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REVIEW ARTICLE

Waste-water Treatment Coupled with Biodiesel Production Using Microalgae: A Bio-refinery Approach

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ABSTRACT

Scarcity and non-renewable nature of petroleum based liquid fuels have made them an un-reliable source of energy. Excessive greenhouse gas emissions and the associated effects on global warming have become noteworthy environmental, economic and social threats. In this milieu, the development of renewable, carbon-neutral and sustainable alternative energy sources has become inevitable. Biofuels have been found a promising alternative and a driving force for modern world. Different feed-stocks have been evaluated for biofuels production to date but microalgae have been found the most attractive due to their higher growth productivity, higher lipid contents, non-competitive nature with human food and their ability to grow on non-arable land using brackish or waste water. However, there are a number of technological barriers that are still questioning the economic feasibility and competitiveness of such biofuels. On the other hand, there are also a number of trade opportunities if we pay attention to the use of integrated system following the bio-refinery concept. Bio-refinery concept reflects the production of value added by-products along with the biofuels, contributing to an overall escalation of the economic feasibility of the whole system. These types of systems may help to progress the competitiveness of biodiesel production using microalgae as a potential feedstock. This paper reviews the most recent and relevant information on such integrated systems. Several aspects related to the treatment of municipal and animal wastewater with simultaneous recovery of microalgae and the potential of biodiesel production are discussed. Bio-refinery concept also presenting new opportunities for the cost-effective production of biodiesel coupled with valuable non-fuel by-products.

Microalgae; potential feed stock for biodiesel production

Fossil fuels are no-more sustainable for transportation and industrial purpose because of their ever-increasing demand and depleting supplies. In addition, buildup of carbon dioxide due to combustion of fossil fuels is the serious environmental threat. These challenges have flashed the pursuit for alternative energy sources to serve as viable replacement to reduce reliance on fossil fuels and develop environmental sustainability. Therefore, renewable raw materials have been explored for the biodiesel production which includes edible plants/seeds such as corn, mustard, canola, soybean, palm oil, sunflower, coconut and non-edible plants/ seeds such as jojoba, castor, pongame, jatropha, *Cucumis melo*, *Citrus reticulate*, *Moringa oleifera* seed oils and waste oils (Rashid et al., 2008; Sharma et al., 2009; Yadav et al., 2010; Rashid et al., 2011; Rashid et al., 2012; Diaz and Borges ; Sumithrabai et al., 2012; Rashid et al., 2013). However, these resources have certainly several limitations, such as; competition with human food, use of arable land, longer cultivation periods, lower yield, use of fresh water for their growth and seasonal production (e.g. once a year). These factors have made plants as un-popular feed-stocks for biodiesel production. Among the many options, microalgae have received massive attention as a source for the production of biofuels. They are being considered to be the cheapest source among all the renewable sources for the biodiesel production (Chisti 2007; Petkov et al., 2012).

Microalgae are tiny photosynthetic biochemical factories. They use photosynthetic processes in the same manner as higher plants for energy and metabolites production. Fortunately, photosynthetic competence of microalgae is remarkably higher than terrestrial plants (Pirt 1986; Shay 1993). Growth rate and oil productivity of many microalgae (Table 1) has greatly exceeds than the oil productivity of the best oil producing crops (Chisti 2007). Microalgae are believed to have up to 300 times more oil productivity for biodiesel production than traditional energy crops (Table 2) on the basis of area usage (Chisti 2007; Schenk et al., 2008; Hu et al., 2008). The average lipid contents of microalgae range between 1-70% but under optimized conditions some species can yield 80% of oil as their dry weight (Schenk et al., 2008; Wu et al., 2012). Moreover, they lack the complex cell structures like lignin as found in higher plants, so are not recalcitrant. Most importantly, they do not compete with food crops, and can be produced using non-arable lands, saline/waste water bodies as well as in compact bioreactors (Musharraf et al., 2012). They can produce useful quantities of polysaccharides (sugars) and proteins along with the lipids, so the left-over biomass after getting the oil for production of biodiesel can be exploited for the production of bio-ethanol, biogas, bio-fertilizers and can potentiate animal feed.

