

# Overview of the potential of microalgae for CO<sub>2</sub> sequestration

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**Abstract** An economic and environmentally friendly approach of overcoming the problem of fossil CO<sub>2</sub> emissions would be to reuse it through fixation into biomass. Carbon dioxide (CO<sub>2</sub>), which is the basis for the formation of complex sugars by green plants and microalgae through photosynthesis, has been shown to significantly increase the growth rates of certain microalgal species. Microalgae possess a greater capacity to fix CO<sub>2</sub> compared to C<sub>4</sub> plants. Selection of appropriate microalgal strains is based on the CO<sub>2</sub> fixation and tolerance capability together with lipid potential, both of which are a function of biomass productivity. Microalgae can be propagated in open race-way ponds or closed photobioreactors. Biological CO<sub>2</sub> fixation also depends on the tolerance of selected strains to high temperatures and the amount of CO<sub>2</sub> present in flue gas, together with SO<sub>x</sub> and NO<sub>x</sub>. Potential uses of microalgal biomass after sequestration could include biodiesel production, fodder for livestock, production of colorants and vitamins. This review summarizes commonly employed microalgal species as well as the physiological pathway involved in the biochemistry of CO<sub>2</sub> fixation. It also presents an outlook on microalgal propagation systems for CO<sub>2</sub> sequestration as well as a summary on the life cycle analysis of the process.

**Keywords** CO<sub>2</sub> sequestration · Flue gas · Life cycle analysis · Microalgae · Photosynthesis

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## Introduction

The impending threat of global climate change has amplified. This has been mainly attributed to greenhouse gas (GHG) emissions of which carbon dioxide (CO<sub>2</sub>) contributes up to 68 % of total emissions (Brennan and Owende 2010; Ho et al. 2011; Kumar et al. 2011). This is partly due to the high-energy usage and the dependence on coal for electricity generation (Kumar et al. 2011). According to a report by the Carbon Dioxide Information Analysis Center (CDIAC), CO<sub>2</sub> emissions worldwide have increased from 3 metric tons in 1,751 to 8,230 metric tons in 2006. Further increases in emissions are projected to permanently alter future climates as well as cause significant dents to the economy. Therefore, it is imperative to develop an appropriate technology to reduce the emissions and accumulation of CO<sub>2</sub>. CO<sub>2</sub> sequestration strategies implemented globally can be divided into physical and biological techniques (Khoo et al. 2011). However, due to numerous challenges associated with physical methods, there is a need to develop other suitable technologies. Biological CO<sub>2</sub> fixation appears to be the only economical and environmentally viable technology of the future (Ho et al. 2011; Kumar et al. 2011). This presents an attractive development option as plants and other photosynthetic organisms naturally capture and use CO<sub>2</sub> as part of their photosynthetic process. Terrestrial plants are able to sequester vast amounts of CO<sub>2</sub> from the atmosphere. However, when compared to terrestrial plants, microalgae and cyanobacteria have faster growth rates, and their CO<sub>2</sub>-fixation efficiency is also between 10 and 50 times higher (Costa et al. 2000; Langley et al. 2012). The biological mitigation of CO<sub>2</sub> using microalgae could therefore offer several advantages. No additional CO<sub>2</sub> is created, while nutrient utilization is achieved in a continuous fashion



leading to the production of biofuels and other secondary metabolites. Therefore, microalgal-mediated CO<sub>2</sub> fixation coupled with biofuel production, and wastewater treatment could present a promising alternative to existing CO<sub>2</sub> mitigation strategies (Wang et al. 2008; Lam et al. 2012). This paper focuses on commonly used microalgal species as well as the physiological pathway involved in the biochemistry of CO<sub>2</sub> fixation. It also presents an outlook on microalgal propagation systems for CO<sub>2</sub> sequestration as well as a summary on the life cycle analysis (LCA) of the process.

### Carbon dioxide sequestration techniques

A number of efforts are currently under way to limit the amount of CO<sub>2</sub> entering the atmosphere (Morais and Costa 2007). Selecting the most appropriate technology has sparked much debate globally. CO<sub>2</sub> mitigation strategies applied worldwide can be divided into physical and biological techniques. Physical-based methodologies often entail three steps: capture, transportation and storage. CO<sub>2</sub> is collected from a fixed source, for instance, from power plants or cement manufacturing facilities. Following capture, the gas mixture is converted to a supercritical fluid. This aids in transportation by pipeline or ship to a place of storage. Storage options often involve injection into deep oceanic or geological trenches and mineralization (Khoo et al. 2011; Pires et al. 2012). These currently employed disposal methods are considered unsustainable as they are expensive, require large amounts of space, are energy intensive and eventually lead to CO<sub>2</sub> leakage over time (Stewart and Hessami 2005). However, even with the aforementioned drawbacks, this still remains a popular technology. It enables societies to maintain their existing carbon-based infrastructure while aiming to reduce the effects of CO<sub>2</sub> on global warming (Pires et al. 2012).

Another option would be to utilize the collected CO<sub>2</sub>. For example, precipitated calcium carbonate (PCC), which is manufactured by controlling the reaction of CO<sub>2</sub> with lime, can be used as a substitute for titanium dioxide or kaolin in the manufacture of paper products. CO<sub>2</sub> could also be employed in the manufacture of paint, plastic, solvent and packaging. However, these industries would consume relatively minute quantities of CO<sub>2</sub>, as compared to the large amounts released annually into the atmosphere.

Biofixation of CO<sub>2</sub> using microalgae could offer a sustainable alleviation technology without the aforementioned shortcomings (Stewart and Hessami 2005). Although terrestrial plants are responsible for fixing around 500 billion tones of CO<sub>2</sub> per annum, they are expected to play a minor role (3–6 %) in the overall reduction in atmospheric CO<sub>2</sub> (Skjanes et al. 2007). Microalgae and cyanobacteria have

come to the forefront of research as they offer greater potential owing to their rapid growth rates, higher CO<sub>2</sub>-fixation ability and tolerance to extreme environments (Ho et al. 2011). Sydney (2010) stated that carbon uptake is often dependent on the metabolic activity of microalgae. Studies have suggested that microalgae exposed to increased levels of CO<sub>2</sub> respond better (on a biomass basis), when compared to microalgae exposed to ambient air only. Microalgae are capable of generating roughly 280 tons of dry biomass per ha per year by utilizing 9 % of the freely available solar energy. During this process, approximately 513 tons of CO<sub>2</sub> can be sequestered (Sydney 2010).

### Research and development

Microalgae have been studied for several decades as a feedstock for renewable energy in an attempt to reduce global warming. These organisms are able to use concentrated amounts of CO<sub>2</sub>, present in power plant flue gases as well as from other sources. Therefore, they represent a powerful GHG mitigation strategy. During the mid-1970s, the US Department of Energy (DOE) began encouraging research pertaining to microalgal wastewater treatment (Benemann et al. 1977). The recovered microalgal biomass was subjected to anaerobic digestion which yielded methane gas. The “Aquatic Species Program” (US) funded by the office of fuels development started out as a project investigating the possibilities of using microalgae to sequester CO<sub>2</sub> emissions from coal power plants (Sheehan et al. 1998). The project screened microalgae that could produce high amounts of oils as well as grow under adverse environmental conditions (extreme temperature, pH and salinity). This program was able to develop a culture collection system as well as support a pilot-scale project consisting of two raceway ponds in New Mexico. It was proposed that sufficient resources would be available in the southwest region of the USA for large-scale microalgal processes capable of capturing several hundred million tons of CO<sub>2</sub> annually (Benemann and Oswald 1996). However, by the end of this program, it was concluded that in order for such a process to be economically feasible, favorable sites that yielded productivities near the theoretical maximum would be required.

During the 1990s in Japan, a major R&D program totaling over \$250 million was carried out. The assignment focused on microalgal biofixation of CO<sub>2</sub> as well as GHG abatement using closed photobioreactors (PBRs). However, owing to the high costs associated with PBRs (which still pose a problem today), the Japanese R&D initiative did not continue. The use of PBRs was then confined to inoculum production only (Lipinsky 1992; Nakajima and Ueda 2000).



