A MINI REVIEW: WASTE WATER TREATMENT BY MICROALGAE AND RENEWABLE RESOURCE PRODUCTION

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ABSTRACT

The waste water demands large amounts of energy and material inputs. As a result of the negative impacts wastewater effluents high in nutrient content may have on receiving waterways, nutrient removal have become more stringent in recent years. In order to wastewater treatment to be more sustainable, the wastewater treatment can be coupled with micro algal photobioreactors (PBRs).Microalgae is gaining specific interest as a source of biofuel because it is rapidly renewable and unlike first generation biofuels, does not compete with food supplies. In the coupled system, nutrient such as nitrogen (N) and phosphorous (P) from the wastewater effluent can be fed to the microalgae, eliminating the needs for synthetic nutrients. Through the industrial symbiosis and the reuse of products that are usually wasted, the coupled system may provide a sustainable option for the advanced treatment of wastewater and production of renewable products including biofuels, electricity, and other value-added products.

Key Words: *Microalgae, Wastewater, Photobioreactor, Treatment, Biofuel*

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1. INTRODUCTION

Since hundreds of years, macroalgae have been one of the eminent sources as food, fodder, nutraceuticals, and fertilizers. Ancient record shows that people collected macroalgae for food as long as 500 B.C. in China and one thousands of years later in Europe. Currently, there are 42 countries in the world with reports of commercial macroalgae cultivation activity. China holds first rank in macroalgae production, with *Laminaria sp.* accounting for most of its production followed by North Korea, South Korea, Japan, Philippines, Chile, Norway, Indonesia, US and India. These top ten countries contribute about 95% of the world's commercial macroalgae based products. About 90% macroalgae comes from culture based practices. The most cultivated macroalgae is the kelp Laminaria japonica, which alone accounts for 60% of the total cultivated macroalgae production (Laura and Paolo, 2006). The main use of kelp Laminaria japonica is to prevent obesity and diabetes (Miyuki and Tomoyuki, 2011). A Laminaria stick can be used as an osmotic dilator of the cervix when induction of pregnancy is necessary. Wastewater that contains of carbon, nitrogen, phosphorous and other metals can be effectively used by microalgae. The centrate, which is generated from activated sludge, is one of the viable alternatives for the cultivation media of the microalgae (Esther et al., 2013). First, the concentrations of carbon, nitrogen and phosphorus are higher in the centrate than in any other wastewater streams obtained from a wastewater treatment plant, which can provide sufficient nutrients for algae growth. Second, centrate contains a variety of minerals such as K, Ca, Mg, Fe, Cu, and Mn, which are essential micronutrients for algae growth and metabolism. Third, the volume of the centrate produced daily is extremely large with no availability problem all year 2 round, and the centrate need to be recycled to the activated sludge process for further treatment to avoid environmental contamination, which adds extra load for the treatment process, especially the high concentration of phosphorus. Thus the use of the centrate for algae cultivation could serve the dual role of waste reduction and biomass/bioenergy production. After primary and secondary treatment of wastewater, effluent is discharged to the nearby water bodies. However, the effluent still contains considerable nitrogen, phosphorus and pathogens. Especially, in anaerobic wastewater treatment, nitrogen, and phosphorus are not removed much, and raised concern in several places in India. These nutrients leads to eutrophication in lakes and causes harmful microalgal blooms and have considered P and N to be the key elements behind the eutrophication. In this regard polishing of anaerobically treated wastewater using algae can absorb nitrogen and phosphorus from wastewater, increases the dissolved oxygen content and helps to reduce pathogens present in the treated wastewater. Symbiotic association of algae and microorganisms present in the effluent could satiate the mutual need of CO_2 and O_2 of algae and microorganisms and thus reduces the need of external sources of CO_2 or O_2 for polishing the wastewater. Alkaline pH of the algal culture due to limitation of CO_2 transfer from air and from microbial respiration could help to kill the pathogens. However, algal growth in the wastewater is severally restricted due to light penetration (colored wastewater absorbs a part of the incident light).

The main objective of this literature review with respect to algae based wastewater treatment is:

1. Synthesizing the current knowledge and applications.

2. Evaluating nutrient removal performance.

3. Characterizing environmental variables affecting growth.

2. Microalgal cultivation systems

There are three main groups of system for cultivation of microalgae. They are open system, closed system and immobilized system. Open is simpler to conduct and cheaper. However, open system is expose to the environmental factors such as temperature and light intensity. Closed system cultivation is more complex to conduct but it allows condition control for cultivation. The third is immobilized system where algae are trapped in a solid medium.