Furthermore, a microalgae-based biofuel industry has tremendous potential to capture CO_2 that can be as high as 99%, if the system works with high efficiency (Zeiler et al., 1995), effectively capturing 1.8 kg of CO_2 per kg of dry biomass (Wang et al., 2008). Although CO_2 captured in this way will be absorbed by the plants that will eventually be released upon combustion, this would displace the emission of fossil CO_2. The remaining biomass can be subjected to a downstream carbon sequestration processes. For example, sequestering carbon into bio-char via pyrolysis can be used to improve soil fertility by reintroducing durable carbon back into the soil mitigating the global climate change (Lim et al., 2012).

Transesterification of algal oil to biodiesel

Lipids from microalgae can be converted to biodiesel through transesterification reactions (Fig. 1) after the extraction (Durrett et al., 2008; Johnson and Wen 2009; Demirbasa 2009). Where, catalyst is very important to achieve efficient reaction. Traditionally, acid and alkali catalyst are called homogeneous catalysts because they act in the same liquid phase of the reaction mixture. Due to their trouble-free usage and less time required for lipid hydrolysis, they govern the biodiesel industry. However, the downstream processing is complicated in this case and feedstock (lipids) must by highly pure (Borges and Diaz 2012). So, alternative catalysis systems are getting attention, particularly the enzyme based catalysis. The catalytic process using super-critical methanol and porous titania microspheres to catalyze the transesterification of lipids and esterification of free fatty acids simultaneously was studied to reach conversion efficiencies up to 85% (Krohn et al., 2010). In another study, a process involving simultaneous extraction and transesterification of wet algal biomass containing almost 90% of water under supercritical methanol conditions was presented (Patil et al., 2010). However, there is still lot to do to develop environmental friendly and economically sustainable catalytic system (e.g. enzymatic) for the transesterification of lipids to biodiesel.

Integrated biomass production; the biorefinery concept

The term "*Biorefinery*" is defined as "*the sustainable processing of biomass into a spectrum of marketable products and energy*". It is a system that integrates biomass conversion processes and equipment to produce fuels, power, materials and/or chemicals from biomass (Cherubini 2010; Singh and Gu 2010) on sustainable basis integrating the green chemistry (Cherubini 2010) of microalgae-based biorefinery (Fig. 2) with the production of wide spectrum of valuable products (Singh and Gu 2010; Subhadra 2010).

Although, microalgae are believed to be the most promising feedstock for biodiesel production yet large-scale commercial production has several limitations. For instance, harvesting of the algal biomass is one of the energy intensive processes that can account for as much as 30% of the total cost of production (Barbosa 2003; Wiley et al., 2009; Ferrell and Sarisky-Reed 2010). On the other hand, water and nutrients have been found the most critical variables in algal biomass production (Stephens et al., 2010). It very fortunate that algae can be grown in both fresh and seawater depending upon the species selected (Schenk et al., 2008), and can raise biodiesel yields much higher than the current in-use energy crops (Table 2). The nitrogen, phosphorous and a number of micronutrients including potassium are required for algal growth (Becker 1994). Algae take up these nutrients along with atmospheric CO_2 and produce biomass via photosynthesis. To maintain the cost of production of biodiesel using microalgae feedstock, we have to design integrated systems keeping "*The Bio-refinery Concept*" in view. Few examples of such systems have been reviewed in the following part of this paper.

