Feasibility studies on the utilization of fresh water microalgal species in Carbon dioxide sequestration

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by

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CERTIFICATE

This is to certify that the thesis entitled **"Feasibility studies on the utilization of fresh water microalgal species in carbon dioxide sequestration"**, submitted to the University of Calicut by Mrs. Karthika S. Menon, in partial fulfillment of the award of the degree of Doctor of Philosophy in Botany is a bonafide record of the research work carried out by her under my guidance and supervision.

No part of the present work has formed the basis for the award of any other degree or diploma, previously.

University of Calicut
10th June 2018

Dr. C. C. Harilal
(Supervising Teacher)

DECLARATION

The thesis entitled **"Feasibility studies on the utilization of fresh water microalgal species in carbon dioxide sequestration"** submitted by me in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Botany of the University of Calicut is an original research work carried out by me under the guidance and supervision of Dr. C.C. Harilal, Associate Professor, Department of Botany, University of Calicut. No part of the work has formed the basis for the award of any other Degree or Diploma of any University.

University of Calicut **Karthika S. Menon**

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ABBREVIATIONS

CO_2	:	Carbon dioxide
BB medium	:	Bolds Basal medium
PBR	:	Photo bio reactor
DO	:	Dissolved oxygen
EDTA	:	Ethylenediaminetetraacetic acid
HCl	:	Hydrochloric acid
NTU	:	Nephelometric Turbidity Unit
^{o}C	:	Degree Celsius
µs	:	micro seimens
kΩ	:	kilo ohm
µm	:	micrometer
ml	:	milliliter
ppm	:	parts per million
mg/l	:	milligram per litre
mg/ml	:	milligram per millilitre
nm	:	Nanometer

General Introduction

Uncontrolled greenhouse gas emissions due to human activities have contributed substantially to global warming and climate change. The greenhouse gases mainly include Carbon dioxide (CO_2), Methane (CH_4) and oxides of Nitrogen (NOx). Of these, CO_2 is of major concern today, owing to its higher concentration in the atmosphere.

Among various strategies for CO_2 sequestration, biological sequestration using photosynthetic microalgae have received considerable attention in recent times. Microalgae, one of the most important living resources of both fresh and marine systems can be employed for CO_2 sequestration, as they have higher photosynthetic efficiency, higher biomass production and faster growth rate, compared to other energy crops. They can readily be incorporated into engineered systems.

The present study is an attempt to assess the potentialities of indigenous freshwater microalgal species in carbon dioxide sequestration. The specific objectives outlined in the present study in this direction include:

- Maintenance of pure cultures of microalgal species using standard methods and selection of microalgal species which are active under culture conditions.
- Monitoring the responses of selected microalgal species under varying dosages of carbon dioxide supply.
- Determining the optimum pH favouring maximized growth of selected micro algal members.

- Assessment of the CO_2 assimilation efficiency of selected micro algal members in PBR under controlled conditions.

For a meaningful elucidation of the objectives, the study has been undertaken in three stages and their outcomes are depicted in three chapters. They include:

I. Screening of microalgae for CO_2 assimilation efficiency

II. pH specific modification of culture medium for growth maximization of *Chlamydomonas globosa* and *Acutodesmus obliquus*

III. PBR based feasibility studies on the carbon sequestration efficiency of selected microalgal members

Chapter I is dealing with microalgal culturing and subsequent assessment of their CO_2 assimilation efficiency. In this study, microalgal samples were collected from heterogeneous fresh water environments and were subjected to culturing in Bolds Basal medium. Accordingly pure cultures of *Chlamydomonas grovei, Chlamydomonas globosa, Desmodesmus opliensis, Monoraphidium contortum* and *Acutodesmus obliquus* were maintained in the laboratory. Screening studies were carried out on these microalgal members with respect to their efficiencies in assimilating varying levels of carbon dioxide supply like 10, 15, 20, 25, 30, 40, 60, 80 and100 bubbles/2 hours. The results were analyzed and the microalgal members, which were effective in CO_2 assimilation, were listed out.

Chapter II is dealing with studies which were carried out to maximize the biomass production of selected microalgal members by altering culture conditions. As pH was noted to be an important factor influencing the growth of microalgal members, an experimental approach has been initiated with an objective to evaluate the optimum pH favouring the maximized growth of *C.*

globosa and *A. obliquus* in Bolds Basal medium which has identified as excellent candidates for carbon sequestration. For experimentation, treatments were maintained using Bolds Basal medium in which the pH of culture medium was adjusted from 3-12, with a gradation of 0.5. The results were analyzed and the optimum pH influencing the growth and multiplication of micro algal members like *C. globosa* and *A. obliquus* were worked out.

As *C. globosa* and *A. obliquus* exhibited better growth performances in higher dosages of carbon dioxide, an attempt has been carried out to assess their efficiencies in carbon dioxide accumulation using a proto type Photo Bio Reactor (PBR). For satisfying this objective, a laboratory scale closed vertical bubble column PBR having a size of 10 x 10 x 45 cm and a volume of 4.5 L was designed using acrylic material. Here separate experimentations were carried out for *C. globosa* and *A. obliquus* and the results concerning their efficiency are depicted in Chapter III.

Chapter I

Screening of Microalgae for CO_2 Assimilation Efficiency

Introduction

Unprecedented greenhouse gas emissions due to human activities have contributed substantially to global warming and climate change. The greenhouse gases mainly include Carbon dioxide (CO_2), Methane (CH_4) and oxides of Nitrogen (NOx). Of these, CO_2 is of major concern today, owing to its higher concentration in the atmosphere (Ramanathan, 1988; Cheng et al., 2013). Industrial revolution of the 20th century has pumped huge volumes of carbon dioxide into the atmosphere and the magnitude of such emissions have grown exponentially from 280ppm before industrialization to 367ppm in 1999, 379 ppm in 2005 and to the current levels of 403.64 ppm (2017), as reported by Mauna Loa Observatory, Hawaii, USA. According to Cape Grim Baseline Air Pollution Station, a joint venture of the Bureau of Meteorology and the Commonwealth Scientific and Industrial Research Organization (CSIRO), the levels of atmospheric carbon dioxide has noticed to be 403.26 ppm in 2017. The situation will worsen that by the end of the 21st century, we could expect to see carbon dioxide concentrations of anywhere from 490 to 1260 ppm, 75-350% above the pre-industrial concentration (Ebi et al., 2003). The release of carbon dioxide into the atmosphere is far more rapidly than it is being removed, and this imbalance causes increased carbon dioxide concentrations in the atmosphere. This unbalanced emission of atmospheric carbon dioxide constitutes a major challenge to global sustainability.

Both natural and anthropogenic sources contribute to elevated CO_2 emissions into the atmosphere. The natural sources include ocean release, decomposition of organic matters, respiration process, forest fires, emissions pertaining to volcanic eruption etc. Anthropogenic releases are said to be due the dependency on fossil fuels to meet energy needs in various sectors (Chang and Yang, 2003; Hansen et al., 2008). Other reasons include deforestation, unscientific agricultural practices, unsustainable land use changes etc. First World Climate Conference by UNEP and WMO (1979) observed anthropogenic activities as contributing factors to increased carbon dioxide concentrations in the atmosphere. The summit by WMO, UNEP and the International Council of Scientific Unions (ICSU) in Austria (1985) also accepted the reality of anthropogenic global warming due to accelerated release of carbon dioxide. The Kyoto Protocol (1997) and Paris Agreement (2016) also called for a reduction in GHG emissions and boosted climate change discussions.

The ill effects of increased levels of carbon dioxide in the atmosphere and its resultant complications are many. Higher emission may result in increased concentration of CO_2 in our atmosphere, where it remains for 100 to 200 years, leading to a raise in temperature. The temperature increase has been unequivocally proven and is occurring at an unprecedented rate. As per studies of NASA on Earth's global surface temperature, year 2017 has been declared as the second warmest year since 1880. It is further stated that atmospheric temperatures could rise by 1.1 to 6.4 °C (2.0 to 11.5 °F) during the 21st century and sea levels would probably rise by 18 to 59 cm (IPCC, 2007).

There are global concerns on the release of GHGs by various nations. International initiatives are in progress over decades to check CO_2 emissions into the atmosphere. In 2016, the largest CO_2 emitting countries/regions

showed reduced emission trends, notably in China (-0.3%), United States (-2.0%), Russian Federation (-2.1%), Brazil (-6.1%) and the United Kingdom (-6.4%). Conversely an increased emission trend was noticed in India (+4.7%) and some of the developing countries (Olivier et al., 2015). This is pointing to the urgent measures, which are to be undertaken by the nation to control its greenhouse gas emissions. If the current emission trend continues, it will results in probable adverse impacts including changes in the quantity, timing and distribution of rain; sea level rise; increased frequency and intensity of wildfires; floods; droughts; storms and disturbances pertaining to coastal, marine and other ecosystems; expanding deserts; heat waves; increasingly severe weather; loss of agricultural productivity etc.

Considering the ill effects of elevated levels of CO_2 in the atmosphere, identification of technically, economically and eco-friendly feasible strategies to reduce CO_2 emissions are of great urgency. Recently, many research and development efforts are on to mitigate CO_2 emissions, worldwide, and the most recent alternative for managing carbon dioxide is carbon sequestration.

Carbon sequestration refers to all means that is natural or artificial, by which CO_2 is either confiscated from the atmosphere or redirected from emission sources and stored in oceans, terrestrial environments and geologic formations. It is the process by which the atmospheric carbon is captured and converted to various forms that are unable to contribute to global warming (Nogia et al., 2013). The goal of deliberate carbon sequestration is to reduce the net flux of CO_2 emissions to the atmosphere by trapping/sequestering carbon dioxide in heterogeneous environments. This itself is the agenda of Kyoto Protocol, to reduce the greenhouse gases emissions by 18%, below 1990 level, by 37 European Community and industrialized countries, within the period of 2013 - 2020.

Various physical, chemical and biological methods have been applied to capture and sequester CO_2 (Abu-Khader, 2006). Physical methods include capturing of CO_2 using PSA (Pressure Swing Adsorption) or PTSA (Pressure and Temperature Swing Adsorption) method (Ohta, 1997). A "molecular basket" using nanoporous solid adsorbent was also used to collect CO_2 in the condensed form. Polyethylenimine (PEI)-modified mesoporous molecular sieve of MCM-41 type (MCM-41-PEI) has also been tested as a CO_2 adsorbent (Xu et al., 2002; Abu-Khader, 2006).

Chemical reaction-based methods include cyclic carbonation/de-carbonation reaction (Gupta and Fan, 2002) and gas-absorption process in which the separation of carbon dioxide from a gas mixture is effected by washing with aqueous amine solution (Resnik, et al., 2004). The chemical reaction-based CO_2 sequestration includes processes like separation, transportation and sequestration, which are expensive. Both the technologies mentioned above are not only costly and energy-consuming, but also offers marginal mitigation benefits (Wang et al., 2008). These methods, also requires considerable space for storage, coupled with elevated costs for monitoring, maintenance and operation and create serious concerns regarding CO_2 leakage (Bilanovic et al., 2009). Although the capturing and transportation of carbon dioxide is a feasible strategy and are technically proven, further investigation pertaining to the reliability and safety in terms of long-term storage remains necessary (Bonenfant et al., 2003; Yeh et al., 2001).Thus it is compulsory to develop cost-effective, environment friendly, sustainable methodologies /alternatives to curb the soaring emission rate. Many research works have been, and are being conducted to address this crisis.

Biosequestration is the capture and storage of atmospheric carbon dioxide by biological processes. This can be by increased photosynthesis (through practices such as reforestation / preventing deforestation and genetic

engineering techniques); by enhanced soil carbon trapping in agriculture; or by the use of algal bio sequestration (through aquatic systems or by algal bioreactors) to mitigate the carbon dioxide emissions from coal, petroleum (oil) or natural gas-fired electricity generation. Biological CO_2 fixation through the production of biomass energy by the process of photosynthesis has attracted much attention as an alternative strategy (Ragauskas et al., 2006; Kondili and Kaldellis, 2007). Many researchers consider photosynthesis as the best near intermediate-term solution to the problem of carbon emissions (Bayless, 2001).

Among several strategies of biological sequestration, utilizing the photosynthetic efficiencies of microalgae has been receiving significant attention as it offers energy-saving and eco-friendly technology (Skjanes et al., 2007; Wang et al., 2008). Apart from the dual role in biofuel production by controlling the GHG emissions and sequestering carbon, the terrestrial plants are expected to contribute 3-6% reduction in CO_2 emissions (Ho et al., 2011; Kao et al., 2014). However, the capabilities of photosynthetic microalgae to fix CO_2 are 10–50 times more efficient than terrestrial plants (Cheng et al., 2013; Lam et al., 2012). It was also proven that the algal species confining to fresh water and marine environments are responsible for 50% of total photosynthetic primary production (Giordano et al., 2005).

Thus microalgae are of particular interest to researchers seeking methods to mitigate carbon due to their rapid growth rates, compared to other energy crops (Dote, 1994; Minowa, 1995; Miao and Wu, 2006; Mutanda et al., 2011), potential for higher-efficiency solar conversion than terrestrial plants (Herzog et al., 1997), higher photosynthetic efficiency, higher biomass production, CO_2 fixation ability, production of biodiesel and other by-products through biomass process technologies, better ability to handle in extreme environments that favour them for an easy incorporation into

engineered systems, shorter growth cycle and weight doubling time of about three to five days etc. (Chen et al., 2011). According to Chisti (2007), during every 24 hours, algal species double their biomass; but during the exponential phase, the doubling time can be as short as three-and-a-half hours. Apart from these, microalgae seem to be the most available eco-friendly alternative for all finite oil and food resources. Approximately one kilogram of algal dry cell utilizes around 1.83 kg of CO_2 (Kumar et al., 2011), which in other words imply that per kilogram microalgae could capture nearly 1.83 kg CO_2 (Brennan and Owende, 2010).

In this scenario, the strategy of employing potential microalgal members seems to be more economically competitive and environment friendly, compared to other short-term carbon capture and storage technologies. However, most of the reports on the responses of microalgae to higher dosages of CO_2 under laboratory conditions confines to a limited number of species like *Chlorella, Scenedesmus* and *Spirulina* etc. Several other members like *Botryococcus, Chlorobotrys, Chlamydomonas, Chlorococcum, Chlorogleopsis, Dunaliella, Emiliania, Eudorina, Haematococcus, Oocystis*, *Nannochloropsis, Synechococcus, Thermosynechococcus* etc. have been employed in carbon dioxide sequestration and have reported to exhibit efficiencies in various capabilities.

In Kerala, heterogeneous aquatic systems including kole wetlands are known for its immense micro algal diversity. The unique climatic conditions, water quality and availability of adequate nutrients provide an appropriate habitat for the profound growth and multiplication of microalgae, of which the major fraction remains still unexplored. Hence an issue based or point source based carbon dioxide mitigation can be made possible through aquatic sequestration using natively isolated, potential microalgal members. The capabilities of such isolated members can be employed in other engineered systems too. The

present study is an attempt to bring/ isolate indigenous freshwater microalgal members that can be employed in this direction.

The objectives of the present study are thus outlined as:

- Maintenance of pure cultures of microalgal species using standard methods and selection of microalgal species which are active under culture conditions.

- Monitoring the responses of selected micro algal species under varying dosages of carbon dioxide supply.

Review of literature

The potential for efficient photosynthesis (Miao and Wu, 2006; Basu et al., 2013), rapid multiplication (Mutanda et al., 2011; Cheng et al., 2013), higher growth rates compared to energy crops (Dote, 1994; Minowa, 1995; Miao and Wu, 2006; Wang et al., 2008), direct capturing of atmospheric gases from point sources (Kadam, 1997; Packer 2009; Chiu et al., 2011; Lam et al., 2012), conversion of atmospheric gases to glucose for growth requirements (Kurano et al.,1995; Ho et al., 2011; Ho et al., 2014; Maity et al., 2014), synthesis of a wide range of biomolecules (Tang et al., 2011; Maity et al., 2014; Cheah et al., 2015), wide tolerance to extreme environments (Kurano et al., 1995; Demidov et al., 2000; Zeng et al., 2011; Zhao and Su, 2014) etc. make microalgae more interesting in their utilization for a wide range of purposes. Biosequestration using microalgae thus received much attention as an eco-friendly approach in recent times.

Microalgae were attempted to fix CO_2 from different sources including atmosphere, industrial flue gases and soluble carbonates ($NaHCO_3$ / Na_2CO_3) (Wang et al., 2008; Vidyasankar et al., 2013). Previous studies have established the ability of microalgae employed in the management of flue

gases emissions pertaining to municipal waste incinerators (Douskova et al., 2009), gas boilers (Doucha et al., 2005), simulated flue gases (Lee et al., 2000), industrial heater (Chae et al., 2006), coal-fired power plants (McGinn et al., 2011) etc. The mechanism of passing of flue gases directly through aqueous medium, in which the cultured microalgae seems to fix the CO_2 is an efficient method in capturing process (Kadam, 1997). Moreover, for the growth and development of many microalgal species, the high purity carbon dioxide is not essential, which in turn reduces the pre-treatment cost (Olaizola et al., 2003), though it imposes extreme conditions to the microalgae employed.

The selection of suitable microalgal species for carbon dioxide mitigation has an important role in the efficiency and cost reduction of the selected bio mitigation mechanism (Brennan and Owende, 2010). Biomass production seems to be the most significant/ critical factor in the selection of appropriate microalgal species for the successful bioconversion of carbon dioxide (Cheng et al., 2006; Cheah et al., 2015). Most of the research works quantified carbon fixation in terms of biomass production (Chae et al., 2006).

In addition to tolerance to higher levels of CO_2 species with higher assimilation ability, increased tolerance to toxic components, adaptability with respect to changing temperature, pH and nutrient conditions seems to be ideal characteristics of an adaptive species (Singh and Ahluwalia, 2013). Fulke et al. (2010) and Eloka-Eboka and Inambao (2017) reported increased carbon fixation ability and lipid production as promising candidates for sequestration. In the case of large scale process, the species that can utilize the CO_2 present in flue gas and exhibit increased growth under the natural day-night cycle are preferred (Benemann, 1993; Stewart and Hessami, 2005).

The CO_2 acclimation of microalgae to higher concentrations is a complex process comprising various adaptation techniques (Sergeenko et al., 2000;

Muradyan et al., 2004). Depending on the microalgal species and CO_2 concentrations, presence of lag period was noticed in some tolerant species (Satoh et al., 2002) like *Chlamydomonas* (Baba et al., 2011) and *Botryococcus braunii* (Yoo et al., 2010). For the reduction of the lag phase during the carbon sequestration studies and improving the carbon fixation efficiencies under higher dosages, acclimatization of inoculums with CO_2 lower dosages under same conditions were performed (Yun et al., 1997).

The microalgae also exhibit higher CO_2 concentration tolerance by redistribution of certain cellular organelles and adjusting their structural anatomy (Papazia et al., 2008). In species of *Chlorella* and *Scenedesmus obliquus*, Miyachi et al. (1986) observed developed pyrenoid surrounded by thick starch granules, in low CO_2 cells than the high CO_2 cells. Similar observations were also reported by Tsuzuki et al. (1986) in *Dunaliella tertiolecta* and the chloroplast in low-CO_2 cells was located near the plasma membrane while that in high- CO_2 cells was positioned in the inner region of the cells.

Structural malformations like decrease in the cell volume (Jian-Rong and Kun-shan, 2002), increase in vacuole size and disintegration of thylakoid membranes (Khairy et al., 2014), increase in volume (Pronina et al., 1993; Faria et al., 2012; Khairy et al., 2014) and vacuolization of the cytoplasm (Sasaki et al.,1999) with response to elevated CO_2 concentrations was also reported. Morphological alterations in *Scenedesmus* occur in response to stress was also reported by Hangata et al. (1992).

In the CO_2 tolerant *Chlorococcum littorale*, as an adaptation technique, Demidov et al. (2000) noticed a rapid shift of the PSA, when subjected to elevated CO_2 state. This transition of the PSA from state I to II was observed to enhance the cyclic electron transport above (PS) I and furthermore to maintain the pH homeostasis, the generation of additional ATP was

mandatory. By using the additionally generated ATP via cyclic electron transport, the V-ATPase maintains the pH homeostasis (Miyachi et al., 2003).

The physiological responses of microalgae to elevated levels of CO_2 may results in the accumulation several other value added by-products including lipids, carbohydrates, proteins, pigments, carotenoids and vitamins, which are active ingredients in pharmaceuticals, food additives, feed supplements (Ho et al., 2010; Wijffels and Barbosa, 2010; Kumar et al., 2011; Milledge, 2011; Šoštaricˇ et al., 2012; Toledo-Cervantes et al., 2013) etc. The carotenoids like lutein, canthxanthin, astaxanthin and β-carotene are of commercial importance and mainly used as ingredients of fish / poultry feed to enhance the reddish color of fish and yellowish color of egg yolk (Lorenz and Cysewski, 2000). Due to the increasing demand of β-carotene as pro-vitamin A, it is used in multivitamin preparations and in healthy food formulations (Krinsky and Johnson, 2005; Spolaore et al., 2006). Their antioxidant activity can safeguard the algal cells from photooxidative damage (Siefermann-Harms, 1985), which in turn can inhibit carcinogenesis in other organisms (Kumar et al., 2014). The fatty acids like oleic acid has significant role in the reduction of cholesterol content and thereby checking cardio vascular diseases (Beyhan et al., 2011) and the lauric, palmitic, linoleic, oleic, stearic, and myristic acids have their antibacterial and antifungal properties (Agoramoorthy et al., 2007).

There are several reports regarding the tolerance of species and strains toward elevated CO_2 concentrations. It has been noticed that the CO_2 tolerance efficiency and optimum CO_2 concentration of micro algae differ significantly (Singh and Ahluwalia, 2013; Toledo-Cervantes et al., 2013; Cheah et al., 2015) within and between species. There were some general conclusions regarding tolerance efficiency that the CO_2 concentrations above 5% may impart toxic effects to microalgal growth (Ramanan et al., 2010; Zhao and Su,

2014). However several authors also claimed that CO_2 concentrations above 5% can improve the growth of microalgal strains (Silva et al., 1984; Lee and Tay, 1991).

Among the promising candidates, *Chlorella* and *Scenedesmus* have been the most studied species to date. *Scenedesmus* was better able to tolerate very high CO_2 concentrations than *Chlorella* (Hanagata, 1992). The species and strains of *Chlorella* employed in carbon sequestration includes *Chlorella* sp. (Chiu et al., 2008; Velea et al., 2009; Fluke et al., 2010; Devgoswami et al., 2011; Kao et al., 2012; Zhao et al., 2015; Suali et al., 2017), *Chlorella sorokiniana* (Kumar et al., 2014), *Chlorella* HA-l (Watanabe et al., 1992), *Chlorella* PY-ZU1 (Cheng et al., 2013), *Chlorella* sp. NTUH15 and *Chlorella* sp. NTUH25 (Chang and Yang, 2003), *Chlorella vulgaris* (Brown, 1996; Yun and Park, 1997; Keffer and Kleinheinz, 2002; Jeong et al., 2003; Chen et al., 2010; Anjos et al., 2013), *Chlorella vulgaris* ARC-1 (Chinnasamy et al., 2009), *Chlorella vulgaris* LEB-104 (Sydney et al., 2010), *Chlorella kessleri* (de Morais and Costa, 2007a,b), *Chlorella pyrenoidosa* (Yang and Gao, 2003), *Chlorella pyrenoidosa* SJTU-2 (Tang et al., 2011), *Chlorella vulgaris* BEIJ 1890 (Kastanek et al., 2010), *Chlorella vulgaris* LEB-104 (Sydney et al., 2010), *Chlorella* sp. UK00l (Hirata et al., 1996), *Chlorella minutissima* (Papazia et al., 2008) and *Chlorella* ZY-1 (Yue and Chen, 2005).

Similarly, the species and strains of *Scenedesmus* include general *Scenedesmus* sp. (Hanagata et al.,1992; De Morais and Costa, 2007a,b; Velea et al., 2009; Ho et al., 2010; Yoo et al., 2010; Devgoswami et al., 2011; Maraskolhe et al., 2012; Nayak et al., 2013), *Scenedesmus acuminatus* (Minillo et al., 2013) *Scenedesmus dimorphus* and *Scenedesmus quadricauda* (Jiang et al., 2013; Vidyashankar et al., 2013), *Scenedesmus obliquus* (Yang and Gao, 2003; Tang et al., 2011), *Scenedesmus obliquus* SA1 (Basu et al., 2013), *Scenedesmus obliquus* CNW-N (Ho et al., 2010), *Scenedesmus*

obliquus WUST4 (Li et al., 2011), *Scenedesmus bajacalifornicus* BBKLP-07 (Patil and Kaliwal1, 2017) and *Scenedesmus* sp. ISTGA1 (Tripathi et al., 2 015).

Several attempts were also been carried out in this direction using other members like *Acutodesmus* sp. (Varshney et al., 2016), *Ankistrodesmus* (Salim, 2013), *Botryococcus braunii* (Sawayama et al., 1995; Yoo et al., 2010), *Botryococcus braunii* SAG-30.81 (Sydney et al., 2010), *Chlamydomonas* sp. MGA 161 (Miura et al., 1993), *Chlamydomonas reinhardtii* (Yang and Gao, 2003; Packer, 2009), *Chlorocuccum littorale* (Kodama et al., 1993; Kurano et al., 1995; Ota et al., 2009), *Chlorobotrys* sp. (Velea et al., 2009), *Chlorogleopsis* sp. (Ono and Cuello, 2007), *Cyanidium caldanum* (Seckbach et al., 1970), *Desmodemus* sp. F2 (Ho et al., 2014), *Dunaliella* sp. (Eloka-Eboka and Inambao, 2017), *Dunaliella tertiolecta* (Nagase et al., 1998), *Dunaliella tertiolecta* SAD-13.86 (Sydney et al., 2010), *Emiliania huxleyi* (Takano and Matsunaga, 1995), *Eudorina* sp. K17 20 (Hanagata et al., 1992), *Haematococcus* (Devgoswami et al., 2011), *Limnothrix redekei* and *Geitlerinema sulphureum* (Manjre and Deodhar, 2013), *Monoraphidium minutum* (Zeiler et al., 1995), *Nannochloropsis* (Negoro et al., 1991; Chiu et al., 2009; Jiang et al., 2011), *Nannochloropsis oculata* (Hsueh et al., 2009), *Oocystis* sp. (Takeuchi et al., 1992), *Spirulina* sp. (De Morais and Costa, 2007b), *Spirulina platensis* (Watanabe and Hall, 1995), *Spirulina platensis* LEB-52 (Sydney et al., 2010), *Synechococcus* sp. (Miyairi,1995) and *Tetraselmis* sp. (Matsumoto et al., 1995),

There are several challenges behind the idea of microalgal sequestration becoming practical. An important task in this direction is the identification of a species that can cope up with very high CO_2 concentrations, as their growth rates are negatively influenced by increasing levels of CO_2 (Lee and Lee, 2003). As CO_2 supply contributes to the lowering of pH of the culture

medium, due to carbonic acid formation (Lam and Lee, 2011), the species that survive excellently in acidic environments are generally considered for CO_2 sequestration studies. The strains suitable for CO_2 sequestration must also have low risk of contamination and should produce high value products (Lopez et al., 2009). The selection of high-performance microalgal species with increased tolerance/adaptability to CO_2, rapid growth rate, high photosynthetic efficiency, higher lipid content etc. maximizes microalgal CO_2 fixation and biomass production (Zhao and Su, 2014). In this light, the present study has been carried out with the objective of screening indigenous microalgal members with respect to their efficiencies for assimilating carbon dioxide.

Materials and Methods

In the present study, pure cultures of microalgae were prepared, maintained in the laboratory and screened for their efficiencies in assimilating external supply of carbon dioxide. Changes in growth pattern of the algae due to carbon dioxide supply were assessed through micrometry, culture turbidity, cell count and biomass. Similarly variations in gaseous components like Dissolved Oxygen (DO), free carbondioxide and other attributes of the culture media like pH, conductivity, resistivity, alkalinity and temperature were also monitored at regular intervals during the treatment period. The comparative efficiencies of algal members were worked out and the results are interpreted. The entire study has been consolidated into (1) collection (2) culturing and (3) screening of microalgae.

Collection of microalgae

The present study has been carried out during March 2014 to December 2016. Microalgal samples were collected using plankton nets from heterogeneous fresh water environments of Malappuram, Palakkad and Thrissur districts of

Kerala state. The collected organisms were identified using standard manuals, literatures (Ramos et al., 2012; Philipose, 1967; Prescott, 1951; Snow, 1902) and also with the aid of experts.

