Biotechnology and Bioprocess Engineering 21: 236-249 (2016)
DOI 10.1007/s12257-015-0388-2

REVIEW PAPER

Integrated Algal Engineering for Bioenergy Generation, Effluent Remediation, and Production of High-value Bioactive Compounds

Mohd Azmuddin Abdullah, Ashfaq Ahmad, Syed Muhammad Usman Shah, Sanaa Mahmoud Metwally Shanab, Hamdy Elsayed Ahmed Ali, Mervat Aly Mohamed Abo-State, and Mohd Fariduddin Othman

Received: 15 June 2015 / Accepted: 23 February 2016

Abstract Increased demand for energy worldwide has resulted in increasing interest in alternative renewable sources of biofuels. Demand for improved systems of bioenergy generation, environmental remediation, and co-production of high value bioactive compounds has led to the potential use of algae in biomass utilization. In Malaysia, palm oil industries generate high amount of solid wastes. Palm Oil Mill Effluent (POME) is estimated to be three times of the amount of crude palm oil produced. POME is a heavily polluting wastewater due to its high chemical oxygen demand (COD), high biochemical oxygen demand (BOD), and high contents of minerals such as nitrogen and phosphorus that can cause severe pollution to the environment and water resources. A combination of wastewater treatment and renewable bioenergy co-generation with recovery of high-value biochemicals would benefit the palm oil industry.

Keywords: microalgae, bioenergy, biocompounds, palm oil mill effluent, bioremediation

Mohd Azmuddin Abdullah*
Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
Tel: +609-668-3104; Fax: +609-668-3105
E-mail: azmuddin@umt.edu.my

Ashfaq Ahmad
Department of Chemical Engineering, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak, Malaysia

Syed Muhammad Usman Shah
Department of Biosciences, COMSATS Institute of Information Technology, Park Road 44000, Islamabad, Pakistan

Sanaa Mahmoud Metwally Shanab
Department of Botany and Microbiology, Faculty of Science, Cairo University, 12613 Giza, Egypt

Hamdy Elsayed Ahmed Ali, Mervat Aly Mohamed Abo-State
Department of Microbiology, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Egypt

Mohd Fariduddin Othman
Department of Freshwater Fisheries Research Division, Fisheries Research Institute GlamiLemi, Jelebu, 71650 Titi, Negeri Sembilan, Malaysia

1. Introduction

Due to increasing demand for energy, interests in alternative renewable sources of fuels are increasing worldwide [1]. The challenge in biofuel production is the competition between energy crop for fuel and food which could affect the food prices [2,3]. The solution may lie in the application of microalgae as source of bioenergy. Microalgae have the capability of using inorganic carbon dioxide and wastewater components as nutrients while producing the biomass. In addition, microalgal biomass can be source of high-value products for health, pharmaceuticals, nutraceuticals, pigments, animal feeds, and biofuels [4-6].

Algae are one of the most abundant and primitive life forms on earth. They are predominantly single-celled microscopic organisms. Categorized as plants because of their photosynthetic ability, algae are now placed within diverse kingdom Protista of eukaryotes [7]. However, they are less complex than plants. Eukaryotic chlorophyll-containing algae cells can be a single cell, colonies, filament of cells, or kelp tissues. Microalgal cells have growth rate that can be 100 times faster than land-based plant. They can double their biomass in less than one day [8]. Under favorable growth conditions, they can double once every 3 ~ 4 h, but mostly every 1 ~ 2 days [9].

Microalgae are ideal green energy source because they

do not interfere with world food chain or the eco-population of animals and plants. Different types of biofuels can be developed using microalgae, including biodiesel from lipid, bioethanol from fermentation of biomass, biomethane from anaerobic digestion of biomass, and photo-biologically produced biohydrogen [6,10,11]. The major challenge to commercialize microalgal biofuels is the high energy input required for the production, especially in the upstream processes. For biodiesel production, the required amount of algae biomass is huge. To be economically feasible, the production cost needs to fall below $400/tonne of algae biomass. However, the costs for a medium-scale plant are still 173 times more expensive than the target price [12,13]. Negative energy balance has been reported in a life cycle assessment (LCA) of microalgae for biodiesel production, especially in the harvesting and drying process [14]. Co-generation of bioenergy with effluent remediation and the recovery of high-value biocompounds are effective strategies to off-set the high production cost. Algae adds an advantage to effluent treatment by increasing the performance of degradation, improving CO_2 balance, and lowering energy demand for oxygen supply in the aerobic treatment stage. Algae can assimilate plant nutrients and support bacteria growth with oxygen. Bacteria, in turn, are involved in the degradation of organic material in wastewater, the same process utilized to activate sludge [15,16].

In aquatic natural products, the following three areas of research have emerged in the last three decades: toxins, bioproducts, and chemical ecology. Algae can provide novel biochemically active substances. More than 15,000 novel compounds in algae have been chemically determined [17-20]. Algae have also developed significant structural–chemical diversities from different metabolic pathways to survive in competitive freshwater and marine environment [21,22]. They contain pigments, antioxidants, β-carotenes, polysaccharides, triglycerides, fatty acids, vitamins, and the biomass. Species such as Cyanobacteria *Phormidium cebennse*, *Oscillatoria raciborskii*, *Scytonema burmanicum*, *Calothrix elenkinii*, and *Anabaena variabilis* have sulfolipids with anti HIV-1 activity. Hydrocolloids, alginate, agar, and carrageenan produced from seaweeds are largely used as viscosity-modifying agents in foods and pharmaceuticals [23]. As they are largely used as bulk commodities in industrial sectors such as pharmaceuticals, cosmetics, nutraceuticals, functional foods, and biofuels, readily available supply of extracts, fractions, and pure compounds are of prime importance [23].

Malaysia has a huge supply of biomass resource mainly derived from palm oil, wood industry, and agro-industries. At present, most palm biomass comes from the oil extraction process. It contains mesocarp fiber, shell, and empty fruit bunches (EFB). Mesocarp fiber and shell are burnt within the boiler to generate steam for electricity, while EFB is used as fertilizer or soil mulching in oil palm plantation. POME has not been commercially re-used by the industry extensively. However, it has high contents of carbohydrates (29.55%), proteins (12.75%), nitrogenous compounds, lipids, and a considerable amount of cellulose and non-toxic minerals that can provide good source for microbial fermentation [24,25].

Effluent treatment methods currently used by the Malaysian palm oil industry include anaerobic/facultative ponds, tank digestion and mechanical aeration, tank digestion and facultative ponds, decanter, physicochemical treatment, and biological treatment. Membrane technology, up-flow anaerobic filtration, up-flow anaerobic sludge blanket, and up-flow anaerobic sludge fixed film bioreactor have also been reported for POME treatment. At present, 85% of POME treatment is based on anaerobic and facultative pond system followed by open tank digester attached with extended aeration in a pond [123]. Since POME contains high levels of organic matters, the anaerobic digestion stage can convert bulk of wastes into biomethane. The treated effluent is further exposed to aerobic treatment in order to meet the required discharge standards [26,27].

The conventional POME treatment method releases significant amount of methane into the open air. Worldwide, approximately 590 ~ 880 million tons of methane are released into the atmosphere through microbial activity annually, majority (90%) of coming from biogenic sources [28]. Methane is over 20 times more effective in trapping heat in the atmosphere than carbon dioxide over a 100-year period [28]. Sustainable energy management in palm oil mill has entered a new dynamic era with the opportunity to culture microalgae using POME [29], to capture biogas for later use as bioenergy, and to recover biomass for value-added products. Most palm oil millers favor the idea of culturing microalgae as a tertiary treatment before POME is discharged due to its practicality, low cost, and high efficiency. Most nutrients such as nitrate and ortho-phosphate that are not removed during anaerobic digestion can be further treated in microalgae pond. The cultured microalgae can be used as diet supplement for live feed culture [30].

This review discusses engineering issues of microalgae for lipid and biomethane production in combination with effluent treatments and the recovery of valuable biocompounds with specific reference to the use of POME.

2. Algal Engineering

With more knowledge on algal biology and technological advances, algal biofuel production and commercialization

will be feasible in the not too distant future, provided that the issues related to large-scale methods for cultivating and harvesting algal cells, extracting lipid, and converting them into diesel are properly addressed. Manipulating the processing conditions such as temperature, salinity, light, pH, nutrients, and culturing duration can modulate cell growth, the biochemical and lipid compositions for subsequent optimization of overall yield, and the productivity of algae [6,31,32].

2.1. Engineering considerations

2.1.1. Growth conditions

Microalgae are naturally acclimatized to a range of aquatic habitats. Autotrophic or photoautotrophic microalgae require photosynthesis to produce complex organic compound from simple inorganic molecules like salts [33]. Heterotrophic microalgae do not carry out photosynthesis but use organic substrates like glucose or acetate as their carbon and energy source to stimulate growth. However, mixotrophic microalgae derive energy from both photosynthesis and chemical oxidation. They combine both photoautotrophic and heterotrophic mechanism to sustain growth [29]. Heterotrophic microalgae can utilize organic carbon source for growth in the dark, thus eliminating the requirement for light [34]. In the presence of a fixed carbon source (glucose), some microalgae can have higher biomass (> 20 g/L) and oil productivity (> 50% of the dry weight as lipid) than photoautotrophic production [35]. For green *Chlorella zofingiensis,* heterotrophic cells fed 30 g/L of glucose has higher oleic acid content (increased from 17.9 to 35.2% in total fatty acids) than photoautotrophic cells. In addition, the oils produced from heterotrophic *C. zofingiensis* appear to be more suitable for biodiesel production [36].

2.1.2. Oil productivity

Oil productivity as high as 1,00,000 L/ha/yr or 27.5 mL/m^2/day in a shallow open pond system (raceway pond) has been reported [10,37]. This would actually require a biomass productivity of 36.7 g/m^2/day with 75% oil content, or 50 g/m^2/day with 50% oil content, or 91.6 g/m^2/day with 30% oil content. However, none of these has been reported. *Botryococcus brauni* accumulates 70 ~ 80% of cell weight as lipid. However, it has low biomass productivity of only 3 g/m^2/day [4,10]. Other fast growing strains may achieve areal productivity of 12 ~ 47.6 g/m^2/day. However, their intracellular lipids levels are typically lower than 30% [124].

