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Importance of Algae in Carbon Sequestration Technology Mohd. Zahid Rizvi, Murtaza Abid* and M.M. Abid Ali Khan

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ABSTRACT

The enhanced human activities and increased consumption of fossil fuels recently resulted in the increase in concentration of CO_2 levels. Being a greenhouse gas, CO_2 contributes towards global warming and alteration of climatic conditions. Therefore there is urgent need to reduce CO_2 emission and devise and implement efficient CO_2 capture and storage technologies worldwide. Capture of CO₂ through biological means (biosequestration) employing microalgae could be an efficient viable technology having more benefits as compared to CO₂ sequestration employing higher plants and ocean fertilization. Microalgae are simple phototrophic living microentities. They are primary producers having minimal nutritional needs. Microalge are photosynthetically more efficient (10-20%) as compared to many terrestrial plants (1-2%). The characters of microalgae putting forward them at the forefront in CO₂ bioseqestration are: low light intensity needs, resistance to enhanced CO₂ concentration (flue gas), adding towards generation of valueadded products and bioenergy and environmental sustainability. Biosequestration through algae and biomass generation of algae involves autotrophic production through open pond or closed photobioreactor systems. The integrated biorefinery system may be sustainable solution towards efficient intertwining and scaling-up of biomass generation and biosequestration employing algae where valuable components are used in myriad of ways along with biosequestration thus making biosequestration employing algae, a commercially viable system. In the present study, significant microalgae-based CO2 sequestration techniques and the hurdles and opportunities in biosequestration technology employing algae are discussed. This study also takes into account the biosequestration through microalgae in the context of integrated bio-refinery system and the novel bio-products generated by microalgal biomass.

Keywords: Algae, biofuels, biorefinery, carbon-sequestration, global warming, waste water.

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INTRODUCTION

Climate change has become a prevalent phenomenon nowadays and threat it is posing needs action on a priority basis globally. Change in climate and subsequent global warming cause worldwide enhancement in average temperature (Dhanwantri et al., 2014). During past century, average temperature has enhanced by $1.4^{\circ}F$ worldwide ($0.72^{\circ}C$) and it has been estimated to further enhance by another 2 to $11.5^{\circ}F$ over next century (NCAR, 2014). Enhancements in atmospheric levels of carbon dioxide and other greenhouse gases is major reason behind increase in average global temperature (Shah, 2015). The CO₂ absorbs infrared rays part of solar radiation mainly, and therefore helps in stabilizing earth's temperature.

Natural phenomenon and human activities are the main reasons behind the enhancements in levels of greenhouse gases including CO_2 (Dhanwantri et al., 2014). Continued enhanced yearly emissions of CO₂ and other greenhouse gases may be the reason behind increase in global temperature at the end of this century which may be 5 degrees Fahrenheit warmer than the 1901-1960 average, and possibly as much as 10.2 degrees warmer (U.S.G.C.R.P., Climate Science Special Report, 2017). The enhanced CO₂ levels in atmosphere cause abnormal balance of atmospheric oxygen to carbon dioxide ratio and subsequently changes in climate worldwide. Due to abnormalities in carbon cycle, biogeochemical cycles of various components are altered that negatively impacts ozone generation in oxygen cycle, thus subsequently impacting ozone layer. Ozone, an important gas found at higher levels in stratosphere layer of atmosphere plays an important role in protecting living organisms on earth by blocking harmful ultraviolet (UV) rays from arriving on surface of earth. Industrial activities, usage of fossil fuels like natural gas, coal and oil, cutting of forests are chiefly responsible for enhancement in atmospheric CO_2 (Le et al., 2013). Coal burning is the highest contributor to increased CO₂ levels as compared to other fossil fuels (DEFRA, London, UK, 2014). Global warming leads to the phenomenon like enhancement in sea level, elevated heat waves, warming of atmosphere and ocean and melting of ice sheets (Meinshausen et al., 2009; Dawson et al., 2011; De Silva et al., 2015). The harmful effects of above mentioned phenomenon manifests in abnormal pattern of precipitation, enhanced spread of diseases and negative health effects of heat waves (Costello et al., 2009). Climate change is sustainable alteration in the average weather patterns that make up earth's local, regional and global climates (Shaftel, 2022). Alterations in environment affect function and distribution of plants in a major way and also exert huge effects on biodiversity pattern (Fitzpatrick et al., 2008). Change in climate has been observed to enhance extinction of some species, it also causes alterations in reproduction timings and distribution of affected species, the growing season of plants is also altered by climate change (W.W.F. Climate change, nature at risk. Threatened species, http://www.panda.org/about wwf/whatwedo/climate_change/problems/impacts/species/index.cfm). The enhancement in average temperature worldwide is affecting genetic architecture, survival and abundance of species. Decline in vegetation is affecting CO_2 levels in atmosphere because vegetation act as absorbers of CO_2 and reduce CO_2 level in atmosphere. Because CO_2 occupies the major portion of greenhouse gases (GHGs) in the atmosphere, efforts should be done in a sustainable manner to curtail emission of CO2 and remove emitted CO2 from the atmosphere. Therefore further emission of CO_2 should be stopped and use of alternative environment friendly fuels such as biofuels should be encouraged.