Culturing of microalgae

The collected microalgal samples were subjected to sub culturing and pure cultures of various organisms were maintained in Bolds Basal medium (Bold, 1949; Bischoff and Bold, 1963). The constituents of Bolds Basal medium is given below:

Table 1.1. Constituents of BB medium

Components	400 mL stock solution	1 Liter Stock Solution	Added quantity per liter of medium	Molar Concentration in final medium
Major Stock Solutions				
$NaNO_3$	10 g L^{-1} dH_2O	25.00 g L^{-1} dH_2O	10 ml	2.94 x 10^{-3}M
$CaCl_2 \cdot 2H_2O$	1 g L^{-1} dH_2O	2.50 g L^{-1} dH_2O	10 ml	1.70 x 10^{-4} M
$MgSO_4 \cdot 7H_2O$	3 g L^{-1} dH_2O	7.50 g L^{-1} dH_2O	10 ml	3.04 x 10^{-4} M
K_2HPO_4	3 g L^{-1} dH_2O	7.50 g L^{-1} dH_2O	10 ml	4.31 x 10^{-4} M
KH_2PO_4	7 g L^{-1} dH_2O	17.50 g L^{-1} dH_2O	10 ml	1.29 x 10^{-3} M
NaCl	1 g L^{-1} dH_2O	2.50 g L^{-1} dH_2O	10 ml	4.28 x 10^{-4} M
Alkaline EDTA Stock Solution			added 1 ml of this solution per liter of medium	
EDTA anhydrous		50 g L^{-1} dH_{2O}		4.28 x 10^{-4} M
KOH		31 g L^{-1} dH_2O		1.38 x 10^{-3} M
Acidified Iron Stock Solution			added 1 ml of this solution per liter of medium	
$FeSO_4 \cdot 7H_2O$		4.98 g L^{-1} dH_2O		4.48 x 10^{-5} M
H_2SO_4		1.0 mL		
Boron Stock			added 1 ml of this solution per liter	

Components	400 mL stock solution	1 Liter Stock Solution	Added quantity per liter of medium	Molar Concentration in final medium
Solution			of medium	
H_3BO_3		11.42 g L^{-1} dH_2O		4.62 x 10^{-4} M
Trace Metal Stock Solution			added 1 ml of this solution per liter of medium	
$ZnSO_4 \cdot 7H_2O$		8.82 g L^{-1} dH_2O		7.67 x 10^{-5} M
$MnCl_2 \cdot 4H_2O$		1.44 g L^{-1} dH_2O		1.82 x 10^{-5} M
MoO_3		0.71 g L^{-1} dH_2O		1.23 x 10^{-5} M
$CuSO_4 \cdot 5H_2O$		1.57 g L^{-1} dH_2O		1.57 x 10^{-5} M
$Co(NO_3)_2 \cdot 6H_20$		0.49 g L^{-1} dH_2O		4.21 x 10^{-6} M

Preparation of Bolds Basal medium (BB medium)

For the preparation of medium, 10 ml of the first six stock solutions together with 1 ml each of alkaline EDTA, acidified iron, boron and trace metals solutions were added and the resultant solution was made upto 1 litre with glass distilled water and autoclaved. The pH of the medium was maintained at 6.6.

The cultures were periodically monitored through an image analyzer (Leica DFC 295 and Olympus BX43) and their photographs were taken (Olympus DP27). The pure cultures were maintained in fresh water micro algal culture collection, maintained in the Department of Botany, University of Calicut. Viability of cultures was maintained through adequate supply of culture medium.

Of various microalgal members maintained in culture collection, *Chlamydomonas grovei, Chlamydomonas globosa, Desmodesmus opliensis, Monoraphidium contortum* and *Acutodesmus obliquus* were taken for further

study. Their selection was based on their viability and rapid growth and multiplication under culture conditions. The photographs concerning the micro algal members are given in plate 1. The descriptions of microalgal members taken for study are given below:

1. *Chlamydomonas grovei* G.S.West

Division	-	Chlorophyta
Class	-	Chlorophyceae
Order	-	Chlamydomonadales
Family	-	Chlamydomonadaceae
Genus	-	*Chlamydomonas*
Species	-	*grovei*

General environment

This is a freshwater species found in all forms of stagnant water such as tanks, ditches, damp soil and pools. It is also adapted to moist soil and habitats rich in salts of ammonium.

Morphology

Motile unicellular algae. Cells almost spherical, 2.5-4.5 µm long and 2.5-4 µm broad, with a pair of anterior flagellum of 6-5-10 µm long. Chloroplast parietal, cup shaped, hollowed out at the anterior end which does not contain pyrenoids and nucleus, but contains bands composed of a variable number of photosynthetic thylakoids, which are not organized into grana-like structures.

Reproduction

Asexual reproduction is by longitudinal division of protoplast into two, four or eight protoplast and liberation occurs by a gelatinization of the parent cell wall. The sexual reproduction occurs under unfavourable conditions. *Chlamydomonas* may also form aplanospores and akinetes.

2. *Chlamydomonas globosa* J.W.Snow

Division	-	Chlorophyta
Class	-	Chlorophyceae
Order	-	Chlamydomonadales
Family	-	Chlamydomonadaceae
Genus	-	*Chlamydomonas*
Species	-	*globosa*

General environment

This is a freshwater species seen in stagnant water bodies including tanks, puddles, pond and lakes.

Morphology

In the motile form, the cells are slightly ellipsoidal or spherical, 5.2 to 7.8 μm in diameter**,** anterior beak absent. Presence of two flagella which are longer or slightly longer than the cells. Pyrenoid is enveloped by a thick layer of starch. Chloroplast is thickened at the posterior end and extends to the extreme anterior end of the protoplast. A single lively vacuole can be distinguished in the anterior end. Presence of numerous oil globules in the anterior section of the cells seen.

Reproduction

Asexual reproduction takes place mainly by zoospores under favourable conditions. The unfavourable conditions like desiccation, scarcity of nitrogen etc. leads to sexual reproduction, which involves gametogenesis, syngamy, zygospore formation and germination.

3. *Desmodesmus opoliensis* (P.G.Richter) E. Hegewald

Division - Chlorophyta

Class - Chlorophyceae

Order - Sphaeropleales

Family - Scenedesmaceae

Genus - *Desmodesmus*

Species - *opoliensis*

General environment

This is a freshwater species. The distribution is ubiquitous and mainly seen in the paddy fields, lakes, shallow still waters, ponds and tanks.

Morphology

Coenobia with a single row of linearly arranged 2,4 or 8 cells. Cells 9-18.5 µm long and 3-7µm broad, oblong-cylindrical with round ends. Cell wall smooth, without ridges. Poles of terminal cells with a long, more or less straight or curved spines. Inner cells oblique to straight with straight walls with short spines.

Reproduction

Reproduction occurs by non-motile autospores. The parental protoplast undergoes longitudinal and transverse division to form non-motile autospores.

***4. Monoraphidium contortum* (Thuret) Komárková-Legnerová**

Division	-	Chlorophyta
Class	-	Chlorophyceae
Order	-	Sphaeropleales
Family	-	Selenastraceae
Genus	-	*Monoraphidium*
Species	-	*contortum*

General environment

Monoraphidium is usually found within the plankton groups of fresh water lakes and ponds and sometimes inhabits artificial ponds and even waterfalls.

Morphology

Cells 7.5 - 12 µm length and 1.5 - 2 µm width, uninucleate, solitary, irregularly curved with long, narrow, spindle shaped, gradually tapered apices, pointed ends, sigmoid, undulate to helicoidally twisted. Single parietal chloroplast without pyrenoids. Presence of 2,4,8 autospores in each cells.

Reproduction

Asexual reproduction occurs by autospores released by longitudinal or transverse rupturing of parental wall. Flagellated stages and sexual reproduction are unknown.

5. Acutodesmus obliquus (Turpin) Hegewald & Hanagata

Division	-	Chlorophyta
Class	-	Chlorophyceae
Order	-	Sphaeropleales
Family	-	Scenedesmaceae
Genus	-	*Acutodesmus*
Species	-	*obliquus*

General environment

This is a freshwater species seen floating in sluggish streams and lakes.

Morphology

Coenobium of 2,4 and 8 cells with linear or slightly alternating irregular arrangement. Cells 7 to 35 µm length, 1.5 to 8 µm in width, fusiformly arranged with long axes parallel, adjoined throughout the lateral walls with truncated tapering ends. Chlorophyll is distributed throughout the cytoplasm. Outer free walls concave; cell poles narrowed and directed away from the centre of the colony.

Reproduction

Reproduction always takes place by the formation of autocolonies within the mother cell wall. The formation of zoospores or motile gametes was never reported in this species.

Experimental layout of screening studies

The screening of microalgal members with regard to their efficiencies in assimilating carbon dioxide at various bubbling ratios like 10, 15, 20, 25, 30, 40, 60, 80 and100 bubbles/2 hours, were worked out. The general layout of the experiment is given in Plate 2.

For experimentation, each time, 12 litres of BB medium was prepared and to this, 4 litres of culture medium containing pure cultures of the test organism was added and kept for incubation for a period of 24 hours. 16 litres of the resultant microalgal culture medium was then transferred to 16 conical flasks, each with a capacity of one litre, after through mixing. These were then separated into three sets of five conical flasks each. The first set of five flasks was treated as control set, and was maintained as such. To the second set containing 5 flasks, ambient air was supplied in the form of bubbles at regular intervals of two hours and this was treated as aerated set. To the third set containing five conical flasks, carbon dioxide from a cylinder was bubbled at a regular interval of two hours and was considered as CO_2 treated set.

The culture contained in 16th conical flask was treated as the initial control set and was used to work out all parameters concerning the initial day of treatment. All the three sets were kept at illumination during the daytime from 6 am to 6pm. The experimentation was started at 6 am on the initial day. The control set was kept idle and to the other sets (aerated and CO_2 treated) air and CO_2 has been supplied at specific flow rates from 6am to 6 pm at an interval of 2 hours. Sampling and analyzing of cultures for pH, conductivity, resistivity, micrometry (cell size), turbidity, cell count, biomass content, dissolved Oxygen, free carbon dioxide and alkalinity were carried out at 6 am, every day. Monitoring of the cultures was carried out for 5 days (120 hours).

The significance of various parameters studied and methods of estimation are given below.

pH

Estimation of the pH of culture media gives information regarding the metabolic status of organisms contained within it. It is also significant to have adequate information pertaining to the impact of various gaseous influxes on to the culture media. In the present study, pH of the treatments sets was analyzed using a digital pH meter (Systronics, 6373)**.**

Conductivity

Conductivity is the measure of the ability of an aqueous solution to conduct electric current. This ability is directly related to the presence, total concentration, mobility and valence of dissolved ions present in the culture medium. The conductivity values of the treatments sets were estimated using a digital water quality analyzer (Eutech Cyberscan, PCD 650) and the results are reported in micro Siemens (µS).

Resistivity

Resistivity is the opposition of the solution to the flow of an electrical current over a distance, which is directly related to the amount of additives; usually in the form of dissolved salts, alkaline substances, chlorides, sulphates and carbonate compounds. Resistivity values of the treatments sets were recorded using digital water quality analyser (Eutech, Cyberscan - PCD 650) and represented in kΩ.

Dissolved oxygen (DO)

Estimation of Dissolved Oxygen (DO) of culture medium gives valid information on the nature, type and metabolic state of organisms contained

within it. In the present study, DO is estimated using Winkler's method as given below:

Reagents used:

1. Winkler A: 100g of KOH and 50g of KI dissolved in 200ml of pre-boiled distilled water.

2. Winkler B: 100g of $MnSO_4.4\ H_2O$ was dissolved in 200ml of distilled water. This has been heated to dissolve maximum salt; filtered after cooled.

3. Concentrated H_2SO_4

4. Sodium thiosulphate: 0.025N

5. Starch: 1.0g of starch powder was dissolved in 100ml distilled water. This was then heated for complete dissolution.

Procedure:

Each sample was taken in DO bottles without bubbling and added with 1.0 ml each of Winkler A and B solution, using separate pipettes. Extra care was taken to ensure that no air bubble is trapped while adding the reagents. After placing the stopper, the bottle was shaken and kept undisturbed for allowing the precipitate to settle down. Then the stopper was carefully removed and added with 1-2ml of concentrated H_2SO_4. The stopper was replaced and the bottle was shaken to dissolve the precipitate. 50ml sample from the DO bottle was transferred to conical flask for titration. The sample was then titrated against 0.025N Sodium thiosulphate, using starch as the indicator. The end point was recorded when initial dark brown / blue colour changes to colourless. The titration was repeated until concordant values were obtained.

DO was then calculated using the following equation and the results are reported in mg/L:

$$\text{DO (mg/l)} = \frac{\text{ml of sodium thiosulphate used x N of sodium thiosulphate x 8x 1000}}{\text{ml of sample used}}$$

Free carbon dioxide

Free carbon dioxide in water accumulates due to microbial activity and respiration of organisms. Like DO, estimation of free carbon dioxide gives reliable estimate of the photosynthetic /respiratory status of organisms contained within it. In the present study, free carbon dioxide was estimated titrimetrically as follows.

Reagent used:

1. 0.05 N NaOH
2. Phenolphthalein reagent

Procedure:

A definite quantity of the sample was titrated with 0.05 N NaOH using Phenolphthalein as indicator. The end point was characterized by the appearance of a pink color. Free carbon dioxide was calculated from the following equation and the results are reported in mg/L:

$$\text{Free carbon dioxide (mg/l)} = \frac{\text{(ml x normality) of NaOH x 1000 x 44}}{\text{ml of sample used}}$$

Alkalinity

Alkalinity is the capacity to neutralize a strong acid and is characterized by the presence of all hydroxyl ions capable of combining with the hydrogen ion.

Reagent used:

1. 0.01 N HCl
2. Phenolphthalein reagent
3. Methyl orange reagent

Procedure:

To 100 ml of the sample, drops of phenolphthalein were added. If the colour changed to pink, it was titrated with 0.1 N HCl until the colour disappeared at end point. This was kept as phenolphthalein alkalinity (PA). Now added 2-3 drops of methyl orange to the same sample and continued the titration, until the yellow colour changed to pink at the end point. The total alkalinity (TA) was then calculated from the following equation. The results are reported in mg/L:

$$\text{PA mg/L} = \frac{\text{ml of HCl used with phenolphtalein x N of HCl x 1000 x 50}}{\text{ml of sample used}}$$

$$\text{TA mg/L} = \frac{\text{ml of HCl used with phenolphtalein and methyl orange x N of HCl x 1000 x 50}}{\text{ml of sample used}}$$

Temperature

The measurement of temperature associated with the culture medium was important for assessing its effects on the biochemistry, rate of photosynthesis and biochemical reactions of micro algae. A mercury thermometer having a scale marked for every $0.1^{o}C$ was taken and immersed in conical flask up to the level of mercury in the capillary column. The results are expressed in degree Celsius (ºC).

Micrometry (cell size)

Micrometry involves the measurement of minute objects with a microscope using stage micrometer and ocular micrometer. In the present study, micrometry is used to assess the changes in cell size of micro algae in the culture media due to external gaseous inputs. In Micrometry, the stage micrometer, which is mounted on the stage, has linear scale of length 1 mm, which is precisely divided into 100 equal divisions. One small division is equal to 0.01mm. The ocular micrometer, which is placed on the top of the field diaphragm of eyepiece has 100 equal divisions. The magnification of the microscope is calibrated by aligning both micrometers parallel to each other. Stage micrometer is positioned so that its initial division coincides with initial divisions of ocular micrometer. The number of divisions on both scales was counted up to the next point, where the divisions of two scales again coincide. The size of organisms was estimated by the following equation and the results are reported in µm.

$$\text{Value of one ocular micrometer} = \frac{\text{Number of divisions on the stage micrometer}}{\text{Number of divisions on the ocular micrometer}} \times 10$$

Turbidity

Turbidity is the measure of the amount of suspended particles in the sample solution. Assessment of the turbidity gives information about the density of the microalgal culture medium. Turbidity readings were taken using Nephelometer (EI-341) using distilled water as blank and the results are represented in NTU. Calibration of the instrument was done using standards prepared using Hydrazine sulphate and Hexa methylene tetramine.

Cell count

Estimation of cell count provides information regarding the growth status of algal cells in culture medium. The cell count of algal members in the present

study was carried out using a Haemocytometer. For estimation of cell count, a drop of well agitated sample was placed on to the counting chamber and mounted using a cover glass. Using a compound microscope the cell count was taken and represented in cells x 10^4.

Biomass

Algal biomass production is directly proportional to the efficiency with which the algal cells assimilate through photosynthesis. The biomass of microalgae was estimated by centrifuging 100ml of culture media containing sample in a pre -weighed centrifuge tube at 4000 rpm for 5 minutes. After centrifugation, the tube was blotted with a filter paper. After discarding the supernatant, it was kept undisturbed for 15 minutes for air drying. The weight of the tube with microalgae was then estimated. The weight of the microalgae was then calculated from the differences in weight of the centrifuge tube with microalgae and the weight of the centrifuge tube alone. The results are reported in g.

Results and Discussion

Currently, biological fixation of CO_2 through photosynthesis has received much attention due to simultaneous production of biomass energy (Kondili and Kaldellis, 2007; Ragauskas et al., 2006). The rapid growth rate of microalgae (up to 10 times that of higher plants) and potential for higher-efficiency solar conversion than land plants has accelerated their search for CO_2 sequestration activities. The main challenge in this regard is the identification of ideal species that can thrive under extreme conditions, owing to extraneous supply of CO_2.

In the present study, an attempt has been carried out to assess the potentialities of selected microalgal species like *Chlamydomonas grovei, Chlamydomonas globosa, Desmodesmus opliensis, Monoraphidium contortum* and *Acutodesmus obliquus* belonging to Chlorophyceae, with regard to their efficiencies in carbon dioxide assimilation. Preliminary studies

were carried out on individual species for determining their optimum levels carbon dioxide assimilation.

Accordingly, 10, 15, 25 bubbles / 2 hours were supplied for *C. grovei* (Table 1.2) and 10, 15, 20, 40, 60 and 80 bubbles / 2 hours for *C. globosa* (Table 1.3). The potentials of *D. opoliensis* were monitored under 4 bubbling frequencies like 20, 25, 30 and 40 bubbles / 2 hours (Table 1.4) and the bubbling frequencies of 10, 15 and 20 bubbles / 2 hours (Table 1.5) were attempted for *M. contortum*. The efficiencies of *A. obliquus* were worked out under CO_2 supply of 20, 40, 60, 80 and 100 bubbles / 2 hours (Table 1.5).

Results pertaining to each treatment sets were analyzed and the final median values were worked out. Consolidated results concerning *C .grovei, C. globosa, D. opliensis, M. contortum* and *A. obliquus* with respect to control and various bubbling frequencies of air and CO_2 are depicted in Tables 1.2 – 1.6, respectively. Statistical analyses of the data were carried out and the results are given in Tables 1.7 and 1.8.

An attempt has also been carried out to standardize the bubbling frequencies (10, 15, 20, 25, 30, 40, 60, 80, 100 bubbles / 2 hours) in accordance with the extent of CO_2 dissolved per litre of Bolds Basal medium. This has been carried out by bubbling CO_2 gas from the source at specific frequencies through the medium (1.0 litre) taken in a conical flask and closed with a cotton plug. The resultant free CO_2content associated with the medium was then analyzed titrimetrically using phenolphthalein and 0.05N NaOH and the results are represented in mg/L.

Accordingly, it has been noticed that a bubbling frequency of 10, 15, 20, 25, 30, 40, 60, 80 and 100 bubbles / 2 hours in a litre of Bolds Basal medium retained 66.0, 72.6, 79.2, 85.8, 92.4, 105.6, 132.0, 158.4 and 184.8 mg/L of free CO2 content, respectively, within BB medium.

Table1.2. Consolidated results on the responses of *Chlamydomonas grovei to* various dosages of CO_2 (Results are represented as median values of respective treatment sets).

Sl. No.	PARAMETERS ANALYSED	CO_2 supply in bubbles / 2 hours of interval		
		10	15	25
1	**pH**			
	Control	6.74	6.74	6.8
	Aerated	6.74	6.72	6.77
	CO_2 treated	6.75	6.74	6.81
2	**CONDUCTIVITY(µs)**			
	Control	764	774.9	754.7
	Aerated	762	773.9	755
	CO_2 treated	763	773.4	754.6
3	**RESISTIVITY(kΩ)**			
	Control	1.28	1.262	1.295
	Aerated	1.283	1.264	1.295
	CO_2 treated	1.283	1.264	1.296
4	**MICROMETRY(µm)**			
	Control	36	31.5	33
	Aerated	37.5	31.5	31.9
	CO_2 treated	36	31.5	31.9
5	**TURBIDITY(NTU)**			
	Control	4.25	10.5	3.4
	Aerated	3.6	10.45	4.9
	CO_2 treated	4.55	10.45	4.65
6	**CELL COUNT($\times 10^4$cells/ml)**			
	Control	8.25	6.38	6.55
	Aerated	6.91	4.38	8.1
	CO_2 treated	9.67	5.75	7.5
7	**BIOMASS (gm)**			
	Control	0.008	0.007	0.01
	Aerated	0.011	0.008	0.018
	CO_2 treated	0.012	0.007	0.017
8	**DISSOLVED OXYGEN(mg/L)**			
	Control	7.9	8.7	8.6
	Aerated	7	7.8	7
	CO_2 treated	7.7	8.2	6.7
9	**FREE CO_2(mg/L)**			
	Control	52.8	52.8	66
	Aerated	55	52.8	68.2
	CO_2 treated	57.2	52.8	74.8
10	**ALKALINITY(mg/L)**			
	Control	75	75	100
	Aerated	80	67.5	90
	CO_2 treated	75	72.5	80

(Temperature of the medium during experimentation ranged from 29 – 32˚C)

Table 1.3. Consolidated results on the responses of *Chlamydomonas globosa* to various dosages of CO_2 (Results are represented as median values of respective treatment sets).

Sl. No.	PARAMETERS ANALYSED	CO_2 supply in bubbles / 2 hours of interval					
		10	15	20	40	60	80
1	**pH**						
	Control	6.91	6.87	6.82	6.6	7.34	6.93
	Aerated	6.88	6.85	6.79	6.6	7.34	6.94
	CO_2 treated	6.86	6.84	6.83	5.71	5.73	5.62
2	**CONDUCTIVITY(µs)**						
	Control	784.5	779	753.6	821	946.6	870.7
	Aerated	782.6	777.65	753.3	821.3	945.7	870.1
	CO_2 treated	782.7	776.35	753.2	807.6	934.95	855.55
3	**RESISTIVITY(kΩ)**						
	Control	1.248	1.255	1.297	1.192	1.033	1.124
	Aerated	1.25	1.257	1.298	1.19	1.034	1.124
	CO_2 treated	1.249	1.26	1.298	1.21	1.047	1.143
4	**MICROMETRY(µm)**						
	Control	23.25	22	33	33.75	31.88	35.6
	Aerated	24.5	22	32.7	37.5	31.88	37.5
	CO_2 treated	23.25	27.5	34.5	37.5	33.75	37.5
5	**TURBIDITY(NTU)**						
	Control	4.2	4.55	4.35	14.1	9.3	9.55
	Aerated	3.95	4.5	3.9	13.6	9.25	9.75
	CO_2 treated	4.2	4.5	4.15	15.25	9.55	9.65
6	**CELL COUNT ($\times 10^4$ cells/ml)**						
	Control	34	23.5	29.5	277.5	150.5	186.5
	Aerated	29	22.5	25	267	157	146
	CO_2 treated	34.5	28	33.75	302.5	179	109.5
7	**BIOMASS(gm)**						
	Control	0.011	0.015	0.013	0.047	0.042	0.032
	Aerated	0.014	0.015	0.013	0.047	0.042	0.028
	CO_2 treated	0.018	0.018	0.019	0.052	0.05	0.028
8	**DISSOLVED OXYGEN(mg/L)**						
	Control	9.2	8	8.8	11	10.4	10.3
	Aerated	8.5	8.2	7.7	10.5	10.3	9.9
	CO_2 treated	8.7	7	8.6	12.8	12.3	10.2
9	**FREE CO_2 (mg/L)**						
	Control	48.4	52.8	50.6	11	8.8	22
	Aerated	48.4	52.8	52.8	17.6	8.8	26.4
	CO_2 treated	48.4	55	50.6	63.8	88	118.8
10	**ALKALINITY(mg/L)**						
	Control	95	107.5	112.5	127.5	102.5	102.5
	Aerated	92.5	100	107.5	122.5	100	110
	CO_2 treated	90	90	112.5	132.5	105	100

(Temperature of the medium during experimentation ranged from 29.5 – 31˚C)

Table 1.4. Consolidated results on the responses of *Desmodesmus opoliensis* to various dosages of CO_2 (Results are represented as median values of respective treatment sets).

Sl. No.	PARAMETERS ANALYSED	CO_2 supply in bubbles / 2 hours of interval			
		20	25	30	40
1	**pH**				
	Control	6.76	7.29	6.61	6.68
	Aerated	6.74	7.21	6.6	6.69
	CO_2 treated	6.76	7.24	6.65	6.67
2	**CONDUCTIVITY(µs)**				
	Control	762.3	805.8	775.4	777.5
	Aerated	760.9	803.3	774.4	776.4
	CO_2 treated	761.5	803.2	774.4	774.7
3	**RESISTIVITY(kΩ)**				
	Control	1.284	1.213	1.26	1.258
	Aerated	1.287	1.216	1.264	1.259
	CO_2 treated	1.285	1.218	1.262	1.263
4	**MICROMETRY(µm)**				
	Control	36	40.5	41.25	38.25
	Aerated	38.3	33.75	41.25	33.75
	CO_2 treated	40.5	36	39.88	36
5	**TURBIDITY(NTU)**				
	Control	0.85	6.6	8.6	9.65
	Aerated	0.75	6.05	8.4	9.6
	CO_2 treated	0.9	7.65	8.9	10.35
6	**CELL COUNT($\times 10^4$cells/ml)**				
	Control	36	37.5	23.63	33.5
	Aerated	30	37	21.13	39
	CO_2 treated	41	47.5	22.46	38.13
7	**BIOMASS (gm)**				
	Control	0.029	0.039	0.029	0.036
	Aerated	0.03	0.035	0.032	0.043
	CO_2 treated	0.032	0.043	0.033	0.039
8	**DISSOLVED OXYGEN(mg/L)**				
	Control	8.7	8.4	8.3	7.2
	Aerated	8.3	7.3	7.2	7.8
	CO_2 treated	8.9	9.6	8	7.4
9	**FREE CO_2 (mg/L)**				
	Control	66	57.2	61.6	110
	Aerated	63.8	70.4	59.4	101.2
	CO_2 treated	63.8	79.2	59.4	103.4
10	**ALKALINITY(mg/L)**				
	Control	80	100	65	77.5
	Aerated	70	90	70	72.5
	CO_2 treated	72.5	92.5	60	72.5

(Temperature of the medium during experimentation ranged from 29.5 – 34°C)

Table 1.5. Consolidated results on the responses of *Monoraphidium contortum* to various dosages of CO_2 (Results are represented as median values of respective treatment sets).

Sl. No.	PARAMETERS ANALYSED	CO_2 supply in bubbles/2 hours of interval		
		10	15	20
1	**pH**			
	Control	6.74	6.85	6.76
	Aerated	6.75	6.86	6.77
	CO_2 treated	6.76	6.81	6.78
2	**CONDUCTIVITY(µs)**			
	Control	772.6	768.2	769.4
	Aerated	772.4	766.6	770.3
	CO_2 treated	772.5	767.2	770.6
3	**RESISTIVITY(kΩ)**			
	Control	1.265	1.273	1.27
	Aerated	1.267	1.276	1.27
	CO_2 treated	1.266	1.274	1.27
4	**MICROMETRY(µm)**			
	Control	2.25	72	32.63
	Aerated	1.875	76.5	32.5
	CO_2 treated	4	76.5	31.5
5	**TURBIDITY(NTU)**			
	Control	7.65	8.45	3.65
	Aerated	6.9	8.2	3.95
	CO_2 treated	8	8.75	3.8
6	**CELL COUNT($\times 10^4$cells/ml)**			
	Control	17	18.5	12
	Aerated	14	17	14
	CO_2 treated	21	21	13.5
7	**BIOMASS(gm)**			
	Control	0.009	0.007	0.01
	Aerated	0.008	0.008	0.013
	CO_2 treated	0.01	0.01	0.012
8	**DISSOLVED OXYGEN(mg/L)**			
	Control	8.2	7.3	8.1
	Aerated	7.8	7	7.8
	CO_2 treated	8.2	8.1	7.9
9	**FREE CO_2 (mg/L)**			
	Control	52.8	52.8	57.2
	Aerated	52.8	55	52.8
	CO_2 treated	52.8	52.8	57.2
10	**ALKALINITY(mg/L)**			
	Control	80	95	85
	Aerated	80	87.5	80
	CO_2 treated	75	82.5	80

(Temperature of the medium during experimentation ranged from 30 – 31˚C)

Table 1.6. Consolidated results on the responses of *Acutodesmus obliquus* to various dosages of CO_2 (Results are represented as median values of respective treatment sets).