2.1 Open ponds

Open pond cultivation offers a simple and cost effective approach. The idea of the open pond was derived from use of artificial lagoons and oxidation ponds in wastewater treatment (Sharma et al. 2013). Most open pond growing units are based on the race-way pond design first proposed by Oswald (1969). The most commonly used open systems include large shallow ponds, tanks, circular ponds and raceway ponds. A raceway pond is most often a rectangular canal with algal culture current flowing from a supply end to an exit end (Chisti 2007).

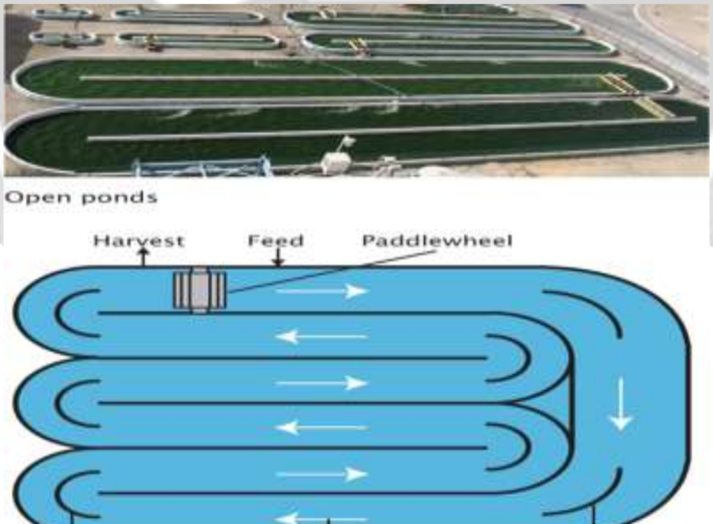

Fig -2.1: Open pond system

The length to width ratio is an important parameter in designing a raceway pond. Larger width may result in weak current speed, which is not desirable for mixing and mass transfer. The length and depth is determined by the light penetration and the amount of culture volume a unit can hold. Each pond contains a paddle wheel to make the water flow continuously around the circuit. The raceway pond is set in a meandering configuration with paddle wheel mixers that exert low shearing forces. In this system, water level is kept no less than 15 cm, and algae are cultured according to the optimum growth condition by supplying needed nutrient to the medium. The raceways are typically made from poured concrete to prevent the ground from soaking up the liquid. The fresh feed (containing nutrient including nitrogen, phosphorus and inorganic salt) is added in front of the paddle wheel and algal broth is harvested behind the paddle wheel. Although the open system is cost effective, it has some disadvantages. Among the disadvantages are it requires large land areas for a considerable biomass yield. Moreover, because this cultivation technology is carried out in the open air, the water level can be effected by from evaporation and rainfall. .

2.2 Closed system

Closed systems are designed to overcome the problems associated with open pond cultivation systems (Chisti 2007). A PBR is defined as a closed (or mostly closed) vessel for phototrophic production, where energy is supplied via electric lights (Andersen 2005). A PBR design should use light efficiently; illumination should be uniform, reduce mutual shading and should provide a fast mass transfer of CO_2 and O_2. A typical PBR is comprised of a four-phase system: solid phase (microalgal cells), liquid phase (growth medium), gaseous phase (CO_2 and O_2) and superimposed light-radiation field (Posten 2009). Hence, in order to design an efficient PBR, an understanding of the World J Microbiol Biotechnol complex interaction between biomass production and associated environmental parameters (e.g., fluid dynamics and light transfer) within the reactor is required. Based on the illuminated surface, PBRs are categorized as flat plate (Sierra et al. 2008; Slegers et al. 2011), tubular (Molina et al. 2001), and column (Eriksen 2008). Bases on their mode of liquid flow, PBRs can be grouped as stirred type, bubble column and airlift reactor. Ideal PBRs should have high transparent surface, minimal non illuminated part, high mass transfer rates and should attain high biomass growth. Moreover, PBR design should be suitable for cultivation various microalgal species universally and prevent fouling of the reactor. Various types of PBRs are discussed in following section