The US DOE-NETL promoted microalgae R&D using closed PBRs. Other international participants of R&D pertaining to microalgae CO<sub>2</sub> abatement included the following: Arizona Public Services, ENEL Produzione Ricerca, EniTecnologie, ExxonMobil and Rio Tinto. Due to the initiative of these governmental and private industries, the International Network on Microalgae Biofixation of CO<sub>2</sub> and GHG Abatement was formed in 2000 in an effort to bring together the limited technical expertise in this field (Pedroni et al. 2001).

Past research initiatives suggest that practical CO<sub>2</sub> utilization using microalgae still requires innovative scientific and technological breakthroughs to render this a feasible technology. Unless coupled with other technologies or co-processes, investments into microalgae R&D are unlikely to make a considerable contribution to solving the CO<sub>2</sub> problem globally. The use of microalgae can be classified as a direct CO<sub>2</sub> mitigation technology. Direct strategies usually encompass much higher economic projections, going into billions of dollars, as opposed to indirect approaches. Therefore, for this technology to be a success, future R&D should focus on achieving higher biomass productivities, culture stability over long periods of time, economical harvesting techniques and improved biomass-to-fuels conversion technologies. The economics of microalgal CO<sub>2</sub> utilization may be improved by integrating this procedure with other co-processes. Potential co-processes include wastewater treatment, production of useful metabolites, as well as biofuels, animal feed and biofertilizer manufacturing. Wastewater treatment as a co-process has emerged as a viable approach as process requirements and objectives overlap significantly. Municipal wastewater treatment is more favorable when compared to agricultural wastewater treatment as it could yield an animal-feed co-product. This would greatly aid in the economics of the entire process as well as contribute to GHG abatement by not producing additional fossil fuel that is generally required for product formation (Ho et al. 2011).

## Microalgae

Microalgae which are primitive, unicellular, microscopic (2–200 μm) organisms that can also be classified as thalphytes have an important ecological role (Khan et al. 2009; Brennan and Owende 2010; Greenwell et al. 2010; Mutanda et al. 2011). Besides the fact that they can serve as food and feed source for people and animals as they belong to the bottom of the food chain, they are the principal producers of O<sub>2</sub> on earth (Khan et al. 2009). They can be autotrophic or heterotrophic, and in some cases even both. Autotrophic microalgae require inorganic compounds, salts

and an appropriate light source for growth, whereas heterotrophic microalgae utilize external sources of organic compounds as well as nutrients, which are used as an energy source. Microalgae are mainly categorized based on their basic cellular structure, pigmentation and life cycle. Due to novel genetic and ultra-structural information constantly emerging, the evolutionary history and taxonomy of microalgae is complex (Brennan and Owende 2010; Mutanda et al. 2011). Microalgae can be classified into two prokaryotic divisions and nine eukaryotic divisions (Khan et al. 2009; Mutanda et al. 2011). These organisms show much promise for the production of value-added products and biofuels, as they are rich in minerals, vitamins, oils and fatty acid methyl esters (Spolaore et al. 2006; Del Campo et al. 2007; Khan et al. 2009; Mutanda et al. 2011). Microalgae are able to endure high concentrations of CO<sub>2</sub>, and this inherent ability makes them very advantageous in utilizing CO<sub>2</sub> from flue gases of power plants. They are fast growers with biomass volumes that double within 24 h. At a flow rate of 0.3 L/min of air with 4 % CO<sub>2</sub> concentration, most microalgal strains are able to achieve a carbon-fixation rate of roughly 14.6 gcm<sup>-2</sup>/day (Farrelly et al. 2013).

## Sampling for highly CO<sub>2</sub> tolerant microalgal strains

For effective CO<sub>2</sub> sequestration using microalgae, the crucial step would be to search, collect and identify hyper-CO<sub>2</sub>-tolerant strains (Khan et al. 2009; Huang et al. 2010). Sampling for these microalgal strains is largely influenced by environmental factors as well as the aquatic system (Mutanda et al. 2011). Brackish aquatic environments appear to be ideal areas to sample for superior carbon sequesters as they are rich in dissolved CO<sub>2</sub>, O<sub>2</sub> and dissolved salts. Also, most CO<sub>2</sub> sources, such as power plants, are located along coastal areas. Proper sampling techniques need to be in place in order to ensure success, as damaged or dead cells often lead to failure. Temporal and spatial collection is often employed to offset any mishaps that may occur at the sampling site. The success of any microalgal mass culture ultimately depends on fast-growing, productive strains that are adapted to the local climatic conditions (Mutanda et al. 2011).

It is imperative that certain factors are measured on-site so that these conditions can be simulated when culturing the specimens under laboratory conditions. Some such parameters would be the light quality and quantity, water temperature, nutrient concentration, dissolved CO<sub>2</sub> and O<sub>2</sub>, as well as pH and salinity. Over the years, microalgal collection and selection processes have been well established. Sampling equipment should include a knife, mesh net, scooping jar, vessels for sample collection, scalpels, dissolved CO<sub>2</sub> and O<sub>2</sub> analyzer, light meter, GPS, salinity meter and a multi-probe system (measuring pH,

temperature, turbidity, conductivity and light intensity) (Mutanda et al. 2011). No definite sampling procedure has been recognized in literature. Researchers are encouraged to follow simple and cheap techniques when collecting microalgal samples (Mutanda et al. 2011).

Once samples are brought back to laboratories, microalgae are usually identified by means of microscope-based techniques. For species-level identification, conventional light microscopy has been extended to include fluorescence microscopy, phase-contrast microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Conventional microscope techniques, however, could often prove misleading in precise microalgal identification as many cell types lack morphological markers. Microalgae are also known to alter cell size and shape during their life cycle (Godhe et al. 2001; Mutanda et al. 2011). A proper identification is impossible as most microalgae fail to survive fixation or in some cases shrink, lose pigmentation and flagella. Microalgal identification from field samples using microscopy is also time-consuming and requires significant experience in technical and taxonomic skills. Molecular-based techniques developed in recent years have led to rapid and precise monitoring, identification and quantification of microalgal species. Commonly analyzed DNA regions for phylogenetic purposes include mitochondria genes, ribosomal RNA genes (rRNA), internal transcribed sequences (ITS), plastid genes (rbcL) and microsatellite DNA sequences. Therefore, molecular-based techniques using species-specific molecular probes offer a powerful technology for rapid screening and identification of microalgal species when compared to conventional identification techniques.

### Propagation systems

After selecting a strain of interest, the step to follow is appropriate cultivation of the organism. Artificial cultivation of microalgae ought to reproduce and enhance the optimum natural growth conditions (Brennan and Owende 2010; Vasumathi et al. 2012). Two systems that have been extensively proposed are based on open pond and closed PBR technologies (Molina et al. 2001; Suh and Lee 2003; Chisti 2008; Brennan and Owende 2010). However, there is ongoing debate pertaining to which of the open pond or closed PBR would be a better system for CO<sub>2</sub> sequestration. Raceway ponds are the most commonly used artificial growth systems because they are cost-effective, but significant CO<sub>2</sub> losses to the atmosphere occur. Open systems are also known to utilize CO<sub>2</sub> much less efficiently than PBRs (Borowitzka 1999; Chisti 2007; Brennan and Owende 2010).

Open ponds are typically constructed of a closed-loop, oval-shaped recirculation channels, generally between 0.2

and 0.5 m deep (Chisti 2007; Brennan and Owende 2010). Mixing and circulation, which is required to stabilize microalgal growth and productivity, is maintained within the pond by a paddlewheel (Borowitzka 1999; Chisti 2007; Brennan and Owende 2010). The paddlewheel is a continuous operation that prevents sedimentation. Flow can be guided around bends by baffles placed in the flow channel (Chisti 2007). Open systems are built in concrete or compacted earth, and are usually lined with white plastic (Borowitzka 1999; Chisti 2007; Ugwu et al. 2008; Brennan and Owende 2010). For a continuous production cycle, microalgal broth and nutrients are introduced in front of the paddlewheel and circulated through the loop to the harvest extraction point. Broth is harvested behind the paddlewheel, on the completion of the circulation loop (Chisti 2007). In raceways, cooling is solely achieved by evaporation. Temperature fluctuates seasonally. Considerable loss of water due to evaporation can occur. Due to significant losses to the atmosphere, raceway ponds are known to use CO<sub>2</sub> much less efficiently than PBRs (Chisti 2007). Microalgae present in open ponds usually obtain their CO<sub>2</sub> requirement from the surface air, but submerged aerators have been known to be installed to enhance CO<sub>2</sub> absorption (Brennan and Owende 2010).

