration, and the algae utilize the available excess photosynthates for biomass development. Mixotrophic cultivation process shows higher growth rates and lesser photo-inhibition (Chojnacka and Noworyta, 2004).

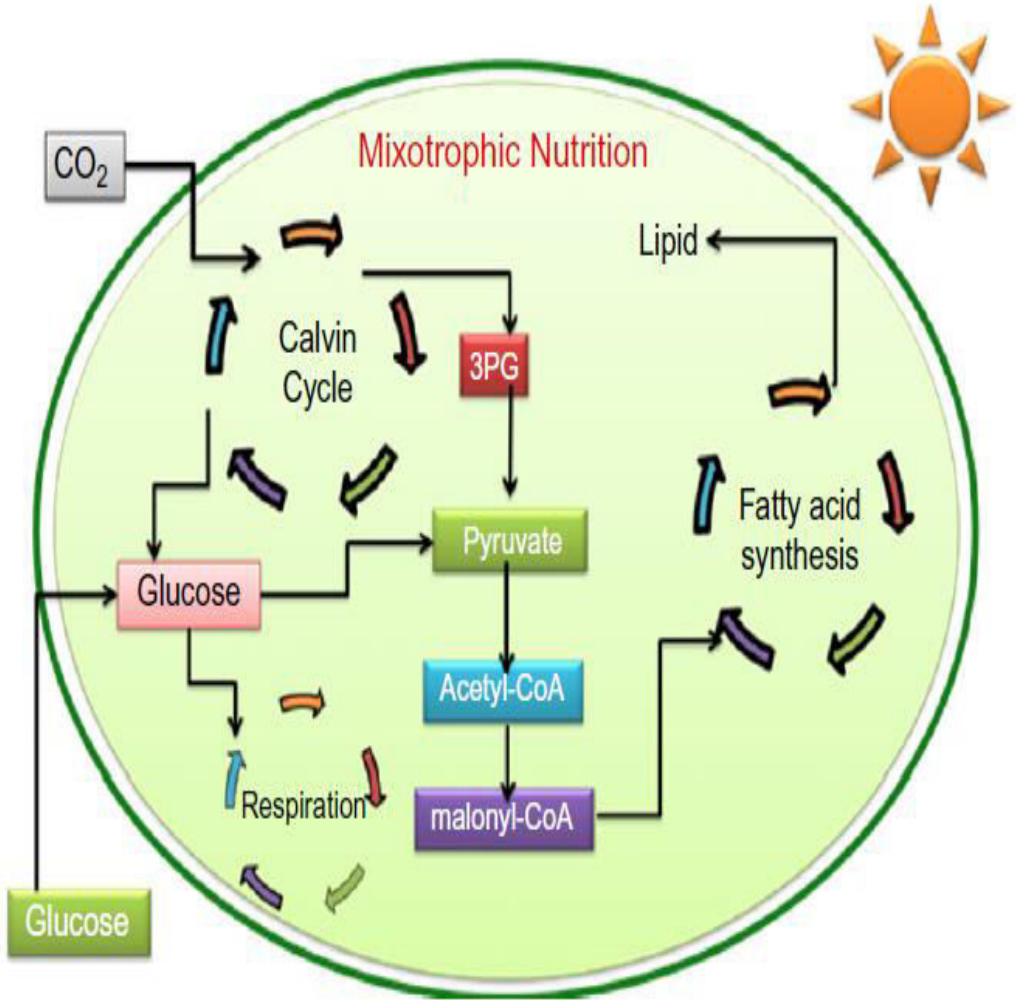


Figure 13: Mixotrophic mode of nutrition in algal cells towards CO_2 fixation and glucose assimilation for lipid biosynthesis.

Microalgae are flexible switch mode of nutrition based on substrate availability and light condition. If simpler carbohydrates are present in the system, algae shift towards heterotrophic nutrition from autotrophic mode to save energy. Scenedesmus obliquus specie is freely adapted in dark conditions to heterotrophic growth utilizing glucose (Abeliovich and Weisman, 1978).

Heterotrophic microalgae cells differs considerably from photoautotrophic cells culture with respect to several physical and biological properties such as CO_2 rate of photo-assimilation and the rate of utilization of carbon and chlorophyll deliberation(Abeliovich and Weisman, 1978). Bacteria play insignificant role in BOD reduction in high oxidation ponds and production of substrate for microalgae cells by degrading biopolymers.

The advantages of mixotrophic nutrition are its independence in terms of both photosynthesis and growth substrates (Kong et al., 2012). Mixotrophic growth mechanism is different than heterotrophic, where respiration and photosynthesis process take place at same time and CO_2 and organic source of carbon are concurrently conformed (Kaplan et al., 1986; Lee, 2004; Perez-Garcia et al., 2011).

Mixotrophic is often observed in ecological water bodies, where the function of living organisms systems are supported by physical, chemical and their organic activities that balance the ecological status. Generally ecosystem of water consist of nutrients and organic source of carbon as essential parts (Mohan et al., 2009), where microalgae and other living cells function together towards growth process.

There are some species of microalgae which are not mixotrophic still they have ability to switch to mixotrophic depending on the conditions of environment (Kaplan et al., 1986). Microalgae species accumulating higher lipid contents are mostly grown in natural water sources which make them potential resources for microalgae growth. Study has focused on the economical method of lipid production using domestic sewage treatment. Microalgae showed highest growth in mixotrophic culture process (Bhatnagar et al., 2010; 2011).

Hence mixotrophic cultivation has proven to be good approach to obtain higher biomass productivity and growth rates in shorter duration of time (Ogawa and Aiba, 1981; Lee and Lee, 2002), with the extra benefit of generating the photosynthetic metabolites to enhance productivity (Chen, 1996; Perez-Garcia et al., 2011).

Solazyme, a renewable oil company in the United States, has developed an integrated algal cultivation process by dark heterotrophic mechanisms, giving carbon sources externally. The company is using various forms of waste material as feedstock for the cultivation of algae in fermenters and harnessing almost 75% of oil on the basis of dry cell weight. The company is anticipating in selling algal oil to commercial refineries by the end of 2013.

CHAPTER 5

GROWTH MACHANISM OF MICROALGAE

5.1 Growth phases of microalgae in batch culture

Growth curve is the graphical representation of microalgae cells concentration as a function of culturing time. In the culture process inoculated with initial small amount of microalgae cells are supplied with limited amount of nutrient. Cells keep on growing until some of the nutrients component is depleted or until some environmental changes in process occurs which acts to prevent growth. Concentration of produced biomass can be defined in terms of total counts of cell number in (cells/ml) or by dry weight measurements mostly in unit of (g/L).

The basic equation which describes the microalgae growth is described as follows:

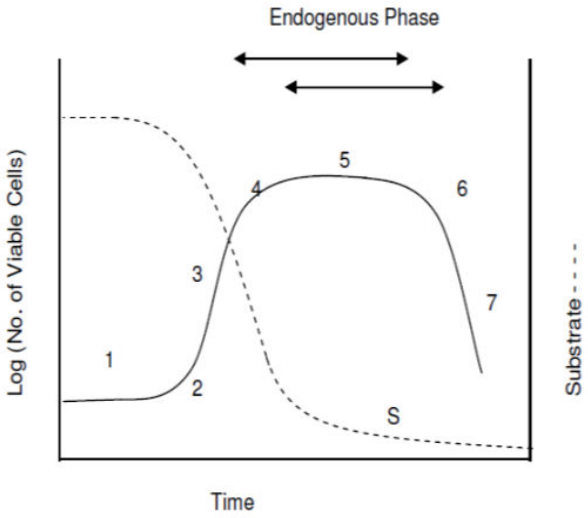
$$\frac{dx}{dt} = \frac{ax}{b} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{A}$$

Where "x" represents the growth of biomass during time interval "t" while "a", "b" are particulars factors affecting the growth rate. Equation shows that rate of biomass production is directly proportional to factor "a" which could be growth substrate, temperature or CO_2 concentration but "x" is inversely related to factor "b" which could be any growth inhibitor. These factors are independent of time "t" and could be ignored initially. In early stage of growth it follows autocatalytic first order pattern depending on initial number of cells.

The growth cycle describes overall growth pattern of microalgae cells in batch culture process. It should be noted that growth cycle is not a property of microorganism somewhat a consequence of decrease in fundamental nutrients supply or accumulation of inhibitor elements in closed batch system.

the microalgae cells growth process cycle is divided into different phases which could be described as:

1. Lag phase (initial phase)
2. Growth accelerating phase
3. Log or exponential growth phase
4. Growth declining phase
5. Stationary growth phase
6. Declining growth or death phase
7. Log or accelerated death phase



Each one of these phases is categorized by different growth rates of cells and can be influenced by different factors: these phases were clearly recognized by using the following Most clearly, phases were recognized by the use of the following estimation function [Buchanan, 1918]

$$N = N_0 e^{(mF(t)t)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where "N" represents the number of microalgae cells at any time, N_0 represents the value of N at the start of different phases, and "t" is growth time. The empirical function F(t) has different forms for different cells growth phases:

i. Initial stationary phase ⟶ F(t)=0

ii. Lag phase ⟶ $F(t)=t_n-1$,

iii. Logarithmic growth ⟶ F(t)=1

iv. Negative growth acceleration ⟶ F(t)= t-t-1

v. Maximum stationary ⟶ F(t)=0

vi. Accelerating death ⟶ $F(t)=t_n-1$

vii. Logarithmic decrease ⟶ F(t)=-1

Many microalgae cultures process characteristics of metabolism activities, agglomeration and resistance to harsh conditions are found to be directly related to changes occurring during growth phases, (Müller, 1895; Sherman and Albus, 1923).

5.1.1 Lag growth phase

Due to change of growth environment cells growth and multiplication is delayed for some time depending upon the adaptability of cells to new medium environment. First cells rearrange themselves to be ready for metabolic activities in different conditions of growth and environment.

- During this initial lag phase cells adapt to new medium and environment of culture without multiplying.
- During this phase number of cells remains same but their size is increased.
- During this phase cells are physiologically very active and are synthesize their activating factors.

In this nutrients medium is integrated into nutrients required for cells growth in term of ease uptake of C, N, and P. the outcome of this phase is the cells which biochemically vibrant i.e. having capability of converting chemicals of medium into biomass in term of growth. Generally this is not desirable phase is should end as early as possible due to its higher time consumption and cost as well. Inoculum strongly affect the lasting period of this phase (Spencer, 1954).

The healthy inoculum taken from the exponential growing phase of culture most probably will not have any lag phase under similar condition of growth in term of light, medium temperature and salinity. The lag phase may remain from hours to days. The dynamics of lag phase may be determined from the following equation whether it is occurred or for how long duration.

$$\frac{log\ n\ - \log n_0}{t - L} = \frac{\log 2}{T} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Where "n" represents the total number of cells at given time "t" after the beginning of culture, "n_0" represents the initially present number of cells and "T" is the generation time or doubling time. As the length of initial phase depends on the inoculum and type of nutrients medium so it could be controlled up to some extent. Mostly its duration is negligible when culture is transferred from same medium.

5.1.2 Accelerating growth phase

This phase occurs when cells divisions starts and cell metabolism increases sharply. This phase could be localized in between lag phase and exponential phase. Here cells increases

in size and numbers noticeably and its considered transition period from where exponential growth periods starts.

5.1.3 Log or exponential growth phase

The growth rate of a microalgae biomass is measured from the cells growth in exponential phase. Relative ecological success of any biological species is measured from its growth rate in adapting experimental or natural environmental conditions enacted upon it. In this growth phase the cells multiply rapidly in numbers and their growth rate is almost independent of medium nutrients concentration, as their concentrations are in excess.

The exponential growth phase is categorized by most rapid and fast growth under applied conditions of nutrients and culture. During this growth phase, the increasing number of cells becomes proportional to cells at any time in culture process. Following are main characteristics of exponential phase.

- Exponentially cells grows during this growth phase, microalgae cells divide frequently at a continuous rate
- The logarithmic plot of cell number against time is almost straight line or concave up for higher efficient medium.
- Maximum utilization rate of substrate in term of nutrients removal and CO_2 fixing
- A maximum of growth rate is found in optimal operating and environmental conditions
- Excess amount of food is present and rate is limited only by ability to utilize food
- Sometimes maximum growth rate is constant called "0-order growth" rate.

The time for each cell division is known as generation time or doubling time. If initially inoculated number of cells is "n_0" in suitable medium and growth is exponential then number of cells after one generation will be "$2n_0$" and after two generation number of cells becomes "$4n_0$" and finally after "z" number of generation total number of cells becomes "$2^z n_0$" where "z" is the number of generation. Duration of this phase could be found from following equation.

$$\frac{log\ n\ - \log n_0}{t - L} = \frac{\log 2}{T} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Where "n" represents total cells numbers at given time "t" after the beginning of culture, "n_0" represents initial cells number and "T" being the generation time or doubling time. After plotting the different growth phases (time "t" on x-axis while biomass "n" on logarithmic on y-axis) carefully exponential growth phase is determined. Two different points, N_1 and N_2, at the extremes of exponential phase are taken and switched into the following equation.

$$Growth\ rate: K = \frac{\ln(N_2/N_1)}{t_2 - t_1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

Whereas N_1 and N_2 is biomass concentration at any times t_1 and t_2 respectively at the any point of exponential growth phase. Duration of this phase depends on following factors:

- The inoculum size fed to culturing medium
- The growth rate of microorganism in term of cells
- Medium capacity to support growth process
- Culturing process conditions to support microalgae growth

The exponential phase ends due to accumulation of toxic contaminants, depletion of nutrients and cells concentration saturation limit reaching.

5.1.4 Declining growth phase

Decline in growth usually take place during cultivation process when some growth requirement is limiting or accumulation of toxic elements which inhibit the growth rate. In this growth phase biomass growth is very high and depletion of a nutrient salt concentration, CO_2 or limitation of light becomes the prime reasons of decline in growth.

With the increase in biomass during exponential growth phase supply of CO_2 and air is balanced with growth rate as others factors are not limiting at that time. With the decline in growth higher amount of CO_2 may cause the decrease in pH which further inhibit growth rate. With higher cell densities growth is proportional to the input amount of CO_2 and limitations of CO_2 can only cause linear growth rate instead of exponential if any growth occurs during this process.

- It represents the switch period to the stationary growth phase.
- Decrease in growth rate happens due to depletion of necessary nutrients
- Increase of toxic accumulation which inhibits the growth
- Growth rate limitations occur either due to toxic metabolic products accumulation or by limitations of nutrients.
- Mostly due to batch process food to the cells becomes limiting factor and biomass and concentrations become dependent on nutrients.

At the higher cells concentration light limitations occurs due to higher absorbance of incidence light which cause the self-shading of cells resulting in decline of growth.

However cells may survive in lower light conditions for long period of time but overall growth is declined due to light limitations

5.1.5 Stationary phase

Stationary phase is a state in which there is no net growth of cells even if some cells grow. In this phase number of cells being produced becomes balanced by number of cells dying. Several reasons exits which contribute to the establishment of stationary phase. One of the most common reasons is the complete depletion of energy and carbon source. When all the carbon sources is finished still some growth exist, ass the dying cells provide nutrients source by their decomposition.

Second reason for establishment of stationary phase is the accumulation of toxic and waste products which inhibits the overall growth. As a result of nutrients source depletion and accumulation of toxic products produce stress on cells in stationary phase and cells become smaller in size than exponential phase. Following are main characteristics of stationary phase which are commonly observed during growth process.

- Total number of cells remains same due to cessation of cell production.
- New cells growth is balanced by old cells death.
- No further increase in cell biomass showing stable phase.
- Net growth rate = 0 due to equal death of cells and food limitations

If we consider by assuming the death kinetics similar to that of growth kinetics then specific rate (б) of cells death could be described mathematically as follows:

$$\frac{dN}{dt} = (\mu - 6)N \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5$$

It clearly indicates that if “μ” is greater than “ϭ” then cell will be growing at a rate of “μ-ϭ”. On the contrary if “ϭ” is greater than “μ” then cells are dying at the rate of “ϭ-μ” and if both “μ” and “ϭ” have equal values then condition of stationary phase is reached.

When the net growth of cells become zero culture enters in the stationary growth phase, where dramatically changes in cell occurs biochemically and nature of this change is dependent on the limitation factor. Limitation of nitrogen results in reduction of protein contents of cells and accordingly lipids and carbohydrate also changes. Limitation of light intensity results in increase of pigment content.

5.1.6 Death or declining growth phase

With the exhaustion of all the nutrients sources, cells metabolic activities almost become zero and cells death accelerates often termed as culture crash. At this stage due to unavailability of food and toxic accumulation cells death rate become higher than growth of viable cells. The death phase at this stage become exponential and loss of produced biomass occurs as the starving cells starts decomposing other cells to produce nutrition.

- Total concentration of viable cells decreases due to accelerating death rate.
- Complete exhaustion of nutrients toxic accumulation occurs.
- Mostly death of cells occurs due to food limitations.

5.1.7 Log death phase

Exponential or log death - "wholesale die-off" system is dead even if food is added, no further growth is observed due to crash of cell structure and capability of revival.

5.1.8 Endogenous phase

Near the food shortage condition that part of the microalgae growth curve including some portion of declining and stationary phases where cells starvation occurs is mostly termed as endogenous growth phase. Mostly CO_2 fixation and wastewater treatment reactor systems are operated in endogenous phase to utilize maximum CO_2 as they are starving for food. Food is limited and microorganisms become forced in metabolizing the products and dead cells with higher dependency on CO_2 utilization. Here cells growth is not ceased but net growth rate become negative due to larger death rate.

CHAPTER 6

EXPERIMENTAL METHODOLOGY

6.1 Introduction

Research methodology was initiated with twelve week literature study performed aimed at providing relevant background information within the subject of carbon dioxide capture through microalgae and growth kinetics mechanism. Within our research Frame of Reference, main focus was also put upon scrutinizing existing culture systems and methods culturing microalgae. Based on literature review and objective of our research methodology for this research thesis project was scheduled deliberately during the proposal preparation and was refined continuously during the execution of the project.

Subsequently a selection of potential biomass end use and CO_2 fixation was formed in correlation with the selection of microalgae. Inoculums of the selected algae were grown at the same time the selection of culture system, design and construction of the experimental equipment was performed. The equipment was installed at the Biochemical lab and the growth test initiated. The results from the growth were then used to elaborate the set of objectives.

A key goal throughout the process was to build a system that would enable batch growth of the microalgae. The batch system would then be monitored to identify the conditions which gave the best performance, regarding CO_2 fixation, microalgae growth and nutrient uptake. The overview of experimental methodology is shown in the Figure14.