Table 1: Lipid contents in the dry biomass of various species of microalgae (Wu et al., 2012)

Sr. #	Microalgae	Lipid Contents (% Dry mass basis)
01	*Anabaena cylindrical*	4–7
02	*Botyococcus braunii*	25–80
03	*Chlamydomonas reinhardtii*	21
04	*Chlorella emersonii*	28–32
05	*Chlorella protothecoides*	57.9
06	*Chlorella pyrenoidosa*	2
07	*Chlorella vulgaris*	14–22
08	*Crypthecodinium cohnii*	20
09	*Cylindrotheca* sp.	16–37
10	*Dunaliella bioculata*	8
11	*Dunaliella primolecta*	23
12	*Dunaliella salina*	6
13	*Dunaliella tertiolecta*	35.6
14	*Euglena gracilis*	14–20
15	*Hormidium* sp.	38
16	*Isochrysis* sp.	25–33
17	*Monallanthus salina*	>20
18	*Nannochloris* sp.	30–50
19	*Nannochloropsis* sp.	31–68
20	*Neochloris oleoabundans*	35–54
21	*Nitzschia* sp.	45–47
22	*Phaeodactylum tricornutum*	20–30
23	*Pleurochrysis carterae*	30–50
24	*Porphyridium cruentum*	9–14
25	*Prymnesium parvum*	22–38
26	*Scenedesmus dimorphus*	16–40
27	*Scenedesmus obliquus*	12–14
28	*Schizochytrium* sp.	50–77
29	*Spirogyra* sp.	11–21
30	*Spirulina maxima*	6–7
31	*Spirulina platensis*	4–9
32	*Synechoccus* sp.	11
33	*Tetraselmis maculata*	8
34	*Tetraselmis sueica*	15–23

Table 2: Biodiesel production ability of different energy crops (Wu et al., 2012)

Sr. #	Crop	Oil yield (L ha^{-1})
01	Corn	172
02	Soybean	446
03	Canola	1,190
04	Jatropha	1,892
05	Coconut	2,689
06	Palm Oil	5,950
07	Microalgae (70% oil in biomass)	136,900
08	Microalgae (30% oil in biomass)	58,700

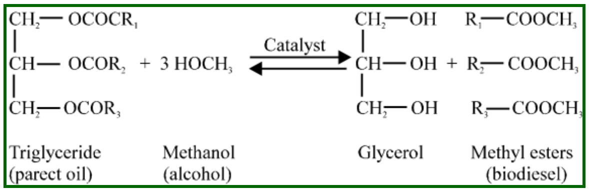

Fig. 1: Catalytic conversion of lipids to biodiesel

Waste-water treatment along with algal biomass production

Microalgae based bioremediation have been extensively studied to remove pollutants from water in the past four decades (Ryther et al., 1972; Kuyucak and Volesky 1988; Romero-Gonzalez et al., 2001; Fu and Wang 2011). An alternative and practical approach to algal bioremediation in which algae were cultured directly in the waste water stream has been studied recently (Saunders et al., 2012). Three species of microalgae were grown in water that was contaminated with heavy metals coupled with either nutrient addition or without nutrients. All species achieved high concentrations of heavy metals (to 8% dry mass). Interestingly, growth rates were doubled in contaminated water as compared to fresh water (Saunders et al., 2012). In another study, the potential of cultivating algae using wastewater as a nutrient medium was assessed. The consortium of algal species, including *Scenedesmus* sp. and *Chlorella* sp. grew favorably on anaerobic sludge centrate demonstrating the higher nutrient uptake for phosphorous and ammonia. The average growth productivity obtained was 3.3±1.5 g dry wt m^{-2} d^{-1} in this case (Dalrymple et al., 2013). Similar findings were reported in another study, where growth rate was 3 g dry wt m^{-2} d^{-1} for *Chlorella* sp. grown on wastewater (Woertz et al., 2009). In addition, a remarkably higher growth rate 13 g dry wt m^{-2} d^{-1} was reported. Moreover, it was shown that at the end of 14-day batch culture 94% ammonia, 89% total nitrogen and 81% total phosphorous was removed by the microalgae (Li et al., 2011a). In a more recent study, algae grown on anaerobic sludge centrate has shown growth productivity rate of 12.8 g dry wt m^{-2} d^{-1} (Zhou et al., 2012b). The lipid contents reported in both studies were close to 11% (Li et al., 2011a; Zhou et al., 2012b). Generally, *Chlorella* sp. can produce lipid contents up to 30% (Table 1) but growing algae, especially *Chlorella* sp., in high strength nitrogen media the lipids contents have been decreased by two folds. It is believed that the caloric content which is linked to lipid production is significantly reduced under such situations (Illman et al., 2000). In general, high lipid contents are achieved when the microalgae are “starved” of nitrogen (Illman et al., 2000; Chisti 2007).