Sl. No.	PARAMETERS ANALYSED	CO_2 supply in bubbles / 2 hours of interval				
		20	40	60	80	100
1	**pH**					
	Control	6.99	6.38	5.63	7.17	6.97
	Aerated	7.01	6.39	5.77	7.13	6.84
	CO_2 treated	7.02	6.43	5.49	5.82	5.04
2	**CONDUCTIVITY(µs)**					
	Control	784.6	766.65	747.75	834.85	850.5
	Aerated	783	766.7	745.2	834.75	845.6
	CO_2 treated	782.5	765.4	740.35	827.65	828.5
3	**RESISTIVITY(kΩ)**					
	Control	1.247	1.276	1.307	1.171	1.151
	Aerated	1.248	1.276	1.313	1.171	1.157
	CO_2 treated	1.251	1.277	1.321	1.181	1.18
4	**MICROMETRY(µm)**					
	Control	45	39	46.88	45	46.88
	Aerated	45	33	46.25	46.5	52.5
	CO_2 treated	45	34.5	49.38	50.25	48.75
5	**TURBIDITY(NTU)**					
	Control	3.95	8.25	11.8	9.05	7.95
	Aerated	4.05	8.35	13.3	9	8.65
	CO_2 treated	4.2	8.65	14.5	10.3	8.2
6	**CELL COUNT ($\times 10^4$ cells/ml)**					
	Control	24	28	53	25.5	24.5
	Aerated	25.13	28.63	59.5	27.5	19.25
	CO_2 treated	30.67	33.75	60.38	30.75	19.75
7.	**BIOMASS (gm)**					
	Control	0.043	0.032	0.042	0.04	0.043
	Aerated	0.041	0.031	0.053	0.047	0.04
	CO_2 treated	0.045	0.034	0.061	0.051	0.034
8	**DISSOLVED OXYGEN (mg/L)**					
	Control	9.4	9.2	8.5	10	10.5
	Aerated	9.9	9.1	10.3	10.1	9.4
	CO_2 treated	9.9	10.1	15	13.8	13.9
9	**FREE CO_2 (mg/L)**					
	Control	28.6	39.6	39.6	44	44
	Aerated	30.8	39.6	37.4	44	44
	CO_2 treated	33	35.2	48.4	41.8	129.8
10	**ALKALINITY(mg/L)**					
	Control	90	90	97.5	110	130
	Aerated	90	92.5	92.5	110	112.5
	CO_2 treated	92.5	97.5	95	100	130

(Temperature of the medium during experimentation ranged from 28 – 30˚C)

For monitoring the carbon dioxide sequestration potentialities of each microalgal member, the results pertaining to major growth parameters like turbidity of the medium owing to microalgal growth, together with cell count and biomass content of micro algal members were considered.

Upon comparing the carbon dioxide assimilation efficiencies of five microalgal members, with *C. grovei ,* higher turbidity (4.55 NTU), cell count (9.67 cells x 10^4 per ml) and biomass (0.012 gm) was noticed in the CO_2 treatment set of 10 bubbles / 2 hours, than control and aerated set. Comparing the growth parameters of *C. globosa,* increased turbidity (9.55 NTU), cell count (179 cells x 10^4 per ml) and biomass content (0.05 gm) was observed in CO_2 treatment set of 60 bubbles / 2 hours. With *D. opoliensis,* higher turbidity (0.9 NTU), cell count (41 cells x 10^4 per ml) and biomass (0.032 gm) was noticed in the CO_2 treatment set of 20 bubbles / 2 hours. Concerning *M. contortum,* maximum turbidity (8.75 NTU), cell count (21 cells x 10^4 per ml) and biomass (0.01 gm) was noticed in the CO_2 treatment set of 15 bubbles / 2 hours, whereas with *A. obliquus,* increased turbidity (10.3 NTU), cell count (30.75 cells x 10^4 per ml) and biomass content (0.051) was observed in CO_2 treatment set of 80 bubbles / 2 hours, compared to control and aerated set.

On an overall assessment of the growth performances of micro algal members under heterogeneous culture conditions, it has been noticed that in higher CO_2 treatment sets of *A. obliquus* (bubbling frequencies of 60, 80 and 100 per 2 hours) and *C. globosa* (bubbling frequencies of 40, 60 and 80 per 2 hours), higher free CO_2 content was noticed in the culture media, compared to control and aerated set.

pH is an important attribute which can affect both gas absorption and nutrient availability in aqueous systems (Vonshak, 1997). In the present study, upon comparison of the pH values pertaining to the treatments sets, it has been noticed that treatment sets of *A. obliquus* (60 bubbles/2 hours and 80 bubbles/2 hours) and *C. globosa* (40 bubbles/2 hours and 60 bubbles/2 hours) fed with intermittent CO_2 supply exhibited pH in acidic ranges. It is being

stated that at higher CO_2 concentration, the culture pH decreases due to the formation of high amount of bicarbonate buffer (Sobczuk et al., 2000).

Devgoswami et al. (2011) reported that when CO_2 dissolve in water, depending on pH of the solution, three inorganic species can be formed. The carbonic acid formation or free CO_2 molecules in pH below 4.5, HCO_3- at pH 8.5 and carbonate at pH above 8.5. Usually the HCO_3 fails to diffuse across cell membrane and the carbonic anhydrase enzyme present in the cell surface, aid in conversion of HCO_3 to CO_2 and consequently transported to cells for assimilation process (Tsuzuki and Miyachi, 1989). The carbonic anhydrase facilitate microalgae in carbon concentrating mechanism (CCM) to satisfy the carbon requirements.

The decreasing pH due to the presence of higher CO_2 concentration seems to be lethal to algal species (Kumar et al., 2010). It is reported to be a limiting condition, influencing photosynthesis and inhibiting carbon dioxide biofixation (Cheng et al., 2006). In the present study, the microalgal members (*A. obliquus* and *C. globosa*) are surviving in acidic range of pH in treatment sets fed with CO_2 owing to their adaptabilities with respect to changing environmental conditions. The capabilities of micro algal members like *Chlorella* sp. and *Chlorococcum littorale* to tolerate acidic pH was also reported (Chang and Yang, 2003; Yue and Chen, 2005; Kodama et al., 1993; Zeng et al., 2011; Zhao and Su, 2014). The better growth performances of some of the micro algal members at acidic pHs and the maintenance of culture pH within a range are indicative of their adaptability and CO_2 assimilation capabilities. The results pertaining to the research work of Olaizola et al. (2004) also indicate that the pH of the medium is a key determinant of CO_2 capture efficiency and the species that survive excellently in acidic environments are generally considered as more tolerant to higher CO_2 concentrations (Singh and Ahluwalia, 2013 and Eloka-Eboka and Inambao, 2017).

Also, upon comparing the conductivity values of the CO_2 treatment sets pertaining to *A. obliquus* (60 bubbles/2 hour and80 bubbles/2 hour) and *C.*

globosa (40 bubbles/2 hour and 60 bubbles/2 hour) it was noticed to be lower than both control and aerated set. In the case of resistivity values of CO_2 treatment sets, it was noticed to be higher than control and aerated set.

Responses of microalgal cells to external stimuli vary. Here in the treatment sets of *A. obliquus* (60 and 80 bubbles/2 hours) and *C. globosa* (15, 20, 40, 60 and 80 bubbles/2 hours) increased cell size was noticed in CO_2 treated set, compared to the cells of both control and aerated set. Hanagata et al. (1992) stated that the morphological modifications assist in imparting tolerance to elevated CO_2 levels. The increase in the cell size of microalgae pertaining to the CO_2 treated sets can be considered as the adaptation techniques with response to the elevated CO_2 dosages. Faria et al. (2012) noticed increased cell volumes of *Chlorella* sp. due to the accumulation of reserve substances, while cultured under CO_2. An increase in size and number of vacuoles with elevated CO_2 levels has been reported by Pronina et al. (1993). The cell enlargement in *Chaetoceros gracilis* in response to elevated CO_2 concentrations was also reported (Khairy et al., 2014).

Increases in turbidity and cell count in most cases are considered to be an index of growth. In the present study, the results of turbidity and cell count was found to be increased in treatment sets of *A. obliquus* (60 and 80 bubbles/2 hour) and *C. globosa* (40 and 60 bubbles/2 hour) fed with intermittent supply of $CO_{2.}$Hence it can be concluded that the intermittent supply of CO_2 on *A. obliquus* (80 bubbles/2 hour) and *C. globosa* (60 bubbles/2 hour) have accelerated the growth of both microalgal members.

According to literature, biomass production seems to be the most significant factor used for assessing the capabilities of microalgae in the successful bioconversion of carbon dioxide (Cheng et al., 2006; Cheah et al., 2015). Most of the research works quantified the carbon fixation in terms of biomass production (Chae et al., 2006). Here in the present study also the results pertaining to biomass content was considered mainly as the major growth parameter to ensure the optimum species and dosage in which maximum CO_2

assimilation occurred. Results with *A. obliquus* (80 bubbles/2 hour) and *C. globosa* (60 bubbles/2 hour) are highly promising and indicating with higher growth potential, as evidenced by results of carbon dioxide treated set compared to control and aerated set.

On a comparative assessment of the DO of the culture medium, it was noticed that CO_2 treated sets of *A. obliquus* (60 and 80 bubbles/2 hour) and *C. globosa* (40 and 60 bubbles / 2 hour) maintained higher levels of DO than their respective control and aerated set. The microalgae can produce oxygen as by product of photosynthesis with respect to simultaneous carbon dioxide uptake (Kumar et al., 2010; Suali et al., 2017).

Different algae exhibits varied responses to high oxygen concentrations (Pope, 1975; Salih, 2011). When O_2 concentration increases, the CO_2 concentration decreases successively, which in turn will hinder the photosynthetic process of microalgae and leads to the declined microalgal growth and CO_2 fixation ability. Kumar et al. (2010) observed that DO concentration >35 mg/l inhibits the microalgal growth. Here in the present study, the DO recorded in the CO_2 treated sets was lower than 35 mg/l and the increased DO content is an indicative of the active photosynthetic process of microalgae in the presence of available CO_2.

Alkalinity content of culture medium in the treatment sets of *A. obliquus* (60 and 80 bubbles/2 hour) was higher in the case of control and aerated set, while with *C. globosa* (40 and 60 bubbles/2 hour) increased alkalinity content in CO_2 treated set was noticed.

The efficiency of CO_2 removal and fixation mainly depends on the physiological conditions of microalgae, the potentiality for cell growth and ability for CO_2 metabolism (Yoo et al., 2010).The optimum CO_2 concentrations and the tolerance range of algae to higher carbon dioxide in the medium are species-specific (Seckbach et al., 1971; Kodama et al., 1993; Sergeenko et al., 2000). The CO_2 concentration of 1–5% was reported as optimal for majority of photosynthetic microorganisms (Sergeenko et al.,

2000). Varshney et al. (2016) observed maximum growth rate for *Acutodesmus* sp. at 10 % CO_2 and no inhibitory effects were noticed in higher CO_2 concentrations. Yun et al. (2016) noticed increased growth rate of *Acutodesmus obliquus* KGE 30 at 14.1 % CO_2. The maximum biomass production of *Acutodesmus obliquus* in 20 % CO_2 and higher tolerance to 50 % CO_2 concentration was also reported (Chen et al., 2016). Tang et al. (2011) observed increased growth of *Acutodesmus* (*Scenedesmus*) *obliquus* SJTU-3 under CO_2 concentrations of 5 to 30 % and Jiang et al. (2013) reported 10 % CO_2as optimum for *Tetradesmus* (*Scenedesmus*) *dimorphus*. However there were no reports regarding the CO_2 fixation efficiencies of *C. globosa.*

In spite of slightly acidic pH and higher free CO_2 in the culture medium, microalgal members like *A. obliquus* (80 bubbles/2 hour) and *C. globosa* (60 bubbles/2 hour) exhibited higher rate of DO production, higher turbidity, higher cell count and higher biomass production in treatment sets supplied with free CO_2, which characteristically indicates their efficiencies in mitigating gaseous CO_2. The species *C. grovei, M. contortum* and *D. opoliensis* also exhibited moderate efficiencies in carbon dioxide assimilation, but only next to *A. obliquus* and *C. globosa*.

Statistical Analysis

The results pertaining to the optimum bubbling frequencies of *A. obliquus* (80 bubbles of CO_2 per 2 hour) and *C. globosa* (60 bubbles of CO_2 per 2 hour) were subjected to statistical analysis using One Way Anova method. Throughout the analysis, significant variation among different parameters, that are likely to influence the growth and survival of these microalgal members under 3 treatment sets were identified by considering the F value. The CD values were worked out for significant parameters to draw conclusions regarding the variations among 3 treatment sets and the results are reported in Tables1.7 and Table 1.8.

Table 1.7. Comparative study of various parameters of *Acutodesmus obliquus* with respect to 80 bubbles of CO_2 per 2 hour.

Sl. No.	TREATMENT SETS	Average	F- value	CD
pH				
1	Control set	7.34	6415.678*	0.0582
2	Aerated set	7.407		
3	Carbon dioxide treated set	5.323		
Conductivity				
1	Control set	834.2	66.736*	4.0696
2	Aerated set	832.8		
3	Carbon dioxide treated set	818.9		
Resistivity				
1	Control set	1.173	17.4*	3.4991
2	Aerated set	1.174		
3	Carbon dioxide treated set	1.194		
Turbidity				
1	Control set	13.23333	11.03*	0.6182
2	Aerated set	13		
3	Carbon dioxide treated set	14		
Cell Count				
1	Control set	32	1.75	NS
2	Aerated set	28		
3	Carbon dioxide treated set	34		
Micrometry				
1	Control set	45	3.681	NS
2	Aerated set	48.75		
3	Carbon dioxide treated set	52.5		
Dissolved oxygen				
1	Control set	9.666	3.88	NS
2	Aerated set	9.6		
3	Carbon dioxide treated set	14		
Free carbon dioxide				
1	Control set	44	64.5*	9.9427
2	Aerated set	44		
3	Carbon dioxide treated set	79.226		
Alklainity				
1	Control set	130	42*	9.2532
2	Aerated set	110		
3	Carbon dioxide treated set	140		
Biomass				
1	Control set	0.043	171.2727*	0.0043
2	Aerated set	0.045		
3	Carbon dioxide treated set	0.069		

*** Significant at 5% level; NS: not significant (critical value of F at 5% level for 3 treatments and 3 replications =5.143)**

Table 1.8. Comparative study of various parameters of *Chlamydomonas globosa* with respect to 60 bubbles of CO_2 per 2 hour.

Sl No	TREATMENT SETS	Average	F- value	CD
pH				
1	Control set	7.77	5598.412*	0.0621
2	Aerated set	7.776		
3	Carbon dioxide treated set	5.716		
Conductivity				
1	Control set	946.233	554.656*	1.4898
2	Aerated set	946.2		
3	Carbon dioxide treated set	930.733		
Resistivity				
1	Control set	1.033	15.073*	3.1732
2	Aerated set	1.033		
3	Carbon dioxide treated set	1.050		
Turbidity				
1	Control set	10.4	202.048*	0.4238
2	Aerated set	11.1		
3	Carbon dioxide treated set	13.95		
Cell Count				
1	Control set	146	137.139*	13.1516
2	Aerated set	155		
3	Carbon dioxide treated set	218		
Micrometry				
1	Control set	37.5	3	NS
2	Aerated set	37.5		
3	Carbon dioxide treated set	33.75		
Dissolved oxygen				
1	Control set	10.4	1089*	0.4524
2	Aerated set	10.4		
3	Carbon dioxide treated set	17		
Free carbon dioxide				
1	Control set	11	2773.59*	4.0740
2	Aerated set	11		
3	Carbon dioxide treated set	105.666		
Alklainity				
1	Control set	105	13*	11.3327
2	Aerated set	100		
3	Carbon dioxide treated set	120		
Biomass				
1	Control set	0.052	17.310*	0.0064
2	Aerated set	0.049		
3	Carbon dioxide treated set	0.062		

*** Significant at 5% level; NS: not significant (critical value of F at 5% level for 3 treatments =5.143)**

In the case of *A. obliquus,* while comparing the results at 5% level, the mean pH values of the three sets showed significant variations when compared with the CD value = 0.0582 (F value = 6415.68, P value = 0.000). The mean pH values of carbon dioxide treated set of *C. globosa* exhibited significant variations between control set and aerated set and there were no significant variations between control set and aerated set while comparing the results with CD value 0.0621 (F value = 5598.412, P value = 0.000).

Upon comparing the results of the mean conductivity values of *A. obliquus* and *C. globosa,* the carbon dioxide treated set showed significant variations with both control set and aerated set with the CD value 4.0696 and 1.4898 (F value = 66.736,554.6555; P value = 0.000, 0.000), respectively. There were no significant variations between the control and aerated set in the case both microalgal species.

While analyzing the results of *A. obliquus* and *C. globosa* pertaining to mean resistivity values, it was noticed in that there were no significant variations between the three treatment sets, when compared to the CD values 3.4991 and 3.1732 (F value = 17.4, 15.07386; P value = 0.003, 0.004), respectively.

In the case of *A. obliquus,* the mean turbidity values of the carbon dioxide treated set showed significant variations with both control and aerated set with the CD value 0.6182 (F value = 11.03,P value = 0.009). However, there were no significant variations between control set and aerated set. Upon comparing the with CD value (0.4238) it was noticed that the *C. globosa* exhibited significant variations between three treatment sets (F value =202.0476, P value = 0.000633).

Upon analyzing the F value (1.75) of the cell count pertaining to *A. obliquus,* it was observed that there were no significant variations among the three treatment sets and considered as non-influencing / non-significant parameter.

However the mean cell count results of *C. globosa* revealed the significant variations of carbon dioxide treated set with both control set and aerated set with the CD value 13.1516 (F value = 137.1386, P value = 0.000) and there were no significant variations between the control and aerated set.

Upon comparing the F value of the treatment sets of *A. obliquus* (3.681) and *C. globosa* (3), micrometry was noted to be anon-significant parameter.

Comparison of the mean values with F value (3.88) of *A. obliquus,* dissolved Oxygen content was considered as non-significant parameter. The mean values of Dissolved Oxygen content of carbon dioxide treated set of *C. globosa* showed significant variations when compared to both control and aerated set with CD value 0.4524 (F value = 1089 and P value = 0.000).

The mean free carbon dioxide values of carbon dioxide treated set in both treatment sets exhibited highly significant variations with control and aerated set for *both A. obliquus* (CD value = 9.9427, F value = 64.5 and P value = 0.00) and *C. globosa* (CD value = 4.0740, F value = 2773.59 and P value = 0.000). However there were no significant variations between control and aerated set.

While comparing the mean alkalinity values of *A. obliquus* (CD value=9.253, F value= 42 and P value=0.000), it was noticed that there were significant variations between control set, aerated set and carbon dioxide treated set. In the case of *C. globosa,* the carbon dioxide treated set exhibited significant variations with control set and aerated set (CD value = 9.2532,F value =13 and P value = 0.006) and there were no significant variations between control set and aerated set.

Upon comparing the F value of *A. obliquus* (171.27) and *C. globosa* (17.31), the biomass content was considered as a significant parameter. Here the carbon dioxide treated set of both *A. obliquus* (CD value = 0.0043, F value =

171.2727 and P value = 0.000) and *C. globosa* (CD value = 0.0064, F value = 17.31081 and P value = 0.003) exhibited significant variations with both control and aerated set.

Upon consolidation of the statistical analysis pertaining to the effect of optimum bubbling ratios on the physico-chemical and biological parameters of both microalgal species, the parameters like pH, conductivity, resistivity, turbidity, free carbon dioxide, alkalinity and biomass were noticed to be significant. However apart from these parameters, the cell count and dissolved oxygen content were also noticed to be significant for *C. globosa.* From the above observations it can be also concluded that among the growth parameters of both species, turbidity and biomass was noted to be significant.

Summary and Conclusion

Among various strategies for CO_2 sequestration, biological sequestration using photosynthetic microalgae have received considerable attention in recent times. Microalgae, one of the most important living resources of both fresh and marine systems can be employed for CO_2 sequestration, as they have higher photosynthetic efficiency, higher biomass production and faster growth rate, compared to other energy crops. Also they can readily be incorporated into engineered systems.

In light of the above, the present study has been carried out with the objective of assessing the CO_2 assimilation capabilities of five indigenous micro algal species belonging to Chlorophyceae, namely *Chlamydomonas grovei, Chlamydomonas globosa, Desmodesmus opliensis, Monoraphidium contortum* and *Acutodesmus obliquus*. These micro algal samples were collected from heterogeneous environments and their pure cultures were maintained in the laboratory using Bolds Basal medium. They were then individually subjected to CO_2 assimilation studies.

For assimilation studies, 12 litres of Bolds Basal medium was prepared and to this, four litres of culture medium containing pure cultures of the respective microalgal species was added. After incubation, the microalgal culture was transferred to 16 one litre conical flasks and then separated into three sets of five conical flasks. The first set of five flasks were treated as control and was maintained as such. To the culture containing conical flasks of the second set, ambient air has been bubbled at an interval of two hours and treated as aerated set. To the third set, carbon dioxide from a cylinder has been bubbled at an interval of two hours and was considered as CO_2 treated set. The culture contained in the last conical flask (16th) was treated as the initial control set and has been used to analyze all parameters meant for the initial day of treatment.

The experimentation was initiated at 6 am on the initial day and all the three sets were kept at illumination during the day time (6 am to 6 pm). Sampling and analyzing of culture has been carried out at 6 am of each day. pH, conductivity, resistivity, dissolved oxygen, free carbon dioxide and alkalinity content of the algal culture together with cell size (micrometry), turbidity, cell count and biomass content of the micro algal members were worked out. Monitoring of the cultures was carried out for a period of 120 hours.

For monitoring the carbon dioxide sequestration potentials of each microalgal member, the results pertaining to major growth parameters like turbidity of the medium owing to micro algal growth, together with cell count and biomass content of micro algal members were considered. While comparing the carbon dioxide assimilation efficiencies of five microalgal members under study, in the case of *C. grovei,* higher turbidity, cell count and biomass content was noticed in the CO_2 treatment set of 10 bubbles/2 hours. With *C. globosa,* increased turbidity, cell count and biomass content was observed in CO_2 treatment set of 60 bubbles/2 hours. In the case of *D. opoliensis,* higher

turbidity, cell count and biomass was noticed in the CO_2 treatment set of 20 bubbles/2 hours. Concerning *M. contortum,* maximum turbidity, cell count and biomass was noticed in the CO_2 treatment set of 15 bubbles /2 hours, while with *A. obliquus,* increased turbidity, cell count and biomass content was observed in CO_2 treatment set of 80 bubbles/2 hours.

Results of the present study indicated that all the microalgal members under study exhibited varied ranges of tolerance to CO_2 supply. The results of *A. obliquus* (80 bubbles/2 hours) and *C. globosa* (60 bubbles/2 hours) are highly promising which exhibited, higher turbidity, cell count and biomass production in CO_2 treatment sets. The species *C. gorvei* (10 bubbles/2 hours), *M. contortum* (15 bubbles/2 hour) and *D. opoliensis* (20 bubbles/2 hours) also exhibited moderate efficiencies in carbon dioxide assimilation.

There are several reports regarding the feasibility of utilizing microalgal species in CO_2 assimilation like *Chlorella, Scenedesmus, Botryococcus, Euglena gracilis*, *Nannochloropsis oculata* etc. The present study also testifies the efficiency of five microalgal members (*C. grovei, C. globosa, D. opliensis, M. contortum* and *A. obliquus*) in CO_2 assimilation. The response of microalgal members to various bubbling ratios were monitored through individual treatment sets. The comparison and consolidation of results revealed the possibilities of utilizing two highly promising microalgal species, *A. obliquus* and *C. globosa* in CO_2 assimilation / sequestration studies, evidenced by higher turbidity, high cell count and high biomass in CO_2 treatment sets.

Chapter II

pH specific modification of culture medium for growth maximization of *Chlamydomonas globosa* and *Acutodesmus obliquus*

Introduction

Microalgae are extensively been used for varying purposes. Their basic features like diversity, abundance, minimal growth requirements, survivability in extreme environments, potential for the synthesis of biomolecules, biofuels, bioadsorption of heavy metals and pollutants, bio sequestration of greenhouse gases, generation of valuable byproducts, better ability to handle in engineered systems etc. are unique. Due to these captivating properties, researchers consider them as a promising tool for multi-orientated research purposes.

One among the major challenges in utilizing microalgae for specific purposes is their biomass production in required quantities in specific time intervals. Most of the microalgal members, which are abundant and virulent under natural habitats, seem to be slow growing under culture conditions, both in an out of laboratories. This can be due to lack of optimum climatic conditions, inadequate nutrient strength, lack of proper aeration and gaseous influx, insufficient light intensities and duration and other culture conditions.

Apart from this, the risk of developing pure cultures without contamination is another major constraint in their utilization for multidimensional purposes. Many mass multiplication techniques/protocols fail to maintain pure cultures of target species due to invading organisms. The major contaminants, apart from unwanted algal members include fungi, bacteria, yeast, molds and a

wide category of zooplanktons, of which most of them are predators (Goldman and Ryther, 1976; Goldman, 1979, 1980).

Thus, one of the major problems faced in the utilization of microalgae as a raw material for various industrial purposes is the lack of cost effective and efficient mass multiplication techniques/protocols, which ensures contamination free population within a short span of time. It is quite disturbing to note that among 30,000 species of microalgae, only a limited number of them are maintained in laboratories and studied for their utilization for various purposes (Sharmila et al., 2014). Therefore, standardization of conditions offering mass multiplication of microalgal species in limited time interval is a challenge to the scientific community.

In recent times, several media have been formulated for microalgal culture, which includes BG-11 Medium, Bolds Basal medium, C medium, Chu-10medium, Combo medium, Forsberg medium 11, URO Medium etc. Later, most of them were modified for meeting required targets and few of them were developed by analyzing the water samples collected from their respective habitats, together with a detailed estimation on the nutrient requirements of respective microalgae. Still the problems associated with culture media preparation are persisting, which include faulty formulations, erroneous ionic concentration represented by pH, contamination, precipitate formation etc.

Considering these constraints, many research activities are undertaken to develop protocols for rapid multiplication of microalgal members. As the growth preferences of microalgal members vary in accordance with their species characteristics, a unified protocol for the mass multiplication of microalgae is inappropriate. Several studies proved that species-specific modification of culture conditions could contribute to the mass multiplication of microalgal members.

Modification of the physical and chemical conditions of the microalgal cultures results in differences in cell composition, production of higher biomass and synthesis of bioactive metabolites. An experimental approach has been initiated in the present study with an objective to evaluate the responses and growth pattern of *Chlamydomonas globosa* and *Acutodesmus obliquus* in Bolds Basal medium at varying ranges of pH. This has been carried out as part of maximizing their biomass production by altering culture conditions and enabling them to be utilized for various commercial/industrial purposes. The specific objective outlined in this study includes:

- Determining the optimum pH favouring maximized growth of selected micro algal members like *Chlamydomonas globosa* and *Acutodesmus obliquus*, cultured in Bolds Basal medium.

Review of Literature

Research and developmental activities based on algal mass culturing initiated in Germany during 1940's (Harder and Witsch, 1942). Later on, many other countries including Netherlands (Wassink et al., 1953), Japan (Tamiya, 1957), France (Clement, 1975), India (Becker and Venkataraman, 1980), South Africa (Toerien and Grobbelaar, 1980) have initiated studies on micro algal culturing and their mass multiplication.

In large-scale cultivation and laboratory level culturing of microalgae, several environmental factors need to be considered. Some of them include pH, light and temperature (Khalil et al., 2010; Gong et al., 2014; Khatoon et al., 2014; Sukenik et al., 1993; Rocha et al., 2003; VanWagenen et al., 2012; Doan et al., 2011; Moazami et al., 2012; Bartley et al., 2013; Rai and Rajasekhar, 2014). These factors not only influence the growth, multiplication and photosynthetic activities, but also alter the carbon fixation and distribution of carbon into different types of macromolecules (Juneja et al., 2013). Through

optimization of these environmental factors, biomass maximization and subsequent usage can be achieved. However, the optimum and tolerance range of parameters tend to be species specific and may vary.

Among various factors, hydrogen ion concentration is an important factor for the optimal growth of microalgae (Khalil et al., 2010). It has an important role in influencing the microalgal growth, functioning of proteins and enzymes, nutrient absorption, carbon and trace metal utilization, lipid and antioxidant production etc. (Borowitzka and Borowitzka, 1988; Chen and Durbin 1994; Pelizer et al., 2002; Sakarika and Kornaros, 2016; Razzak et al., 2015). The pH of the culture medium also influences the photosynthetic activity of microalgae. In high and low pH, the photosynthetic activity decreases (Bakuei et al., 2015) and the high pH ranges alters the nutrient absorption. The low pH also performs as the enzyme inhibitor during photosynthesis (Bitong et al., 2011). Hence, it is essential to maintain the pH of the culture medium at optimum ranges, as extreme pH conditions may lead to the disruption of cellular activity and collapsing of cultured organism.

