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# Microalgae: a robust "green bio-bridge" between energy and environment

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# Abstract

Microalgae are a potential candidate for biofuel production and environmental treatment because of their specific characteristics (*e.g.* fast growth, carbon neutral and rich lipid accumulations). However, several primary bottlenecks still exist in current technologies, including low biomass conversion efficiency, bio-invasion from external environment, limited or costly nutrient sources, and high energy and capital input for harvest, stalling its industrial progression. Coupling biofuel production with environmental treatment makes microalgae a more feasible feedstock. This review focuses on microalgal biotechnologies for both bioenergy generation and environmental treatment (e.g. $CO_2$ sequestration and wastewater reclamation). Different intelligent technologies have been developed, especially during the last decade, to unclog the bottlenecks, including mixotrophic/heterotrophic cultivation, immobilization, and co-cultivation. It has been realized that any single purpose for the cultivation of microalgae is not an economically feasible option. Combinations of applications in biorefineries are gradually reckoned to be necessary as it provides more economically feasible and environmentally sustainable operations. This presents microalgae as a special niche occupier linking the fields of energy and environmental sciences and technologies. The integrated application of microalgae is also proven by most of the life-cycle analysis (LCA) studies. This study summarizes the latest development of primary microalgal biotechnologies in the two areas that will bring researchers a comprehensive view towards industrialization with an economic perspective.

# 1. Introduction

Microalgae, prokaryotic or eukaryotic unicellular microorganisms, are capable of growing in terrestrial, freshwater, brackish water and seawater habitats. Compared to higher plants, the simpler unicellular structure makes microalgae grow relatively faster with usually a larger pool of specific compounds like lipids (*1*), carbohydrates (especially starch (*2*)), pigments (*3*), and antioxidants (*4*). The thriving biodiversity enables microalgae to be specially applicable in a variety of fields, including aquaculture, food, pharmacy, environmental engineering as well as biofuel production (*5-8*). Existence of algae can be dated back to billions of years (*9*). They are ubiquitous with high adaptivity even under harsh environments such as high temperature or high salinity (*10*). Due to their robust nature, microalgae find application in the production of different sustainable biofuels, and treatment of various environmental problems.

Nowadays, one major focus on microalgae is in using them as renewable materials for biofuels. Fossil derived fuels is a depleting resource and a predominant contributor to global warming and climate change (*11*). It is evidenced that the largest source of carbon dioxide is fossil fuel burning, taking up around three quarters of the total anthropogenic emissions (*12*). The increasing demand for energy and our heavy reliance on carbon based fossil fuel combustion has resulted in this crisis (*13*). The unsustainability of deriving renewable energy from food crops is increasingly becoming apparent (*14*). Microalgae can offer carbon-neutral biofuels, *e.g.* bio$H_2$ (*15*), biogas (*16*), bioethanol (*17*), biodiesel (*14*) and bio-oil (*18*), more efficiently without adversely affecting the supply of food reserves (*19*). However, several primary bottlenecks still exist, including low biomass conversion efficiency, bio-invasion form external environment, limited or costly

nutrient sources, and high energy and capital input for harvest, stalling industrial progression. Coupling biofuel production with environmental treatment gives extra benefits making microalgal biofuels more feasible.

Carbon mitigation is now a serious environmental concern and countries around the world have now pledged their intentions to cut carbon dioxide emissions towards alleviating global warming and climate change concerns resulting from greenhouse gas emissions. The recently concluded COP21 (the 21st yearly session of the Conference of the Parties) saw pledges made by key emitters to cut carbon dioxide emissions. The UK Government has committed to reducing its territorial greenhouse gas emissions to 80% of 1990 levels, by 2050 (*20*). China pledged to peak carbon emissions by 2030 and source 20% of its primary energy from non-fossil carbon sources by 2030. USA is aiming to get 26-28% domestic reduction in greenhouse gases by 2025 compared to 2005, and the EU is aiming for upward of 40% domestic reduction in GHG emissions by 2030 compared to 1990 (*21*). On the basis of photosynthesis, development of microalgae-based technologies will have a significant role to play in future carbon dioxide mitigation strategies.

Microalgae have also been used for reclamation of wastewater. Wastewater from municipality or industry usually contains considerable nitrogen, phosphorus and other pollutants. Its discharge into a natural water body does not only pose a threat to the ecosystems but also supplies a rich source of nutrients leading to eutrophication and algal bloom (*22*). Phytoremediation, using green plants to remove or reduce pollutants, is regarded as a simple low-cost clean up technology for wastewater treatment (*23, 24*). To do this, many principles for selecting a potential plant should be taken into consideration, such as growth rate, nutrient-removal efficiency and tolerance to the toxic pollutants (*23*).

From this aspect, a number of microalgal species, such as *Chlorella zofingiensis, Chlorella sorokiniana and Scenedesmus obliquu,* have been found to be capable of growing in wastewater to remove specific pollutants (*25-29*).

There have been a substantial number of articles that reviewed the microalgae-based biofuels and various associated bioreactors (*7, 30, 31*). In contrast, the potential of microalgal cultivations for both energy production and environmental treatment together is rarely reviewed and is yet to be widely practiced in industry, even though industrial cultivation of microalgae have been employed in the production of selected products, such as pigments like astaxanthin or special lipids as omega-3 fatty acids (*32*). Therefore, this article critically reviews the research progress in applying microalgae (both biotic and abiotic) for biofuel production and environmental treatment, as well as the current advaned and combined technologies (e.g. trophic modes, immobilized systems and co-cultivation) for solving current bottlenecks. The life cycle assessment with the economic feasibility for the technologies is also discussed.

# 2. Environmental treatment

## 2.1 $CO_2$ sequestration

The fast−growing microalgae have a distinguishing ability to sequester $CO_2$ with a superior efficiency that is 10 to 50 times greater than that of terrestrial plants (*33*). Microalgae can theoretically capture up to 9% of the incoming solar energy, *via* photosynthesis, to produce 280 tons of dry biomass $ha^{-1}$ $year^{-1}$ whilst consuming around 513 tons of $CO_2$ (*34*). Due to the low level of $CO_2$ in the atmosphere (0.04%), the available $CO_2$ gas is ra-

ther low and underfeeds microalgae. Using carbon dioxide from flue gases for microalgae alleviates the related environmental issues like global warming, and has the potential for cost reduction in biomass and biofuel production. Both artificial and real exhaust gases have been tested (Table 1).

Many microalgal species can fix high concentrations of $CO_2$. As listed in Table 1, *Botryococcus braunii*, *Chlorella kessleri*, *Chlorococcum littorale*, *Chlorella sp.*, *Scenedesmus obliquus*, *Scenedesmus sp*., and *Spirulina* sp. appear to have good potential for $CO_2$ sequestration. *Spirulina* sp. is one of the potential candidates with a high ability for biomass production as well as carbon dioxide fixation (*45, 54*). When it was cultivated at 30 °C in a three-stage serial tubular photobioreactor (*45*), the maximum specific growth rate, maximum productivity rate and maximum cell concentration were 0.44 $d^{-1}$, 0.22 g $L^{-1}$ $d^{-1}$ and 3.50 g dry cell $L^{-1}$, respectively, with both $CO_2$ concentrations 6% and 12% (v/v). Yun *et al*.(*44*) cultivated a freshwater microalga, *Chlorella vulgaris*, in wastewater discharged from a steel−making plant to remove ammonia from wastewater and $CO_2$ from flue gas simultaneously. The high $CO_2$ concentration of 15% in the simulated flue gas significantly improved the growth of the algae with a $CO_2$ fixation rate of 26.0 g $m^{-3}$ $h^{-1}$ and an ammonia removal rate of 0.92 g $m^{-3}$ $h^{-1}$. *Chlorococcum littorale*, a marine alga, exhibited spectacular tolerance to up to 40% of high $CO_2$ concentration (*55*). There are also some algae which can tolerate pure $CO_2$ like *Chlorella* sp. but under these conditions they show a limited growth rate (*43, 47*).