2.1.3. Tolerance to extreme culture conditions

Lipids in eukaryotic photoautotrophic cells are structural components of cell membranes. They can modulate cellular activity and serve as energy storage compounds. Under stressed conditions, microalgae can accumulate high concentrations of carbon in the form of neutral lipids triacylglycerol (TAG) [38,39]. These high-carbon lipids are potential source for sustainable oil production. One of the main biological functions of TAG is to provide energy for immediate or delayed metabolic requirements. Once the stationary phase of the growth cycle is reached and nutrients are depleted, microalgae may switch cell metabolism and store energy in the form of lipids for future response to environmental stresses [38,40]. The switch can be protracted without net biomass productivity. The estimated biochemical compositions for single species of chlorophyta *Chlorella*, *Botryococcus braunii*, and *Dunaliella salina* are 30 ~ 50% protein, 20 ~ 40% carbohydrate, and 8 ~ 15% lipids. Under stressed conditions, these species can accumulate 80% fatty acid, 80% hydrocarbon, and 40% glycerol on dry weight basis [41]. However, under nutrient deficient condition, oil productivity might be lower than that in the control (at 5 g/m^2/day) [38].

2.2. Factors for optimal production

2.2.1. Temperature and light

Light and temperature can influence the compositions of algae, including lipid, carbohydrate, and biocompounds. Carbohydrate and other bioactive contents of microalgae can be increased by irradiance and temperature variations [42]. Light intensity could stimulate fatty acid and membrane lipid biosynthesis, mainly in the chloroplast. Under 24 h illumination, it has been reported that *Pavlova lutheri* can achieve the maximum specific growth rate (μ_{max}) at 0.12/day and maximum lipid content at 35% as compared to 0.1/day and 15% lipid content in the dark [43]. However, high light intensities may lead to oxidative damages to polyunsaturated fatty acids (PUFA). In *Nannochloropsis* sp., the levels of unsaturation of fatty acids are decreased when irradiance is increased, especially the level of total n-3 fatty acids is decreased from 29 to 8% in total fatty acids mainly due to decreased Eicosapentaenoic acid (EPA) [44]. It has been reported that increasing the mean light intensity from 215 to 330 mol/m^2/sec can significantly enhance starch content from 8.5 to 40% [45].

Temperature affects biochemical composition, the quantity of cellular lipid, fatty acid classes, and fatty acid unsaturation in membrane lipids. Lipid changes can alter the physical properties of membranes involved in photosynthesis, respiration, and membrane transport [46,47]. The optimal temperatures for most microalgae strains (either freshwater or saline) are 16 ~ 28ºC, although, some can survive extreme temperatures such as -5ºC and above 90ºC [48]. Using a 12:12 h light:dark cycle, higher lipid accumulation (19.3%) in *P. cruentum* has been demonstrated at 25°C as compared

to 35°C [49]. Temperature below optimal range can increase the content of unsaturated lipids in the membrane, thus improving the stability and fluidity of cell membranes, especially thylakoid membrane. Increased contents of unsaturated fatty acids can prevent the photosynthetic machinery from photo-inhibition at low temperatures [50].

2.2.2. Nutrients limitation

Under deficiency conditions at 10 ~ 65 g/L KNO_3, 3 ~ 7.5 g/L Na_2HPO_4, and 2.5 g/L $FeCl_3$, highest lipid accumulation at 37.3, 23.6, 28.3, and 37.2% are obtained but with slightly reduced cell growth at 0.64, 0.49, 0.54, and 0.38 g/L for *N. oculata*, *T. suecica*, *I. galbana*, and *P. lutheri,* respectively [32]. When algal growth slows down as a result of nutrient deficiency, there is no need to synthesize new membrane compounds because algae cells can transfer fatty acids into storage lipids before conditions improve. The highest lipid cell content at 0.4 g/g in *Neochloris oleoabundans* has been obtained at the lowest sodium nitrate concentration of 3 mM, whereas higher lipid productivity at 0.133 g/L/day has been achieved at 5 mM $NaNO_3$ with lipid content of 0.34 g/g [51]. Therefore, nutrient limitation is an important modulator in algal lipid biosynthesis.

2.2.3. Salt concentrations and pH

Some algae exhibit excellent ability to tolerate high salt concentrations. Lipid content in *D. Salina* cells can be manipulated by salt stress and nitrogen limitation. It can reach 38% content when grown at 16% NaCl with 2.5 mM unspecified nitrogen salts [52]. Salt stress can also increase the relative proportions of PUFAs, particularly C18:3n-3 and C16:4n-3 fatty acids. An increase of the initial NaCl concentration from 0.5 to 1.0 M has resulted in an increase of intracellular lipid content in *D. tertiolecta* from 60 to 67% [53]. The optimum salinity and pH for cell growth and lipid content (34 ~ 36%) for *P. lutheri* have been suggested to be 30 ~ 40 ppt and pH 8 ~ 9, respectively [43]. Alkaline pH stress may increase TAG accumulation but decrease the relative level of membrane lipids [43].

2.2.4. CO_2 supplement

Addition of CO_2 is required for autotrophic growth. Sufficient supply of CO_2 is one of the key factors that influence the accumulation of carbohydrate in microalgae, although low level of CO_2 may be adequate for lipid production. Increasing dissolved CO_2 from 3 to 186 mol/L in *C. pyrenoidosa* and *C. reinhardtii* cultivation can elevate the carbohydrate content from 9.3 to 21% and from 3.2 to 7.4%, respectively [54]. However, *N. oculata* grows the best in a semicontinuous system aerated with just 2% CO_2 and operated by 1-day medium replacement for long-term biomass production and higher lipid yield [55].

Maximum lipid content (56% of dry weight) has been reported after 6 days of nitrogen depletion without CO_2 supplementation in *Neochlorisole abundans* UTEX#1185 [56]. Fatty acid synthesis in a highly CO_2-tolerant *Chloroccoccum littorale* is increased at low CO_2 after nitrate depletion with a controlled HCO_3/CO_2 ratio. The relative FA content is 34% at temperature of 22°C, light intensity of 170 mmol photons m^2/sec, and 5% CO_2 with O_2-free gas [57]. Such FA content is comparable to plant seed oils [57]. Therefore, suitable addition of CO_2 may improve autotrophic growth of microalgal cells, protein content, and carbohydrate accumulation with appropriate stress conditions [58].

2.2.5. Open and closed system

An effective culture system should consist of the following: (1) effective lighting area, (2) optimal gas–liquid transfer, (3) easy to operate, (4) low contamination level, (5) low capital and production cost, and (6) the least land area requirement [59]. For commercialization of microalgae cultivation to produce high-value nutritious products for animal feed, raceway pond may be suitable because it has a paddle wheel for agitation and CO_2 sparging at the bottom [60,61]. Raceway pond is relatively easy to operate. It does not consume too much energy. However, high contamination level by undesired microorganisms can ultimately risk the survival of microalgae. In addition, it has high water losses due to evaporation [62]. Therefore, closed systems might be more appropriate choice for biofuels and high-value biocompounds production. Volumetric productivity in closed photobioreactors (PBRs) has been reported to be more than 30 times higher than that in open pond raceways [10]. Due to high mass productivity, harvesting costs can be significantly reduced if closed PBRs are used.

2.3. Reactor engineering

2.3.1. Growth kinetics and productivity

Kinetics of cell growth and lipid production in 250 mL shake flask and 1 ~ 30 L batch cultures at optimized conditions are shown in Table 1. At different optimized conditions, cell densities of 64.6×10^6, 40.8×10^6, 15.5×10^6, and 14.5×10^6 cells/mL were achieved in 250 mL flask for *N. oculata*, *T. suecica*, *I. galbana*, and *P. lutheri*, respectively. Lower cell densities of 52.6×10^6 and 10.7×10^6 cells/mL were obtained in 30 L tank for *N. oculata* and *P. lutheri*, respectively. Cell densities of 36.4×10^6 and 13.4×10^6 cells/mL were obtained in 5 L vessel for *T. suecica* and *I. galbana*, respectively. Only *N. oculata* and *P. lutheri* showed higher lipid content (at 32 ~ 37%).

At optimal illumination for 19.3 ~ 24 h with light intensity

of 162 ~ 198 μmol photons/m^2/sec, the highest cell density and biomass achieved by *N. oculata* are 82.6 × 10^6 cells/mL and 0.96 g/L in 5 L PBR, respectively, as compared to 63.7 × 10^6 cells/mL and 0.72 g/L biomass in 300 L open tank [32]. Lipid content (at 40 ~ 42%) in 5 L PBR is also higher than that (30 ~ 32%) in 300 L open tank. For *N. oculata* in PBR, contents of palmitic acid C16:0 (22%), heptadecanoic acid C17:0 (13.7%), palmitoleic acid C16:1 (9.9%), and oleic acid C18:1 (7.4%) are high. For *P. lutheri* in PBR, the levels of palmitic acid C16:0 (34.4%), palmitoleic acid C16:1 (21.3%), eicosapentanoic acid (EPA) C20:5 (8.4%), and docosahexaenoic acid (DHA) C22:6 (6.9%) are high. Fatty acid profiles of *N. oculata* and *P. Lutheri* in PBR have revealed comparable levels of total saturated fatty acids (SFA) (57% *vs*. 47.9%), monounsaturated fatty acids (MUFA) (17.7% *vs*. 30.9%), and PUFA (22.3% *vs*. 18.9%) [32].

2.3.2. Mixing

Good hydrodynamic condition with cell exposure to light, nutrients, and CO_2 will determine culture performance. Optimum flow velocity for different PBRs is essential to ensure that cells remain in suspension. Flow velocity can be determined based on sinking rate of cells and medium depth. Adding a stirrer may increase the flow rate [63], especially for mass cultivation. However, mixing speed should not cause excessive shear stress. A velocity of 10 ~ 30 cm/sec is effective because higher velocities will consume more energy [64].