As an important factor in carbon sequestration studies, the pH determines the solubility and availability of CO_2 and other nutrients (Juneja et al., 2013; Razzak et al., 2015; Varshney et al., 2016). While initiating carbon dioxide sequestration experimentations, the carbon dioxide discharge to culture medium results in shifting of medium pH to acidic ranges, which negatively influences the growth (Lee and Lee, 2003) and only a few microalgal members can thrive in this extreme pH ranges (Singh and Ahluwalia, 2013; Eloka-Eboka and Inambao, 2017). As a result of growth and multiplication of the microalgal cells in acidic pH, the depletion of inorganic carbon content of culture medium occurs, which in turn leads to increase in pH values (Razzak et al., 2015). The higher pH ranges restricts the availability of carbon from CO_2 and reduces the affinity of microalgae to free CO_2 (Azov, 1982; Rotatore

and Colman, 1991; Chen and Durbin, 1994; Juneja, et al., 2013), which leads to algal growth inhibition (Azov, 1982; Chen and Durbin, 1994).

Certain factors like buffering capacity and composition of the culture medium, metabolic functions associated with microalgae etc. also influences the pH of the culture medium (Dineshkumar et al., 2017). Previous research works proved that the microalgal members like *Scenedesmus* (Nalewajko et al., 1997) and *Chlorella* (Khalil et al., 2010) have pH-buffering capacity, which differs among the species or genera.

Most of the previous studies were based on the manipulations of the initial pH (Watanabe et al., 1992; Yue and Chen, 2005). Several studies have been carried out to assess the effect of changing pH on the growth of microalgae (Moss, 1973; Dubinsky and Rotem, 1974; Lane and Burris, 1981; Tubea et al., 1981; Van der Westhuizen and Eloff, 1983; Gehl et al., 1987; Chen and Durbin, 1994; Mayo, 1997). On the other hand, most of the recent experimentations are attempted to increase the lipid content (Gardner et al., 2011; Sharma et al., 2012; Shah, 2014; Bartley et al., 2014; Khatoon et al., 2014; Mandotra et al., 2016) and enhancing antioxidant potentialities (Ismaeil et al., 2016; Guedes et al., 2011) of microalgal members.

Modification of the pH of the culture medium normally results in metabolic inhibition (Goldman, 1982) and disruption of cellular processes (Razzak et al., 2015), which leads to limited growth and ultimately death and collapse of culture. The acidic pH of the culture medium can alter the nutrient uptake (Gensemer et al., 1993) and induce metal toxicity. The higher alkaline pH range also limits the availability of carbon from carbon dioxide and results in suppression of growth (Chen and Durbin, 1994; Azov, 1982).

The decline in growth at pH greater than 9 was also reported by many (Taraldsvik and Myklestad 2000; Munir et al., 2015; Mandotra et al., 2016;

Al- Safaar et al., 2016). On the other hand, Garcia et al. (2000) observed reduced growth rate above pH 8 and Chen and Durbin (1994) noticed reduced growth above pH 8.8. Aggregation of *Chlorella vulgaris* in pH 9.5, and little growth on extreme acidic and alkaline conditionswas noticed by Sakarika and Kornaros (2016). Varshney et al. (2016) observed growth inhibition in *Acutodesmus* sp. above and below pH ranges 4 and 11 were the color of species changed from green to white within 48 hours ultimately resulted in cell death.

Many report variations in the pH of culture medium due to external stimulus. The common trend of shifting the medium pH to alkaline ranges was noticed during microalgal growth (Armstrong and Calder, 1978; Zang et al., 2014). Moheimani (2013) reported the increasing pH levels during day time. The photosynthesis seems to be the established / general conclusion regarding the increasing pH in culture medium (Verduin, 1964; Zhang et al., 2014). During daytime, utilization of carbon dioxide for photosynthesis increases the pH levels and the respiration process during nighttime decreases the pH levels (Bartley et al., 2014). The photosynthetic activity was also influenced by the pH of the culture medium that the photosynthetic rate reduces at extreme higher and lower pH ranges (Bakuei et al., 2015). Dubinsky and Rotem (1974) also noticed similar variations in the pH values of culture medium. The pH variation observed in the culture medium may be due to the nature of the metabolites secreted by microalgae with response to the nutrients of the culture medium (Al-Shatri et al., 2014).

Several researchers reported on the cellular level adaptations and mechanisms of microalgae to resist and prevail over the extreme pH conditions (Lane and Burris, 1981; Malis-Arad and MC Gowan, 1982 a, b; Guckert and Cooksey, 1990; Visviki and Santikul, 2000). The ultra structural monitoring of the

microalgal cells provides information about the structural changes and adaptation techniques to cope with extreme pH ranges.

The acid tolerant species and acidophilic species can maintain neutral cystosolic pH for broad ranges of external pH (Lane and Burris, 1981; Gerloff-Eliaset al., 2005). However, the maintenance of neutral cystosolic pH was an energy consuming mechanism that the energy required to eliminate and neutralize the hydrogen ions, otherwise used for cell growth results in decreased photosynthetic rates (Nixdorf et al., 2003; Kamjunke et al., 2005). In spite of the external pH, growth of microalgae canbe achieved through optimizing the intracellular pH to 7.5 (Razzak et al., 2015).

Raven (1981) stated that the pH difference in the culture medium also affect the metabolic activities and membrane transport. The acidophilic algae possess an inner positive electric potential difference across plasmalemma, which reduces the proton influx. To survive in low pH, it is essential to decrease these proton influx and increase the efficiency of proton pump (Gross, 2000).

As an adaptation technique, increased membrane fluidity by altering the fatty acid composition was observed in *Euglena mutabilis* while exposed to acidic environment (Halter et al., 2012). Jiang et al. (2012) proposed the method of acid adaptation to enhance the acid resistance of microalgae exposed to the extreme acidic conditions. However the acid tolerance of microalgae mainly depends on the extent of adaptive period and the pH to which microalgae subjected for adaption (Davis et al., 1996).

There were only a few reports regarding the morphological changes of microalgae subjected to extreme environments like highly acidic and alkaline conditions. Visviki and Santikul (2000) conducted ultra-structure monitoring to reveal the adaptation techniques of *Chlamydomonas applanata* to extreme

acidic environments. On exposure of *Chalmydomonas* cells to acidic pH (4.4), cells with larger pyrenoids, decreased volume of starch granules and palmelloid colonies were observed. In pH 3.4, authors observed clumping and sticky palemelloid colonies with excessive mucilage production, abnormal cell division, loss of mobility and death of both single cells and colonies. Moreover, the existing single cells were 30 % larger than control cells with thicker cell walls. Authors also reported that the cell wall thickening act as an appropriate barrier for restricting the passive hydrogen ion uptake and thereby ensure the survival of microalgae treated in acidic stress.

In the alkaline pH ranges, the flexibility of the mother cell wall increases consecutively, which prevents the rupturing and autospore release and thereby extends the time for cell cycle completion (Malis-Arad and MC Gowan, 1982 a,b) Moreover, the cell division reduces due to the stress attributed by the culture medium and one stage of the cell cycle will be more sensitive than other stages (Guckert and Cooksey, 1990). Guckert and Cooksey (1990) while conducting experiments using *Chlorella* CHORI observed autosporangial stage as more sensitive stage. They also stated that the longer cell cycle results in the lower cell densities in extreme pH conditions. Gardner et al. (2011) also reported the presence of larger cells with irregular conformations in higher alkaline cultures especially in pH 10.3.

The adaptation of a microalgal species to specific environment plays a supreme role in its yield (Sakarika and Kornaros, 2016). Any differences or modifications in the pH values can cause changes in the quantity and composition of pigments, protein, carbohydrate, glycerol and secondary carotenoids or may cause cell death (Liu and Lee, 2000; Khalil et al., 2010; Khatoon et al., 2014).

Huang and Su (2014) reported pH as a significant factor influencing the chlorophyll content. Abe et al. (1999) observed the inhibition of chlorophyll synthesis at extreme pH (nearly 3). Beklioglu and Moss (1995) stated that the increasing pH causes decreasing of chlorophyll-*a*. However, Poza-carrion et al. (2001) and Sharma et al. (2014) declared the significant increase of phycobiliproteins in cyanophycean members with respect to increasing pH. There were also reports regarding the enhanced chlorophyll production in alkaline pH ranges (Ismaiel et al., 2016; Richmond and Grobbelaar 1986).

Several researchers (Jimenez and Niell 1991; Celekli and Donmez, 2006; Liu and Lee, 2000) reported the significant improvement in accumulation and production of β-Carotene and secondary carotenoids in microalgae due to effect of pH. Abalde et al. (1991) reported that the cells subjected to the extreme pH conditions exhibit hindered cell division, which consecutively leads to carotenoid accumulation. Conversely, Del Campo et al. (2000) observed limited accumulation of carotenoids at higher and lower ranges of pH.

The pH of the microalgal culture medium has a supreme role in the accumulation of lipid that the key enzyme for the lipid synthesis (ACCase) is pH dependent (Thampy and Wakil, 1985; Mandotra et al., 2016). During the adverse conditions, the growth reduces and the deviated energy was stored in the form of lipids (Abu-Rezq et al., 1999). Several authors (Guckert and Cooksey, 1990; Moheimani, 2013; Khatoon et al., 2014; Rai et al., 2015; Mandotra et al., 2016) discussed the influence of pH on the enhanced lipid accumulation. However Bartley et al. (2014) disagree with the role of pH on the enhanced lipid accumulation and Rai et al. (2015) commented that the mechanism behind this accumulation of lipid was unknown.

The morphological observations performed by Guckert and Cooksey (1990) on *Chlorella* CHORI proved that the alkaline pH inhibits the autospore

release and results in reduced growth and the redirected energy was utilized in the biosynthesis of TAG. Similar observations made by Gardner et al. (2011) stated that the actual mechanism of TAG accumulation due to pH was unknown. According to Bartley et al. (2014), pH have no effect on the fatty-acyl profile. On the other hand Mandotra et al. (2016) claimed that pH have marked effect on the FA profile of algae.

During acidic pH stress, as an adaptation technique to control the osmotic imbalance, *Dunaleilla acidophila* accumulated glycerols (Fuggi et al., 1988). While the *Pinnilaria braunii* and *Chlamydomonas* sp. accumulated triacylglycerides (Tatsuzawa et al., 1996). Tatsuzawa et al. (1996) reported the ability of microalgae to increase saturated fatty acid content under acidic conditions to inhibit elevated proton concentrations and to decrease membrane fluidity. Poerschmann et al. (2004) observed such a mechanism in *Chlamydomonas* sp.

pH also influences the biochemical configuration of micro algae. Guedes et al. (2011) conducted experiments to evaluate the individual and combined effects of pH and temperature on the growth rate and antioxidant production of *Scenedesmus obliquus* through a full factorial design. The results revealed that the *Scenedesmus obliquus* exhibited augmented antioxidant production under higher pH ranges. Ismaiel et al. (2016) studied the effect of pH on the activity and production of various antioxidants developed by *Spirulina platensis* and observed the production of several antioxidants in the extreme conditions to alleviate the harmful effects of reactive oxygen species.

The formation of precipitate in microalgal cultures maintained at extreme pH ranges were discussed by several authors (Becker, 1994; Visviki and Santikul, 2000; Sirisansaneeyakul et al., 2011; Dineshkumar et al., 2017). These precipitations also influence the microalagal cell cycle (Malis-Arad and MC Gowan, 1982 a,b).

As stated in previous session, the contamination of micro algal cultures can happen due to various reasons. While initiating microalgal mass culturing attempts, the lack of appropriate controlling mechanisms of contaminations due to other microorganisms appears to be a major problem (Das et al., 2011). The major contaminants includes fungus, virus, protozoa, golden algae, bacteria and zooplanktons (Moreno-Garrido and Can˜avate, 2001; Shi et al., 2006; Zhou et al., 2011; Peng et al., 2015; Touloupakis et al., 2015; Ma et al., 2017). The cross contaminations due to other microalgae (Piazzi and Ceccherelli, 2002) was also a major setback.

Among various environmental factors influencing biomass production, adjusting the pH of the microalgal medium can restrict the contaminations due to invading organisms (Wang et al., 2013). Liu and Lu (1990) recommend shifting of pH values to 3 for reducing flagellate populations in algal culture. Maintaining the culture medium pH in acidic range (pH-3) for nearly 1-2 hours can reduce the contaminations occurred due to rotifers (Becker, 1994). Ma et al. (2017) also confirmed this approach of lowering pH values to reduce contamination.

In order to prevent the proliferation of invading microbes, the pH of cyanobacterial cultures can be increased to alkaline ranges (Mc Ginn et al., 2011). In spite of such extreme pH conditions, cyanobacteria can grow without altering the biochemical composition (Touloupakis et al., 2015). The ability of microalgae to develop in alkaline pH seems to enhance the interest in outdoor cultivation by reducing the risk of biological contaminations (Muthuraj et al., 2014). Considering the chance of growth inhibition of microalgae at extreme pH ranges, it is important to seek the optimum pH range, at which the proliferation of contaminants and maximization of specific microalgal species takes place.

Generally, algae may have different growth responses with respect to varying ranges of pH. Certain factors like metabolic effects of the microalgal cells or the chemical influences on the medium determine the pH tolerance limit (Azov, 1982). Even though many microalgal members grow near neutral pH ranges (7.0-7.4), the optimum pH at which maximum growth occurs may vary (Sakarika and Kornaros, 2016). Ho et al. (2011) and Dineshkumar et al. (2017) considered pH ranges 7-9 as optimum. There were also reports regarding the optimum pH as the initial culture pH at which microalgae adapted to grow (Visviki and Santikul, 2000 and Hansen, 2002). Celekli and Donmez (2006) and Gong et al. (2014) stated that initial pH has little impact on the growth of microalgae.

Most of the available reports regarding an augmentation in the growth of micro algae based on pH specific medium modification confines to their exploitation in the perspective of lipid production/biodiesel production through stress induction. While compiling the available literature, it was noticed that the responses of microalgal members to varying ranges of pH confines only to a limited microalgal species having industrial significance. Preliminary studies in our laboratory proved the efficiencies of *Chlamydomonas globosa* and *Acutodesmus obliquus* as excellent candidates for carbon sequestration. However, the production of biomass in sufficient quantities seems to be a significant factor in determining the selectivity of such species for specific purposes. There were no reports regarding the pH specific medium modification of *C. globosa.* Literature related to *A. obliquus* is scanty. In this light, the present work has been attempted with an objective of determining the optimum pH favouring the maximized growth of selected micro algal species like *C. globosa* and *A. obliquus* cultured in Bolds Basal medium.

Materials and Methods

As pH was noted to be an important factor influencing the growth of microalgal members, an attempt has been carried out to assess the optimum pH favouring the maximum growth and multiplication of *Acutodesmus obliquus* and *Chlamydomonas globosa*, under culture conditions. Such a standardization is appropriate as their biomass production in sufficient quantities is a pre-requisite for various studies, including carbon dioxide sequestration.

Experimental design

For the present study, pure cultures of *C. globosa* and *A. obliquus* maintained in BB medium was used. The preparation of BB medium was carried out as per standard methods mentioned in Chapter I.

Each algal member has been attempted separately. For experimentation, treatment sets were maintained with conical flasks of 100 ml capacity (21 nos), each containing 50 ml Bolds Basal medium. The pH of culture medium was adjusted from 3-12, with a gradation of 0.5 using 0.5 N NaOH and 0.05 N HCl. After adjusting to the required pH, 5 ml of pure cultures of the respective micro algal members were added to the respective conical flasks and the changes in pH, if any, was adjusted. The control set was maintained at pH 6.6, which represents the original pH of the culture medium. Detailed work plan is depicted in Plate 3.

Monitoring of the treatment sets were carried out for a period of 7 days. The culture conditions like pH, temperature, conductivity and resistivity and micro algal growth parameters like turbidity, cell count, cell size, and biomass were monitored throughout the treatment period. The methodology, as depicted in Chapter I was followed for monitoring the culture conditions and growth performances of micro algal members. The biomass estimation was carried

out on the initial and final days of treatment. All the sets were kept at illumination during day time (6 am – 6 pm) at a light intensity of 40 µmol m^2 s^1. Throughout experimentation, the temperature and humidity associated with culture medium of treatment sets were monitored. The temperature of the medium during experimentation ranged from 26 – 29.8˚C while the humidity ranged from 48 - 54 %. Every day after observation, the altered pH was readjusted to their experimental condition. The entire study has been carried out during March to September, 2016. The results are reported.

Results and Discussion

In the present study, an attempt has been carried out to assess the growth performances of selected microalgal members (*C. globosa* and *A. obliquus*) in Bolds Basal medium under varying culture conditions altered by pH. Results of conductivity, resistivity, cell size, turbidity, cell count and biomass content of the micro algal members in response to different ranges of pH are given in tables 2.1(a) – 2.2(g). The results are represented as median values of three trials. Responses of microalgal members to varying ranges of pH on the first day (initial day) and seventh day (final day) are represented in plate 4. Similarly, changes in turbidity, cell count and biomass associated with the microalgal members under different ranges of pH are represented as Figures 1a – 3b in plate 5.

During the treatment period, at higher alkaline pH ranges (pH 9 and above), a precipitation was noticed. Several researchers (Becker, 1994; Visviki and Santikul, 2000; Sirisansaneeyakul et al., 2011 and Dineshkumar et al., 2017) reported precipitate formation in cultures on account of pH exceeding 9. Hence, in the present study, median values of major growth parameters above pH 9 were neglected.

pH

The natural changes in pH accomplished by the treatment sets in response to the pre adjusted pH are worked out. In the case of *C. globosa* all the treatment sets with altered pH showed a tendency to move towards neutral range. The pH values increased in acidic ranges and decreased in alkaline ranges to attain a neutral range. Here in the control set, the pH values ranged from 6.82 on the first day to 7.78 on the seventh day. A similar trend was noticed with *A. obliquus* and the pH values associated with control ranged from 6.63 on the first day to 7.19 on the seventh day.

Similar changes were also noticed by Zhang et al. (2014). There are various reasons attributed to this process. Verduin (1964) reported that algae have the ability to increase the pH of the culture media through photosynthesis. Similar reasons were also attributed by Dubinsky and Rotem (1974), while monitoring the changes in pH due to algae in relation to initial pH and temperature. The mechanisms like photosynthesis and respiration leads to day and night pH fluctuations (Bartley et al., 2014). Furthermore, Al-Shatri et al. (2014) stated that the variation in the pH of the culture medium was due to secretion of metabolites by microalgae in response to the nutrients present in the medium. Al-Qasmi et al. (2012) discussed the capabilities of algae to manipulate the growth and biochemical composition with respect to altered physiochemical conditions.

In general, algal species exhibit different ranges of tolerance to pH (Ying et al., 2014). However, the changes in the pH values indicate the adaptabilities of the micro algal members to survive in modified environments by altering the pH to optimum levels. The capacity to survive in extreme environments by acclimatizing the metabolism corresponding to changing environmental conditions makes microalgae more interesting candidates for research (Chiranjeevi and Mohan, 2016).

Table 2.1(a). Variation in pH noticed in cultures of *Chlamydomonas globosa.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	6.82	6.97	7.01	7.51	7.56	7.61	7.78	7.51
pH-3	4.86	3.47	3.35	3.38	3.4	3.38	3.41	3.4
pH-3.5	5.19	4.23	4.04	3.97	3.88	4.04	3.88	4.04
pH-4	5.25	4.55	4.44	4.4	4.4	4.41	4.44	4.44
pH-4.5	5.4	4.83	4.72	4.7	4.73	4.68	4.63	4.72
pH-5	5.57	5.16	5.16	5.05	5.06	5.12	5.07	5.12
pH-5.5	5.87	5.52	5.55	5.54	5.54	5.53	5.54	5.54
pH-6	6.32	6.04	6.03	6.05	6.05	6.04	6.03	6.04
pH-6.5	6.76	6.56	6.57	6.58	6.57	6.58	6.55	6.57
pH-7	7.14	7.03	7.11	7.08	7.13	7.11	7.2	7.11
pH-7.5	7.37	7.37	7.41	7.48	7.54	7.54	7.52	7.48
pH-8	7.51	7.59	7.67	7.72	7.81	7.78	7.79	7.72
pH-8.5	7.62	7.71	7.84	7.91	8.04	8.16	7.99	7.91
pH-9	8.06	8.04	8.16	8.32	8.41	8.49	8.35	8.32
pH-9.5	8.3	8.51	8.66	8.87	9.01	9.05	8.85	8.85
pH-10	8.59	9.11	9.3	9.42	9.59	9.59	9.46	9.42
pH10.5	8.83	9.02	9.66	9.76	9.87	9.91	9.94	9.76
pH-11	9.1	10.23	10.34	10.3	10.43	10.35	10.41	10.34
pH-11.5	11.03	11.17	11.2	11.19	11.14	11.13	11.2	11.17
pH-12	11.87	11.85	11.94	11.92	11.9	11.93	11.93	11.92

Table 2.2(a).Variation in pH noticed in cultures of *Acutodesmus obliquus.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	6.63	6.64	6.65	6.68	6.79	6.95	7.19	6.68
pH-3	3.21	3.04	2.96	3.06	2.99	3.03	3.03	3.03
pH-3.5	4.35	3.6	3.49	3.54	3.47	3.58	3.48	3.54
pH-4	5.41	4.29	4.01	4.13	3.99	4.08	4.05	4.08
pH-4.5	5.68	4.99	4.75	4.71	4.62	4.66	4.66	4.71
pH-5	5.87	5.24	5.17	5.2	5.13	5.17	5.1	5.17
pH-5.5	6	5.67	5.63	5.54	5.58	5.6	5.54	5.6
pH-6	6.25	6.08	6.06	6.08	6.05	6.06	6.06	6.06
pH-6.5	6.54	6.58	6.56	6.58	6.5	6.53	6.55	6.55
pH-7	7.09	7.07	7.07	7.09	6.96	7	7.03	7.07
pH-7.5	7.49	7.5	7.55	7.58	7.49	7.55	7.5	7.5
pH-8	7.75	7.86	7.95	7.93	7.89	8.01	8	7.93
pH-8.5	7.89	8.01	8.14	8.23	8.09	8.25	8.28	8.14
pH-9	7.97	8.2	8.3	8.43	8.38	8.5	8.54	8.38
pH-9.5	8.2	8.48	8.61	8.71	8.72	8.96	8.97	8.71
pH-10	8.53	8.91	9.15	9.26	9.31	9.6	9.58	9.26
pH10.5	8.5	9.19	9.5	9.73	9.68	10.11	9.99	9.68
pH-11	9.11	9.87	10.18	10.28	10.19	10.5	10.45	10.19
pH-11.5	10.12	10.65	10.82	10.9	10.7	11.1	10.89	10.82
pH-12	11.59	11.7	11.81	11.69	11.54	11.79	11.81	11.7

Conductivity (µS)

In the case of *C. globosa* maximum conductivity was observed in pH 12 (8842 µS) on the sixth day and minimum in pH 6 (745.1 µS) on the first day. In control set, the conductivity ranged from 789.2 µS on the first day to 851.6 µS on the seventh day. Upon comparing the final median values the maximum conductivity was recorded in pH 12 (8373 µS) and minimum in pH 6 (753.2 µS).

Throughout the experimental period, the treatment set containing *A. obliquus* showed maximum conductivity in pH 12 (5552 µS) on the sixth day and minimum in pH 5.5 (746.4 µS) on the first day. The conductivity values of the control set ranged from 762.4 µS on the first day to 831.9 µS on the seventh day. While analyzing the final median values, the maximum conductivity was noticed in pH 12 (3880 µS) and minimum in pH 6 (754.9 µS).

The conductivity of a solution depends on the concentration of ions present in it. Generally, conductivity value of solution increases with increasing concentration of ions. Here in the present study, pH was maintained in varied ranges from 3-12 with a gradation of 0.5 using 0.5 N NaOH and 0.05 N HCl. Moreover, every day after observation, the altered pH was readjusted to their initial pH range. Therefore, to monitor the changes in the ionic concentration of culture medium pertaining to treatment sets, the conductivity values were recorded.

In all the treatment sets, conductivity values were noted to be higher towards higher alkaline ranges (pH 12) and lower towards near neutral ranges (pH 6 and 6.5). During experimentation, a gradual increase in conductivity was observed in all treatment sets, including control. From the initial day to final day, the conductivity increased more rapidly in the treatment sets than control, which may be due to the increased ionic influx owing to the addition of NaOH / HCl to adjust pH.

Table 2.1(b). Variation in conductivity (µS) noticed in the cultures of *Chlamydomonas globosa.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	789.2	791.7	824.3	830.5	853.2	855.5	851.6	830.5
pH-3	838.1	856.7	864.4	880.7	884.2	898.5	903.6	880.7
pH-3.5	781.2	791.2	802.5	811.5	815.1	822.3	828.3	811.5
pH-4	758.8	779.7	788.2	798.9	806.8	812.3	818.3	798.9
pH-4.5	754.5	763.8	767.4	771.6	778.1	779.8	788.3	771.6
pH-5	758.7	771.7	774.7	776.8	791.2	794.5	801.4	776.8
pH-5.5	748.8	753.7	755.5	758.1	763.6	765.3	767.5	758.1
pH-6	745.1	748.1	749.3	753.2	755.1	758.1	761.7	753.2
pH-6.5	806.1	809.3	808.3	812.9	815	817	820.3	812.9
pH-7	826.2	827.5	830.4	834.1	836	838.3	835.9	834.1
pH-7.5	851.8	865.7	867.3	873.8	873	879.8	870.9	870.9
pH-8	861	884.6	892	902.3	909.5	917.9	925.6	902.3
pH-8.5	851.8	875.9	892.2	918.7	937.6	972	985.6	918.7
pH-9	875.1	927.7	962.7	1005	1043	1085	1132	1005
pH-9.5	894.1	965.1	1051	1150	1240	1349	1435	1150
pH-10	954	1148	1304	1484	1709	1953	2119	1484
pH10.5	1016	1193	1546	1828	2125	2386	2683	1828
pH-11	1176	1593	1986	2293	2634	2975	3350	2293
pH-11.5	2133	2894	3409	3827	4160	4484	4962	3827
pH-12	6496	6948	8153	8493	8373	8842	8762	8373

Table 2.2(b).Variation in conductivity (µS) noticed in the cultures of *Acutodesmus obliquus.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	762.4	764.4	764	769.7	804.9	826.2	831.9	769.7
pH-3	852.2	913.9	917.6	914.2	929.7	937.6	964.4	917.6
pH-3.5	764.9	818.1	828.4	823.1	832.8	829.6	834.1	828.4
pH-4	778.7	802.7	809.8	809.2	840.4	842.5	840.1	809.8
pH-4.5	753.3	760.3	778.8	784.7	829.3	835	837.1	784.7
pH-5	751.3	760.6	763.1	765.4	786.6	792.2	798	765.4
pH-5.5	746.4	751.7	752.3	811.5	814.1	819.5	821.8	811.5
pH-6	748.5	751.6	752.4	755	754.9	772.5	775.9	754.9
pH-6.5	753.2	760.5	765.1	770.4	773.4	776.1	775.9	770.4
pH-7	793.3	796.7	799.3	813.9	814.9	818	816.9	813.9
pH-7.5	831.3	832.3	833.9	835.5	835.4	840.8	842.2	835.4
pH-8	859	873.4	883.4	886	899.6	909.1	910.8	886
pH-8.5	872.4	905.5	923.9	959	972.5	981.6	991.1	959
pH-9	878.8	909.8	931.2	950.6	1033	1049	1066	950.6
pH-9.5	894.3	947.3	978.1	1016	1048	1078	1131	1016
pH-10	921.1	988.3	1064	1145	1247	1393	1495	1145
pH10.5	920	1062	1238	1398	1568	1853	2010	1398
pH-11	956.8	1220	1556	1791	2060	2560	2725	1791
pH-11.5	1163	1599	2023	2447	2721	3243	3410	2447
pH-12	2349	2931	3663	3880	4115	5552	5069	3880

Resistivity (kΩ)

In the treatment set containing *C. globosa,* the maximum resistivity was observed in pH 6 (1.312 kΩ) on the first day and the minimum in pH 12 (0.104 kΩ) on the seventh day. In the control set, resistivity values ranged from 1.239 kΩ on the first day to 1.188 kΩ from the seventh day. While comparing the final median values of the treatment set, maximum resistivity was noticed in pH 6 (1.298 kΩ) and minimum in pH 12 (0.117 kΩ).