If the temperature of flue gases cool down to an appropriate range, microalgae can be directly exposed to the flue gases with moderate levels of SOx and NOx (up to 150 ppm) (*56*). Table 1 also gives some pilot practice of microalgae in $CO_2$ sequestration

from exhaust gases of various industrial plants, including coal−fired thermal power plant, oil−fired power plant, coke oven, cement plant, and steel plant. Compared with air, the growth rate of microalgae is enhanced indeed by the higher $CO_2$ content in the flue gases. A thermal− and $CO_2$−tolerant mutant strain, *Chlorella* sp. MTF−7 was isolated and its on−site bioremediation potential was studied by direct aeration with the flue gas (25% $CO_2$) from a steel plant (*49*). The biomass concentration, and growth rate were 2.87 g $L^{-1}$ and 0.52 g $L^{-1}$ $d^{-1}$, respectively.

Besides, some cyanobacteria also exhibit thermal− and/or $CO_2$−tolerant behaviours, such as *Cylindrospermopsis raciborskii* (*57*), *Synechocystis* sp. (*58*), *Planktothrix* sp. (*59*), *Spirulina platensis* (*54*). Under high temperature with sufficient supply of nutrients, cyanobacteria are able to compete with other algal species and become the dominant one (*57, 59*). Cyanobacteria are capable of carrying out different strategies in response to different $CO_2$ levels (*60, 61*). At low $CO_2$ levels, efficient $CO_2$−concentrating mechanism (CCM) would be aroused to elevate $CO_2$ concentration in the vicinity of RuBisCO active centers (Ribulose bisphosphate carboxylase oxygenase, a key enzyme for $CO_2$ fixation). At high $CO_2$ levels, cyanobacteria would bloom by constitutively expressing both the low− and high−affinity $CO_2$ uptake genes, yet downregulating *cmpA* encoding the high−affinity bicarbonate uptake system BCT1 (*e.g. Microcystis*) (*60, 61*).

More details about the application of microalgae in $CO_2$ sequestration have been discussed by several reviews (*62, 63*). However, the amount of fixed carbon is such an important parameter that should be studied carefully. Most results show that only a relatively low proportion (around 10−20%) of $CO_2$ can be captured, which means 80−90% of $CO_2$ is released to the atmosphere (*49, 51*). This is mainly because most of the current

photobioreactors cannot maintain a reactor $CO_2$ residence time of around 4h that is required for significant $CO_2$ removal (*64*). As such, an ideal bioreactor should be designed to reduce the $CO_2$ release and enhance the $CO_2$ sequestration efficiency. A double−set photobioreactor system was developed and employed to culture *Chlorella* sp. MTF−7 with intermittent flue gas aeration (*49*). This reached up to 60% of average $CO_2$ removal efficiency, 70% and 50% of NO and $SO_2$ removal efficiencies, respectively. A strain *Scenedesmus obliquus* (*S. obliquus* WUST4) mutated by UV was grown in a 100 L air−lift photobioreactor to capture $CO_2$ from a flue gas emitted from the combustion chamber in a coke oven (*52*). The flue gas was composed of 18% $CO_2$, 2% $O_2$, 200 ppm or below SOx, 150 ppm or below NOx. As a result, a very high $CO_2$ removal efficiency was reached (67%) under the optimal operation conditions.

## 2.2 Wastewater reclamation

Even though flue gases can supplement the limited atmospheric $CO_2$, the growth of microalgae still needs nitrogen, phosphorus, and other nutrients. Artificial addition of these nutrients from commercial market will increase the capital cost especially in a large scale cultivation (*65*). A solution to this is using wastewater to cultivate microalgae (*66*). Typically, a wastewater contains rich nitrogen and/or phosphorus. Direct discharge of wastewater into a water body can result in severe eutrophication and even vital disorder of its ecosystem (*67*). The use of wastewater as a nutrient source seems to be an inevitable option when culturing microalgae for biofuel (*65*). This conserves the cost of nutrients and earns an extra benefit from wastewater purification.

As displayed in Table 2, many microalgae species, such as *Botryococcus brauinii*, *Chlorella*, *Nannochloris*, *Scenedesmus*, *Spirulina,* can be used for wastewater treatment to remove nutrients, heavy metals and organic carbon. Some of these microalgae have been grown for nutrient−removal (*e.g.* N/P) from artificial wastewater. Two nanoplanktonic microalgal species, *i.e*. *Nannochloris* sp. and *Scenedesmus intermedius* Chod. isolated from different sources of pig manure, were studied for their growth rate, and nitrogen and phosphorus uptake (*68*). These autochthonous species exhibited excellent performance with respect to the N/P uptake rates, compared with the commercial species. For *S. intermedius*, the uptake rates of nitrogen and phosphorous were 0.022 mg $h^{-1}$ and 0.014 mg $h^{-1}$, respectively; whilst for *Nannochloris* sp., they were 0.011 mg $h^{-1}$ and 0.006 mg $h^{-1}$, respectively.

Real wastewaters, such as piggery wastewater, urban wastewater and wastewater treatment plant effluents, have also been tested for pollutant removal by microalgae. Sometimes, higher biomass productivity can be reached in wastewater than that in artificial media in laboratories. An *et al*. (*69*) cultivated *Botryococcus braunii* UTEX 572 in pretreated piggery wastewater at 25°C with 1% of $CO_2$, and obtained up to 80 % of nitrogen removal with an extraordinary biomass productivity of 8.5 g $L^{-1}$ and hydrocarbon level of 0.95 g $L^{-1}$. It needs to be pointed out that the potential toxicity of wastewaters to microalgae should be pre−tested before being used for cultivation. If the wastewater contains concentrated chemicals or exhibits toxicity to the growth of microalgae, a pre−treated or dilution procedure is usually required. Olguín *et al*. (*75*) grew *Spirulina* (Arthrospira) in swine wastewater in outdoor raceways with high concentration of nutrients (1519 mg $L^{-1}$ N and 620 mg $L^{-1}$ P). The anaerobic effluents from digested pig waste

were diluted with untreated seawater in a proportion of 2% (v/v), followed by freshwater (1:4) and supplemented with 2 g $L^{-1}$ $NaHCO_3$. The semi−continuous cultures produced an average biomass productivity of 11.8 g $m^{-2}$ $d^{-1}$, with 84−96% and 72−87% of $NH_4$−N and P removals, respectively. Alternatively, tolerant and adapted microalgae can be screened naturally or isolated through a period of acclimation cultivation in wastewater, *e.g. Chlorella luteoviridis*, *Parachlorella kessleri* (*76*). *Chlorella* is one of the genera that can grow in a variety of wastewaters (*77*). For instance, a *Chlorella* isolate has shown high tolerance and removal ability to polybrominated diphenyl ethers from wastewater treatment plants (*78*). Besides, *Chlamydomonas mexicana* has been found to be able to degrade herbicide atrazine and thus, can be employed for the remediation of atrazine-contaminated streams (*79*).

Microalgae have also been proposed as a promising tool to remove heavy metals, which are a common type of contaminants in industrial wastewater (*80*). The presence of heavy metal ions in aquatic food chains can cause severe health problems for humans, such as damage to the nervous system (lead) and kidney (lead and cadmium), and carcinogenic (nickel). Considering the nutrient requirements for organisms, heavy metals can be divided into two groups: (1) essential but only at trace amount (*e.g.*, Cr, Co, Cu, As, Ni, Se, Va, and Zn); (2) highly poisonous without any known nutritional value (*e.g.* Pb, Hg, Cd, Ur, Ag, and Be) (*81*). Conventional methods for removing metals from contaminated waters (like reverse osmosis, electrodialysis, ultrafiltration, ion exchange, chemical precipitation and phytoremediation *etc*.) have drawbacks, such as high reagent and energy requirements, generation of toxic sludge, and incomplete removal (*82*). In contrast, using microalgae for heavy metal removal is an ecologically safer, cheaper, and

more efficient means (*82*). Owing to the various benefits of using microalgae for heavy metals removal, many studies have been carried out to explore this technology and its research and development have been thoroughly reviewed, including the species selection, removal mechanisms as well as the influencing factors (*80, 82, 83*).