2.2.1. Stirred tank PBRs

Stirred tanks are the conventional aerated bioreactor. The mixing is achieved by mechanical agitation (Fig.2.1). The core component of the stirred tank bioreactor is the agitator or impeller, which performs a wide range of functions, including: heat and mass transfer, aeration, and mixing for homogenization (Doran 2013). This requires a relatively high input of energy per unit volume. are used in stirred reactors to reduce vortexing. Typically, only 70–80 % of the volume of stirred reactors is filled with liquid. This allows adequate headspace for disengagement of liquid droplets from the exhaust gas and to accommodate any foam that may develop. In order to prevent foaming, supplementary impellers called foam breakers are installed. CO_2 enriched air is bubbled at the bottom to provide a carbon source for the growth of algae. Stirred tanks PBRs have very effective stirring mechanism; hence, mass transfer rates and light dispersion are very high. This leads to a lower incidence of dark zones inside the reactor and higher biomass productivity. However, the main disadvantage of this system is low surface area to volume ratio, which in turn decreases light harvesting efficiency (Franco-Lara et al. 2006). Moreover, mechanical agitation generates much more heat than the sparging of compressed gas; hence, stirred PBRs are expensive to operate and maintain.

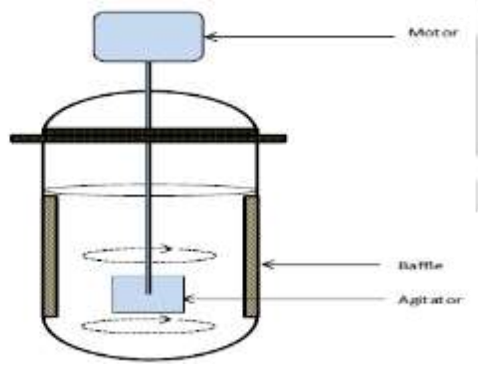

Fig -2.2.1: Stirred tank photobioreactor

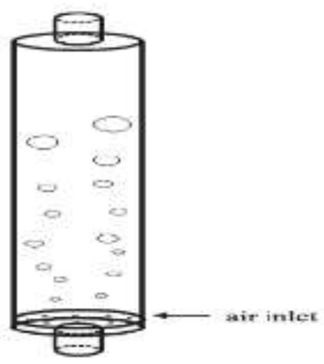

Fig -2.2.2: Bubble column photobioreactor

2.2.2 Bubble column PBRs

Bubble column PBRs are an alternative to stirred reactor, in which the agitation and mixing is achieved by gas sparging. Bubble column reactors are extensively used commercially for production of baker's yeast, beer, vinegar and the treatment of wastewater. The design is simple, with a height greater than twice the diameter (Fig. 2.2.2).

2.2.3 Airlift PBRs

Airlift PBRs differ from the bubble column PBRs due to the physical separation of the two interconnecting zones the riser (up flowing) and the down comer (down flowing) streams (Fig. 2.2.3). Gas is sparged through the riser, resulting in gas holdup, thereby decreasing fluid density and eventually causing liquid in the riser to move upward. As gas bubbles disengage from the liquid at the top of the vessel, heavier bubble-free liquid is left to recirculate through the down comer. Thus, liquid circulation in airlift reactors results from the density difference between the riser and down comer (Doran 2013).

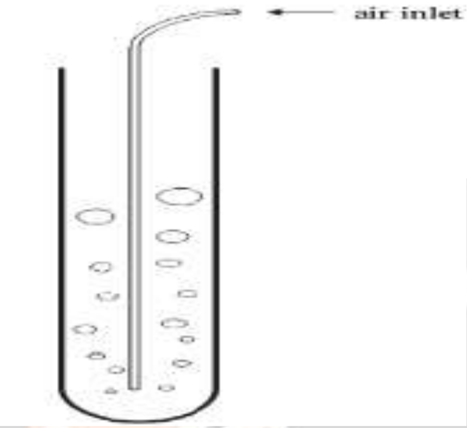

Fig -2.2.3: Schematic representation of airlift tubular reactor

3. Algal growth affecting parameters

Successful treatment with the microalgae requires a thorough knowledge of various parameters that affect the growth. If one of the factors varies drastically then the whole process can go wrong in terms of algal productivity and treatment efficiency. The growth rate of the algae depends on the various factors such as physical, chemical as well as the biological. Abiotic factors such as light are one of the crucial parameter that algae survive. Examples of physical factors are light and temperature. Chemical factors can be availability of nutrients and carbon dioxide, and biological factors are e.g. competition between species, and virus infections. Operational factors such as mixing, dilution rate, depth, addition of bicarbonate, harvesting frequency also affect the growth.