2.3.3. Light path

Many types of closed PBRs have been designed, including tubular, column, and flat plate PBRs. The major limitation for mass cultivation is lighting. Light path is therefore an important design factor directly related to the depth of the medium that affects the probability of microalgae exposure to light. A maximum biomass productivity of 1.6 g dry weight L^{-1} day^{-1} has been reported in the 4 cm PBR, while biomass productivity decreases by 35% and 53% in 8 and 12 cm PBRs, respectively. Cellular oil content of 39% and 18% (w/w) are achieved on day 11 in 4 and 8 cm PBRs, respectively, but the oil content in 12 cm PBR is less than 10% (w/w), indicating that suitable light path length is important for oil production [65]. Photoinhibition often occurs under high light intensity with a short light path. It can affect growth rate and lipid content. Additional light especially artificial light can enhance photosynthetic efficiency. However, it can also lead to high energy consumption.

2.3.4. Reactor configuration

To enhance lighting in an open pond raceway, many methods have been suggested [61,66]. However, effective solution is currently unavailable. Transparent rectangular chambers (TRCs) have been used to achieve deep light into open tank PBR to improve photosynthetic efficiency. TRC has the highest illumination surface-to-volume ratio. It can result in the highest cell density (3.745 g/L) at biomass productivity of 0.34 g/L/day. The total biomass from TRC is 56% more than a standard open tank system without TRCs [66]. Therefore, using TRCs is an effective method with low cost [61,66]. Flat-plate PBRs (FPPBRs) have been widely used for microalgal cultivation because of their large surface area to volume ratio, short light/dark cycles, simple structure, and easy scale-up [67]. Novel waved baffles have been introduced into FPPBR to improve light gradient mixing [67,68].

Tubular PBRs can be aligned horizontally, vertically, inclined, or as a helix. The diameters of these tubes are generally 2.5 ~ 5 cm [69,70]. The length of these tubes depends on the potential O_2 accumulation and CO_2 depletion that can limit the scale. To increase the scale, these tubes have to be arrayed horizontally fence-like to improve light

Table 1. Kinetics of cell growth and lipid production in 250 mL shake flask or 1 ~ 30 L batch cultures at optimized conditions

Reactor	*N. oculata*[a]				*T. suecica*[b]				*I. galbana*[c]				*P. lutheri*[d]			
	X'_{max} (g/L/day)	μ_{max} (/day)	t_d (day)	Lipid (%)	X'_{max} (g/L/day)	μ_{max} (/day)	t_d (day)	Lipid (%)	X'_{max} (g/L/day)	μ_{max} (/day)	t_d (day)	Lipid (%)	X'_{max} (g/L/day)	μ_{max} (/day)	t_d (day)	Lipid (%)
250 mL	0.134	0.15	4.62	36.8	0.120	0.16	4.33	26.8	0.128	0.15	4.72	26.3	0.124	0.15	4.74	37.1
1 L	0.117	0.14	4.83	35.3	0.115	0.15	4.72	25.4	0.136	0.14	4.77	28.2	0.120	0.14	4.77	35.8
5 L	0.126	0.14	4.83	34.7	0.113	0.14	4.77	25.1	0.108	0.13	5.21	24.4	0.110	0.13	5.21	34.3
30 L	0.115	0.13	5.21	32.4	0.136	0.17	3.98	27.2	0.134	0.14	4.81	25.7	0.085	0.13	5.33	34.5

[a]*N. oculata*: pH 8, Salinity (35 ppt), photoperiod (24 h), light intensity (188 μmol photons/m^2/sec) KNO_3 (10 g/L), Na_2HPO_4 (6 g/L), and $FeCl_3$ (2.53 g/L).
[b]*T. suecica*: pH 7.9 Salinity (32 ppt), photoperiod (24 h, light intensity (196.5 μmol photons/m^2/s), KNO_3 (13.7 g/L), Na_2HPO_4 (5.6 g/L), and $FeCl_3$ (2.50 g/L).
[c]*I. galbana* : pH 9, Salinity (39.2 ppt), photoperiod (20.5 h), light intensity (188.7 μmol photons m^2/s), KNO_3 (75.4 g/L), Na_2HPO_4 (8.9 g/L), and $FeCl_3$ (2.8 g/L).
[d]*P. lutheri* : pH 7.9, Salinity (35.5 ppt), photoperiod (24 h), light intensity (198 μmol photons m^2/s), KNO_3 (62.5 g/L), Na_2HPO_4 (3.92 g/L), and $FeCl_3$ (2.63 g/L).

exposure and space utilization. However, this will increases operational cost. The wall of these tubes has direct effect on light permeability. However, washing of the wall of these tubes is difficult. At present, mechanical cleaning is the main method to do the washing. Maximum productivities of 40 and 600 mg/L/day have been achieved in *Chlorella vulgaris* cultured in helix tubes with 3.12 cm diameter and straight tubes with 3.2 cm diameter, respectively [71,72].

Column PBRs are generally 2 ~ 2.5 m high with 20 ~ 50 cm diameter. They are similar to conventional fermentation tanks. The only difference between the two is the requirement for internal or external light for microalgae. Simple materials for construction can help reduce the cost. However, it is difficult to obtain high cell density using column PBRs, thus limiting cell growth and the scale. In addition, light utilization efficiency of stirred column PBRs is relatively low. Airlift column PBR uses a draft tube to achieve good mixing. Mixing with CO_2 bubbling can maximize CO_2 capture and further minimize cost [61,73].

Combinations of open and closed systems are the most effective configuration for algal mass cultivation. Combinations can be made between open and closed systems or between autotrophic and heterotrophic cultivation. Abundant microalgae with low density can be cultured in open pond raceways first, which can then be cultured in closed PBRs at high density. Two stage processes can be used to increase the productivity and lipid content in alage [74]. Combination of open pond raceway and flat plate PBRs (or bubbling column) can enhance the production of *Nannochloropsis* by 75% [61]. The capital and operating costs as well as land requirements for combined systems are likely to be significantly higher than those required for one reactor [36].

To improve the volumetric productivity and CO_2 uptake, coupling membrane with the bioreactor can be done in two ways: (1) the membrane can be used to retain biomass in the reactor by continuously extracting water from the bioreactor; and (2) membranes can be used to deliver CO_2 into the cultivation medium either through contact or as sparger. Membrane carbonation photobioreactor (C-MPBR) (Fig. 1) is a new and effective concept proposed for microalgae cultivation and pre-harvesting. The bioreactor is coupled to membrane filtration where membranes can completely retain *C. vulgaris* biomass and then partly recycle them into the bioreactor to maintain high biomass concentration, thus enhancing the flexibility and robustness of the system. MPBR can operate at higher dilution with higher growth rates, resulting in 9-fold higher biomass productivity. In addition, pre-harvesting can be achieved by applying variable concentration factors in the filtration stage. The permeate is recycled to the reactor as feed medium without affecting cell growth, which offers a substantial reduction (77%) in water footprint [75].

3. Harvesting

High recovery efficiency with economically low energy consumption are the main aims in algal harvesting. Macroalgae can be harvested manually. However, microalgae harvesting and dewatering need techniques such as sedimentation, filtration, flocculation, filteration, centrifugation, and drying. Dewatering efficiency can be directly proportional to investment made on the technology used. Natural flocculation methods (*Ecotan* and *Tanfloc*) have been applied to microalgae grown in a high rate algal pond to treat urban

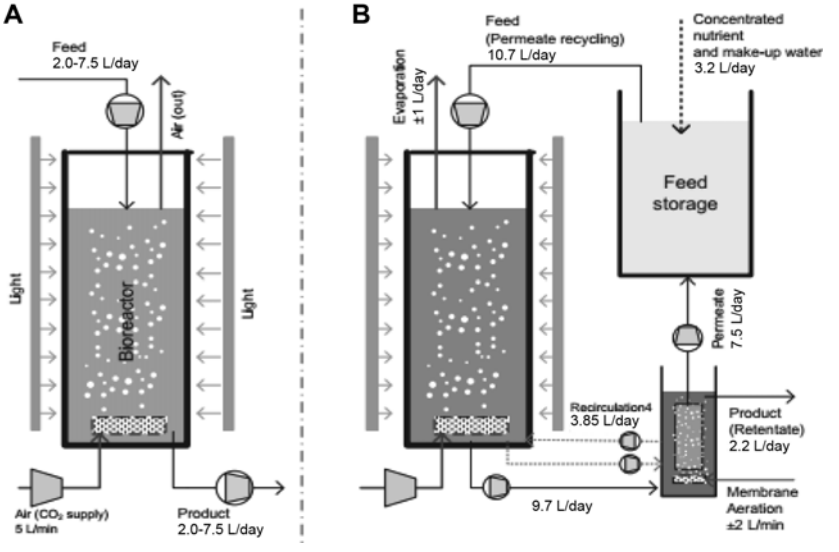

Fig. 1. Schematic illustration of PBR (A) and MPBR (B) set-up [75].

wastewater [76]. Both flocculants at 10 and 50 mg/L doses can increase the settling velocity in the settling tank with fast and efficient biomass recovery (90% in 10 ~ 20 min) [76].

Flocculation of negative-zeta-potential microalgae with positive-zeta-potential metal oxides by electrostatic attraction followed by magnetic separation has been reported [77]. Harvesting of freshwater (*Botryococcus braunii* and *Chlorella ellipsoidea*) and marine (*Nannochloropsis maritime*) microalgae using nanometer-sized magnetic particles (Fe_3O_4) can achieve 95% of harvesting efficiencies within 5 min [78,79]. Continuous magnetic separation of *C. ellipsoidea* can entail the capture of Fe_3O_4 nanoparticle aggregates on the surface of a rotating permanent magnet drum followed by collection with a scraper blade [80]. However, when microalgae in solution concentration and flow rate are increased to 0.5 ~ 2 g/L and 100 ~ 1,140 mL/min, respectively, the harvesting efficiencies are significantly reduced from 95.0 and 95.4% to 82.0 and 85.6% [80].

