Article:

Chen, Y., Xu, C. and Vaidyanathan, S. orcid.org/0000-0003-4137-1230 (2017) Microalgae: a robust “green bio−bridge” between energy and environment. Critical Reviews in Biotechnology. ISSN 0738-8551

https://doi.org/10.1080/07388551.2017.1355774

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Journal:	*Critical Reviews in Biotechnology*
Manuscript ID	BBTN-2017-0010.R1
Manuscript Type:	Review Article
Date Submitted by the Author:	08-May-2017
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Keywords:	Microalgae, Environmental treatment, Biofuel production, Integrated application, Economic feasibility

Paper 16 BBTN-2017-0010.R1 spell checked to GGS June 5

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

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Keywords

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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.* bioH_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 rather 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 ammonium 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 biofuel 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 treatment 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 requirements 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 technologies (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.

Acknowledgement

We gratefully acknowledge SOA Fundamental Research Funds (HE160302-16(2)), China Postdoctoral Science Foundation (2016M602055), Xiamen Science and Technology Program (3502Z20161162) and "13th Five-Year" Xiamen National Marine Economic Innovation Development Demonstration (16CZP017SF06) for the funding support that made this work possible.

Reference

1. D'Alessandro EB, Antoniosi Filho NR, Concepts and studies on lipid and pigments of microalgae: A review. Renewable and Sustainable Energy Reviews 2016; 58: 832-841.
2. Choix FJ, de-Bashan LE, Bashan Y, Enhanced accumulation of starch and total carbohydrates in alginate-immobilized *Chlorella* spp. induced by *Azospirillum brasilense*: II. Heterotrophic conditions. Enzyme and Microbial Technology 2012; 51: 300-309.
3. Simon DP, Anila N, Gayathri K, Sarada R, Heterologous expression of β-carotene hydroxylase in *Dunaliella salina* by *Agrobacterium*-mediated genetic transformation. Algal Research 2016; 18: 257-265.
4. Guedes A, Amaro HM, Malcata FX, Microalgae as sources of high added‐value compounds—a brief review of recent work. Biotechnology Progress 2011; 27: 597-613.
5. Pulz O, Gross W, Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology 2004; 65: 635-648.
6. Mata TM, Martins AA, Caetano NS, Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews 2010; 14: 217-232.
7. Chisti Y, Biodiesel from microalgae. Biotechnology Advances 2007; 25: 294-306.
8. Sendra M, Sánchez-Quiles D, Blasco J, Moreno-Garrido I, Lubián LM, Pérez-García S, Tovar-Sánchez A, Effects of TiO_2 nanoparticles and sunscreens on coastal marine microalgae: Ultraviolet radiation is key variable for toxicity assessment. Environment International 2017; 98: 62-68.
9. Bengtson S, Belivanova V, Rasmussen B, Whitehouse M, The controversial "Cambrian" fossils of the Vindhyan are real but more than a billion years older. Proceedings of the National Academy of Sciences 2009; 106: 7729.
10. Chen CH, Berns DS, Comparison of the stability of phycocyanins from thermophilic, mesophilic, psychrophilic and halophilic algae. Biophysical Chemistry 1978; 8: 203-213.
11. Peters GP, Marland G, Le Quéré C, Boden T, Canadell JG, Raupach MR, Rapid growth in CO_2 emissions after the 2008-2009 global financial crisis. Nature Climate Change 2012; 2: 2-4.
12. Ravishankara AR, Rudich Y, Pyle JA, Role of Chemistry in Earth's Climate. Chemical Reviews 2015; 115: 3679-3681.
13. Balat M, Balat H, Recent trends in global production and utilization of bio-ethanol fuel. Applied Energy 2009; 86: 2273-2282.
14. Chisti Y, Biodiesel from microalgae beats bioethanol. Trends in Biotechnology 2008; 26: 126-131.
15. Torzillo G, Scoma A, Faraloni C, Giannelli L, Advances in the biotechnology of hydrogen production with the microalga *Chlamydomonas reinhardtii*. Critical Reviews in Biotechnology 2015; 35: 485-496.

16. Elsayed S, Boukis N, Patzelt D, Hindersin S, Kerner M, Sauer J, Gasification of Microalgae Using Supercritical Water and the Potential of Effluent Recycling. Chemical Engineering & Technology 2016; 39: 335-342.

17. John RP, Anisha G, Nampoothiri KM, Pandey A, Micro and macroalgal biomass: a renewable source for bioethanol. Bioresource technology 2011; 102: 186-193.

18. Cho S-H, Kim K-H, Jeon YJ, Kwon EE, Pyrolysis of microalgal biomass in carbon dioxide environment. Bioresource Technology 2015; 193: 185-191.

19. "Sustainable Development of Algal Biofuels," (USA National Academy of Sciences, NW Washington, http://www.nap.edu, 2012).

20. Serrenho AC, Mourão ZS, Norman J, Cullen JM, Allwood JM, The influence of UK emissions reduction targets on the emissions of the global steel industry. Resources, Conservation and Recycling 2016; 107: 174-184.

21. Ondrey G, Progress to Limit Climate Change. Chemical Engineering 2016; 123: 16-19.

22. Chisholm JR, Fernex FE, Mathieu D, Jaubert JM, Wastewater discharge, seagrass decline and algal proliferation on the Cote d'Azur. Marine Pollution Bulletin 1997; 34: 78-84.