3.1 Carbon

Carbon is one of the important sources for microalgae for cell growth as inorganic carbon dioxide, in autotrophic mode of cultivation. It would be organic carbon source for heterotrophic and mixotrophic mode of cultivation. Heterotrophic algal cultivation has been reported to provide oil content as well (Miao and Wu 2004; Xu et al. 2006; Li et al. 2007). Microalgae are considered as more photosynthetically efficient than terrestrial plants for fixing CO_2. In the process of fixation, microalgae use CO_2 as an inorganic carbon source, while water acts as an electron donor for production of glucose, which is further transformed to various complex sugar forms such as carbohydrate, starch etc. (Figure 1). Many microalgae species are able to utilize carbonates such as Na_2CO_3 and $NaHCO_3$ for cell growth (Wang et al., 2009). Some studies have indicated that about 25–50 % of the algal carbon in high rate algal ponds is derived from heterotrophic utilization of organic carbon. The organic carbon sources can be assimilated either chemo- or photoheterotrophically. In the first case, the organic substrate is mused both as the source of energy (through respiration) and as carbon source, while in the second case, light is the energy source. In several algal species, the mode of carbon nutrition can be shifted from autotrophy to heterotrophy when the carbon source is changed; this is the case with e.g. the green algae *Chlorella* and *Scenedesmus* (Becker and E.W., 1994)

3.2 Nitrogen and Phosphorous

After consideration of the Carbon, Nitrogen and Phosphorous are key components for the algae for nutrient assimilation. The nitrogen mainly comes from the sewage by the metabolic conversion and more than 50% phosphorous comes from the detergents. The principal forms in which they occur in wastewater are NH_4 (ammonia), NO_2 (nitrite), NO_3 (nitrate) and PO_4 (orthophosphate). Together these two elements are known as nutrients and their removal is known as nutrient stripping (Horan 1990). Microalgal culture offers a cost-effective approach for removing nutrients from wastewater (tertiary wastewater treatment) (Evonne et al., 1997). Microalgae have a high capacity for inorganic nutrient uptake and they can be grown in mass culture in outdoor solar bio-reactors (Joel de et al., 1992). Biological processes appear to perform well compared to the chemical and physical processes, which are in general, too costly to be implemented in most places and which may lead to secondary pollution (Joel de et al., 1992). The widely used microalgae cultures for nutrient removal are species of Chlorella (Lee and Lee, 2001; Gonzales et al., 1997), Scenedesmus (Martinez et al., 1999, 2000), and Spirulina (Olgun et al., 2003). It was pointed out through research that Scenedesmus sp. is very common in all kinds of fresh water bodies, which play an important role as primary producers and contributes to the purification of eutrophic waters (Mohamed, 1994). The interest in microalgal cultures stems from the fact that conventional treatment processes suffer from some important disadvantages: (a) variable efficiency depending upon the nutrient to be removed; (b) costly to operate; (c) the chemical processes often lead to secondary pollution; and (d) loss of valuable potential nutrients (N, P) (De la.,1992). The last disadvantage is especially serious, because conventional treatment processes lead to incomplete utilization of natural resources (Guterstan and Todd, 1990).

3.3 Light

Microalgae are phototrophes, which mean that they obtain energy from light. However, some algae are able to grow in the dark using simple organic compounds as energy and carbon source. The light energy is converted to chemical energy in the photosynthesis, but large parts are lost as heat. It was reported that in outdoor ponds, more than 90 % of the total incident solar energy can be converted into heat and less than 10 % into chemical energy (Oswald and W.J.,1988). It was also reported that the conversion efficiency of sunlight energy into chemical energy of only 2 % (Fontes et al., 1987). The easiest way to prevent algal cultures from light limitation is to decrease the depth of the culture vessel. The productivity in light limited ponds is inversely correlated to the depth .Generally, depths of between 15 and 50 cm are recommended. However, during winter shallower depths are recommended due to the lower light conditions, and depths greater than 20 cm markedly decreases production (Fontes et al., 1987).