However, in suspension cultures the presence of microalgae as free cells makes them vulnerable to the native microorganisms already present in the wastewaters. It has been noted that the population of microalgae directly cultivated in unsterile wastewater may be significantly lower than that in sterile wastewater (*84*). Moreover, the harvest of free microalgae cells from the wastewater or culture medium is another stubborn problem because of the small size of microalgae cells and the diluted cultures leading to a large capital expenditure (*85*). For these reasons, immobilization of microalgae in special materials is drawing more attention with the attempt to solve these problems occurring in the suspension culture mode (see next section).

## 3. Promising technologies for unclogging bottlenecks

To date, there are several primary bottlenecks existing that inhibit the large-scale industrial application of microalgae, including low biomass conversion efficiency, bio-invasion from external environment, limited or costly nutrient availability, and high harvest cost. For this reason, many researchers have developed various intelligent technologies with the attempt to unclog the bottlenecks and make it economically feasible. Bioreactor design like raceway pond, tubular photobioreactor and other photobioreactors is one method, which has been widely studied and not discussed here, but elsewhere (*7, 86*). Mixotrophic

and heterotrophic cultivations are used to enhance biomass or lipids production due to the low efficiency in photoautotrophy. Immobilization is culturing algal cells on surfaces of special materials or in tiny enclosed environments to prevent bio-invasion from other microorganisms and to easily harvest the cells. Co-cultivation is culturing target microalga with other microalgal specie(s) or other microorganisms for nutrient complementation or bioflocculation.

## 3.1 Mixotrophic & Heterotrophic cultivation

Heterotrophy is using organic compounds for growth, without the need for light or inorganic carbon; whist, mixotrophy is a combination of autotrophy and heterotrophy (*87, 88*). Compared to autotrophy, heterotrophy can result in an extraordinary increase in biomass; mixotrophy usually lies between the two but the benefit of producing photosynthetic metabolites remains (*89*). As such, mixotrophic and heterotrophic cultivation of microalgae are employed to surmount bottlenecks of autotrophy, including limitation in $CO_2$ availability and light distribution, suppression by photosynthetic $O_2$ in the medium, less efficient production, among others. This strategy allows the use of most industrial fermenters to produce high densities of microalgae cells, which is regarded as an economically feasible method for large-scale biomass production (*90*).

There are a variety of saccharides and other organic carbon sources that can be utilized by microalgae, such as glucose, glycerol, and acetate. Among saccharides, monosaccharides are more frequently used than disaccharides. Particularly, glucose is the most common carbon source for the majority of heterotrophic algae, followed by galactose and fructose. With glucose, higher rates of growth and respiration are obtained than with any

other substrate like acetate, which may be owing to the higher energy it can produce (~2.8 KJ $mol^{-1}$) than acetate (~0.8 KJ $mol^{-1}$) (*91*). Although acetate is also a common carbon source (e.g. for *Chlamydomonas reinhardtii*) and has the effect of buffering high pH levels in the culture, its high concentrations could be toxic for many microorganisms (*92*), indicating that fed-batch cultivation at a low level of acetate is a good choice. In contrast, glycerol, which is an osmoticum that keeps the osmotic equilibrium in cells, exhibits almost no toxic effects on many species even at high concentrations (*e.g.* 5 g $L^{-1}$), such as *Neochloris oleabundans, Botryococcus braunii*, *Dunaliella* sp. and *Scenedesmus* sp. (*93, 94*). Similar to acetate for *C.reinhardtii*, strains in genus *Chlorella* also had a wide variation in response to glycerol, which can be explained by phylogenetic analysis that showed high diversity within the *Chlorophyta* phylum (*95*). This diversity implies that a desired strain is possible to be screened out or domesticated for a specific organic compound, especially when it is used for wastewater treatment that supplies cheap carbon sources (*95, 96*).

Apart from carbon, nitrogen is another important element that can contribute from 1 to 10% dry weight to microalgal cells (*90*). Nitrogen plays a critical role on microalgal metabolism, and various nitrogen sources have been tested, mainly including nitrate, ammonium, urea, among others. Nitrate is a primary source of nitrogen and can be assimilated by most algae. The assimilation of nitrate requires reduction to ammonia and large amounts of energy, accompanied with an increase in pH (*97*). Ammonium is also a preferred nitrogen form for algae because its assimilation does not involve a redox reaction leading to less energy consumption (*98, 99*). Nitrate seems to be friendlier to microalgae than ammonium as high concentration of nitrate does not show toxicity to cells, but am-

monium does (*100*). Toxicity of high ammonium concentration in algal culture can be caused by both the unionized ammonia ($NH_3$) and the ionized ammonium ($NH_4^+$), which can result in remarkable disturbances to both extracellular and intracellular pH, and cause damages like pigment degradation and even cell lysis (*101*). In general, the declining order of tolerance was found to be: Cyanophyceae > Diatomophyceae > Raphidophyceae > Prymnesiophyceae > Dinophyceae (*101*). Urea is considered to be a low-cost and efficient nitrogen source for algal growth as urea contains approximately 46.7% nitrogen content. Urea and other organic nitrogen like amino acids (*e.g.* glutamine) have been reported to have a positive influence in the growth of some species, such as *Chlorella* sp., *Coccomyxa acidophila* and *Chlorella variabilis* (*102-104*).

Microalgal preference for a nutrient is not only algae-dependent but also affected by other nutrients. Selection of a nitrogen source can be affected by the carbon source used because their metabolisms are intimately associated (*90*). For example, It is worth noting that the uptake rates of ammonium could be improved by the addition of acetate under mixtrophic regimen, but not under heterotrophic conditions (*105*).

Although mixotrophic and heterotrophic growth of microalgae can produce higher biomass or lipids than autotrophic growth, they also have several major limitations, including (a) less species for heterotrophy, (b) higher costs associated with the addition of organic substrate, (c) contamination and competition with other microbes, (d) inhibited growth by excess organic substrate, (e) loss of some autotrophic metabolites, and (f) deeper footprint on environment by increased wastes (*89*). If a suitable species can be found, then wastewater should be given a priority as a low-cost nutrient source. Perez-Garcia *et al.* pointed out that using microalgae for wastewater treatment followed by bio-

fuel production from the produced biomass might offer a strategy to overcome some limitations of these mixotrophic and heterotrophic technologies, making them economically acceptable (*90*). Otherwise, applying the co-culture system of microalgae and bacteria under heterotrophic conditions could give even higher potential for wastewater treatment and/or biofuel production (see section 3.3 Co-culture) (*106*).

## 3.2 Immobilized systems

By being fastened in biological or inert matrices, immobilized microalgae (or other microorganisms) can also be used to remove nutrients, heavy metals and other industrial pollutants (*107, 108*). Six types of immobilization have been developed: adsorption, affinity immobilization, covalent coupling, confinement in liquid−liquid emulsion, capture behind semi−permeable membrane, and entrapment (*109*). Generally, the immobilization process consists of two steps: I) mixing the microbial suspension with macromolecule monomers of polymer; II) solidifying the mixture to form a polymeric gel with diverse shapes (*24*). Although natural polymers (*e.g.* algal polysaccharides, chitosan) are less stable in wastewater than synthetic polymers (acrylamide, polyurethane, polyvinyl, resins), the most popular polymers used are the natural polymers alginate and carrageenan (*110, 111*). There are two types of algal immobilization in the treatment of wastewater, i.e. immobilized in alginate films on polyester mesh and encapsulated in alginate beads.

Immobilization of microalgae in the treatment of wastewater has been reviewed by several articles (*24, 111*) and the advances in this area since 2010 is updated in Table 3. Immobilized microalgae can have higher nutrient removal rate than free cells but the leakage of cells to the medium is a common issue (*112*). This problem depends on the

material of capsules and can be overcome by using sodium cellulose sulphate/poly-dimethyl-diallyl-ammonium chloride (NaCS−PDMDAAC) (*71*). Immobilization usually has a disadvantage of lower biomass productivity. This is because the entrapped microalgal cells in the capsules are exposed to a higher pressure, weaker mass transfer, and higher viscosity environment than free cells and thus, require more energy and nutrient to maintain normal physiology (*71*). An exception has been reported where compared with free cells, the immobilized *Chlorella sorokiniana* GXNN 01 in alginate had not only similar biomass productivity but also higher removal efficiencies of both ammonium and phosphate under different conditions especially micro−aerobic condition (*113*).