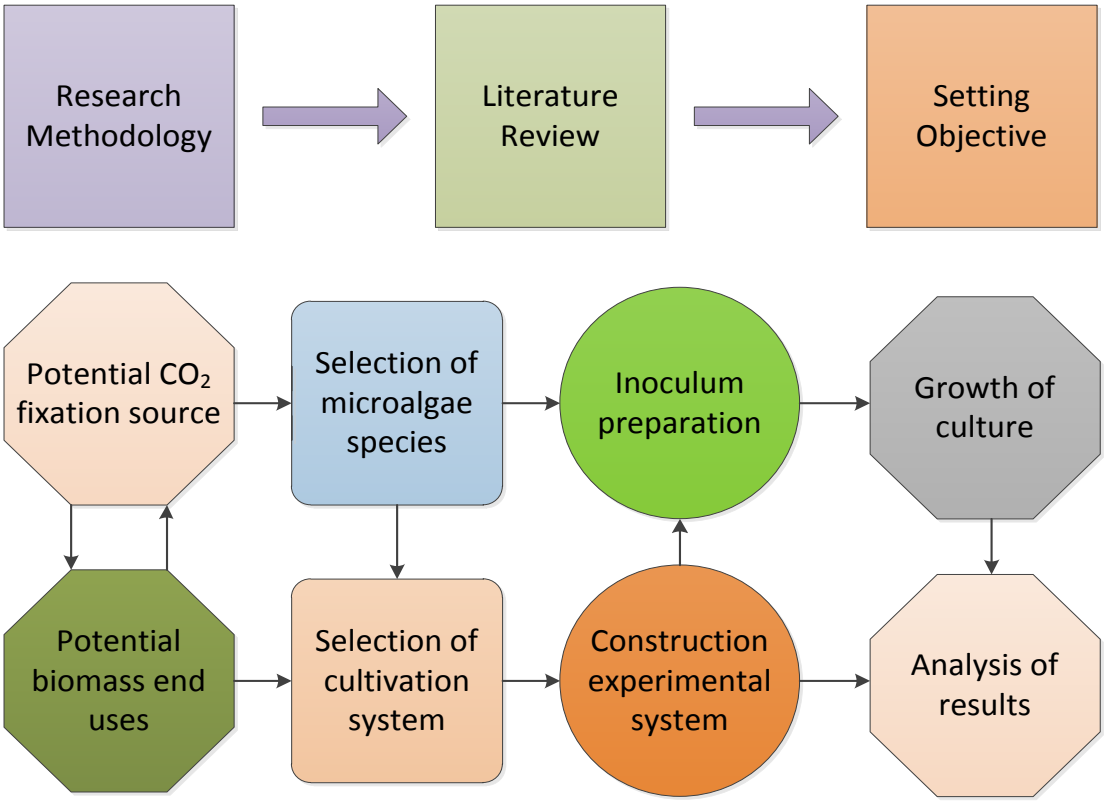


Figure 14: The overview of research methodology plan for experimentation process.

A goal for any future continuous system would be to apply and maintain these optimum growth conditions. The proposed method was finally applied for construction of photo-bioreactor system, experimental operation of equipment and analysis of performed results.

6.1.1 Selection of potential biomass use

A decision was made during selection stages of the project regarding the potential use of the algal biomass. This decision was closely related to the selection of microalgae, and was based on information collected in the Literature Frame of Reference. For the purposes of this project, esterification of the biomass to produce lipids and gasification to produce biogas, whereby a biodiesel and gas is produced from the biomass, has been identified as the most applicable end use, for a number of reasons.

Firstly, this process is relatively flexible in that it can use any biomass as a feedstock to produce green fuel which means some of the limitations and problems associated with the high oil yielding microalgae strains used for producing biodiesel from microalgae are not an issue as faster growing and more robust microalgae strains that can be used.

Secondly, Saudi Arabia has existing infrastructure for energy production based on the combustion of biogas produced through anaerobic digestion, and potential expansion of that infrastructure to incorporate sustainable natural gas produced from algal biomass through gasification would most likely be easier and more efficient to implement than a completely new process.

6.1.2 Selection of microalgae species

Selection of a suitable algal species was performed and specimens to produce inoculums were performed. This selection was closely related to the CO_2 bio-fixation capability, wastewater treatment potential and choice of potential biomass application, as discussed in the previous section.

For the purposes of this research project two microalgae strains called *Chlorella Vulgaris* and *Nannochloropsis Oculata*, provided by the American Algae Depot Culture Collection, were chosen. These microalgae were selected for a number of reasons. A primary goal of this project was to capture CO_2 as much as possible which equates to growing as much biomass as quickly as possible. These species are widely known as some of the fastest growing strains of freshwater microalgae (Borowitzka, 1999b; E. et al. Stephens, 2010; Mata et al., 2010).

Chlorella vulgaris has also been used in many trials to sequester CO_2 and bio-remediate different wastewater substances such as nitrates (Wang et al., 2008), phosphates (Mata et al., 2010) and heavy metals (Das et al, 2008). A review of different trials using single microalgae strains, multiple microalgae strains and immobilizing surfaces is presented by de-Bashan (Bashan et al., 2010). *Chlorella vulgaris* is also known to grow under photoautotrophic, heterotrophic and mixotrophic conditions (Mata et al., 2010).

Aresta states that microalgae have received more attention than macro-algae for CO_2 sequestration largely because more research has been performed on microalgae strains for biofuel particularly biodiesel production purposes. Microalgae are also considered easier to grow in ponds or bioreactors (Aresta et al, 2005).

6.1.3 Inoculum preparation

A culture of *Chlorella vulgaris* and *Nannochloropsis Oculata* were obtained from www.algaeDepot.com America commercial company. The culture cells of the specified specie were grown in F/2 and Synthetic wastewater culture medium using deionized water. The F/2 and Synthetic wastewater culturing medium composition is defined in appendices [1].

Stack cultures of *Chlorella vulgaris* and *Nannochloropsis Oculata* were prepared using 1tsp of sea salt and two drops of F/2 food concentrate per 400ml of distilled water used to prepare the nutrients medium solution. Using 300ml of nutrients solution in two 500ml flasks and 2ml of sample of algae culture were used. Foam stopper were placed half exposing to air to avoid contamination of culture and this was placed in artificial light. Samples were mixed three times on daily basis for seven days. These samples were used for our desired F/2 culture medium experiments.

Same procedure was adopted for second experimental set for Synthetic wastewater media inoculum preparation for both *Chlorella vulgaris* and *Nannochloropsis Oculata study* in Synthetic wastewater media. Synthetic wastewater media was prepared according to the recipe given in appendices [1]

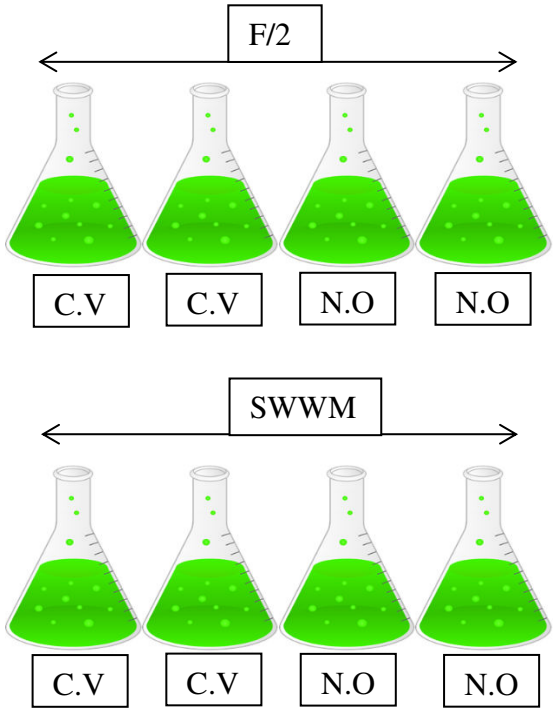


Figure 15: Inoculum of *Chlorella vulgaris (C.V)* and *Nannochloropsis Oculata (N.O)* in F/2 and Synthetic wastewater (SWWM)

6.1.4 Experimental setup for batch reactors

The proposed photo-bioreactor belongs to vertical tubular class of photo-bioreactor having novel gas sparging mechanism which acts as agitator as well to keep liquid in moving phase. Externally photo-bioreactor is equipped with light distribution, gas mixing and monitoring mechanism and liquid circulation pump assembly.

Experimental batch reactor consists of a transparent vertical bubble column with Sparger at the bottom of column to bubble CO_2 through the culture medium. Gas bubble rises through the liquid where mass transfer from gas to liquid take place and microalgae cells uptake CO_2 from liquid continuously rest of CO_2 bubbles out at the top where some of its part goes to CO_2 sensor through flow meter for analysis, major part of it is recycled or sent to atmosphere. Close to bottom of column sample point is provided for homogeneous sampling.

CO_2 from the source is mixed with filtered air in gas mixer in different ratios according to requirements of operation. Two CO_2 sensors are provided at inlet and outlet for the detection of CO_2 in air mixture. Both sides of photo-bioreactor are provided with white artificial light necessary for photosynthesis process. All the setup with accessories of batch photobioreactor is shown in the following Figure 16.

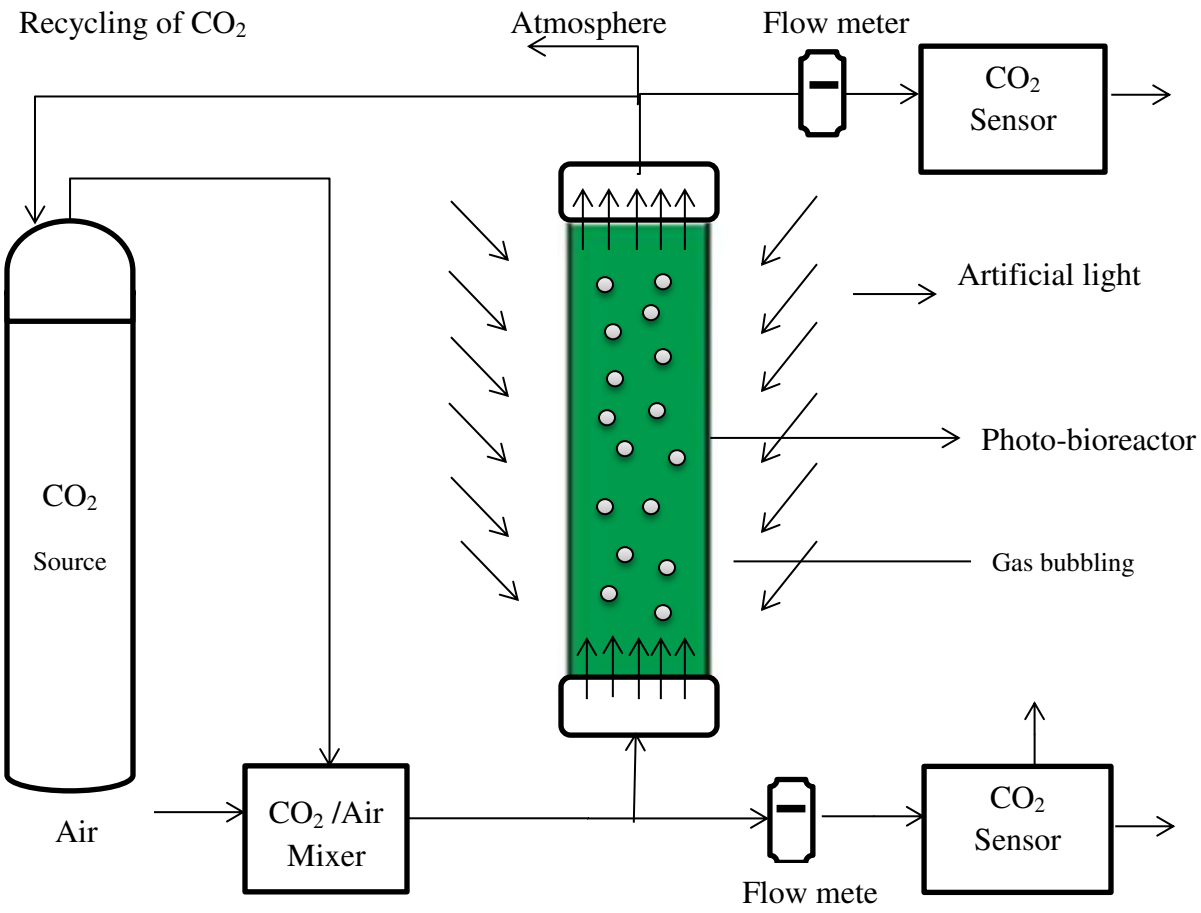


Figure 16: Representation diagram of batch photo-bioreactor for the experimentations on CO_2 reduction

6.1.5 Experimental setup for series reactors

Semi-continuous culturing system with four batch reactors in series was setup with continuous CO_2 supply from reactor one to fourth reactor step by step. Each reactor in series act as a batch reactor and CO_2 concentration mixed with air feed to each reactor. All of the four reactors were air tighten with cork having one inlet and one outlet streams for gas.

The CO_2 mixed air stream from gas mixing device was split up into two streams one feed to reactors and other with small controlled flow rate using glass Rota-meter sent to CO_2 sensing device for inlet CO_2 concentration measurement. The second stream from first reactor goes to second and from second to third and third to fourth reactor and finally outlet stream is split into two streams one to CO_2 measuring device through glass Rota-meter and other to recycling or exhaust air through water tank to provide necessary pressure for CO_2 sensing device stream. The series batch photobioreactors setup is shown in Figure 17.

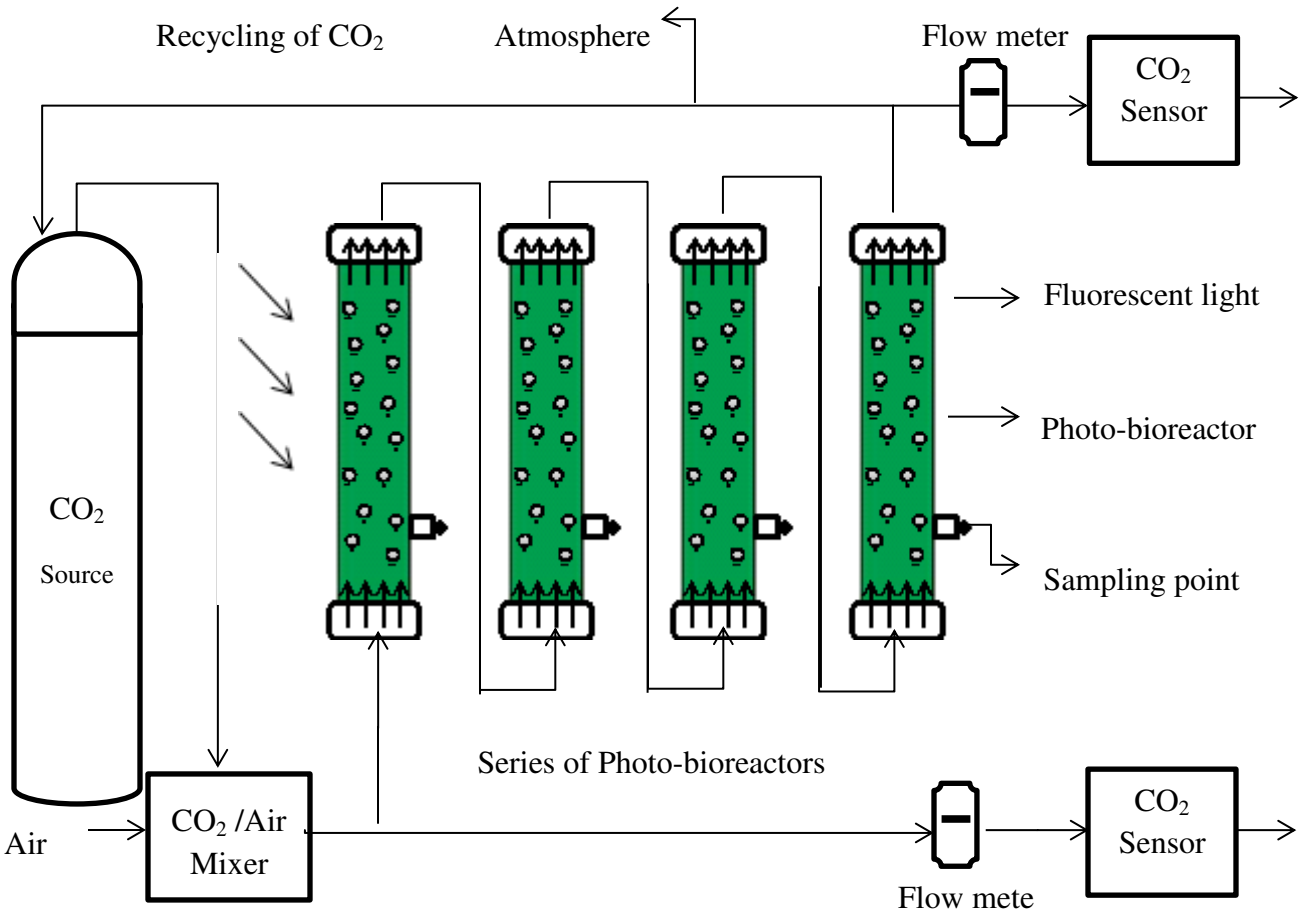


Figure 17: Representation diagram of series batch photo-bioreactor for the experimentations on CO_2 reduction. The streams of different CO_2 concentrations mixed with air were used and determined through CO_2 sensor.

6.1.6 Experimental startup procedure

The micro algal species samples obtained from starter culture was incubated in four different batch photo bioreactors with 1800ml working volume of each reactor. The culture was placed in fume hood with provision of fluorescent light of equal strength to all the four cultures from both sides continuously with the intensity in the range of 3200-4000 Lux at the surface of all the batch reactors. The ambient air after filtration was mixed with CO_2 using gas mixing device. The CO_2 concentration of 2%, 4%, 6%, 8%, 10% and 12% was mixed with four outlets streams provision. There were two batch reactors of each species with same initial cell concentrations and each species was fed with 2%, 4%, 6%, 8%, 10% and 12% CO_2 concentrations. Light intensity, temperature and pH of all the samples of culture were monitored on daily basis.

Four batch photo bioreactors were filled with 1800ml of F/2 nutrients medium and 100ml of cultured *Chlorella vulgaris* starter was added with initial cells concentration as specified in table. Air was mixed with CO_2 in concentration of 2%, 4%, 6%, 8%, 10% and 12% CO_2 for four different batch reactors. Fluorescent light was provided at the surface of reactors. Cultures were set for 15days, first three days samples were analyzed twice a day and after that once a day to determine the optimal cell density, cell concentration, and dry weight, effect of CO_2 utilization by two different species, pH changes, and growth rate measurements were made.

6.1.7 Growth by cells counting

Direct measurement of cells growth by microscope (Fisher Scientific™ Micromaster Microscope) was employed to measure the cell concentration using Hymo-cytometer.10 µmL sample of culture was analyzed for cell counting on daily bases. With the increase in cell density necessary dilution of the sample was made to avoid cell clustering and make easier and accurate counting. The measured cell concentrations with different CO_2 concentrations for *Chlorella vulgaris and Nannochloropsis Oculata* growth study

Figure 18: Microscopic method for cell counting.

6.1.8 Growth by dry biomass finding

Dry biomass was calculated by vacuum filtration method using 5ml of homogeneous sample. Filter paper was first tarred at 50C to remove trapped water molecules for 24 hours after that it was weighted and sample was filtered with the help of vacuum and wet filter papers containing biomass were dried for 24 hours and weighted for dry weight calculations. Finally 5mL weight was changed to per liter basis using calculations.

Figure 19: Filtration method for biomass finding

Second method for dry biomass finding is by centrifugation and freeze drying under high vacuum application. At the completion of growth period the cultured samples is centrifuged at very high speed of 1200 rpm for 5 minutes in order to find out biomass. Samples are washed using deionized water again centrifuged. After centrifuge by adding some amount of deionized water the extract is first freeze to -40^{0}C with using deep freezer and the dried using freeze dryer at very high vacuum and low temperature of -80^{0}C in order to keep cells alive. When all the water evaporates the sample of biomass is ready for future analysis.

Figure 20: Freeze drying under high vacuum

6.1.9 pH, light intensity and temperature measurements

The pH value of cultures was monitored on daily basis using desktop pH meter (Fisher Scientific Accumet® Basic AB15 Plus pH Meter). Light intensity was also monitored on daily basis using light meter (Fisher Scientific™ Traceable™ Dual-Display Light Meter). Temperature of the cultures was recorded on daily basis using digital temperature sensor (Fisher Scientific™ Digital Thermometers with Stainless-Steel Probe on Cable) in degree Celsius scale.

Figure 21: Desktop pH meter, Dual-Display Light Meter and Digital Thermometers

6.2 Growth and CO_2 fixation analysis methods

6.2.1 Growth kinetics analysis

Cell density measured is converted into dry biomass of micro algal cell per liter basis of culture grown for cultivations. Biomass calculations were made on the basis of per liter dry weight produced from calculations (gL^{-1}).