On the other hand excess CO_2 has to be captured, stored properly and used again to produce useful products thus adding towards commercial sustainability of this process (Tokgoz, 2010). An international environmental treaty was established by United Nations Framework Convention on Climate Change (UNFCCC) to counter ill effects of human activities on climate by stabilizing GHGs levels in the atmosphere [United Nations Framework Convention on Climate Change (UNFCCC), United Nations, 1992: http://unfccc.int/resource/docs/convkp/conveng.pdf]. Stabilizing GHGs levels, will need changing habits to produce and use energy judiciously. The Kyoto Protocol and the Paris Agreement (2015), are important agreements as far as taking necessary measures by participating countries to tackle negative effects of climate change is concerned. For achieving these goals, decreased use of fossil fuels, enhanced capture of carbon and carbon sequestration need to be done (Cheah et al., 2016). The Intergovernmental Panel on Climate Change (IPCC) in its Sixth Assessment Report (2022) on climate change said that emission of GHGs must reach to their highest level before 2025 at the latest and reduce 43% by 2030 in case global warming has to be limited to 1.5 °C (2,7°F); (The Guardian, 2022; IPCC, 2022). Carbon sequestration seems to be good approach to regulate levels of CO₂ in atmosphere (Department of Energy, 1999, Washington, DC). Carbon sequestration includes natural as well as intentional methods to capture and remove CO2 (carbon) from atmosphere or separate CO₂ from emission source and store it in geological formations, oceans or in terrestrial vegetations or other suitable terrestrial environments. The methods adopted for carbon sequestration may be to enhance the amount and rate by which carbon is sequestered by living organisms and decrease the amount and rate of decomposition or combustion of carbon in forests and soils [U.S. Environmental Protection Agency (U.S. EPA), 1999]. Carbon sequestration includes geological carbon sequestration and natural carbon sequestration. Geological carbon sequestration covers geological storage of carbon dioxide while capture of carbon by forests, wood products, atmosphere and soil are examples of natural carbon sequestration (Dhanwantri et al., 2014).

CO₂ Capture and Sequestration Techniques

Myriad of carbon capture and sequestration methods involving physico-chemical approaches are being practiced such as transformation of CO₂ into stable liquids or solids through chemical methods, generation of carbonate salts (Benson and Cook, 2005) and separating CO₂ from point sources and injecting into geological structures (Yang et al., 2008), using alkaline solid wastes for CO₂ sequestration or direct mineral carbonation of steelmaking slag (Revathy et al., 2016). Approaches like transforming biomass into biochar (Hielmann et al., 2010), depositing algal biomass and carbon rich portions from algal biomass in geological structures (Sayre, 2010) and conversion of the biomass into biochar (Hielmann et al., 2010) are also being followed. Above mentioned methods come under umbrella of carbon capture and storage (CCS) technologies. But these techniques are appropriate only for capture of CO₂ from sources harbouring elevated levels of CO₂ (Nouha et al., 2015). Additionally they are becoming less favourable due to possibility of long term CO₂ leakage and high cost of operation and transportation (Lam et al., 2012; De Silva et al., 2015). Another trend is CO₂ capture though biological methods. These methods chiefly involve sequestration of CO₂ through land and aquatic photosynthetic organisms. Enhancement of CO_2 capture by phytoplanktons through deposition of iron and other nutrients into ocean is another technique (Williamson et al., 2012).

Terrestrial sequestration techniques such as afforestation, reforestation, restoration of wetlands and mined lands and betterment of farming methods of crops and livestock can also be employed for carbon sequestration (Farrelly et al., 2013; Cheah et al., 2016). Carbon sequestration through cultivation of algae is a trend followed nowadays (Cheah et al., 2016; Zhou et al., 2017). This review takes into account current updates, various hurdles and future scope of application of microalgae for carbon sequestration. The present review also emphasizes on the scope of microalgae mediated carbon capture through biorefinery and manufacturing of important bio-products through microalgae.

Role of Algae in Carbon Sequestration

Algae perform activities of photosynthesis and carbon sequestration efficiently. Algae, although present at most of the places globally, harbour fresh and marine water environments predominantly (Chen et al., 2009). Algal species transform CO₂ to complex organic compounds taking the route of Calvin-Benson cycle and help of enzyme RuBisCo. Algae can be divided into 2 main categories: microalgae and macroalgae. Macroalgae are eukaryotic, multicellular and macroscopic organisms made up of single differentiated cells generating energy employing chromophores (CEN/TC 454 - Algae and algae products. 2020). Macroalgae, also called as seaweeds, are marine photosynthetic organisms. Their morphology resemble plants having large dimensions. Their structure is made up of a blade or lamina, the stipe and holdfast. Holdfast helps in attaching algal body to substrates (US DOE, 2010, National Algal Biofuels Technology Roadmap, http://biomass.energy.gov). Macroalgae include algae from the prokaryotic algal group-Cyanophyta or from eukaryotic groups of algae-Chlorophyta, Rhodophyta and Phaeophyceae (Littler and Littler, 2011). Due to good lipid content, Macroalgae are useful for biodiesel production. The microalgae consist of algae belonging to groups such as Chlorophyta, Euglenoids, Rhodophyta, Phaeophyceae, Chrysophyta and Cyanophyta. They can be employed for carbon capture from various sources (Chen et al., 2009; Fulke et al., 2013) and carbon sequestration. A general estimate of their capacity for carbon sequestration is that about 1 kg biomass of microalgae fix 1.84 kg of atmospheric carbon dioxide. The carbon dioxide fixed by Chlorella vulgaris is 6.24 g/L/d and by Anabaena is 1.46 g/L/d. The CO_2 captured/sequestered by algae is used to produce biomass and bioenergy (Chen et al., 2009; Cheah et al., 2015).

Regarding different sources of CO₂, the atmosphere which is a natural source of CO₂, contains CO₂ levels ranging from 0.03% to 0.06% (v/v) while another source of CO₂, flue gas emanating largely due to anthropogenic activities, may have CO₂ levels varying from 6% to 15% (v/v); (Rahaman et al., 2011). The name flue gas may have taken from the word "Flue" which may be a chimney, pipe or channel from which combustion product gases from furnace, boiler, steam generator, oven or fireplace goes outside to the atmosphere. The sources of flue gas are natural gas, coal, fuel oil, other fossil fuels or wood which generate flue gas when combusted. The important constituents of flue gas are nitrogen, carbon dioxide (CO₂) and water vapour as well as excess oxygen remaining from the intake combustion air. The minor constituents of flue gas are nitrogen oxides, sulphur oxides, carbon monoxide, and particulate mercury matter (https://en.citizendium.org>wiki>Flue_gas). Therefore important sources of CO₂ that may be employed for microalgal cultivation are atmospheric CO_2 and CO_2 from flue gas (Pires et al., 2012; Brilman et al., 2013). As compared to CO_2 in atmospheric air, CO_2 obtained from flue gas emissions from fossil fuel based power plants are better for carbon sequestration