With improved techniques, immobilization in alginate beads becomes one of the most popular methods enabling high-density cultivation, which is not affected by the thickness of beads and the supply of $CO_2$. For instance, *Botryococcus braunii* (Kützing) and *Chlorellavulgaris* (Beijerinck) were entrapped in low−sodium silica gels in a novel photosynthetic $CO_2$ bioconvertor for $CO_2$ assimilation (*116*). The mesoporosity of the hybrid gels enabled diffusion of both nutrients and gases. Although there are still scarce investigations in using this technique for $CO_2$ mitigation or other applications, the potential capacity for this purpose can be envisioned.

Immobilization in polymers poses an extent of pressure on microalgae and their metabolism. Nevertheless, many benefits have been observed for the entrapped microalgae, including resistances to aggressive zooplankton or other undesirable organisms, improvements in cellular function and behavior, no secondary pollution (environmentally friendly), no filtration of the treated wastewater, resistances to toxic compounds, and co−immobilization with other microorganism for different purposes (*24, 84, 117*).

## 3.3 Co-culture

Over the last decade, more and more researchers have diverted their attention from single cell cultures to co-culture systems of microalgae and other microorganisms to explore novel strategies. This is because many expected and unexpected benefits can be obtained *via* the interaction between the microorganisms in the system, even though it is usually very complicated and difficult to untangle. The objective of the mixed culture is mainly based on the complementation of each member of the consortium to form a symbiosis, by using one species to produce renewable and low-cost nutrients, to improve the production of specific bioproducts (like biomass, lipid or pigments), or to harvest the other, as shown in Table 4.

### *Co-culture with microalgae*

On the basis of the microbial type, there are three major groups that can be defined, namely microalgae, bacteria, yeast and molds (*106*). Co-cultivation of more than two microalgal species is primarily carried out to produce more lipids or biomass (*118, 119*). One of the species is usually oleaginous and its lipid productivity is largely caused by the more biomass produced, because the lipid content (%) in co-culture system is not the highest, compared to mono-cultures. For example, the lipid content (%) in the co-culture of *Chlorella* sp. and *Monoraphidium* sp. (47.79%) is higher than that in the mono-culture of *Chlorella* sp. (32.03%), but lower than that in the mono-culture of *Monoraphidium* sp. (51.72%) (*119*). Owing to the higher biomass productivity of the co-culture (62 mg $L^{-1}$ $d^{-1}$) than that of the two mono-cultures (58.4 and 35.60 mg $L^{-1}$ $d^{-1}$, respectively), its eventual lipid productivity was also higher than that of the two monocultures. It

indicates that the photosynthetic efficiency of the co-culture was higher, with a fast consumption of nutrients for growing biomass leading to an earlier arrival of stationary phase. However, it was observed that high density of *Chlorella* sp. suppressed the growth of *Monoraphidium* sp. over the entire experimental period (*119*). Admittedly, there must be some beneficial effects of each member of the co-culture to each other, but this strategy of co-cultivation of two (at least) microalgal species should be carefully considered and verified because they are nutrient competitors.

## *Co-culture with other microbes for bioflocculation*

Generally, the principle of co-culture between microalgae and other microorganisms is based on no inhibition on each other at first, then cooperation with complementary functions. One kind of cooperation is using a microorganism for harvesting microalgae. Conventional harvesting can account for up to 50% of the total cost of biodiesel production, making it unfeasible for the microalgal industry due to the increased energy requirements and the addition of chemicals (*131*). For this reason, bacteria (*e.g. Solibacillus silvestris* and *Bacillus* sp.) and yeast (*Aspergillus fumigatus*) have been employed to function as bio-flocculants to harvest microalgal cells (*120-122, 131*). The flocculation efficiency varies from 50 to 90% with a time course from 10 days to 30 s. However, when microalga was co-cultivated under heterotrophic conditions, competition and growth inhibition were observed especially for long-term co-cultivation (*122*).

## *Co-culture with other microbes for wastewater treatment*

Photoautotrophic microalgae can supply photosynthetic $O_2$, fixed carbon for bacteria, yeasts and molds, which returns with respiratory $CO_2$, vitamins and minerals *etc*. Moreover, this kind of cooperation forms a robust team especially for wastewater reclamation: microalgae are able to remove nitrogen and phosphorous nutrients; whist the others are efficient to degrade chemical oxygen demand (COD) (*27, 124, 125*). For example, de-Bashan *et al*. co-cultivated *Chlorella vulgaris* or *Chlorella sorokiniana* with *Azospirillum brasilense* in a wastewater collected from a municipal wastewater treatment plant, reaching removal of up to 100% ammonium, 15% nitrate, and 36% phosphorous within 6 days, plus enhanced growth of microalgae (*27*). The phosphorous removal could be further improved up to 72% by starvation of algae in a saline solution for several days (*124*). Bacteria of the genus *Azospirillum* are well-known plant growth-promoting bacteria (PGPB) used as inoculants for control of phytopathogens and for plant growth promotion, which has been also found to be microalgae growth-promoting bacteria (MGPB) (*27, 124, 125*).

## 4. Integrated applications

Investigation of a single application of microalgae is conceivable in specific cases but unlikely to be sustainable and economically feasible when taken into practice. Culturing microalgae for $CO_2$ sequestration or wastewater reclamation takes the role as an "environmental detergent", but without appropriate control and management the produced biomass will be a second pollutant. Likewise, culturing microalgae simply for biofuel production provides a renewable energy source with a potential to replace the traditional fossil fuel in the future, but using artificial and commercial nutrients is not sustainable.

Therefore, a microalgae−based "N−dimensional" combination of various applications is required, where N stands for the number of applications connected. To reduce the algae cultivation costs and sufficiently utilize the produced microalgae biomass, it is better to combine the upstream application (wastewater/exhaust gas treatment) with the downstream application (biofuel production). For this reason, Table 5 shows the integrated systems but does not involve the integration of $CO_2$ sequestration and wastewater treatment, both of which are the upstream applications.

Many researchers have conducted two−dimensional combination, between biofuel production and $CO_2$ sequestration or wastewater treatment (Table S1), although the performance varies over species. *Dunaliella tertiolecta* can be a good candidate as a $CO_2$ capturer and simultaneously a biofuel producer (*147*). Under high saline condition without sterilization, its $CO_2$ fixation rate, productivity of maximum biomass and productivity rate of biomass were 0.313 g $L^{-1}$ $d^{-1}$, 2.3 g $L^{-1}$ and 0.17 g $L^{-1}$ $d^{-1}$, respectively (*147*). Besides, this species contained 10% (w/w) of glycerol and the produced oil by thermo−chemical liquefaction was 36% (w/w). The rich nutrients in the wastewater appear to be not in favor of lipid accumulation that is widely found under nitrogen−deficient conditions (*148*). Therefore, post-treatment is usually required to induce lipid accumulation.

Alternatively, wastewater can still be used to grow lipid-deficient microalgae but with high biomass productivity for other biofuel production such as biogas and bio-oil, among others. For this purpose, fast accumulation of biomass rather than lipids becomes the first priority enabling mixed cultivation of different microalgal species in wastewater. Passos *et al* (*149*) cultivated several microalgae, including *Monoraphidium* sp, *Stigeoclonium* sp., *Scenedesmus* sp. and *Nitzchia* sp., in a high rate algal pond for secondary treat-

ment of domestic wastewater. The biomass was harvested for anaerobic digestion in continuous anaerobic reactors, yielding 0.12-0.14 L $CH_4$/L day of the methane production rate. A microwave pretreatment enhanced the methane yield by 30% at 15 days HRT (hydraulic retention time) and 58% at 20 days HRT, but also increased the energy consumption. Another mixed culture algae from wastewater was used as feedstock of hydrothermal liquefaction for bio-crude oil production (*150*).