Specific growth rate (μ) it is defined as the increase in cell mass per unit time, e.g., grams cells (g) per gram cells (g) per hour. The specific growth rate is commonly given by the symbol, μ (mu), and the most common units are in reciprocal hours (h−1).

$$\mu = \frac{ln(X_m/X_0)}{t_n - t_0} \dots\dots\dots\dots\dots\dots 1$$

Where X_m and X_0 are biomass calculated and t_n and t_0 are the time of sample measurement on day basis here in this research we have specifically used biomass (gL^{-1}) for all the samples for calculations.

Biomass productivity (*p*) productivity or production refers to the rate of generation of biomass in an during microalgae growth and found as:

$$p = \frac{X_m - X_0}{t_n - t_0} \dots\dots\dots\dots\dots\dots 2$$

Absolute Growth Rate (AGR) By plotting growth in term of size or mass versus cultivation time we get a constantly increasing growth curve. By calculations of slope between any two points on this curve we get absolute growth rate, which represents the

actual change in growth with time. On each pair of time interval we get different AGR which indicates the change of growth rate over time.

$$AGR = \frac{X_2 - X_1}{t_2 - t_1} \dots\dots\dots\dots\dots\dots 3$$

AGR yields average slope over that time interval where values are selected.

Relative Growth Rate (RGR) If we plot the logarithmic values of growth in term of size, mass or number versus time duration, a linear profile is obtained. By calculations of slope between any two points on this line we get relative growth rate, which represents the relative change in growth with time. As the growth line is linear, we get the same RGR, irrespective of time interval selection to calculate the slope.

$$RGR = \frac{lnX_2 - lnX_1}{t_2 - t_1} \dots\dots\dots\dots\dots\dots 4$$

RGR Yields constant slope during logarithmic phase for any consecutive value selected.

Doubling Time is the time required to double the quantity of biomass that is growing exponentially.

$$td = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \dots\dots\dots\dots\dots 5$$

Specific growth rate (μ) can be defined as any point during the growth cycle as described earlier. During the exponential growth period is constant.

6.2.2 CO_2 bio-fixation rate

The CO_2 bio fixation rate R_{CO2} ($gL^{-1}d^{-1}$) was calculated according to the method described as

$$R_{CO_2} = C_{carbon} P \left(\frac{M_{CO_2}}{M_c}\right) \ldots\ldots\ldots\ldots 6$$

Where C_{carbon} carbon content of microalgae species *Chlorella vulgaris* was determined using TOC analyzer (Teledyne Tekmar® Torch Combustion TOC/TN Analyzer): M_{CO_2} is the molecular weight of CO_2: M_c is the molecular weight of carbon: P is the biomass productivity as described earlier. The amount of CO_2 to be supplied to the algal culture depends on the efficiency of gas sparging, CO_2 loss from algal culture into ambient atmosphere and CO_2 consumption by algal cells.

Figure 22: TOC Analyzer

6.2.3 Nutrients uptake analysis

Analysis in term of nutrients uptake is made by measuring the nitrate, ammonia, phosphate and COD for the base medium on daily basis using spectrophotometer (DR 3900 Bench-top Spectrophotometer) and digital reactor (DRB200: Digital Reactor)for coking .

As the growth increase, the consumption of these nutrients from the medium indicates the treatment of wastewater component uptake by microalgae. This analysis would be helpful to predict the wastewater treatment potential of microalgae in real process.

Figure 23: DR 3900 Bench-top Spectrophotometer and DRB200: Digital Reactor for coking.

6.2.4 Nitrate (0.2 to 30mg/L NO3-1 - N)

The method used for nitrate analysis is termed as Chromotropic Acid Method and has application in wastewater treatment, described as:

i. Selection of test from Spectrophotometer "344N, Nitrate HR, TNT" for nitrates
ii. Preparation blank sample by adding 1.0mL of sample in "NitrVer X Reagent" test vial
iii. Mixed the sample and placed in cell holder on Spectrophotometer and made zero reading.
iv. Prepared sample using reagent powder pillow and 1.0mL sample to test vial
v. Mixed and set for five minutes and the placed in cell holder to read the values in mg/L.
vi. Required dilution is done to make reading in range of instrument.

6.2.5 Ammonia (0.4 to 50mg/L NH3 - N)

The method used for Ammonia analysis is termed as Salicylate Method and has application in wastewater treatment, described as:

i. Selection of test from Spectrophotometer "343N, Ammonia HR, TNT".
ii. Preparation blank sample by adding 1.0mL of ammonia free water in "AmVer Reagent" test vial.
iii. Prepared sample using adding 1.0mL of sample in "AmVer Reagent" test vial.
iv. Added the contents of one Ammonia Salicylate and Ammonia Cyanurate to each test vial

v. Mixed the sample by and hold for 20minutes and placed blank sample in cell holder on Spectrophotometer and made zero reading.

vi. Placed the sample in cell holder to read the values in mg/L.

vii. Required dilution is done to make reading in range of instrument.

6.2.6 Total phosphate (1.0 to 100mg/L PO43-)

The method used for Phosphate analysis is termed as Molybdovanadate Method with Acid Persulphate Digestion and has application in wastewater treatment, described as:

i. Selection of test from Spectrophotometer "542P, Total HR, TNT".

ii. Preparation blank by adding 5.0mL of deionized water in "Total 'P'" test vial.

iii. Prepared sample using adding 5.0mL of sample in "Total 'P'" test vial.

iv. Added the contents of one Potassium Persulphate to each test vial and shake well

v. Mixed the sample by and hold for 30minutes in DRB200 Reactor at 150 ^{0}C.

vi. Cool the vials and add 2mL of Sodium Hydroxide and 0.5mL of Molybdovanadate.

vii. Let the sample for 7minutes for reaction to take place

viii. Placed blank sample in cell holder on Spectrophotometer and made zero reading.

ix. Placed the sample in cell holder to read the values in mg/L.

x. Required dilution is done to make reading in range of instrument.

6.2.7 COD Analysis (3.0 to 150mg/L COD)

The method used for COD analysis is termed as Reactor Digestion Method and has application in wastewater treatment, described as:

i. Selection of test from Spectrophotometer "430COD LR".

ii. Preparation blank sample by adding 2.0mL of deionized water in COD test vial.

iii. Prepared sample using adding 2.0mL of sample in COD test vial.

iv. Mixed the sample by shaking and hold for 2Hours in DRB200 Reactor at 150 ^{0}C.

v. Let the sample to cool to room temperature.

vi. Placed blank sample in cell holder on Spectrophotometer and made zero reading.

vii. Placed the sample in cell holder to read the values in mg/L.

viii. Required dilution is done to make reading in range of instrument.

CHAPTER 7

RESULTS AND DISCUSSION

Before discussing results in detail first here in Figure 24 we have described the overall experimental process, with reactor types, culture mediums used and compositions of CO_2 mixed with air for feed inputs for cultivation process.

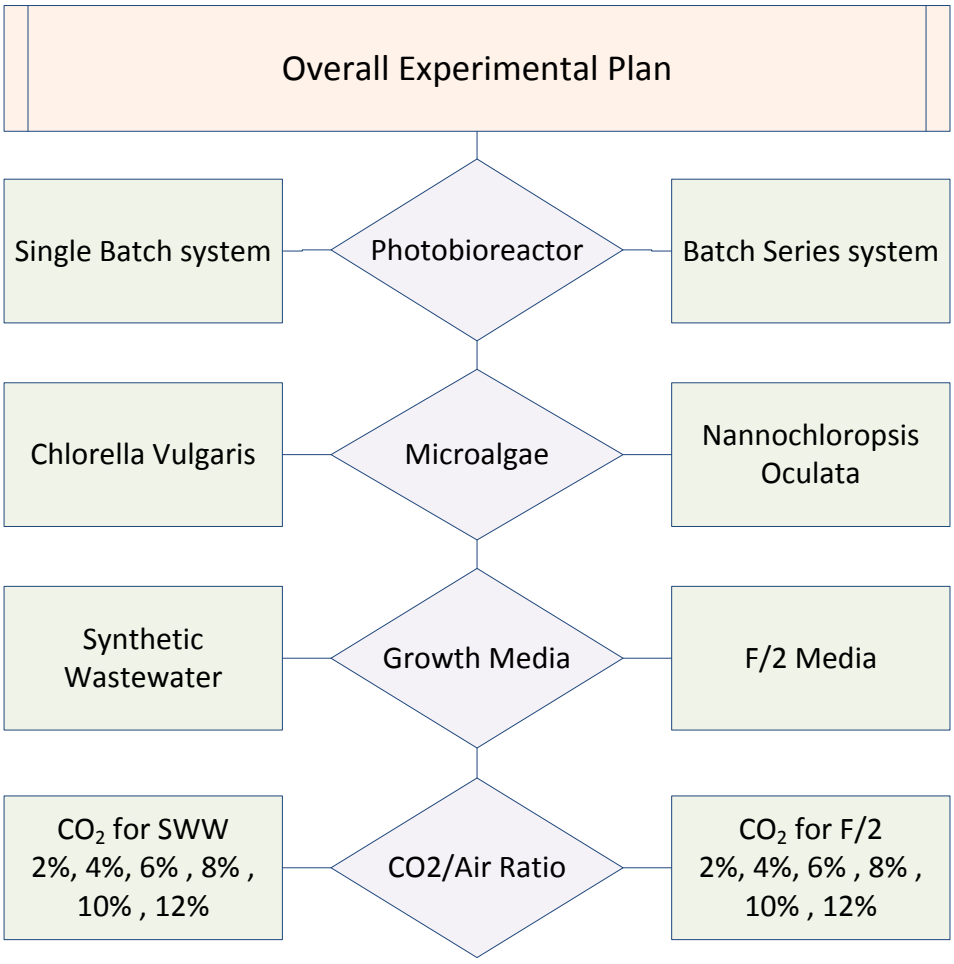


Figure 24: Represents the overall experimental plan for cultivation process.

7.1 *Chlorella vulgaris* growth in F/2 media

Chlorella vulgaris was cultivated in batch photo-bioreactor using F/2 media to see the effect of photoautotrophic growth especially for CO_2 fixation according to recipe give in research methodology appendices. Semi-continuous series cultivation of *Chlorella vulgaris* was also conducted to investigate the potential of CO_2 mitigation in series photo-bioreactor systems. The effect of CO_2 concentration was investigated and rate of CO_2 fixation was compared.

7.1.1 Growth mechanism

The growth of *Chlorella vulgaris* was divided into three regions as shown in the Figure 25. First region is initial growth region called "lag region" specified as "A" in Figure. When the culture is incubated in fresh media, it takes some time for the cells to adjust with new environment during which cell metabolism increases and cell size is increased but cell division is not taking place at this stage. The length of this phase depends on the previous growth media for sample and nutrients concentration in culturing medium. When cells are added from nutrient poor medium to nutrient rich medium then cells adjust with new medium immediately and starts multiplying so lag period is decreased.

The second region of growth is exponential growth or logarithmic growth specified as "B" in Figure. During this region the microalgae cells are rapidly growing and dividing which is the result of their increased metabolic activity rate. During this stage they utilize maximum of their nutrients and food elements which mean more CO_2 utilization. The growth medium concentration is decreased at maximum rate, culture growth rate reaches at maximum value with cells concentration increasing exponentially and cells division

take place according to rule (2, 4, 8……..2n where "n" is the number of generations) which results in balanced growth. The time taken by the microalgae cells to double in number during a specified time period is known as the generation time. The duration of this exponential phase for *Chlorella vulgaris* depends on i) size of inoculum ii) growth rate of cells iii) medium and nutrients concentration iv) CO_2 provision and other culture conditions. As from the Figure 25 it shows that it starts from 5^{th} day and remains until 14^{th} day of culture.

The stationary phase is most important one for nutrients utilization for wastewater treatment, CO_2 consumption rate biomass productivity and cell concentration. The exponential growth rate can be found from plot by taking two points on exponential growth line with corresponding time by using the following relation $\mu = \frac{ln(M_2/M_1)}{t_2 - t_1}$

Where M_2 and M_1 are biomass calculated and t_1 and t_2 is the time of sample measurement in day.

The third region which is known as stationary region is described as part "C" of the curve in Fig.25 for *Chlorella vulgaris*. There are several factors which are considered responsible for stationary growth i) exhaustion of nutrients ii) limiting CO_2 during exponential growth CO_2 supplied is balanced with growth and nutrients consumption while at low cell densities CO_2 lowers the PH growth is depressed iii) light limitation due to self-shading effect of cells iv) accumulation of waste material iv) toxic metabolic compounds. All these factors creates unfavorable culturing conditions for cells as a results their reproduction rate slows down and cells division approaches to cells death, finally growth rate become stabilized as in plot.

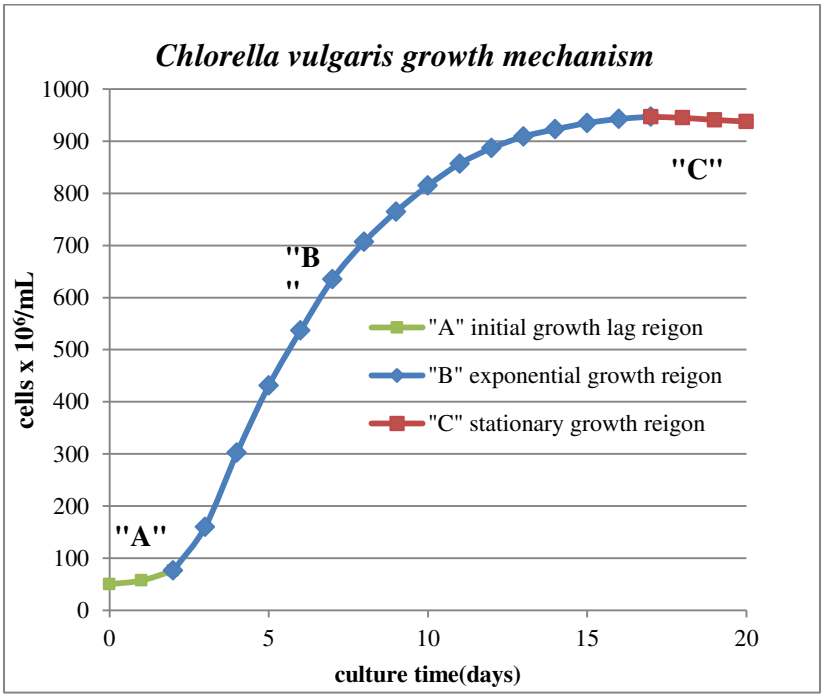


Figure 25: Growth mechanism of *Chlorella vulgaris* in batch photo-bioreactor.

At these points, most of the nutrients are consumed and cells are starving on CO_2 and remaining waste so this is the cells saturation point they can't consume any more CO_2. At this point if cells are not provided with fresh medium they starts to die and biomass is decreased.

7.1.2 Effect of different CO_2 concentration on growth

The investigation of the effect of CO_2 on growth of *Chlorella vulgaris* was studied in batch and series photo-bioreactor cultured for twelve (12) days in temperature range of 20.8^0C to 23.2 ^{0}C and light intensity of 3755-3800 (Lux) with CO_2 concentrations of 2%, 4%, 6%, 8%, 10% and 12% mixed with air with flow rate of 350 cm^3min^{-1}. Sampling was made on daily basis and results were analyzed and growth kinetics was investigated.

As shown in Figure 26 for six different CO_2 concentrations the growth of cells and biomass produced increases rapidly first till it reach a plateau up to this point growth is almost same with little variation of CO_2 percentage between 2%, 4%, 6%, 8%, 10% and 12% but when growth reaches the plateau after that growth rate decreases more rapidly for the culture with lower CO_2 provision which shows that at this growth point cells have still capacity to utilize the CO_2 for growth to produce more biomass but up to this point maximum capacity is reached.

From the Figure *Chlorella vulgaris* was found to grow in higher CO_2 concentrations favorably where growth rate is accelerated by shifting towards higher CO_2 concentration and again decreased when CO_2 concentration exceeds from 4% CO_2. As an example the biomass of *Chlorella vulgaris* increased from 0.58 g L^{-1} to 0.90 g L^{-1} (increment of 55.7%) when CO_2 concentration saturated with air was shifted from 12% to 4% CO_2 and

decreased to 0.83 g L^{-1} when CO_2 concentration shifted from 4% to 2% CO_2. Similar results were found for multiplication of cell concentration. In addition to biomass acceleration, the biomass productivity is also increased from 0.045 $gL^{-1}day^{-1}$ to 0.118 $gL^{-1}day^{-1}$ with an increment of 162.2% when CO_2 concentration shifted from 12% to 4% CO_2 and decreased to 0.098 $gL^{-1}day^{-1}$ when further CO_2 decreased to 2%.

Therefore we can conclude that with higher CO_2 inputs most of the CO_2 is released from photo-bioreactor before having the opportunity to be dissolved in liquid and utilized by microalgae. In other words supply of CO_2 in different concentration ranging from 2%-12% can enhance the dissolved CO_2 but not all of it is dissolved in liquid due to poor solubility of CO_2 in water at atmospheric conditions. When higher amount of CO_2 is applied keeping other conditions same then higher growth in 4% CO_2 shows more favorable to grow for this specific culture in lower CO_2 values. After reaching a specific plateau all of them become constant with similar growth rate.

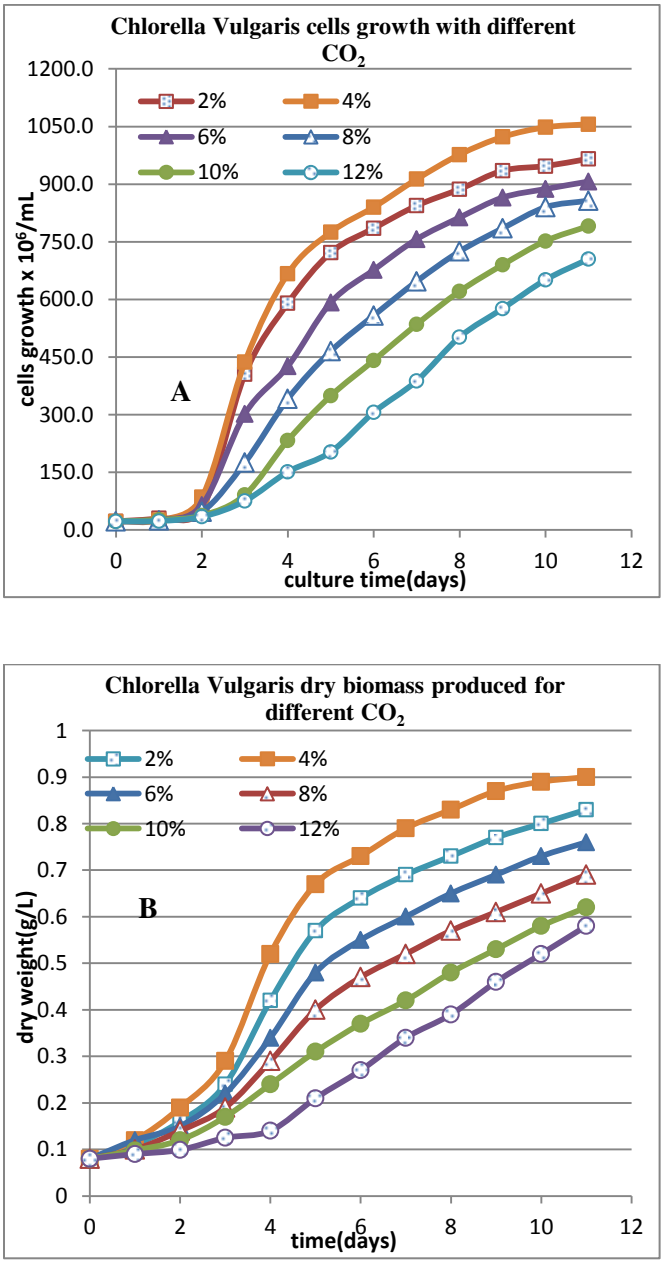


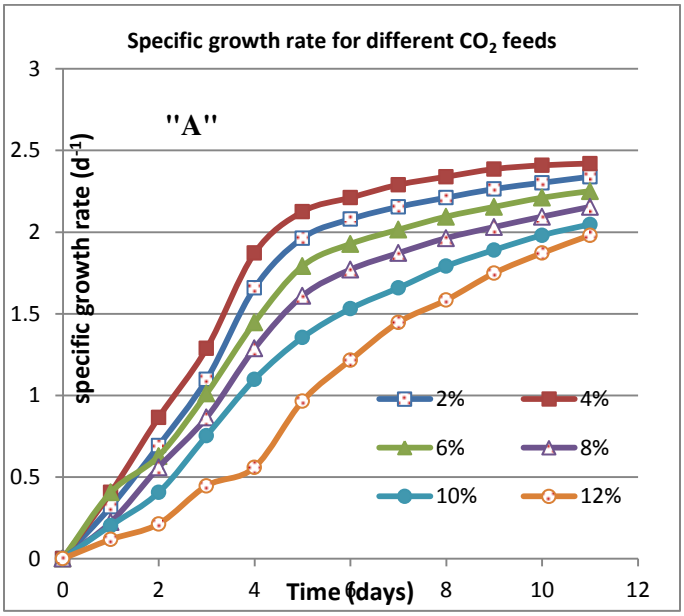
Figure 26: Shows the trends of growth rate of *Chlorella vulgaris* in term of cell concentration (A) and dry biomass (B).

