Research in Biotechnology, 3(1): 47-60, 2012 ISSN: 2229-791X
www.researchinbiotechnology.com

Review Article

An integrated process for Industrial effluent treatment and Biodiesel production using Microalgae

Arjun R.Krishna[1], Lipin Dev[1] and V.Thankamani[2]*

[1]School of Biosciences and Technology (SBST), VIT University, Vellore, 632014
[2]Department of Biotechnology, University of Kerala, Kariavattom, Trivandrum
*Corresponding author: dr.thankamani@gmail.com

The present day necessities and requirements have emphasized the need for a renewable and alternate energy source is very high. Besides, the present day energy resources posse potential threat to the environment by emitting Greenhouse gases (GHGs) etc. An integrated process which involves a model of the wastewater High Rate Algal Ponds (HRAPs) near the industries and establishment of Biodiesel plants nearer to these ponds to produce algal biodiesel along with other byproducts is elucidated. Wastewater HRAPs also help in sequestering the CO_2 emitted by the industries and is used by the microalga for their photosynthesis in turn providing oxygen to the bacterial population which accumulates and degrades the toxic compounds present in the industrial effluents. This integrated process involving cheaper treatment of industrial effluents, production of algal biodiesel, accumulation of toxic compounds, sequestration of CO_2 and various other non-fuel applications contribute to an effective energy management system.

1. Introduction

In our day to day life, the need for energy is increasing rapidly. We depend on various sources of energy like electricity and fuels for industries, automobiles, household activities and many other basic needs. Among these sources, the fossil fuels account for about 88% of primary energy production (Brennan and Owende, 2010). Fossil fuels being limited resources of energy are fast depleting due to the continuous exploitation by mankind (Srivastava and Prasad, 2000). Studies reveal that, these energy sources are expected to be extinct by the year 2042 (Shafiee and Topal, 2009). Also, the combustion of fossil fuels is responsible for 73% of the CO_2 production (Ragauskas et al., 2006; Demirbas and Demirbas, 2007; NM Verma et al., 2010). So, the alternate energy sources like Biofuels which are renewable and capable of maintaining environmental and economic sustainability (Prasad et al., 2007 a, b; Brennan and Owende, 2010; Singh et al., 2010 a, b) are the need of the hour.

Biofuels refer to the energy derived from the biological sources. They can be used in vehicles as an alternative to the petroleum based fuels with little change to current technologies (Carere et al., 2008). Reduction in the long term CO_2 emissions makes them both sustainable and environment friendly energy sources (Yuan et al., 2008). The food and oil crops (viz. rapeseed oil, palm oil, sugarcane, sugar beet, wheat, barley, maize, etc.) as well as animal fats contribute to the first-generation biofuels which have been mainly extracted using conventional technology (Nigam and Singh, 2010). The second generation biofuels are produced from lignocellulosic biomass including agricultural residues, forest harvesting residues, wood processing wastes and non-edible components of corn or sugarcane that are not food resources (Brennan and Owende, 2009). The food v/s fuel competition of the first generation biofuels (Nigam and Singh, 2010) and the costlier technologies for the large scale production of the second generation biofuels have led to major controversy and debate on their sustainability (Goh and Lee, 2010) and a need for an alternative third generation biofuels. The algae are a potential source of the third generation biofuels that could be a promising renewable source of energy. (The draft National Algal Biofuels Technology Roadmap, Department of energy, US, 2009).

Algae are considered as the only alternative to current biofuel crops such as corn and soybean as they do not require arable land (Chisti, 2007; Hu et al., 2008; Singh et al., 2010c). They can be grown in the submerged area and also in the sea water (Singh et al., 2010c). Microalgae store more neutral lipids as compared to macroalgae. The microalgae such as *Chlorella protothecoides* has higher heating value (25.1 MJ/Kg) than the macroalgae like *Chlorella fracta* (21.1 MJ/Kg) and contributes better as an alternative energy source (Demirbas, 2008). Microalgae have excellent heavy metal scavenging property besides being cost effective and easy to handle. (Dayananda et al., 2005; Sazdanoff, 2006; Chisti, 2007; Huntley and Redalje, 2007; Li et al., 2008; Schenk et al., 2008; Tan et al., 2009).