Different microalgae species have been studied under various experimental designs with respect to nutrient starvation and heterotrophic conditions to evaluate their lipid contents and the lipid productivity. However, the *Chlorella* sp. has been found the most suitable for such systems (Chu WL 2009; Bhatnagar et al., 2010). Among these, *Chlorella kessleri* was found to produce a very high biomass density (2.01 g L^{-1}) when cultivated using municipal wastewater (Li et al., 2011b). Naturally, a consortium of microalgae genera (*Chlorella, Micractinium* and *Actinastrum*) may be

Fig. 2: Integrated Bioenergy System; Biorefinery concept
Circle 1: Represents the growth of microalgae using waste-water; 1A: Lipids from microalgae may be supplied to biodiesel plant, 1B: Algal biomass after lipid extraction may be used as fertilizer to grow other energy crops. **Circle 2:** Represents the growth of oil seed crops using waste materials from Biodiesel plant; 2A*:* Use of seed oil for biodiesel production, 2B: Extraction of seed oil for human consumption. **Circle 3:** Represents the Seed oil extracted from either food or non-food crops; 3A: Oil can be converted to biodiesel, 3B: Plant biomass after lipid extraction can be used to produce bio-methane, bio-hydrogen, or other biological gases for industrial consumption. **Circle 4:** Biogas production; 4A: Use of waste from Biodiesel plant for biogas production, 4B: Supply of biogas to end user. **Circle 5:** Consumption of bio-diesel; 5A: Biodiesel supplied to the transport system, 5B: Carbon dioxide emission will be re-cycled by the field grown microalgae.

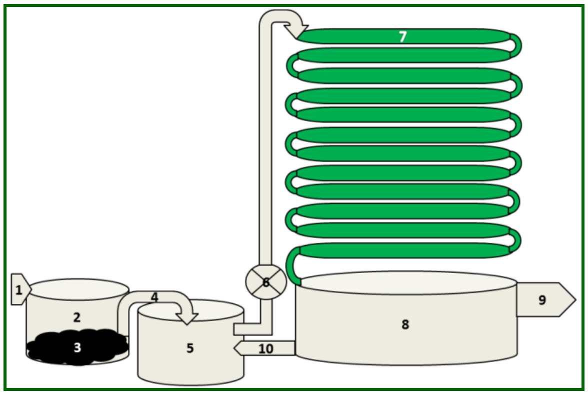

Fig. 3: Schematic diagram of a proposed waste-water treatment plant using microalgae
Where; 1: City waste water supplied to the plant, 2: Waste-water accumulated in a large pond, 3: solid materials are sedimented at the bottom, which can be dried and may be directly subjected to combustion for electricity production, 4: Water from pond-1 is supplied to next pond, 5: waste-water from previous pond is inoculated with selected microalgal strains, 6: water is pumped to flat-panel photo-bioreactors, 7: Microalgae grows in flat-panel photo-bioreactors for 7-10 days depending upon the growth rate of the selected strains, 8:Water collected along with algal culture, 9: Water may be either used for irrigation purposes or may be subjected to water-purification plants or may recycled (10) depending upon the quality of water.