23. Ansari AA, Gill SS, Khan FA, Naeem M, in *Eutrophication: Causes, Consequences and Control.* (Springer, 2011), pp. 239-248.

24. de-Bashan LE, Bashan Y, Immobilized microalgae for removing pollutants: review of practical aspects. Bioresource Technology 2010; 101: 1611-1627.

25. Zhu L, Wang Z, Shu Q, Takala J, Hiltunen E, Feng P, Yuan Z, Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water Research 2013; 47: 4294-4302.

26. Abdelaziz AE, Leite GB, Belhaj MA, Hallenbeck PC, Screening microalgae native to Quebec for wastewater treatment and biodiesel production. Bioresource Technology 2014; 157: 140-148.

27. de-Bashan LE, Hernandez J-P, Morey T, Bashan Y, Microalgae growth-promoting bacteria as “helpers” for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. Water Research 2004; 38: 466-474.

28. Ruiz-Marin A, Mendoza-Espinosa LG, Stephenson T, Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresource technology 2010; 101: 58-64.

29. Perez-Garcia O, Bashan Y, in *Algal Biorefineries: Volume 2: Products and Refinery Design,* A. Prokop, R. K. Bajpai, M. E. Zappi, Eds. (Springer International Publishing, Cham, 2015), pp. 61-131.

30. Williams PJlB, Laurens LML, Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetics & economics. Energy & Environmental Science 2010; 3: 554-590.

31. Ajeej A, Thanikal JV, Narayanan CM, Kumar RS, An overview of bio augmentation of methane by anaerobic co-digestion of municipal sludge along with microalgae and waste paper. Renewable and Sustainable Energy Reviews 2015; 50: 270-276.

32. Ruiz J, Olivieri G, de Vree J, Bosma R, Willems P, Reith JH, Eppink MHM, Kleinegris DMM, Wijffels RH, Barbosa MJ, Towards industrial products from microalgae. Energy & Environmental Science 2016; 9: 3036-3043.

33. Li Y, Horsman M, Wu N, Lan CQ, Dubois‐Calero N, Biofuels from microalgae. Biotechnology Progress 2008; 24: 815-820.

34. Bilanovic D, Andargatchew A, Kroeger T, Shelef G, Freshwater and marine microalgae sequestering of CO_2 at different C and N concentrations – Response surface methodology analysis. Energy Conversion and Management 2009; 50: 262-267.

35. Wang T-H, Chu S-H, Tsai Y-Y, Lin F-C, Lee W-C, Influence of inoculum cell density and carbon dioxide concentration on fed-batch cultivation of *Nannochloropsis oculata*. Biomass and Bioenergy 2015; 77: 9-15.

36. Murakami M, Ikenouchi M, The biological CO_2 fixation and utilization project by rite (2) — Screening and breeding of microalgae with high capability in fixing CO_2 —. Energy Conversion and Management 1997; 38: S493-S497.

37. de Morais MG, Costa JAV, Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Conversion and Management 2007a; 48: 2169-2173.

38. Kasiri S, Abdulsalam S, Ulrich A, Prasad V, Optimization of CO_2 fixation by *Chlorella kessleri* using response surface methodology. Chemical Engineering Science 2015; 127: 31-39.

39. Iwasaki I, Hu Q, Kurano N, Miyachi S, Effect of extremely high-CO_2 stress on energy distribution between photosystem I and photosystem II in a high-CO_2 'tolerant green alga', *Chlorococcum littorale* and the intolerant green alga *Stichococcus bacillaris*. Journal of Photochemistry and Photobiology B: Biology 1998; 44: 184-190.

40. Ota M, Takenaka M, Sato Y, Smith RL, Inomata H, Variation of photoautotrophic fatty acid production from a highly CO_2 tolerant alga, *Chlorococcum littorale*, with inorganic carbon over narrow ranges of pH. Biotechnology Progress 2015; 31: 1053-1057.

41. Ota M, Kato Y, Watanabe H, Watanabe M, Sato Y, Smith Jr RL, Inomata H, Effect of inorganic carbon on photoautotrophic growth of microalga *Chlorococcum littorale*. Biotechnology Progress 2009; 25: 492-498.

42. Sakai N, Sakamoto Y, Kishimoto N, Chihara M, Karube I, *Chlorella* strains from hot springs tolerant to high temperature and high CO_2. Energy Conversion and Management 1995; 36: 693-696.

43. Maeda K, Owada M, Kimura N, Omata K, Karube I, CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae. Energy Conversion and Management 1995; 36: 717-720.

44. Yun YS, Lee SB, Park JM, Lee CI, Yang JW, Carbon dioxide fixation by algal cultivation using wastewater nutrients. Journal of Chemical Technology and Biotechnology 1997; 69: 451-455.

45. De Morais MG, Costa JAV, Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. Journal of Biotechnology 2007; 129: 439-445.

46. Bagchi SK, Mallick N, Carbon dioxide biofixation and lipid accumulation potential of an indigenous microalga *Scenedesmus obliquus* (Turpin) Kützing GA 45 for biodiesel production. RSC Advances 2016.