From these observations we can conclude that specified microalgae specie can tolerate higher CO_2 concentration but its growth rate is declined above certain limits. The maximum growth in term of biomass and cell concentration is achieved at 4% CO2 in our case). Also reducing the flow rate of CO_2/Air stream in batch photo-bioreactor could enhance the residence time which increases the CO_2 utilization efficiency.

From the Fig.26 shown above one on dry biomass basis and other on cell concentration shows same trends for same species and CO_2 concentration. When overall omparison is made for chlorella then growth rate of 4%CO_2 is higher which reveals that chlorella has higher CO_2 reduction capacity when lower amount of CO_2 is provided. From these two plots its concluded that we can reduce CO_2 from environment by using this green technology, we can produce biomass which can reduce the cost of CO_2 capture by producing biodiesel and valuable co-products. Growth of *Chlorella vulgaris* also uptakes nutrients from medium in term of nitrates, ammonia and phosphate which are major component of dairy and muncipal wastewater which results in treatment of wastewater.

7.1.3 Specific and relative growth of *Chlorella vulgaris*

The specific and relative growth rates curve can be divided into three regions as shown in the Figure. The first region is where the both specific and relative growth rates are increasing, in the initial phase where there is very small number of cell divisions, growth rate is small while after 4 –5 days when there is sufficient number of cells, they have enough food available in term of nutrients and CO_2 and all the condition are favorable at this time their cell division and growth rate is maximum as shown in the figure 27.



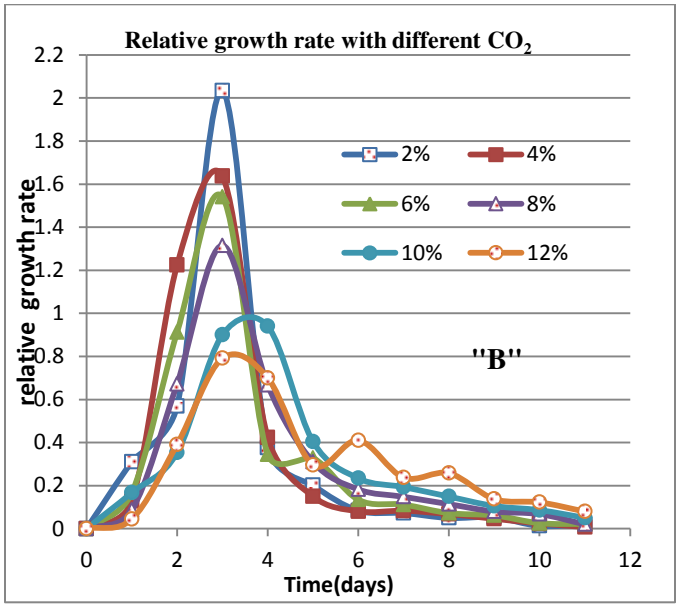


Figure 27: Shows the trends of specific and relative growth rate of *Chlorella vulgaris* at 2%, 4%, 6%, 8%, 10%, and 12% CO_2.

The second region is where the growth rate decreases while the number of cells is still increasing but not at much higher rate as at its peak. Here decline in growth rate could be due to several possible reasons i) due to shortage of food for microorganisms ii) due to self-shading provision of insufficient light for photosynthesis iii) due to increased dying rate of cells. In third region growth rate become negative and number of cells decreases. At this time cells are striving for food and their food become limited only excess CO_2 is present, due to shortage of nutrients cells only survive on CO_2 and eventually they start eating each other and number of cells decreases. At this point productivity is highly affected and culture must be harvested before this declining growth period.

As shown from the Fig.27 the growth rate of *Chlorella vulgaris* increased from 1.98 day^{-1} to 2.42 day^{-1} when CO_2 concentration saturated with air was shifted from 12% to 4% CO_2 and again growth rate is declined to 2.33 day^{-1} with further reduction of CO_2 to 2%. Similar results were found for relative growth rate variation with CO_2 concentrations.

7.1.4 CO_2 bio-fixation rate

Analysis of carbon contents using TOC analyzer showed that presence of carbon contents in *Chlorella vulgaris* did not signaficantly change with different CO_2 concentrations and its value found was 20-22% on average for all the CO_2 concentrations. The CO_2 biofixation rate was determined using the equation described in methodoligy and results for six differents CO_2 concentrations are shown in fig.28. As showen from the Figure Chlorella vulgaris shows higher CO_2 fixation rates under 2% to 4% CO_2 concentrations . the maximum CO_2 biofixation rate found was 0.086 g L^{-1} d^{-1} with CO_2 concentration of 4% at day 5 of culture (fig. 28).

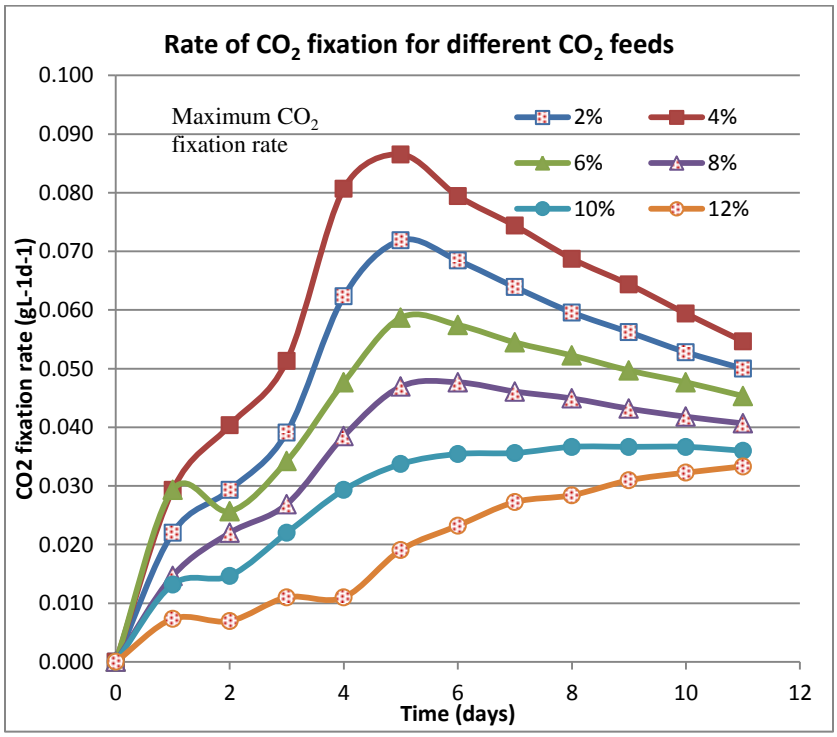


Figure 28: Shows CO_2 biofixation rate with time for different CO_2 concentrations.

Simillar trend of CO_2 biofixation was observed for all CO_2 concentrations. CO_2 fixation rate is first incresed as the growth procedes with msximum growth rate it reaches to maximum values afer that with the decline in growth CO_2 fixation rate is also decresed. Specifically for this the examined microalgae specie showed the promising CO_2 biofixation abilities under different CO_2 concentrations from 2% to 12% CO_2 concentration and performed the best CO_2 fixation at 4% CO_2.

7.1.5 Max. of productivity, CO_2 fixation rate & biomass yield

As it has been investigated the growth of *Chlorella vulgaris* with six different CO_2 operating conditions of 2%, 4%, 6%, 8%, 10% and 12% of CO_2 mixed with air and feed for growth of culture. As growth rate and biomass was measured on daily basis and analysis was made, either higher values of CO_2 is favorable for growth or lower one. Fig.29 shows the results of maximum biomass, productivity and CO_2 bio-fixation rate during operation for each CO_2 concentration value using batch column reactor with continuous CO_2 supply mixed with air at a flow rate of $350cm^3min^{-1}$. From these observations of results it shows increase of defined parameters of growth with increase CO_2 from $_2$% to 4% mixed with air and decline with further CO_2 increment from 4% to 12 %. Overall productivity, biomass and CO_2 fixation rate observed is maximum up to 4% CO_2 exceeding this limit results in decline of growth.

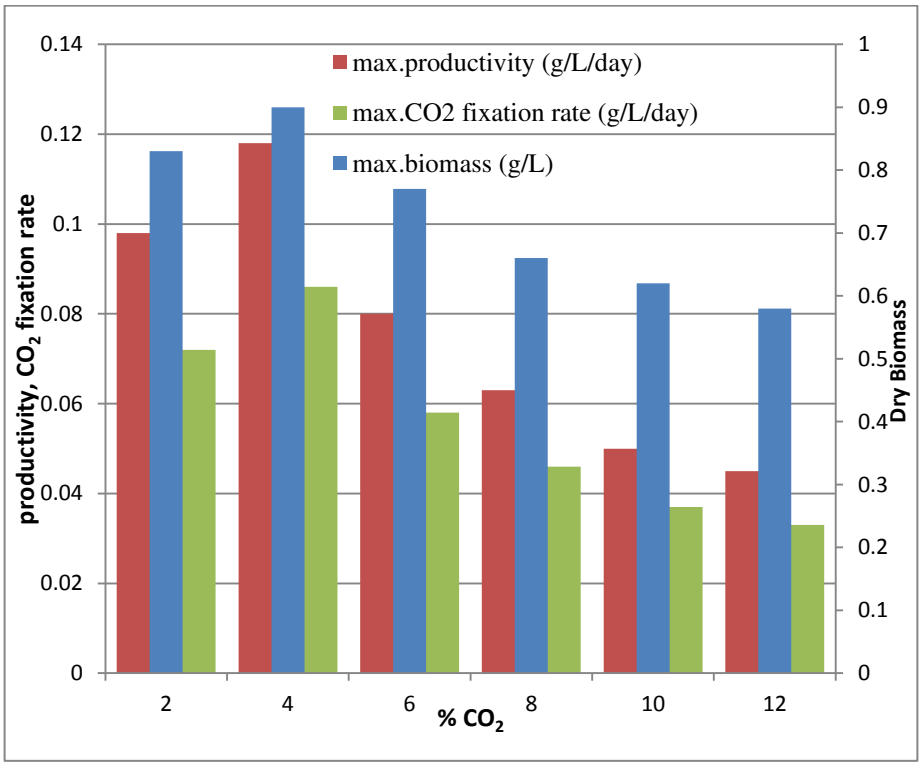


Figure 29: Shows maximum biomass, productivity and CO_2 biofixation rate with shifting CO_2 concentration from 2% - 12% in air mixed stream

Table 5: Maximum biomass, productivity and CO_2 bio fixation rate

CO_2 conc.	Max. Biomass	Biomass Increment	Max. Productivity	Yield Increment	Specific Growth	R_{CO2} fixation
%	X_{max} g L^{-1}	% change	P_{max} g L^{-1} d^{-1}	% change	u_{max} d^{-1}	R_{maxCO2} g L^{-1} d^{-1}
2	0.83	43.10	0.098	117.78	2.33	0.072
4	0.9	55.17	0.118	162.22	2.42	0.086
6	0.77	32.76	0.08	77.78	2.26	0.058
8	0.66	13.79	0.063	40.00	2.11	0.046
10	0.62	6.90	0.05	11.11	2.05	0.037
12	0.58	0.00	0.045	0.00	1.98	0.033

Table.5 represent the maximum biomass, maximum productivity and CO_2 fixation rate with percent increment of these values with shift of CO_2 concentration from 2% - 12%. For the maximum values of biomass produced increment is 55.17% for 4% CO_2 in reference to 12% CO_2 biomass produced and productivity increses 162.22% when CO_2 concentration changed from 12% to 4% in air stream.

From these trends of results predicted from chart and table for *Chlorella vulgaris*, it could be concluded that with the optimization of certain parameters by further research we can make microalgae as practical potential CO_2 mitigation method coupling with other applications like wastewater treatment and making co-products from biomass as extra environmental friendly source of biofuel.

7.1.6 Series photobioreactors analysis

From the results of batch photobioreactor for different CO_2/Air mixing feeds it was concluded maximum CO_2 biofixation and growth was found at 4% CO_2. Using 4% CO_2 growth kinetics and CO_2 biofixation was studied in four series semibatch photobioreactor results are shown in Fig.30 and Table.7. From the Table.7 the results are almost simillar to that for individual batch photobioreactor for 4% CO_2 .

From the Figure it shows that for same inputs feed of CO_2/Air ratio the CO_2 fixation rate becomes four time passing from reactor one to four and biomass produced is also four time more tha single reactor . we can conclude that more reactor in series more the CO_2 fixation and biomass production. This could be made industrial pratical solution by installing reactors in series to improve production and CO_2 fixation efficiency, this in term will reduce the cost of operation as well.

Figure 30: Shows maximum growth achieved in four series photo-bioreactor for *Chlorella vulgaris*

Table 6: Maximum productivity and CO_2 bio fixation rate in series PBRs

Photo-bioreactor	Max. of Biomass	Max. Productivity	Specific Growth	Max. CO_2 fixation rate
No.	X_{max} g L^{-1}	P_{max} g L^{-1} d^{-1}	u_{max} d^{-1}	R_{max} g L^{-1} d^{-1}
1	0.93	0.084	2.45	0.062
2	0.91	0.079	2.42	0.058
3	0.9	0.08	2.43	0.057
4	0.87	0.071	2.39	0.053
Average	0.9025	0.0785	2.4225	0.0575

7.1.7 Effects of pH changes with CO_2 Concentration variation

The photoautotrophic growth of the microalgae needs important factors as: light, nutrients as N, P, Fe, etc. and a carbon source, usually carbon dioxide. When the microalgae uptake CO_2 and nitrates, the pH of the culture increases (Hulatt and Thomas, 2011)

Mostly microalgae are found to grow in neutral pH whereas some of species are found to be tolerant to higher and few for lower pH range. The relation between pH and CO_2 concentration is complex in photo-bioreactors, due to chemical equilibrium in the species CO_2, H_2CO_2, HCO_3, and CO_3^{-2}. Productivity could be increased by higher CO_2 concentrations but pH will be decreased and physiology will be affected. The increase in pH can be beneficial for inactivation of pathogens in microalgae wastewater treatment, but can also inhibit microalgae growth. Similarly, the speciation of NH_3 and NH_4^+ in microalgae bioreactors is strongly dependent on pH – NH_3 uncouples electron transport in the microalgae photosystem and competes with water molecules in oxidation reactions, thus leading to release of O_2.

During the growth time in the presence of light microalgae uptakes CO_2 from water during photosynthesis promoting cell growth of algae. Removal of CO_2 from water results in reduction of carbonate and bicarbonate from medium which increases the pH level of medium. This depletion of inorganic carbon from medium by algae results in higher pH values, which is evidenced from the plots of pH given below.

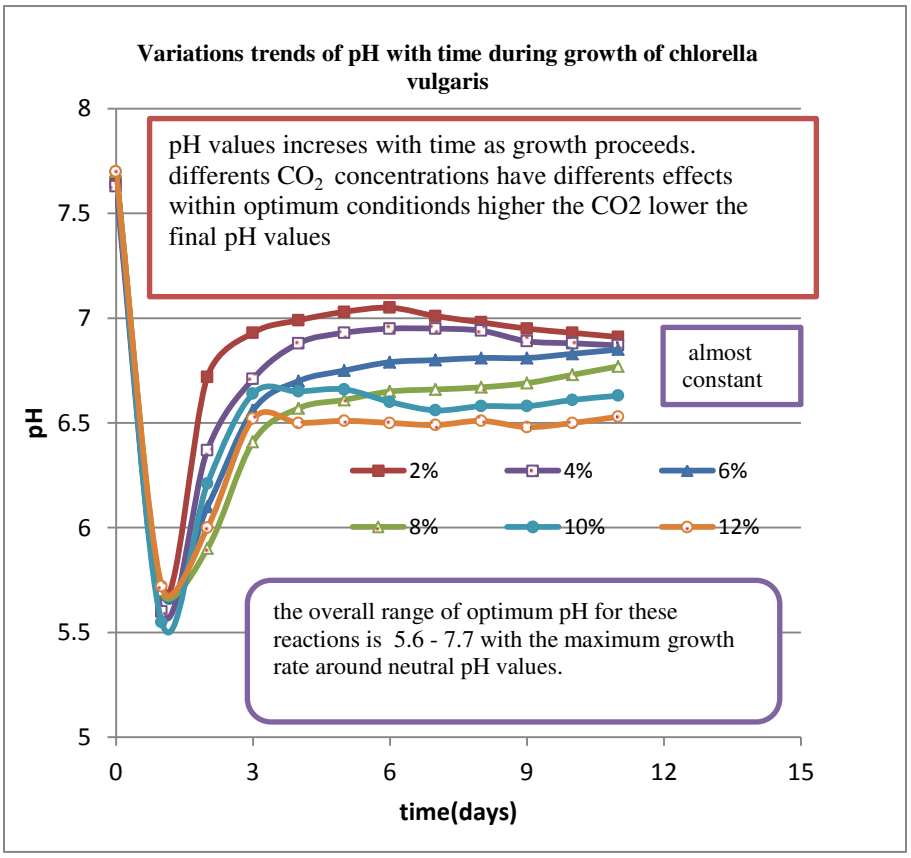


Figure 31: Effect of pH on growth rate and CO_2 fixation with changing CO_2 concentration

In the case of pH changes first pH of medium is decreased sharply according to amount of CO2 injected which is dissolved in medium with no utilization at initial phase. As the growth of cells starts abrupt increasing trend is observed, with passage of time as pH decreases and ultimately it becomes constant when growth reaches its plateau where there is no further significant growth. The below equation represents the different CO_2 form in water available for *Chlorella vulgaris*

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{-2}$$

Equation shows the different forms of CO_2 that can be available in water, the amount of each form that can be found in a solution at any given time will depend greatly on the pH of the solution. In a poorly buffered system, the absorption of CO_2 by the growing microalgae causes a shift on the equilibrium shown on equation which results in an increase of pH values due to the excretion of OH^- by the algae into the media. It is important to maintain pH within an adequate range to avoid the loss of carbon dioxide present in the media.

7.1.8 Concluding remarks on *Chlorella vulgaris* growth in F/2 Media

Semi-continuous and batch culture of *Chlorella vulgaris* was conducted to investigate the potential of CO_2 mitigation in the photo-bioreactor systems. Prior to operation of reactor with our culture, photo-bioreactor was tested with 2%, 4%, 6%, 8%, 10%, and 12% CO_2 without microalgae sample to ensure non-presence of any abiotic factor for CO_2 removal. During this test CO_2 concentrations measured at inlet and outlet were same values ensuring that no any biotic factor was involved in CO_2 reduction mechanism. The amount of CO_2 fixation mixed with air in different concentrations was investigated in batch and

semi-continuous photo-bioreactor during a period of 13-days operation. All the experiments performed for each run and on each day influent and effluent CO_2 concentrations were consistent and showed the similar pattern. The overall CO_2 removal efficiency was maximum at 4% CO_2 mixed in air stream. Also increasing the residence time of CO_2 in photo-bioreactor could also enhance the CO_2 reduction efficiency significantly. Therefore efficiency of CO_2 removal could be increased by increasing the retention time of air in the photo-bioreactor.