Water intensive industries like pulp and paper, agro industries, tanneries, distilleries, textile industries are the major contributors of effluents (Chandralata et al., 2008) and usually contain very high concentrations of total N and total P concentration as well as toxic metals, making their treatment costlier (Gasperi et al., 2008). In addition, these effluents are also rich in cellulose, hemicelluloses, starch, carbohydrates and other organic and inorganic compounds (Lara et al., 2003) and hence can act as sustainable growth medium for the algal feedstock (Oswald et al., 1957; de la Noue et al., 1992; Green et al., 1995). In such ambience, microalgae can grow effectively accumulating nutrients and metals, making them sustainable and suitable for low cost wastewater treatment. (Hoffmann, 1998; Mallick, 2002; de-Bashan and Bashan, 2010). Moreover, species like *Chlorella vulgaris* has shown promising results with the highest lipid content (42%) when grown in wastewaters (Feng, Li a, Zhang, 2011). This suggests a setup of wastewater High Rate Algal Ponds (HRAPs) near the industrial areas to trap sustainable and renewable source of energy. Wastewater treatments HRAPs are presently the only economic and eco-friendly systems to produce biofuels (Park, Craggs and Shilton, 2011). We present a paper on integration of efficient energy management system and production of biodiesel with other value added products like glycerol from algae grown on wastewater HRAPs.

The wastewater released from different origins has different amounts of BOD (Biological Oxygen Demand) and TSS (Total Solid Substrate) as described in Table 1. The application of the microalgae in the wastewater treatment has shown better results as compared to the conventional chemical processes (Pedroni et al., 2001). One kg of BOD removed by photosynthetic oxygenation requires no energy inputs and produces enough algal biomass for fuel generation. Whereas in the activated sludge process, one kWh of electricity is required for aeration to remove one kg of BOD ,besides producing one kg of fossil CO_2 from power generation (Oswald, 2003). The BOD in the ponds may be loaded as heavily as 350 kg/ha-day in the tropics. At this loading rate, one may expect the effluent to have a filtered BOD of less than 20 mg/I when treating sewage (Dodd, 1980).

Table 1: Levels of BOD and TSS from various industries

Origin of waste	BOD Kg per ton product	TSS Kg per ton product
Domestic sewage	0.025 (kg/day/person)	0.022 (kg/day/person)
Dairy industry	5.3	2.2
Starch & glucose industry	13.4	9.7
Textile industry	30-314	55-196
Pulp & paper industry	4-130	11.5-26
Beverage industry	2.5-220	1.3-257
Tannery industry	48-86	85-155

Source: *Industrial Wastewater Treatment Plants Self-monitoring Manual, Chapter 2, 2002*

Studies reveal their ability to hold up the potentially toxic associated SOx, NOx gases present in fuel gas and also to sequester atmospheric CO_2 (Bajhaiya et al., 2010; Ono and Cuello, 2006; Hsueh et al., 2007; Jacob-Lopes et al., 2008). *Chlorella sps* has been reported to grow in condition with CO_2 concentration from 20% to up to even 40% (Hanagata et al., 1992). It is also found that the protein and chlorophyll content have been elevated up to 6% due to an elevated level of CO_2 concentration at 30^{o}C (Chinnasami et al., 2009). *Chlorella sps* also help in fixing CO_2 released into the atmosphere. They have shown a lipid productivity of 23.0 mg/L/d at different CO_2 concentration (0.03%, 3%, 10% and 15%) (Abhay et al., 2010**)**. A biodiesel plant can thus be set up near an industry so that the algae can get the Carbon source and the industry can have an efficient and environment friendly waste management system. It's also found that microalgae including *Chlorella sps* can fix nitrogen, sulphur etc apart from CO_2. The entire set up of algae based wastewater treatment is carbon negative as the CO_2 consumed by algae is higher than the amount released into the atmosphere. Various companies and industries already use algae as a source to sequester CO_2 to reduce global warming. The main purpose of the algae in these companies is to sequester CO_2 (*www.oilgae.com*). But it can be extended to heavy metal accumulation and biodiesel production by the construction HRAPs and biodiesel plants nearby these industries. The production of biodiesel (and electricity) from algae is beneficial, with a reduction in greenhouse gas output of between 63.1 and 108.8 g t^{-1} km^{-1} (Campbell et al., 2011).

The functional groups present on the cell surface are able to bind to metal ions which makes microalgae excellent heavy metal scavengers. Exposure of *Chlorella vulgaris* to elevated concentrations of copper, chromium, nickel and zinc led to intracellular accumulation of high concentrations of these metals. Accumulation of Proline has occurred based on the concentration of external medium or in the cell, which increased based on the accumulation of the metal in the cell (Mehta and Gaur, 2002). This excellent capability of microalgae gives it a huge potential to be used in waste water treatment plants. The waste water containing heavy metals can be directed towards a biodiesel plant thus coupling an excellent waste management system along with biodiesel production.