because of higher levels of CO₂ in flue gas emitted from fossil fuel based power plants (Stepan et al., 2002; Bilanovic et al., 2009). There are numerous benefits of employing microalgae for carbon dioxide sequestration as compared to other organisms. Some of these benefits are as follows: (i) arable land not needed; (ii) can grow in many types of environments ranging from land, wastewater to saline-alkaline water; (iii) simple growth requirements (Wang et al., 2008; Bhakta et al., 2015); (iv) high CO₂ fixation rates; (v) elevated photosynthetic efficiency (Kassim and Meng, 2017); (vi) growth rates higher than many crop plants (Kassim and Meng, 2017); (vii) environment friendly and biodegradable; (viii) generates economically useful byproducts and bioenergy while performing carbon sequestration (Pires et al., 2012; Ullah et al., 2015). There are some requirements for an ideal algal species to perform optimum CO₂ sequestration and generate optimum biomass. Some important requirements are as follows: (i) high CO₂ levels toleration rate; (ii) high carbon capture and carbon fixation rate; (iii) can grow in high temperature and fluctuating pH levels; (iv) proper utilization of limited nutrients (Rahaman et al., 2011).

Microalgae Resistant to Carbon Dioxide

Resistance to CO₂ especially high CO₂ levels is an important aspect to be considered while choosing algal species for carbon sequestration because it has a large impact on costeffectiveness and competency of CO₂ sequestration (Brennan and Owende, 2010). The maximum CO₂ tolerance levels differ in various microalgae (Ono and Cuello, 2003; Table 1). In the study of Hanagata et al. (1992), it was reported that *Scenedesmus* sp. exhibited more resistance to CO_2 than *Chlorella* sp. when exposed to high CO_2 levels (10–80%), although both species have the capacity to grow in lower CO₂ levels (10-30%). S. dimorphus was observed to exhibit good resistance to stream gas harbouring high concentrations of CO_2 (2– 20%), SO₂ (100 ppm) and NO (150–500 ppm); (Jiang et al., 2013). In the study of Kao et al. (2014), Chlorella sp. MFT-15 was reported to efficiently utilize the CO₂, NO_X, and SO₂ found in the various flue streams from power plant in a steel plant, hot stove and a coke oven. The maximum lipid production and average specific growth were observed to be 0.81 g/L and 0.77/day respectively. Nannochloropsis sp. when exposed to 15% (v/v) CO₂, exhibited an enhanced growth rate for 58%, which is from 0.33 to 0.52 per day (Jiang et al., 2011). *Chlorella* sp., which is a microalga, can grow in 40% (v/v) CO₂ at pH 5.5–6.0 and 30°C temperature (Chen et al., 2014). It was found that the species that exhibit more resistance to high CO₂ levels, usually show good survival in acidic scenarios in growth medium because CO₂ decreases the pH of a solution. Viridella and Galderia sp. are some species of algae exhibiting affinity to more acidic cultures (Oilgae, 2011). It was found that high CO₂ levels enhance the photosynthetic efficiency of microalgae thus causing enhanced and faster growth. But, it was observed that consistently high CO₂ levels may inhibit algal growth. CO₂ concentration in excess of 5% (v/v) was considered harmful for growth of some microalgae species (Lee et al., 2000). Usually CO₂ levels required for optimum algal growth are less as compared to maximum CO₂ tolerance levels. Scenedesmus sp. in accordance with this exhibited to be capable of growing under 80% CO₂ levels, but the optimum cell mass was observed when the CO₂ level was 10-20% (Hanagata et al., 1992). Euglena gracilis is tolerant to CO₂ levels up to 45%, but the optimum growth was exhibited with 5% CO₂ level (Nakano et al., 1996).

| S. No. | Algal Species | Level of Tolerance to CO ₂ | References |
|--------|--------------------------------|---------------------------------------|----------------------------|
| 1. | Anabaena sp. | 10% | Chiang et al., 2011 |
| 2. | Botryococcus braunii | 10% | Yoo et al., 2010 |
| 3. | Chlorella sp. | 10-80% | Hanagata et al., 1992 |
| 4. | Chlorella sp. T-1 | 100% | Maeda et al. <i>,</i> 1995 |
| 5. | Chlorella sp.ZY-1 | 70% | Yue and Chen, 2005 |
| 6. | Mutant of <i>Chlorella</i> sp. | 70% | Sung et al., 1999 |
| | (strain KR-1) | | |
| 7. | Chlorella sp. | 40% | Chen et al., 2014 |
| 8. | Chlorella sp. | 40% | Kuo et al., 2016 |
| 9. | Chlorella minutissima | 15% | Sankar et al., 2011 |
| 10. | Chlorococcum littorale | 70% | Ota et al., 2009 |
| 11. | <i>Cyanidium</i> sp. | 100% | Graham and Wilcox, 2000 |
| 12. | Cyanidium caldarium | 100% | Seckbach et al., 1971 |
| 13. | Desmodemus sp. | 100% | Kativu et al., 2012 |
| 14. | Dunaliella tertiolecta | 15% | Nagase et al., 1998 |
| 15. | Euglena gracilis | 45% | Nakano et al., 1996 |
| 16. | Haematococcus pluvialis | 34% | Huntley and Redalje, 2007 |
| 17. | Nannochloropsis sp. | 15% | Jiang, et al., 2011 |
| 18. | Scenedesmus sp. K34 | 80% | Hanagata et al. ,1992 |
| 19. | Scenedesmus dimorphus | 2-20% | Jiang et al., 2013 |
| 20. | Scenedesmus incrassatulus | 0.03% | Fulke et al., 2013 |
| 21. | Scenedesmus obliquus | 10% | Tang et al., 2011 |
| 22. | Synechococcus elongatus | 60% | Miyairi, 1995 |

Table 1: Algal Species tolerant to High Concentrations of CO₂.