The pH of the medium without microalgae culture is around 7 when culture was incubated with microalgae sample and CO_2 with different concentration was fed to the culture its pH drops due to CO_2 absorption in aqueous phase, with time it again increases as the growth is started to increase. Overall average of pH for 13-days operation is in decreasing order with increasing order of CO_2 % mixed with air but this decrease is not significant which showed that aqueous CO_2 dissolved in medium is almost constant it could not dissolve any further increase in CO_2 inlet stream beyond 4% of CO_2 as indicated from the results. This showed that most of the CO_2 at influent directly flowed out of the culture medium reactor when CO_2 concentration was increased beyond 4% of CO_2 in air mixed stream.

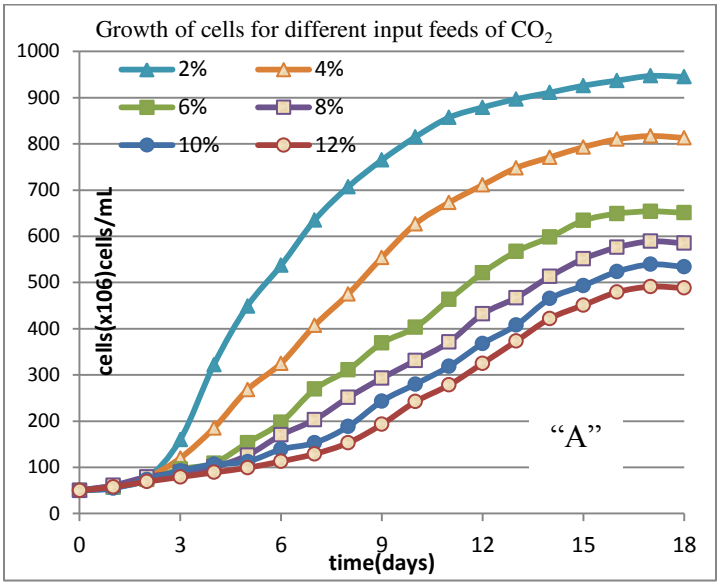
The efficiency of CO_2 fixation in closed photo-bioreactor system is mainly dependent on the following i: microalgae species ii: CO_2 concentration iii: medium selection iv: photo-bioreactor selection v: residence time of gas streams in culture (Cheng et al., 2006). The dependence of CO_2 fixation on microalgae species may be due to the potential of growth of that specie and CO_2 metabolism activity.

7.2 Results of *Nannochloropsis Oculata* in F/2 medium

Batch culture of *Nannochloropsis Oculata* was conducted to investigate the potential of CO_2 mitigation in the photo-bioreactor systems. Prior to operation of reactor with our culture, photo-bioreactor was tested with 2% CO_2 without microalgae sample to ensure non-presence of any abiotic factor for CO_2 removal. During this test CO_2 concentrations measured at inlet and outlet were same values ensuring that no any biotic factor was involved in CO_2 reduction mechanism. All the experiments performed for each run and on each day influent and effluent CO_2 concentrations were consistent and showed the similar pattern.

7.2.1 Effect of different CO_2 concentration on growth

To investigate the effect of CO_2 on growth of *Nannochloropsis Oculata* was studied in batch reactor culture for eighteen (18) days in CO_2 concentrations of 2%, 4%, 6%, 8%, 10% and 12% mixed with air at flow rate of 350 cm^3min^{-1}. Fig.32 shows the effect of CO_2 concentration towards the cell growth and biomass yield of *Nannochloropsis Oculata*. From the Figure *Nannochloropsis Oculata* was found to grow in lower CO_2 concentrations favorably where growth rate is accelerated by shifting towards lower CO_2 concentration.



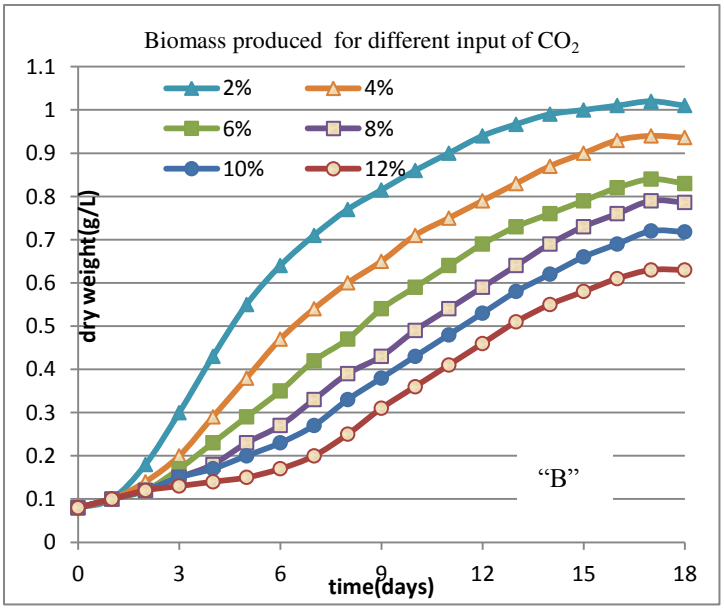


Figure 32: Shows the trend of growth kinetics "A" represents the cell concentration and "B" represents the dry biomass

As an example the growth rate of *Nannochloropsis Oculata* increased from 2.05 day^{-1} to 2.54 day^{-1} (increment of 23.9%) when CO_2 concentration saturated with air was shifted from 12% to 2% CO_2. Similar results were found for multiplication of cell concentration. In addition to growth rate acceleration, the biomass productivity is also increased from 0.034 $mgL^{-1}day^{-1}$ to 0.094 $mgL^{-1}day^{-1}$ with an increment of 180% when CO_2 concentration shifted from 2% to 12% CO_2.

From these observations we can conclude that specified microalgae species can tolerate higher CO_2 concentration but its growth rate is declined. The maximum growth in term of biomass and cell concentration can be achieved with lower CO_2 percentage (2% in our case). Also reducing the flow rate of CO_2/Air stream in batch photo-bioreactor could enhance the residence time which increases the CO_2 utilization efficiency.

Therefore we can conclude that with higher CO_2 inputs most of the CO_2 is released from photobioreactor before having the opportunity to be dissolved in liquid and utilized by microalgae. In other words supply of CO_2 in different concentration ranging from 2%-12% can enhance the dissolved CO_2 but not all of it is dissolved in liquid due to poor solubility of CO_2 in water at atmospheric conditions.

7.2.2 CO_2 bio-fixation rate

Analysis of carbon contents using TOC analyzer showed that presence of carbon contents in *Nannochloropsis Oculata* did not signaficantly change with different CO_2 concentrations and its value found was 23-27% on average for all the CO_2 concentrations. The CO_2 biofixation rate was determined using the equation described in methodoligy and results for six differents CO_2 concentrations are shown in fig.33. As showen from the Figure *Nannochloropsis Oculata* shows higher CO_2 fixation rates under 2% to 4% CO_2 concentrations .

The maximum CO_2 biofixation rate found was 0.086 g L^{-1} d^{-1} with CO_2 concentration of 2% at day 5 of culture (Figure 33). Simillar trend of CO_2 biofixation was observed for all CO_2 concentrations. CO_2 fixation rate is first incresed as the growth procedes with msximum growth rate it reaches to maximum values afer that with the decline in growth CO_2 fixation is also decresed. Specifically for this the examined microalgae specie showed the promising CO_2 biofixation abilities under different CO_2 concentrations from 2% to 12% CO_2 concentration and performed the best CO_2 fixation at 2% CO_2.

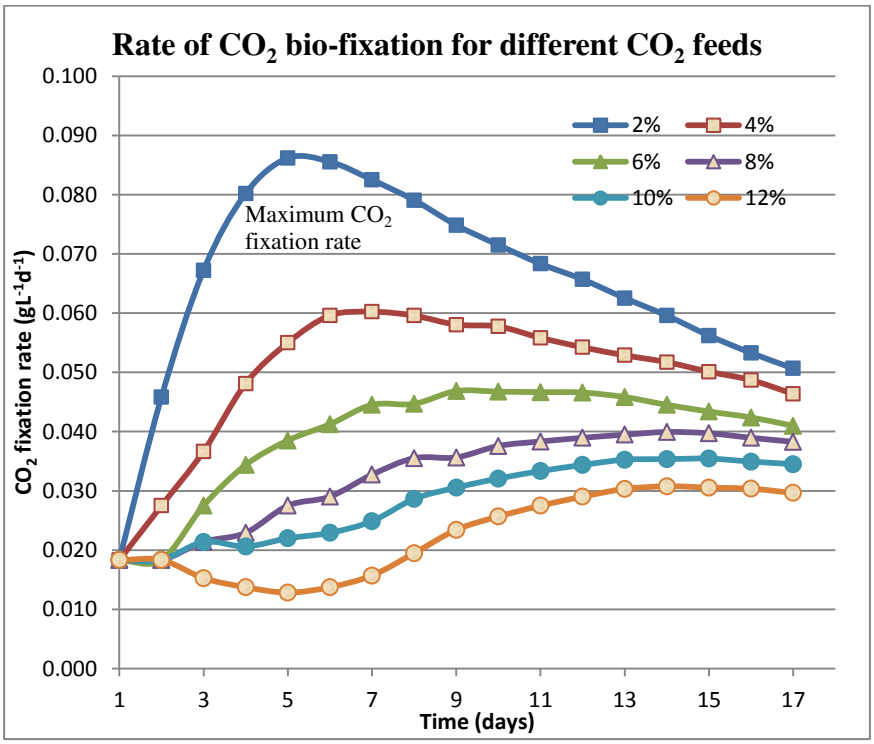


Figure 33: Shows the CO_2 biofixation rate with time for different CO_2 concentrations.

7.2.3 Max. of productivity, CO_2 fixation rate & biomass yield

As it has been investigated the growth of *Nannochloropsis Oculata* with six different CO_2 operating conditions of 2%, 4%, 6%, 8%, 10% and 12% of CO_2 mixed with air for growth of culture. As growth rate and biomass was measured on daily basis and analysis was made, to analyze either higher values of CO_2 is favorable for growth or lower one. Figure 3 shows the results of maximum biomass, productivity and CO_2 bio-fixation rate during operation for each CO_2 concentration value using batch column reactor with continuous CO_2 supply mixed with air at a flow rate of 350cm^3/min . From these observations of results it shows decline with increase CO_2 from 2% to 12% mixed with air. Overall productivity, biomass and CO_2 fixation rate observed is maximum up to 2% CO_2 exceeding this limit results in decline of growth.

Table 7 represent the maximum biomass, maximum productivity and CO_2 fixation rate with percent increment of these values with shift of CO_2 concentration from 2% - 12%. For the maximum values of biomass produced increment is 61.9% for 2% CO_2 in reference to 12% CO_2 biomass produced and productivity increses 176.47% when CO_2 concentration changed from 12% to 2% in air stream. From these trends of results predicted from chart and table for *Nannochloropsis Oculata,* it could be concluded that with the optimization of certain parameters by further research we can make microalgae as practical potential CO_2 mitigation method with other wastewater treatment and co-products from biomass as extra environmental friendly source of biofuel.

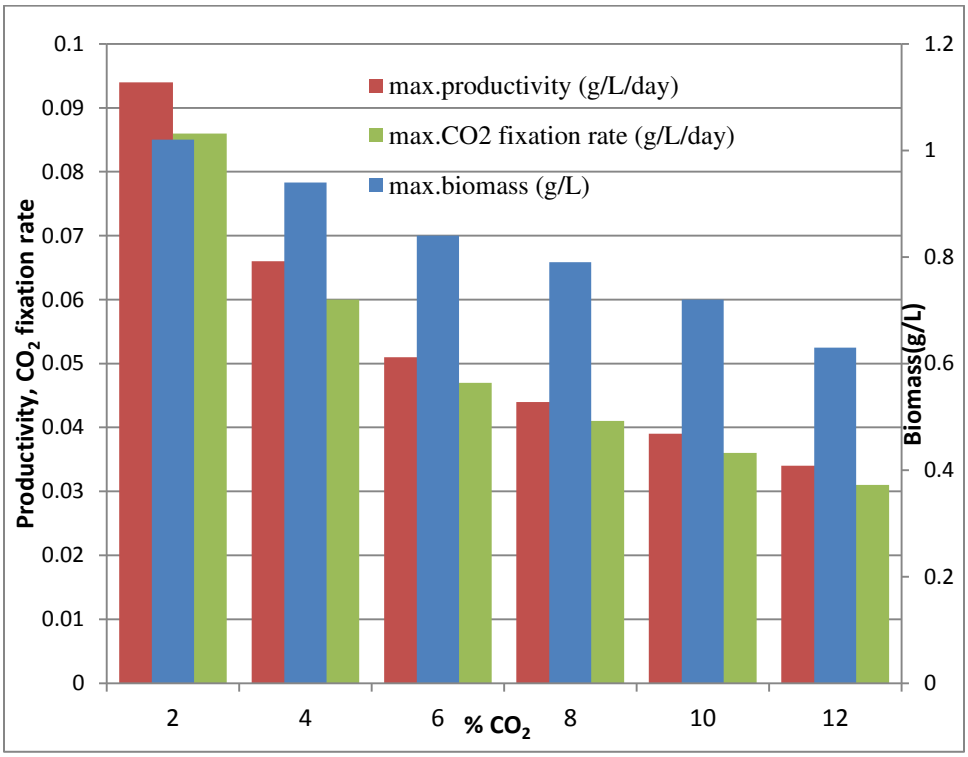


Figure 34: Shows the maximum growth and CO_2 biofixation rate achieved for *Nannochloropsis Oculata* with different CO_2 input feeds.

Table 7 Maximum productivity and CO_2 fixation rate for *Nannochloropsis Oculata*

CO_2 conc.	**Max. Biomass**	**Biomass Increment**	**Max. Productivity**	**Yield Increment**	**Specific Growth**	**R_{CO2} fixation**
%	X_{max} g L^{-1}	% change	P_{max} g L^{-1} d^{-1}	% change	R_{max} d^{-1}	g L^{-1} d^{-1}
2	1.02	61.90	0.094	176.47	2.544	0.086
4	0.94	49.21	0.066	94.12	2.46	0.06
6	0.84	33.33	0.051	50.00	2.35	0.047
8	0.79	25.40	0.044	29.41	2.28	0.041
10	0.72	14.29	0.039	14.71	2.19	0.036
12	0.63	0.00	0.034	0.00	2.06	0.031

7.2.4 Concluding remarks on N. Oculata growth in F/2 Media

From these results it is concluded that CO_2 can be reduced by growing different microalgae species with different CO_2 compositions which reveals that biological CO_2 fixation in the countries like Saudi Arabia which has vast CO_2 production sources like power plants, wastewater treatment plants as source of nutrients for microalgae and round the year sunlight and coastal area of different water compositions is more viable and economical towards the green and CO_2 free environment.

From these experimental results for experimental specie of microalgae it is clear that it has CO_2 fixation potential and increasing growth rates within specified optimal range of temperature, light intensity and in controlled pH conditions. In this case of *Nannochloropsis Oculata* its shows more growth with lower CO_2 mixture with air as compared to higher CO_2 values. For investigations of more efficient CO_2 reduction species specifically used for these experiments it's clearly identified that *Nannochloropsis Oculata* has CO_2 reduction potential with biomass production which leads to valuable products. From these experiments we conclude that biological CO_2 reduction is a potential field of technology with its additional advantages of green fuel production from dry biomass and waste water treatment as well.

7.2.5 Comparison of C. vulgaris and N. Oculata in F/2 media

From the comparison results of *Chlorella vulgaris* and *Nannochloropsis Oculata* in both grown in F/2 Media, the biomass productivity is higher in case of *Chlorella vulgaris* and most importantly it's utilizing more CO_2 which is our key objective study. Concluding it more sustainable to CO_2 concentrations and fluctuations of pH values associated with CO_2 concentration variation.

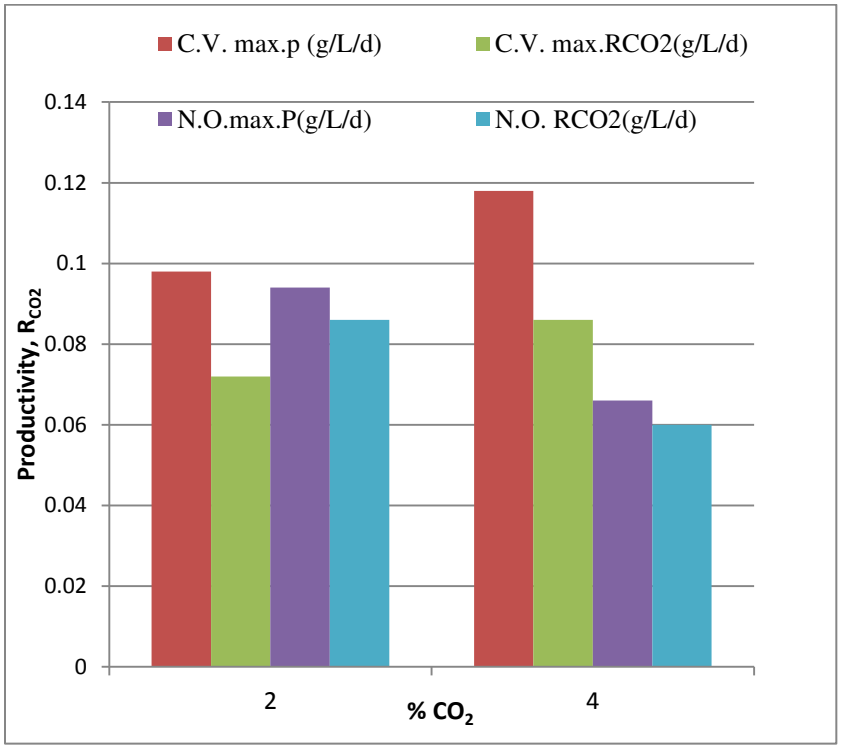


Figure 35: Comparison of *Chlorella vulgaris* and *Nannochloropsis Oculata* in F/2 Media

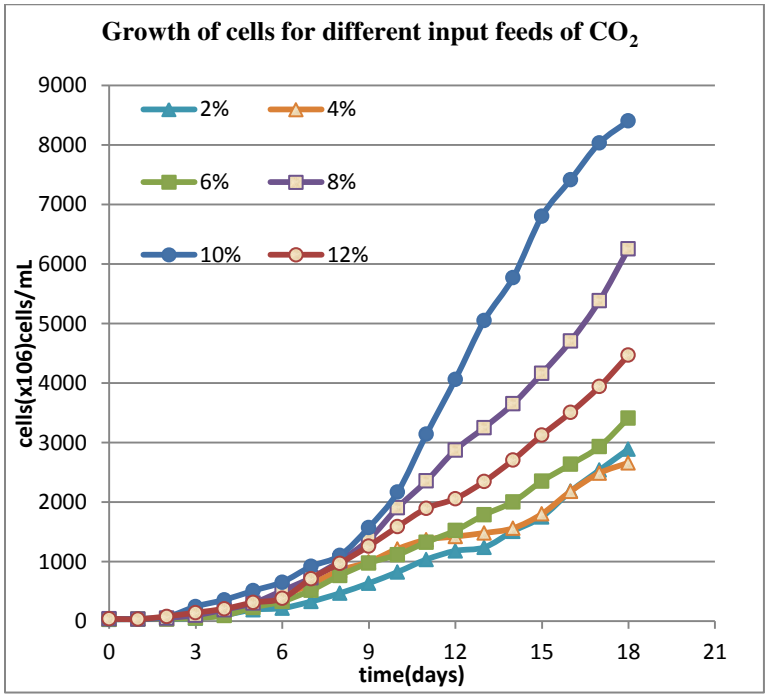
7.3 *Chlorella vulgaris* growth in SWWM

Chlorella vulgaris was cultivated in batch photo-bioreactor using SWW Media modified with organic carbon source to see the effect of mixotrophic growth especially for wastewater treatment according to recipe give in research methodology appendices. The effect of CO_2 concentration was investigated and rate of CO_2 fixation was compared as well as uptake of nutrients for wastewater treatment purpose. It was assumed that presence of organic carbon source may help the treatment of wastewater rich in organic carbon source such as dairy wastewater and municipal wastewater.