Rapid separation for cyanobacteria *Microcystis aeruginosa* has been achieved by using magnetic nanoparticles (MNPs) with 99.6% efficiency at 0.58 g MNPs/g/dry-biomass. However, the efficiency is gradually decreased to 59.1% when MNPs are reused 5 times continuously. MNPs can be effectively reactivated with ultrasonic chloroform: methanol solvent treatment with 60% efficiency. After 5 times of reactivation, the efficiency can remain above 93% at 0.2 g MNPs/g dry-biomass dosage. The cyanobacteria-MNPs complex can be disrupted by ultrasonic chloroform:methanol solvent treatment so that zeta potential can be recovered for MNPs electrostatic attraction [81]. Such recycling technique could achieve low energy separation while reducing consumption of MNPs by 67% [81].

Electrochemical harvesting (ECH) by non-sacrificial carbon electrodes have been used to overcome the cost issue and metallic contamination as well as the effect of electrolyte on harvesting efficiency. ECH process has not shown any deteriorating effect on the lipid extraction process or fatty acid composition. Addition of electrolyte (NaCl) can increase the recovery of *C. sorokiniana* from 65.99 to 94.52% with energy consumption of 1.6 kWh/kg [82]. Ozoflotation can achieve high biomass recovery (79.6% as TSS) at ozone dose of 0.23 mg/mg dried biomass. The amounts of lipid extracted and FAME recovered are doubled at ozone doses of 0.12 ~ 0.23 mg/mg dried biomass as compared to centrifugation [83]. The oxidative stability of biodiesel can be enhanced by the degree of FAME saturation caused by ozone doses [83].

4. Product Extraction and Purification

Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are non-toxic and green methods for the extraction of cell lysates [84]. UAE and MAE are efficient for quality extraction with short processing time [85,86]. *Undaria pinnatifida* and *Sargassum fusiforme* separated by MAE can be purified with non-aqueous two-phase solvent system consisting of n-hexane–acetonitrile–methanol (5:5:3, v/v/v). Extractions of fucosterol (13 mg), 24-methylenecholesterol (1.5 mg), and phytol (10.7 mg) have been obtained from 15 g of *U. pinnatifida*. Extractions of fucosterol (4.6 mg), 24-methylenecholesterol (0.3 mg), and (3.5 mg) phytol have been obtained from 15 g of *S. fusiforme* after 220 min treatment [87]. The purities of all products are more than 97% as determined by HPLC. These results demonstrate that MAE coupled with high-speed counter-current chromatography is a feasible, economical, and efficient technique for rapid extraction, separation, and purification of effective compounds from natural products [87].

Super critical carbon dioxide ($SCCO_2$) method is another alternative green technology for oil extraction without solvent assistance [84]. $SCCO_2$ is safe for extraction due to its low toxicity, low flammability, and lack of reactivity [88]. Super critical methanol for direct conversion of oil from wet algae to alkyl esters has also been investigated [89]. However, its optimal operation conditions are at temperature of 50°C with 200 ~ 250 bar pressure which have too high energy consumption to be considered for algae processing. To eliminate the oil extraction step in biodiesel production, direct conversion or *in situ* transesterification of oil in biomass has been proposed [90,91]. Co-solvent modified supercritical CO_2 ($SCCO_2$) extraction of lipids from *N. oculata* with $SCCO_2$ anti-solvent precipitation of carotenoids from the extracts followed by the purification of Zeaxanthin has been reported [92]. Continuous modification by ethanol of $SCCO_2$ extractions has shown that the addition ratio is important for extraction efficiency of lipids and carotenoids. $SCCO_2$ extraction at 350 bar, 323K, and 16.7 wt% of ethanol addition can yield 239.7 mg of triglycerides and 7.61 mg of carotenoids per gram extract with a total yield of 15.5%. The content of Zeaxanthin in the precipitate is greater than that by fraction with normal phase column chromatography [92]. The purest Zeaxanthin (93.8%) has been successfully isolated from the purified fraction by using a reverse-phase HPLC column chromatography [92].

Lipid extraction from *Chlorella vulgaris* with initial fatty acids content of 292.2 mg/ g cell by using ionic liquid (IL) blends has been investigated. The yield using single IL has been compared to that with organic solvents and IL mixtures. Among 12 ILs used, 1-ethyl-3-methyl imidazolium acetate, 1-ethyl-3-methyl imidazolium diethylphosphate, 1-ethyl-3-methylimidazolium tetrafluoroborate, and 1-ethyl-3-methyl imidazolium chloride have been found to have high lipid extraction yields (> 200.0 mg/ g cell). Although

the yields of 1-ethyl-3-methyl imidazolium ethyl sulfate and 1-ethyl-3-methyl imidazolium thiocyanate are only 60.5 and 42.7 mg/g cell, respectively, the yield of their mixture (weight ratio of 1:1) is 158.2 mg/g cell. The yield of 1-ethyl-3-methyl imidazolium hydrogen sulfate is only 35.2 mg/g cell. However, its mixture with 1-ethyl-3-methyl imidazolium thiocyanate (weight ratio of 1:1) has a yield of 200.6 mg/g cell [93]. The synergistic effect of IL mixtures with different anions can improve the extraction yield [93]. A microalgae-to-biofuel route including *C. vulgaris* extraction and CO_2 capture has been proposed with wet algae input and delivery outputs of water, biodiesel, pyrolysis oil, proteins, sugars, biogas, and glycerol [94]. Lipids extraction from *C. vulgais* by IL in combination with CO_2 capture by ILs can compensate for energy consumption required for the extraction. The addition of CO_2 to [BMIM][BF_4] can increase lipid yield of *C. vulgaris* from 68 to 76%. However, protein denaturation and degradation are found during the lysis of algae cells [94]. Approximately 82.2 wt. % of total extracted proteins could be precipitated during algae lysis and supernatant liquid drying [94].

5. POME Remediation

Algal treatment that can replace the conventional tertiary POME treatment in needed to offer oxygenated effluent and ecologically safe, less expensive, and more efficient mean to remove nitrogen, phosphorus, and metals that are not removed during anaerobic digestion. This can reduce eutrophication at point sources better than conventional treatment [95,96]. During digestion, bacteria can consume oxygen released by microalgae to decompose organic matter, producing CO_2, ammonia, and phosphates that can be assimilated by the microalgae while releasing methane as energy. Sludge from wastewater treatment plant can be co-cultured with algae to enhance remediation. However, unlike activated sludge for secondary effluents treatment, algae do not require organic carbon [97]. Harvested microalgae can be used as diet supplements for live feed culture [30,51] or for biodiesel production.

POME treatment utilizing *N. oculata*, and *Chlorella* sp. can achieve the highest removal efficiencies of COD (95 ~ 98%), BOD (90 ~ 98%), TOC (80 ~ 86%), and TN (80%) after 7 days of anaerobic treatment compared to POME treatment without microalgae [98,99]. When *T. suecica* is used in POME treatment, the removal efficiencies of COD, BOD, TOC, and TN are 87 ~ 95%, 84 ~ 95%, 67 ~ 90%, 73 ~ 80%, respectively, which are slightly lower compared to those when *N. oculata*, and *Chlorella* sp. are used. The lowest removal efficiencies of COD (53%), BOD (73%), TOC (49%), and TN (48%) are achieved on day 3 of aerobic treatment without microalgae [100]. Filtered POME composition in sea water at different levels (1, 5, 10, 15, and 20%) has also been explored as alternative medium to enhance cell growth and lipid accumulation. At 10% POME, *N. oculata* and *T. suecica* can achieve the maximum specific growth rate of 0.21 and 0.20/day, respectively. The lipid contents are 39 and 27%, respectively, after 16 days cultivation in 250 mL flask. Algal cultivation in POME/seawater media can also remove COD (93.6 ~ 95%), BOD (96 ~ 97%), TOC (71 ~ 75%), TN (78.8 ~ 90.8%), and oil/grease (92 ~ 94.9%) [101].

The major fatty acids in lipid recovered from *N. oculata* and *T. suecica* cultivated in 10% POME in sea water are total SFA (pentadecanoic acid (C15:0), palmitic acid (C16:0), and stearic acid (C18:0)) and MUFA (palmitolic acid (C16:1) and oleic acid (C18:1)). Total SFA (59.2%, 68.7%), MUFA (15.1%, 12.3%), and PUFA (9.1%, 8.9%) have been obtained from *N. oculata* and *T. suecica*. *N. oculata* contains high contents of palmitic acid (C16:0 at 28.2%) and palmitolic (C16:1 at 9.4%) while *T. suecica* contains high levels of palmitic acid (C16:0 at 36.5%) and pentadecanoic acid (C15:0 at 9.2%). In PUFA profiles, the highest percentages of linolenic acid (C18:3) found in *N. oculata* and *T. suecica* are 4.5 and 5.1%, respectively. Therefore, cultivation of *N. oculata* and *T. suecica* in 10% POME composition with sea water is suitable for cell growth and the production of MUFA and PUFA. With high SFA and MUFA, *N. oculata* and *T. suecica* are potential candidates for biodiesel production [101].

6. Bioenergy Co-generation

Anaerobic co-digestion of mono-algal species with POME and EFB for biomethane production is shown in Table 2. With *Chlorella* sp. after 3 days Hydraulic retention Time (HRT), the highest biomethane rate (5,276 mL/L POME/day) and specific biogas production rate (0.129 m^3/kg COD/day) are achieved at 2 mL/mL POME with EFB of 0.12 g/mL POME. With *N. oculata*, biomethane rate remains high (4,812 mL/L POME/day). However, the specific biogas production rate is consistent (0.126 m^3/kg COD/day) [98-100]. With reduced amount of EFB (0.06 g/mL POME) but high mono-algal *N. oculata* and *Chlorella* sp. (2 mL/mL POME), comparable biomethane yield (4,443 ~ 4,524 mL CH_4/L POME/day) and biogas production rate of 0.120 ~ 0.122 m^3/kg/COD/day are obtained. At lower amount of *Chlorella* sp. (1 mL/mL POME) but higher EFB (0.12 g/mL), the biomethane rate (3,816 mL CH_4/L POME/day) and specific biogas production rate (0.105 m^3/kg COD/day) are lower but comparable to those *N. oculata* cultured separately.