3.4 Temperature

Temperature is also one of the crucial parameter for the growth of algae in countries where fluctuation of the temperature is high. Increased temperature is good for the growth of algae up to certain range, after that critical temperature growth is ceased (Monique and Jean,2013).The temperature at which culture are maintained should ideally be close as possible to the temperature at which the organism were collected. Most commonly cultured species of microalgae tolerate temperature between 16 and 27 degree celsius (Laura and Paolo, 2006). Overheating of the algae can reduce growth rate especially in the humid country where evaporation is inhibited. The effects of temperatures which exceed thresholds for optimal growth are described as more deleterious than lower temperatures. Indeed, this is visualized by the asymmetrical growth curve versus temperature. Beyond optimal temperatures, growth rate decrease is linear and reaches critical temperatures more or less abruptly depending on the species (Butter Wick et al., 2005).

4. Coupled waste water treatment

In the coupled system, nutrients such as nitrogen (N) and phosphorus (P) from the wastewater effluent can be fed to the microalgae, eliminating the need for synthetic nutrients (Mallick 2002; Aresta, Dibenedetto et al. 2005) which have a high environmental impact when commercially produced. As the microalgae consume the N and P, the wastewater can be treated with the potential for reuse in appropriate applications (Mallick 2002; Johnson and Wen 2010). By coupling the wastewater treatment system with algae cultivation and harvesting for useful products, fewer chemicals, fertilizers, water, and energy are needed for an expanded system. There is a possibility that life cycle environmental impacts can be reduced when system expansion is considered due to the replacement of conventional processing techniques for products such as electricity, fuels, plastics, etc. When considering the coupled system scenario in Figure 1.1, the environmental impacts from conventional product manufacturing can be considered as avoided impacts and can be subtracted from the environmental impacts of the coupled system. In industries, the reuse of products that are usually wasted, the coupled system may provide a sustainable option for the advanced treatment of wastewater and production of renewable products including biofuels, electricity, and other value-added products.

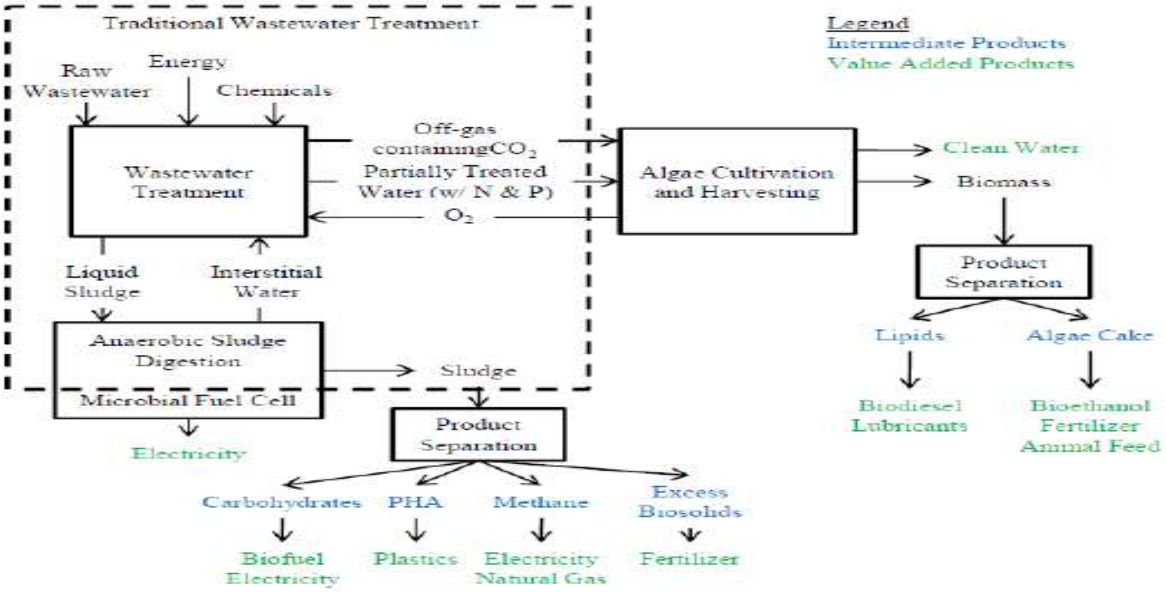

Fig -4: Coupled system scenario

5. Harvesting Techniques

Algae growing in open waste ponds can reach biomass levels of up to 300 mg dry weight per liter. Harvesting of microalgae is therefore crucial for wastewater treatment in order to separate both nutrients and BOD from the water. However, this is not easily achieved, and is consequently a cost expensive part of the cultivating process. Even though harvesting effectively can be accomplished by e.g. filtration or centrifugation, such methods may be too difficult or costly to implement. There are some methods that are used for the harvesting of microalgae as sedimentation, floatation, filtration, centrifugation, biological filtration etc.