In *A. obliquus,* maximum resistivity was observed in pH 5.5 and pH 6 (1.31 kΩ) on the first day and the minimum in pH 12 (0.18 kΩ) on the sixth day. In control set, the resistivity values ranged from 1.29 kΩ on the first day to 1.17 kΩ on the seventh day. Upon comparing the final median values, maximum resistivity was recorded in pH 6 (1.3 kΩ) and minimum in pH 12 (0.25 kΩ).

Resistivity is directly related to the amount of additives, mainly dissolved salts, alkali, chlorides, sulphates and carbonate compounds present in the medium. If the sample solution contains high concentration of ions, it exhibits low resistivity. In the present study, the respective pH ranges were maintained and readjusted daily during experimentation using 0.5 N NaOH and 0.05 N HCl. Hence, for monitoring the changes associated with ionic concentrations due to the addition of NaOH and HCl to the culture medium, the resistivity values of the treatment sets were registered.

Upon comparing the final median values, the resistivity pertaining to cultures under treatment was found to be high in neutral ranges and low at high alkaline ranges. The resistivity values of cultures were noted to be in agreement of conductivity in all treatments of both microalgae retained at varying pHs.

Table 2.1(c). Variation in resistivity (kΩ) noticed in the cultures of *Chlamydomonas globosa.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	1.239	1.235	1.186	1.162	1.146	1.144	1.188	1.186
pH-3	1.166	1.141	1.131	1.109	1.103	1.086	1.085	1.109
pH-3.5	1.251	1.236	1.218	1.205	1.199	1.189	1.18	1.205
pH-4	1.288	1.254	1.24	1.22	1.212	1.203	1.195	1.22
pH-4.5	1.296	1.28	1.274	1.267	1.259	1.253	1.24	1.267
pH-5	1.288	1.267	1.262	1.258	1.236	1.23	1.22	1.258
pH-5.5	1.305	1.297	1.294	1.289	1.283	1.277	1.274	1.289
pH-6	1.312	1.307	1.305	1.298	1.295	1.289	1.283	1.298
pH-6.5	1.212	1.208	1.209	1.2	1.199	1.197	1.194	1.2
pH-7	1.183	1.181	1.177	1.172	1.169	1.166	1.169	1.172
pH-7.5	1.148	1.129	1.127	1.119	1.12	1.111	1.122	1.122
pH-8	1.135	1.107	1.096	1.083	1.073	1.065	1.056	1.083
pH-8.5	1.148	1.116	1.096	1.064	1.043	1.006	0.99	1.064
pH-9	1.117	1.054	1.015	0.973	0.942	0.901	0.864	0.973
pH-9.5	1.096	1.013	0.93	0.848	0.788	0.726	0.681	0.848
pH-10	1.025	0.852	0.75	0.659	0.572	0.507	0.462	0.659
pH10.5	0.999	0.819	0.632	0.535	0.49	0.41	0.363	0.535
pH-11	0.831	0.614	0.493	0.427	0.371	0.329	0.29	0.427
pH-11.5	0.458	0.338	0.287	0.256	0.235	0.218	0.186	0.256
pH-12	0.151	0.141	0.12	0.115	0.117	0.111	0.104	0.117

Table 2.2(c).Variation in resistivity (kΩ) noticed in the cultures of *Acutodesmus obliquus.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	1.29	1.28	1.28	1.27	1.21	1.18	1.17	1.27
pH-3	1.14	1.07	1.06	1.07	1.05	1.05	1.02	1.06
pH-3.5	1.28	1.2	1.18	1.19	1.17	1.18	1.17	1.18
pH-4	1.26	1.22	1.21	1.21	1.16	1.16	1.16	1.21
pH-4.5	1.3	1.29	1.26	1.25	1.18	1.17	1.17	1.25
pH-5	1.3	1.29	1.28	1.28	1.24	1.24	1.23	1.28
pH-5.5	1.31	1.3	1.3	1.21	1.2	1.19	1.19	1.21
pH-6	1.31	1.3	1.3	1.3	1.29	1.27	1.26	1.3
pH-6.5	1.3	1.28	1.28	1.27	1.26	1.26	1.26	1.27
pH-7	1.23	1.23	1.23	1.2	1.2	1.19	1.2	1.2
pH-7.5	1.18	1.17	1.17	1.17	1.17	1.17	1.16	1.17
pH-8	1.14	1.12	1.11	1.1	1.08	1.07	1.07	1.1
pH-8.5	1.12	1.08	1.06	1.02	1.01	0.99	0.99	1.02
pH-9	1.11	1.07	1.05	1.03	0.95	0.93	0.91	1.03
pH-9.5	1.09	1.03	1	0.96	0.93	0.91	0.87	0.96
pH-10	1.06	0.99	0.92	0.86	0.78	0.7	0.65	0.86
pH10.5	1.07	0.92	0.79	0.7	0.62	0.53	0.49	0.7
pH-11	1.02	0.8	0.63	0.55	0.47	0.38	0.36	0.55
pH-11.5	0.84	0.61	0.48	0.4	0.36	0.31	0.29	0.4
pH-12	0.42	0.33	0.27	0.25	0.24	0.18	0.19	0.25

Turbidity (NTU)

In the treatment set containing *C. globosa*, the maximum turbidity was noticed in pH 8 (21.2 NTU) on the seventh day and minimum was recorded in pH 3 (4.7 NTU) on the first day. In the control set, turbidity ranged from 5.5 NTU on the first day to 11 NTU on the seventh day. In the present experimentation, minimum turbidity was noticed in acidic pH ranges (3-4.5). While comparing the final median values, maximum turbidity was noticed in pH 9 (10.4 NTU) and the minimum in pH 4 and 5 (5.6 NTU).

In the case of *A. obliquus,* maximum turbidity was monitored in pH 9 (5.9 NTU) on the seventh day and minimum in pH 3.5, 4.5 and 5 (1.4 NTU) on the second day. The turbidity values of the control set ranged from 1.7 NTU on the first day to 3.9 NTU on the seventh day. Upon comparing the final median values of the treatment set, maximum turbidity was noticed in pH 9 (4.6 NTU) and minimum in pH 3 (2.1 NTU).

Assessment of turbidity helps in estimating the microalgal cell densities in culture medium. Here, while comparing the final median values, both species exhibited maximum turbidity in alkaline ranges and minimum in acidic ranges, which is indicative of their growth enhancement in alkaline culture conditions. However, during experimentation, in higher alkaline ranges, formation of precipitate was noticed. Hence, for drawing conclusions regarding the optimum pH range in which maximum turbidity occurred, pH ranges above 9 were neglected.

Table 2.1(d). Variation in turbidity (NTU) noticed in the cultures of *Chlamydomonas globosa.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	5.5	5.2	7.1	7.3	9.5	11.9	11	7.3
pH-3	4.7	4.9	5.7	5.3	6	6.9	6.2	5.7
pH-3.5	4.9	4.9	5.8	5.5	6	6.9	6.3	5.8
pH-4	4.9	4.8	5.6	5.1	5.8	6.9	6	5.6
pH-4.5	4.9	4.9	5.8	5.5	5.9	6.8	6.8	5.8
pH-5	5	5	5.6	5.2	5.9	6.9	6.9	5.6
pH-5.5	5.1	5.3	6.1	5.4	6.3	7.5	7	6.1
pH-6	5.5	5.6	7	4.4	7.2	9.2	9.6	7
pH-6.5	5.2	6.9	7.3	7.9	9	10.3	11.1	7.9
pH-7	5.3	6.9	7.6	8.2	10.1	12.4	14.3	8.2
pH-7.5	5.3	7.2	8.4	8.4	10.4	13.4	20.1	8.4
pH-8	5.8	8.1	8.9	10	12	16.5	21.2	10
pH-8.5	6.2	7.9	9.1	10.2	11.7	16.3	20.6	10.2
pH-9	6.1	8.8	9.9	10.4	11.6	15.1	18.8	10.4
pH-9.5	6.6	8.9	10.2	10.6	11.8	14.8	19.2	10.6
pH-10	7.2	10	11.2	11.2	11.9	13.7	17.2	11.2
pH10.5	6.2	8.3	9.1	10.4	11.2	12.2	14.2	10.4
pH-11	7.2	9	8.4	9.3	10.4	12.4	13.2	9.3
pH-11.5	7	8.6	8.4	8.2	8.3	8.8	9	8.4
pH-12	6.1	7.3	6.5	6.5	6.6	7.1	7.1	6.6

Table 2.2(d).Variation in turbidity (NTU) noticed in the cultures of *Acutodesmus obliquus*.

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	1.7	1.8	3.2	3.6	3.3	3.3	3.9	3.3
pH-3	1.7	1.9	2.2	2.9	2.1	2.2	2	2.1
pH-3.5	1.8	1.4	2.3	3	2.3	2.2	2	2.2
pH-4	1.8	1.8	2.6	3.2	2.7	3	2.2	2.6
pH-4.5	1.9	1.4	2.7	3.3	2.9	3.6	2.5	2.7
pH-5	1.9	1.4	2.8	3.5	3.1	3.5	2.8	2.8
pH-5.5	2	1.6	2.7	3.6	2.8	3.7	2.6	2.7
pH-6	2	1.7	2.5	3.6	3	3.9	3	3
pH-6.5	1.6	1.9	2.1	3.4	2.9	3.5	2.8	2.8
pH-7	1.9	1.6	2.5	2.6	3.4	3.5	2.5	2.5
pH-7.5	2	1.8	2.9	3	2.7	4.1	2.9	2.9
pH-8	2.2	1.7	3.1	4.5	3.6	4.1	3.5	3.5
pH-8.5	2.2	2.1	3.5	4.3	4.7	5.2	5.2	4.3
pH-9	2.2	2.1	3.8	5.3	4.9	5.9	4.6	4.6
pH-9.5	2.6	3	5	6.3	5	6	5	5
pH-10	3	3.3	5.4	5.6	5.6	6	5.3	5.4
pH10.5	2.8	2.8	4.3	6	5.1	5.9	5.4	5.1
pH-11	3	3.3	4.7	6.3	5.8	5.4	4.7	4.7
pH-11.5	4	3.5	5	5.4	4.3	5.5	3.7	4.3
pH-12	4.2	3.9	4.6	4.1	4.3	4.2	3.1	4.2

Cell count

In the treatment set containing *C. globosa,* the maximum cell count was observed in pH 8 (170.25 cells x 10^4 per ml) on seventh day and the minimum in pH 12 (19 cells x 10^{4} per ml) on the second and seventh day of the treatment. During the treatment period, cell count in control set ranged from 49.25 cells x 10^4 per ml on the first day to 158.26 cells x 10^{4} per ml on the seventh day. While comparing the final median values, the maximum cell count was noticed in pH 8 (83.5 cells x 10^{4} per ml) and minimum in pH 3 (32 cells x 10^{4} per ml). The optimum range at which the maximum cell count was noted is pH 8 (83.5).

In the case of *A. obliquus*, the maximum cell count was noticed in pH 5 (24.75 cells x 10^{4} per ml) on the fifth day and minimum in pH 12 (2 cells x 10^{4} per ml) on the seventh day. In control set, the cell count ranged from 11 cells x 10^{4} per ml on the first day to 9 cells x 10^{4} per ml on the seventh day. While comparing the final median values, maximum cell count was observed in pH 5 (20 cells x 10^{4} per ml) and minimum in pH 3 (4 cells x 10^{4} per ml). Here the cell count was significant in acidic ranges (pH 5 and pH 5.5) and the optimum pH in which the maximum cell count occurred was five.

The estimation of cell count at regular intervals provides information regarding the growth of microalgae under varying treatment conditions. In the present study, *C. globosa* exhibited maximum cell count in alkaline range (pH 8), whereas that of *A. obliquus* was in acidic range (pH 5). The cell count obtained by both the microalgal members under specific pH was higher than the control. Varshney et al. (2016) conducted studies on the effects of pH on the growth of *Acutodesmus* species and reported that the algae exhibited high growth rates over a wide range pH (5–10). There are no reports regarding the growth rate of *C. globosa* with respect to varying pH ranges.

Several researchers also noticed decline in the growth of microalgal species under higher alkaline ranges (Garcia et al., 2000; Taraldsvik and Myklestad, 2000; Munir et al., 2015; Al-Safaar et al., 2016; Mandotra et al., 2016). In the higher alkaline pH , the increased flexibility of the mother cells inhibits the rupturing of cells and cell release from autospore and thereby prevents or lags the cell cycle which results in declined growth (Malis-Arad and MC Gowan, 1982 a,b; Guckert and Cooksey 1990; Rai et al.,2015).

Table 2.1(e). Variation in cell count (cells x 10^4 per ml) noticed in the cultures of *Chlamydomonas globosa.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	49.25	50.25	67.5	73.5	81.75	138.25	158.26	73.5
pH-3	31	32	25.75	38.25	32.61	33.25	28.2	32
pH-3.5	34.25	35.5	44.5	41.26	33	39.28	38.2	38.2
pH-4	35	39.46	37.25	37.25	36.45	36.45	42.56	37.25
pH-4.5	40	36.95	38.75	49.82	46.25	56.38	66.2	46.25
pH-5	34.75	39.5	38.25	38.5	49.25	55.23	68.2	39.5
pH-5.5	37.5	39.25	53.5	48.9	45	59	72.45	48.9
pH-6	36.5	40.25	60.75	65.6	63.25	79.25	82.56	63.25
pH-6.5	45	48	67	72	80.25	118.32	122.68	72
pH-7	49	54.82	67.5	80.75	97.38	124.62	146.3	80.75
pH-7.5	48.5	59.65	72	79.5	87	140.23	160.23	79.5
pH-8	49.75	60.28	67	83.5	92.64	142.03	170.25	83.5
pH-8.5	49.25	56.25	66.5	68.5	92	139.6	160	68.5
pH-9	39.75	51	67.75	62.75	95.41	139.25	150.23	67.75
pH-9.5	50.66	50.26	64	75.25	94.5	140	146.25	75.25
pH-10	45	46.5	56.5	49.75	68	135.69	135.62	56.5
pH10.5	48.75	49.25	49.5	66.39	60.25	118.25	120.36	60.25
pH-11	39	38	50.2	55	55	114.02	110.25	55
pH-11.5	37.25	40	31.25	34	35	88.72	99.56	37.25
pH-12	32	19	22.5	22	22	22	19	22

Table 2.2(e).Variation in cell count (cells x 10^4 per ml) noticed in the cultures of *Acutodesmus obliquus*.

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	11	8.5	10	7	13.25	14.05	9	10
pH-3	5.75	4.25	3.25	4.75	3	2.75	4	4
pH-3.5	5.25	4	7.5	5.25	8.5	4	8.75	5.25
pH-4	10	10.25	8.25	7.5	7.5	10.75	9.75	9.75
pH-4.5	9.5	12	11.75	10.75	17	11.25	17.75	11.75
pH-5	10	15.25	20	18.25	24.75	24	23	20
pH-5.5	10	13	12.25	18.5	19.75	21.5	19.25	18.5
pH-6	9	10.25	10	9	11.25	14.75	13.25	10.25
pH-6.5	11.25	11.75	8.75	7.75	8.8	9	13.5	9
pH-7	8.75	10.25	8.5	6.75	17.5	9	15.5	9
pH-7.5	9	10.75	7.75	8	8	11.75	14	9
pH-8	8.75	10.25	11.25	10.75	8.5	13.5	15.25	10.75
pH-8.5	10	9.25	9.5	10.75	14.25	4	19.5	10
pH-9	9.5	11	9.75	10.75	11.75	12	19.25	11
pH-9.5	8.5	11.25	11.5	10.5	10.75	12.25	18.75	11.25
pH-10	7.25	9.75	10.5	9	10.75	8.5	10.75	9.75
pH10.5	8.5	6.5	7.5	6.25	11.75	9.25	7.5	7.5
pH-11	4.5	6.5	7.75	6	5.25	4.5	8.8	6
pH-11.5	6.75	5.8	6	4.5	5.75	3	3.75	5.75
pH-12	4.25	4.2	3.5	3.25	6.5	4.25	2	4.2

Cell size

In the experiment using *C. globosa*, the maximum cell size was noticed in pH 5 (38.5 µm) on the first day and minimum in pH 5, 6, 7.5, 9.5, 10.5, 11 and 12. In the control set, the cell size ranged from 24.75 on the first day to 27.45 on the seventh day. While comparing the final median values, the maximum cell size was noticed in acidic pH 4.5 (29.5 µm) and minimum in higher alkaline pH 12 (22 µm).

In the treatment sets of *A. obliquus* the maximum cell size was noticed in pH 5.5 (55 µm) on the first day and minimum in pH 5 (22 µm) on the seventh day. In the control set, the cell size remained without change during experimentation. Here in the treatment sets, the maximum and minimum cell size was observed in acidic range of pH. In the case of control set, there has a gradual decrease in cell size until fifth day and then an increase in the sixth day. While evaluating the final median values of treatment set, maximum cell size was monitored in pH 9 (46.5 µm) and minimum in pH 12 (33 µm).

Structural changes like shrinkage or enlargement in cell size owing to the stress imparted by the addition of 0.5 N NaOH and 0.05 N HCl has been monitored through micrometry. While comparing the final median values, *C. globosa* showed increased cell size in acidic range, whereas *A. obliquus* showed an increase in cell size in near neutral to alkaline range. Here both species exhibited decreased cell size in higher alkaline range (pH 12). Moreover, in the case of *A. obliquus,* increased cell size was noticed in the control set when compared to that of treatment sets.

The presence of microalgal cells with increased cell size in acidic ranges were already reported (Visviki and Santikul, 2000; Hargreaves and Whitton, 1976). During morphometric analysis of *Chlamydomonas applanata* in pH 3.4, Visviki and Santikul (2000) noticed single cells with thicker cell walls, which

were 30 % larger than control cells. Authors reported that during extreme acidic conditions, the cell volume increases due to the proliferation of the cell wall by the enhanced deposition of cell wall polysaccharides. This cell wall thickening can ensure the survival of microalgae in acidic conditions by acting as an appropriate barrier in restricting the passive hydrogen ion uptake. On the other hand, while conducting experimentations using *Scenedesmus* species, Gardener et al. (2011) observed the presence of large cells in pH 9.3 with majority of single cells and two-cell grouping. The authors also noticed larger cells with irregular conformations in pH 10.3.

There were no reports regarding decrease in cell size owing to higher alkaline ranges of pH (pH 12). Thus, cell size cannot be considered as a parameter to assess the growth of a micro algal species as undivided cells are likely to appear in large size and newly formed cells in small size under culture conditions.

Table 2.1(f). Variation in cell size (µm) noticed in the cultures of *Chlamydomonas globosa.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	24.75	24.75	22	24.75	24.75	29.5	27.45	24.75
pH-3	27	24.75	27.5	29.24	24.75	29.5	27.5	27.5
pH-3.5	30.25	30.25	30.25	27.6	24.75	27.5	24.75	27.6
pH-4	33	33	28.4	24.5	24.75	29	29.5	29
pH-4.5	33	30.25	30.25	24.75	25.42	24.75	29.5	29.5
pH-5	38.5	33	27.5	22	27.5	24.5	22.45	27.5
pH-5.5	24.75	33	27.5	24.75	22.35	30.5	27.5	27.5
pH-6	27.5	27.5	27.5	29.5	22	30	27.5	27.5
pH-6.5	24.75	27.5	27.5	24.75	24.75	24.75	29.5	24.75
pH-7	24.75	24.75	30.25	27.25	27.5	24.75	24.5	24.75
pH-7.5	22	29.5	30.25	24.75	27.5	22	29.5	27.5
pH-8	27.5	33	27.5	27.25	27.5	24.75	27.75	27.5
pH-8.5	27.5	30.25	35.8	29.25	27.5	24.75	29.95	27.5
pH-9	27.5	29.25	33	27.5	26.5	27	30.25	27.5
pH-9.5	27.5	27.5	24.75	27.5	30.25	22	26.75	27.5
pH-10	27.5	27.5	24.75	24.5	24.75	24.75	28	24.75
pH10.5	27.5	27.5	24.75	22	22	22	32.25	24.75
pH-11	27.5	27.5	22.75	24.25	27.5	22	22.45	24.25
pH-11.5	27.5	27	22.5	24.5	24.75	22.45	22.75	24.5
pH-12	27.5	27	22	22	22	22	22	22

Table 2.2(f).Variation in cell size (µm) noticed in the cultures of *Acutodesmus obliquus.*

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	46.75	46.75	41.25	38.5	44	49.5	46.75	46.75
pH-3	49.5	44	35.75	38.5	38.5	37.5	46.75	38.5
pH-3.5	46.75	41.25	44	27.5	38.5	33	44	41.25
pH-4	41.25	47	38.5	38.5	38.5	37.5	44	38.5
pH-4.5	41.25	47	44	35.75	30.25	27.5	35.75	35.75
pH-5	52.25	38.5	35.75	41.25	24.75	27.5	22	35.75
pH-5.5	55	44	41.25	38.5	27.5	27.5	27.5	38.5
pH-6	49.5	46.75	41.25	38.5	35.75	38.5	44	41.25
pH-6.5	46.75	44	44	38.5	35.75	38.5	46.75	44
pH-7	52.25	44	38.5	41.25	38.5	38.5	38.5	38.5
pH-7.5	46.75	44	41.25	38.5	38.5	46.75	44	44
pH-8	41.25	46.5	41.25	41.25	38.5	46.75	38.5	41.25
pH-8.5	46.75	46.5	41.25	44	38.5	44	41.25	44
pH-9	46.75	46.5	44	49.25	38.5	38.5	46.75	46.5
pH-9.5	41.25	46.5	46.75	44	41.25	38.5	44	44
pH-10	41.25	46.5	44	41.25	35.75	38.5	46.75	41.25
pH10.5	38.5	46.5	41.25	41.25	33	44	35.75	41.25
pH-11	38.5	41.225	41.25	41.25	33	33	35.75	38.5
pH-11.5	41.25	38.5	38.5	41.25	33	30.25	35.75	38.5
pH-12	41.25	30.25	30.25	41.25	33	41.25	27.5	33

Biomass

In trials with *C. globosa*, maximum biomass was noticed in pH 9 (0.062g) and minimum in pH 4.5 (0.013g). In the control set, the biomass obtained was 0.031gm.

With *A. obliquus*, maximum biomass production was noticed in pH 9 (0.034 g) and minimum in acidic pH, ranging from 5.5 - 6.5 (0.014 g). In control set, the biomass obtained was noted to be 0.018 g.

Table 2.1(g). Variation in biomass (g) noticed in the cultures of *Chlamydomonas globosa.*

Experimental condition	Trial 1	Trial 2	Trial 3	Median
Control	0.026	0.03	0.031	0.031
pH-3	0.013	0.02	0.017	0.015
pH-3.5	0.016	0.02	0.015	0.016
pH-4	0.021	0.02	0.018	0.018
pH-4.5	0.019	0.01	0.013	0.013
pH-5	0.015	0.01	0.018	0.015
pH-5.5	0.028	0.02	0.019	0.019
pH-6	0.019	0.02	0.021	0.019
pH-6.5	0.019	0.03	0.027	0.027
pH-7	0.019	0.03	0.035	0.027
pH-7.5	0.028	0.04	0.036	0.036
pH-8	0.03	0.04	0.038	0.038
pH-8.5	0.038	0.06	0.056	0.056
pH-9	0.055	0.07	0.062	0.062
pH-9.5	0.052	0.08	0.053	0.053
pH-10	0.054	0.05	0.075	0.054
pH10.5	0.057	0.05	0.07	0.057
pH-11	0.06	0.07	0.072	0.07
pH-11.5	0.091	0.08	0.082	0.082
pH-12	0.099	0.08	0.08	0.08

Table 2.2(g).Variation in biomass (g) noticed in the cultures of *Acutodesmus obliquus.*

Experimental condition	Trial 1	Trial 2	Trial 3	Median
Control	0.03	0.02	0.02	0.018
pH-3	0.017	0.02	0.01	0.017
pH-3.5	0.012	0.02	0.02	0.016
pH-4	0.014	0.02	0.02	0.017
pH-4.5	0.018	0.03	0.01	0.018
pH-5	0.024	0.03	0.02	0.024
pH-5.5	0.014	0.02	0.01	0.014
pH-6	0.013	0.01	0.02	0.014
pH-6.5	0.013	0.01	0.03	0.014
pH-7	0.013	0.02	0.02	0.024
pH-7.5	0.018	0.02	0.02	0.018
pH-8	0.016	0.03	0.03	0.032
pH-8.5	0.026	0.03	0.03	0.028
pH-9	0.03	0.03	0.04	0.034
pH-9.5	0.044	0.04	0.04	0.04
pH-10	0.05	0.05	0.05	0.05
pH10.5	0.051	0.06	0.07	0.06
pH-11	0.06	0.06	0.05	0.06
pH-11.5	0.06	0.05	0.08	0.06
pH-12	0.122	0.07	0.06	0.07

Interestingly biomass content of respective microalgae in optimum ranges seems to be double that of the biomass content obtained in their respective control sets. Throughout experimentation, the biomass obtained from treatment sets above pH 9 was high, compared to other pH ranges, which was mainly due to precipitate formation. This was evidenced by low cell count and the white coloured precipitate formation within treatment sets.

Precipitation in culture medium owing to higher pH (pH 9 and above) was also confirmed in several reports (Becker, 1994; Visviki and Santikul, 2000; Sirisansaneeyakul et al., 2011; Dineshkumar et al., 2017). Such a situation is reported to influence the microalgal cell cycle adversely (Malis-Arad and MC Gowan, 1982 a,b). Lane and Burris (1981) and Gensemer et al. (1993) reported that extreme range of pH influences the growth, photosynthetic activity and nutrient assimilation of algae. Sakarika and Kornaros (2016) observed cell lysis in extreme acidic (pH 3 and 4) and alkaline ranges (pH 11) during the initial days of cultivation. Studies by Garcia et al. (2000), Taraldsvik and Myklestad (2000), Munir et al. (2015), Mandotra et al. (2016); Al-Safaar et al. (2016) noticed decline in growth at higher alkaline pH ranges. The reduction in growth at extreme range of pH can be due to the decrease in the rate of certain significant biochemical reactions as well as changes in cell membrane properties (Taraldsvik and Myklestad, 2000).

Throughout experimentation, the temperature pertaining to the culture medium of control and treatment sets were monitored. This has been carried out in a general view that microalgal growth fluctuates with an increase or decrease in temperature from the optimum range.

In the case of *C. globosa,* higher temperature was recorded on the fifth day (29.8ºC) and the lower temperature on the first and seventh day (29ºC). Higher temperature of 29 ºC was observed on the sixth day and lower temperature of 26 ºC on the fourth day of the treatment set pertaining to *A. obliquus*. It was also noticed that there were no variations in the temperature among acidic, neutral and alkaline conditions. Moreover analysis of data on temperature envisaged that the temperature prevalent in the treatment sets were almost within the range to support the growth of micro algal members under study. Acién et al. (2013) reported 25-35˚C as the optimal temperatures

range for freshwater microalgae and stated that for a short period, freshwater microalgae can tolerate temperature up to 40˚C.

Each species of microalgae has a specific pH range in which appropriate growth and multiplication takes place (Sakarika and Kornaros, 2016). However, the optimal and tolerance ranges may vary between species. Various literatures suggest that the maximum algal growth occurs around neutral pH (pH 7 to 7.6) and the optimum pH is the initial culture pH at which microalgae adapted to grow (Visviki and Santikul, 2000 and Hansen, 2002). On the other hand, Gong et al. (2014) reported that the initial pH ranges have little impact on cell density. However, the optimum pH ranges pertaining to microalgal species may differ.

There were several reports regarding the optimum pH ranges of *Chlorella* species. The results of the research works pertaining to Yeh et al. (2010) suggested pH 8.5 as optimum range for *Chlorella vulgaris* ESP-3. On the other hand, Gong et al. (2014) observed maximum growth of *Chlorella vulgaris* at pH ranges 10- 10.5. At the same time, Sakarika and Kornaros (2016) recorded maximum biomass production of *Chlorella vulgaris* in pH 7.5. The experimentations of Khalil et al. (2010) confirmed enhanced growth of *Chlorella ellipsoidea* under alkaline conditions, especially at pH 10. Ma et al. (2017) observed sustainable biomass production of *Chlorella sorokiniana* GT-1 at pH 6.5. The investigations of Moheimani (2013) reported augmented biomass production of *Chlorella* sp. in pH 7. While Ponnuswamy et al. (2014) recommended the pH ranges of 4-7 as optimum. On the other hand, Rai et al. (2015) monitored higher biomass production in pH 7.

Attempts were also conducted to identify the optimum pH of *Scenedesmus* species. Xiao et al. (2011) recommended pH range of 6.5 - 8.5 as ideal for maximized growth of *Scenedesmus quadricauda*. Similarly Mandotra et al.

(2016) observed increased biomass production of *Scenedesmus abundans* in pH 8.