7.3.1 Effect of different CO_2 concentration on growth

Chlorella vulgaris was cultivated in Modified Bolds Bessel Media at room temperature under different CO_2 concentrations. The initial concentration of inoculum was (31, 34, 37 cells/mL), 0.08 g/L dry biomass and pH was 6.84 in initial medium. The effect of CO_2 concentration on growth of *Chlorella vulgaris* in term of cell concentration and dry biomass production with time is shown in the Figure 36.

The investigation of the effect of CO_2 on growth of *Chlorella vulgaris* was studied in batch and series photo-bioreactor cultured for twelve (12) days in temperature range of 20.8^0C to 23.2 ^{0}C and light intensity of 3755-3800 (Lux) with CO_2 concentrations of 2%, 4%, 6%, 8%, 10% and 12% mixed with air with flow rate of 350 cm^3min^{-1} . The culture was grown to stationary phase around 20days period of cultivation.



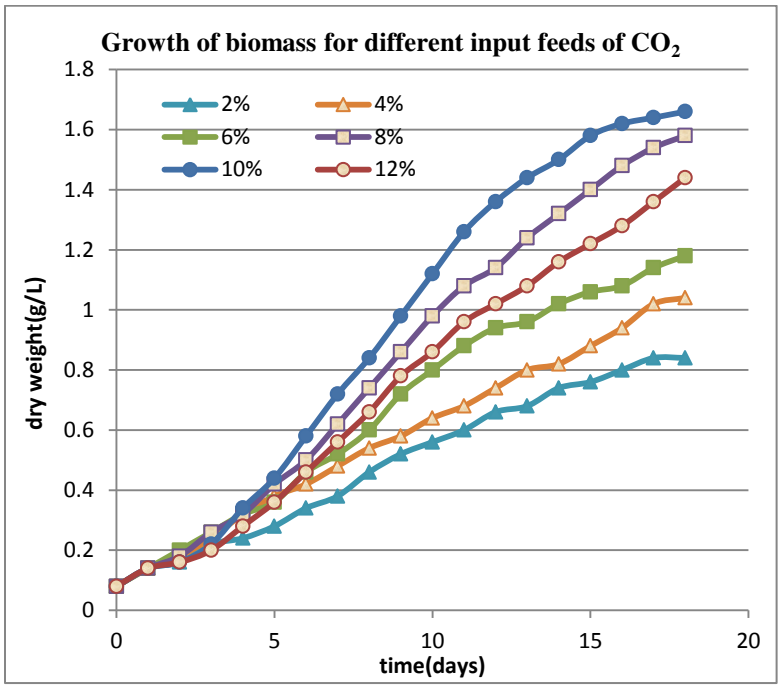


Figure 36: Shows the trends of cell concentration and dry biomass produced in SWWM medium

From the Figure 36 *Chlorella vulgaris* was found to grow in higher CO_2 concentrations favorably where growth rate is accelerated by shifting towards higher CO_2 concentration and again decreased when CO_2 concentration exceeds from 10% CO_2. As an example the biomass of *Chlorella vulgaris* increased from 1.04 g L^{-1} to 1.66 g L^{-1} (increment of 59.2%) when CO_2 concentration saturated with air was shifted from 2% to 10% CO_2 and decreased to 1.44 g L^{-1} when CO_2 concentration shifted from 10% to 12% CO_2. Similar results were found for multiplication of cell concentration as shown in the Figure.

For these experiments it showed promising growth results of *Chlorella vulgaris* in higher CO_2 concentrations of 8%, 10% and 12 %. In addition to biomass acceleration, the biomass productivity is also increased from 0.050 $gL^{-1}day^{-1}$ to 0.107 $gL^{-1}day^{-1}$ with an increment of 78.79% when CO_2 concentration shifted from 2% to 10% CO_2 and decreased to 0.078 $gL^{-1}day^{-1}$ when further CO_2 increased to 12%. The optimum productivity obtained was 0.091 $gL^{-1}day^{-1,}$ 0.107 $gL^{-1}day^{-1}$ and 0.078 $gL^{-1}day^{-1}$ at 8%, 10% and 12 % CO_2 concentrations respectively.

From these observations we can conclude that specified microalgae specie can tolerate higher CO_2 concentration but its growth rate is declined above certain limits. The maximum growth in term of biomass and cell concentration can be achieved with higher CO_2 percentage (10% in our case). Also reducing the flow rate of CO_2/Air stream in batch photo-bioreactor could enhance the residence time which increases the CO_2 utilization efficiency.

7.3.2 Specific growth rate for different CO_2 concentration

Chlorella vulgaris growth in SWW Media was studied for different CO_2 concentrations. Culture was sampled on daily basis until a stationary phase was reached and further reasonable growth was not observed. Specific growth rate was calculated for each batch of culture for CO_2 variation from 2-12% and result is shown in the Figure 37

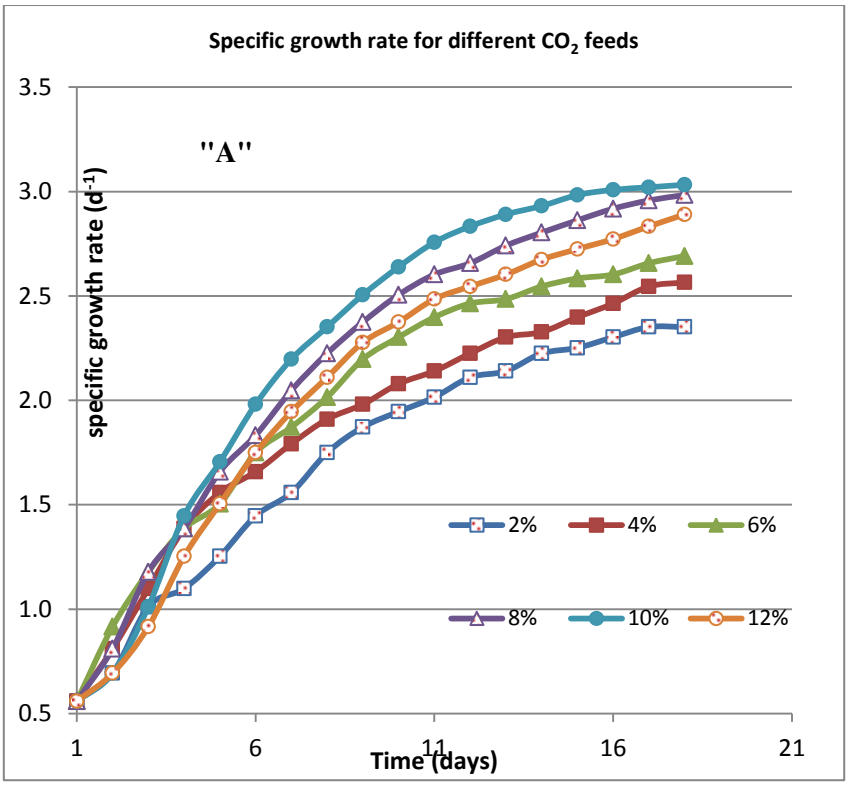


Figure 37: Shows specific growth rate cultivation in SWWM medium

The specific growth rate curve can be divided into different regions as shown in the Figure. The first region is where the both specific growth rates is increasing, in the initial phase where there is very small number of cell divisions, growth rate is small while after 4 – 5 days when there is sufficient number of cells, they have enough food available in term of nutrients and CO_2 and all the condition are favorable at this time their cell division is at its climax and growth rate is maximum after that growth rate becomes constant and finally decreases.

As shown from the Figure the specific growth rates for exponential growth phase found were 2.56. 2.69, 2.3, 2.98, 3.03 and 2.89 day^{-1} in 2, 4, 6, 8, 10, and 12% CO_2 mixed with air used for cultivation in SWWM medium. These results show that at higher CO_2 concentration microbial cells grow faster without any stress. The specific growth rate of *Chlorella vulgaris* increased from 2.56 day^{-1} to 3.03 day^{-1} when CO_2 concentration saturated with air was shifted from 2% to 10% CO_2 and again growth rate is declined to 2.89 day^{-1} with further increased CO_2 concentration to 12% and still these values are very high which indicate the growth potential of *Chlorella vulgaris* in higher CO_2 concentrations.

7.3.3 CO_2 bio-fixation rate for different CO_2 concentration

Batch culture of *Chlorella vulgaris* was conducted to investigate the potential of CO_2 mitigation in the photo-bioreactor systems. Prior to operation of reactor with our culture, photo-bioreactor was tested with 2% CO_2 without microalgae sample to ensure non-presence of any abiotic factor for CO_2 removal. During this test CO_2 concentrations measured at inlet and outlet were same values ensuring that no any biotic factor was involved in CO_2 reduction mechanism. The amount of CO_2 fixation mixed with air in

different concentrations was investigated in batch photo-bioreactor during a period of 18-days operation.

Analysis of carbon contents using TOC analyzer showed that presence of carbon contents in *Chlorella vulgaris* did not signaficantly change with different CO_2 concentrations and its value found was 20-22% on average for all the CO_2 concentrations. The CO_2 biofixation rate was determined using the equation described in methodoligy and results for six differents CO_2 concentrations are shown in Figure 38.

As showen from the Figure *Chlorella vulgaris* shows higher CO_2 fixation rates under 8% to 12% CO_2 concentrations . the maximum CO_2 biofixation rate found was 0.0983 g L^{-1} d^{-1} with CO_2 concentration of 10% at day 11 of culture as shown in the Figure. Simillar trend of CO_2 biofixation was observed for all CO_2 concentrations. CO_2 fixation rate is first incresed as the growth procedes with msximum growth rate it reaches to maximum values afer that with the decline in growth, CO_2 fixation rate is also decresed. Specifically for this the examined microalgae specie showed the promising CO_2 biofixation abilities under different CO_2 concentrations from 2% to 12% CO_2 concentration and performed the best CO_2 fixation at 10% CO_2.

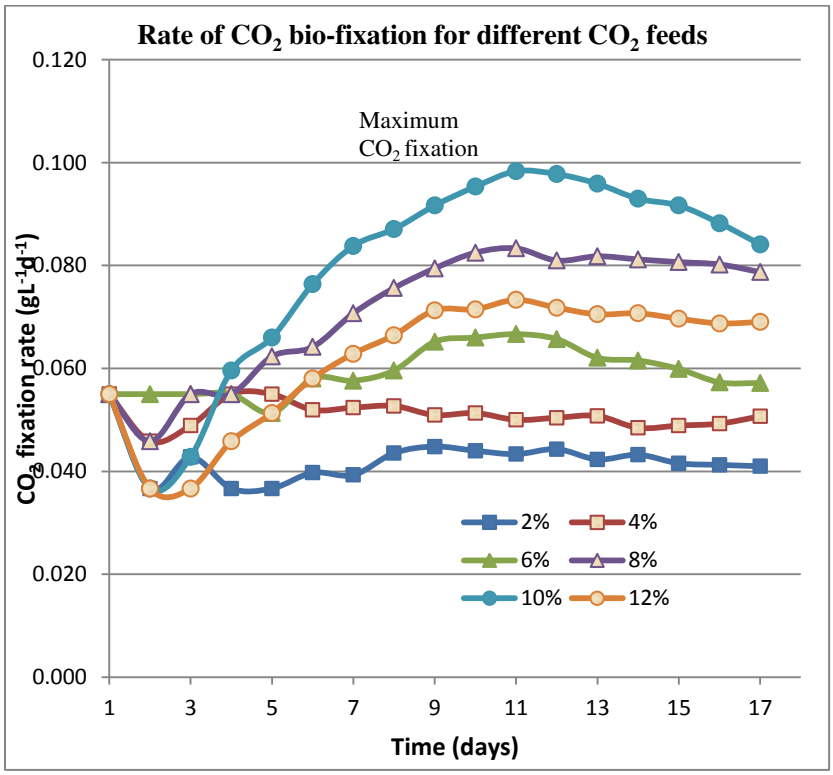


Figure 38: Shows the CO_2 biofixation rate in different CO_2 concentrations

7.3.4 Max. of productivity, CO_2 fixation rate & biomass yield

Analysis of carbon content as determined using TOC showed that carbon contents did not vary significantly with CO_2 concentration and bio-fixation rate was calculated using equation as defined in methodology. Figure 39 shows the results of maximum biomass, productivity and CO_2 bio-fixation rate during operation for each CO_2 concentration value using batch column reactor with continuous CO_2 supply mixed with air at a flow rate of $350 cm^3 min^{-1}$.

From these observations of results it shows increase of defined parameters of growth with increase CO_2 from 2% to 4% and 8% to 12% mixed with air and decline with further CO_2 increment from 4% to 6 % and 10% to 12%. Overall productivity, biomass and CO_2 fixation rate observed is maximum up to 12% CO_2 exceeding this limit results in decline of growth.

Also Table 8 represent the maximum biomass, maximum productivity and CO_2 fixation rate with percent increment of these values with shift of CO_2 concentration from 2% - 12%. For the maximum values of biomass produced increment is 59.2% for 10% CO_2 in reference to 2% CO_2 biomass produced and productivity increses 78.78% when CO_2 concentration changed from 2% to 10% in air stream and with further increse in CO_2 concentration to 12% productivity is decresed 30.56% of value at 2% CO_2.

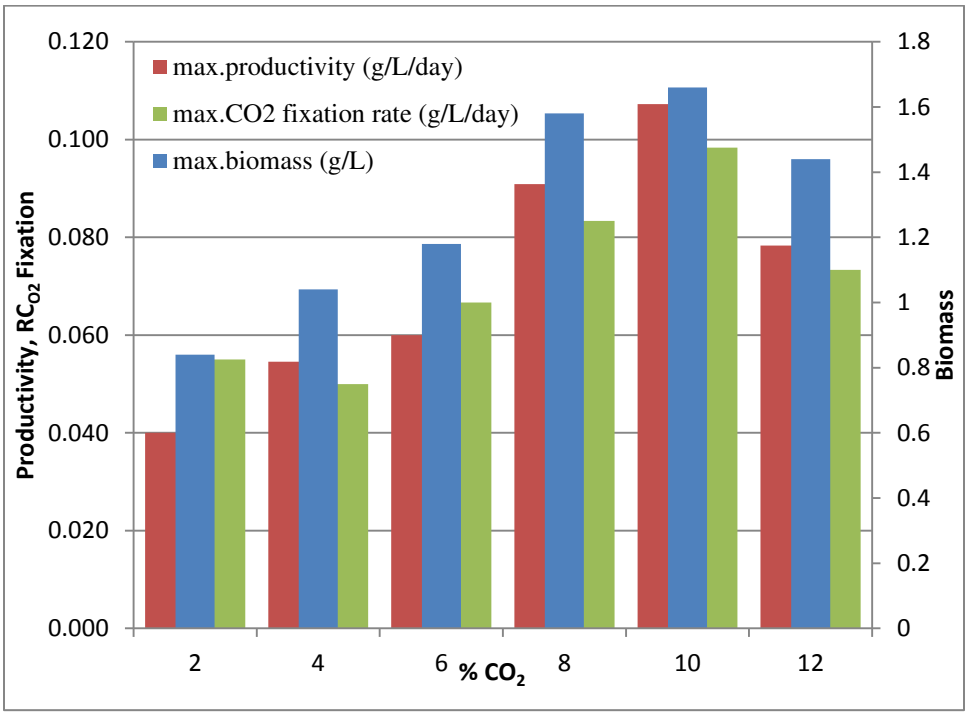


Figure 39: Shows the maximum growth achieved in SWWM media

Table 8: Maximum productivity and CO_2 bio fixation rate for C.V in SWWM

CO_2 conc.	**Max. Biomass**	**Biomass Increment**	**Max. Productivity**	**Yield Increment**	**Specific Growth Rate**	**R_{CO2} fixation**
%	X_{max} g L^{-1}	% change	P_{max} g L^{-1} d^{-1}	% change	R_{max} d^{-1}	R_{maxCO2} g L^{-1} d^{-1}
2	0.84	0.00	0.040	0.00	2.3514	0.055
4	1.04	23.81	0.055	36.36	2.5649	0.0500
6	1.18	40.48	0.060	50.00	2.6912	0.0667
8	1.58	88.10	0.091	127.27	2.9832	0.0833
10	1.66	97.62	0.107	168.18	3.0325	0.0983
12	1.44	71.43	0.078	95.83	2.8904	0.0733

7.3.5 Nutrients uptake analysis

As its well-known that wastewater is rich in organic and inorganic nutrients which could be removing for treatment purpose to produce cleaner waster for different uses. The process of wastewater treatment could reduce the freshwater and nutrients requirement which in term reduces the cost of CO_2 capture and biomass production. As we have seen in synthetic wastewater two types of growth mechanisms are involved photoautotrophic using inorganic and heterotrophic utilizing organic carbon and nutrients to produce higher biomass production rates.

To find out the wastewater treatment analysis and utilization of nutrients and their effects on growth rates nutrients analysis was made and results are shown in the Figure. As clearly from the Figure the uptake of nitrogen source is almost reached to zero level at the end of cultivation period. Initially nitrogen present in the synthetic wastewater in term of nitrates was 49.2 mg/L which is decreased to 1.5 mg/L during a course of 21 days, while in term of ammonia it was initially 187mg/L and reduced to 0.0 mg/L showing 100% removal efficiency.

The removal and utilization of phosphate was done relatively low from 189mg/L to 125 mg/L during cultivation period it could be utilized more by allowing more time for cultivation or adjusting the concentration and composition of medium. In case of COD its value is first decreased from 162mg/L to 73mg/L and then gradually increases due to the presence of organic carbon source heterotrophic growth starts which in term utilize organic sources by decomposing and COD starts increasing from 73mg/L to 141mg/L at the time of harvesting.

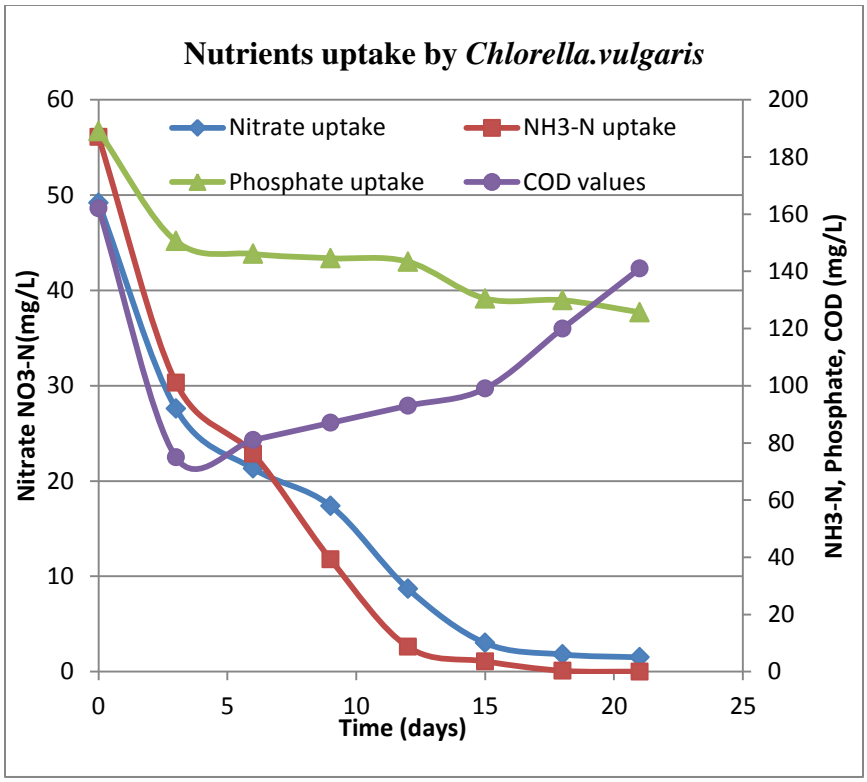


Figure 40: Shows maximum nutrients uptake from synthetic wastewater

7.3.6 Concluding remarks on *Chlorella vulgaris* growth in SWW media

The maximum growth in term of biomass and cell concentration can be achieved with higher CO_2 percentage (10% in our case). The specific growth rate of *Chlorella vulgaris* increased from 2.56 day^{-1} to 3.03 day^{-1} when CO_2 concentration saturated with air was shifted from 2% to 10% CO_2 and again growth rate is declined to 2.89 day^{-1} with further increased CO_2 concentration to 12% and still these values are very high which indicate the growth potential of *Chlorella vulgaris* in higher CO_2 concentrations.