Microalgal production using closed PBR technology has been implemented to overcome some of the key problems associated with the above-described open pond production systems. A major advantage of PBRs when compared to open raceway systems is that they permit culture of single species of microalgae for prolonged durations with lower risk of contamination (Brennan and Owende 2010). Harvesting costs may also be significantly reduced owing to the higher cell mass productivities attained, and CO<sub>2</sub> is also utilized more effectively (Chisti 2007; Brennan and Owende 2010; Vasumathi et al. 2012). Despite the fact that a great deal of work has already been done to develop PBRs for microalgal cultures and effective CO<sub>2</sub> utilization, more efforts are still required to improve PBR technologies and know-how of microalgal cultures. Photobioreactor design and development is perhaps one of the first major steps that should be undertaken for efficient mass cultivation of microalgae for carbon mitigation (Ugwu et al. 2008). Light distribution and higher biomass productivities are more efficient in PBRs that have a larger optical cross-sectional area. PBRs having special designed light systems have been investigated for the effective CO<sub>2</sub> sequestration and biomass production (Lee 2001). In 2003, Suh and Lee designed and operated an internally illuminated airlift PBR. This reactor was employed to study the light distribution in an attempt to maximize the photosynthetic efficiency and hence carbon uptake of a *Synechococcus* sp. A flat-plate PBR was constructed by Zijffers et al. (2010). In this design, sunlight was focused on the top of the reactor



by dual-axis positioning of lenses. Solar radiation was captured by vertical plastic light guides and then distributed into the PBR. This design allowed for a more uniform distribution of light throughout the reactor and hence, more effective light utilization (Kumar et al. 2011).

Volumetric gas transfer coefficient is another important characteristic that should be taken seriously when designing reactors. Gas transfer and cell growth rate vary at different regions of liquid flow. Liquid flow within reactors is often divided into three regions: bubble flow, transitional flow and a heterogeneous zone. These zones are often dependent on gas velocity. The bubble flow region is an area in which gas hold-up, interfacial area as well as the volumetric gas transfer coefficient is proportional to the gas superficial velocity. Increases in the gas transfer coefficient promote cell growth rate. However, toward the end of the transition zone, a reduction in growth rate can be observed. The drop in specific growth rate could be attributed to shear stress (Kumar et al. 2011). In 2002, Zhang et al. conducted a series of experiments to comparatively analyze gas transfer in different PBRs at various CO<sub>2</sub> percentages. They were able to conclude that the gas transfer coefficient increases with a decrease in the CO<sub>2</sub> concentration from the inlet gas stream.

Ascertaining the CO<sub>2</sub> solubility within the cultivation media is essential as this will determine the amount of carbon available for growth of microalgal cells. The solubility and speciation of CO<sub>2</sub> in the medium is dependent on pH, temperature and nutrient concentration. Henry's law states that CO<sub>2</sub> dissolves in water to an extent determined by its partial pressure (PCO<sub>2</sub>), temperature, as well as the interaction of dissolved CO<sub>2</sub> with other solutes in the water (Carroll and Mather 1992). CO<sub>2</sub> solubility is known to increase with increasing pressures and decrease with increasing temperatures. Due to the thermodynamics of the reaction, CO<sub>2</sub> becomes more soluble at lower temperatures. Solubility of CO<sub>2</sub> in freshwater is also significantly higher as opposed to solubility in salt water (Carroll and Mather 1992).

A study by Borkenstein et al. (2011) investigated the cultivation of *C. emersonii* for 30 days using both flue gas and pure CO<sub>2</sub> in 5.5 L airlift PBRs. The experimental setup is shown in Fig. 1. Results showed that the *Chlorella* sp. supplied with CO<sub>2</sub> containing flue gas resulted in a biomass yield of 2.00 g L<sup>-1</sup>; when cultivated with pure CO<sub>2</sub>, it yielded 2.06 g L<sup>-1</sup> biomass. There was no significant difference in biomass yields. When supplied with flue gas, *C. emersonii* was able to grow as successfully as when supplied with pure CO<sub>2</sub>. It can be concluded that the concentrations of the components within the flue gas together with the size and proximity of the culture vessel and characteristics of the microalgal species are imperative in developing an effective CO<sub>2</sub> remediation technology.

## Microalgal species employed for carbon bio-mitigation

Ambient CO<sub>2</sub> levels are generally low (approximately 0.036 %) when compared to industrial areas, which release large amounts of flue gases into the atmosphere. CO<sub>2</sub> concentrations present in flue gases vary from industry to industry, but are normally within the range of 3–30 %. Microalgae are able to fix CO<sub>2</sub> from a range of sources, such as the atmosphere, industrial exhaust gases (flue and flaring gas) as well as in the form of soluble carbonates (NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>) (Wang et al. 2008).

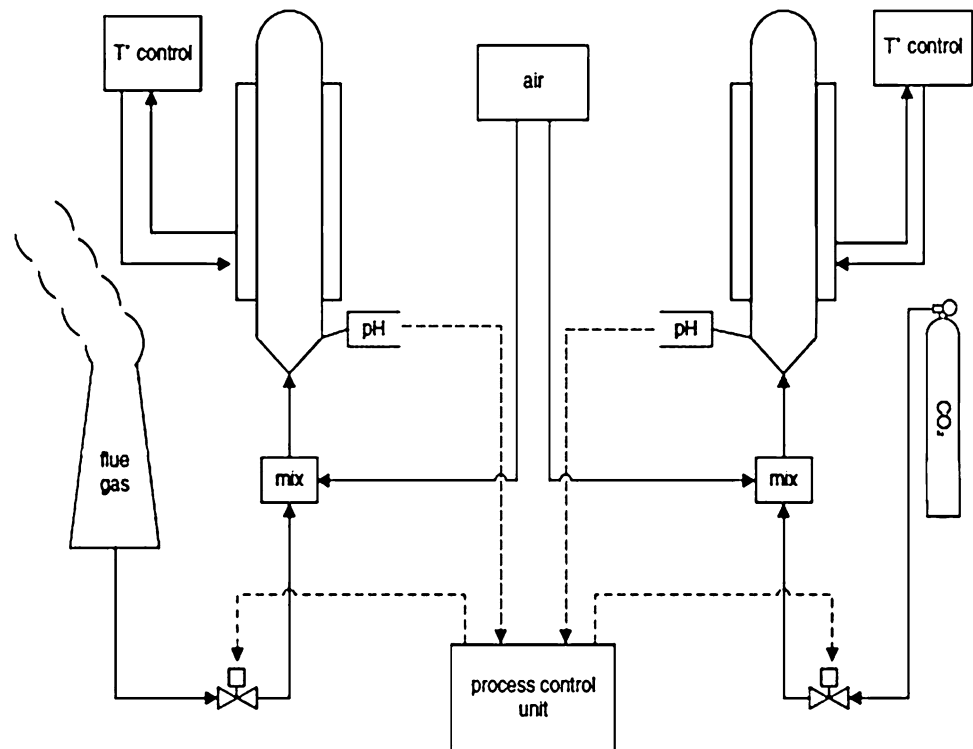
Carbon dioxide (CO<sub>2</sub>) is an essential nutrient for all photosynthetic life forms. Terrestrial plants naturally capture and utilize CO<sub>2</sub> from the atmosphere. Microalgae, however, possess a greater capacity to fix CO<sub>2</sub> (Kumar et al. 2011). This can be attributed to their photosynthetic apparatus and chlorophyll being present within a single microalgal cell permitting rapid biomass generation. Listed below in Table 1 are some advantages of using microalgae over higher plants for CO<sub>2</sub> sequestration.

In the context of propagation, atmospheric CO<sub>2</sub> (0.036 %) is most often not sufficient to support microalgal growth as microalgae requires an enriched point source of carbon. Due to this, biofixation of CO<sub>2</sub> from power plants and other point sources using microalgae has emerged as a likely alternative to combat the growing concerns of global warming due to CO<sub>2</sub> emissions (Ho et al. 2011; Kumar et al. 2011).