47. Hanagata N, Takeuchi T, Fukuju Y, Barnes DJ, Karube I, Tolerance of microalgae to high CO_2 and high temperature. Phytochemistry 1992; 31: 3345-3348.

48. Borkenstein CG, Knoblechner J, Frühwirth H, Schagerl M, Cultivation of *Chlorella emersonii* with flue gas derived from a cement plant. Journal of Applied Phycology 2011; 23: 131-135.

49. Chiu SY, Kao CY, Huang TT, Lin CJ, Ong SC, Chen CD, Chang JS, Lin CS, Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using Chlorella sp. cultures. Bioresource Technology 2011; 102: 9135-9142.

50. Mortensen LM, Gislerød HR, The growth of *Chlorella sorokiniana* as influenced by CO_2, light, and flue gases. Journal of Applied Phycology 2016; 28: 813-820.

51. Zimmerman WB, Zandi M, Hemaka Bandulasena H, Tesar V, James Gilmour D, Ying K, Design of an airlift loop bioreactor and pilot scales studies with fluidic oscillator induced microbubbles for growth of a microalgae *Dunaliella salina*. Applied Energy 2011; 88: 3357-3369.

52. Li FF, Yang ZH, Zeng R, Yang G, Chang X, Yan JB, Hou YL, Microalgae capture of CO_2 from actual flue gas discharged from a combustion chamber. Industrial & Engineering Chemistry Research 2011; 50: 6496-6502.

53. Yoshihara KI, Nagase H, Eguchi K, Hirata K, Miyamoto K, Biological elimination of nitric oxide and carbon dioxide from flue gas by marine microalga NOA-113 cultivated in a long tubular photobioreactor. Journal of Fermentation and Bioengineering 1996; 82: 351-354.

54. Chen H-W, Yang T-S, Chen M-J, Chang Y-C, Eugene I, Wang C, Ho C-L, Lai Y-J, Yu C-C, Chou J-C, Purification and immunomodulating activity of C-phycocyanin from Spirulina platensis cultured using power plant flue gas. Process Biochemistry 2014; 49: 1337-1344.

55. Iwasaki I, Hu Q, Kurano N, Miyachi S, Effect of extremely high-CO_2 stress on energy distribution between photosystem I and photosystem II in a high-CO_2 tolerant green alga, *Chlorococcum littorale* and the intolerant green alga *Stichococcus bacillaris*. Journal of Photochemistry and Photobiology B: Biology 1998; 44: 184-190.

56. Matsumoto H, Hamasaki A, Sioji N, Ikuta Y, Influence of CO_2, SO_2 and NO in flue gas on microalgae productivity. Journal of Chemical Engineering of Japan 1997; 30: 620-624.

57. Thomas MK, Litchman E, Effects of temperature and nitrogen availability on the growth of invasive and native cyanobacteria. Hydrobiologia 2016; 763: 357-369.

58. Červený J, Sinetova MA, Zavřel T, Los DA, Mechanisms of High Temperature Resistance of *Synechocystis* sp. PCC 6803: An Impact of Histidine Kinase 34. Life 2015; 5: 676-699.

59. Wang X, Liu X, Qin B, Gu Z, Wu Z, Xu H, Zhu H, Cheng G, Liu H, Green algae dominance quickly switches to cyanobacteria dominance after nutrient enrichment in greenhouse with high temperature. Journal of Ecology and Environment 2015; 38: 293-305.

60. Kupriyanova EV, Samylina OS, CO_2-concentrating mechanism and its traits in haloalkaliphilic cyanobacteria. Microbiology 2015; 84: 112-124.

61. Sandrini G, Jakupovic D, Matthijs HCP, Huisman J, Strains of the harmful cyanobacterium *Microcystis aeruginosa* differ in gene expression and activity of inorganic carbon uptake systems at elevated CO_2 levels. Applied and Environmental Microbiology 2015; 81: 7730-7739.

62. Skjånes K, Lindblad P, Muller J, BioCO_2–a multidisciplinary, biological approach using solar energy to capture CO_2 while producing H_2 and high value products. Biomolecular Engineering 2007; 24: 405-413.

63. Wang B, Li Y, Wu N, Lan CQ, CO_2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology 2008; 79: 707-718.

64. Judd S, van den Broeke LJP, Shurair M, Kuti Y, Znad H, Algal remediation of CO_2 and nutrient discharges: A review. Water Research 2015; 87: 356-366.

65. U.S.DOE, National algal biofuels technology roadmap. Report no.: DOE/EE-0332. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program. 2010. May.

66. Carney LT, Reinsch SS, Lane PD, Solberg OD, Jansen LS, Williams KP, Trent JD, Lane TW, Microbiome analysis of a microalgal mass culture growing in municipal wastewater in a prototype OMEGA photobioreactor. Algal Research 2014; 4: 52–61.

67. Correll DL, The role of phosphorus in the eutrophication of receiving waters: A review. Journal of Environmental Quality 1998; 27: 261-266.

68. Jimenez-Perez M, Sanchez-Castillo P, Romera O, Fernandez-Moreno D, Pérez-Martínez C, Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. Enzyme and Microbial Technology 2004; 34: 392-398.

69. An J-Y, Sim S-J, Lee JS, Kim BW, Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. Journal of Applied Phycology 2003; 15: 185-191.