established during wastewater treatment and a maximum lipid productivity of 24 mg $L^{-1}d^{-1}$ have been reported (Woertz et al., 2009). Other microalgae, for instance, *Botryococcus braunii* is widely distributed on all continents in freshwater, brackish and saline lakes and is able to accumulate unsaturated long-chain hydrocarbons at a concentration of 15% to 75% of its dry weight (An et al., 2003; Orpez et al., 2009) but still we need optimize the desired conditions for each water source. An interesting strain of *Scenedesmus* sp. LX1 showed biomass yield (0.11 g L^{-1}), lipid contents (31–33%) and a lipid productivity (8 mg L^{-1} d^{-1}) in a batch culture using secondary effluent as growth medium (Li et al., 2010b) which are encouraging results. Moreover, LX1 was shown to have considerable removal of total nitrogen (90.4%) and total phosphorus (nearly 100%) in another study. When ammonium was used as the nitrogen source, LX1 reached a very high specific growth rate of 0.82 d^{-1} (Li et al., 2010a). Similar findings have been reported in another more recent study (Zhou et al., 2012b; Zhou et al., 2012a). *Chlamydomonas reinhardtii* is another microalga with the potential to treat wastewater along with oil production. It was cultivated in wastewaters taken from three different stages of a municipal wastewater treatment plant (influent, effluent and centrate). In this case, a lipid productivity of 505 mg $L^{-1}d^{-1}$ was achieved, which may be the highest lipid productivity reported for microalgae in wastewater (Kong et al., 2010). The olive mill waste-water was used as media for biomass production from *Scenedesmus dimorphus* and *Arthrospira platensis* it found promising strategy (Cicci et al., 2013).

Keeping in view the bio-extraction potential of microalgae of contaminants along with biomass production, such integrated systems that use microalgae for treating wastewater and producing oil for biodiesel and chemical products are gaining interests. Recently, it was found that dual-use microalgae cultivation for wastewater treatment coupled with biofuel generation is an attractive option for reducing energy, fertilizer and freshwater costs, as well as reducing greenhouse gas emissions (Pittman et al., 2011; Park et al., 2011;

Menger-Krug et al., 2012). A multi-national group of researchers have also claimed that microalgae-mediated CO_2-fixation coupled with bio-fuel production is more sustainable if we integrate biomass production with wastewater treatment (Kumar et al., 2010). Actually, cultivation of microalgae consumes more fertilizers as compared to the most common oleaginous plants. For instance, N-fertilizer consumption in the range of 0.29 to 0.37 kg kg^{-1} oil is reported, which is higher than that for Jatropha (0.24 kg kg^{-1} oil) and palm oil (i.e. 0.048 kg kg^{-1} oil) (Lam and Lee 2012). So, use of waste-water enriched with nutrients will decrease cost of production. Another major advantage of microalgae over higher plants as a fuel source is their environmental benefits. Despite having to grow in an aquatic medium, microalgae production may require less water than terrestrial oleaginous crops and can make use of saline, brackish, and/or coastal seawater (Kliphuis et al., 2010: Rodolfi, 2009). This allows the production of microalgae without competing for valuable natural resources such as arable land, biodiverse landscapes and freshwater. The microalgae *Nannochloropsis* sp., *Dunaniella salina, Chlorella* sp. and *Etraselmis* sp. were found as suitable candidates for a multiple-product algae crop. The tropical and subtropical coastal microalgae display a variety of fatty acid profiles that offer a wide scope for several oil-based bioproducts, including biodiesel and omega-3 fatty acids (Lim et al., 2012). A biorefinery approach for microalgae would make economical production more feasible but challenges remain for efficient harvesting and extraction processes for some species.

Associated Challenges using waste-water as growth media

One problem associated with such system is the availability of production locations (Slade and Bauen 2013) because we can only integrate biomass production with city waste water sites. Overall, it is clearly highlighted that decreasing the energy and fertilizer consumption of the process makes algae an ideal feedstock in such integrated systems as compared to other biofuel feed-stocks, such as corn, canola and switchgrass (Clarens et al., 2010) because the requirement of fresh water and nutrients may be decreased to 90-100% by using waste water (Li et al., 2010b; Udom et al., 2013) as growth media. However, there are several challenges associated with harvesting of microalgae when grown in waste water.