An increase in the maximum growth rate of microalgal species due to higher CO<sub>2</sub> concentrations has been investigated by many researchers (Cheng et al. 2006; Ono and Cuello 2006; López et al. 2010; Ho et al. 2011; Kumar et al. 2011). Microalgal-CO<sub>2</sub> fixation occurs via photoautotrophic growth. Therefore, the CO<sub>2</sub>-fixation potential of microalgal species should positively correlate with their light utilization efficiency and cell growth rate. Increases in temperature (>20 °C) can cause significant reduction in CO<sub>2</sub> solubility, which eventually leads to a decline in the photosynthetic efficiency.

Strains often favored are those that can directly utilize CO<sub>2</sub> from industrial flue gas, those that grow well under natural day-night cycles, strains with high productivities that are easy to harvest and most importantly those that produce biomass that can be used in the production of desirable co-products. Unfortunately, it is rare for a single microalgal strain to possess all these attributes. Most often, a highly productive strain functions poorly in dense mass culture, while a robust strain could have low growth rates (Farrelly et al. 2013). In recent years, there has been talk of genetically manipulating microalgal strains to enhance their properties pertaining to carbon mitigation and mass culture (Farrelly et al. 2013). Over the years, research has largely focused on single effective carbon sequestrators and

**Fig. 1** Schematic representation of an airlift photobioreactor (Borkenstein et al. 2011)



trying to upscale these strains for mass culture. However, it would appear that a more effective approach would be to isolate strains from nature that thrive in mass culture, and subsequently manipulate these strains to grow in dense culture while altering their physiology to increase their productivity and ease of harvesting (Farrelly et al. 2013).

Microalgae and cyanobacterial species routinely used for CO<sub>2</sub> mitigation include *Anabaena* sp., *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Chlorella* sp., *Chlorococcum littorale*, *Scenedesmus* sp., and *Spirulina* sp. (de Morais and Costa 2007; Ota et al. 2009; Packer 2009; Chen et al. 2010; Chiang et al. 2011; Ho et al. 2011). Table 2 represents commonly employed carbon sequesters cultivated in different bioreactors.

Green microalgae that are effective carbon sequesters generally belong to the genera *Chlorococcum*, *Chlorella*, *Scenedesmus* and *Euglena*. In 1970, Seckbach and Libby isolated species from the aforementioned genera that were able to survive after exposure to pure (100 %) CO<sub>2</sub>. Experiments on the *Scenedesmus* sp. revealed that this strain thrived under 100 % CO<sub>2</sub> concentration and cell concentration increased for up to 30 days, reaching 3.65 g L<sup>-1</sup>. This was a significant increase in cell concentration, when compared to the 1.19 g L<sup>-1</sup> obtained under conditions of atmospheric CO<sub>2</sub> (0.036 %). *Chlorococcum littorale* represents a fast-growing species that is able to tolerate high CO<sub>2</sub> concentrations. In another

experiment, Seckbach and Libby (1970) demonstrated that *C. caldarium* could tolerate 10 atm of CO<sub>2</sub> but not 50 atm of CO<sub>2</sub>. They were able to conclude that growth was inhibited due to the effect of high CO<sub>2</sub> concentrations and not due to the high pressure. These species, however, are known to have low growth rates, and hence, productivity and CO<sub>2</sub> recovery were found to be low (Satoh et al. 2001).

A *Chlorella* Tx 71105 strain was supplied with pure CO<sub>2</sub> at a rate of 3.3 mL min<sup>-1</sup> over a 28-day period. During the first 6 days, the effluent gas contained more than 96 % O<sub>2</sub>. On the 12th day of cultivation, a spike in temperature from 37 to 39 °C was observed. On the 13th day, a 32 % CO<sub>2</sub> concentration was noted in the effluent gas. The gas flow was then turned off for 3.5 h. Upon re-addition of CO<sub>2</sub>, the effluent gas then contained 97.8 % O<sub>2</sub>. During the last 6 days, no alterations were made to the experiment, and over this period, it was noted that the effluent gas contained 18 % CO<sub>2</sub>. When this strain was cultured under 41, 71 and 100 % CO<sub>2</sub>, the mean biomass concentration recorded was 3.15, 2.71 and 2.49 g L day<sup>-1</sup>, respectively. These cell concentrations are reasonably similar to those obtained with other *Chlorella* species (Table 2) (Geckler et al. 1962).

In another study, Zhao et al. (2011) did a comparative study of the growth and CO<sub>2</sub> biofixation of a *Chlorella* sp. under two different cultivation modes. Findings showed that closed cultivation substantially enhanced microalgal



**Table 1** Advantages of using microalgae as opposed to terrestrial plants for CO<sub>2</sub> biofixation

Factors	Microalgae	Terrestrial plants	References
Resilience to adverse climatic conditions	High	Low	Mata et al. (2010)
Conventional agricultural food production	No competition	Direct competition	Radakovits et al. (2010) and Demirbas and Demirbas (2011)
Photon conversion efficiencies	High (8–10 %)	Low (0.5 %)	Aresta et al. (2005) and Sharma et al. (2011)
CO <sub>2</sub> -fixation efficiency	High	Low	Kumar et al. (2011)
Growth rate	Rapid	Slow	Greenwell et al. (2010) and Mata et al. (2010)
Production and harvesting	All year round	Seasonal	Kumar et al. (2011)
Acid deposition	None	Fair	Sharma et al. (2011)
Scale-up	Easy	Difficult	Clarens et al. (2010)
Carbon-rich biomass	High	Low	Chisti (2007)
Cultivation costs	Low	High	Kumar et al. (2011)
Direct CO <sub>2</sub> sequestration from power plants	High	Low	Ho et al. (2011)

performance with regard to growth and carbon biofixation. Specific growth rate and CO<sub>2</sub>-fixation rate during closed cultivation were observed to be 1.78 and 5.39 times higher than that of open cultivation, respectively. Closed systems also allow for effective gas bubble motion, which plays a vital role in reducing dissolved O<sub>2</sub> build-up. Under the proper cultivation mode, *Chlorella* sp. exhibit much potential as effective carbon sequestrators.

A study by Kurano et al. (1995) showed that *C. littorale* was able to reach a maximum cell concentration of 4.9 g L<sup>-1</sup> at a 20 % CO<sub>2</sub> concentration. When exposed to CO<sub>2</sub> concentrations of more than 20 %, a short lag phase was observed prior to active photosynthesis. It must be noted that the performance of microalgal strains does not solely depend on CO<sub>2</sub> concentrations, but also on culture and experimental conditions, such as culture medium, temperature, light intensity as well as reactor design. Variation in any of these conditions could have an effect on the CO<sub>2</sub>-fixation efficiency of the strains (Ho et al. 2011).

### Physiological pathway involved in the biochemistry of CO<sub>2</sub> fixation

Photosynthesis (Fig. 2) within microalgae cells occurs in two stages. The first stage involves light-dependent or light reactions that only take place when cells are illuminated. This step exploits light energy to form the energy-storage molecules adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH). Carbon-fixation or dark reactions that form the second stage of photosynthesis transpire both in the presence and in the absence of

light. Energy-storage products generated during light reactions are used at this step to capture and reduce CO<sub>2</sub> (Calvin 1989).

Photosystem I (PSI) and photosystem II (PSII) are major photoactive complexes responsible for transferring sunlight into the electron transport chain via the excited chlorophyll dimer (Calvin 1989; Iverson 2006; Ho et al. 2011). Photosynthesis begins in the PSII complex. Once a chlorophyll molecule at the core of the PSII complex attains ample excitation energy, an electron is transferred to the primary electron acceptor molecule. This process is often termed photo-induced charge separation. Electrons are then transported across the membrane through an electron transport chain. PSI accepts the electrons transferred from PSII and transports them via the P700 dimer of chlorophyll, which is oxidized from light-excited antenna chlorophyll to strongly reducing ferredoxin and NADPH (Cerveny et al. 2009; Ho et al. 2011). Energy harvested via the light reaction can be stored by the formation of ATP during photophosphorylation (Yang et al. 2000). Research has concluded that 1.3 ATP molecules are formed per pair of electrons moving through the photosynthetic electron transport chain (Yang et al. 2000). NADPH that is the main reducing agent in chloroplasts is responsible for supplying electrons to fuel other reactions. Due to its formation, a deficit of electrons exist in the chlorophyll of PSI. These electrons are replaced from the electron transport chain by plastocyanin.