70. Worms IAM, Traber J, Kistler D, Sigg L, Slaveykova VI, Uptake of Cd (II) and Pb (II) by microalgae in presence of colloidal organic matter from wastewater treatment plant effluents. Environmental Pollution 2010; 158: 369-374.

71. Zeng X, Danquah MK, Zheng C, Potumarthi R, Chen XD, Lu Y, NaCS–PDMDAAC immobilized autotrophic cultivation of *Chlorella* sp. for wastewater nitrogen and phosphate removal. Chemical Engineering Journal 2012; 187: 185-192.

72. Mendez L, Sialve B, Tomás-Pejó E, Ballesteros M, Steyer JP, González-Fernández C, Comparison of *Chlorella vulgaris* and cyanobacterial biomass: cultivation in urban wastewater and methane production. Bioprocess and Biosystems Engineering 2016; 39: 703-712.

73. Álvarez-Díaz PD, Ruiz J, Arbib Z, Barragán J, Garrido-Pérez MC, Perales JA, Wastewater treatment and biodiesel production by *Scenedesmus obliquus* in a two-stage cultivation process. Bioresource Technology 2015; 181: 90-96.

74. Martínez ME, Sánchez S, Jiménez JM, El Yousfi F, Muñoz L, Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. Bioresource Technology 2000; 73: 263-272.

75. Olguín EJ, Galicia S, Mercado G, Pérez T, Annual productivity of *Spirulina* (Arthrospira) and nutrient removal in a pig wastewater recycling process under tropical conditions. Journal of Applied Phycology 2003; 15: 249-257.

76. Osundeko O, Dean AP, Davies H, Pittman JK, Acclimation of Microalgae to Wastewater Environments Involves Increased Oxidative Stress Tolerance Activity. Plant and Cell Physiology 2014; 55: 1848-1857.

77. Chiu S-Y, Kao C-Y, Chen T-Y, Chang Y-B, Kuo C-M, Lin C-S, Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource. Bioresource Technology 2015; 184: 179-189.

78. Deng D, Tam NF-y, Isolation of microalgae tolerant to polybrominated diphenyl ethers (PBDEs) from wastewater treatment plants and their removal ability. Bioresource Technology 2015; 177: 289-297.

79. Kabra AN, Ji M-K, Choi J, Kim JR, Govindwar SP, Jeon B-H, Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas mexicana*. Environmental Science and Pollution Research 2014; 21: 12270-12278.

80. Suresh Kumar K, Dahms H-U, Won E-J, Lee J-S, Shin K-H, Microalgae – A promising tool for heavy metal remediation. Ecotoxicology and Environmental Safety 2015; 113: 329-352.

81. Inthorn D, Removal of heavy metal by using microalgae. Photosynthetic Microorganisms in Environmental Biotechnology 2001; 310: 111-135.

82. Zeraatkar AK, Ahmadzadeh H, Talebi AF, Moheimani NR, McHenry MP, Potential use of algae for heavy metal bioremediation, a critical review. Journal of Environmental Management 2016; 181: 817-831.

83. Mehta SK, Gaur JP, Use of Algae for Removing Heavy Metal Ions From Wastewater: Progress and Prospects. Critical Reviews in Biotechnology 2005; 25: 113-152.

84. Covarrubias SA, de-Bashan LE, Moreno M, Bashan Y, Alginate beads provide a beneficial physical barrier against native microorganisms in wastewater treated with immobilized bacteria and microalgae. Applied Microbiology and Biotechnology 2012; 93: 2669-2680.

85. Delrue F, Setier PA, Sahut C, Cournac L, Roubaud A, Peltier G, Froment AK, An economic, sustainability, and energetic model of biodiesel production from microalgae. Bioresource Technology 2012; 111: 191-200.

86. Posten C, Design principles of photo-bioreactors for cultivation of microalgae. Engineering in Life Sciences 2009; 9: 165-177.

87. Wan M, Liu P, Xia J, Rosenberg JN, Oyler GA, Betenbaugh MJ, Nie Z, Qiu G, The effect of mixotrophy on microalgal growth, lipid content, and expression levels of three pathway genes in *Chlorella sorokiniana*. Applied Microbiology and Biotechnology 2011; 91: 835-844.

88. Turon V, Trably E, Fouilland E, Steyer JP, Potentialities of dark fermentation effluents as substrates for microalgae growth: A review. Process Biochemistry.

89. Chen F, High cell density culture of microalgae in heterotrophic growth. Trends in Biotechnology 1996; 14: 421-426.

90. Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y, Heterotrophic cultures of microalgae: Metabolism and potential products. Water Research 2011; 45: 11-36.

91. Boyle NR, Morgan JA, Flux balance analysis of primary metabolism in *Chlamydomonas reinhardtii*. BMC Systems Biology 2009; 3: 4.

92. Cheirsilp B, Torpee S, Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. Bioresource Technology 2012; 110: 510-516.

93. Choi H-JL, Seung-Mok, Biomass and oil content of microalgae under mixotrophic conditions. Environmental Engineering Research 2015; 20: 25-32.

94. Pancha I, Chokshi K, Mishra S, Enhanced biofuel production potential with nutritional stress amelioration through optimization of carbon source and light intensity in *Scenedesmus* sp. CCNM 1077. Bioresource Technology 2015; 179: 565-572.