From these observations of results it shows increase of defined parameters of growth with increase CO_2 from 2% to 4% and 8% to 12% mixed with air and decline with further CO_2 increment from 4% to 6 % and 10% to 12%. Overall productivity, biomass and CO_2 fixation rate observed is maximum up to 10% CO_2 exceeding this limit results in decline of growth.

From these trends of results for *Chlorella vulgaris*, growth is promising as two type of growth is involved one is photoautotrophic utlizing CO_2 as carbon source and second is heterotrophic growth mechanism which is utlizing organic carbon source present in the media and produced by photoautotrophic mechanism. Integration of CO_2 capture with wastewater treatment rich in organic carbon source may enhance biomass productivity twofold and wastewater treatment as well. it could be concluded that with the optimization of certain parameters by further research we can make microalgae as practical potential CO_2 mitigation method with other wastewater treatment and co-products from biomass as extra environmental friendly source of biofuel.

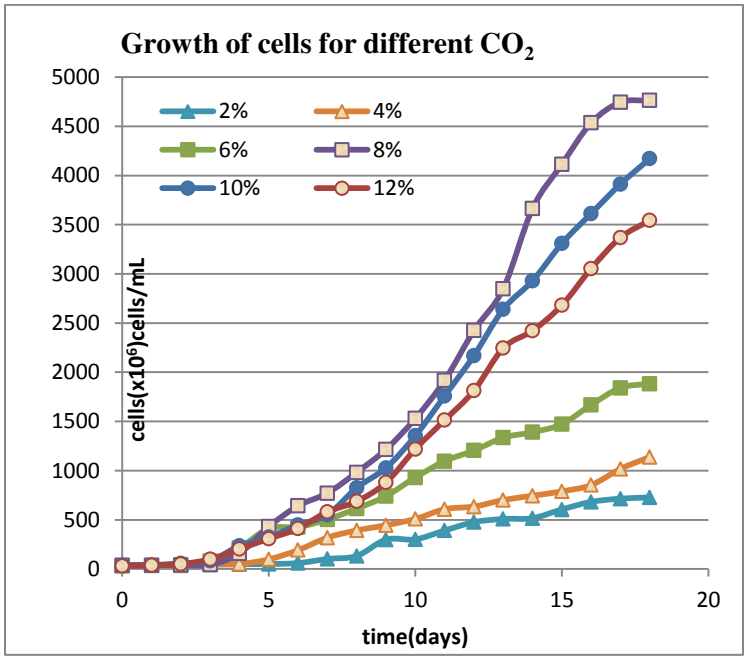
7.4 Results of *Nannochloropsis Oculata* in SWWM

Nannochloropsis Oculata was cultivated in batch photo-bioreactor using SWW Media modified with organic carbon source to see the effect of mixotrophic growth especially for wastewater treatment according to recipe give in research methodology appendices. The effect of CO_2 concentration was investigated and rate of CO_2 fixation was compared as well as uptake of nutrients for wastewater treatment purpose. It was assumed that presence of organic carbon source may help the treatment of wastewater rich in organic carbon source such as dairy wastewater and municipal wastewater.

7.4.1 Effect of different CO_2 concentration on growth

Nannochloropsis Oculata was cultivated in SWW Media at room temperature under different CO_2 concentrations. The initial concentration of inoculum was (30, 35, 39 cells/mL), 0.08 g/L dry biomass and pH was 6.58 in initial medium. The effect of CO_2 concentration on growth of *Nannochloropsis Oculata* in term of cell concentration and dry biomass production with time is shown in the Figure 41.

The investigation of the effect of CO_2 on growth of *Nannochloropsis Oculata* was studied in batch photo-bioreactor cultured for twelve (20) days in temperature range of 20.8^0C to 23.2 ^{0}C and light intensity of 2755-3800 (Lux) with CO_2 concentrations of 2%, 4%, 6%, 8%, 10% and 12% mixed with air with flow rate of 350 cm^3min^{-1} . The culture was grown to stationary phase around 20days period of cultivation.



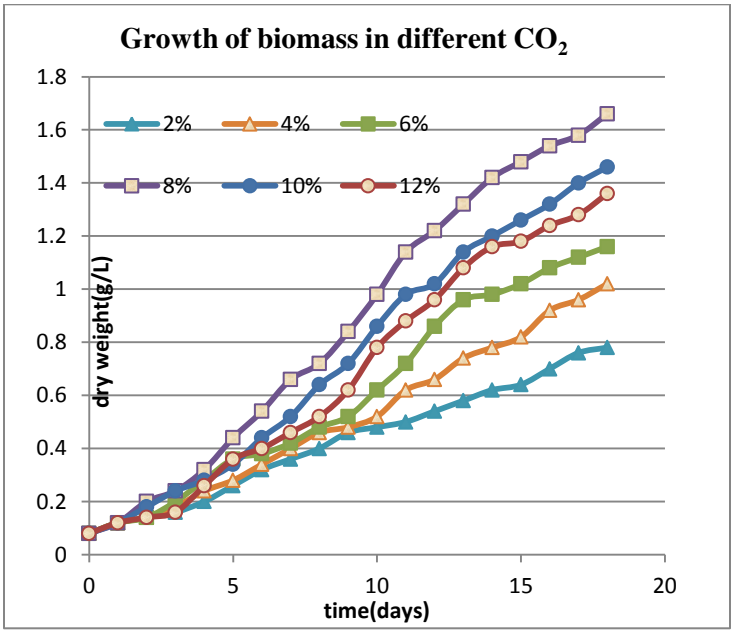


Figure 41: Growth rate of *Nannochloropsis Oculata* in SWW medium

From the Figure *Nannochloropsis Oculata* was found to grow in higher CO_2 concentrations favorably where growth rate is accelerated by shifting towards higher CO_2 concentration and again decreased when CO_2 concentration exceeds from 8% CO_2. As an example the biomass of *Nannochloropsis Oculata* increased from 0.78 g L^{-1} to 1.66 g L^{-1} (increment of 118.2%) when CO_2 concentration saturated with air was shifted from 2% to 8% CO_2 and decreased to 1.46 g L^{-1} when CO_2 concentration shifted from 8% to 10% CO_2 and a further decrease to 1.36 g L^{-1} was observed when CO_2 concentration was increased to 12%. Similar results were found for multiplication of cell concentration as shown in the Figure.

For these experiments it showed promising growth results for *Nannochloropsis Oculata* in higher CO_2 concentrations of 8%, 10% and 12 %. In addition to biomass acceleration, the biomass productivity is also increased from 0.038 $gL^{-1}day^{-1}$ to 0.96 $gL^{-1}day^{-1}$ with an increment of 148.8% when CO_2 concentration shifted from 2% to 8% CO_2 and decreased to 0.082 $gL^{-1}day^{-1}$ and 0.077 $gL^{-1}day^{-1}$ when further CO_2 increased to 10% and 12 % respectively. The optimum productivity obtained was 0.096 $gL^{-1}day^{-1,}$ 0.82$gL^{-1}day^{-1}$ and 0.077 $gL^{-1}day^{-1}$ at the CO_2 concentrations of 8%, 10% and 12 % respectively. From these observations we can conclude that specified microalgae specie can tolerate higher CO_2 concentration but its growth rate is declined above certain limits. The maximum growth in term of biomass and cell concentration can be achieved with higher CO_2 percentage (8% in our case). Also reducing the flow rate of CO_2/Air stream in batch photo-bioreactor could enhance the residence time which increases the CO_2 utilization efficiency.

7.4.2 Specific growth rate for different CO_2 concentration

Nannochloropsis Oculata growth in Modified Bolds Bessel Media was studied for different CO_2 concentrations. Culture was sampled on daily basis until a stationary phase was reached and further reasonable growth was not observed. Specific growth rate was calculated for each batch of culture for CO_2 variation from 2-12% and result is shown in the Figure 42.

The specific growth rate curve can be divided into different regions as shown in the Figure. The first region is where the both specific growth rates is increasing, in the initial phase where there is very small number of cell divisions, growth rate is small while after 4 – 5 days when there is sufficient number of cells, they have enough food available in term of nutrients and CO_2 and all the condition are favorable at this time their cell division is at its climax and growth rate is maximum after that growth rate becomes constant and finally decreases.

As shown from the Figure the specific growth rates for exponential growth phase found were 2.28, 2.55, 2.67, 3.03, 2.90 and 2.83 day^{-1} in 2, 4, 6, 8, 10, and 12% CO_2 mixed with air used for cultivation in SWWM medium. These results show that at higher CO_2 concentration microbial cells grow faster without any stress. The specific growth rate of *Nannochloropsis Oculata* increased from 2.28 day^{-1} to 3.03 day^{-1} when CO_2 concentration saturated with air was shifted from 2% to 8% CO_2 and again growth rate is declined to 2.90 and 2.83 day^{-1} with further increased CO_2 concentration from 10 to 12% respectively and still these values are very high which indicate the growth potential of *Nannochloropsis Oculata* in higher CO_2 concentrations.

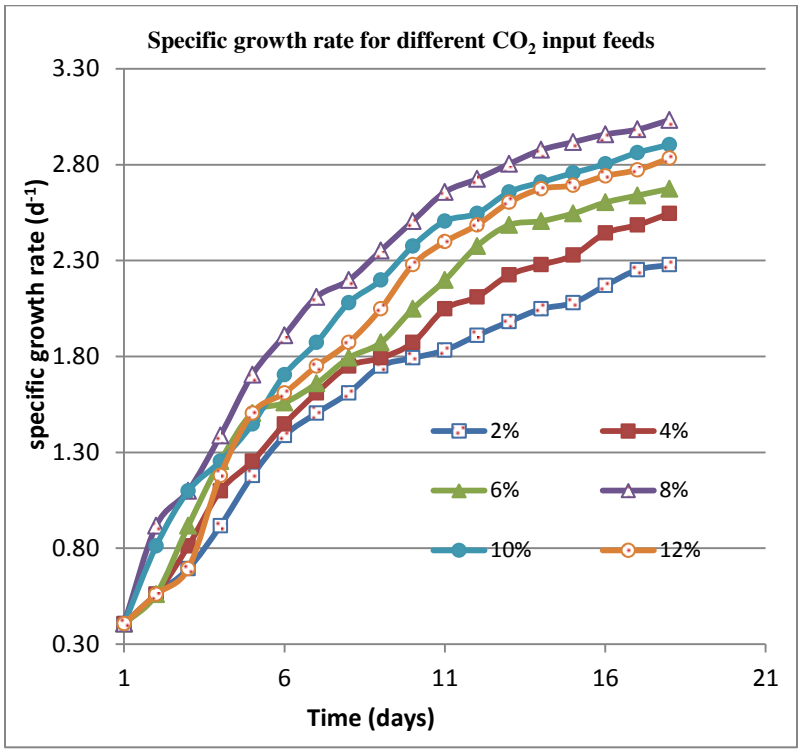


Figure 42: Specific growth rate of *Nannochloropsis Oculata* in SWW medium

7.4.3 CO_2 bio-fixation rate for different CO_2 concentration

Batch culture of *Nannochloropsis Oculata* was conducted to investigate the potential of CO_2 mitigation in the photo-bioreactor systems. Prior to operation of reactor with our culture, photo-bioreactor was tested with 2% CO_2 without microalgae sample to ensure non-presence of any abiotic factor for CO_2 removal. During this test CO_2 concentrations measured at inlet and outlet were same values ensuring that no any biotic factor was involved in CO_2 reduction mechanism. The amount of CO_2 fixation mixed with air in different concentrations was investigated in batch photo-bioreactor during a period of 18-days operation.

Analysis of carbon contents using TOC analyzer showed that presence of carbon contents in *Nannochloropsis Oculata* did not signaficantly change with different CO_2 concentrations and its value found was 20-22% on average for all the CO_2 concentrations. The CO_2 biofixation rate was determined using the equation described in methodoligy and results for six differents CO_2 concentrations are shown in Figure 43.

As showen from the Figure *Nannochloropsis Oculata* shows higher CO_2 fixation rates under 8% to 12% CO_2 concentrations . the maximum CO_2 biofixation rate found was 0.0883 g L^{-1} d^{-1} with CO_2 concentration of 8% at day 11 of culture as shown in the Figure. Simillar trend of CO_2 biofixation was observed for all CO_2 concentrations. CO_2 fixation rate is first incresed as the growth procedes with msximum growth rate it reaches to maximum values afer that with the decline in growth, CO_2 fixation rate is also decresed. Specifically for this the examined microalgae specie showed the promising CO_2 biofixation abilities under different CO_2 concentrations from 2% to 12% CO_2 concentration and performed the best CO_2 fixation at 8% CO_2.

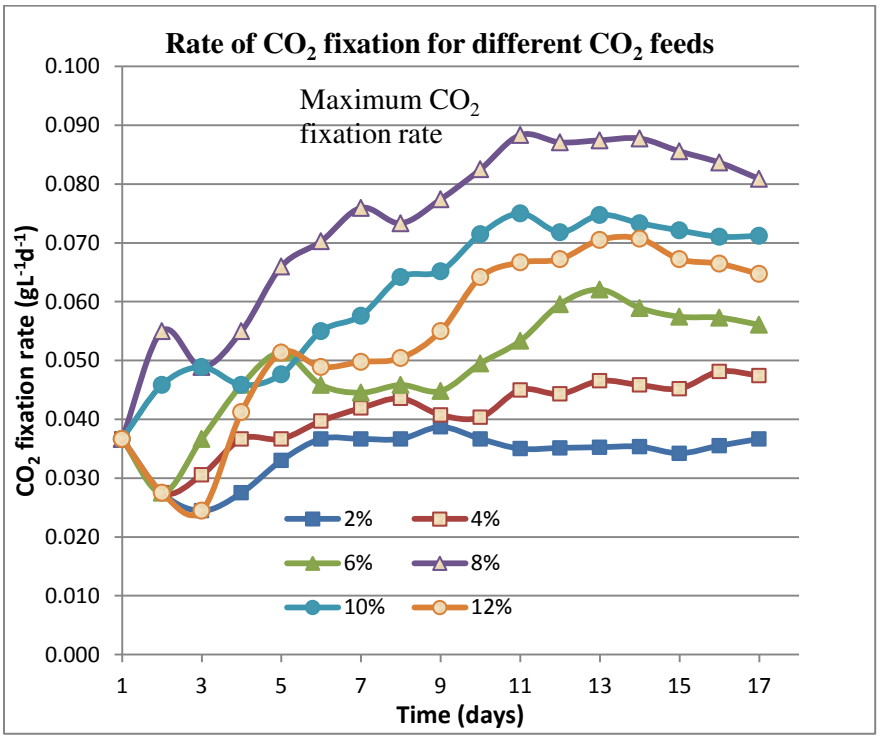


Figure 43: Shows the trends of CO_2 biofixation rate in SWW medium

7.4.4 Max. of productivity, CO_2 fixation rate & biomass yield

Analysis of carbon content as determined using TOC showed that carbon contents did not vary significantly with CO_2 concentration and bio-fixation rate was calculated using equation as defined in methodology. Figure 44 shows the results of maximum biomass, productivity and CO_2 bio-fixation rate during operation for each CO_2 concentration value using batch column reactor with continuous CO_2 supply mixed with air at a flow rate of $350 cm^3 min^{-1}$.

From these observations of results it shows increase of defined parameters of growth with increase CO_2 from 2% to 4% and 8% to 12% mixed with air and decline with further CO_2 increment from 4% to 6 % and 10% to 12%. Overall productivity, biomass and CO_2 fixation rate observed is maximum up to 8% CO_2 exceeding this limit results in decline of growth.

Also Table 9 represent the maximum biomass, maximum productivity and CO_2 fixation rate with percent increment of these values with shift of CO_2 concentration from 2% - 12%. For the maximum values of biomass produced increment is 112.82% for 8% CO_2 in reference to 2% CO_2 biomass produced and productivity increses 148.85% when CO_2 concentration changed from 2% to 8% in air stream and with further increse in CO_2 concentration to 10 and 12% productivity is increses 112.73% and 100% respectively compared to value at 2% CO_2.

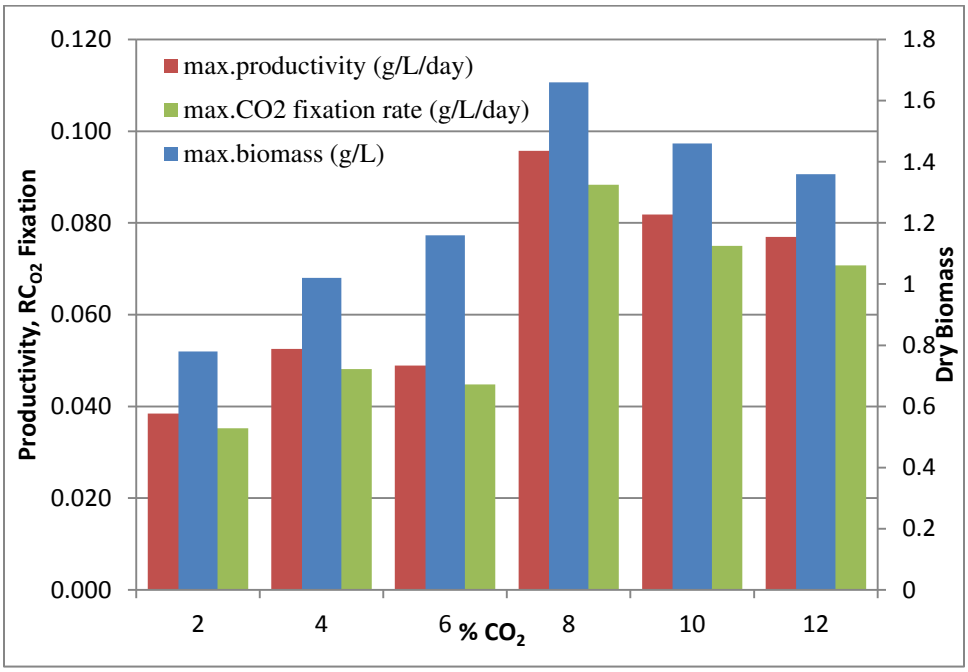


Figure 44: Shows the maximum growth achieved for *Nannochloropsis Oculata* in SWWM media.