2. An Industrial effluent treatment coupled with Biodiesel production using Microalgae

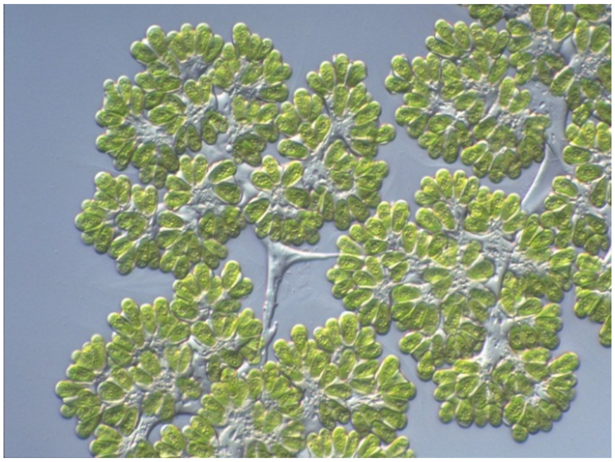
This model emphasises to meet the energy requirements as an alternative to fossil fuels besides reducing GHGs. The model proposes the setting up of HRAPs & Biodiesel plants near to the large CO_2 emitting industries. The algae help in CO_2 sequestration and reducing other flue gases emitted from the industries. This provides an added benefit of monetizing the carbon credits simultaneously producing biodiesel and presenting many interesting opportunities.

2.1. *Species selection:*

Many species of microalgae grow effectively in wastewater (Oswald et al., 1957). The selection of the algal strain depends on the type of wastewater where it grows. Microalgal species like *B. Braunii*, *Chlorella saccharophila* and *Pleurochrysiscarterae* grow well particularly in the untreated wastewaters from industries (Chinnasamy et al., 2010). Efficient growth and effective uptake of NO_3 have been observed in green alga like *Botryococcus braunii* growing in the piggery wastewater (An et al., 2003). In case of wastewaters from dairy industries, the potential growth of benthic freshwater algal species like *Microsporawilleana, Ulothrix sp.* and *Rhizocloniumhierglyphicum* have been found (Mulbry and Wilkie, 2001; Wilkie and Mulbry, 2002; Mulbry et al., 2008). Other species which dominate in the wastewater treatment HRAPs include *Micractinium sp., Actinastrum sp., Pediastrum sp., Dictyosphaerium sp., Coelastrum sp* and often form large colonies (50–200 lm) (Benemann et al., 1978, 1983; Benemann, 1986; Park and Craggs, 2010; Craggs et al., in press) making easier to harvest. *Chlorella* and *Scenedesmus* genus are particularly tolerant to sewage effluents and have shown tolerance and efficiency in accumulation of nutrients from the wastewater (Aslan and Kapdan, 2006; Gonzalez et al., 1997; Ruiz-Marin et al., 2010, Masseret et al., 2000, Bhatnagar et al., 2010; Lau et al., 1995; Shi et al., 2007; Wang et al., 2010, in press). Besides, they are efficient in almost complete removal of ammonia, nitrate and total Phosphorus during the tertiary wastewater treatment (Martinez et al., 2000; Ruiz-Marin et al., 2010; Zhang et al., 2008). Recent studies on *Chlorella sps* have shown good growth even in very raw wastewaters (Wang et al., 2010). Species like *Chlorella minutissima* are identified in the wastewater oxidation ponds in India (Bhatnagar et al., 2010), which will be a good candidate for setting up wastewater HRAPs. Also, species like *Scenedesmus obliqus* has a high potential for CO_2 capture and lipid production (Mandal and Mallick., 2009) and have shown better growth in municipal wastewater (Ruiz-Marin et al., 2010). Table 2: shows the list of various algal species growing at various habitats with their lipid content and lipid productivity.