Carbon-fixation reactions (dark reactions) involve the Calvin cycle (Calvin 1989; Iverson 2006; Yang et al. 2000). During the Calvin cycle, CO<sub>2</sub> is converted into sugar with the aid of ATP by the carboxylase activity of the

**Table 2** Commonly employed microalgal species used for CO<sub>2</sub> sequestration under varying cultivation modes

Strain	Propagation system	Temperature (°C)	pH	Light intensity	Supplied CO <sub>2</sub> (%)	Growth rate (g/L/day)	CO <sub>2</sub> -fixation rate (g/L/day)	CO <sub>2</sub> -fixation efficiency (%)	Ref <sup>d</sup>
<i>Chlorella</i> sp.	Open pond	30	10	30 <sup>a1</sup>	10	–	–	46	1
<i>Chlorella</i> sp.	PBR	26	–	–	2	1.21	0.261	58	2
<i>Chlorella kessleri</i>	Conical flask PBR	30	–	–	0.038	0.090	–	–	3
<i>Chlorella vulgaris</i>	Tubular PBR	25	–	–	0.036	0.4	0.075	–	4
<i>Chlorococcum littorale</i>	Flat-plate reactor	25	6.1.7.2	2000 <sup>a1</sup>	5	–	200.4 <sup>b1</sup>	–	5
<i>Dunaliella tertiolecta</i>	Bubble column reactor	25	7.2 ± 0.2	3500 <sup>a2</sup>	5	–	0.271	–	6
<i>Dunaliella</i> sp.	PBR	3	–	–	27	0.17	0.313	–	7
<i>Botryococcus braunii</i>	Bubble column reactor	25	7.2 ± 0.2	3500 <sup>a2</sup>	5	–	0.497	–	6
<i>Scenedesmus obliquus</i>	Tubular PBR	30	–	–	6	0.10	–	28.08	8
<i>Scenedesmus obliquus</i>	Tubular PBR	30	–	–	12	0.14	–	13.56	8
<i>Nannochloropsis oculata</i>	PBR	26	–	–	15	0.372	0.393 <sup>b2</sup>	15	9
<i>Haematococcus pluvialis</i>	Tubular PBR	16–18	–	–	0.036	0.076	0.143	–	10
<i>Aphanathece m. Nageli</i>	Airlift Reactor	25	6.8	150 <sup>a1</sup>	15	–	14.5	–	11
<i>Aphanathece m. Nageli</i>	Bubble column reactor	35	–	11.000 <sup>a2</sup>	15	–	2.621	–	12
<i>Thermosynechococcus</i> sp.	Bubble column reactor	55	–	10.000 ± 350 <sup>a2</sup>	10	2.7	–	–	13
<i>Spirulina</i> sp.	Tubular PBR	30	–	–	12	0.33	–	45.61	7
<i>Spirulina platensis</i>	Open pond	30	10	30 <sup>a1</sup>	10	–	–	39	1
<i>Anabaena</i> sp.	Bubble column reactor	27	8.5	900 <sup>a3</sup>	0.036	–	1.45	–	14
<i>Chlorogleopsis</i> sp.	PBR	50	–	–	5	–	20.45 <sup>b3</sup>	–	15
<i>Synechococcus</i> sp.	Airlift reactor	30	6.8	8000 <sup>a2</sup>	5	–	0.6	–	16

a<sup>1</sup> μmol m<sup>-2</sup> s<sup>-1</sup>a<sup>2</sup> luxa<sup>3</sup> μE m<sup>-2</sup> s<sup>-1</sup>b<sup>1</sup> g m<sup>-2</sup> d<sup>-1</sup>b<sup>2</sup> g/hb<sup>3</sup> mgC/L/day

<sup>d</sup> Ref: 1- (Ramanan et al. 2010); 2- (Chiu et al. 2008); 3-(Morais and Costa 2007); 4- (Seragg et al. 2002); 5- (Hu et al. 1998); 6- (Sydney 2010); 7- (Wang et al. 2008); 8- (Morais and Costa 2007); 9- (Chiu et al. 2009); 10- (Huntley and Redalje 2007); 11- (Jacob-Lopes et al. 2009); 12- (Jacob-Lopes et al. 2008); 13- (Hsueh et al. 2009); 14- (López et al. 2010); 15- (Ono and Cuello 2007); 16- (Heasman et al. 2000)





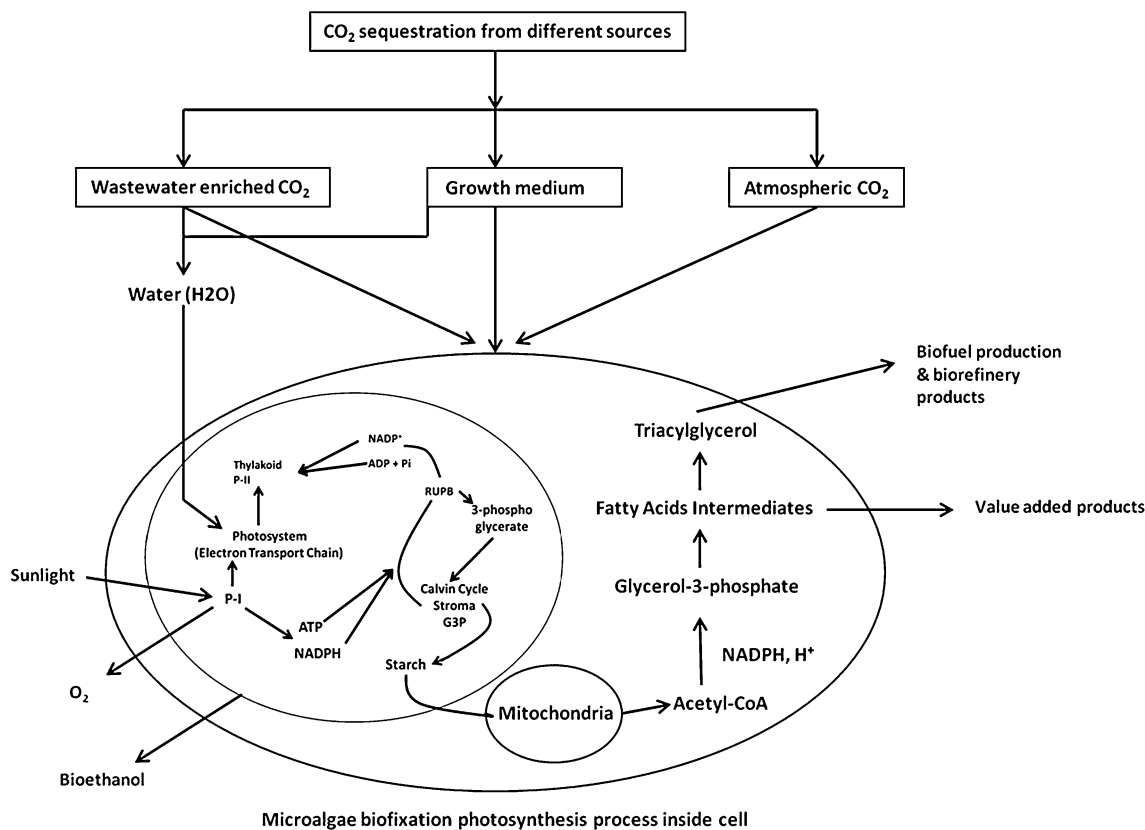


Fig. 2 Microalgal photosynthesis: modified from Zeng et al. (2011)

enzyme RuBisCO (Ribulose 1,5-bisphosphate carboxylase/oxygenase). Ribulose 1,5-bisphosphate carboxylase/oxygenase that has low affinity for CO<sub>2</sub> also carries out oxygenase activity and produces glycolate 2-phosphate as an end product. This end product is of no use to the cell and its synthesis consumes considerable amounts of cellular energy. It is also responsible for releasing previously fixed CO<sub>2</sub> by the carboxylase activity of RuBisCO. The oxygenase activity of RuBisCO can hinder around 50 % of biomass formation (Giordano et al. 2005; Kumar et al. 2011).