Another challenge with the municipal wastewater is the presence of compounds which are potentially toxic to microalgae, especially when municipal waste water is being mixed with industrial wastewater. Heavy metals are potent inhibitors to important enzymes in photosynthetic pathways of microalgae (Kumar et al., 2010). A significant reduction in specific growth rate and biomass productivity of *B. braunii* was observed when cultivated in secondary effluents of a municipal sewage treatment plant and it was found that could be due to the presence of phenolic compounds and heavy metals in the wastewater (Orpez et al., 2009). Very fortunately some microalgae (*Scenedesmus* and *Pseudochlorococcum.*) have shown tolerance to higher concentrations (80-100 $mgmL^{-1}$) Pb^{2+}. On the other hand, Hg^{2+} showed a strong inhibition of chlorophyll biosynthesis even at the lower concentrations (5–10 mg mL^{-1}) and a complete destruction of the algal cell at concentration above 20 mg mL^{-1} (Shanab et al., 2012). This growth inhibition in microalgae is related to the amount of heavy metal ions bound to the algal cell surface, and also, to the amount of intracellular heavy metal ions (Franklin et al., 2001). However, for zinc, the growth inhibition may be related to extracellular zinc concentration (Wilde et al., 2006). In the algal stabilization ponds there was 72% removal of Zn^{2+} and 73% removal of Pb^{2+} by *Chlorella* sp. (Kumar and Goyal 2010). These studies have shown that there are important benefits to be derived from integrating algal production systems with nutrient-rich waste water streams and million gallons and kilograms of oil and gas can be produced using a single lake containing waste water per annum, respectively (Dalrymple et al., 2013).

Although, people argue that using wastewater for algal biomass production may pose contamination risks yet Life Cycle Analysis (LCA) studies have confirmed that it is a very fruitful approach to ensure the viability and the sustainability of the whole biofuels production process, in economic terms (Kumar et al., 2010). An LCA study was carried out to assess the energy balance and environmental impacts from biomass production to biodiesel combustion. It was revealed that significant (>50%) cost reductions may be achieved if CO_2, nutrients and water can be obtained at lower cost, i.e. from waste water (Lardon et al., 2009).

Heterotrophic growth of microalgae; use of biodiesel-derived glycerol

Heterotrophic cultivation without light and with the controlled addition of a source of carbon and energy is similar to procedures established with bacteria or yeasts. To date, only a small number of microalgal species have been cultured heterotrophically in conventional bioreactors (Chen 1996; Perez-Garcia et al., 2011). The few commercialized processes in which microalgae are grown under heterotrophic conditions and have been focused for the manufacture of polyunsaturated fatty acids (PUFA) in 100 m^3 scale (Behrens 2005). These biotechnological processes represent a sustainable alternative to the extraction of PUFA from fish oil (Barclay et al., 1994; Apt and Behrens 1999; Mendes et al., 2009; Wynn et al., 2010). Several other heterotrophic processes that utilize microalgae have been established at laboratory scale to deliberately enrich the biomass with compounds such as pigments and antioxidants (Pulz and Gross 2004; Spolaore et al., 2006; Raja et al., 2008). L-Ascorbic acid (Running et al., 1994) and polysaccharides are examples of commercially valuable extracellular products obtained from microalgae. Classes of compounds that are found in microalgae and that exhibit desirable properties for treating inflammation, tumors and viral or microbial infections are attracting new interest (Guedes et al., 2011). Moreover, research in the rapidly expanding field of biofuels (Wijffels 2008) provides a valuable source of fundamental information on the physiology and biochemistry of microalgae, producing high-value compounds (Xiong et al., 2008; Branyikova et al., 2011). The growing interest in microalgae, either non-recombinant or with appropriate genetic modification (Potvin and Zhang 2010; Specht et al., 2010), suggests that heterotrophic microalgal processes offer significant commercial opportunities (Rosenberg et al., 2008). The opportunities for heterotrophic processes with microalgae have been considered in published literature (Chen 1996; Apt and Behrens 1999; Borowitzka 1999; Lee 2001; Chen and Chen 2006) as well as more recently (Perez-Garcia et al., 2011).