5.1 Filtration

This process consist a rotating fine-mesh screen and backwash to harvest microalgae. It is proved that, by using microstraining for harvesting microalgae, almost 20-fold of concentration, or higher can be achieved (Benemann J.R., 1979). For the filtration harvesting method, rotary vacuum and the chamber filter appears to be commonly employed type of filter in fairly large size of microalgae (Mohn, 1980). The advantages of these filters are they can be used incontinuous operation and useful when sterility and contaminant is not reviewed. However, filtration is only suitable for harvesting fairly large microalgae (e.g spirulina platensis) and not succeeds to separate bacteria size microalgae like scenedesmus, dunaliella or chlorella species.

5.2 Sedimentation and Floatation

Using sedimentation or flotation, the biomass can be concentrated already in the water, which in turn can be decanted. Sedimentation without addition of chemicals is the most common method in full-scale facilities .Flotation processes operate more efficiently and rapidly than sedimentation and achieve a higher solids fraction (up to 7 %) in the concentrate, but these on the other hand can be more expensive (Borowitza and M.A.,1988). Many algal species are particularly difficult to sediment without treatment due to their natural tendency to float in order to catch enough light. Flotation of unicellular algae without flocculation may also be very difficult due to the hydrophilic cell surface on which air bubbles will not attach (personal unpublished experiences). Algae can be flocculated by addition of various chemical flocculants such as alum, lime, FeCl3, cationic polyelectrolytes, and Ca(OH)$_2$ (de la et al.,1992) . A major disadvantage of adding these chemicals, however, is that they can cause secondary pollution. Some toxically safe flocculating agents recognized are e.g. potato starch derivatives and chitosans, and these are suitable for initiating sedimentation**.**

5.3 Centrifugation

Centrifugation harvesting method can be applied to almost every type of microalgae. Centrifugation is using the same sedimentation principal but with addition with enhanced gravitational force to increase the sedimentation rate (Knuckey et al., 1986) .There is some types of centrifugation harvesting method and it is depending on the particle size ranges. Tubular bowl centrifugation provides the most efficient result in harvesting but the capacity is very limited. This type of method is more preferably done in small laboratory scale.

5.4 Biological filtration

Biological filtration means feeding of easily harvested filter feeders with algae, and is consequently a form of aquaculture. Well-known filter feeders are mussels and cladocerans like *Daphnia* spp. (de la, 1992). Complete food chains starting with wastewater have been studied in order to develop integrated systems able to generate useful biomass simultaneously with effluent purification (Etnier and B., 1991). Pathogen safety of such biomass does not appear to be of major concern although more complete and systematic monitoring of pathogens should be made before the final edible biomass (fish in general) is available for human consumption (de la.,1992).

6. Microalgal biofuels

Microalgae are a promising replacement for first generation biofuels (Li, Horsman et al. 2008; Patil, Tran et al. 2008; Griffiths and Harrison 2009) and a promising option for the contribution of renewable fuels to the existing fuel infrastructure (Rosenberg, Oyler et al. 2008; Schenk, Thomas-Hall et al. 2008; Pienkos and Darzins 2009; Pittman, Dean et al. 2011). Microalgal biomass may be used to produce biofuels without the negative environmental and agricultural impacts associated with first generation, land-based biofuels (Dismukes, Carrieri et al. 2008). Microalgal biomass is a source of renewable energy (Aresta, Dibenedetto et al. 2005; Amin 2009) which can be converted into useful energy sources such as biofuel oil and gas (Amin2009). Algal biodiesel could provide a greater amount of energy than any other oilseed crop (Sheehan, Dunahay et al. 1998; Patil, Tran et al. 2008) and is theoretically capable of meeting the existing energy demand (Chisti 2007). Microalgal biofuels have great potential to progressively replace fossil fuels (Brennan and Owende 2010), and will become even more competitive as petroleum supplies decrease and associated costs of fuel increase (Campbell 2008).