The rate of CO_2 fixation capacity by microalgae may vary between different species or even mutated strains of the same species (Table 2).

| Table 2: CO2 Fixation Rates of Various Algae. | | | |
|---|---------------------------|---------------------------------------|---------------------|
| S. | Algal Species | CO ₂ Fixation/Assimilation | References |
| No. | | Rate (g/L/day) | |
| 1. | Anabaena sp. | 1.01 | Chiang et al., 2011 |
| 2. | Botryococcus braunii SAG- | 0.496 | Sydney et al., 2010 |
| | 30.81 | | |
| 3. | Chlorococcum littorale | 0.902 | Kurano et al., 1995 |
| 4. | Chlorella sp. | 2.33 | Kuo et al., 2016 |
| 5. | Chlorella pyrenoidosa | 0.26 | Tang et al., 2011 |
| 6. | Chlorella sorokiniana | 0.33* | Lizzul et al., 2014 |

Table 2: CO₂ Fixation Rates of Various Algae.

| 7. | Chlorella vulgaris | 0.26** | Larsson and |
|-----|-----------------------------|----------|---------------------|
| | | | Lindblom, 2011 |
| 8. | C. vulgaris LEB-104 | 0.251 | Sydney et al., 2010 |
| 9. | Desmodesmus sp. | 1.58 | Xie et al., 2014 |
| 10. | Dunaliella tertiolecta SAD- | 0.272 | Sydney et al., 2010 |
| | 13.86 | | |
| 11. | Scenedesmus obliquus | 0.288 | Tang et al., 2011 |
| 12. | S. obliquus | 0.252 | Basu et al., 2013 |
| 13. | Mutant strain of S. | 0.922*** | Li et al., 2011 |
| | obliquusWUST4 | | |
| 14. | Spirulina platensis LEB-52 | 0.318 | Sydney et al., 2010 |

* g/L after 96 h exposure

**g/L/h

***g/L under 10% CO₂

It was found that a mutant strain of *Scenedesmus obliquus*WUST4 generated high biomass (0.922 g/L) when exposed to lesser CO₂ levels (10% CO₂ from flue gas), as compared to nonmutant *S. obliquus* (0.653 g/L biomass), exposed to higher levels of CO₂ (20%); (Li et al., 2011).

Algal Culture

Various cultivation methods and physicochemical processes significantly affect carbon dioxide fixation and generation of algal biomass. The selection of appropriate microalgal species/strain for cultivation is an important factor that influences the CO₂ sequestration and biomass generation (Brennan and Owende, 2010). An ideal algal species should have high CO₂ sequestration and biomass production rate, enhanced tolerance to high CO₂ levels (Singh and Ahluwalia, 2013), tolerance to vast range of temperatures and simple nutritional requirements. An ideal algal species should also have enhanced photosynthetic efficiency, rapid productivity cycle and in addition to CO₂ sequestration, should also generate commercially valuable products. As far as various cultivation systems are concerned, an ideal algal species in photobioreactor cultivation should be able to cope with shear stress while in open pond cultivation systems, ideal algal species should be able to overcome competition with wild strains. But presently no algal strain seems to be possessing all these qualities of an ideal algal strain (Singh and Ahluwalia, 2013). Chlorella vulgaris, Scenedesmus incrassatulus, Scenedesmus obliguus,, Spirulina sp. and Haematococcus pluvialis have the potential for efficient CO₂ sequestration and thus aiding towards reduction of CO₂ emission from various CO₂ emitting sources (Chakrabarti et al., 2014).

The factors in culture medium upon which the microalgal growth is dependent, consist of temperature, pH, light, salinity, dissolved oxygen, nutrient status of culture medium, viruses and competition from other species (Kumar et al., 2010). Factors of operating processes influencing microalgal growth rates consist of gas transfer and mixing and harvesting rates due to their effect on exposure to light, availability of CO₂ and shear rates. Carbon (C), nitrogen (N) and phosphorus (P) are the import nutrient elements for fulfilling nutrient requirements of microalgal culture (Becker, 1994). Rapidly-growing microalgae are inclined towards ammonium instead of nitrate as primary N source. Due to its good bioavailability,

phosphorus should be provided in culture predominantly as phosphates (Kumar et al., 2010). Trace elements and vitamins should also be part of microalgal culture medium (Becker, 1994). Light intensity and how efficiently it is utilized, are also important in microalgal culture. In high-density cultures, light does not penetrate effectively to depths and light intensity decreases. But high light intensity periods also negatively affect algal growth due to excess generation of DO (Singh and Ahluwalia, 2013). Another important factor affecting microalgal growth is pH of culture medium (Li et al., 2003). neutral pH is preferred by many microalgal species while some species resist lower pH (e.g. *Chlorococcum littorale* at pH 4) or higher pH (e.g. *Spirulina platensis* at pH 9); (Oilgae, 2011).