Currently, in use strategies are based on experimental data collected for non-recombinant microalgae which are also applicable to genetically improved strains. Lipid content has been reported to increase under nutrient-deprived conditions such as low concentrations of nitrogen (Griffiths and Harrison 2009; Jakobsen et al., 2008; Lv et al., 2010), phosphorus (Lynn et al., 2000; Rodolfi et al., 2009) and silicon (Griffiths and Harrison 2009; Lynn et al., 2000). The *C. protothecoides* has shown to contain higher lipid contents (53.8%) at lower C:N as compared to (25.2%) when grown using a high C:N. This increase in lipid contents was accompanied by a drop in protein content from 25.8% to 10.5%, when grown at lower C:N and higher C:N, respectively (Xiong et al., 2010).

Although various waste-derived carbon sources have been studied for biomass production using micro-algae (Bhatnagar et al., 2011) yet biodiesel-derived glycerol has shown to be a promising substrate for mixotrophic cultivation of oleaginous microalgae. However, for the economic feasibility, the potential of using glycerol and glucose as the complex carbon substrate to produce microalgal biomass is still need to be studied deeply. It was shown that *C. vulgaris* could utilize glycerol as a sole carbon substrate. However, the use of glycerol-glucose mixture could enhance the growth rate and biomass productivity. So, the lower biomass productivity due to glycerol as the sole organic carbon source can be overcome using mixotrophic culture medium (WeiBao et al., 2013). Mixotrophic microalgae grow faster when compared with the photoautotrophic control culture, providing higher productivities of biomass, lipids contents, starch and proteins (Abreu et al., 2012).

However, the following future research should be taken into account; (1) the characterization of microalgae suitable for heterotrophic cultivation, (2) optimization of the chemical composition of mineral growth media along with C:N ration, (3) strategies to enhance the cell-density (4) use of biodiesel-derived glycerol as a carbon source (6) optimization of growth conditions to get enhanced lipid productivity. Thus, we can make the biofuels production more economical and sustainable using heterotrophic growth systems.