Clear purpose for three−dimensional combination of $CO_2$ sequestration, wastewater treatment and biofuel production is rarely seen in the literature. If assuming that all microalgae used in the two-dimensional combination of $CO_2$ sequestration and wastewater treatment contain a specific amount of lipids, the content still needs to be present for helping the selection of ideal species. Table 6 also displays a few examples in this type of three-dimensional combination. A consortium of 15 native algae were cultured at 6% of $CO_2$ in a wastewater composed of 85−90% carpet industry effluents and 10−15% municipal sewage (*140*). Most of the nutrients were removed (>96%) with relatively lower lipid content of 6.82%. In contrast, *Nannochloropsis* seems to be a desirable species of efficient capability in these three aspects (*139*). By growing *Nannochloropsis* sp. in the municipal wastewater at high $CO_2$ concentration of 15%, the maximum productivity of biomass and the lipid content reached as high as 2.23 g $L^{-1}$and around 60% (w/w), respectively.

It needs to be pointed out that microalgae have wide applications in various fields. The number of applications is more than the three combinations discussed above, including fields such as productions of valuable chemicals for human/animal nutrients. Nevertheless, the practical adoption of application should take the local conditions and re-

quirements into account. The local properties include weather/climate conditions (*e.g.* sunlight, temperature, arid or rainy), water types (freshwater or seawater), nutrient source availability (*e.g.* carbon, either gaseous $CO_2$ or aquatic carbonate or organic carbon), nitrogen and phosphorus (artificial or waste water, what type if wastewater). A critical basic notion of microalgae−based application should be considered: "LECEM", short for Locality-adapted, Environment−friendly, Cost−minimized, Efficiency−maximized and Mass−maximum utilized. From this viewpoint, a four−dimensional combination of microalgal application is proposed in Figure 1. This integration is composed of using flue gas as the rich $CO_2$ source, using wastewater as the rich nutrient source, bio−$H_2$ collection and/or extraction of lipids and/or other valuable compounds, and the production of biomethane or bioethanol or others like PHA from the biomass waste or residues.

In general, the product biomass is given priority to extract valuable products, and then the residue of biomass is used to generate other biofuel like biogas or bio-fertilizers. This two-stage of separating products maximizes the utilization of biomass that is also supported by the life-cycle analysis (*151*). Not limited by this, when wastewater also contains other nutrients such as organic chemicals, these chemicals may be utilized by microalgae *via* heterotrophy (*152*). A bigger system if allowed can also be expanded to combine autotrophic and heterotrophic or even mixotrophic microalgae cultivation.

## 5. Life-cycle analysis

Life-cycle analysis or assessment (LCA) is a technique used to assess the environmental impacts caused by all the stages of a service or product's life according to the ISO14040

standard, therefore which is also called as cradle-to-grave analysis (*153*). The analysis should engage all of the relevant energy and material inputs, environmental releases, and evaluate the potential impacts. LCA has become a fundamental element in designing a microalgae system and pipeline towards the end product.

Since microalgae are widely accepted as a promising candidate in various fields, many LCA have been carried out especially in recent years. An environmental LCA was conducted to compare algae with other biofuel feedstocks including corn, switchgrass and canola (*154*). By using a stochastic life cycle model, algae perform favorably in total land required and eutrophication potential but exhibit higher environmental impacts than the conventional crops in greenhouse gas emissions, energy and water use. The incorporation of flue gas and wastewater treatment seems to be inevitable to offset the cost burdens and to reduce the large environmental footprint of algae cultivation. In a study, integrating microalgae systems at municipal wastewater treatment plants for energy production has been proved to considerably improve the energy balance (*155*). An analysis of water types, operation with or without recycling, algal species and geographic distributions in the US revealed the water footprint of biodiesel production (*156*). Under freshwater without recycling, 0.33 Kg N and 0.71 Kg P per Kg biodiesel are required. The water and nutrients usage can be reduced by 84% and 55%, respectively, when recycling the harvested water. A significant reduction in water requirement of up to 90% was obtained with total elimination of nutrients except P using sea/wastewater. Another LCA evaluates the environmental influence of wastewater-based algal biofuels (*157*). Of 16 pathways examined by combining different nutrient sources (municipal wastewater, centrate from the sludge drying process, swine manure, and freshwater) and several biomass conversion technolo-

gies (microwave pyrolysis, combustion, wet lipid extraction, and hydrothermal liquefaction), only the centrate cultivation with wet lipid extraction pathway and the centrate cultivation with combustion pathway have smaller footprints than petroleum diesel in all environmental categories examined (fossil fuel use, greenhouse gas emission, eutrophication potential, and consumptive water use). Before biofuel production, however, downstream dewatering operations under optimal economy are very important for large-scale processing. Co-cultivation with some special microorganisms like bacteria or fungi appears to be a good strategy for bioflocculation. This was demonstrated by a recent LCA that by using bioflocculation coupled with flow filtration, total energy input of 0.041 kWh, 0.05 kg $CO_2$ and a cost of $ 0.0043 for producing 1 kg of microalgae biomass were achieved (*158*).

An LCA study in biodiesel production from microalgae in ponds under Australian conditions showed lower GHG emission by algae (-27.6 ~ 18.2 g $CO_2$-e/t km) than that by canola (35.9 g $CO_2$-e/t km) and ULS diesel (ultra-low sulfur, 81.2 g $CO_2$-e/t km) (*159*). However, the cost of algae is not favorable (2.2 ~ 4.8 /t km) compared with canola (4.2 /t km) and ULS diesel (3.8 /t km). This indicates that culturing microalgae simply for biodiesel production without considering the cost of energy and fertilizer input is not economically feasible. Jorquera *et al*. (*160*) conducted an energy LCA for biomass production by culturing the oil-rich microalgae, *Nannochloropsis* sp. in open ponds and photobioreactors. The obtained net energy ratio (NER, energy outputs divided by energy inputs) indicated that flat-plate photobioreactors (PBR) and raceway ponds (both NER > 1) are more economically feasible than horizontal tubular PBR (NER < 1).

Using algae for biodiesel production is more suitable for oleaginous species. For lipid-limited species but with a fast growth rate, the energy content of algal biomass can be regarded as an alternate to lipid extraction and biodiesel production. An analysis was undertaken for energy balance of microalgal production in open ponds coupled with nutrient removal from wastewater (*161*). The results show that even without an energy credit for nutrient removal, culturing algae in open pond reactors for biofuel production is still energetically favorable when utilizing wastewater as a nutrient source. If the lipid content of dry biomass (*e.g.* 10%) is lower than the ideal scenario in lab scale reactors (50-60%), direct combustion of algal biomass seems to be a more viable energy source than biodiesel production (*161*). Net energy conversion efficiencies for biomass combustion power are usually in the range of 20-40% (*162*). A higher efficiency can be obtained in larger systems or when biomass is co-combusted in coal fired power plants (*162*). An LCA of coal-algae co-firing demonstrated that coal-algae co-firing could reduce GHG emissions and air pollution (*163*). Alternatively, the produced biomass can be converted to bio-jet fuel *via* hydrothermal liquefaction (HTL) and its LCA study presents a reduction of 76% in GHG emissions by sitting HTL at a wastewater treatment plant compared to conventional jet fuel (*164*).

There is another interesting LCA study performed to study the biogas production from microalgae (*151*). The authors found that the impacts formed by the production of methane strongly depend on the electric consumption. Nevertheless, great progress can be achieved by decreasing mixing costs, or by combining lipid extraction from biomass with methane production from the biomass residue. It needs to be noted that these LCAs still do not include the profit earned from the $CO_2$ sequestration, which will definitely further

increase the economic feasibility of the microalgae-based application. Besides, results from LCA studies are recognized to be largely inconclusive because modeling assumptions and system boundaries, the basis of LCA, are diverse (*165*). This issue can be solved by model-normalization with a generic pathway, the results of which show that algae-based biodiesel is on par with existing biofuel options (*e.g.* corn ethanol, soy biodiesel) in energy consumption and GHG emissions (*165*). Lack of comprehensive uncertainty analysis is another drawback in many LCA studies (*166*). A Monte Carlo approach can be employed to estimate ranges of expected values of LCA metrics by incorporating parameter variability with empirically specified distribution functions (*166*). Sills *et al* suggest that reporting results from LCA models as ranges, instead of single values, will more reliably inform industry and policy makers on expected energetic and environmental performance of algae-based biofuels (*166*).