In 1997, Zhang et al. observed increased growth of *Chlorococcum* sp. in pH 8. For the maximized growth of *Chlamydomonas applanata,* Visviki and Santikul (2000) reported pH 7.4 as optimum. During experimentations, regarding the influence of pH on the growth of *Spirulina platensis,* Panday et al. (2010) noticed maximum biomass production at pH 9. The effect of pH on the growth of *Tetraselmis suecica* was analyzed by Moheimani (2013) and suggested pH 7.5 as most favorable range for enhanced biomass production. Bartley et al. (2014) observed pH 8–9 as ideal for enhanced growth of *Nanochloropsis salina*.

In the present study, while comparing the final median values of major growth parameters pertaining to the treatment sets containing *C. globosa,* maximum cell count was noticed at pH 8. In addition, increased turbidity and biomass content were obtained in pH 9. This indicates that the optimum pH requirements of *C. globosa* were ranging from 8-9.

Upon comparing the final median values of major growth parameters associated with *A. obliquus*, highest turbidity was noticed in pH 9, cell count in pH 5 and biomass in pH 9. Here, the algal member is showing a wide range of pH (5-9) ideal for growth and multiplication. Varshney et al. (2016) reported pH 8 as the optimum range for *Acutodesmus* sp. The results are indicative of the adaptability of *A.obliquus* even to acidic ranges and thereby its applicability for carbon sequestration experiments. This property of *A. obliquus* is significant as most micro algal members fails to develop in culture conditions, where there will be a drastic shift in pH to acidic conditions due to extraneous supply of carbon dioxide/ flue gas.

Correlation analysis

Correlation analysis is a prominent tool, which helps in identifying the degree of relationship existing between the variables. In the present study, the relationship between pH (control set and optimum pH ranges) and major growth parameters like turbidity, cell count and biomass of two microalgal species have been analyzed statistically using Microsoft Excel. The significance of the each parameter was calculated using the t-value and the results are represented in Tables 2.3 and 2.4.

Table 2.3. Correlation of pH with respect to major growth parameters in *Chlamydomonas globosa*.

Experimental set	Turbidity	Cell count	Biomass
Control set	4.10*	3.42*	1.42
pH 8	2.84*	2.69	4.91*
pH 8.5	2.68	2.87*	0.79
pH 9	2.42	3.02*	3.12*

(* Significant at 5% level; NS: not significant (t-value at 5% level for 3 treatments and 3 replications =2.776)

In the present study, the relationship (t-value) between the optimum pH range and their respective growth parameters of ***Chlamydomonas globosa*** were worked out. Similarly, the t-values of control set with respect to growth parameters were also analyzed. Here in the case of treatment sets, the turbidity content of the pH 8, cell count values of both pH 8.5 and pH 9 and biomass content pertaining to pH 8 and pH 9 exhibited significant positive relation. In the case of control set, the turbidity content and cell count values exhibited significant positive correlation with control pH.

Table 2.4. Correlation of pH with respect to major growth parameters in *Acutodesmus obliquus*.

Experimental set	Turbidity	Cell count	Biomass
Control set	1.87	0.46	4.41*
pH 5	-2.57	-3.54*	-2.34
pH 9	3.89*	1.81	7.5*

(* Significant at 5% level; NS: not significant (t value at 5% level for 3 treatments and 3 replications =2.776)

Here, with ***Acutodesmus obliquus***, the t-values between the optimum pH ranges and their respective growth parameters were worked out. Likewise, the t-values of control set were also analyzed. While analyzing the relationship between pH 9and growth parameters the turbidity content and biomass content exhibited significant positive relation. Here in the case of pH 5, the cell count showed a significant negative relationship. In the case of control set, the biomass content exhibited significant positive relation with control pH. The present work thus confirms the capabilities of *Acutodesmus obliquus* to grow in acidic ranges and thereby its utilization in carbon sequestration experimentations. For mass multiplication and subsequent utilization of *Chlamydomonas globosa* and *Acutodesmus obliquus* in Bolds Basal medium, the optimum pH range of respectively 8-9 and 5-9 can be followed.

Summary and conclusion

In recent times, microalgae have been employed for a wide range of beneficial purposes. For exploiting their potentials for such purposes, their biomass is required in sufficient quantities.

One among the major constraints in their utilization for such purposes is that most of the members, which are most abundant and virulent in natural conditions, seem to be slow growing in culture conditions. Moreover, the risk of maintaining cultures without contamination is another constraint in their

utilization for multidimensional purposes. Hence, standardization of species specific culture conditions is a prerequisite for meeting the required targets.

pH is noted to be an important factor influencing the growth and multiplication of micro algal species in culture conditions. pH of the medium is also significant in carbon sequestration studies, as it determines the availability and solubility of CO_2 and other nutrients. In the present study, an attempt has been carried out to assess the growth performances of selected microalgal members (*Chlamydomonas globosa* and *Acutodesmus obliquus*) in Bolds Basal medium under varying culture conditions, altered by pH.

For experimentation, treatment sets were maintained with conical flasks of 100 ml capacity (21 nos), each containing 50 ml Bolds Basal medium. The pH of culture medium was adjusted from 3-12, with a gradation of 0.5 using 0.5 N NaOH and 0.05 N HCl. After adjusting to the required pH, 5 ml of pure cultures of the respective micro algal members were added to the respective conical flasks and the changes in pH, if any, was readjusted. The control set was maintained at pH 6.6 (original pH of the culture medium).

The culture conditions like pH, temperature, conductivity and resistivity and micro algal growth parameters like turbidity, cell count, cell size, and biomass were monitored throughout the treatment period. The biomass estimation was carried out on the initial and final days of treatment. All the sets were kept at illumination during daytime (6 AM – 6 PM) and retained under light intensity of 40 µmol m^2 s^{-1}. The temperature of the medium during experimentation ranged from 26 – 29.8˚C while the humidity ranged from 48 - 54 %. Every day after observation, the altered pH was readjusted to their experimental condition. Monitoring of the treatment sets were carried out for a period of 7 days.

For the confirmation of appropriate pH level at which maximum growth of both microalgal members under the study had occurred, the median values of the 7 days triplicate data were worked out and compared. However, during experimentation, in higher alkaline ranges, formation of precipitate was noticed. Hence, for drawing conclusions regarding the optimum pH range in which maximum turbidity occurred, the final median values of pH ranges above 9 were neglected.

Upon comparing the final median values of major growth parameters pertaining to the treatment sets containing *Chlamydomonas globosa,* the maximum cell count was recorded at pH 8, whereas increased turbidity and biomass content were noticed in pH 9. While analyzing the final median values of major growth parameters pertaining to *Acutodesmus obliquus*, increased turbidity and biomass content were noticed in pH 9, while maximum cell count was observed in pH 5.

The present findings thus confirms that the optimum range of pH favouring the growth of *Chlamydomonas globosa* in BB medium was noted to be 8-9 and that of *Acutodesmus obliquus* at a wide range of 5-9. Hence for ensuring better biomass production of these micro algal members in BB medium, the above mentioned pH ranges can be followed.

Chapter III

PBR based feasibility studies on the carbon sequestration efficiency of selected micro algal members

Introduction

Screening studies carried out in Chapter I proved the efficiencies of micro algal species like *Chlamydomonas globosa* and *Acutodesmus obliquus* in assimilating extraneous supply of carbon dioxide. Attempts were also carried out to assess the optimum pH favouring the enhanced growth and development of *C. globosa* and *A.obliquus* in chapter II. As these species are sustaining in higher dosages of carbon dioxide, an attempt has been carried out to assess their better efficiencies in carbon dioxide accumulation using a proto type Photo Bio Reactor (PBR), which can ensure optimum conditions of algal growth and carbon assimilation.

PBRs can be defined as closed (or nearly closed) vessels for phototrophic production, comprising of a solid phase (microalgal cells), liquid phase (growth medium), gaseous phase (CO_2 and O_2) and super imposed light-radiation field (Posten, 2009). Based on the illuminated surface, PBRs are categorized as flat plate (Sierra et al., 2008; Slegers et al., 2011), tubular (Molina et al., 2001) and column type (Eriksen, 2008). Based on their mode of liquid flow, PBRs can be grouped as stirred type, bubble column and airlift reactor type (Gupta et al., 2015). Also there are wide ranges of hybrid type PBRs (Kumar et al., 2011).

Screening studies on carbon dioxide assimilation efficiencies of micro algae revealed the potentialities of *C. globosa* and *A. obliquus* under laboratory conditions. Their efficiencies were further assessed in a laboratory scale

closed vertical bubble column PBR for optimization of their growth conditions and system efficiency for ensuring their industrial/commercial applicability. Though there are approaches on system designs for various micro algal members, including *A. obliquus*, no such efforts have ever been carried out on *C. globosa*, which are found to be effective in CO_2 sequestration under present laboratory conditions.

Review of literature

The first laboratory 'PBR', capable of controlling critical parameters like light, nutrients and temperature was developed in early 1940's (Myers and Clark, 1944; Ketchum et al., 1949). Since then, so much of R & D activities were carried out for their effective utilization in various sectors. The use of photo bioreactors for microalgal CO_2 sequestration has gained significant attention in recent times, as it offers increased microalgal productivity due to controlled environmental conditions, optimized space utilization and exhibits higher photosynthetic efficiency compared to open systems.

Several factors need to be considered while designing a photobioreactor for carbon sequestration. For better performance of the system, suitable candidate species that can manage extremes of pH, temperature, shear stress (Kumar et al., 2011) and having high growth rate and low risk of contamination are preferred. Moreover, proper mixing should be ensured for efficient light distribution to cells (Kumar et al., 2011). The continuous removal of oxygen is also essential, as excessive concentration of dissolved oxygen inhibits photosynthesis (Molina et al., 2001). The selection of optimum cell concentration for efficient CO_2 sequestration is also vital. Below the optimum cell concentration, not all the supplied light energy is captured by the cells (Zhang et al., 2001) though, highly dense culture makes cells more tolerant to high CO_2 concentration (Chiu et al., 2008). The identification of suitable light

source may avoid the problem of availability of light to the culture and also the formation of biofilms.

Until now, several researchers worked on developing novel photobioreactors for improving the CO_2 fixation efficiencies of microalgae by overcoming the existing drawbacks and offering significant yield with high production efficiency and reduction of biological contamination (Chisti, 2007; Huntley and Redalje, 2007; Vasudevan and Briggs, 2008). Some of the attempts in this direction are detailed below.

A gas recycling photobioreactor was developed by Yun and Park (1997) for *Chlorella vulgaris* UTEX -259 with increasing gas retention time and high CO_2 fixation rate. With increased gas retention time, the CO_2 concentrations can be maintained in optimal ranges for algal photosynthesis. It has also been reported that, using the same photobioreactor, microalgal species without CO_2 tolerance can also be cultivated without growth inhibition.

A modified flat-plate photobioreactor has been developed by Hu et al. (1998) for obtaining high cell density under elevated CO_2 concentrations. The reactor was made up of an acrylic plastic plate, with an outer and inner chamber. The outer chamber functions as a temperature regulator and the bubbling tube was placed at the bottom of the inner chamber. Studies using this photobioreactor revealed that, daily replacement of the culture medium was effective in maintaining ultrahigh-cell-density culture of CO_2 tolerant unicellular *Chlorococcum littorale*.

The carbon dioxide fixation efficiencies of *Euglena gracilis* were explored by Chae et al. (2006) by using a laboratory-scale photo-bioreactor (100 L working volume) with baffles stimulated plug-flow of the culture medium. The effect of lightwas minimizedby fixing the width of the photo-bioreactor to 20 cm. A pilot scale novel photo-bioreactor (1000 L working volume)

using sunlight as energy source and flue gas as carbon source was also fabricated for the experimentation. It was designed in such a way that, the dark and light regions were separated using a cover and a scraper for the internal circulation of culture medium between light and dark regions. The experimentations showed an enhanced cell yield of ten times in pilot-scale reactor than the laboratory-scale reactor.

A membrane-sparged helical tubular photobioreactor (MSTR) with a working volume of 800 ml was designed for monitoring the carbon dioxide fixation efficiencies of *Chlorella vulgaris* (Fan et al., 2008). The performance of MSTR, including the light intensity, gas flow rate and characteristics of membrane module on CO_2 fixation were compared with draft tube airlift photobioreactor, a bubble column and a membrane contactor. The results revealed that the limitation of CO_2 removal was improved in MSTR and registered higher CO_2 fixation rate (0.95–5.40 times) when compared to the conventional reactors. Jana et al. (2017) investigated the carbon dioxide bio fixation potential of *Arthrospira* sp. cultured in membrane photobioreactor (MPBR) of cylindrical glass vessel. The authors reported effective CO_2 dissolution and significant removal of dissolved oxygen by bentonite clay derived membrane.

Studies were conducted to improve the CO_2 utilization efficiency of *Chlorella* sp. AG10002 in vertical tubular photobioreactor (Ryu et al., 2009). The work as a whole optimized the bubble size for maximum cell concentration and noticed that, while culturing using large bubbles, the mass transfer may decreases due to reduction in the interfacial area between gas and liquid. In addition, they recommended the utilization of slanted cross-sectional type baffle for increased cell growth. To determine the economic and technical feasibility of micro algae-based carbon sequestration, Wilson et al. (2014)

cultured *Scenedesmus acutus* using flue gas through house designed closed loop, vertical tube photobioreactor.

Fransico et al. (2010) evaluated the carbon dioxide sequestration efficiencies, lipid productivity and biofuel quality of six microalgal strains (*Aphanothece, Dunaliella, Chlorella, Scenedesmus, Phormidium and Phaeodactylum*) in bubble column photobioreactor with working volume of 3.0 L.A dispersion system with 1.5 cm diameter was positioned at the center of the column and the reactor was continuously illuminated. The comparison of the results reported *Chlorella vulgaris* as better strain.

A novel assembled culture system, fermentor-helical combined photobioreactor was designed by Jia et al. (2011). In this reactor, the temperature and pH can be controlled in the fermentor and the CO_2 fixation can be accomplished in the helical tube. CO_2 removal ratio of 95% was achieved in this novel culture system.

A novel graphical integration technique for quantifying the moles of CO_2 sequestered was developed by Kargupta et al. (2015) for evaluating the CO_2sequestration potentialities of *Chlorella pyrenoidosa* and *Scenedesmus abundans* using tubular batch photobioreactor. For experimentation, three completely mixed tubular batch reactors (CMTBRs) were connected in parallel and the gas was continuously supplied through the base of the reactor. Using mirror arrangement, the illumination was provided by a fluorescent lamp. The results showed that continuous bubbling enhanced the biomass productivity and CO_2 sequestration efficiencies of microalgae.

Naderi et al. (2015) evaluated the carbon biofixation efficiencies of *Chlorella vulgaris* under different light intensities using a 3-L bench top bioreactor (New Brunswick's BioFlo/CelliGen 115) of 2-L working volume. The system was enclosed by a water jacket to achieve precise temperature control. The

DO and pH meters were fixed at the top of the bioreactor. Using a Resistance Temperature Detector (RTD), the culture temperature was measured.

A promising approach of integrated use of wastewater and flue gas for CO_2 fixation and biomass production was established by Kuo et al. (2016) through a column-type glass-fabricated photobioreactor with 1 L working volume. The effect of high gas superficial velocity on CO_2 capture from air by *Chlorella vulgaris* using airlift bioreactor was investigated by Sadeghizadeh et al. (2017). The authors commented that *Chlorella vulgaris* exhibited resistance to shear stress and enhanced potentialities in growth and CO_2 capture under high input gas superficial velocities.

A few attempts were also conducted using flask type photobioreactors. A flask culture photobioreactor was developed by Fluke et al. (2015) to screen the efficient microalgal species for high carbon dioxide fixation. The photobioreactor consists of an upper 250 ml Erlenmeyer flask for microalgal growth and a lower flask with buffer mixture for CO_2 generation. The LED based light panels were used as light source and the CO_2 partial pressure was maintained constantly by reloading the buffer at an interval of 48 hours. The CO_2 partial pressure in headspace of the flask was accurately maintained in different concentrations using buffer mixture. The authors recommended culture flask photobioreactor for screening of microalgal strain for CO_2 sequestration efficiency.

Duarte et al. (2017) conducted batch cultivation studies in closed Erlenmeyer flask-type photobioreactors with a working volume of 1.8 L. The gaseous mixture was supplied to the cultures at an interval of 2 hours of the light period for 10 min. through a porous curtain spurger, placed on the base of photobioreactor. The authors confirmed the approach of intermittent flue gas supply to the culture medium, which enhances the growth and CO_2 biofixation by *Chlorella fusca* and *Spirulina* sp.

For improving the CO_2 fixation efficiencies, Ho et al. (2013) cultivated *Scenedesmus obliquus* CNW-N in 1-liter glass vessel, illuminated through external light source. The experimentation showed that the highest CO_2 fixation rate was achieved by 50% replacement of culture medium. The lipid productivity and carbon dioxide fixation rate of *Chlorella protothecoides* were monitored and reported by Binnal and Babu (2017) in a 5L lab scale photobioreactor made up of borosilicate vessel. The authors adopted Response Surface Methodology (RSM) to optimize the environmental condition.

A series of experimentations on the effect of flue gas on the growth of *Scenedesmus dimorphus* were conducted by Jiang et al. (2013) by employing glass columns photobioreactors. The toxicity of flue gas was reduced by adopting strategies like neutralization of culture medium by $CaCO_3$ addition and intermittent spurging by pH feedback control. The authors reported that the obstacles were well controlled and resulted in increased algal cell growth.

Optimization studies on the operational parameters like enhancement of height, intermittent supply of CO_2 and reduction of flow rates to lengthen the CO_2 residence time has been conducted by Basu et al. (2015). For maximizing the CO_2 fixation efficiency of *Scenedesmus obliquus* SA1 (KC733762) the open cylindrical glass tube was employed. The authors fixed 15% CO_2 supply at 0.43 LPH for 12 h per day as an optimum condition.

Literatures are also available on the comparison of the efficiencies of photobioreactors. The CO_2 utilization efficiencies of three types of gas-spurged photobioreactor designs, including a flat plate (working volume of 7.0 dm^3), a polyethene column (working volume of 20 dm^3) and a bubble column (working volume of 1.4 dm^3) were attempted by Lakaniemi et al. (2012). For experimentation, *Chlorella vulgaris* was cultivated. The authors

observed long time CO_2 retention and higher carbon dioxide utilization efficiencies in bubble column.

The carbon dioxide fixation efficiency of *Haematococcus pluvialis* under both indoor (working volume 5 L photobioreactor of 1 column) and outdoor conditions with working volume of 20 L (4 sequential columns) was monitored by Lee et al. (2015). The carbon distribution pattern in the photobioreactor was also investigated. Based on the results, authors stated that, enhanced carbon fixation efficiency of 4 fold under indoor and 3.63 fold under outdoor conditions can be attained through sequential operation system.

The efficiencies of a single reactor and six-parallel photobioreactors were compared for CO_2 mitigation using *Chlorella* species (Chiu et al., 2008). A cylindrical glass reactor (30 cm length, 7 cm diameter) with a working volume of 800 ml was designed and used. The results revealed that the CO_2 reduction, biomass production and lipid production were six times superior in the six-parallel photobioreactors than that of single photobioreactor. However, the CO_2 removal efficiencies were noted to be similar in both cases. The strategy of employing high initial cell density with photobioreactor operation, in series, was adopted by Yadav et al. (2015) towards reducing the hindering effect of pure flue gas and to maximize the CO_2 fixation. A bubble column photobioreactor with a working volume of 500 mL for *Chlorella* species was designed.

Cheng et al. (2013) conducted studies to improve the carbon fixation efficiency of *Chlorella* PY-ZU1 in sequential column bioreactor. By operating in a multi-stage sequential bioreactor, CO_2 was categorized and utilized. The authors reported that the residence time of CO_2 has enhanced exponentially and the multi-capture by microalgae ultimately resulted in increased CO_2 fixation efficiency.

The strategy of internal illumination of the cultures was also carried out. An internally illuminated photobioreactor (IIPBR) of 18-l prototype was designed by Pegallapati and Nirmalakhandan (2011) for cultivating *Nannochloropsis salina and Scenedesmus* sp. The PBR was developed according to the principles of airlift/bubble column. For maximizing biomass productivity, spurging Carbondioxide- Enriched Air (CEA) was attempted. The authors claimed that the light utilization can be improved through internal illumination and the energy for mixing can be reduced through the airlift operations. When compared to normal bubble columns, the IIPBR has the potential to minimize the energy input to 50%.

The idea of internal illumination was also attempted by Kurano et al. (1995). The CO_2 fixation capability of *Chlorococcum littorale* was monitored using PBR of three types of culture vessels. A large vessel illuminated via fluorescent lamps was placed inside the culture medium and other two vessels were illuminated from outside. The authors noticed maximum CO_2 fixation rate in the smallest vessel due to increased irradiation and daily medium exchange by cell harvest.

Research works were also conducted to develop photobioreactors to utilize sunlight as energy source. Hirata et al. (1996) described a photobioreactor equipped with specially designed illumination plate made of Pyrex glass for collecting sunlight. The efficiency of sun light collection and transmission to the algal cells was streamlined. Results obtained from photobioreactor were compared with Roux flasks illuminated with fluorescent or xenon lamp. The results revealed that the cell growth and CO_2 fixation rate achieved in the photobioreactor were lower than Roux flask cultures. The photobioreactors with solar collectors to monitor its feasibility in CO_2 biofixation was also developed by Ono and Cuello (2006). The authors stated that, high initial cost

constraints associated with solar collector and photobioreactor can equalize through the reduction in land-related cost.

Studies pertaining to the outdoor cultivation of microalgal species using various photobioreactors were carried out and reported. An outdoor air lift photobioreactor with working volume 0.1 m^3 was designed for the flue gas reduction using *Scenedesmus obliquus*. The photobioreactor consists of an external pump for circulating flow between the down-comer and riser tube to enhance the CO_2 transfer. The results of experimentation revealed CO_2 removal ratio of 67% in the pilot scale system (Li et al., 2011).

The feasibility of outdoor cultivation of *Chlorella vulgaris* in 80 L (8 L x 10 sets) bubble column PBR using various CO_2 input concentrations was monitored by Guo et al. (2015). The possibility of culturing *Acutodesmus obliquus* in large-scale outdoor tubular photobioreactor (500-L) using gaseous emissions from a methanol plant was reported by Chen et al. (2016). In the OTP, the gas exchange and circulation of the algal suspension was accomplished with the aid of a pneumatic diaphragm pump. The maximum biomass obtained in the OTP was consistent with that of the indoor experimentations. Eloka-Eboka and Inambao (2017) cultured *Chlorella vulgaris, Dunaliella, Scenedesmus quadricauda* and *Synechococcus* sp. in both open pond and closed photobioreactor of model BF-115 Bioflo/celliGen. The open pond consists of working volume 850 L exposed to 12 h continuous sun light. It was reported that, species with lower biomass productivity exhibited increased lipid content. The authors recommended *Dunaliella* as an efficient strain in CO_2 sequestration.

Upon analyzing the literature, it has been noticed that though PBRs were widely used and have several advantages over open systems, still there are major drawbacks that make them uneconomical. These are in the areas of species selection, system design and culture conditions. Lack of appropriate

candidate species for withstanding fluctuations in pH and temperature, inadequate temperature controlling facility, biofilm formation, improper mixing of microalgal culture with devices generating heat, damages due to aeration, inadequate arrangements/positioning of suitable illumination source etc. were noticed as major obstacles in the design and scaling up of PBR technology.

In the present study, a proto type PBR (closed, vertical bubble column type) was designed to assess the carbon sequestration efficiencies of micro algal species like *C. globosa* and *A. obliquus*. A review of the literature revealed that most of the studies regarding engineered design structures for carbondioxide sequestration are confined to limited number of microalgal species like *Chlorella* and *Scenedesmus.* Though there are fragmentary information pertaining to *A. obliquus*, reports regarding photobioreactor design for *C. globosa* is scanty. Thus the present objective of the study is to:

- Assess the CO_2 assimilation efficiency of *Chlamydomonas globosa* and *Acutodesmus obliquus,* cultured in a PBR under controlled conditions.

Materials and methods

The growth performances of *Acutodesmus obliquus* and *Chlamydomonas globosa*, which survived under higher levels of carbon dioxides supply, were assessed for their carbon dioxide sequestration potentialities using a proto type Photo bio reactor maintained at controlled conditions. Description of the PBR used in the study is as follows:

PBR design

A closed vertical bubble column structure having a size of 10 x 10 x 45 cm and a volume of 4.5 L was designed using Acrylic material. The technical details concerning the experimental system is given in Plate 6 a and the

photograph in Plate 6 b. For the supply of both culture medium and carbon dioxide gas, an inlet, with control facility was maintained at the bottom. Similarly an outlet was maintained at the top of the column to permit the gases within the column, if any, to come out. The outlet of the PBR is connected to the inlet of a reservoir of culture medium kept in a closed / sealed bottle (2 L). The outlet of the PBR is retained in the bottom of the reservoir to permit the gases, if any, to bubble out through the medium. A controlled outlet is also provided to the reservoir for the release of gases, if any.

Experimental layout

For experimentation, micro algal cultures, which were maintained in respective pH, as outlined in Chapter II were used. At a time a single experiment was carried out with a particular species and a particular carbon dioxide dosage.

For experimentation, pure cultures of candidate species (3 litres) with an initial concentration of 0.5 OD, maintained in bolds basal medium was transferred to the vertical column of the PBR through its inlet. Care was taken to maintain adequate space for the accumulation of gases in the upper side of the PBR. The other end of the inlet was connected to a cylinder of carbon dioxide, having control facilities. The outlet of the PBR was connected to the inlet of the reservoir containing 2 liters of Bolds Basal medium. The inlet of the reservoir was maintained in such a way to permit bubbling of gases, if any, which passes through it.

The carbon dioxide from the cylinder, having control facilities, was allowed to bubble at regular intervals through the culture medium contained in the column of the PBR. The frequency of carbon dioxide supply was set for 60 or 80 bubbles /2 hours from 6 am to 6 pm. The PBR was maintained at a light

intensity of 100µmol m^{-2} s^{-1} using LED lights and fluorescent tubes. The room temperature was maintained at 28±0.5ºC and humidity at 48%. For monitoring the nature and magnitude of the gas generated at the top chamber of the vertical closed column structure, the reservoir containing BB medium (devoid of microalgae) was monitored for gaseous influxes, specifically with respect to CO_2 and O_2. The carbon sequestration efficiencies of both microalgal members were monitored for 3 and 6 days in separate trials.

During experimentation, CO_2, as per its pre-determined magnitude and frequency was supplied through the inlet of the PBR. The physico-chemical characteristics of the culture medium within PBR (before and after experimentation), together with growth performances and biochemical characteristics of microalgae (before and after experimentation) were carried out. Along with studies on the culture medium contained in PBR, the medium contained in the reservoir was also subjected to the analysis of pH, free carbon dioxide and dissolved oxygen for determining the changes, if any, associated with them due to gaseous influx.

The characteristics of the culture medium were worked out on the first day, before experimentation and after experimentation for the stipulated period of 3/6 days. The physicochemical parameters analyzed include pH, temperature, conductivity, resistivity, free carbon dioxide, dissolved oxygen and alkalinity following Trivedi and Goel (1987). Growth parameters associated with microalgal members, which were attempted before and after experimentation include turbidity, specific growth, biomass productivity, cell count and cell size. The biochemical parameters associated with micro algae like pigments (Shof and Lium,1976), total lipid (Bligh and Dyer, 1959), protein (Lowry et al.,1951), total carbohydrate (Dubois et al., 1956) and mineral composition (Pancha et al., 2015)were worked out on the initial day and final day of experimentation.