However, anaerobic co-cultivation of *T. suecica* with

EFB and POME can only achieve moderate biomethane rate (3965 mL CH_4/L POME/day) and specific biogas production (0.116 m^3/kg COD/day). Without *T. suecica*, high specific biogas production (0.127 m^3/kgCOD/day) is obtained with comparable biomethane yield (3,642 mL CH_4/L POME/day). Without microalgae but high EFB (0.12 g/mL) POME, the highest biomethane rate is 3650.3 mL/LPOME/day with equivalent specific biogas production rate (0.1207 m^3/kg COD/day). These are still much higher than the reported methane production of 573 ~ 1,170 mL/L/day from co-digestion of *Scenedesmus* sp. and *Chlorella* sp. separately with 50% waste paper [102]. Without both algae and sludge inocula, no biomethane is detected. However, specific biogas production rate (0.130 m^3/kg COD/day) and CO_2 (190 mL CO_2/L POME/day) are much higher with some hydrogen (78 mL H_2/L POME/day) detected. Therefore, high microalgae and EFB co-digestion with POME at the correct ratio of POME and sludge inocula not only can enhance POME treatment, but also can increase biomethane production compared to POME treatment without microalgae co-digestion. However, the specific biogas production rate remains consistent between 0.094 and 0.129 m^3/kg COD/day [98-100].

7. High-value Biocompounds

Screening of algae and cyanobacteria for antibiotics and other pharmacologically active compound as potential source for new drugs has received considerable interest [103]. Blue green algae contain nutrients including vitamin B, vitamin E, beta-carotene, manganese, zinc, copper, iron, selenium, and essential fatty acid such as γ-linolenic acid [104]. They are known to produce a wide array of bioactive compounds (secondary metabolites) with different biological activities including antibacterial, antifungal, antiviral, antimalarial, antitumoral, and anti-inflammatory properties. Therefore, they have industrial, therapeutic, and agricultural significance [104]. Cyanobacterial phycobiliproteins (phycocyanin, phycoerythrin and allophycocyanin) are pigments with different pharmaceutical applications. They can be used as antioxidants. They can boost the immune system, decrease the risk of heart disease, prevent onset of cancers, and protect against age related diseases such as cataracts and multiple sclerosis [105].

The ethanolic extract of *Oscillatoria* sp. (Table 3) has the highest antioxidant activity (69.1%). Green *Chlorella* sp. has high phenolic content (39.1 mg GAE/g dry wt.). *Scenedesmus obliquus* possesses higher carotenoid content (3.73 mg/L) than cyanobacterial species [106]. *S. platensis* has relatively high contents of phycobiliprotein (0.16 ± 0.01 mg/mL), total phenolics (21.88 ± 1.67 mg GAE/g), total alkaloids (3.02 ± 0.06%), and terpenoids (0.14 ± 0.00%). The antioxidant activities and total phenolic contents of three cyanobacterial species (*Phormidium* sp., *Oscillatoria* sp., and *Nostocmuscorum*) evaluated suggest that their ethanolic extracts have high phenolic contents (mg GAE/g), with *Phormidium* sp. possessing the highest level (12.66 ± 0.16), followed by *Oscillatoria* sp. (7.9 ± 0.11) and *Nostoc muscorum* (5.04 ± 0.15). Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered as major contributors to the antioxidant properties of plants. They have diverse biological properties such as anti-inflammatory, anti-atherosclerotic, and anti-carcinogenic activities. However, there is no direct correlation between the antioxidant capacity and the phenolic content, suggesting

Table 2. Biogas production by mono-algal co-digestion with OPEFB, POME, and sludge inocula

	Algae (mL/mL POME)	OPEFB (g/mL POME)	Responses					
			Specific biogas production rate (m^3/kg/ COD/day)			Biomethane (mL CH_4/L POME/day)		
			N. oculata	*Chlorella* sp.	*T. suecica*	*N. oculata*	*Chlorella* sp.	*T. suecica*
Without addition of inocula	0	0	0.094	0.046	0.011	ND	ND	ND
	0	0.06	0.104	0.012	0.014	ND	ND	ND
	0	0.12	0.015	0.014	0.014	ND	ND	ND
With addition of inocula (3 mL/mL POME)	0	0	0.104	0.105	0.105	2,703	2,704	2,704
	0	0.06	0.115	0.115	0.115	3,224	3,226	3,026
	0	0.12	0.125	0.120	0.127	3,649	3,650	3,642
Co-digestion of algae and inocula (3 mL/mL POME)	1	0	0.094	0.090	0.094	2,945	3,180	2,441
	1	0.06	0.095	0.092	0.099	3,030	3,266	2,854
	1	0.12	0.121	0.105	0.010	4,020	3,816	3,762
	2	0	0.107	0.106	0.109	3,874	4,132	3,669
	2	0.06	0.120	0.122	0.111	4,450	4,524	3,785
	2	0.12	0.126	0.129	0.116	4,812	5,276	3,965

that phenolic compounds are not major contributors to the antioxidant activities of these cyanobacterial species. Therefore, microalgal biomass might have a great potential as sources of natural antioxidants [107,108].

Aqueous and organic extracts of seven cyanobacterial species (*Oscillatoria* sp., *Nostoc* sp., *N. muscorum, N. piscinale, Phormidium* sp., *A. flos-aquae,* and *S. platensis*) have been screened *in vitro* against eight human bacterial pathogens and five fungal strains. It has been reported that chloroform extracts of the seven cyanobacterial species have the largest antibacterial inhibition zone diameter against Gram-negative (*Escherichia coli, Aeromonas hydrophila, Salmonella enteric S1180, Klebsiella pneumonia K51, Vibrio cholera V116*, and *Salmonella paratyphi*) and Gram-positive (*Staphylococcus aureus* S1426, *Listeria monocytogenes* L49) bacterial pathogens. These chloroform extracts also have antifungal activities against *Aspergillus terreus* F98. However, none of the seven extracts from cyanobacterial species demonstrated any activity against *Tirchoderma viride* F94. Ethanolic extracts of four cyanobacterial species (*Nostoc musorum, Spirulina platensis, Phormidium sp* and *Oscillatoria sp*) possess antifungal activities against yeast strains of *Candida tropicalis* Y26 and *Saccharomyces cerevisiae* Y39. However, ethyl acetate extracts of all cyanobacterial species only showed antifungal activity against *Saccharomyces cerevisiae* YH [109].

Fatty acids, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates, and phenols are the compounds identified to have antimicrobial activities [110]. Among different biologically active compounds, exometabolites include antibacterial diterpenoids in *Nostoc commune* [111] and antifungal peptides in *Tolypotrix byssoidea* have been identified [112]. Some substances in cyanobacteria have been identified as Nostocyclyne A [113]. Microalgae and Cyanobacteria can produce secondary metabolites at the late exponential stage or the beginning of stationary growth phase. Under stressful culture conditions, the production of biocompounds may be enhanced as stress response. These biocompounds exhibit different defensive and biological activities. For example, *Oscillatoria* species (*O. hameli, O. rubescens*, and *O. platensis*) can produce spermine (tetramine) and piperazine derivatives as well as fatty acids, which may individually exhibit antibacterial and/or antifungal activities and may collectively, have synergistic antimicrobial effects [114]. Under salinity stress, *S. platensis* can produce carotenoids and fatty acids with antiviral activities against Hepatitis- A-virus type MBB and Herpes simplex-virus type-1. In addition, they possess anticoagulant activity comparable to heparin and antioxidant activity comparable to synthetic antioxidant butylated hydroxy anisal (BHA) as well as antimicrobial activity [115].

Fatty acids (non-polar) have antibacterial and antifungal activities against a broad spectrum of bacterial and fungal species [116]. Gram-negative bacteria are more resistant to the inactivation by medium and long chain fatty acids than Gram–positive bacteria. This might be due to the impermeability of the outer membranes of Gram–negative bacteria as effective barriers against hydrophobic substances [117]. The inhibitory effect of antifungal compounds may be due to their inhibition on spore germination, B-(1,3)-D-glucan synthesis, or the integral components of fungal cell wall. Such inhibitory effect on fungal cell membranes can alter their permeability [118]. Antifungal compounds may also inhibit lipid synthesis in fungal species by decreasing the ratio of unsaturated to saturated fatty acids or by inhibiting ergosterol biosynthesis [119].

Red algal extracts exert corrosive inhibition on titanium alloy (Ti-6Al- 4 V) surfaces. The inhibition efficiency depends on the type of extract, extract concentration, and immersion time [120]. Microalgal (green and cyanobacteria) aqueous extracts have pronounced antioxidant activities based on

Table 3. Antioxidant activities of different microalgal extracts (225 mg/L)

Algal species	Antioxidant activity (%) Algal extracts						
	Hexane	Chloroform	Ethyl acetate	Ethanol (70%)	Water	Ascorbic acid	BHT
Oscillatoria sp.	47.4 ± 0. 6	55.6 ± 0.8	47.4 ± 0.5	69.1 ± 0.4	50.2 ± 0.4	94.6 ± 0.1	85.8 ± 0.1
Nostoc sp.	52.2 ± 0.4	63.8 ± 0.6	63.9 ± 0.8	51.0±0.6	50.6 ± 0.5		
N. muscorum	31.4 ± 0.5	30.2 ± 0.3	27.0 ± 0.5	26.3 ± 0.7	27.8 ± 0.4		
N. piscinale	48.0 ± 0.5	50.5 ± 0.5	49.2 ± 0.7	55.4 ± 1.1	49.5 ± 0.3		
Phormidium sp.	49.7 ± 0.3	50.7 ± 0.3	50.8 ± 0.3	52.6 ± 0.4	50.9 ± 0.1		
A. flos-aquae	44.9 ± 0.2	48.8 ± 0.2	48.7 ± 0.4	44.9 ± 0.2	46.9 ± 0.1		
S. platensis	44.9 ± 0.1	45.9 ± 0.2	47.5 ± 0.3	43.6 ± 0.4	41.4 ± 0.3		
D. splendida	43.9 ± 0.4	46.3 ± 0.3	45.6 ± 0.2	42.6 ± 0.2	45.4 ± 0.2		
Chlorella sp.	41.0 ± 0.3	30.3 ± 0.3	42.6 ± 0.4	42.0 ± 0.4	42.3 ± 0.2		
S. obliquus	59.8 ± 0.2	61.2 ± 0.1	61.3 ± 0.3	60.7 ± 0.1	64.3 ± 0.2		