# 6. Conclusions

This article primarily reviews the microalgal biotechnologies for both biofuel production and environmental treatment. The algae-based biofuel production is limited by many drawbacks especially the high energy and cost inputs. Owing to the limited biomass conversion efficiency of photosynthetic microalgae, the major problem of $CO_2$ sequestration is that most of $CO_2$ (80-90%) escapes to the air. To reduce the amount of escape, intermittent aeration may be needed and particular bioreactor should be designed to elongate the retained period of gases. Alternatively, gaseous $CO_2$ can be converted to bi-/carbonates and stored in an alkali solution. The high content of $CO_2$ in flue gases maybe

not a problem for many species, but other components like $SO_2$/NO may exhibit toxicity to algal cells or change the culture pH. Wastewater contains a variety of inorganic salts *e.g.* $NO_3^-$/$NH_4^+$/$PO_4^{3-}$ that can be used as microalgal nutrients. It has been found that some special microalgae can also biodegrade particular organic compounds like herbicide atrazine. Besides, the efficiency of heavy metal removal significantly depends on the microalgal species.

Several intelligent technologies have been developed to solve the current bottlenecks, mainly including immobilization, mixotropic and heterotrophic cultivation, and co-cultivation. Immobilization of algal cells on special material surface or in tiny environments can enhance growth rate, prevent bio-invasion from other microorganisms and simplify the harvest process. Mixotrophic and heterotrophic cultivations can enhance biomass or lipids production, compared to photoautotrophy, but the organic nutrients will increase the cost unless a cheap source is available. Co-cultivation with other microalgae or microbes can help the harvest of cells *via* bioflocculation and forms a symbiosis for nutrient complementation. Admittedly, these techniques also have their own limitations, and their seamless coupling with other techniques still needs more researches and verification.

When building a microalgal cultivation system, species screening and applications adopted should take the basic "LECEM" principle into account at least. To date, most of the evidences and LCA studies have approved an integrated system of combining various algal applications but a flexible and different combination is allowed depending on the local requirements. As has been predicted (*167*), microalgae will have enormous potential

if microalgal systems are developed by coupling bioenergy and product diversity with improved efficiency in biorefinery concepts.

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## Figure captions

**Figure 1.** Integrated system of microalgae-based applications, composed of four major parts: feedstock, cell cultivation, product extraction, and biomass waste utilization. The wastewater and $CO_2$ recycling are shown in dashed lines.

**Table 1. Microalgal carbon sequestration under various artificial and real flue gases.** $CO_2$%: $CO_2$ percentage on the basis of volume; T: temperature (°C); $F_{CO2}$: $CO_2$ fixation rate (g $L^{-1}$ $d^{-1}$); $P_{MB}$: Productivity of maximum biomass (g $L^{-1}$); $P_{BR}$: productivity of biomass rate (g $L^{-1}$ $d^{-1}$).

| Species | Inlet conditions | | | | | Performance | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plant type | $CO_2$ % | T °C | SOx ppm | NOx ppm | $CO_2$% captured | $F_{CO2}$ g $L^{-1}$ $d^{-1}$ | $P_{MB}$ g $L^{-1}$ | $P_{BR}$ g $L^{-1}$ $d^{-1}$ | |
| **Artificial flue gases** | | | | | | | | | | |
| *Botryococcus braunii* | – | – | 25–30 | – | – | – | >1.0 | – | 1.1 | (*35, 36*) |
| *Chlorella kessleri* | – | 6 | 30 | – | – | – | 0.163[a] | – | 0.087 | (*37, 38*) |
| *Chlorococcum littorale* | – | 40 | 25 | – | – | – | – | – | – | (*39, 40*) |
| | | 70 | – | | | | – | – | – | (*41*) |
| *Chlorella* sp. | – | 40 | 42 | – | – | – | 1.0 | – | – | (*42*) |
| | | 10 | 35 | | | | – | ~2.0 | ~0.394 | (*43*) |
| | | 100 | 35 | | | | – | ~0.4 | ~0.074 | (*43*) |
| | | 15 | 35 | | | | >1.0 | – | – | (*36*) |
| *Chlorella vulgaris* | – | 15 | 27 | – | – | – | 0.624 | 1.1 | 0.22 | (*44*) |
| *Scenedesmus obliquus* | – | 18 | 30 | – | – | – | 0.26 | – | 0.14 | (*45, 46*) |
| *Scenedesmus* sp. | – | 80 | 30 | – | – | – | – | – | – | (*47*) |
| *Spirulina* sp. | – | 12 | 30 | – | – | – | 0.413[a] | 3.5 | 0.22 | (*45*) |
| **Real flue gases** | | | | | | | | | | |
| *Chlorella emersonii* | Cement plant | 15 | 25 | 7595 | – | – | 3.25 | 2 | – | (*48*) |
| *Chlorella* sp. | Steel plant | 25 | 25 | 87 ± 9 | 78 ± 4 | 13 | – | 2.87 | 0.353 | (*49, 50*) |
| | Coal–fired thermal power plant | 13 | | 10 | 150 | – | – | ~2.0 | ~0.392 | (*43*) |
| *Dunaliella salina* | Steel plant | 20 | 23 | – | – | 14 | – | 0.24 | 0.014 | (*51*) |
| *Scenedesmus obliquus* | Coke oven | 18 | 28 | 200 | 150 | 67 | – | – | – | (*52*) |
| *NOA– 13* | Fuel oil–fired power plants | 15 | 25 | – | 300 | – | 0.875 | ~2.4 | ~0.333 | (*53*) |

[a] Calculated by using the equation: $CO_2$ fixation rate ($Pco_2$)=1.88×biomass productivity (P), which is derived from the typical molecular formula of microalgal biomass, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (*7*).

**Table 2. Wastewater treatment by suspension microalgae.** TN: total nitrogen (mg $L^{-1}$); TP: total phosphor (mg $L^{-1}$); $R_N$, $R_P$, $R_C$, $R_{TN}$, $R_{TP}$%: removal percentage of nitrogen, phosphor, COD, total nitrogen and total phosphor; $P_{MB}$: Productivity of maximum biomass (g $L^{-1}$).

| Species | Inlet conditions | | | Performance | | Ref. |
|---|---|---|---|---|---|---|
| | Water type | T ($^oC$) | N(mg $L^{-1}$) or P(mg $L^{-1}$) | $R_N$, $R_P$, $R_C$, $R_{TN}$, $R_{TP}$ | $P_{MB}$ (g $L^{-1}$) | |
| *Botryococcus braunii* | piggery wastewater | 25 | $NH_4^+$: 4; $NO_3^-$: 788 $PO_4^{3-}$: 40 | $R_{TN}$: 80% | 8.5 | (*69*) |
| *Chlorella kessleri* | wastewater treatment plant effluents | – | Containing Cd(II) and Pb(II) | – | – | (*70*) |
| *Chlorella* sp. | artificial wastewater | 20 | TN: 113.9; TP: 102.48 | $R_{TN}$: 6.9; $R_{TP}$: 3.5 mg $g^{-1}$ biomass $d^{-1}$ | 1.58 | (*71, 72*) |
| *Chlorella vulgaris* | urban wastewater | 25 | $NH_4^+$: 32.5; $NO_3^-$: 2.0 $PO_4^{3-}$: 2.5 | $R_N$: 60.1%; $R_P$ :80.3% | – | (*27*) |
| *Nannochloris* sp. | Artificial medium | 20 ± 2 | – | $R_N$ & $R_P$: 0.011 & 0.006 mg $h^{-1}$ | – | (*68*) |
| *Scenedesmus intermedius* | Artificial medium | 20 ± 2 | – | $R_N$ & $R_P$: 0.022 & 0.014 mg $h^{-1}$ | – | (*68*) |
| *Scenedesmus obliquus* | urban wastewater | 25 | Containing $NH_4^+$ | $R_N$: 98% ; $R_P$: 100% | – | (*73, 74*) |
| *Spirulina* | diluted pig wastewater | – | TN: 1519; TP: 620 | $R_N$: 84–96% ; $R_P$: 72–87% | 11.8 g $m^{-2}$ $d^{-1}$ | (*75*) |