Table 9: Maximum productivity and CO_2 bio fixation rate for N.O in SWWM

CO_2 conc.	Max. Biomass	Biomass Increment	Max. Productivity	Yield Increment	Specific Growth Rate	R_{CO2} fixation
%	X_{max_1} g L^-	% change	P_{max} g L^{-1} d^{-1}	% change	R_{max} d^{-1}	R_{maxCO2} g L^{-1} d^{-1}
2	0.78	0.00	0.038	0.00	2.28	0.035
4	1.02	30.77	0.053	36.50	2.55	0.0481
6	1.16	48.72	0.049	27.11	2.67	0.0448
8	1.66	112.82	0.096	148.86	3.03	0.0883
10	1.46	87.18	0.082	112.73	2.90	0.0750
12	1.36	74.36	0.077	100.00	2.83	0.0707

7.4.5 Nutrients uptake analysis

Coupling of wastewater treatment with CO_2 capture could benefit in term of higher growth rates, mixotrophic growth and commercial viability as well. The process of wastewater treatment could reduce the freshwater and nutrients requirement which in term reduces the cost of CO_2 capture and biomass production. As we have seen in synthetic wastewater two types of growth mechanisms are involved photoautotrophic using inorganic and heterotrophic utilizing organic carbon and nutrients to produce higher biomass production rates.

To find out the wastewater treatment analysis and utilization of nutrients and their effects on growth rates nutrients analysis was made and results are shown in the Figure. As clearly from the Figure the uptake of nitrogen source is almost reached to zero level at the end of cultivation period. Initially nitrogen present in the synthetic wastewater in term of nitrates was 49.2 mg/L which is decreased to 3.1 mg/L during a course of 21 days, while in term of ammonia it was initially 187mg/L and reduced to 0.0 mg/L showing 100% removal efficiency.

The removal and utilization of phosphate was done relatively low from 189mg/L to 152.2 mg/L during cultivation period it could be utilized more by allowing more time for cultivation or adjusting the concentration and composition of medium. In case of COD its value is first decreased from 162mg/L to 78mg/L and then gradually increases due to the presence of organic carbon source heterotrophic growth starts which in term utilize organic sources by decomposing and COD starts increasing from 78mg/L to 219mg/L at the time of harvesting.

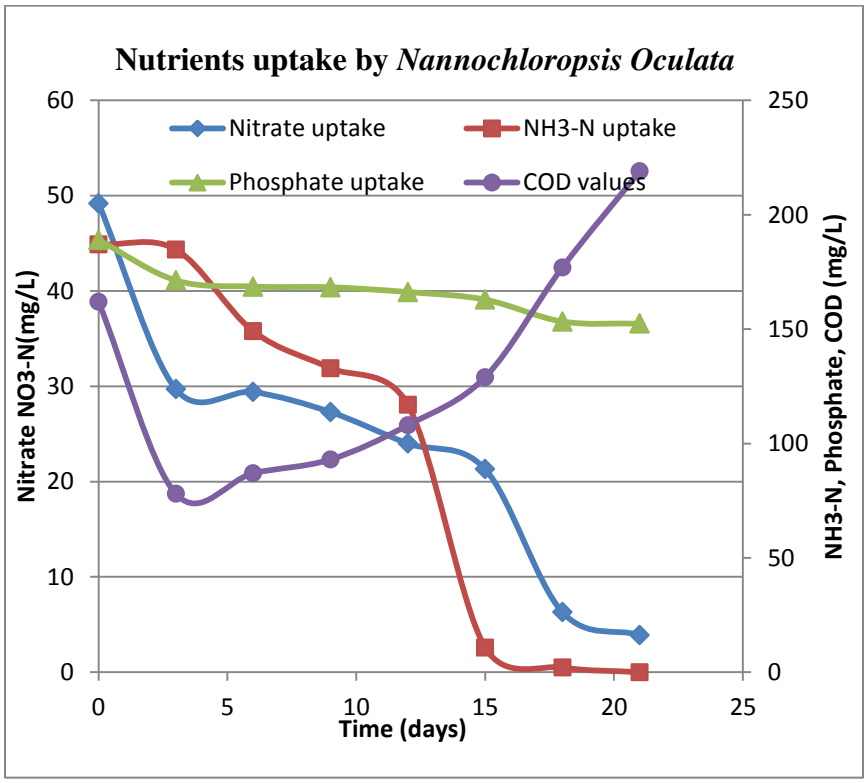


Figure 45: Shows the maximum nutrients uptake from synthetic wastewater for *Nannochloropsis Oculata*

7.4.6 Conclusion on *Nannochloropsis Oculata* growth in SWW media

From these observations of results it shows increase of defined parameters of growth with increase CO_2 from 2% to 4% and 8% to 12% mixed with air and increase with further CO_2 increment from 4% to 6 % and 8% to 12%. Overall productivity, biomass and CO_2 fixation rate observed is maximum up to 8% CO_2 exceeding this limit results in decline of growth.

From these trends of results for *Nannochloropsis Oculata*, growth is promising as two type of growth is involved one is photoautotrophic utlizing CO_2 as carbon source and second is heterotrophic growth mechanism which is utlizing organic carbon source present in the media and produced by photoautotrophic mechanism. Integration of CO_2 capture with wastewater treatment rich in organic carbon source may enhance biomass productivity twofold and wastewater treatment as well. it could be concluded that with the optimization of certain parameters by further research we can make microalgae as practical potential CO_2 mitigation method with other wastewater treatment and co-products from biomass as extra environmental friendly source of biofuel.

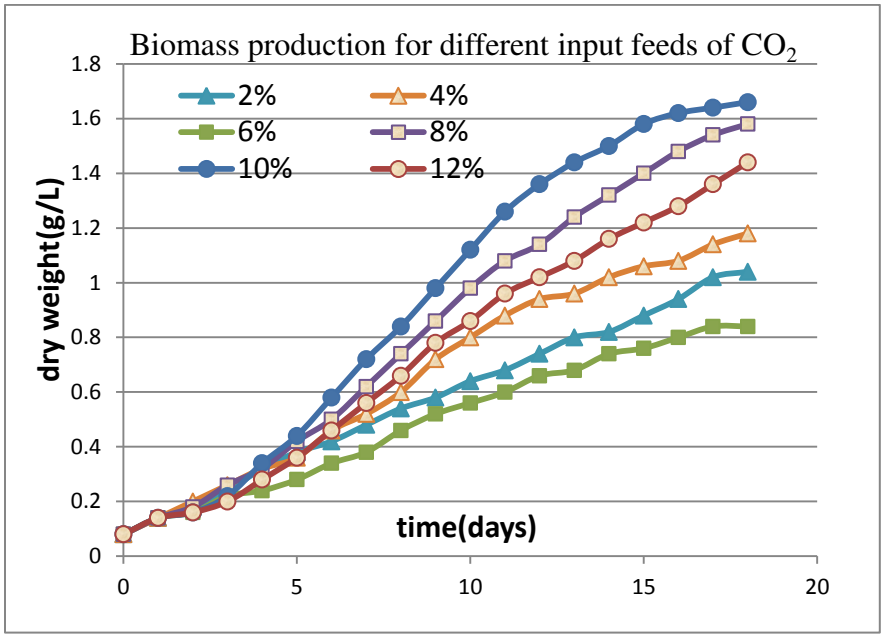
7.4.7 Comparison to literature values:

For comparison of results to literature, when searched we found we little results in the area of CO_2 capture and wastewater treatment only data available. As most of the research in this area was done for the purpose of biofuel production so only data found was for culturing process and growth kinetics.

From the comparison from literature as shown in the Figure 46 for Bolds Bessel Medium they produced maximum of 0.80g/L biomass with the tolerance of 5% CO_2 mixed with in air and in our case of synthetic wastewater media biomass produced is 1.80g/L which is twice higher than BBM media and maximum tolerance of CO_2 achieved in process is 10% which is double than BBM media.

Similarly better results for the rate of CO_2 fixation are determined, which is dependent on the biomass productivity while the carbon content remains same for identical species. In similar manner if we analyze the cultivation time for all the results in our synthetic wastewater medium it reveals maximum growth achieved in shorter time period with higher growth rate.

In addition to biomass production and CO_2 fixation, wastewater treatment could be achieved in Mixotrophic cultivation mechanism: utilization of waste organic carbon and other nutrients could be converted to useful biomass.



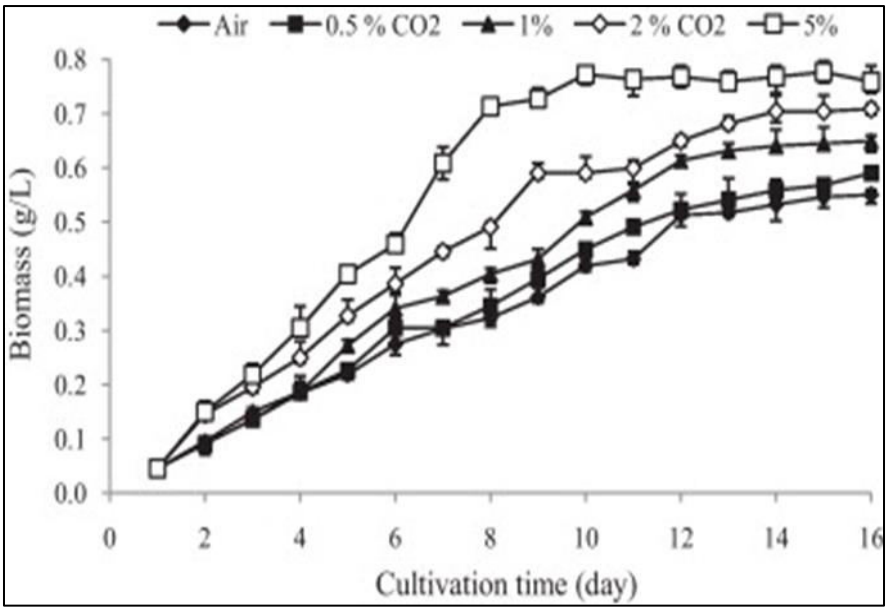


Figure 46: Comparison of experimental results in synthetic wastewater media to literature values in Bolds Bessel Medium for *Chlorella vulgaris* (Lam & Lee, 2013).

7.4.8 Comparison of *C. vulgaris* and *N. Oculata* in SWWM media:

It is well recognized in comparison of commercial growth media (F/2) and synthetic wastewater mediua (SWWM) for both *Chlorella vulgaris* and *Nannochloropsis Oculata* : synthetic wasterwater media (SWWM) is more effective in higher growth rates, CO_2 fixation capability and biomass production in all six input concentrations of CO_2 . To evaluate the better operational parameters and performance of *Chlorella vulgaris* and *Nannochloropsis Oculata* in synthetic wastewater media (SWWM): comparison is made based on same operating conditions for each of them.

As illustracted in Figure 47 indiviual results of growth rate, productivity and CO_2 fixation rate for *Chlorella vulgaris* and *Nannochloropsis Oculata* in synthetic wastewater medium (SWWM): the maximum biomass productivity (0.107 , 0.082) g L^{-1} d^{-1} and CO_2 fixation rates (0.098 , 0.075) g L^{-1} d^{-1} in *Chlorella vulgaris* and *Nannochloropsis Oculata* respectively at 10% inputs concentrations of CO_2 while (0.90 , 0.096) g L^{-1} d^{-1} productivity and (0.083 , 0.088) g L^{-1} d^{-1} CO_2 fixation rates in *Chlorella vulgaris* and *Nannochloropsis Oculata* at 8% inputs concentrations of CO_2 respectively.

From carefull observations of these results, its concluded *Chlorella vulgaris* has higher productivity and tolerance towards input concentration of CO_2 (around10%) in synthetic wastewater media (SWWM) while *Nannochloropsis Oculata* having more carbon accumulation capability in biomass has higher CO_2 fixation rate and lesser tolerance (around 8%) towards input CO_2 concentration as compared to *Chlorella vulgaris*.

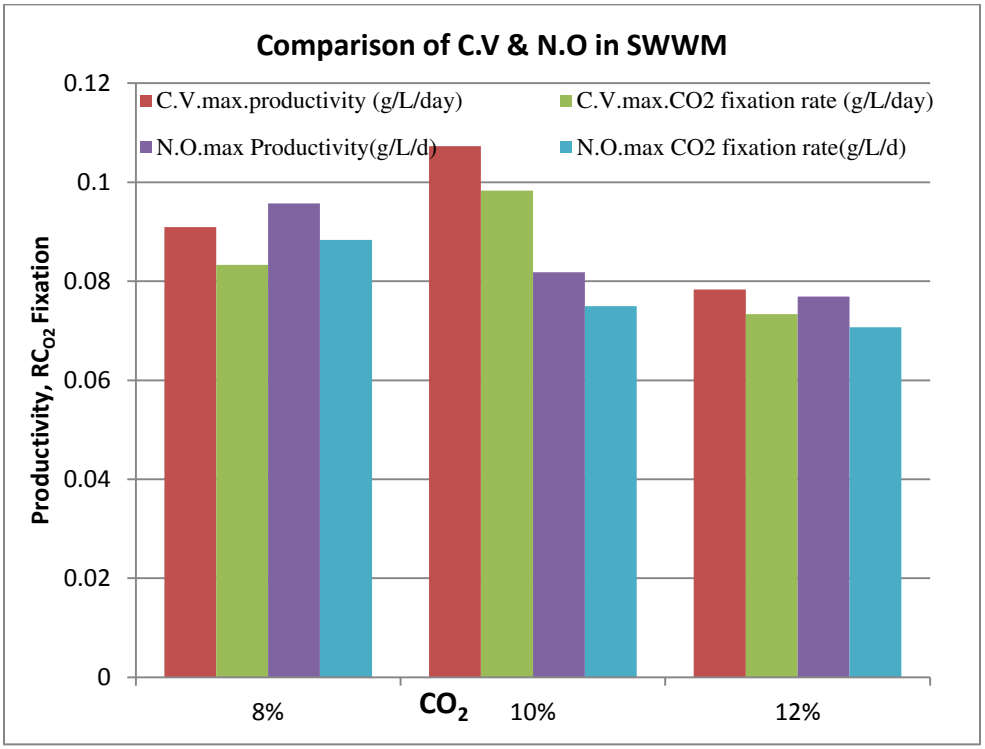


Figure 47: Comparison of *Chlorella vulgaris* and *Nannochloropsis Oculata* in SWWM Media

CHAPTER 8

CONCLUSION AND RECOMMENDATION

8.1 Conclusion:

The results presented in this experimental study leads to better understanding of operational performance of *Chlorella vulgaris* and *Nannochloropsis Oculata,* towards growth behaviours, CO_2 fixation, nutrients uptake and biomass production in commercial (F/2) and synthetic wastewater (SWW) cultur media.

Following are the conclusion of the study:

1. CO_2 capture using microalgae, integrated with wastewater treatment is promissing approach to deal with CO_2 emission issues and contribute to wastewater treatment.
2. pH is the main controlling parameter for microalgage growth, CO_2 capture and biomass production which is directly controlled by input feeds of different CO_2/Air mixture.
3. 4% CO_2 for *Chlorella vulgaris* and 2% CO_2 for *Nannochloropsis Oculata* has been found suitable for maximum growth and CO_2 fixation in F/2 media. The CO_2 fixation rates are 0.087 g L^{-1} d^{-1} and 0.086 g L^{-1} d^{-1} for *Chlorella vulgaris* and *Nannochloropsis Oculata*, respectively.
4. In synthetic wasterwater, 10 % CO_2 for *Chlorella vulgaris* and 8% CO_2 for *Nannochloropsis Oculata*, are found favorable for the maximum growth and CO_2

fixation (0.11 g L^{-1} d^{-1} and 0.098 g L^{-1} d^{-1} for *Chlorella vulgaris* and *Nannochloropsis Oculata*, respectively).

5. The maximum of biomass produced is 1.66 g L^{-1} in synthetic wastewater medium which is alost the double as compared to the commercial F/2 media.
6. The nutrients uptake by the microalgaes from synthetic wastewater showed are found to be significant. In some cases, both the *Chlorella vulgaris* and *Nannochloropsis Oculata* can complete remove nitrogen source and phosphorous based compounds in the wasterwater.
7. Mixtrophic cultivation for wastewater treatment using organic carbon source from wastewater and inorganic from CO_2 is best solution for real applications of CO_2 capture coupled with wastewater treatment.
8. Results showed the use of bubble coloumn photobioreactors are the best design that exist for mass cultivation of microalgae and CO_2 capture.

From these results we can further procede to find out the more details towards pratical solution using pilot scale photobioreactors by applying industrial waste CO_2 and real wastewater to findout wastewater treatment potential, CO_2 uptake efficiency and biomass production. The advancement in proposed integrated process of industrial CO_2 fixation and wastewater treatment may benefit to environment and community.

8.2 Recommendations

Based on results of this study, following are recommended for future study:

i. More focused study based on hydrodynamics and mass transfer of CO_2 to liquid medium and uptake by microalgae cells at defined optimum operating CO_2 concentrations

ii. CO_2 capture and growth kinetics study on larger scale in pilot plant photo-bioreactors for practical application using real wastewater as source of nutrients and investigation of uptakes of different components of wastewater by microalgae cells

iii. Using mixotrophic growth mechanism as introduced here utilizing CO_2 as inorganic carbon source and taking organic source of carbon from wastewater to couple CO_2 capture with wastewater treatment.

iv. Detailed analysis of biomass produced and its conversion to different valuable fuel and non-fuel products to reduce CO_2 capture process cost.

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Appendices:

Table 10: F/2 Medium composition

Component	**Stock Solution**	**Quantity**
$NaNO_3$	75 g/L dH_2O	1 mL
NaH_2PO_4 H_2O	5 g/L dH_2O	1 mL
Na_2SiO_3 $9H_2O$	30 g/L dH_2O	1 mL
trace metal solution	(see recipe below)	1 mL
vitamin solution	(see recipe below)	0.5 mL
Trace metal Comp.	**Primary Stock Sol.**	**Quantity**
$FeCl_3$ $6H_2O$	---	3.15 g
Na_2EDTA $2H_2O$	---	4.36 g
$CuSO_4$ $5H_2O$	9.8 g/L dH_2O	1 mL
Na_2MoO_4 $2H_2O$	6.3 g/L dH_2O	1 mL
ZnSO4 $7H_2O$	22.0 g/L dH_2O	1 mL
$CoCl_2$ $6H_2O$	10.0 g/L dH_2O	1 mL
$MnCl_2$ $4H_2O$	180.0 g/L dH_2O	1 mL
Vitamin Solution	**Primary Stock Sol.**	**Quantity**
Thiamine HCl (vit. B_1)	---	200 mg
Biotin (vit. H)	0.1 g/L dH_2O	10 mL
Cyano-cobalamin	1.0 g/L dH_2O	1 mL

Table 11: Synthetic wastewater medium composition

Component	Molar Mass	Stock solution g/L	Quantity used ml/L
$NaNO_3$	84.99	25	10
$CaCl_2.2H_2O$	147.0154	2.5	10
$MgSO_4.7H_2O$	246.47	7.5	10
K_2HPO_4	174.2	7.5	10
KH_2PO_4	136.09	17.5	10
NaCl	58.44	2.5	10
EDTA	372.24	50	1
KOH	56.1056	31	1
$FeSO_4.7H_2O$	278.0157	4.98	1
H_2SO_4	98.078	1.84	-
H_3BO_3	61.83	11.42	-
$ZnSO_4.7H_2O$	287.56	8.82	-
$MnCl_2.4H_2O$	197.905169	1.44	-
$CuSO_4.5H_2O$	249.69	1.57	-
$CoCl_2.6H_2O$	502.3869	0.49	-
CH_4N_2O	60.06	0.0918	-
$C_{12}H_{22}O_{11}$	342.2965	0.122	-
peptone proteose	-	0.0174	-

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	Investigation of effect of different CO_2/Air ratios on the growth of Chlorella Vulgaris for CO_2 fixation in photo-bioreactor (YAS 2014, France)
	pH Effect on growth mechanism of Nannochloropsis Oculata in the study of CO_2 capture process(Accepted AIChE 2014,USA)
	CO_2 bio-fixation and pH based optimization of *Nannochloropsis Oculata* growth using Photo-bioreactor for environmental remediation (accepted LabTech 2014, Bahrain)