As mentioned previously, light supply (both light intensity and quality) is a key variable that greatly impacts the photosynthetic activity and hence growth kinetics of microalgae. Culture systems are illuminated by sunlight, artificial light or a combination of both. Light/dark cycle durations also play a significant role in microalgal growth (Pires et al. 2012). Grobbelaar et al. (1996) used a *Scenedesmus obliquus* strain to demonstrate that photosynthetic rates increase exponentially with increasing light/dark frequencies. Research suggests that microalgae cells associate a low light/dark cycle with low light conditions and vice versa. It was also observed during this study that the microalgal cells became increasingly more proficient in the overall exploitation of light energy during a longer dark

phase relative to light phase. However, it must be noted that a relatively longer dark phase did not necessarily achieve higher photosynthetic rates nor did the microalgal cells acclimatize to a precise light/dark cycle. High photon flux densities over a long duration of time eventually led to damage to protein D1 in PSII. Damage to protein D1 led to poor trapping of photons. This then causes an overall reduction in the photosynthetic activity. During the dark phase, the photo-induced damage can be repaired by the algae. Airlift reactors are extremely beneficial in this regard, allowing cells to experience adequate amounts of shading in which time they are able to repair damage to PSII. Within airlift reactors, light flux decreases exponentially with the distance from the irradiated surface. As a result of this, cells near the irradiation source are exposed to a high photon density when compared to cells at the center. These cells at the center receive less light owing to shading. Photosynthesis can be measured as rates of carbon accumulation or O<sub>2</sub> evolution. Either measurement can be converted into the other using the photosynthetic quotient (Pires et al. 2012).

The influence of photoperiods on the rates of CO<sub>2</sub> sequestration was also studied by Jacob-Lopes et al. (2010) using a cyanobacterial strain in both BGN medium and refinery wastewater. A linear decrease in biomass

productivity was observed during a longer dark period in BGN medium. A photosynthetic quotient of 0.74 was achieved using refinery wastewater as the growth medium. This essentially means that 1 g of CO<sub>2</sub> consumed corresponds to the release of 0.74 g of O<sub>2</sub>. From this study, it can be concluded that the gas-exchange pattern within a system is greatly influenced by the intermittent light cycle. During dark periods, microalgal cells consume organic carbon through heterotrophic metabolism and release CO<sub>2</sub> in the process. Microalgal growth can, therefore, be enhanced through sequential changing in the light intensity. Furthermore, it is imperative that irradiance be regulated according to the culture density. Low culture densities exposed to high light intensities would lead to photoinhibition of cells; and at high culture densities, light penetration becomes the limiting factor. Studies have also suggested that growth under red light (600–700 nm) enhanced PSII relative to PSI, whereas blue light (400–500 nm) could induce PSI. These findings suggest that blue and red lights are more suitable than others for both microalgal cell growth and CO<sub>2</sub> mitigation (You and Barnett 2004; Ravelonandro et al. 2008).

Microalgae possess a unique, inherent ability of accumulating large quantities of inorganic carbon in their cytoplasm. These concentrations are usually several orders of magnitude higher when compared to that on the outside. This system is called a CO<sub>2</sub>-concentrating mechanism (CCM). CO<sub>2</sub> concentration is an important factor for photosynthesis. Too high a concentration would increase the CO<sub>2</sub> mass transfer mechanism from the gas mixture to the medium, resulting in a pH drop. This sudden reduction in pH hinders the growth of most microalgal species. Flue gases typically contain very high concentrations of CO<sub>2</sub> (sometimes even greater than 30 %). It should also be noted that another key inhibitor of microalgal growth is the O<sub>2</sub> produced during photosynthesis. It is imperative that this gas be removed routinely and not be allowed to accumulate within the system (Pires et al. 2012).

Numerous studies have been documented on improving the CO<sub>2</sub>-fixation rate via the Calvin cycle, PEP carboxylase and/or through synthetic pathways (Rosgaard et al. 2012; Gimpel et al. 2013). These efforts have met with varying degrees of success. They can broadly be classified into these categories: engineering of RuBisCO for increased catalysis rates of carboxylation and reduction of the oxygenation reaction, enhancing the activation state of RuBisCO, enhancing the regeneration phase of the Calvin cycle and CO<sub>2</sub> enrichment around RuBisCO in an effort to inhibit the oxygenase reaction. Findings from most of these studies indicate that the challenge lies in the activity of RuBisCO for carbon flux through the Calvin cycle when CO<sub>2</sub> is not supplemented in the media, or under conditions of high temperature/light. *Chlamydomonas reinhardtii*

strains are RuBisCO deficient and are able to complete their life cycle heterotrophically. This makes them ideal candidates for engineering of RuBisCO. *Chlamydomonas* sp. containing varying amounts of RuBisCO have been engineered for the effective utilization of energy, carbon and nitrogen. This has been achieved using the nuclear genome of an MRL1-deficient strain and expressing the *rbcl* mRNA maturation factor MRL1 at different levels. When compared to the wild type, results for the deficient strain showed that RuBisCO could maintain phototrophic growth even when it was lowered up to 15 %. These findings suggest that based on the culture conditions (light intensity or CO<sub>2</sub> concentration), an inducible promoter for MRL1 could effectively be applied to modify RuBisCO accumulation (Rosgaard et al. 2012; Gimpel et al. 2013).

### Influence of flue gas composition on microalgal cultivation

The ability of microalgae to utilize CO<sub>2</sub> from flue gas and thereby mitigate the amount of carbon discharged into the atmosphere is an attractive idea. However, there are several major challenges that need to be addressed before this idea can be implemented:

When compared to atmospheric air, CO<sub>2</sub> is an effective supplement to stimulate microalgal growth. However, at high concentrations of CO<sub>2</sub> (>5 %), microalgal growth can be suppressed. This is often attributed to acidification of the cellular content which eventually hinders growth (Lee and Lee 2003). A study by Watanabe et al. (2000) showed that strains that grew well at CO<sub>2</sub> concentrations between 5 and 10 % had drastic decreases in their growth rate above CO<sub>2</sub> concentrations of 20 %. At elevated CO<sub>2</sub> concentrations, pH can drop down to 5 or even lower due to the formation of high amounts of bicarbonate buffer. This environmental stress causes a biological reduction in the capacity of microalgal cells to sequester CO<sub>2</sub>. Slight decreases in pH due to increased CO<sub>2</sub> concentrations usually have a minor impact on microalgal growth. Strong pH changes, however, could inhibit all growth (Kumar et al. 2011). Considering that flue gas usually contains between 3 and 30 % CO<sub>2</sub>, it is imperative to identify strains capable of growing under very high CO<sub>2</sub> concentrations. Screening studies have yielded strains that grow well in CO<sub>2</sub> concentrations between 30 and 70 % (Hanagata et al. 1992; Iwasaki et al. 1996; Sung et al. 1999). Findings by Olaiola (2003) indicate that microalgal growth may even be sustained at a 100 % CO<sub>2</sub> concentration by controlling changes in pH and only releasing CO<sub>2</sub> to the microalgae on demand.

Flue gases are often made up of CO<sub>2</sub>, water vapor, NO<sub>x</sub>, SO<sub>x</sub> and heavy metals such as nickel, vanadium and mercury (Packer 2009). CO<sub>2</sub> is a major component of industrial