Biogas production using algal biomass

Oil extracted from microalgae cultured in an integrated system may be subjected to biodiesel production. The left over biomass may be either subjected to direct combustion for energy production or anaerobically digested to produce methane, especially biomass which is supposed to be unsuitable for biodiesel production due to low lipid content. The microalgae such as *Chlorella* and *Scenedesmus* genera commonly dominate wastewater pond ecosystems, both of which are suitable for biodiesel and biogas production. However, wastewater environments do not present algae with nutrient limited conditions, preventing them from accumulating high proportions of triacyle glycerides. It was reported that (Woertz et al., 2009) lipid concentrations within *Chlorella* range between 4.9-11.3% when grown in municipal wastewater, which translates into 1.9 to 4.3 MJ of lipid energy per kilogram of biomass (assuming a lower heating value [LHV] of 38.3 MJ kg^{-1} for algal lipids; (Lardon et al., 2009). In contrast, anaerobically digesting this material would generate between 6.1-16 MJ of methane energy per kilogram of biomass, assuming an LHV of 35.6 MJ m^{-3} for methane (Sialve et al., 2009). Therefore, the lipid content of wastewater algae needs to be somewhere between 16-42% to match the energy content of biogas. Unlike algal biodiesel production, anaerobic digestion processes are capable of producing methane-rich biogas regardless of lipid content. It was estimated that anaerobically digesting algal biomass containing between 2-22% lipid would produce a theoretical methane yield ranging from 0.47 to 0.80 m^3 CH_4 kg^{-1} (Sialve et al., 2009). However, experimental data concerning the anaerobic digestion of algae report actual yields ranging between 0.17-0.45 m^3 CH_4 kg^{-1} (Sialve et al., 2009). Despite the inconsistency between theoretical prediction and the experimental data, methane productivity from anaerobic digestion of microalgae is comparable with experimental values of gas production using pig waste (0.19 m^3 CH_4 kg^{-1} VS), sugar beets (0.21 m^3 CH_4 kg^{-1} VS), wastewater sludge (0.23 m^3 CH_4 kg^{-1} VS), and clover grass (0.34 m^3 CH_4 kg^{-1} VS) (Hansen et al., 1998; Sosnowski et al., 2003; Amon et al., 2007). Furthermore, several acceptable substrates have been found more appropriate for anaerobic digestion as compared with algal biodiesel production (Wiley et al., 2011). It is believed that 1.0 mole of algae biomass produces 47.17 moles of methane. However, algal biomass is not particularly easy to digest having a biogas yield of 29.5%. Therefore, 1 g of algae dry wt. is estimated to generate 62.7 mg methane. So, the attempts to derive biogas from spent biomass after lipid extraction may be more fruitful in economic terms (Batten et al., 2012). Overall, it is encouraging to know that an average sized algal biomass production system using wastewater nutrient sources have a potential to fulfill the energy demands of 500 homes (Slade and Bauen 2013).

However, there are few challenges associated with biogas production using spent algal biomass including, dewatering and oil extraction. Choice of a cheap and environmental solvent friendly solvent to extract lipids is very important. Secondly, recalcitrance of cellulosic cell wall makes the residual biomass unfit for being used as feedstock for biogas production. Because, the recalcitrant cell walls of microalgae may limit their digestibility for bioenergy production. Recently, it was investigated that the bioaugmentation with a cellulolytic and hydrogenogenic bacterium *C. thermocellum* the degradation of *C. vulgaris* biomass was improved. Subsequently, methane and hydrogen production was increased (La et al., 2013). Moreover, it was found that the by-products of biodiesel production, including crude glycerol, oil-pressed cakes and washing water may be exploited as valuable feedstocks for biogas generation (Kolesarova et al., 2011).

Conclusion and future prospects

Following conclusions can be made from the above discussion which will lead us towards future research orientation;

- There are no specific species which we may call "the best" for biomass and lipid productivity when used to treat wastewater. The strain selection depends on various factors, such as the specific characteristics of the wastewater, original habitat of the algal strain and the climatic conditions in the treatment plant. So, the strain selection process should be continued to find the desired strains.
- Naturally, consortia of various microalgae may be established spontaneously while waste water is used as growth media. Thus, use of consortia for biodiesel production, and the bio-compatibility of the selected algal strains in the consortia should be evaluated in future.
- Higher biomass production may be achieved using heterotrophic/mixotrophic growth systems using waste water and biodiesel-derived glycerol. So, more detailed studies should be conducted to evaluate the sustainability of such systems.
- Total lipid contents of microalgae cultivated in wastewater may be lower than the one observed in synthetic medium. So, a cost-benefit analysis should be undertaken to justify in such cases in which the nutrients might not be sufficient for supporting algae growth and nutrient supplementation may be required and the left-over biomass may be characterized for its use in biogas production, as a carrier for biofertilizers, direct application as biofertilizers, biochar production and mixing with animal feed (especially biomass with higher protein contents).
- Microalgal treated wastewater (Fig. 3) may not be perfect for drinking purpose but may be more suitable for irrigation purposes, but only after careful field trials. Moreover, if the waste-water after harvesting the microalgae is subjected to the water treatment plants, it will definitely reduce to operational cost of the water-treatment process.

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