95. Paranjape K, Leite GB, Hallenbeck PC, Strain variation in microalgal lipid production during mixotrophic growth with glycerol. Bioresource Technology 2016; 204: 80-88.

96. Lowrey J, Brooks MS, McGinn PJ, Heterotrophic and mixotrophic cultivation of microalgae for biodiesel production in agricultural wastewaters and associated challenges—a critical review. Journal of Applied Phycology 2015; 27: 1485-1498.

97. Sanz-Luque E, Chamizo-Ampudia A, Llamas A, Galvan A, Fernandez E, Understanding nitrate assimilation and its regulation in microalgae. Frontiers in Plant Science 2015; 6: 899.

98. Cai T, Park SY, Li Y, Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renewable and Sustainable Energy Reviews 2013; 19: 360-369.

99. Kang J, Wen Z, Use of microalgae for mitigating ammonia and CO2 emissions from animal production operations — Evaluation of gas removal efficiency and algal biomass composition. Algal Research 2015; 11: 204-210.

100. He PJ, Mao B, Shen CM, Shao LM, Lee DJ, Chang JS, Cultivation of *Chlorella vulgaris* on wastewater containing high levels of ammonia for biodiesel production. Bioresource Technology 2013; 129: 177-181.

101. Collos Y, Harrison PJ, Acclimation and toxicity of high ammonium concentrations to unicellular algae. Marine Pollution Bulletin 2014; 80: 8-23.

102. Hsieh C-H, Wu W-T, Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. Bioresource Technology 2009; 100: 3921-3926.

103. Casal C, Cuaresma M, Vega JM, Vilchez C, Enhanced Productivity of a Lutein-Enriched Novel Acidophile Microalga Grown on Urea. Marine Drugs 2011; 9: 29.

104. Minaeva E, Forchhammer K, Ermilova E, Glutamine Assimilation and Feedback Regulation of L-acetyl-N-glutamate Kinase Activity in *Chlorella variabilis* NC64A Results in Changes in Arginine Pools. Protist 2015; 166: 493-505.

105. Ogbonna JC, Yoshizawa H, Tanaka H, Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. Journal of Applied Phycology 2000; 12: 277-284.

106. Perez-Garcia O, de-Bashan LE, Hernandez J-P, Bashan Y, Efficiency of growth and nutrient uptake from wastewater by heterotrophic, autotrophic, and mixotrophic cultivation of *Chlorella vulgaris* immobilized with *Azospirillum brasilense*. Journal of Phycology 2010; 46: 800-812.

107. Kalyuzhnyi S, Sklyar V, Epov A, Arkhipchenko I, Barboulina I, Orlova O, Kovalev A, Nozhevnikova A, Klapwijk A, Sustainable treatment and reuse of diluted pig manure streams in Russia. Applied Biochemistry and Biotechnology 2003; 109: 77-94.

108. Moreno-Garrido I, Lubián LM, Blasco J, Sediment toxicity tests involving immobilized microalgae (Phaeodactylum tricornutum Bohlin). Environment International 2007; 33: 481-485.

109. Mallick N, Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 2002; 15: 377-390.

110. Bashan Y, Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnology Advances 1998; 16: 729-770.

111. Moreno-Garrido I, Microalgae immobilization: Current techniques and uses. Bioresource Technology 2008; 99: 3949-3964.

112. Lam MK, Lee KT, Immobilization as a feasible method to simplify the separation of microalgae from water for biodiesel production. Chemical Engineering Journal 2012; 191: 263-268.

113. Liu K, Li J, Qiao H, Lin A, Wang G, Immobilization of *Chlorella sorokiniana* GXNN 01 in alginate for removal of N and P from synthetic wastewater. Bioresource Technology 2012; 114: 26-32.

114. Praveen P, Loh K-C, Photosynthetic aeration in biological wastewater treatment using immobilized microalgae-bacteria symbiosis. Applied Microbiology and Biotechnology 2015; 99: 10345-10354.

115. Petrovič A, Simonič M, The effect of carbon source on nitrate and ammonium removal from drinking water by immobilised *Chlorella sorokiniana*. International Journal of Environmental Science and Technology 2015; 12: 3175-3188.

116. Rooke JC, Leonard A, Sarmento H, Meunier CF, Descy J-P, Su B-L, Novel photosynthetic CO_2 bioconvertor based on green algae entrapped in low-sodium silica gels. Journal of Materials Chemistry 2011; 21: 951-959.

117. Aguilar-May B, del Pilar Sánchez-Saavedra M, Lizardi J, Voltolina D, Growth of *Synechococcus* sp. immobilized in chitosan with different times of contact with NaOH. Journal of Applied Phycology 2007; 19: 181-183.

118. Liu J, Optimisation of biomass and lipid production by adjusting the interspecific competition mode of *Dunaliella salina* and *Nannochloropsis gaditana* in mixed culture. Journal of Applied Phycology 2014; 26: 163-171.

119. Zhao P, Yu X, Li J, Tang X, Huang Z, Enhancing lipid productivity by co-cultivation of *Chlorella* sp. U4341 and *Monoraphidium* sp. FXY-10. Journal of Bioscience and Bioengineering 2014; 118: 72-77.