**Table 3** Approximate annual global CO<sub>2</sub> flue gas emissions per industrial sector (Kuramochi et al. 2012)

Industrial sector	Worldwide CO <sub>2</sub> emissions (Gt/year)
Iron and steel manufacturing	2.3
Petroleum refineries	1.0
Cement production	2.0
Chemical and petrochemical	1.3

flue gases. Table 3 shows the approximate global CO<sub>2</sub> flue gas emissions per industrial sector. The use of flue gas to culture microalgae is advantageous in that a carbon-free nutrient medium could be used as the carbon requirement will be provided for by CO<sub>2</sub>. Pre-treatment costs could be minimized by direct utilization of flue gas; however, this would depend on the presence and quantities of the aforementioned components (Benemann 1993; Ono and Cuello 2007). Some researchers argue that the presence of NO<sub>x</sub> in flue gases pose little or no problem to microalgal growth, while the difficulty arises in the presence of SO<sub>x</sub>, which decreases the pH due to the formation of sulfurous acid (Maeda et al. 1995; Packer 2009; Kumar et al. 2011). Others, however, further argue that some strains are not inhibited by CO<sub>2</sub> with <50 ppm SO<sub>x</sub>, but can be inhibited by CO<sub>2</sub> when NO<sub>x</sub> are also present (Negoro et al. 1993; Lee et al. 2002; Ho et al. 2011). Therefore, denitrification and desulphurization along with cooling and dedusting represent scrubbing options that may be necessary for pre-treatment of flue gases. It should also be noted that nickel concentrations above 1 and 0.1 ppm of vanadium decrease microalgal productivity while mercury can be remediated by certain microalgal species (Packer 2009; Hende et al. 2012). A few tolerant microalgae exist: *Dunaliella tertiolecta* (Nagase et al. 1998), *Tetraselmis* sp. (Matsumoto et al. 1995), *Chlorella* sp.T-1 and *Chlorella* sp. HA-1 (Maeda et al. 1995) are capable of growing at NO<sub>x</sub> concentrations of up to 300 ppm. In these cases, excess NO is absorbed by the cultivation medium and transformed to NO<sub>2</sub>, which is then further utilized as a nitrogen source by the microalgae. Therefore, it must be noted that the tolerance of microalgae to NO<sub>x</sub> and SO<sub>x</sub> varies depending on the species. By buffering the medium, pH drops could be prevented leading to little or no changes in growth rates (Kumar et al. 2011; Hende et al. 2012).

Thermal stability is another essential characteristic required by microalgal strains involved in CO<sub>2</sub> sequestration. Flue gas temperatures are extremely high (around 120 °C) and could have an adverse effect on cells when introduced to the system (Ono and Cuello 2007; Kumar et al. 2011). Therefore, the feasibility of sequestering CO<sub>2</sub> from flue gas would either depend on using thermophilic microalgal species or installing a heat-exchange system.

Over the years, numerous species capable of tolerating temperatures up to 60 °C have been identified. Miyairi (1995) studied the effects of various CO<sub>2</sub> concentrations at different temperatures on the growth of the cyanobacteria *Synechococcus elongates*. The study revealed that a drop in pH at 52 °C with 60 % CO<sub>2</sub> was comparable to a drop in pH at 25 °C with 20 % CO<sub>2</sub>. These findings suggest that the temperature-dependent solubility of CO<sub>2</sub> gives an advantage to the thermophilic microalgae, enabling them to endure a higher concentration of CO<sub>2</sub>. As temperature increases so too does the ratio of O<sub>2</sub> to CO<sub>2</sub> solubility. This leads to considerable O<sub>2</sub> fixation by the oxygenase activity of RuBisCO. Increases in temperature also lead to a decrease in RuBisCOs affinity for CO<sub>2</sub> (Kumar et al. 2011).

Light is one of the principal requirements for photosynthesis. The relationship between light and photosynthesis can be illustrated using the photosynthesis–irradiance response (P–I) curve, which has three distinctive regions: light-limited photosynthesis, light-saturated photosynthesis and photoinhibition (Ralph and Gademann 2003). For effective CO<sub>2</sub> fixation leading to biomass production, optimum light intensity is necessary. Light becomes the limiting factor for microalgal cultivation when it is below the optimum, while exposure of cells to a high light intensity over long periods of time leads to photoinhibition. Photoinhibition is due to damage to the repair mechanism of PSII, which leads to the inactivation of other systems (electron carriers, oxygen evolving systems and the related D1/D2 proteins). Light intensity is dependent on wavelength, cell concentration and the penetrating distance of light as well as the geometry of the system (Kumar et al. 2011).

### Challenges and economics associated with microalgal CO<sub>2</sub> sequestration

There are numerous hurdles that need to be overcome before microalgae can be employed to significantly reduce CO<sub>2</sub> emissions at a commercial level. Strain selection and design of the culturing system are key factors in maximizing CO<sub>2</sub> mitigation rates. Even though open systems are much more cost-effective compared to closed PBRs, it is difficult to maintain culture purity in such systems. Closed systems are efficient vessels for sustaining axenic cultures as well as minimizing CO<sub>2</sub> loss to the atmosphere. However, cleaning and sterilizing of large-scale PBRs is difficult, and this then poses a problem in the production of high value-added products. Land availability for set-up of propagation vessels also becomes a problem in developing countries. For example, Kadam (2001) demonstrated that 1,000 ha of land area will be required for the construction of open ponds to mitigate CO<sub>2</sub> emissions from a 50-MW power plant. Carbon-fixation rates for microalgal cultures



differ under varying operational conditions. Preliminary ideas of these values are required so as to estimate space requirements for reactor implementation for effective CO<sub>2</sub> fixation. A Portuguese cement industry annually produces  $\pm 450$  kt of CO<sub>2</sub>. If two types of reactors (open ponds and light-diffusing optical fiber reactors) are considered for the effective sequestration of CO<sub>2</sub> emitted from this industry, the estimated space required would be very different. Studies showed that under natural day/night cycles, a 4,000 m<sup>3</sup> pond could sequester up to 2.2 kt of CO<sub>2</sub> per year. If ponds were to be scaled up to a height of 30 cm (to prevent dark zones), open ponds occupying an area of  $2.72 \times 10^6$  m<sup>2</sup> would be required to sequester almost all the CO<sub>2</sub> from this cement company. CO<sub>2</sub>-fixation rates for light-diffusing optical fiber reactors were reported to be  $4.44 \text{ g L}^{-1} \text{ d}^{-1}$ . Therefore, a reactor height of 1 m and a culturing area of  $2.78 \times 10^5$  m<sup>2</sup> would be needed to effectively sequester CO<sub>2</sub> from this cement plant (Stewart and Hessami 2005; Pires et al. 2012).

Geographical considerations must also be taken into account: fluctuations in temperature and solar irradiation over the seasons. Tropical areas are often considered most suitable for microalgal cultivation. To maximize the overall economic and environmental efficiency of microalgal CO<sub>2</sub> sequestration, culturing systems should be located as close as possible to the point source. Furthermore, a comprehensive plan should be compiled for the large-scale production of microalgae. This scheme should encompass modeling and LCA of the overall process. Failure in doing so could render many algal production systems unsustainable. It should also be noted that potential leaks from large-scale algal systems could cause ecological damage by eutrophication (Pires et al. 2012; Farrelly et al. 2013).

Bio-mitigation of carbon at large scale may entail the cultivation of non-transgenic as well as transgenic microalgae. Even under the best of conditions, minor releases of microalgae into the surrounding environment are bound to occur. While natural disasters would lead to massive releases. Very little has been discussed in the literature pertaining to minor or massive spills, especially those involving large amounts of non-transgenic microalgae. Possible risks from non-transgenic strains should not be taken lightly as the introduction of many non-native species to terrestrial and aquatic environments could have far-reaching ecological effects. In recent years, public awareness on the possible risks of commercial production of algae (especially non-transgenic algae) has increased. There have been suggestions that detailed scientific risk analysis reports for each strain be compiled, prior to large-scale cultivation (Gressel et al. 2013).

Biomass recovery poses a challenge in microalgal biomass production processes. This phase generally requires

one or more solid–liquid separation steps and usually accounts for 20–30 % of the total costs of production (Wang et al. 2008; Brennan and Owende 2010). Common harvesting practices include flocculation, filtration, flotation and centrifugal sedimentation. Some of these procedures can be highly energy intensive. Selecting an appropriate harvesting technology during microalgal cultivation is crucial to economic production of microalgal biomass (Brennan and Owende 2010).

The choice of harvesting technique is dependent on microalgae characteristics such as size, density and the value of the target products (Packer 2009; Brennan and Owende 2010). According to Brennan and Owende (2010), microalgae harvesting is a two-stage process, involving bulk harvesting and thickening. Bulk harvesting aims at separating biomass from the bulk suspension. Thickening is generally a more energy-intensive step as it involves concentrating the slurry through techniques such as centrifugation, filtration and ultrasonic aggregation (Packer 2009).