DPPH or ABTS assays and anticancer activities against Ehrlich Ascites Carcinoma cell (EACC) and Human hepatocellular Cancer cell line (HepG2), which may be due to synergistic effect between the polar secondary metabolites (phenolic compounds and polysaccharides) and the phycobiliprotein pigments produced in nitrate-based-media. Under nitrogen starvation condition, these activities may either be comparable to control or enhanced due to alteration of metabolic pathways favoring the production of carbon-skeleton-compounds instead of the phycobiliprotein pigments [121]. Regular scavenging of reactive oxygen species (ROS) produced normally from metabolic process has no harmful effect on human body. However, excessive production of ROS under stress conditions without proper scavenging may damage biomolecules and biocompounds such as DNA and proteins, thus leading to severe diseases. Human body uses antioxidants including antioxidant enzymes (catalyases, peroxidases, superoxide dismutases) and low molecular weight antioxidant substances (ascorbic acid, tocopherol and phenolic compounds) to neutralize excessive level of ROS. Antioxidants with natural origins are important for both human and plants. In addition, they can protect food from rancidity (lipid oxidation) [122]. Earlier studies have revealed that not only *Oscillatoria* sp. and *Spirulina* sp. can produce bioactive substances with different biological activities, but also most cyanobacteria species (*Oscillatoria* sp., *Nostoc* sp., *Nostoc muscorum*, *Nostoc piscinale*, *Phormidium* sp., *Anabaena flos-aquae* and *Spirulina platensis*) are rich sources of secondary metabolites (of different polarities) with different antioxidant activities and antimicrobial activities against a broad spectrum of pathogenic bacteria and fungi [106-109].

8. Conclusion

Rapid industrial development, reduced mineral oil resources, and rising atmospheric CO_2 requires carbon-neutral renewable alternatives. Treatment of POME with microalgae could achieve both waste remediation and bioenergy co-generation. In addition, microalgae cells can be harvested to recover valuable biocompounds.

References

1. Rittmann, B. E. (2008) Opportunities for renewable bioenergy using microorganisms. *Biotechnol. Bioeng.* 100: 203-212.
2. Somerville, C. (2007) Biofuels. *Curr. Biol*. 17: 115-119.
3. Rude, M. A. and A. Schirmer (2009) New microbial fuels: A biotech perspective. *Curr.Opin.Microbiol*. 12: 274-281.
4. Mata, T. M., A. A. Martins, and N. S. Caetano (2010) Microalgae for biodiesel production and other applications: A review. *Renew. Sust. Energy Rev*. 14: 217-232.
5. Chisti, Y. (2013) Constraints to commercialization of algal fuels. *J. Biotechnol*. 167.3: 201-214.
6. Abdullah, M. A., S. M. U. Shah, A. Ahmad and H. El-Sayed (2015). Algal biotechnology for bioenergy, environmental remediation and high value biochemicals. In: Thangadurai D, Sangeetha J. Biotechnology and Bioinformatics: Advances and Applications for Bioenergy, Bioremediation, and Biopharmaceutical Research. CRC Press / Apple Academic Press, New Jersey, USA, 301-344.
7. Hollar, S. (2012) *A closer look at bacteria, algae and protozoa*. NY, USA.
8. Tredici, M. R. (2010) Photobiology of microalgae mass cultures: Understanding the tools for the next green revolution. *Biofuels* 1: 143-62.
9. Williams, P. J. L. B. and L. M. L. Laurens (2010) Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetics & economics. *Energy Environ. Sci*. 3: 554-590.
10. Chisti, Y. (2007) Biodiesel from microalgae. *Biotechnol. Adv*. 25: 294-306.
11. Cheenkachorn, K., N. Choosri, A. Chutrapukdeekul, and T. Kangsadan (2011) Computational Modeling of Microalgae Culture Using a Helical Photobioreactor. pp. 300-305. *Proceedings of the 9th IASME / WSEAS International Conference on Fluid Mechanics & Aerodynamics (FMA '11).* August 23-25. Florence, Italy.
12. Chisti, Y. (2010) Fuels from microalgae. *Biofuels* 1: 233-235.
13. Acién, F. G., J. M. Fernández, J. J. Magán, and E. Molina (2012) Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnol. Adv*. 30: 1344-1353.
14. Sander, K. and G. S. Murthy (2010) Life cycle analysis of algae biodiesel. *Int. J. Life Cycle Assess*. 15: 704-714.
15. Kirkwood, A. E., C. Nalewajko, and P. R. Fulthrope (2003) Physiological characteristics of cyanobacteria in pulp and paper waste-treatment systems. *J. Appl. Phyco.* 15: 325-335.
16. Singh, D. (2007). Removal of Ni (II) from Aqueous Solution by Biosorption using two Green Algal Species *Oscillatoria* sp. & *Spirogyra* sp. In *5th WSEAS Int. Conf. on Environment, Ecosystems and Development*. pp. 310-314.
17. Mayer, A. M. S. and M. T. Hamann (2004) Marine pharmacology in 2000: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antituberculosis, and antiviral activities; affecting the cardiovascular, immune, and nervous system and other miscellaneous mechanisms of action. *Mar. Biotechnol.* 6: 37-52.
18. Mayer, A. M. S. and M. T. Hamann, (2005) Marine pharmacology in 2001–2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*.140: 265-286.
19. Singh, S., B. N. Kate, and U. C. Banerjee (2005) Bioactive compounds from cyanobacteria and microalgae: an overview. *Crit. Rev. Biotechnol.* 25: 73-95.
20. Blunt, J. W., B. R. Copp, M. H. G. Munro, P. T. Northcote, and M. R. Prinsep (2005) Marine natural products. *Nat. Prod. Rep.* 22: 15-61.
21. Barros, M. P., E. Pinto, P. Colepicolo, and M. Pedersén (2001) Astaxanthin and peridinin inhibit oxidative damage in Fe^{2+} loaded liposomes: Scavenging oxyradicals or changing membrane permeability? *Biochem. Biophys. Res. Commun.* 288: 225-232.
22. Puglisi, M. P., L. T. Tan, P. R. Jensen, and W. Fenical (2004) Capisterones A and B from the tropical green alga *Penicillus*