**Table 3. Immobilized microalgae in wastewater treatment.** TN: total nitrogen (mg $L^{-1}$); TP: total phosphor (mg $L^{-1}$); $R_N$, $R_P$, $R_{TN}$, $R_{TP}$%: removal percentage of nitrogen, phosphor, total nitrogen and total phosphor; $P_{MB}$: Productivity of maximum biomass (g $L^{-1}$).

| Species | Inlet conditions | | | | Performance | | Ref. |
|---|---|---|---|---|---|---|---|
| | Water type | Immobilized material | T ($^oC$) | N(mg $L^{-1}$) or P(mg $L^{-1}$) | $R_N$, $R_P$, $R_{TN}$, $R_{TP}$ % | $P_{MB}$ (g $L^{-1}$) | |
| *Chlorella* sp. | Artificial wastewater | sodium cellulose sulphate/poly−dimethyl diallyl−ammonium chloride | 20 | TN: 113.9; TP: 102.48 | $R_{TN}$: 12.56; $R_{TP}$: 10.24 mg $g^{-1}$ biomass $d^{-1}$ | 0.6 | (*71*) |
| *Chlorella vulgaris* | Urban wastewater | Sodium alginate | 25 | $NH_4^+$: 32.5; $NO_3^-$: 2.0 $PO_4^{3-}$: 2.5 | $R_N$: 80.0%; $R_P$ :53.3% | − | (*28*) |
| | Artificial wastewater | Calcium alginate beads | 25−28 | TN: 13.09 | − | 0.67 mg $bead^{-1}$ | (*112, 114*) |
| *Chlorella sorokiniana* | synthetic wastewater | Calcium alginate beads | 30 | TN: 42; TP: 12 | $R_N$: 41.46%; $R_P$ :84.84% | $3.78\times10^9$ cells $flask^{-1}$ | (*113*) |
| *Chlorella sorokiniana* + *Azospirillum brasilense* | municipal wastewater | Calcium alginate beads | 28±2 | $NH_4^+$: 2630; $NO_3^-$: 3.01 $PO_4^{3-}$: 100.38 μM | $R_N$: 61%; $R_P$: 53% | $5.2\times10^6$ cells $bead^{-1}$ | (*84, 115*) |
| *Nannochloris* sp. | Artificial medium | Calcium alginate beads | 20 ± 2 | − | $R_N$ & $R_P$: 0.006 & 0.009 mg $h^{-1}$ | − | (*68*) |
| *Scenedesmus intermedius* | Artificial medium | Calcium alginate beads | 20 ± 2 | − | $R_N$ & $R_P$: 0.009 & 0.012 mg $h^{-1}$ | − | (*68*) |

**Table 4. Co-culture system of microalgae and other microorganisms.**

| Microalgae | Microbial partners | Purposes | Results | Ref. |
|---|---|---|---|---|
| | **Microalgae** | | | |
| *Nannochloropsis gaditana* | *Dunaliella salina* | Biomass & Lipid production | Biomass: 1.00 g $L^{-1}$; lipid: 0.383 g $L^{-1}$ | (*118*) |
| *Monoraphidium* sp. | *Chlorella* sp. | Lipid production | lipid: 29.52 mg $L^{-1}$ $d^{-1}$ | (*119*) |
| | **Bacteria** | | | |
| *Nannochloropsis oceanica* | *Solibacillus silvestris* | Bio-flocculation | Flocculation: 90% | (*120*) |
| *Nannochloropsis* sp. | *Bacillus* sp. | Bio-flocculation | Flocculation: 70-95%; Fast process in 30s | (*121*) |
| *Chlorella vulgaris* | *Rhizobium radiobacter* | Bio-flocculation& Energy production | Flocculation: 45-50%; Lipid: 21%; Slightly inhibited growth of algae | (*122*) |
| *Chlorella vulgaris* or *C. sorokiniana* | *Azospirillum brasilense* | B1 release & Wastewater treatment | $NH_4^+$-removal:100%; $NO_3^-$-removal: 15%; P-removal: 36% | (*27, 123*) |
| *Chlorella* spp. | *Azospirillum brasilense* | Wastewater treatment | P-removal: 72% | (*124*) |
| *Synechococcus elongatus* | *Azospirillum brasilense* | Wastewater treatment | Biomass: 2-folds; P-removal: 44.8% | (*125*) |
| *Scenedesmus* sp. | *Anaerobic sludge* | Energy production & Wastewater treatment | $H_2$:1508.3 mL $L^{-1}$; Lipid: 0.36 g $L^{-1}$; COD, TN and TP removal: 80.5%, 88.7% and 80.1% | (*126*) |
| *Lobomonas rostrata* | *Mesorhizobium loti* | Exchange of $B_{12}$ and fixed carbon | Stable equilibrium formed in terms of population numbers | (*127*) |
| *Chlamydomonas reinhardtii* | *Sinorhizobium meliloti* | Enhancement of thermal tolerance by supply of $B_{12}$ | Thermal tolerance was enhanced up to 42 $^oC$ | (*128*) |
| | **Yeast & Molds** | | | |
| *Scenedesmus obliquus* | *Candida tropicalis* | Biomass & Lipid production | Biomass increased by 30.3%, up to 4.5 g $L^{-1}$; Lipid: 97.8 mg $L^{-1}$ $d^{-1}$; | (*129*) |
| *Chlorella* spp. | *Trichosporonoides* | Wastewater treatment; Biomass & | Biomass: 12.2 g $L^{-1}$; lipid: 5.74 g $L^{-1}$ | (*130*) |

| | | | | |
|---|---|---|---|---|
| | *spathulata* | Lipid production | | |
| *11 microalgae* | *Aspergillus fumigatus* | Bio-flocculation; Wastewater treatment; Biomass & Lipid production | Flocculation: 90%; $NH_4^+$-removal: 96%; P-removal: 84%; | (*131*) |

**Table 5. Integrated systems: (a) wastewater & lipids, (b) $CO_2$ & wastewater & lipids, (c) Biomass & other biofuels.** TN: total nitrogen (mg $L^{-1}$); TP: total phosphor (mg $L^{-1}$); $R_A$, $R_N$, $R_P$, $R_C$, $R_{TN}$, $R_{TP}$%: removal percentage of ammonium, nitrogen, phosphor, COD, total nitrogen and total phosphor; $P_{MB}$: Productivity of maximum biomass (g $L^{-1}$).