120. Wan C, Zhao X-Q, Guo S-L, Asraful Alam M, Bai F-W, Bioflocculant production from *Solibacillus silvestris* W01 and its application in cost-effective harvest of marine microalga *Nannochloropsis oceanica* by flocculation. Bioresource Technology 2013; 135: 207-212.

121. Powell RJ, Hill RT, Rapid aggregation of biofuel-producing algae by the *Bacterium Bacillus* sp. strain RP1137. Applied and Environmental Microbiology 2013; 79: 6093-6101.

122. Wang Y, Yang Y, Ma F, Xuan L, Xu Y, Huo H, Zhou D, Dong S, Optimization of *Chlorella vulgaris* and bioflocculant-producing bacteria co-culture: enhancing microalgae harvesting and lipid content. Letters in Applied Microbiology 2015; 60: 497-503.

123. Palacios OA, Bashan Y, Schmid M, Hartmann A, de-Bashan LE, Enhancement of thiamine release during synthetic mutualism between *Chlorella sorokiniana* and *Azospirillum brasilense* growing under stress conditions. Journal of Applied Phycology 2016; 28: 1521-1531.

124. Hernandez J-P, de-Bashan LE, Bashan Y, Starvation enhances phosphorus removal from wastewater by the microalga *Chlorella* spp. co-immobilized with *Azospirillum brasilense*. Enzyme and Microbial Technology 2006; 38: 190-198.

125. Ruiz-Güereca DA, Sánchez-Saavedra MdP, Growth and phosphorus removal by *Synechococcus elongatus* co-immobilized in alginate beads with *Azospirillum brasilense*. Journal of Applied Phycology 2016; 28: 1501-1507.

126. Ren H-Y, Liu B-F, Kong F, Zhao L, Ren N, Hydrogen and lipid production from starch wastewater by co-culture of anaerobic sludge and oleaginous microalgae with simultaneous COD, nitrogen and phosphorus removal. Water Research 2015; 85: 404-412.

127. Kazamia E, Czesnick H, Nguyen TTV, Croft MT, Sherwood E, Sasso S, Hodson SJ, Warren MJ, Smith AG, Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. Environmental Microbiology 2012; 14: 1466-1476.

128. Xie B, Bishop S, Stessman D, Wright D, Spalding MH, Halverson LJ, *Chlamydomonas reinhardtii* thermal tolerance enhancement mediated by a mutualistic interaction with vitamin B12-producing bacteria. ISME J 2013; 7: 1544-1555.

129. Wang RM, Tian Y, Xue SZ, Zhang DM, Zhang QH, Wu X, Kong DZ, Cong W, Enhanced microalgal biomass and lipid production via co-culture of *Scenedesmus obliquus* and *Candida tropicalis* in an autotrophic system. Journal of Chemical Technology and Biotechnology 2016; 91: 1387-1396.

130. Ryu B-G, Kim J, Farooq W, Han J-I, Yang J-W, Kim W, Algal–bacterial process for the simultaneous detoxification of thiocyanate-containing wastewater and maximized lipid production under photoautotrophic/photoheterotrophic conditions. Bioresource Technology 2014; 162: 70-79.

131. Wrede D, Taha M, Miranda AF, Kadali K, Stevenson T, Ball AS, Mouradov A, Co-cultivation of fungal and microalgal cells as an efficient system for harvesting microalgal cells, lipid production and wastewater treatment. PLoS ONE 2014; 9: e113497.

132. Kong Q, Li L, Martinez B, Chen P, Ruan R, Culture of microalgae *Chlamydomonas reinhardtii* in wastewater for biomass feedstock production. Applied Biochemistry and Biotechnology 2010; 160: 9-18.

133. Patel AK, Huang EL, Low-Décarie E, Lefsrud MG, Comparative Shotgun Proteomic Analysis of Wastewater-Cultured Microalgae: Nitrogen Sensing and Carbon Fixation for Growth and Nutrient Removal in Chlamydomonas reinhardtii. Journal of Proteome Research 2015; 14: 3051-3067.

134. Li Y, Chen Y-F, Chen P, Min M, Zhou W, Martinez B, Zhu J, Ruan R, Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresource Technology 2011; 102: 5138-5144.

135. Bhattacharjee M, Siemann E, Low algal diversity systems are a promising method for biodiesel production in wastewater fed open reactors. Algae 2015; 30: 67-79.

136. Feng Y, Li C, Zhang D, Lipid production of *Chlorella vulgaris* cultured in artificial wastewater medium. Bioresource Technology 2011; 102: 101-105.

137. Woertz I, Feffer A, Lundquist T, Nelson Y, Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. Journal of Environmental Engineering 2009; 135: 1115-1122.

138. Wu LF, Chen PC, Huang AP, Lee CM, The feasibility of biodiesel production by microalgae using industrial wastewater. Bioresource Technology 2012; 113: 14-18.

139. Jiang L, Luo S, Fan X, Yang Z, Guo R, Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO_2. Applied Energy 2011; 88: 3336-3341.

140. Chinnasamy S, Bhatnagar A, Hunt RW, Das KC, Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresource Technology 2010; 101: 3097-3105.

141. Rashid N, Lee K, Mahmood Q, Bio-hydrogen production by Chlorella vulgaris under diverse photoperiods. Bioresource Technology 2011; 102: 2101-2104.