capitatus: Unexpected anti-fungal defenses targeting the marine pathogen *Lindra thallasiae*. *Tetrahedron*. 60: 7035-7039.
23. Dos, Santos, M. D., T. Guaratini, J. L. C. Lopes, P. Colepicolo, and N. P. Lopes, (2005) *Plant cell and microalgae culture. In: Modern Biotechnology in Medicinal Chemistry and Industry*. Research Signpost, Kerala, India.
24. Habib, M. A. B., F. M. Yusoff, S. M. Phang, K. J. Ang, and S. Mohamed (1979) Nutritional values of chironomid larvae grown in palm oil mill effluent and algal culture. *Aquacult.* 158: 95-105.
25. Wu, T. Y., A. W. Mohammad, J. M. Jahim, and N. Anuar (2009) A holistic approach to managing palm oil mill effluent (POME): Biotechnological advances in the sustainable reuse of POME. *Biotechnol. Adv.* 27: 40-52.
26. Vijayaraghavan, K., D. Ahmad, and M. Ezani (2007) Aerobic treatment of palm oil mill effluent. *J. Environ. Manag*. 82: 24-31.
27. Subramaniam, V., A. N. Ma, Y. M. Choo, and N. M. N. Sulaiman, (2008) Environmental performance of the milling process of Malaysian palm oil using the life cycle assessment approach. *Am. J. Environ. Sci*. 4: 310-315.
28. Environmental Protection Agency (2010) http://www.epa.gov/methane/.
29. Khan, S. A., H. M. Z. Rashmi, S. Prasad, and U. C. Banerjee (2009) Prospects of biodiesel production from microalgae in India. *Renew. Sust. Energ. Rev.* 13: 2361-2372.
30. Li, Y., M. Horsman, B. Wang, N. Wu, and C. Q. Lan (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Appl. Microbiol. Biotechnol*. 81: 629-636
31. Carvalho, A. P., C. M. Monteiro, and F. X. Malcata (2009) Simultaneous effect of irradiance and temperature on biochemical composition of the microalga *Pavlova lutheri*. *J. Appl. Phycol*. 21: 543-552.
32. Shah, S. M. U. (2014) *Cell culture optimization and reactor studies of green and brown microalgae for enhanced lipid production*. Ph. D. Thesis. Universiti Teknologi PETRONAS, Malaysia.
33. Brennan, L. and P. Owende (2010) Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sust. Energ. Rev.* 14: 557-577.
34. Lee, Y. K. (2001) Microalgal mass culture systems and methods: Their limitation and potential. *J. Appl. Phycol*. 13: 207-315.
35. Li, X., H. Xu, and Q. Wu (2007) Large scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. *Biotechnol. Bioeng*. 98: 764-771.
36. Liu, X., J. Sheng, and R. Curtiss (2011) III: Fatty acid production in genetically modified cyanobacteria. *Proc. Natl. Acad. Sci. USA.* 108: 6899-6904.
37. Peer, M. S., R. Skye, T. Hall, E. S. U. J. Marx, H. Mussgnug, C. Posten, O. Kruse, and B. Hankamer (2008) Second generation biofuels: High-efficiency microalgae for biodiesel production. *BioEnergy Res*. 1: 20-43.
38. Thomas, W. H., T. G. Tornabene, and J. Weissmann (1984) Screening for lipid yielding microalgae: Activities for 1983, Final subcontract report. No. SERI/STR-231-2207. Solar Energy Research Institute. Golden, CO (USA).
39. Converti, A., A. A. Casazza, E. Ortiz, P. Perego, and M. Del Borghi (2009) Effectect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chem. Eng. Pro*. 48: 1146-1151.
40. Sukenik, A. (1999) Production of eicosapentanoic acid by the marine *eustigmatophyte Nannochloropsis*. pp. 41-56. In: Zvi Cohen (ed.). *Chemicals from Microalgae*. Taylor and Francis, London, UK.
41. Richmond, A. (2004) Principles for attaining maximal microalgal productivity in photobioreactors: An overview. *Hydrobiol.* 512: 33-37.
42. De Oliveira, M. A. C. L., M. P. C. Monteiro, P. G. Robbs, and S. G. F. Leite (1999) Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. *Aquacult. Int*. 7: 261-275.
43. Shah, S. M. U., C. M. CheRadziah, Z. S. Ibrahim, F. Latif, M. F. Othman, and M. A. Abdullah (2014) Effects of photoperiod, salinity and pH on cell growth and lipid content of *Pavlova lutheri*. *Annals Microbiol*. 64: 157-164.
44. Fabregas, J., A. Maseda, A. Dominquez, and A. Otero (2004) The cell composition of Nannochloropsis sp. changes under different irradiances in semicontinuous culture. *World J. Microbiol. Biotechnol.* 20: 31-35.
45. Fernandes, B. D., G. M. Dragone, J. A. Teixeira, and A. A. Vicente (2010) Light regime charac- terization in an airlift photobioreactor for production of microalgae with high starch content. *Appl. Biochem. Biotechnol*. 161: 218-226.
46. Masojodek, J. and G. Torzillo (2003) Mass cultivation of freshwater microalgae. *Encyclopedia of Ecol.* 3: 2226-2235.
47. Guschina, I. A. and J. L. Harwood (2008) *Chemical diversity of lipids*. pp. 1-31. Wiley encyclopedia of chemical biology. Wiley, Hoboken.
48. Wong, Y. S. and N. F. Y. Tam (1997) *An Overview of Microalgae as a Wastewater Treatment*. *Wastewater Treatment with Algae*. pp. 203-217.
49. Ohnuma, M., O. Misumi, T. Fujiwara, S. Watanabe, K. Tanaka, and T. Kuroiwa (2009)Transient gene suppression in a red alga, *Cyanidioschyzonmerolae* 10D. *Protoplasma* 236: 107-112.
50. Nishida, I. and N. Murata (1996) Chilling sensitivity in plants and cyanobateria: The crucial contribution of membrane lipids. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 47: 541-568.
51. Li, Y., M. Horsman, N. Wu, C. Q. Lan, and N. Dubois-Calero (2008) Biofuels from microalgae. *Biotechnol. Prog.* 24: 815-820.
52. Abd El-Baky, H. H., F. K. El Baz, and G. S. El-Baroty (2004) Production of lipids rich in omega-3 fatty acids from the halotolerant alga *Dunaliellasalina*. *Biotechnol.* 3: 102-108.
53. Takagi, M., Karseno, and T. Yoshida (2006) Effect of salt concentration on intracellular accumulation of lipids and triacylglycerols in marine microalgae *Dunaliella* cells. *J. Biosci. Bioeng.* 3: 223-226.
54. Xia, J. R. and K. S. Gao (2005) Impacts of elevated CO_2 concentration on biochemical compo- sition, carbonic anhydrase, and nitrate reductase activity of freshwater green algae. *J. Integr. Plant Biol*. 47: 668-675.
55. Chiu, S. -Y., C. -Y. Kao, M. -T. Tsai, S. C. Ong, C. -H. Chen, and C. -S. Lin (2009) Lipid accumulation and CO2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration. *Bioresour. Technol.* 100: 833-838.
56. Gouveia, L., A. E. Marques, T. L. da Silva, and A. Reis (2009) *Neochlorisole abundans* UTEX#1185: A suitable renewable lipid source for biofuel production. *J. Ind. Microbiol. Biotechnol.* 36: 821-826.
57. Ota, M., Y. Kato, H. Watanabe, M. Watanabe, Y. Sato, R. L. Smith Jr, and H. Inomata (2009) Fatty acid production from a highly CO2 tolerant alga, *Chlorococcum littorale*, in the presence of inorganic carbon and nitrate. *Bioresour. Technol*. 100: 5237-5242.
58. Chen, C. Y., X. Q. Zhao, H. W. Yen, S. H. Ho, C. L. Cheng, D. J. Lee, and J. S. Chang (2013) Microalgae-based carbohydrates for biofuel production. *Biochem. Eng. J.* 78: 1-10.
59. Xu, L., P. J. Weathers, X. R. Xiong, and C. Z. Liu (2009) Microalgal bioreactors: Challenges and opportunities. *Eng. Life Sci.* 9: 178-189.

60. Stephenson, A. L., E. Kazamia, J. S. Dennis, C. J. Howe, S. A. Scott, and A. G. Smith (2010) Life-cycle assessment of potential algal biodiesel production in the United Kingdom: A comparison of raceways and air-lift tubular bioreactors. *Energy Fuel* 24: 4062-4077.
61. Junying, Z. H. U., R. O. N. G. Junfeng, and Z. O. N. G. Baoning (2013) Factors in mass cultivation of microalgae for biodiesel. *Chin. J. Catal* 34: 80-100.
62. Schenk P, Thomas-Hall S, Stephens E, Marx U, J. Mussgnug, and C. Posten (2008) Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenerg. Res.* 1: 20-43.
63. Ugwu, C. U., J. C. Ogbonna, and H. Tanaka (2005) Characterization of light utilization and biomass yields of *Chlorella sorokiniana* in inclined outdoor tubular photobioreactors equipped with static mixers. *Proc. Biochem*. 40: 3406-3411.
64. Sheehan, J., T. Dunahay, J. Benemann, and P. Roessler (1998) *A Look Back at the U.S. Department of Energy's Aquatic Species Program-Biodiesel From Algae*. NREL/TP-580-24190. p. 328. National Renewable Energy Institute, Golden, CO.
65. Satoh, A., K. Ichii, M. Matsumoto, C. Kubota, M. Nemoto, M. Tanaka, and T. Tanaka (2013). A process design and productivity evaluation for oil production by indoor mass cultivation of a marine diatom, Fistulifera sp. JPCC DA0580. *Bioresour Technol*. 137: 132-138.
66. Hsieh, C. H. and W. T. Wu (2009) A novel photobioreactor with transparent rectangular chambers for cultivation of microalgae. *Biochem. Eng. J*. 46: 300-305.
67. Wang, L. L. and X. Y. You (2013) Light-gradient mixing performance improvement of the flat plate photobioreactor with waved baffles. *Chem. Biochem. Eng*. Q. 27: 211-218.
68. Yu, G., Y.G. Li, G. Shen, W. Wang, C. Lin, H. Wu, and Z. Chen (2009) A novel method using CFD to optimize the inner structure parameters of flat photobioreactors. *J. Appl. Phycol*. 21: 719-727.
69. Travieso, L., D. O. Hall, K. K. Rao, F. Benítez, E. Sánchez, and R. Borja (2001) A helical tubular photobioreactor producing Spirulina in a semicontinuous mode. *Int. Biodeter. Biodegr*. 47: 151.
70. Miao, J. L., G. F. Kan, B. T. Zhang, Y. H. Jiang, X. G. Hou, and G. Y. Li (2004). Optimum culture of Antarctic ice microalgae Pyramidomonas sp. and Chlorophyceae L-4. *J. Fish Sci. China* 11: 245-252.
71. Scragg, A. H., A. M. Illman, A. Carden, and S. W. Shales (2002) Growth of microalgae with increased calorific values in a tubular bioreactor. *Biomass Bioenerg*. 23: 67.
72. Hulatt, C. J. and D. N. Thomas (2011) Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. *Bioresour. Technol*. 102: 5775-5787.
73. Deb, U. K., K. Chayantrakom, Y. Lenbury, and B. Wiwatanapataphee (2012) Numerical Simulation of Two-phase Laminar Flow for CO_2 and Microalgae Suspension in the HLTP". pp. 53-58. *Proceedings of 11th WSEAS International Conference on System Science and Simulation in Engineering (ICOSSSE '12).* Singapore.
74. Junying, Z. H. U., R. O. N. G. Junfeng, and Z. O. N. G. Baoning (2013). Factors in mass cultivation of microalgae for biodiesel. *Chinese J. Catalysis* 34: 80-100.
75. Bilad, M. R., V. Discart, D. Vandamme, I. Foubert, K. Muylaert, and I. F. J. Vankelecom. (2014) Coupled cultivation and pre-harvesting of microalgae in a membrane photobioreactor (MPBR). *Bioresour. Technol.* 155: 410-417.
76. Gutiérrez, R., F. Passos, I. Ferrer, E. Uggetti, and J. García (2015) Harvesting microalgae from wastewater treatment systems with natural flocculants: Effect on biomass settling and biogas production. *Algal Rese* 9: 204-211.
77. Lee, Y. C., K. Lee, and Y. K. Oh (2015) Recent nanoparticle engineering advances in microalgal cultivation and harvesting processes of biodiesel production: A review. *Bioresour. Technol*. 184: 63-72.
78. Hu, Y. -R., F. Wang, S. -K. Wang, C. -Z. Liu, and C. Guo (2013) Efficient harvesting of marine microalgae *Nannochloropsis maritime* using magnetic nanoparticles. *Bioresour. Technol.* 138: 387-390.
79. Xu, L., C. Guo, F. Wang, S. Zheng, and C. Z. Liu (2011) A simple and rapid harvesting method for microalgae by in situ magnetic separation. *Bioresour. Technol*. 102: 10047-10051.
80. Hu, Y. R., C. Guo, X. Ling, F. Wang, S. -K. Wang, Z. Hu, and C. -Z. Liu (2014) A magnetic separator for efficient microalgae harvesting. *Bioresour. Technol*. 158: 388-391.
81. Lin, Z., Y. Xu, Z. Zhen, Y. Fu, Y. Liu, W. Li, and D. Zhang (2015)Application and reactivation of magnetic nanoparticles in Microcystis aeruginosa harvesting. *Bioresour. Technol.* 190: 82-88.
82. Misra, R., A. Guldhe, P. Singh, I. Rawat, and F. Bux (2014) Electrochemical harvesting process for microalgae by using nonsacrificial carbon electrode: A sustainable approach for biodiesel production. *Chem. Eng. J*. 255: 327-333.
83. Velasquez-Orta, S. B., R. Garcia-Estrada, I. Monje-Ramirez, A. Harvey, and M. O. Ledesma (2014) Microalgae harvesting using ozoflotation: Effect on lipid and FAME recoveries. *Biomass Bioener.* 70: 356-363.
84. Mahanta, P. and A. Shrivastava (2011) Technology development of biodiesel as an energy alternative. Available from: http://www.newagepublishers.com/sample chapter/001305.pdf.
85. Cravotto, G., L. Boffa, S. Mantegna, P. Perego, M. Avogadro, and P. Cintas (2008) Improved extraction of vegetable oils under high-intensity ultra sound and /or microwaves. *Ultrason. Sono Chem*. 15: 898-902.
86. Balasubramanian, S., J. D. Allen, K. Kanitkar, and D. Boldor (2011) Oil extraction from Scenedesmus obliquus using a continuous microwave. *Bioresour. Technol*. 102: 3396-3403.
87. Xiao, X. H., Z. Q. Yuan, and G. K. Li (2013) Preparation of phytosterols and phytol from edible marine algae by microwave-assisted extraction and high-speed counter-current chromatography. *Separat. Purif. Technol.*104: 284-289.
88. Macias-Sanchez, M. D., C. Mantell, M. Rodriguez, E. M. dela Ossa, E. M. Lubian, and O. Montero (2007) Super critical fluid extraction of carotenoids and chlorophylla from *Synechococcus* sp. *J. Super Crit. Fluids* 39: 323-329.
89. Patil, P. D., V. G. Gude, A. Mannarswamy, S. Deng, P. Cooke, and S. Munson-McGee (2011) Optimization of direct conversion of wet algae to biodiesel under super critical methanol conditions. *Bioresour. Technol*. 102: 118-122.
90. Lewis, T., P. D. Nichols, and T. A. McMeekin (2000) Evaluation of extraction methods for recovery of fatty acids from lipid producing micro heterotrophs. *J. Microbiol. Meth.* 43:107-116.
91. Johnson, M. B. and Z. Wen (2009) Production of biodiesel fuel from the microalga *Schizochytrium limacinum* by direct transesterification of algal biomass. *Energy Fuels* 23: 5179-5183.
92. Liau, B. C., C. T. Shen, F. P. Liang, S. E. Hong, S. L. Hsu, T. T. Jong, and C. M. J. Chang (2010) Supercritical fluids extraction and anti-solvent purification of carotenoids from microalgae and associated bioactivity. *J. Supercritic. Fluids* 55: 169-175.
93. Sun-A Cho, You-Kwan Oh, Min-Ji Jeong, Seung Wook Kim, Jin-Suk Lee, and Ji-Yeon Park (2014) Effects of ionic liquid mixtures on lipid extraction from *Chlorella vulgaris*. *Renew. Energ.* 65: 169-174.
94. Yu, X., J. Yang, H. Lu, S. T. Tu, and J. Yan (2015) Energy-efficient extraction of fuel from Chlorella vulgaris by ionic liquid combined with CO2 capture. *Appl. Energy* 16: 648-655.
95. Órpez, R., M. E. Martínez, G. Hodaifa, F. E. Yousfi, N. Jbari, and S. Sánchez (2009) Growth of the microalga *Botryococcus-braunii* in secondarily treated sewage. *Desalination* 246: 625-630.
96. De-Bashan, L. E. and Y. Bashan (2010) Immobilized microal-