Flocculation is a popular technique as it is straightforward and cost-effective. This harvesting mode uses multivalent cations to overcome the overall negative charge present on the surface of microalgae. Multivalent metal salts (ferric chloride, aluminum sulfate and ferric sulfate) and polymers (polyelectrolyte and chitosan) are usually effective flocculants. An ideal flocculant is one that can be applied at low concentrations, inexpensive, non-toxic, and further downstream processing is not adversely affected by its use. For effective microalgal harvesting, flocculation is often combined with “floating.” This simple technique allows microalgae to float on the surface of the medium and can be easily removed as scum (Packer 2009). Dissolved air flotation (DAF) is a costly process that uses fine bubbles, which are injected under high pressure into the water column and then rise to the surface (Packer 2009). Most microalgae can be easily harvested from suspension by centrifugation. However, while centrifugal recovery is quick and easy, it is highly energy intensive (Ho et al. 2011). For low-value products (biofuels or animal feed), gravity sedimentation enhanced via flocculation is probably the most appropriate method (Ho et al. 2011).

Life cycle analysis (LCA) is a methodical environmental technique used for evaluating the input–output inventory of a product system throughout its entire life cycle. This process encompasses acquisition of raw material, production, use and ultimate disposal (from cradle to grave) (Tsoutsos et al. 2010). Such an assessment helps detect problem-shifting during life cycle stages (lower energy utilization during use, attained at a much higher manufacturing energy consumption cost), transfer from one medium to another (lower air emissions, but increased solid waste), identify technological innovation opportunities as well as project the environmental performance based on a

selected functional unit of the product (Gnansounou et al. 2009; Tsoutsos et al. 2010). Energy balance, an essential factor within a microalgal system, is usually calculated by evaluating energy inputs required at each LCA stage, against the total required inputs of the embodied energy related to the specific product/idea of interest. Energy balance may be influenced at each or any life cycle phase. It is thus imperative that each stage be closely monitored so as to prevent any mishaps that could have far-reaching implications on the rest of the chain.

In theory, an efficient GHG capture mechanism would entail the capture of CO<sub>2</sub> flue gas from power stations and its subsequent utilization in promoting the growth of microalgae for biofuel production (Khoo et al. 2011). However, according to Campbell et al. (2011), such a theory is only marginally correct. They propose that permissible carbon credits do not arise from captured flue gas due to the fact that the algae-derived fuel will ultimately be burnt and returned to the atmosphere. Carbon credits arise from the replacement of the fossil fuel that would have been used if the biofuel had not become accessible. Additional carbon credits may become available if the spent microalgal biomass went into the generation of electricity. Hence, the biomass would displace any coal, gas or other materials that would have originally been used for energy production (Campbell et al. 2011). It is necessary to therefore carry out comprehensive life cycle calculations of the processing energy required to create the biofuel. These calculations would allow one to quantify GHG emissions at each stage of the process, enabling the researcher to establish whether the process does in fact emit less CO<sub>2</sub> than the use of fossil fuels and if this is the case to quantify the associated GHG savings (Gnansounou et al. 2009; Khoo et al. 2011). Different microalgal propagation systems can only be compared if they perform the same function. After selection of a shared function, a unit is chosen that will enable one to compare the systems on the same quantitative basis. All energy and mass flows within the operating system will then be standardized to this functional unit (Kadam 2001). When assessing the CO<sub>2</sub> balance of a system, it is necessary to take into account the total discharge from fossil energy versus the CO<sub>2</sub> uptake of the microalgae during cultivation (Khoo et al. 2011).

Life cycle analysis (LCA) essentially covers cultivation, harvesting, lipid extraction and finally product formation. Wastewater emissions as well as waste (solids or wastewater) treatment is generally not covered in a LCA study (Khoo et al. 2011).

Alabi et al. (2009) did a detailed cost analysis on different algal production systems. Findings from this study revealed that capital investment required per liter volume for each production facility was as follows: US\$52L<sup>-1</sup> for raceway ponds, US\$2L<sup>-1</sup> for fermenters and US\$111L<sup>-1</sup>

for PBRs. Production costs (which included labor and running costs) for a kg of dried algal biomass showed that PBRs were the most expensive system to operate (US\$7.32 kg<sup>-1</sup>), followed by raceway ponds (US\$2.66 kg<sup>-1</sup>). Surprisingly, fermenters proved the least expensive, at a cost of US\$1.54 kg<sup>-1</sup>. Another cost analysis study by Molina-Grima et al. (2003) illustrated that it would cost US\$32.16 to produce a kg of microalgal biomass (dry) in a standard PBR. This study further serves to highlight the high costs associated with closed systems. Despite years of research on the commercial viability of closed PBRs, a breakthrough has yet to be developed.

Carbon dioxide (CO<sub>2</sub>) sequestration costs depend largely on microalgal productivity, carbon content of selected strains and overall process efficiency. Stewart and Hessami (2005) investigated the carbon uptake rate of *Synechocystis aquatilis* and found that this strain was able to uptake carbon at a rate of 1.5 g/L/day. It was then proposed that under natural light conditions, this strain could mitigate up to 2.2 kt CO<sub>2</sub>/4,000 m<sup>3</sup> pond/yr. In a separate study, Kadam (2001) demonstrated that a 1,000-ha open raceway pond could mitigate 210,000 t/yrCO<sub>2</sub> of the 414,000 t/yrCO<sub>2</sub> generated by a 50-MW power plant. This suggests that a 50 % reduction in CO<sub>2</sub> from the flue gas emissions could be achieved. Carbon sequestration strategies routinely implemented in the USA entail the injection of carbon in saline aquifers and amine scrubbing of CO<sub>2</sub> from flue gases. Carbon burial in saline aquifers is far cheaper (US\$40 t<sup>-1</sup> CO<sub>2</sub>) as opposed to amine scrubbing (US\$150 t<sup>-1</sup> CO<sub>2</sub>). The combined costs of these 2 processes, however, are still significantly lower when compared to carbon mitigation using microalgal open ponds (US\$793t<sup>-1</sup> CO<sub>2</sub>). As can be seen, this figure is extremely cost intensive. However, mitigation costs using algae could be reduced if recovered biomass was utilized in the production of valuable by-products (Pires et al. 2012; Farrelly et al. 2013).

## Conclusion

The use of microalgae for the purpose of CO<sub>2</sub> sequestration is a unique environmental technology. Microalgae are promising candidates for CO<sub>2</sub> mitigation, which aids in combating GHG-related environmental impacts and has the added benefit of producing renewable biomass. In comparison with terrestrial plants, microalgae are capable of fixing CO<sub>2</sub> at a rate several times higher than plants owing to their high photosynthetic efficiencies. Additionally, selected microalgal strains can assimilate CO<sub>2</sub> from industrial flue gas within various ranges of concentrations from ambient (0.036 % v/v) to extremely high (100 % v/v). CO<sub>2</sub> fixation from industrial flue gas coupled with nutrient recycling from wastewater makes algae ideal



organisms for the production of useful by-products. Several algal strains capable of growing in and consuming high CO<sub>2</sub> concentrations from flue gas streams have been isolated and propagated; however, they are just a fraction of the vast majority of total algal species that have yet to be isolated and exploited for this purpose. An interesting approach would be to utilize a consortium of hyper-CO<sub>2</sub>-tolerant microalgal strains instead of a single strain. For effective CO<sub>2</sub> sequestration, an in-depth knowledge of flue gas composition and biology of microalgal cells would be required. Temperature, pH, SO<sub>x</sub>, NO<sub>x</sub>, heavy metals, light, culture strain and density, as well as CO<sub>2</sub> mass transfer and O<sub>2</sub> accumulation are major factors that affect CO<sub>2</sub> sequestration and biomass production. LCA is imperative to ascertain economic feasibility and environmental sustainability of algal CO<sub>2</sub> sequestration systems. For example, harvesting and dewatering are processes that are highly energy intensive; thus, research efforts should focus on developing an optimal harvesting strategy. Furthermore, strategic engineering decisions should be taken into consideration to realize effective microalgal CO<sub>2</sub> sequestration systems. Microalgal cultivation requires the development of suitable reactors with features such as high S/V ratio, mixing, mass transfer, scalability and ease of operation. Airlift bioreactors that distribute light through optical fibers could be a possible solution (this increases the ratio between the illumination surface and reactor volume).

Technical viability of algal CO<sub>2</sub> sequestration has already been demonstrated in a few systems; however, the major challenges are the strategic and holistic development of technologies that will improve economic feasibility of algal CO<sub>2</sub> sequestration and make this a viable industrial approach to GHG remediation.

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