The methods followed for the estimation of pH, conductivity, resistivity, free carbon dioxide, dissolved oxygen and alkalinity of the culture medium and turbidity, cell count and cell size associated with micro algal growth are already depicted in Chapter 1. Other parameters attempted, like biomass productivity and specific growth, pigments, total lipids, total protein, total carbohydrate and mineral components are as per the details listed below:

Biomass productivity and specific growth (Chen et al., 2016; Guillard and Ryther, 1962)

The biomass productivity during treatment period was determined by recording the optical density of microalgal culture at 684 nm using spectrophotometer (Systronics, 2201).Specific growth rate is the measure of number of generations (the number of doublings) that occur per unit of time. The specific growth rate was obtained using following equation (Guillard and Ryther, 1962):

$$\mu = \frac{\ln (Nt/No)}{Tt - To}$$

Nt = OD value on final day of treatment

No = OD value on intial day treatment

Tt = Final day of treatment

To = Starting day of treatment

Pigments - chlorophyll (Shof and Lium, 1976)

Reagents:

DMSO

Procedure:

Due to the critical "light harvesting" role in photosynthesis, the measurements of photosynthetic pigments have received considerable attention. The pigments like chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were estimated using Shof and Lium (1976) method. To the 0.025 g of fresh microalgal sample, 7 ml of Dimethyl sulphoxide was added and kept in an oven at 60°C for one hour. The extract was then transferred to a graduated tube and made up to a total volume of 10 ml with DMSO (can be assayed immediately or transferred to vials and stored between 0-4°C until required for analysis). The optical density was recorded at 480nm, 645nm and 663nm using a spectrophotometer (Systronics 2201). Quantitative estimation of the pigments was carried out using the following equations:

$$\text{Chlorophyll } a = \frac{12.7 \times OD663 - 2.69 \times OD645}{1000 \times W \times 1} \times V \times df$$

$$\text{Chlorophyll } b = \frac{22.9 \times OD645 - 4.68 \times OD6663}{1000 \times W \times 1} \times V \times df$$

$$\text{Total Chlorophyll} = \frac{20.2 \times OD645 + 8.02 \times OD6663}{1000 \times W \times 1} \times V \times df$$

$$\text{Carotenoids} = OD480 + \left[(0.114 \times OD663) - (0.638 \times OD645)\right] \times \frac{V \times df}{1000 \times W}$$

V = Volume

df = Dilution factor

W = Weight of the microalgae taken

OD = Optical density at a particular wave length

Total Lipids (Bligh and Dyer, 1959)

Reagents used:

Extraction solvent- Chloroform: Methanol: Distilled water (5:10:4)

Procedure:

For estimating the total lipid content, 20 mg fresh microalgal sample was homogenized using extraction solvent (Chloroform: Methanol: Distilled water-5:10:4) and centrifuged at 4000 rpm for 10 minutes. After keeping in room temperature for overnight the solution was again homogenized using 5 ml Chloroform and 5 ml Distilled water and centrifuged. Carefully transferred the lower layer of the centrifuge tube to a pre weighed beaker using syringe or micropipette and kept in room temperature for evaporation. The dried beakers along with extracts were weighed. The total lipids were calculated from the difference in final weight of beaker and the weight of the beaker alone and expressed in percentage.

Total Protein (Lowry et al., 1951)

Reagents used:

1) 10% TCA - Reagent A

2) 1N Sodium Hydroxide- Reagent B

3) Alkaline Sodium Carbonate Solution (Dissolving 2 g of Sodium Carbonate in 100 ml of 0.1N Sodium hydroxide) -Reagent C

4) Copper sulphate-Sodium potassium tartarate solution (Mixing 0.5% Copper sulphate and 1% Sodium potassium tartarate solution) - Reagent D

5) Alkaline copper reagent (Mixing 50 ml reagent C and 1 ml of reagent D) - Reagent E

6) Folin-ciocalteu regent

Procedure:

Total protein content was determined by using the method of Lowry et al. (1951). The microalgal cultures were centrifuged and washed with distilled water. For extraction, 20 mg of fresh microalgal sample was homogenized using reagent A and centrifuged at 5000 rpm for 10 minutes. The pellets were then treated with reagent B and boiled for 30 minutes, cooled and centrifuged. The resulting supernatant was then made up to a known volume. For estimation, to the 1 ml of sample (0.1 ml of the supernatant mixed with 0.9 ml distilled water) 5ml of reagent E were added and incubated for 10 minutes. Finally, 0.5 ml of folin-ciocalteu regent was also added. After 30 minutes of incubation the absorbance at 750 nm was measured. The amount of total protein was calculated from the standard graph prepared using Bovin Serum Albumin (BSA) and the reagents were taken as blank.

Total Carbohydrates (Dubois et al., 1956)

Reagents used:

1) Phosphate buffer 0.1M (pH 6.8)

2) 5% phenol

3) Sulphuric acid

Procedure:

Total carbohydrate contents of microalgae were determined by phenol - H_2SO_4 method (Dubois et al., 1956). 20 mg of fresh microalgal biomass was homogenized in 5ml of sodium phosphate buffer (pH 6.8) and centrifuged at 5000 rpm for 10 minutes. To the 1 ml of the supernatent 5% phenol solution and 5ml of sulphuric acid were added and mixed thoroughly. The sample was then kept for incubation at room temperature for 30 minutes and the

absorbance was measured at 490 nm against a reagent blank using spectrophotometer. Glucose was used as a standard for the preparation of calibration graph.

Mineral composition (Pancha et al., 2015)

Reagents used:

1) Conc. HNO_3

2) $HClO_4$

3) H_2SO_4

4) 2% HCl solution

Procedure:

For estimation of the mineral content, 100 mg of dried microalgal biomass was taken in a beaker and digested using 10 ml of Conc. HNO_3 for overnight. To the digested sample, 2.5 ml $HClO_4$ and 250 µl H_2SO_4 was added on the next day and heated until the elimination of white smoke. The resulting content was then dissolved in 100 ml of 2% HCl solution and filtered using Whatsmann filter paper. Using the flame photometer (Systronics, 128) the analysis of sodium and potassium was carried out.

The results of the above analyses were depicted and interpretations were made in results and discussion. The results of major influencing parameters pertaining to both treatment sets of *C. globosa* and *A. obliquus* were also subjected to statistical analysis using t-test.

Results and discussion

Although microalgal culturing using photobioreactors have been in practice since 1940s, their multi-oriented application and scale up activities are still a

challenge. Efforts are going on, both in field and laboratory conditions, to improve the productivities of various micro algal members at low cost. Here in the present study a prototype of a closed vertical bubble column type PBR was developed under laboratory conditions for maximizing the growth of micro algal species like *C. globosa* and *A. obliquus* under elevated levels of carbon dioxide.

The study has been undertaken during August to November, 2017. Separate studies were carried out for both microalgal members. However optimum conditions of temperature (28±0.5ºC), humidity (48%) and light intensity (100µmol m^{-2} s^{-1}) were followed throughout the study. The parameters selected (physico chemical characteristics of culture medium and growth and biochemical characteristics of micro algal members) were worked out before (control) and after experimentation (3rd or 6th day) and the results are reported.

An attempt has also been carried out to standardize the bubbling frequencies (60 and 80 bubbles/2 hours) in accordance with the extent of CO_2 dissolved in a closed system of Bolds Basal medium. This has been carried out by bubbling CO_2 gas from the source at specific frequencies through the medium (3.0 litres) taken in the closed chamber of the PBR. The resultant free CO_2 content associated with the medium was then analyzed titrimetrically using phenolphthalein and 0.05N NaOH and the results are represented in mg/L.

Accordingly, it has been noticed that a bubbling frequency of 60 and 80 bubbles/2 hours in 3.0 litres of Bolds Basal medium retained 127.6 and 145.2mg/L of free CO2 content, respectively, within BB medium.

Studies on *Chlamydomonas globosa*

The responses of *C. globosa* with respect to optimum flow rate of carbon dioxide (60 bubbles/2 hours) from 6 am to 6 pm has been monitored for 3 and 6 days under optimum conditions. The median values of the results

concerning culture media and morphological / biochemical characteristics of the *C. globosa* are depicted in table 3.1. For drawing conclusions regarding the performances of micro algae in closed vertical bubble column structure under optimum carbon dioxide flow rate, the percentage difference of the major influencing parameters were worked out.

Upon comparing the results of *C. globosa*, a marked decrease in the pH values of both 3 days (4.78%) and 6 days treatment was noticed (9.84%). With respect to free carbon dioxide content, an increase of 249.1% was noticed in 3 days and 181.82 % in 6 days of treatment. In the case of dissolved oxygen content, an increase was noticed in the 3rdday (400%) and the 6th day (386.11%).

In the case of microalgal growth parameters, maximum turbidity was observed after 6 days of treatment (167.27%) than 3 days (156.96%). Upon comparing the results pertaining to biomass productivity a marked increase of 230.5% was recorded in 6 days of treatment than 3 days (156.4%). Also the cell count of 6 days treatment showed significant increase with 237.73% than 3 days of treatment (81.92%).While comparing the results of cell size, higher value of 25% was noticed in 3 days treatment than 6 days of treatment (6.66%), compared to their initial values.

On comparison of the pigment content of microalgae concerning both treatment sets, chlorophyll *a* content of the 3 days treatment showed lesser values (32.675%) than 6 days treatment (36.39%). Similarly a lower value of 39.45% was noted for chlorophyll *b* content in 3 days treatment, whereas a higher value of 41.337% was noticed with 6 days of treatment. Upon comparing the percentage differences of total chlorophyll content, the 3 days treatment exhibited a decrease of 38.72%, while 6 days treatment showed an increase of 38.08%. Here the carotenoid content exhibited a marked decrease of 12.844% in 3 days of treatment and 31.648% in 6 days.

Upon comparison of the biochemical parameters, lipid content showed a significant increase in 3 days of treatment (185.7%) than 6 days (14.32%). An increase of 21.186% of protein content was observed in 3 days of treatment, while it was considerably less (7.2%) in 6 days of treatment. In the case of carbohydrate content, a marked increase was noticed in 6 days of treatment (128.6%), whereas a lower value of 12.5 % was recorded in 3 days of treatment.

Table 3.1. Changes in culture media characteristics and morphological / biochemical responses of *Chlamydomonas globosa* to varying durations of carbon dioxide supply.

Sl. No	Parameters Analysed	Units	CO_2 supply in 60 bubbles / 2 hours of interval for 3 days		CO_2 supply in 60 bubbles / 2 hours of interval for 6 days	
			Initial day	Final day	Initial day	Final day
1.	pH	-	7.12	6.78	7.01	6.32
2.	Conductivity	µs	832.7	784.6	834.5	777.8
3.	Resistivity	kΩ	1.319	1.400	1.316	1.411
4.	Dissolved Oxygen	mg/L	6.8	34	7.2	35
5.	Free Carbon dioxide	mg/L	48.4	168.96	48.4	136.4
6.	Alkalinity	mg/L	120	160	140	145
7.	Turbidity	NTU	46	118.2	50.1	133.9
8.	Specific growth	μd^{-1}	0.136		0.071	
9.	Biomass production	g/L	1.545	3.962	1.495	4.941
10.	Cell Count	cells/ml	0.448×10^6	0.815×10^6	0.387×10^6	1.307×10^6
11.	Cell size	µm	30	37.5	37.5	40
12.	Chlorophyll a	mg/ g^{-1}	8.808	5.93	6.859	9.355
13.	Chlorophyll b	mg/ g^{-1}	4.771	2.889	3.544	5.009
14.	Total Chlorophyll	mg/ g^{-1}	14.36	8.8	10.4	14.36
15.	Carotenoid	mg/ g^{-1}	0.436	0.38	0.534	0.365
16.	Lipid	%	10	12	10	8
17.	Protein	mg/mg	0.118	0.143	0.152	0.141
18.	Carbohydrate	mg/mg	0.008	0.009	0.014	0.032
19.	Sodium	ppm	17.1	10.7	4.3	16
20.	Potassium	ppm	20.5	25.3	26.8	24.9

During experimentation with *C. globosa*, the medium contained in the reservoir was assessed for parameters like pH, free carbon dioxide and dissolved oxygen for estimating the magnitude and nature of release of gases from the PBR. The results are depicted in table 3.2.

While comparing the pH of the bolds basal medium contained in the reservoir for 3 treatment days, an increase was noticed in the final day (6.88), compared to initial day (6.6). On the other hand, in 6 days treatment, the pH of the final day (6.66) exhibited a small decrease with respect to initial value (6.7). Similarly, in the case of 3 days treatment, a decrease in the free carbon dioxide content was observed in the final day (61.6 mg/L), when compared to the initial value (74.8 mg/L). Conversely in 6 days treatment, an increase in the final free carbon dioxide content (74.8 mg/L) was observed with respect to initial value (61.6 mg/L). However, there were no marked differences in the final values of dissolved oxygen content of the 3 days (8 mg/L) and 6 days treatment sets (7.4 mg/L) compared to their initial values of 8 mg/L and 7.4 mg/L, respectively.

Table 3. 2. Results on the analysis of Bolds Basal medium contained in the outlet reservoir of PBR on treatment studies with *Chlamydomonas globosa*.

Sl.No	Parameters Analysed	CO_2 supply of 60 bubbles / 2 hours for an interval of 3 days		CO_2 supply in 60 bubbles / 2 hours for an interval of 6 days	
		Initial day	Final day	Initial day	Final day
1.	pH	6.6	6.88	6.7	6.66
2.	Free Carbon dioxide (mg/L)	74.8	61.6	61.6	74.8
3.	Dissolved Oxygen(mg/L)	8	8	7.4	7.4

Studies with *Acutodesmus obliquus*

Similar to *C. globosa*, the responses of *A. obliquus* with respect to an optimum flow rate of carbon dioxide (80 bubbles/2 hours) has been monitored under ideal conditions. The median values of the results concerning the culture media and morphological / biochemical results concerning *A. obliquus* are depicted in table 3.3.

Analysis of the results regarding pH values showed a significant reduction in both 3 days (23.7%) and 6 days (20.65%) of treatment. In the case of free carbon dioxide content, an increase of 980 % was noticed with 3 days and 900% with 6 days of treatment. In the case of dissolved oxygen content, a significant increase of 200% was noticed in 6 days of treatment and an increase of only 79.51% with 3 days of treatment.

Upon comparing the major growth parameters, in the case of turbidity, marked increase was noticed in 6 days (110.5%), while an average increase of 31.175% was noticed in 3 days of treatment. An increase in biomass productivity was noticed in 6 days (89.686%) and 3 days of treatment (59.6%).Similarly the cell count of micro algae at 6 days of treatment exhibited a significant increase of 200% while an increase of only 79.51% was noticed in 3 days of treatment. Comparing the results, decrease in cell size was noticed in both 3 days (13.33%) and 6 days of treatment (6.66%).

In the case of pigment content, both treatments exhibited percentage decrease with respect to their initial values. The chlorophyll *a* content of the 3 days treatment showed a decrease of 11.189% and 6 days treatment showed a decrease of 11.6%. Likewise the percentage decrease of chlorophyll *b* content of the 3 days and 6 days treatment was noted to be 12.2% and 1.772% respectively. The total chlorophyll content of the 3 days treatment exhibited 7.5% reduction and in the case of 6 days treatment, a decrease of 18.73% was

noticed. In the case of carotenoid content, the 3 days treatment showed 17.32% and 6 days treatment exhibited 6.31 % of decrease.

While analyzing the biochemical parameters like lipid content, an increase of 60% was registered in 6 days and 50 % in 3 days of treatment. An increase of 14.86% of protein content was noticed in the 3 days treatment, while 6.58% of increase was noticed in the 6 days treatment. In the case of carbohydrate content, an increase of 28.57% was observed in 6 days of treatment, while 3 days of treatment exhibited a reduction of 66.67%.

Table 3.3. Changes in media characteristics and morphological / biochemical responses of *Acutodesmus obliquus* to varying durations of carbon dioxide supply.

Sl.No	Parameters Analysed	Units	CO_2 supply in 80 bubbles / 2 hours of interval for 3 days		CO_2 supply in 80 bubbles / 2 hours of interval for 6 days	
			Initial day	Final day	Initial day	Final day
1.	pH	-	7.75	5.91	7.94	6.30
2.	Conductivity	µs	859.3	822.2	854.7	800.4
3.	Resistivity	kΩ	1.278	1.336	1.285	1.351
4.	Dissolved Oxygen	mg/L	9.8	21.8	6.4	24.8
5.	Free Carbon dioxide	mg/L	44	475.2	35.2	352
6.	Alkalinity	mg/L	150	140	100	150
8.	Turbidity	NTU	58.7	77	49.3	103.8
9.	Specific growth	μd^{-1}	0.1069		0.0346	
10.	Biomass production	g/L	1.847	2.948	1.655	3.043
11.	Cell Count	cells/ml	0.122×10^6	0.189×10^6	0.117×10^6	0.351×10^6
12.	Cell size	µm	56.25	48.75	56.25	52.5
13.	Chlorophyll *a*	mg/ g^{-1}	7.436	6.604	6.31	5.579
14.	Chlorophyll *b*	mg/ g^{-1}	2.851	2.503	2.653	2.606
15.	Total Chlorophyll	mg/ g^{-1}	10.28	9.505	10.07	8.183
16.	Carotenoid	mg/ g^{-1}	0.485	0.401	0.428	0.401
17.	Lipid	%	20	30	10	16
18.	Protein	mg/mg	0.148	0.17	0.076	0.081
19.	Carbohydrate	mg/mg	0.009	0.003	0.014	0.018
20.	Sodium	ppm	19.5	19	7	23
21.	Potassium	ppm	18.8	15.7	21	22.5

In the case of *A. obliquus* also, the gaseous release from the PBR was collected in the reservoir containing pure bolds basal medium. The culture medium contained in the reservoir was then subjected to the analysis of parameters like pH, free carbon dioxide and dissolved oxygen for assessing the magnitude and nature of gases released from the PBR. The results are depicted in table 3.4.

Upon comparing the pH values of the 3 days treatment, a decrease in pH was noticed in the final day (6.71) when compared to that of initial day (7.19). Similar trend was also observed in 6 days of treatment, where the pH of the final day (5.30) decreased with respect to initial day (7.07). In the case of free carbon dioxide content of the 3 days treatment, an increase in the final free carbon dioxide content (88 mg/L) was observed with respect to initial value (79.2 mg/L). Likewise in the 6 days treatment, a significant increase in the free carbon dioxide content was noticed in the final day (352 mg/L) when compared to that of initial day (48.4 mg/L). While comparing the dissolved oxygen content of 3 days treatment, a decrease was noticed in the final day (6.6 mg/L) with respect to the initial day (7.8 mg/L). In the case of 6 days treatment also a reduction in the dissolved oxygen content was observed in the final day (6.8 mg/L), when compared to the initial day (8.2 mg/L).

Table 3.4. Results on the analysis of Bolds basal medium contained in the outlet reservoir of PBR on treatment studies with *Acutodesmus obliquus*.

Sl.No	Parameters Analysed	CO_2 supply in 80 bubbles / 2 hours for an interval of 3 days		CO_2 supply in 80 bubbles / 2 hours for an interval of 6 days	
		Initial day	Final day	Initial day	Final day
1.	pH	7.19	6.71	7.07	5.30
2.	Free Carbon dioxide (mg/L)	79.2	88	48.4	352
3.	Dissolved Oxygen(mg/L)	7.8	6.6	8.2	6.8

In the present study, carbon dioxide, at an optimum flow rate as elucidated in chapter 1, has been supplied at regular intervals of 2 hours to the PBR containing candidate species for facilitating increased gas retention within the system. There are several reports regarding the significance of intermittent sparging of carbon dioxide to overcome the growth inhibition on microalgae (Jiang et al., 2013; Radmann et al., 2011; Duarte et al., 2017). This strategic approach of intermittent sparging can not only minimize the acidic inhibition on microalgae, but also maximizes CO_2 consumption efficiency (Jiang et al., 2013). Guo et al. (2015) also recognized that a constant supply of CO_2 was not essential for the increased conversion of CO_2 to biomass.

Upon consolidation of the results, it has been noticed that with both microalgal species, the pH of the medium after PBR studies were in acidic range, which normally happens due to the influx of CO_2 (Chiu et al., 2008; Kumar et al., 2011; Naderi et al., 2015). It was also noticed in the present study that both microalgal species survived in acidic range of pH. This is indicative of their adaptabilities and better growth performances with respect to changing environmental conditions, thereby becoming ideal candidates for CO_2 assimilation.

On an assessment of the DO values of treatments of both microalgal species, 3 days of treatment of *C. globosa* and 6 days of treatment of *A. obliquus* maintained higher levels of DO. With respect to CO_2 consumption and biomass production, the DO content increases (Naderi et al., 2015). Also it is stated that an increase in DO leads to toxic effects like photo-bleaching and reduction in biomass production in controlled systems (Naderi et al., 2015). However in the present study no reduction in biomass production was noticed in the 6 days of treatment of *A. obliquus* with respect to increased DO content. Here the presence of increased DO content was an indication of their active

photosynthetic process (Kumar et al., 2010) in presence of available CO_2 and subsequent release of oxygen.

Estimation of biomass production and growth rate measurements was considered as vital tools in evaluating the bio-sequestration efficiencies of microalgae (Cheah et al., 2015). Upon comparing the results of major growth parameters of 3 days and 6 days of treatment, it was noticed that both microalgal species exhibited maximum turbidity, biomass productivity and cell count in 6 days of treatment. However, the results of 3 days treatment of both microalgae were higher than their respective control/initial values. On the other hand, while comparing the results of specific growth of both microalgal species, increased growth was noticed in the 3 days treatments. Increased growth rate of both microalgal species under intermittent supply of carbon dioxide than their control indicates them as promising candidates for CO_2 biofixation.

Here, both 3 days and 6 days of treatment of *A. obliquus* and 3 days of treatment of *C. globosa* exhibited a decreasing trend in the pigment content with respect to their initial values. At the same time the 6 days of treatment set of *C. globosa* exhibited increased pigment production, compared to their initial values. However the results pertaining to the caroteinoid content of all treatment sets exhibited a decreasing trend. The variation in pigments can be attributed by variation in the metabolic responses of the microalgal members due to varying inputs in culture conditions. Similarly the higher CO_2 concentration in culture medium inhibited the growth of microalgae by disturbing the pigment contents (Gordillo et al., 1998). Jana et al. (2017) also observed a decline in chlorophyll *a* content under elevated CO_2 levels and commented that the presence of lower chlorophyll content may be due to the acclimatization of microalgae to higher CO_2 rich environments.

Upon comparison of the results of the biochemical components of *C. globosa,*an increase in lipid content and protein content was noticed in 3 days of treatment, while maximum carbohydrate content was noticed in 6 days of treatment. In the case of *A. obliquus,* the increased lipid content and carbohydrate, content was recorded in the 6 days of treatment and the maximum protein content was observed in 3 days of treatment.

While comparing the lipid content of both microalgal species, increased production was noticed in 3 days and 6 days of treatment of *A. obliquus*. Tang et al. (2011) also reported the efficiency of *Acutodesmus obliquus* SJTU-3 to accumulate increased lipid content under various CO_2 concentrations, ranging from 0.03 to 50%. The ability of *A. obliquus* to accumulate high lipid content was considered as a significant factor in CO_2 assimilation (Solovchenko and Khozin-Goldberg, 2013). Moreover, Fransico et al. (2010) commented that the lipid-rich species exhibit lower biomass productivity and established that the increased biomass productivity and lipid content were mutually exclusive. Here in the present study also lower biomass productivity was noticed with treatments of *A. obliquus* when compared to the treatments of *C. globosa*.

Higher carbohydrate content in both *C. globosa* and *A. obliquus* was recorded in 6 days of treatment. On the other hand, increased protein content was recorded in 3 days of treatment in both *C. globosa* and *A. obliquus.* Many researchers consider that elevated concentrations of CO_2 stimulate the production of relevant proteins, which can influence cell physiology (Chen et al., 2013). Here in the treatments also increased protein content was recorded in 3 days in both microalgal members, in which increased free carbon dioxide content was noticed.

Throughout the treatment tenure of both microalgal species, the outlet gas from the PBR was trapped in a reservoir containing Bolds Basal medium (devoid of microalgae) and were subjected to analysis to have an idea of the

gas trapped within it. The analytical results concerning media in the reservoir were compared with the results of the media contained in the PBR.

While comparing the results of the reservoir of *C. globosa*, it was noticed that during 3 days of treatment, the final pH increased (4.24%) and free carbon dioxide decreased (17.64%) with respect to their initial values. However there were no differences between the initial and final DO content (0%). It can be assumed that the supplied CO_2 concentration to the PBR (60 bubbles / 2 hours) might have been in an optimum range, leading to proper assimilation and a resultant influx of oxygen into the reservoir, contributing to an increase in pH to the alkalinity range. To support this, an increased DO accumulation (400%)in the culture medium contained in the PBR was also noticed. It can be assumed that excess of oxygen transfer to the reservoir might have contributed to an increase in pH. However, a small decrease in pH (4.78%) was noticed in the PBR, compared to their initial values.

In the case of 6 days treatment with *C. globosa,* the final pH decreased (0.59%) and free CO_2 content increased (21.42%) in the reservoir, compared to their initial values. However there were no differences between the initial and final DO content (0%). Here, the prolonged supply of carbon dioxide (60 bubbles / 2 hours) above the saturation limit of the culture medium contained in the PBR might have resulted in decrease in pH and increase in accumulation of carbon dioxide in the reservoir. To support this, a decrease in pH (9.84%) to the acidic range was noticed in the in the culture medium contained in PBR during 6 days of treatment. Likewise, while comparing the DO content of PBR, a decrease was noticed in 6 days of treatment (386.11%) than 3 days.

Upon comparing the results of *A. obliquus* confining to the reservoir, in 3 days treatment, the final pH values decreased (6.68%), free carbon dioxide content increased (11%) and the DO content reduced (15.38%) with respect to

their initial values. The prolonged supply of carbon dioxide (80 bubbles / 2 hours) above the optimum levels of assimilation by the culture medium contained in PBR might have resulted in an influx of excess of carbon dioxide to the reservoir. A marked decrease in pH (23.7%), significant increase in free carbon dioxide content (980%) and reduced DO content (122.4%) was also noticed in the PBR containing *A. obliquus* pertaining to 3 days of treatment.

In the case of 6 days of treatment with *A. obliquus*, there observed a decrease in the final pH value (25.03%), significant increase in the free carbon dioxide content (627.27%) and reduction in DO content (17.07%) in the reservoir, with respect to the initial values. As stated above, the continuous supply of carbon dioxide (80 bubbles / 2 hours) to the PBR above the limit of assimilation might have attributed to such a super saturation level. To support this, a reduction in pH (20.65%), decrease in free carbon dioxide content (900%) and increased DO content (287.5%) was noticed in the PBR containing *A. obliquus* pertaining to 6 days of treatment.

Statistical analysis

The results pertaining to both treatment sets of *C. globosa* and *A. obliquus* were subjected to statistical analysis using t-test. Making use of this analysis, the variations between the t values of each influencing parameter concerning *C. globosa* and *A. obliquus* were worked out and are depicted in Tables 3.5 and 3.6, respectively.

Table 3.5. Results of t-test on the responses of *Chlamydomonas globosa* to optimum dosage of carbon dioxide.

Sl.No	Parameters Analysed	3 days treatment (t value)	6 days treatment (t value)
1.	Dissolved Oxygen	211.01	68.57
2.	Turbidity	58.5	114.4
3.	Biomass productivity	21.01	123.07
4.	Cell Count	33.95	14.38
5.	Chlorophyll a	48.12	75.63
6.	Chlorophyll b	159.49	149.49
7.	Total Chlorophyll	21.00	25.98
8.	Lipid	2.27	2.06
9.	Protein	0.82	1.009
10.	Carbohydrate	0.030	0.54

t for n-1(=2) degree of freedom at 5% level=9.93

While comparing the results of *C. globosa* at 5% level, t-values of the DO content of both treatments exhibited highly significant variation, while in the case of turbidity, significant variation was with 6 days of treatment (114.4). In the case of biomass productivity the t-values pertaining to 6 days of treatment (123.07) exhibited highly significant variation, whereas the cell count showed variations in 3 days of treatment (33.95).

As far as the t-values of chlorophyll *a* concerned, the significant variations was observed in 6 days treatment set (75.63). With chlorophyll *b*, both 3 days treatment (159.49) and 6 days treatment (149.49) exhibited highly significant variation. Similarly in the case of total chlorophyll, significant variation was observed in both 3 days (21.00) and 6 days of treatment (25.98).

Table 3.6. Results of t-test on the responses of *Acutodesmus obliquus* to optimum dosage of carbon dioxide.

Sl.No	Parameters Analysed	3 days treatment (t value)	6 days treatment (t value)
1.	Dissolved Oxygen	45.36	64.79
2.	Turbidity	40.36	99.50
3.	Biomass production	14.99	56.90
4.	Cell Count	19.70	26.96
5.	Chlorophyll a	4.89	18.89
6.	Chlorophyll b	2.99	2.86
7.	Total Chlorophyll	5.21	225.13
8.	Lipid	6.002	4.02
9.	Protein	2	1.80
10.	Carbohydrate	11.11	0.83

t for n-1(=2) degree of freedom at 5% level=9.93

Upon comparing the results of *A. obliquus* at 5% level, t values of the DO content of both treatment sets showed variations in both 3 days (45.36) and 6 days (64.79) of treatment. Here in the case of turbidity (99.50) and biomass productivity (56.90), the 6 days treatment set exhibited highly significant variation. As far as the cell count concerned, the 6 days treatment set (26.96) also exhibited variation. With chlorophyll *a,* variation was observed in 6 days of treatment (18.89), while no variation was observed in both treatments set for chlorophyll *b*. In the case of total chlorophyll, highly significant variation was noticed in 6 days of treatment (225.134).

There were no significant variations in the biochemical parameters of both microalgal members in both the treatment sets that the t-values of the treatments of both microalgal species was noticed to be below 9.93, the degree of freedom at 5% level.

Upon comparing the results of major influencing parameters of *C. globosa*, increased biomass productivity, turbidity, cell count, pigment production and carbohydrate production were experienced in the 6 days of treatment. The enhanced production of dissolved oxygen, lipid content and protein content were recorded in 3 days of treatment. While analyzing the results pertaining to *A. obliquus* the enhanced dissolved oxygen content, biomass productivity, turbidity, cell count, lipid content and carbohydrate production were noticed in 6 days of treatment and the increased production of protein was registered in 3 days of treatment.