gae for removing pollutants: Review of practical aspects. *Bioresour. Technol*. 101: 1611-1627.
97. Aslan, S. and I. K. Kapdan (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol. Eng*. 28: 64-70.
98. Ahmad, A., S. M. U. Shah, M. F. Othman, and M. A. Abdullah (2015). Aerobic and anaerobic co-cultivation of *Nannochloropsis oculata* with oil palm empty fruit bunch for enhanced biomethane production and palm oil mill effluent treatment. *Desalination Water Treatment* 56: 2055-2065.
99. Ahmad, A., S. M. U. Shah, M. F. Othman, and M. A. Abdullah (2014) Enhanced palm oil mill effluent treatment and biomethane production by aerobic and anaerobic co-cultivation of *Chlorella* sp., *Can. J. Chem. Eng*. 92: 1636-1642.
100. Ahmad, A., S. M. U. Shah, A. Buang, M. F. Othman, and M. A. Abdullah (2014) Evaluation of aerobic and anaerobic co-digestion of *Tetraselmis suecica* and oil palm empty fruit bunches by response surface methodology. *Adv. Mat. Res.* 925: 243-247.
101. Shah, S. M. U., A. Ahmad, M. F. Othman, and M. A. Abdullah (2016) Effects of palm oil mill effluent media on cell growth and lipid content of Nannochloropsis oculata and Tetraselmis suecica. *Int. J. Green Energ*. 13: 200-207.
102. Yen, H. W. and D. E. Brune (2007) Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresour. Technol*. 98: 130-134.
103. Schlegel, I., N. T. Doan, N. De Chazol, and G. D. Smith (1999) Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *J. Appl. Phycol.* 10: 471-479.
104. Gupta, V., S. K. Ratha, A. Sood, V. Chaudhary, and R. Prasanna (2013) New insights into the biodiversity and applications of cyanobacteria (blue-green algae)-Prospects and challenges. *Algal Res.* 2: 79-97.
105. Larsson, S. C., L. Bergkvist, I. Naslund, J. Rutegard, and A. Wolk (2007) Vitamin A, retinal, and carotenoids and the risk of gastric cancer: A prospective cohart study. *Am. J. Clin. Nutrition* 85: 497-503.
106. Ali, H. E. A., S. M. M. Shanab, M. A. M. Abo-State, E. A. A. Shalaby, U. M. N. El Demerdash, and M. A. Abdullah (2014) Screening of microalgae for antioxidant activities, carotenoids and phenolic contents. *Appl. Mech. Mat*. 625: 156-159.
107. Ali, H. E. A., S. M. M. Shanab, M. A. M. Abo-State, E. A. A. Shalaby, U. M. N. El Demerdash, and M. A. Abdullah (2014) Determination of antioxidant activities and total phenolic contents of three cyanobacterial species. *Appl. Mech. Mat*. 625: 826-829.
108. Ali, H. E. A., S. M. M. Shanab, M. A. M. Abo-State, E. A. A. Shalaby, U. M. N. El Demerdash, and M. A. Abdullah (2014) Evaluation of antioxidants, pigments and secondary metabolites contents in spirulina platensis. *Appl. Mech. Mat*. 625: 160-163.
109. Abo-State, M. A. M., S. M. M. Shanab, H. E. A. Ali, and M. A. Abdullah (2015) Screening of antimicrobial activity of selected Egyptian cyanobacterial species. *J. Eco. Heal. Env*. 3: 7-13.
110. Kannan, R. R. R., R. Arumugam, and P. Anantharaman (2010) *In vitro* antioxidant activities of ethanol extract from Enhalus acoroides (L.F.) Royle. *Asian Pac. J. Trop. Med.* 3: 898-901.
111. Jaki, B., J. Orjala, J. Heilmann, A. Linden, B. Vogler, and O. Sticher (2000) Novel extracellular diterpenoids with biological activity from the cyanobacterium *Nostoc Commune*. *J. Nat. Prod.* 63: 339-343.
112. Jaki, B., O. Zerbe, J. Heilmann, and O. Sticher (2001) Two novel cyclic peptides with antifungal activity from the cyanobacterium Tolypothrix byssoidea (EAWAG 195). *J. Nat. Prod.* 64: 154-158.
113. Ploutno, A. and S. Carmeli (2000) *Nostocyclyne A*, a novel antimicrobial cyclophan from the cyanobacterium *Nostoc* sp. *J. Nat. Prod.* 63: 1524-1526.
114. Shanab, S. M. M. (2007) Bioactive allelochemical compounds from *Oscillatoria* species (Egyptian isolates) *Int. J. Agri. Biol.* 9: 617-621.
115. Shalaby, E. A. and S. M. M. Shanab (2010) salt stress enhanced of antioxidant and antiviral efficiency of *Spirulina platensis*. *J. Med. Plant Res.* 4: 2622-2632.
116. Agoramoorthy, G., M. Chandrasekaran, V. Venkatesala, and M. J. Hsu (2007) Antibacterial and antifungal activites of fatty acid methyl esters of the B lind-your-Eye mangrove from India. *Braz. J. Microbiol.* 38: 739-742.
117. Sheu, C. W. and E. Freese (1973) Lipopoly saccharide layer protection of Gram-negative bacteria against inhibition by long chain fatty acids. *J. Appl. Bacterial.* 115: 869-875.
118. Gupta, C. P., R. S. Dabey, S. C. Kany, and D. K. Mahashwari (2001) Antibiosis mediated recrotrophic effect of Pseaudomonas GRC2 against two fungal plant pathogens. *Curr. Sci.* 81: 91-94.
119. Georgopapadakou, N. H., B. A. Dix, S. A. Smith, J. Frrendenberger, and P. T. Funke (1987) Effect of antifungal agents on lipid biosynthesis and membrane integrity in Candida albicans. *Antimicrob. Agents Chemother.* 3: 46-55.
120. Ameer, M. A., A. M. Fickry, and S. M. Shanab (2011) Electrochemical behavior of Titanium Alloy in 3.5% NaCl containing natural product substances. *Int. J. Electrochem. Sci*. 6: 1572-1585.
121. Shanab, S. M. M., S. S. M. Mostafa, E. A. Shalaby, and G. M. Ibrahim (2012) Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. *Asian Pacific J. Tropical Biomed.* 2: 608-615.
122. Shalaby, E. A. and S. M. M. Shanab (2013) Review. Antioxidant compounds, assays of determination and mode of action. *African J. Pharm. Pharmacol.* 7: 528-539.
123. Poh, P. E. and M. F. Chong (2009) Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. *Bioresour. Technol*. 100: 1-9.
124. Das, P. (2010) Development of microalgal biomass for biodiesel production. PhD dissertation. National University of Singapore.