Algal Cultivation Methods

Photoautotrophic (needs inorganic carbon and light), heterotrophic (needs organic carbon and light) and mixotrophic (photosynthesisis in autotrophic mode and assimilation is heterotrophic type) are the various types of algal production mechanisms encountered. For culturing algae for economic purpose, enclosed bioreactors and openraceway ponds are important methods (Jimenez et al., 2003). According to Brennan and Owende (2010), photobioreactors are made up of straight glass or plastic tubes while closed loop, oval shaped recirculation channels are part of raceway ponds (Ugwu et al., 2008; Fulke et al., 2010). In open pond method contamination by other algal species or microbes is a big problem (Benemann and Oswald, 1996). Employing algal strains tolerant of extreme culture environment can be a good choice as far as production rates are concerned e.g. Chlorella sp., Spirulina, Dunaliella, are cultivated in high nutrition, alkalinity and salinity respectively (Harun et al., 2010). In open methods, high downstream processing cost has a negative effect on cost-effectiveness of the process. While in closed photoreactors, contamination is reduced and other factors in culture such as temperature, carbon dioxide levels, light intensity and nutrients concentration can be tightly regulated leading towards enhanced biomass production (Miron et al., 1999; Harun et al., 2010). Some innovative models of photobioreactors exhibiting enhanced productivity have fibre-optic delivery of light to various parts of the photobioreactor and solar concentrators (Packer, 2009). While Kumar et al. (2011) has suggested to combine two reactors (hybrid reactors) for eliminating shortcomings of a single photobioreactor. In the study of Cheng et al. (2006), it was observed that enhancing CO₂ retention time in photobioreactor has a positive effect on efficiency of CO₂ fixation. Ketheesan and Nirmalakhandan (2012) investigated an airliftdriven raceway reactor for microalgal culture with the highest CO₂ consumption. Many researchers have investigated CO₂ fixation through microalgae employing closed photobioreactors (Cheng et al., 2006; Fulke et al. 2010). Chojnacka and Noworyta (2004) observed growth of Spirulina sp. in 3 types of cultures i.e. heterotrophic, photoautotrophic and mixotrophic cultures. Fu et al. (2019), in its investigation of growth of *Chlorella vulgaris* in LEDs based photobioreactors, observed that glucose at low levels caused an enhancement in photoautotrophic growth-dependent biomass production and capture of CO₂ by 10% and this increase correlated with enhancement of given photon flux. The best biomass productivity obtained in this study was 30.4% increased than the initial productivity of purely autotrophic culture. Pourjamshidian et al. (2019) studied CO₂ fixation by the microalgae Chlorella sp. for various CO₂ levels and gas flow rate scenarios in a bubble column reactor. The highest biomass productivity rate (at 0.17 g/L/day) was obtained for a sample with 1.75% CO₂ having gas flow rate of 70 ml/min.

The highlight of this study was observation that low concentration of CO₂ and high flow rate may stimulate a reduction in CO₂ fixation efficiency by *Chlorella* sp. Valdovinos-García et al. (2020) assessed techno-economic evaluation of microalgae biomass generation, taking into account technologies having potential of scaling at industrial level. Consumption of energy and cost of operation were taken as parameters for assessment. The CO₂ capture from a thermoelectric plant was investigated as a carbon source for microalgal cultivation. Low biomass productivity, 12.7 g/m²/day, was taken into account, obtaining 102.13 tons of CO₂ capture/year in 1 ha for the cultivation area. The process having centrifugation and vacuum filtration steps has highest energy consumption. The range of cost of operation was from US 4.75-6.55/kg of dry biomass. How biomass is finally utilized, impacts the selection of optimum scenario.

Flue Gas in Carbon Sequestration

Flue gases emitted from power plants are important source of CO₂ globally (Sakai et al., 1995). The flue gas is rather cheap source of CO_2 . Extracting CO_2 from flue gas is a costeffective method. Although flue gases emitted from power plant enhance biomass of algae but it is an energy intensive costly process (Lee Jeong et al., 2003). The process of CO₂ capture by algae is dependent upon factors such as temperature, chemical reactions in pond and physiology of algae (Keffer and Kleinheinz, 2002). Thermophilic algae which can survive in high temperatures, can be very useful in CO₂ sequestration employing high temperature waste gases from thermal power plants (Bayless et al., 2001). Employing thermophilic algae has advantage of decreased cooling costs in CO₂ sequestration and they produce valuable products such as secondary metabolites also which make the CO₂ sequestration process cost-effective. It should be taken care that concentration of CO₂ should not be so much that it inhibits growth of algal species while it should not be below a minimum level that is limiting for the algal growth (Rados et al., 1975). There is variability amongst various algal species as far as maximum and minimum levels of CO₂ are concerned. Ponds for algal culture may be established near the sources of CO₂ which may decrease cost of transportation of CO₂, heat emanated from power plant can be employed for warming ponds in winter besides giving benefit of carbon credits. Therefore an integrated algal pond and power plant (CO₂ source) set up can make the CO₂ sequestration process cost-effective and efficient (Kadam, 1997). Elevated levels of Ci in the form of real or simulated flue gas, soluble carbonate (bicarbonate) or pure gaseous CO_2 may enhance carbon dioxide sequestration and biomass generation. (Sydney et al., 2010; Singh et al., 2014; Thomas et al., 2016; Aslam et al., 2017; Kuo et al., 2017; Vuppaladadiyam et al. 2018). The fixed carbon may act as source of primary compounds for protein, sugars and lipids (Sydney et al., 2010). To make the microalgae based carbon sequestration and production systems more costeffective and commercially feasible, the integrated biorefinery system should be adopted where there is optimum extraction, processing and valorization of important biomass components (Chew et al., 2017).

Fermentation is a photosynthetic biogas upgrading procedure, which is amalgamation of CO_2 capture by carbonate solution and carbonate regeneration by employing aquatic microbial oxygenic photoautotrophs (i.e., algae, diatoms and cyanobacteria). This process may provide a potential alternative to the commercial processes employed for gaseous biofuel upgrading. For the betterment of upgrading performance, in the study of Ye et al. (2020),

the effects of NaHCO₃ levels and light intensity on the growth and HCO₃⁻ transformation of alkaliphilic and halophilic *Spirulina platensis* were assessed. It was observed that 0.05 to 0.6 M NaHCO₃ range was suitable for growth of *S. platensis* and 0.1 M NaHCO₃ was optimal concentration for maximum biomass generation (1.46 gL⁻¹) and this was 65.9% higher than at 0.05 M NaHCO₃. Biocarbonate utilization efficiency was 42%. Enhancing light intensity upto 210 µmol m⁻² s⁻¹ gave better results as far as *S. platensis* growth and photosynthetic pigment aggregation are concerned. It was reported that biogas stream at a flow rate of 800 m³ h⁻¹ could produce biomass up to 344 kg h⁻¹, relating to an energy value of 5591 MJ h⁻¹.