142. Chader S, Mahmah B, Chetehouna K, Amrouche F, Abdeladim K, Biohydrogen production using green microalgae as an approach to operate a small proton exchange membrane fuel cell. International Journal of Hydrogen Energy 2011; 36: 4089-4093.

143. Hallenbeck PC, Fermentative hydrogen production: Principles, progress, and prognosis. International Journal of Hydrogen Energy 2009; 34: 7379-7389.

144. Yan Q, Zhao M, Miao H, Ruan W, Song R, Coupling of the hydrogen and polyhydroxyalkanoates (PHA) production through anaerobic digestion from Taihu blue algae. Bioresource Technology 2010; 101: 4508-4512.

145. Harun R, Danquah MK, Forde GM, Microalgal biomass as a fermentation feedstock for bioethanol production. Journal of Chemical Technology and Biotechnology 2010; 85: 199-203.

146. Khoo HH, Koh CY, Shaik MS, Sharratt PN, Bioenergy co-products derived from microalgae biomass via thermochemical conversion–life cycle energy balances and CO_2 emissions. Bioresource Technology 2013; 143: 298-307.

147. Kishimoto M, Okakura T, Nagashima H, Minowa T, Yokoyama SY, Yamaberi K, CO_2 fixation and oil production using micro-algae. Journal of Fermentation and Bioengineering 1994; 78: 479-482.

148. Liu J, Yuan C, Hu G, Li F, Effects of Light Intensity on the Growth and Lipid Accumulation of Microalga *Scenedesmus* sp. 11-1 Under Nitrogen Limitation. Applied Biochemistry and Biotechnology 2012; 1-11.

149. Passos F, Hernandez-Marine M, García J, Ferrer I, Long-term anaerobic digestion of microalgae grown in HRAP for wastewater treatment. Effect of microwave pretreatment. Water Research 2014; 49: 351-359.

150. Chen W-T, Zhang Y, Zhang J, Yu G, Schideman LC, Zhang P, Minarick M, Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil. Bioresource Technology 2014; 152: 130-139.

151. Collet P, Hélias A, Lardon L, Ras M, Goy R-A, Steyer J-P, Life-cycle assessment of microalgae culture coupled to biogas production. Bioresource Technology 2011; 102: 207-214.

152. Bell G, Experimental evolution of heterotrophy in a green alga. Evolution 2013; 67: 468-476.

153. Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, Smith AG, Biodiesel from algae: challenges and prospects. Current Opinion in Biotechnology 2010; 21: 277-286.

154. Clarens AF, Resurreccion EP, White MA, Colosi LM, Environmental life cycle comparison of algae to other bioenergy feedstocks. Environmental Science & Technology 2010; 44: 1813-1819.

155. Menger-Krug E, Niederste-Hollenberg J, Hillenbrand T, Hiessl H, Integration of microalgae systems at municipal wastewater treatment plants: implications for energy and emission balances. Environmental Science & Technology 2012; 46: 11505-11514.

156. Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y, Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresource Technology 2011; 102: 159-165.

157. Mu D, Min M, Krohn B, Mullins KA, Ruan R, Hill J, Life cycle environmental impacts of wastewater-based algal biofuels. Environmental Science & Technology 2014; 48: 11696-11704.

158. Soomro RR, Ndikubwimana T, Zeng X, Lu Y, Lin L, Danquah MK, Development of a two-stage microalgae dewatering process – a life cycle assessment approach. Frontiers in Plant Science 2016; 7: 113.

159. Lardon L, Hélias A, Sialve B, Steyer JP, Bernard O, Life-cycle assessment of biodiesel production from microalgae. Environmental Science & Technology 2009; 43: 6475-6481.

160. Jorquera O, Kiperstok A, Sales EA, Embiruçu M, Ghirardi ML, Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. Bioresource Technology 2010; 101: 1406-1413.

161. Sturm BSM, Lamer SL, An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Applied Energy 2011; 88: 3499-3506.

162. Demirbaş A, Biomass resource facilities and biomass conversion processing for fuels and chemicals. Energy Conversion and Management 2001; 42: 1357-1378.

163. Kadam KL, Environmental implications of power generation via coal-microalgae cofiring. Energy 2002; 27: 905-922.

164. Fortier M-OP, Roberts GW, Stagg-Williams SM, Sturm BSM, Life cycle assessment of bio-jet fuel from hydrothermal liquefaction of microalgae. Applied Energy 2014; 122: 73-82.

165. Liu X, Clarens AF, Colosi LM, Algae biodiesel has potential despite inconclusive results to date. Bioresource Technology 2012; 104: 803-806.

166. Sills DL, Paramita V, Franke MJ, Johnson MC, Akabas TM, Greene CH, Tester JW, Quantitative uncertainty analysis of life cycle assessment for algal biofuel production. Environmental Science & Technology 2012; 47: 687-694.

167. Larkum AWD, Ross IL, Kruse O, Hankamer B, Selection, breeding and engineering of microalgae for bioenergy and biofuel production. Trends in Biotechnology 2012; 30: 198-205.

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 (P_{CO_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 (°C)**	**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 kesslerii	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 (°C)**	**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%	3.78×10^9 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%	5.2×10^6 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 °C	(*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 (°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*)
Chlorococum 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

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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 (°C)**	**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*)

mus sp.	fied petroleum gas

a, semi-continuous culture.