On an overall assessment of above stated results, it can be concluded that both microalgal species were found to be effective in sequestering carbon dioxide. Inspite of acidic pH and higher free carbon dioxide content, both microalgal species exhibited increased biomass productivity, turbidity, cell count in 6 days of treatments. This characteristically indicates their efficiencies in mitigating gaseous carbon dioxide in newly designed PBR, under controlled laboratory conditions. The 6 days of treatment of *C. globosa* are also highly promising with increased pigment and carbohydrate production. Moreover maximized lipid content and carbohydrate production were noticed in 6 days of treatment of *A. obliquus*. The capability of *A. obliquus* to accumulate lipid is considered as an important factor of CO_2 tolerance (Solovchenko and Khozin-Goldberg, 2013), in which lipid biosynthesis represents a sink for the excess products of carbon fixation.

Various literatures suggest that *Chlorella* and *Scenedesmus* can be considered as promising candidates to fix CO_2 and also for handling in adequate engineering systems. Several photobioreactor designs have been developed for the optimization of enhanced CO_2 fixation under varying conditions. The results of research works of Chen et al. (2016) on *Acutodesmus obliquus*

claimed that the species can tolerate higher CO_2 concentrations of 50% and the maximum biomass production was obtained at 20 % CO_2.

The efficiency of *A. obliquus* in assimilating external supply of CO_2 in photobioreactor was already reported. However, there were no reports on sequestration efficiencies and photobioreactor designs for *C. globosa*. The present study highlights the excellent efficiencies of both *C. globosa* and *A. obliquus* in sequestering carbon dioxide. The CO_2 fixation efficiencies of both microalgal species was maximized in the present laboratory scale closed vertical bubble column PBR and cultured using optimum CO_2 concentration. The present venture constitutes an important step in the development of an environmentally sustainable solution/strategy to mitigate CO_2 from small to medium scale point sources through a biological approach, using *C. globosa* and *A. obliquus*.

The presence of excess of CO_2 and their resultant influxes in the reservoir concerning PBR of *C. globosa* and *A. obliquus* (except 3 days treatment with *C. globosa*) are indicative of an alteration in the design or possibility of coupling Photo Bio Reactors to accommodate / assimilate the excess quantities/unused share of CO_2 generated within them.

Summary and conclusion

In the present study, an attempt has been carried out to assess the efficiencies of *Acutodesmus obliquus* and *Chlamydomonas globosa* in carbon dioxide sequestration using a proto type Photo Bio Reactor (PBR), which can ensure optimum conditions of algal growth and carbon assimilation. Such type of standardization will help in the development of small to medium scale commercial installations which can effectively confiscate the released carbon dioxide using effective micro algal members.

For fulfilling the target, a closed vertical bubble column structure having a size of 10 x 10 x 45 cm and a volume of 4.5 L was designed using acrylic material. An inlet, with control facility was maintained at the bottom for the supply of both culture medium and carbon dioxide gas. Likewise an outlet was maintained at the top of the structure to allow the gases within the column, if any, to come out. The outlet of the PBR was connected to the inlet of a reservoir of Bolds Basal medium kept in sealed bottle. The outlet of the PBR was kept at the bottom of the reservoir to permit the gases, if any, to bubble out through the medium. A controlled outlet was also provided to the reservoir for the release of gases, if any.

For experimentation, 3 litres of pure cultures of candidate species with an initial concentration of 0.5 OD, was transferred to the vertical column of the PBR. The carbon dioxide from the cylinder, having control facilities, was allowed to bubble at regular intervals through the culture medium contained in the column. The frequency of carbon dioxide supply was set for 60 or 80 bubbles/2 hours from 6 am to 6 pm. The PBR was retained at a light intensity of 100 µmol $m^{-2} s^{-1}$ using LED light and fluorescent tubes. The room temperature was maintained at 28±0.5ºC and humidity at 48%. The carbon sequestration efficiencies of both microalgal members were monitored for 3 and 6 days in separate trials. Moreover for analyzing the nature and magnitude of the gas generated at the top of the vertical closed column structure, the reservoir containing pure BB medium was monitored for gaseous influxes, specifically with respect to CO_2 and O_2.

The physico-chemical attributes like pH, conductivity, resistivity, free carbon dioxide, dissolved oxygen, alkalinity and growth performances including turbidity, specific growth, biomass productivity, cell count, cell size were worked out on initial day and final day of the experimentation. The biochemical characteristics of micro algae including pigment contents, total lipid content, protein content, total carbohydrate and mineral composition were also analyzed before and after experimentation. The Bolds Basal

medium contained in the reservoir was also subjected to the analysis of pH, free carbon dioxide and dissolved oxygen for determining the changes associated with them due to gaseous influx during experimentations in PBR.

While analyzing the results of major influencing parameters of *C. globosa*, increased biomass productivity, turbidity, cell count, pigment production and carbohydrate production were monitored in the 6 days treatment while enhanced dissolved oxygen content, lipid content and protein content were noticed in the 3 days treatment. Upon comparing the results pertaining to *A. obliquus,* the increased dissolved oxygen content, biomass productivity, turbidity, cell count, lipid content and carbohydrate production were observed in the 6 days treatment and the enhanced protein content was recorded in the 3 days treatment.

Here, inspite of acidic pH and higher free carbon dioxide content, both microalgal species exhibited increased biomass productivity, turbidity, cell count in 6 days of treatments which characteristically indicates their capabilities in mitigating gaseous carbon dioxide in newly designed PBR, under controlled laboratory conditions. The 6 days of treatment of *C. globosa* were also promising with increased pigment and carbohydrate production. Moreover maximized lipid content and carbohydrate production were noticed in 6 days of treatment of *A. obliquus.*

The present study highlights the capabilities of both *C. globosa* and *A. obliquus* in sequestering carbon dioxide. The CO_2 fixation efficiencies of both microalgal species has maximized in the present laboratory scale, closed vertical bubble column PBR, while cultured using optimum CO_2 concentration. The present venture constitutes an eco-friendly approach in developing a sustainable solution for the mitigation of CO_2 emission from small to medium scale point sources by utilizing the carbon fixation efficiencies of *C. globosa* and *A. obliquus*.

General Summary and Conclusion

Uncontrolled greenhouse gas emissions due to various anthropogenic activities have contributed substantially to global warming and climate change. The greenhouse gases mainly include Carbon dioxide (CO_2), Methane (CH_4) and oxides of Nitrogen (NOx). Of these, CO_2 is of major concern today, owing to its higher concentration in the atmosphere.

Among various strategies for CO_2 sequestration, biological sequestration using photosynthetic microalgae have received considerable attention in recent times. Microalgae, one of the most important living resources of both fresh and marine systems can be employed for CO_2 sequestration, as they have higher photosynthetic efficiency, higher biomass production and faster growth rate, compared to other energy crops. They can easily be incorporated into engineered systems.

The present study is an attempt to assess the potentialities of indigenous freshwater microalgal species in carbon dioxide sequestration. The specific objectives outlined in the present study include:

- Maintenance of pure cultures of microalgal species using standard methods and selection of microalgal species which are active under culture conditions.
- Monitoring the responses of selected microalgal species under varying dosages of carbon dioxide supply.
- Determining the optimum pH favouring maximized growth of selected microalgal members.

- Assessment of the CO_2assimilation efficiency of selected microalgal members in PBR under controlled conditions.

For a meaningful elucidation of the objectives, the study has been undertaken in three stages and their outcomes are depicted in three chapters. They include:

Chapter I. Screening of microalgae for CO_2 assimilation efficiency

Chapter II. pH specific modification of culture medium for growth maximization of selected micro algal species

Chapter III. Photo Bio Reactor based feasibility studies on the carbon sequestration efficiency of selected micro algal members

Chapter I

The study has been carried out with the objective of assessing the CO_2 assimilation capabilities of five indigenous microalgal species belonging to Chlorophyceae, namely *Chlamydomonas grovei, Chlamydomonas globosa, Desmodesmus opliensis, Monoraphidium contortum* and *Acutodesmus obliquus*. These micro algal samples were collected from heterogeneous environments and their pure cultures were maintained in the laboratory using Bolds Basal medium. They were then individually subjected to CO_2 assimilation studies.

For assimilation studies, 12 litres of Bolds Basal medium was prepared and to this, four litres of culture medium containing pure cultures of the respective microalgal species was added. After incubation, the microalgal culture was transferred to 16 one litre conical flasks and then separated into three sets of five conical flasks. The first set of five flasks was treated as control and was maintained as such. To the culture containing conical flasks of the second set, ambient air has been bubbled at an interval of two hours and treated as aerated

set. To the third set, carbon dioxide from a cylinder has been bubbled at an interval of two hours and was considered as CO_2 treated set. The culture contained in the last conical flask (16th) was treated as the initial control set and has been used to analyze all parameters meant for the initial day of treatment.

The experimentation was initiated at 6 am on the initial day and all the three sets were kept at illumination during the day time (6 am to 6 pm). Sampling and analyzing of culture has been carried out at 6 am of each day. pH, conductivity, resistivity, dissolved oxygen, free carbon dioxide and alkalinity content of the algal culture together with cell size (micrometry), turbidity, cell count and biomass content of the micro algal members were worked out. Monitoring of the cultures was carried out for a period of 120 hours.

For monitoring the carbon dioxide sequestration potentials of each microalgal member, the results pertaining to major growth parameters like turbidity of the medium owing to micro algal growth, together with cell count and biomass content of micro algal members were considered. While comparing the carbon dioxide assimilation efficiencies of five microalgal members under study, in the case of *C. grovei,* higher turbidity, cell count and biomass content was noticed in the CO_2 treatment set of 10 bubbles / 2 hours. With *C. globosa,* increased turbidity, cell count and biomass content was observed in CO_2 treatment set of 60 bubbles / 2 hours. In the case of *D. opoliensis,* higher turbidity, cell count and biomass was noticed in the CO_2 treatment set of 20 bubbles / 2 hours. Concerning *M. contortum,* maximum turbidity, cell count and biomass was noticed in the CO_2 treatment set of 15 bubbles / 2 hours, while with *A. obliquus,* increased turbidity, cell count and biomass content was observed in CO_2 treatment set of 80 bubbles / 2 hours.

Results of the present study indicated that all the micro algal members under study exhibited varied ranges of tolerance to CO_2 supply. The results of *A. obliquus* (80 bubbles/2 hours) and *C. globosa* (60 bubbles/2 hours) are highly promising which exhibited, higher turbidity, cell count and biomass production in CO_2 treatment sets. The species *C. gorvei* (10bubbles/2 hours)*,* *M. contortum* (15 bubbles/2 hours)and *D. opoliensis* (20bubbles/2 hours) also exhibited moderate efficiencies in carbon dioxide assimilation.

Chapter II

In recent times, microalgae have been employed for a wide range of beneficial purposes. For exploiting their potentials for such purposes, their biomass is required in sufficient quantities.

One among the major constraints in their utilization for such purposes is that most of the members, which are most abundant and virulent in natural conditions, seem to be slow growing in culture conditions. Moreover, the risk of maintaining cultures without contamination is another constraint in their utilization for multidimensional purposes. Hence, standardization of species specific culture conditions is a prerequisite for meeting the required targets.

pH is noted to be an important factor influencing the growth and multiplication of micro algal species in culture conditions. pH of the medium is also significant in carbon sequestration studies, as it determines the availability and solubility of CO_2 and other nutrients. In the present study, an attempt has been carried out to assess the growth performances of selected microalgal members (*C. globosa* and *A. obliquus***)** in Bolds Basal medium under varying culture conditions, altered by pH.

For experimentation, treatment sets were maintained with conical flasks of 100 ml capacity (21 nos), each containing 50 ml Bolds Basal medium. The pH of culture medium was adjusted from 3-12, with a gradation of 0.5 using

0.5 N NaOH and 0.05 N HCl. After adjusting to the required pH, 5 ml of pure cultures of the respective microalgal members were added to the respective conical flasks and the changes in pH, if any, was readjusted. The control set was maintained at pH 6.6 (original pH of the culture medium).

The culture conditions like pH, temperature, conductivity and resistivity and microalgal growth parameters like turbidity, cell count, cell size, and biomass were monitored throughout the treatment period. The biomass estimation was carried out on the initial and final days of treatment. All the sets were kept at illumination during daytime (6 am – 6 pm) and retained under light intensity of 40 μmol m^2 s^{-1}. The temperature of the medium during experimentation ranged from 26-29.8˚C while the humidity ranged from 48 - 54%. Every day after observation, the altered pH was readjusted to their experimental condition. Monitoring of the treatment sets were carried out for a period of 7 days.

For the confirmation of appropriate pH level at which maximum growth of both microalgal members under the study had occurred, the median values of the 7 days triplicate data were worked out and compared. However, during experimentation, in higher alkaline ranges, formation of precipitate was noticed. Hence, for drawing conclusions regarding the optimum pH range in which maximum turbidity occurred, the final median values of pH ranges above 9 were neglected.

Upon comparing the final median values of major growth parameters pertaining to the treatment sets containing *C. globosa,* the maximum cell count was recorded at pH 8, whereas increased turbidity and biomass content were noticed in pH 9. While analyzing the final median values of major growth parameters pertaining to *A. obliquus*, increased turbidity and biomass content were noticed in pH 9, while maximum cell count was observed in pH 5.

The present findings thus confirms that the optimum range of pH favouring the growth of *C. globosa* in BB medium is 8-9 and that of *A. obliquus* is 5-9. Hence for ensuring better biomass production of these microalgal members in BB medium, the above mentioned pH ranges can be followed.

Chapter III

In this chapter, an attempt has been carried out to assess the efficiencies of *A. obliquus* and *C. globosa* in carbon dioxide sequestration using a proto type Photo Bio Reactor (PBR), which can ensure optimum conditions of algal growth and carbon assimilation. Such type of standardization will help in the development of small to medium scale commercial installations which can effectively confiscate the released carbon dioxide using effective micro algal members.

For fulfilling the target, a closed vertical bubble column structure having a size of 10 x 10 x 45 cm and a volume of 4.5 L was designed using acrylic material. An inlet, with control facility was maintained at the bottom for the supply of both culture medium and carbon dioxide gas. Likewise an outlet was maintained at the top of the structure to allow the gases within the column, if any, to come out. The outlet of the PBR was connected to the inlet of a reservoir of Bolds Basal medium kept in sealed bottle. The outlet of the PBR was kept at the bottom of the reservoir to permit the gases, if any, to bubble out through the medium. A controlled outlet was also provided to the reservoir for the release of gases, if any.

For experimentation, 3 litres of pure cultures of candidate species with an initial concentration of 0.5 OD, was transferred to the vertical column of the PBR. The carbon dioxide from the cylinder, having control facilities, was allowed to bubble at regular intervals through the culture medium contained in the column. The frequency of carbon dioxide supply was set for 60 or 80

bubbles /2 hours from 6 am to 6 pm. The PBR was retained at a light intensity of 100 µmol $m^{-2} s^{-1}$ using LED light and fluorescent tubes. The room temperature was maintained at 28±0.5ºC and humidity at 48%. The carbon sequestration efficiencies of both microalgal members were monitored for 3 and 6 days in separate trials. Moreover for analyzing the nature and magnitude of the gas generated at the top of the vertical closed column structure, the reservoir containing pure BB medium was monitored for gaseous influxes, specifically with respect to CO_2 and O_2.

The physico-chemical attributes like pH, conductivity, resistivity, free carbon dioxide, dissolved oxygen, alkalinity and growth performances including turbidity, specific growth, biomass productivity, cell count, cell size were worked out on initial day and final day of the experimentation. The biochemical characteristics of microalgae including pigment contents, total lipid content, protein content, total carbohydrate and mineral composition were also analyzed before and after experimentation. The bolds basal medium contained in the reservoir was also subjected to the analysis of pH, free carbon dioxide and dissolved oxygen for determining the changes associated with them due to gaseous influx during experimentations in PBR.

While analyzing the results of major influencing parameters of *C. globosa*, increased biomass productivity, turbidity, cell count, pigment production and carbohydrate production were monitored in the 6 days treatment while enhanced dissolved oxygen content, lipid content and protein content were noticed in the 3 days treatment. Upon comparing the results pertaining to *A. obliquus,* the increased dissolved oxygen content, biomass productivity, turbidity, cell count, lipid content and carbohydrate production were observed in the 6 days treatment and the enhanced protein content was recorded in the 3 days treatment.

Here, inspite of acidic pH and higher free carbon dioxide content, both microalgal species exhibited increased biomass productivity, turbidity, cell count in 6 days of treatments which characteristically indicates their capabilities in mitigating gaseous carbon dioxide in newly designed PBR, under controlled laboratory conditions. The 6 days of treatment of *C. globosa* were also promising with increased pigment and carbohydrate production. Moreover maximized lipid content and carbohydrate production were noticed in 6 days of treatment of *A. obliquus.*

The present study highlights the capabilities of both *C. globosa* and *A. obliquus* in sequestering carbon dioxide. The CO_2 fixation efficiencies of both microalgal species has maximized in the present laboratory scale, closed vertical bubble column PBR, while cultured using optimum CO_2 concentration. The present venture constitutes an eco-friendly approach in developing a sustainable solution for the mitigation of CO_2 emission from small to medium scale point sources by utilizing the carbon fixation efficiencies of *C. globosa* and *A. obliquus*.

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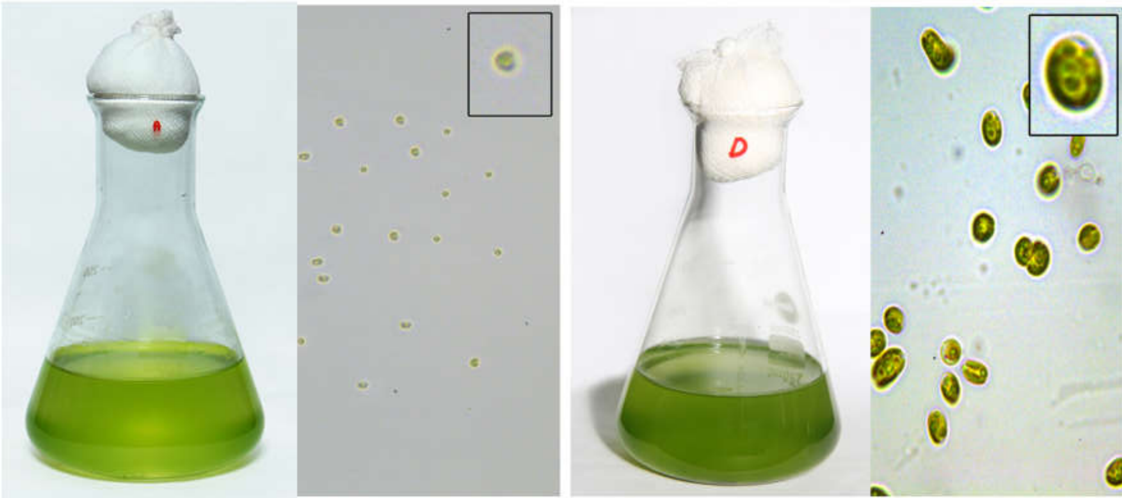
PLATE 6B
Photobioreactor

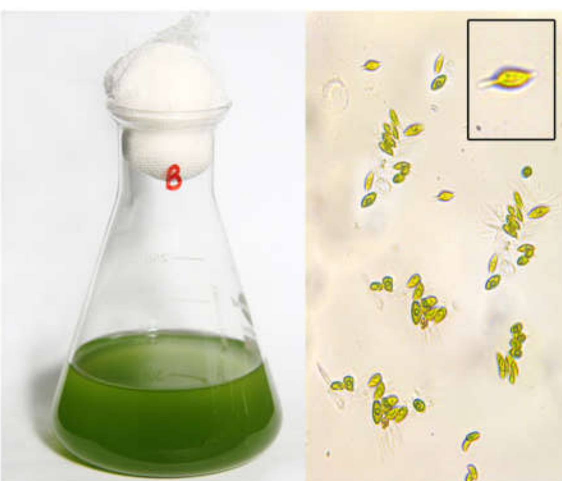
PLATE 1
Pure cultures of microalgae

1a. Culture of *Chlamydomonas grovei* **1b. Culture of *Chlamydomonas globosa***



1c. Culture of *Desmodesmus opoliensis* **1d. Culture of *Monoraphidium contortum***

1e. Culture of *Acutodesmus obliquus*



1f. Culture rack

PLATE 3
Experimental setup

PLATE 4

Response of microalgae to varing range of pH

Chlamydomonas globosa

3a. First day

3b. Seventh day

Acutodesmus obliquus

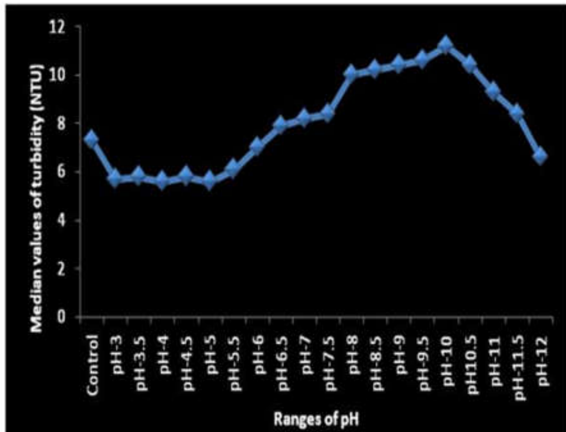
3c. First day

3d. Seventh day

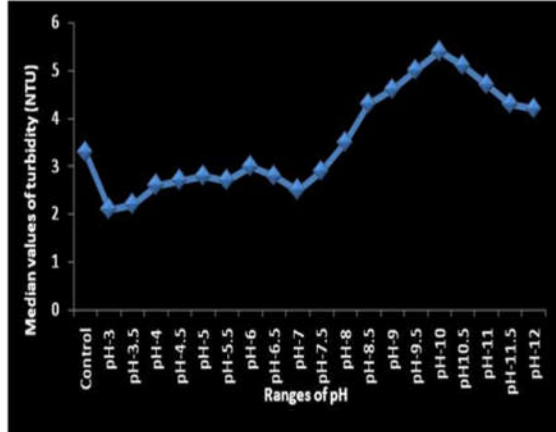
PLATE 5

Figures representing the growth responses of *Chlamydomonas globosa* (a) and *Acutodesmus obliquus* (b) to varying pH ranges.

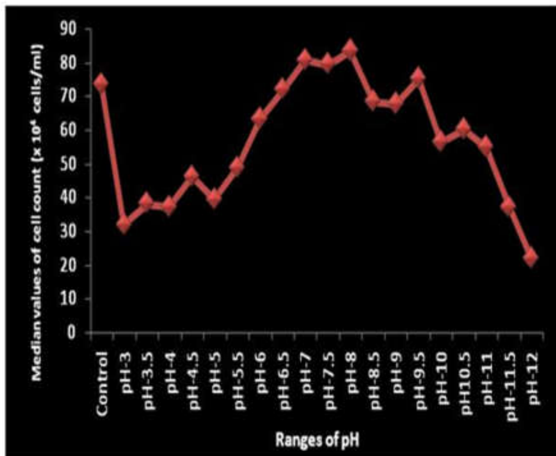
1a. turbidity



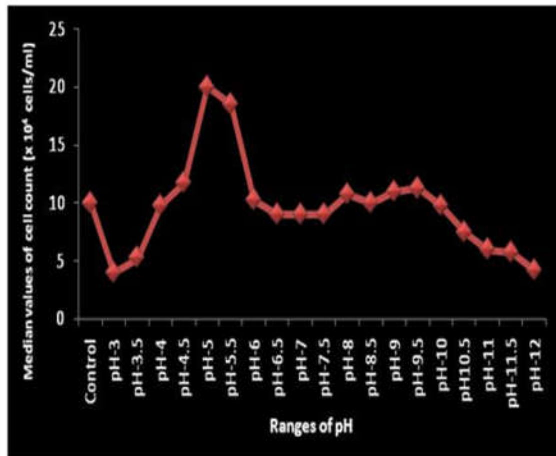
1b. turbidity



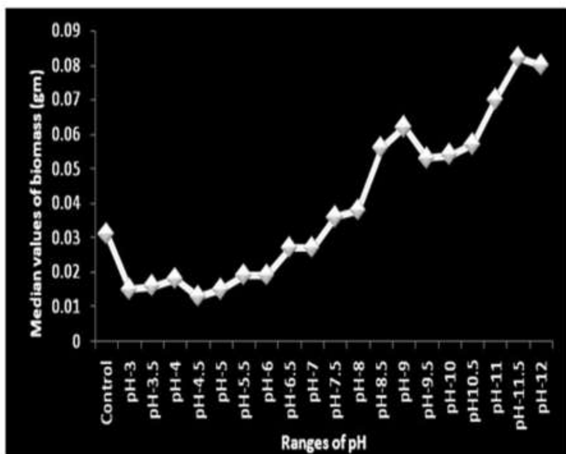
2a. cell count



2b. cell count



3a. biomass



3b. biomass

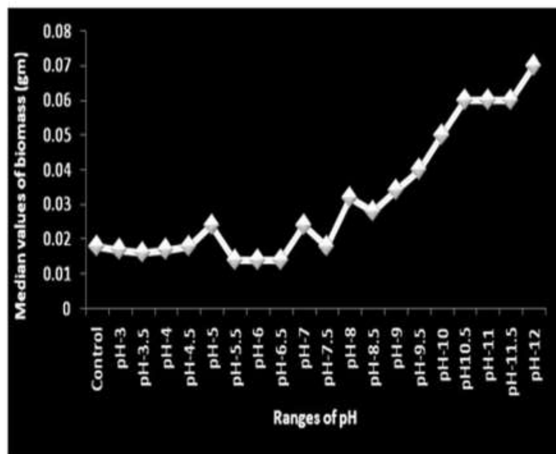
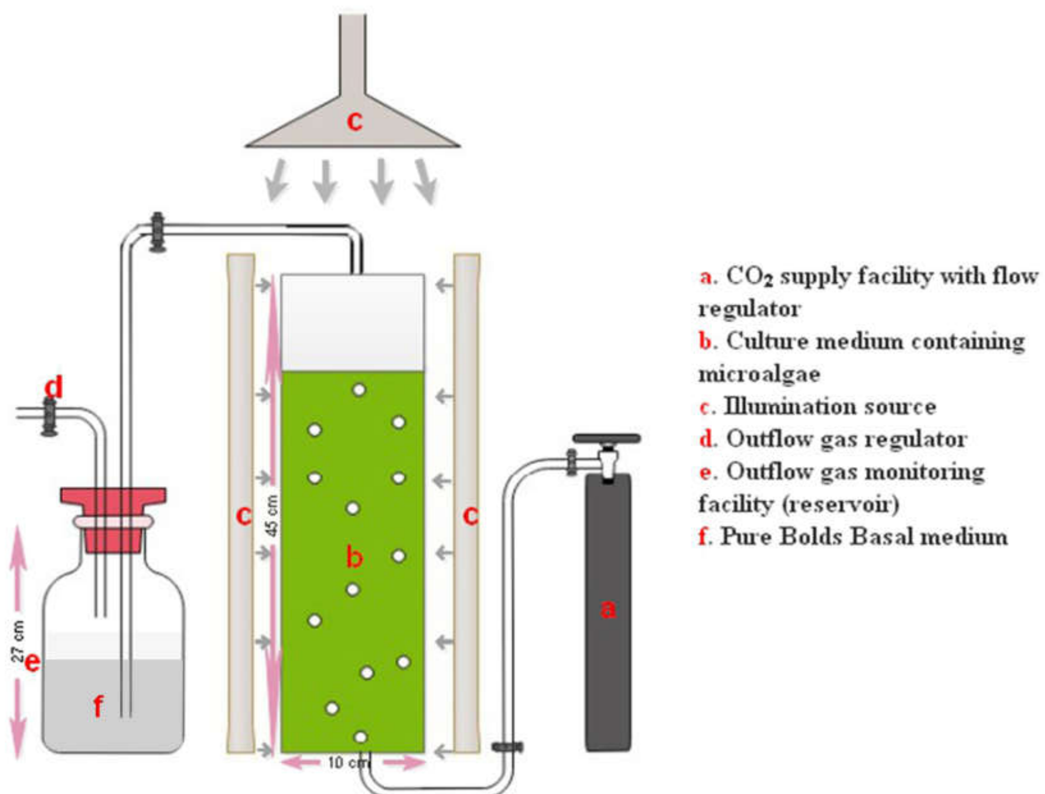


PLATE 6A

Technical specification of the PBR facility used for the present study



a. CO_2 supply facility with flow regulator
b. Culture medium containing microalgae
c. Illumination source
d. Outflow gas regulator
e. Outflow gas monitoring facility (reservoir)
f. Pure Bolds Basal medium