Use of Wastewater

Wastewater nutrients cause eutrophication of water bodies like rivers, lakes, and seas. Different wastewater sources such as industrial and agricultural run-off waste, municipal sewage and waste matter from concentrated animal feed processes serve as source of N, P and minor nutrients for microalgae which on one hand supports growth of algae and also perform bioremediation of wastewater (Wang et al., 2010; Koutra et al., 2018). Microalgae can also be employed to extract heavy metals from wastewaters and negative charge on surface of microalgae helps in this process (Munoz and Guieysse, 2006). Microalgae use wastewater for their growth thereby making the process of wastewater bioremediation and production cost-effective which happens due to use of wastewater and cultivation of nutrient-ladden microalgae at the same time. In this way CO_2 sequestration employing algae may become less costly, when integrated with wastewater treatment process (Kuo et al., 2016; Collotta et al., 2018). High Rate Algal Ponds (HRAP) process is a cost-effective method for wastewater treatment and production of algal biomass. This system has a photobioreactor and intensified oxidation ponds where microalgae act as source of oxygen for bacteria. On the other hand, bacteria transforms mineral compounds (e.g., ammonium to nitrate) that supply nutrients for microalgae. This system helps in decreasing bacteria and biological oxygen demand (BOD) thus improving quality of water. The difference between HRAP and conventional oxidation ponds is that in HRAP due to deep algal photosynthesis, oxygen saturation is provided to start the aerobic treatment conditions and use of wastewater nutrients into generating algal biomass (Moghazy et al., 2022). Arthrospira, Scenedesmus, Oscillatoria and Micractinium are some of the algal species that exhibit good growth in HRAP systems and their harvesting is also simple. Removal of toxic minerals such as Pb, Hg, As, Br, Sn and Cd ions employing algae have also been reported (Abdel-Raouf et al., 2012). Microalgae based CO₂ sequestration can generate valuable economically important products from biomass of algae which can make process of CO₂ mitigation costeffective. Waste gases such as CO_2 and oxides of sulphur and nitrogen, obtained from flue gas; inorganic and organic carbon, phosphorus, nitrogen and other pollutants from industrial and agricultural sources can be processed with help of algae into biofuels and other environment-friendly products (Pires et al., 2012; Singh and Thakur, 2015). Aslam et al. (2017) and Vuppaladadiyam et al. (2018) have discussed these aspects in detail.

Microalgal Biofuel Systems

It was reported that microalgae harbour sizeable amount of lipids, proteins and carbohydrates (Sialve et al., 2009). Due to the prescence of these compounds, microalgae can generate biogas, biodiesel and other biofuels such as biobutanol and bioethanol during process of carbon sequestration (Lively et al., 2015; Nayak, et al., 2016; Table 3).

| S. No. | Algae | Products | References |
|--------|-------------------------------|---------------|-------------------------------|
| 1. | Bacillariophyta sp. | Biooil | Huang et al., 2016 |
| 2. | Chlorella vulgaris | Biooil | Biller et al., 2012 |
| 3. | Chlorogloeopsis fritschii | Biooil | Biller et al., 2012 |
| 4. | <i>Cyanobacteria</i> sp. | Biooil | Huang et al., 2016 |
| 5. | Desmodesmus | Biooil | Chen et al., 2020 |
| 6. | Dunaliella | Biooil | Chen et al., 2020 |
| 7. | Nannochloropsis oculata | Biooil | Biller et al., 2012 |
| 8. | Scenedesmus | Biooil | Chen et al., 2020 |
| 9. | Spirulina | Biooil | Chen et al., 2020 |
| 10. | Spirulina platensis | Biooil | Biller et al., 2012 |
| 11. | Tetraselmis sp. | Biooil | Eboibi et al., 2014 |
| 12. | Dunaliella salina | Biogas | Mussgnug et al., 2010 |
| 13. | Euglena gracilis | Biogas | Mussgnug et al.,2010 |
| 14. | Scenedesmus obliquus | Biogas | Mussgnug et al., 2010 |
| 15. | Anabaena sp. | Biohydrogen | Nayak et al., 2014 |
| 16. | Chlamydomonas reinhardtii | Biohydrogen | Wei et al., 2017; Wei et al., |
| | | | 2020 |
| 17. | <i>Laminaria digitata</i> and | Bio hydrogen | Ding et al., 2018 |
| | Arthrospira platensis | (fermentative | |
| | | hydrogen) and | |
| | | methane | |
| 18. | Arthrospira maxima | Methane | Inglesby and Fisher, 2012 |
| 19. | Euglena gracilis | Methane | Nguyen et al., 2015 |
| 20. | S. obliquus | Methane | Zamalloa et al., 2012 |
| 21. | Spirulina sp. | Methane | Zamalloa et al., 2012 |
| 22. | Chlorella vulgaris | Oil/gas/char | Wang et al., 2015 |
| 23. | Nannochloropsis oculata | Syngas | Duman et al., 2014 |
| 24. | Saccharina latissimi | Syngas | Onwudili et al., 2013 |
| 25. | Tetraselmis sp. | Syngas | Alghurabie et al., 2013 |
| 26. | Botryococcus braunii | Biodiesel | Yoo et al., 2010 |
| 27. | Chlorella pyrenoidosa | Biodiesel | Huang et al., 2015 |
| 28. | Desmodesmus quadricaudatus | Biodiesel | Saad et al., 2018 |
| | and Chlorella sp. | | |
| 29. | Oscillatoria sp. | Biodiesel | Saad and Shafik , 2017 |
| 30. | Scenedesmus sp. | Biodiesel | Huang et al., 2012 |
| 31. | Spirulina sp. | Biodiesel | El-Shimi et al., 2013 |
| 32. | Arthrospira | Bioethanol | Mathushika and Gomes, |
| | | | 2021 |
| 33. | C. reinhardtii | Bioethanol | Choi et al., 2010 |
| 34. | Dunaliella | Bioethanol | Mathushika and Gomes, |
| | | | 2021 |

Table 3: Some Important Algae for Generation of Biofuels.

| 35. | Gracilaria | Bioethanol | Mathushika and Gomes, |
|-----|----------------------------|------------|---------------------------|
| | | | 2021 |
| 36. | Spirulina | Bioethanol | Mathushika and Gomes, |
| | | | 2021 |
| 37. | Ulva lactuca | Bioethanol | Nikolaison et al., 2012 |
| 38. | Ascophylum nodosum and | Bioethanol | Obata et al., 2016 |
| | Laminaria digitata | | |
| 39. | Chlorella sp. | Biobutnol | Yeong et al., 2018; Onay, |
| | | | 2018 |
| 40. | S. obliquus | Biobutnol | Yeong et al., 2018 |
| 41. | Tetraselmis subcordiformis | Biobutnol | Yeong et al., 2018 |