References

1. Ge Y, Liu J, Tian G, Growth characteristics of *Botryococcus braunii* 765 under high CO_2 concentration in photobioreactor. Bioresource Technology 2011; 102: 130-134.
2. Yoo C, Jun SY, Lee JY, Ahn CY, Oh HM, Selection of microalgae for lipid production under high levels carbon dioxide. Bioresource Technology 2010; 101: S71-S74.
3. Illman A, Scragg A, Shales S, Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. Enzyme and Microbial Technology 2000; 27: 631-635.
4. Lv JM, Cheng LH, Xu XH, Zhang L, Chen HL, Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions. Bioresource Technology 2010; 101: 6797-6804.
5. Tang D, Han W, Li P, Miao X, Zhong J, CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. Bioresource Technology 2011; 102: 3071-3076.
6. Fan J, Xu H, Luo Y, Wan M, Huang J, Wang W, Li Y, Impacts of CO_2 concentration on growth, lipid accumulation, and carbon-concentrating-mechanism-related gene expression in oleaginous *Chlorella*. Applied Microbiology and Biotechnology 2015; 99: 2451-2462.
7. Kishimoto M, Okakura T, Nagashima H, Minowa T, Yokoyama SY, Yamaberi K, CO_2 fixation and oil production using micro-algae. Journal of Fermentation and Bioengineering 1994; 78: 479-482.
8. Huntley ME, Redalje DG, CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitigation and Adaptation Strategies for Global Change 2007; 12: 573-608.
9. Chiu SY, Kao CY, Tsai MT, Ong SC, Chen CH, Lin CS, Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration. Bioresource Technology 2009; 100: 833-838.
10. Wang T-H, Chu S-H, Tsai Y-Y, Lin F-C, Lee W-C, Influence of inoculum cell density and carbon dioxide concentration on fed-batch cultivation of *Nannochloropsis oculata*. Biomass and Bioenergy 2015; 77: 9-15.
11. Chiu SY, Kao CY, Huang TT, Lin CJ, Ong SC, Chen CD, Chang JS, Lin CS, Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using Chlorella sp. cultures. Bioresource Technology 2011; 102: 9135-9142.

12. Li T, Xu G, Rong J, Chen H, He C, Giordano M, Wang Q, The acclimation of *Chlorella* to high-level nitrite for potential application in biological NOx removal from industrial flue gases. Journal of Plant Physiology 2016; 195: 73-79.

13. Zimmerman WB, Zandi M, Hemaka Bandulasena H, Tesar V, James Gilmour D, Ying K, Design of an airlift loop bioreactor and pilot scales studies with fluidic oscillator induced microbubbles for growth of a microalgae *Dunaliella salina*. Applied Energy 2011; 88: 3357-3369.

14. Li X-k, Xu J-l, Guo Y, Zhou W-z, Yuan Z-h, Effects of simulated flue gas on components of *Scenedesmus raciborskii* WZKMT. Bioresource Technology 2015; 190: 339-344.

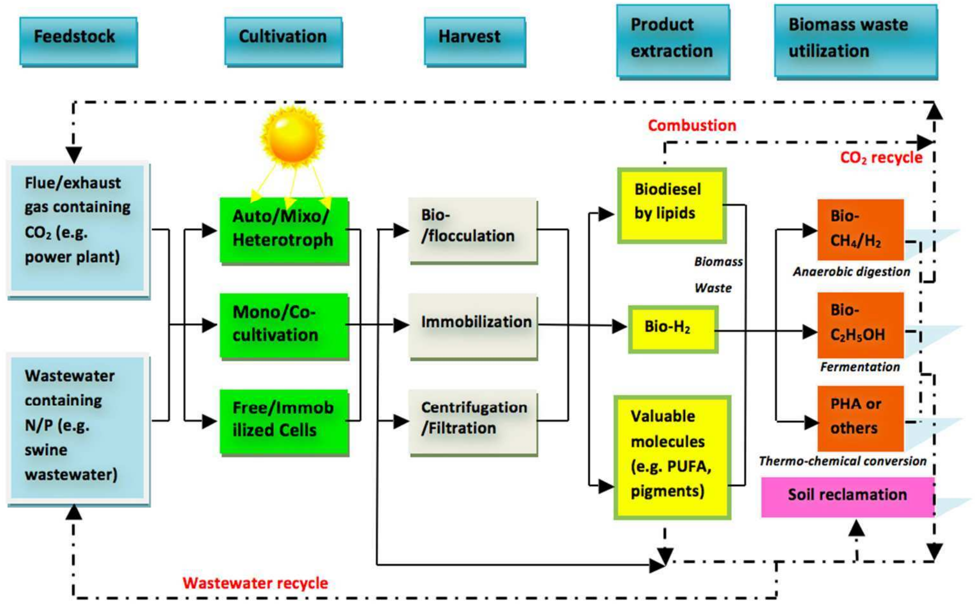

Figure 1. Integrated system of microalgae-based applications, composed of four major parts: feedstock, cell cultivation, product extraction, and biomass waste utilization. The wastewater and CO2 recycling are shown in dashed lines.

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.