| **(a) Wastewater & lipids** | **Inlet conditions** | | | **Performance** | | | **Ref.** |
|---|---|---|---|---|---|---|---|
| | **Water type** | **T ($^o$C)** | **Others** | **$R_N$, $R_P$ , $R_C$, $R_{TN}$, $R_{TP}$** | **$P_{MB}$ (g $L^{-1}$)** | **Lipids %** | |
| *Chlamydomonas* | municipal wastewater (centrate) | 25±1 | $NH_4^+$: 67; TP: 120.60 mg $L^{-1}$ | $R_{TN}$: 55.8; $R_{TP}$: 17.4 mg $L^{-1}$ $d^{-1}$ | 2 | 25.25 | (*132, 133*) |
| *Chlorella* sp. | municipal wastewater (semi continuous) | 25 | $NH_4^+$: 82.5; TN: 116.1; TP: 212; COD: 2304 mg $L^{-1}$ | $R_{TN}$: 89.1; $R_{TP}$: 80.9; $R_C$: 90.8% | – | 11.4 | (*134, 135*) |
| *Chlorella vulgaris* | artificial wastewater | | $NH_4^+$: 20; TP: 4; COD: 400 mg $L^{-1}$ | $R_A$:97; $R_P$: >96; $R_C$: 86% | 0.69 | 42 | (*77, 136*) |
| *Scenedesmus obliquus* | urban wastewater | 25 | – | $R_N$:100; $R_P$: 83.3% | – | 16 | (*28, 135*) |
| *Algae consortium* | 25% dairy wastewater | ~32 | $NH_4^+$: 30.5; TN: 81; TP: 2.1 mg $L^{-1}$ | $R_A$:96; $R_P$: >99% | 0.9 | 10–29 | (*137*) |
| **(b) $CO_2$ & Wastewater & lipids** | | | | | | | |
| *Chlamydomonas* sp. TAI–2 | industrial wastewater | – | $CO_2$: 5% | $R_N$ :100; $R_P$ :33% | – | 18.4 | (*138*) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Nannochloropsis* sp. | municipal wastewater | 26 | $CO_2$: 15% | – | 2.23 | 59.9 | (*35, 139*) |
| 15 native algal consortium | carpet industry effluents with municipal sewage | – | $CO_2$: 6% | >96% nutrient removal | 9.2–17.8 tons $ha^{-1}$ $year^{-1}$ | 6.82 | (*140*) |
| **(c) Biomass & other biofuels** | | | | | | | |
| *Chlorella vulgaris* | Immobilization with addition of glucose under sulfur limited condition | | | $H_2$ production increased 18-folds (34.8 ml/h/l) | | | (*141*) |
| | Coupled with a proton exchange membrane fuel cell | | | Maximum current of 8.9 mA with 27.09 ml of hydrogen | | | (*142*) |
| Algal biomass | Anaerobic digestion by *Bacillus cereus* | | | Production of PHA, $H_2$ and biomethane | | | (*143, 144*) |
| *Chlorococcum sp.* | Fermentation of lipid-extracted microalgae debris | | | 3.8g $L^{-1}$ bioethanol from 10 g $L^{-1}$ of biomass | | | (*145*) |
| Algal biomass | Thermochemical conversion of lipid-depleted residual microalgae | | | 1.67 MJ/MJ (bio-oil) and 7.01 MJ/MJ (gas) | | | (*16, 146*) |

**Microalgae: a robust "green bio−bridge" between environment and energy**

*Yimin Chen, Changan Xu* and *Seetharaman Vaidynanthan*

# Supplementary material

**Table S1. Integrated systems: combination of $CO_2$ &biofuels.** $F_{CO2}$: $CO_2$ fixation rate (g $L^{-1}$ $d^{-1}$); $P_{MB}$: Productivity of maximum biomass (g $L^{-1}$); $P_{BR}$: productivity rate of biomass (g $L^{-1}$ $d^{-1}$); Lipid%: lipid content on the basis of dry biomass weight.

| $CO_2$ &biofuels | Inlet conditions | | | Performance | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | $CO_2$% | T ($^oC$) | Flue gas type | $CO_2$% captured | $F_{CO2}$ (g $L^{-1}$ $d^{-1}$) | $P_{MB}$ (g $L^{-1}$) | $P_{BR}$ (g $L^{-1}$ $d^{-1}$) | Lipid% | |
| ***Artificial flue gases*** | | | | | | | | | |
| *Botryococcus braunii* | 20 (0.2 vvm) | 25 | – | – | – | 2.31 | 0.092 | 12.71 | (*1*) |
| | 10 | 25 | – | – | – | – | 0.027 | 20.75 | (*2*) |
| *Chlorella emersonii* | 5 | 25 | – | – | 0.053 | – | 0.028 | 29 | (*3*) |
| *Chlorella vulgaris* | 1 | 25 | – | >15 | >1.0 | – | – | 20 | (*4*) |
| | 10 | 25 | – | – | – | – | 0.105 | 6.6 | (*2*) |
| *Chlorella pyrenoidosa* | 10 (0.25 vvm) | 25 | – | – | ~0.22 | ~1.52 | ~0.126 | 24.25 | (*5, 6*) |
| *Dunaliella tertiolecta* | 10 | 27 | – | – | 0.313 | 2.3 | 0.17 | 36 | (*7*) |
| *Haematococcus pluvialis* | 16–34 | 20 | – | – | 0.143 | 0.076 | – | 35 | (*8*) |
| | 50 (0.25 vvm) | 25 | – | – | ~0.075 | ~0.65 | ~0.054 | 26.75 | (*5*) |
| *Nannochloropsis oculata* | 2[a] | 26 | – | 47 | 6.33 | – | 0.48 Max | 29.7 | (*9, 10*) |
| | 15[a] | 26 | – | 11 | 11.79 | – | 0.37 Max | 22.7 | (*9*) |
| *Scenedesmus obliquus* | 10 (0.25 vvm) | 25 | – | – | ~0.25 | ~1.8 | ~0.128 | 19.25 | (*5*) |
| | 50 (0.25 vvm) | 25 | – | – | ~0.1 | ~0.8 | ~0.057 | 24.4 | (*5*) |
| *Scenedesmus* sp. | 10 | 25 | – | – | – | – | 0.218 | 9.49 | (*2*) |
| ***Real flue gases*** | | | | | | | | | |
| *Botryococcus braunii* | 5.5 | 25 | Burning liquefied petroleum gas | – | – | – | 0.077 | 24 | (*2*) |
| *Chlorella* sp. | 23 ± 5 (0.05vvm) | 25 | Coke oven of a steel plant | 13 | – | 2.87 | 0.353 | 25.2 | (*11, 12*) |
| *Dunaliella salina* | 20 | 23 | Steel plant | 14 | – | 0.24 | 0.014 | 31–75 | (*13*) |
| *Scenedes-* | 5.5 | 25 | Burning lique- | 24 | – | – | 0.203 | 18 | (*2, 14*) |

| | | |
|---|---|---|
| a, semi-continuous culture. | *mus* sp. | fied petroleum gas |

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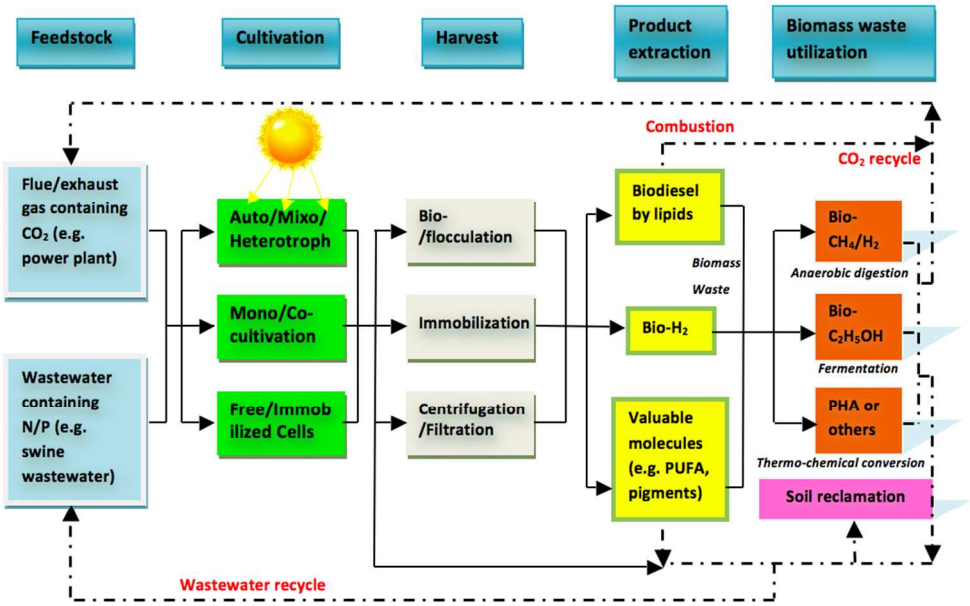


Figure 1. Integrated system of microalgae-based applications, composed of four major parts: feedstock, cell cultivation, product extraction, and biomass waste utilization. The wastewater and CO2 recycling are shown in dashed lines.

186x114mm (150 x 150 DPI)

**Figure captions**

**Figure 1.** Integrated system of microalgae-based applications, composed of four major parts: feedstock, cell cultivation, product extraction, and biomass waste utilization. The wastewater and $CO_2$ recycling are shown in dashed lines.