Some of the high lipid containing diatom species are Navicula pellicusa, Chaetoceros muelleri, Phaeodactylum tricornutum, Cyclotella cryptica and Chaetoceros gracilis (Hu et al., 2008; Pratiwi et al., 2009; Mata et al., 2010). Some important algal species suitable for production of biofuels are Scenedesmus obliquus, Botryococcus braunii, Chlorella vulgaris and Nannochloropsis oculate (Pires et al., 2012; Singh and Ahluwalia, 2013). Macroalgae or seaweed are also important as far as generation of bioenergy during carbon capture is concerned. Although they have low lipid content but high amounts of carbohydrates. They can be employed for generation of biofuels such as isobutanol, bioethanol and methanol. Some of algal species produce elevated amounts of carobohydrates e.g. Laminaria digitata, Laminaria hyperborean and Sacchorhiza polyschides. Ulva and Laminaria macroalgal species have good potential as far as generation of bioenergy is concerned. Ulva lactuca, Catenella repens, Enteromorpha intestinalis, Gracilariopsis longissima and Sargassam wightii harbour good amount of lipids (Bastianoni et al., 2008; Muralidhar et al., 2010). Generation of biofuels employing algae has certain positive points such as decreasing atmospheric CO₂ emissions, land used for growing food producing crops can be spared, better yield and greater biomass production efficiency as compared to terrestrial systems (Mata et al. 2010) and algal biomass production systems are free of seasonal dependence which is not the case with land crops. Many Algal systems such as unicellular algae have high biofuel production efficiency (Weyer et al., 2010). In algal systems for biofuel production, many important parameters such as CO₂ and nutrient levels, pH and temperature can be precisely monitored, regulated and optimized for high biofuel production. In algal pond systems, quality (wavelength) and quantity of light can be regulated or frequency shifting fluorophores can be employed to enhance photosynthetically active radiation. These parameters cannot be regulated in terrestrial plant systems where plants are fixed in soil (Wang et al. 2009, Stephens et al. 2010). Another positive point is that CO_2 emitted from algal biofuels can be recycled and reused for microalgal cultivation thus making the net carbon emission of the system zero which will be a big advantage for environment such as helping in reducing global warming (Slade and Bauen, 2013).

Biodiesel

Some important microalgae like *Isochrysis, Dunaliella, Chlorella, Nannochloropsis, Schizochytrium and Porphyridium* have significant lipid content that may be elevated further by optimization of process and environmental variables and genetic manipulation of the concerned algal strain (Mata et al., 2010).

Stressful conditions and addition of sugar and glycerol enhance generation of lipids in algae. Deficiency of nitrogen also stimulates enhancement in relative content of oleic acid and aggregation of triacylglycerol (TAG); (Choi et al., 2011). Other factors which are important in enhancing lipid levels of many microalgae are temperature, salinity (Wu and Hsieh, 2008), CO₂ percentage (de Morais et al., 2007; Chiu et al., 2009), harvesting process (Chiu et al., 2009; Widjaja et al., 2009) and intensity of light (Weldy and Huesemann, 2007). It was observed that enhancement in aggregation of lipid and lipid productivity are not much linked with each other therefore a better approach would be processing algae into biofuels instead of first extracting oil and then processing. This approach also enhances costeffectiveness of the process. Wet algal biomass can be transformed to liquid fuels through process of liquefaction with direct hydrothermal (where pressure is used to keep water in liquid state above 100°C temperature); (Patil et al., 2008). In the study of Fulke et al. (2010), photosynthetic capacity of various microalgae causing calcite formation and enhanced fixation of CO₂ alongwith their capacity to generate biodiesel precursors was assessed. Botryococcus braunii was observed to be the most appropriate biodiesel producer under CO₂ growth scenario amongst various microalgal species assessed (Yoo et al., 2010). Linked processes of extraction of biodiesel from microalgae and CO₂ sequestration can make the biodiesel extraction from microalgae cost-effective (Wang et al., 2008).

Bioethanol

Since many microalgal species possess decent amounts of carbohydrates, they can be employed for generation of bioethanol following process of fermentation. Due to absence of lignin in the microalgae, they are more preferred for the production of bioethanol (Jambo et al., 2016). Some important algae employed for the generation of carbohydrates are *Chlorella* sp., *Porphyridium cruentum, Isochrysis galbana, Spirogyra* sp. and *Nannochloropsis oculate* (Markou and Nerantzis, 2013).

Biobutanol

Biobutanol as a biofuel is more appropriate than bioethanol or biomethanol due to its high energy density. Microalgae can also serve as source of biobutanol (Yeong et al., 2018). But few studies have been done on transformation of microalgal biomass to biobutanol (Gao et al., 2016; Wang et al., 2016). Microalgal strains appropriate for biobutanol generation should have high starch and convertible sugar levels. Some microalgae suitable for biobutanol production are *Chlorella reinhardtii, Chlorella vulgaris, Tetraselmis subcordiformis* and *Scenedesmus obliquus* (Yeong et al., 2018).

Biogas

Biogas production form microalgae is achieved through either microalgal biomass or lipid isolated from biomass (Passos et al., 2014). Biomass may be a good source for the generation of biogas, including hydrogen, methane and biohythane (5-25% hydrogen and methane); (Ghimire et al., 2017). Due to its good conversion efficiency, non-polluting nature and potential for recycling, bio-hydrogen may be preferred over other fuels (Batista et al., 2015). Selection of microalgal strain, pretreatment of biomass, culture methods and factors influencing anaerobic digestion (AD) impact the cost-effectiveness of process (Jankowska et al., 2017). Using recycled AD effluents initially in AD in closed-loop production process are beneficial for the environmental sustainability and commercial viability of the process (Jankowska et al. (2017).