Table -6.1: Comparison of properties of biodiesel, diesel fuel, and ASTM standard Taken from Amin (2009)

Properties	Biodiesel from microalgae oil	Diesel fuel	ASTM biodiesel standard
Density (kg/l)	0.864	0.838	0.86-0.90
Viscosity (mm^2/s, cSt at 40°C)	5.2	1.8-4.1	3.5-5.0
Flash point (°C)	115	75	Min 100
Solidifying point (°C)	-12	-50 to 10	-
Cold filter plugging point (°C)	-11	-3.0 (max -6.7)	Summer max 0 Winter max <-15
Acid value (mg KOH/g)	0.374	Max 0.5	Max 0.5
Heating value (MJ/kg)	41	40-45	-
H/C ratio	1.81	1.81	-

7. Advantages of Microalgae for Biofuels

The advantages of using microalgae for biofuel production are numerous. Microalgae contain an efficient biological system for harvesting solar energy (Vonshak 1990; Aresta, Dibenedetto et al. 2005; Schenk, Thomas-Hall et al. 2008), making microalgae more efficient harvesters of solar energy than terrestrial crops (Dismukes, Carrieri et al. 2008). Microalgae have simple reproductive organs with a simple cell division cycle (Vonshak 1990; Li, Horsman et al. 2008), they have a high growth rate (Behzadi and Farid 2007; Li, Horsman et al. 2008) and there is the potential to produce a high volume of biomass through algae cultivation (Campbell 2008). Algal cells generally contain a high lipid content (Pienkos and Darzins 2009), but algal species can also be engineered to produce large concentrations of carbohydrates, lipids, proteins, or pigments (Vonshak 1990) depending on the intended use of the algal biomass. The minimal land requirements for algae cultivation also make microalgal biofuels advantageous over terrestrial crops. The cultivation of microalgae for biofuel production would use a smaller amount of land than terrestrial biofuels (Dismukes, Carrieri et al. 2008), and marginal lands could be used for cultivation (Campbell 2008). According to Chisti (2007), biofuels from microalgae are the only realistic option capable of replacing petroleum fuels based on land use alone (Chisti 2007). Among the greatest advantages of using microalgae for biofuels is the potential for CO_2 fixation (Aresta, Dibenedetto et al. 2005; Behzadi and Farid 2007; Schenk, Thomas-Hall et al. 2008; Wang, Li et al. 2008; Amin 2009). The ability for microalgae to consume CO_2 for growth results in an overall process that is carbon neutral (Aresta, Dibenedetto et al. 2005; Campbell 2008) because CO_2 is released back into the atmosphere when the biofuel is combusted. It is important to note that CO_2 is also produced at other points in the life cycle of the system, so the global warming potential (GWP) is positive over the entire life cycle. Finally, there is potential for the cost-effective production of microalgal biofuels (Campbell 2008).

Table-6.2: Comparison of some sources of biodiesel.Taken from Chisti (2007)

Crop	Oil Yield (L/ha)	Land area neede (M/ha)
Corn	172	1540
Soya bean	446	594
Canola	1190	223
Jatropha	1892	140
Coconut	2689	99
Oil palm	5950	45
Microalgae	136900	2

9. CONCLUSIONS

Studies have shown that the use of wastewater to supply nutrients for algae cultivation reduces GWP and EP in the cultivation stage when compared to using synthetic fertilizers (Clarens, Resurreccion et al. 2010; Soratana and Landis 2011). Algae can be used in wastewater treatment for a range of purposes, including; reduction of BOD, removal of N and/or P, inhibition of coliforms, removal of heavy metals. Harvesting of algae for biofuel production may be limited due to the high energy requirements associated with harvesting the treated water by centrifugation. Harvesting techniques are well developed; but they often result in the highest energy use and environmental impacts in the process (Oswald and Golueke 1960; Bich, Yaziz et al. 1999; Sander and Murthy 2010). The impacts of replacing other advanced nutrient removal technologies with algae cultivation for nutrient removal may reveal a greater energy savings. This study shows that nutrients can be removed from wastewater while simultaneously producing carbohydrates and lipids for biofuels in an algal PBR. In general, the nutrient concentration in the system decreases while the concentration of algal biomass increases. However, there was no statistical significance between nutrient removal, algae growth, or lipid and carbohydrate content in the system. A harvesting method which uses less energy and effectively separates solids from water must be developed for a coupled system which creates useful products while treating wastewater to be feasible.

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