Algae as Source of Commercially Important Products

Many commercially important compounds e.g. β -carotene, c-phycocyanin, chlorophyll a and b, astaxanthin and phycobilins have been generated from microalgae which have myriad of uses in nutraceuticals and pharmaceuticals, anti-oxidant and neuroprotective compounds, cosmetics and dyes (Begum et al., 2016; Kiran and Venkata Mohan, 2021; Mishra et al., 2021). Microalgae provide amino acids, vitamins, minerals, carbohydrates and omega fatty acids which have diverse applications such as in medicines, health supplements and food additives. Muller-Feuga et al. (2003) reported bactericides production using algae of class Ulvophyceae, Charophyceae and Spirogyra. Microalgae also serve as source of polyhydroxyalkanoates (PHAs) and industrially important extracellular polymeric substances (EPSs). PHAs may be employed in generation of biodegradable bioplastics (Markou and Nerantzis, 2013; Koller et al., 2014; Singh et al., 2021). Investigators have devised a technology for withdrawing CO_2 from atmosphere. In this process algae transform CO_2 from steel processing and power plants and atmosphere into biofuel (algal oil) which is subsequently employed to generate commercially important carbon fibers. Carbon fibers may serve as source of high-strength and lightweight materials (Arnold et al., 2018a, b). Lately carbon fibers may be stored in empty coal seams, thus permanently removing the associated carbon dioxide equivalents from atmosphere. This technology is cost-effective and better than carbon capture and storage (CCS) in the underground (Arnold et al., 2018a, b).

Algal Biorefinery

A biorefinery is a place where biomass transformation processes and equipments are amalgamated to generate fuel, power and economically important products/chemicals from biomass (Taylor, 2008; Espinoza Pérez et al., 2017). The integration of many processes may be useful in making the system cost-effective besides optimum utilization of biomass. Generation of economically important products from microalgal biomass employing biorefinery has been reported but cost-effectiveness of this process needs to be worked out (Zhou et al., 2017; 't Lam et al., 2018). The important aspects of biorefinery process such as upstream processing (USP) and downstream processing (DSP) needs to be worked out to make the process simple and all steps of process well connected. The important steps of the USP which are instrumental in deciding efficiency of the process, are selection of appropriate strain of microalgae, culture conditions (light and temperature) and supply of nutrients (CO₂, P and N); (Vanthoor-Koopmans et al., 2013). While, the steps which are instrumental in cost-intensive DSP operations are harvesting, cell disarrangement, and extraction. Selection of suitable algae is very important step but it is time consuming. Recently screening techniques such as 96-well microplate swivel system (M96SS) have made rapid screening of microalgae possible ('t Lam et al., 2018). Further improvements in microalgal strains can be done by exposing them to environmental stresses and through metabolic and genetic engineering (Chen et al., 2017; Ng et al., 2017; Schüler et al., 2017; Yang et al., 2017; Jagadevan et al. 2018). Nutrient supply during large level microalgal cultivation needs significant commercial input. Biofilm base attached cultivation method has become important recently which can be helpful in reducing low biomass productivity and large energy and water requirements in conventional methods (Kesaano and Sims, 2014; Wang et al., 2017). The processes involved in DSP are harvesting, disarrangement and extraction.

Usually for harvesting microalgae, extraction is employed. But high cost inputs are involved in centrifugation and therefore it is not appropriate for massive systems. Flocculation which involves cationic chemical and polymeric flocculants, is a cost-effective method (Brennan and Owende, 2010), but the downside of this process is that it can negatively affect the biomass toxicity and output water (Ryan, 2009). An efficient fungal mediated bioflocculation method was reported employing fungal spores for harvesting in algal culture by ordinary filtration (Zhou et al., 2012). Attached culture may be employed for harvesting (Wang et al., 2017). Methods for disarrangement generally employed such as use of enhanced pressure, homogenizers, high temperature, bead beating, use of chemicals or enzymes for lysis; are not cost-effective and may be detrimental for generation of multi-products in biorefinery set-up. Physical methods for disarrangement e.g. pulsed electric field (PEF) offer advantages like low temperature operating range, low shear generation and removal of hydrophobic components of the biomass. (Goettel et al., 2013; 't Lam et al., 2018). Ionic liquids (ILs) are non volatile organic salts. Extraction employing ILs has an edge over use of conventional solvents. Imidazolium-based ILs are used for lipid extraction from microalgal biomass (Orr and Rehmann, 2016).

CONCLUSIONS

Algae especially microalgae mediated carbon sequestration technology can aid towards the effective reduction of greenhouse gases concentration in the atmosphere in a sustainable manner. Microalgae are advantageous for CO₂ sequestration as compared to crops, forests and other vegetation because of their properties such as enhanced photosynthetic efficiency, utilization of waste matter such as nutrients from effluents and flue gas simultaneously providing many economically important products and faster growth. Integration of processes such as fixation of CO₂ obtained from flue gas, withdrawal of nutrients from wastewater and generation of biomass will make CO₂ sequestration commercially viable and competitive with other CO_2 capture technologies. Research is being conducted with generation of useful new information on selecting suitable algal strains for culture, adopting updated culture techniques for efficient CO₂ absorption, minimizing adverse effect of shear stress on algal growth and working out requirements of optimal light intensity for algal growth. Adoption of these updated methods may make algae-based carbon sequestration technology cost-effective and suitable for large-scale algae based biorefinery systems. Making steps involved in DSP process simpler and properly integrated with each other will reduce the energy intensive operations and make the DSP process costeffective especially in large-scale algae-based biorefineries. Delivery of light in various parts of PBR should be optimized and innovative technologies such as fibre optics and solar concentrators may aid towards optimum delivery of light and enahnced generation of biomass. Hybrid bioreactors with better designs may be employed for optimizing productivity. Algae based CO₂ sequestration and productivity of algae may be enhanced by updating information on algal biosynthetic processes and algal responses under stressful conditions. This information can be employed for efficient value-added products generation and could be very useful in enhancing market acceptability of algal biorefinery based CO₂ sequestration processes.

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