Table 2: Representation of algal species apt for growing in wastewater

Waste water type	Micro algal species	Biomass (DW) productivity (mg L^{-1} day^{-1})	Lipid content (% DW)	Lipid productivity (mg L^{-1} day^{-1})	References
Municipal sewage	*Scenedesmus obliquus*	26	31.4	8	Martinez et al., 2000
	Botryococcus braunii	345.6	17.85	62	Orpez et al., 2009
Agricultural	*Botryococcus braunii*	700	Nd	69	An et al., 2003
	Chlorella sp.	2.6 g m^{-2} day^{-1}	9	230img m^{-2} day^{-1}	Johnson and Wen, 2010
	Mix of *Microsporawilleana, Ulothrix sps, Rhizoclo-niumhieroglyphicum*	5.5 g m^{-2} day^{-1}	Nd	Nd	Wilkie and Mulbry, 2002
Industrial	*B. braunii*	34	13.2	4.5	Chinnasamy et al., 2010
	Chlorella saccharophila	23	18.10	4.2	
	Pleurochrysiscarterae	33	12	4	



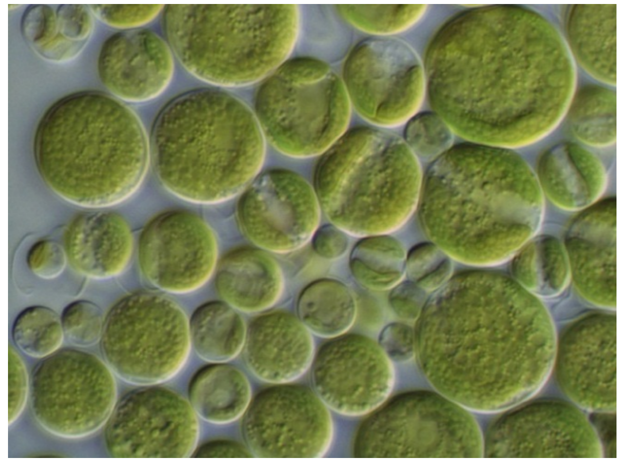


Fig 1(a): *Botryococcus braunii* **Fig 1(b):** *Chlorella protothecoides*

Fig 1(a) and 1(b)-Courtesy: http://www.shigen.nig.ac.jp

2.2. *Growth in HRAPs (open raceways):*

Algae culture in open raceways is more fitted to mass production than photo bioreactors, even if the growth rate of algae is lower in open ponds than in photo bioreactors (Chisti, 2007). Actually the Net Energy Ratio (NER) for total biomass is higher in raceway ponds than in flat-plate photo bioreactors (Jorquera et al., 2009). In addition, the economic cost of photobioreactors is almost one order of magnitude higher than the cost of open raceways (Campo et al., 2007).

The ideal attributes of algal species for use in wastewater treatment in High Rate Algal Ponds (HRAPs) are high growth rate, adaptive to seasonal and diurnal variations in outdoor growth conditions, aggregate formation and thereby enabling simple gravity harvest. High levels of valuable algal cell components (e.g. lipid for biodiesel) are also desirable. The other factors are like temperature, light, pH, nutrient composition and concentration, hydraulic retention time, pre-adaptation and seeding, gazers and parasites (Sheehan et al., 1998; Benemann, 2003).

Fig 3: An aerial view of HRAP
Courtesy: http://www.aquagy.net

Fig 4: Paddle wheel system in HRAPs
Courtesy: http://www.makebiofuel.co.uk

2.3. *Influence of light, temperature, pH, CO_2 and nutrients:*

Algae grow proportionally with the light intensity, when grown in the absence of nutrient limitation. The proportionality reaches a stand still when maximum algal growth is occurred at the light saturation point (Bouterfas et al., 2002; Macedo et al., 2002; Torzillo et al., 2003; Richmond, 2004). Studies in some green algae reveal that, neutral lipid accumulation was high at elevated light intensity which triggered the moderate photo inhibition of photosynthesis (Yantao Li et al., 2011). In the typical HRAPs designed with the depth of about 30 cm, the algal biomass is intermittently exposed to the light by the turbulent eddies and paddle wheel mixing. This paddle wheel system can be used to agitate the water, besides increasing the productivity over 30% higher than the other conventional techniques (Agustin G. Fontes, José Moreno, and M. Angeles Vargas, 2004), it also promotes algal cells to flocculate and settle down (Benemann, J.R et al., 1980).

The effect of temperature on growth of a living being is vital. In case of many algae, maximal growth rate are observed at optimum temperatures between 28°C and 35° (Soeder et al., 1985), above which result in decreased productivity (Tillett, 1988; Sheehan et al., 1998; Pulz, 2001). Sometimes, temperature also can affect the ionic equilibrium, pH and gas (Oxygen/CO_2) solubility (Bouterfas et al., 2002).

Growth and metabolic activities of many freshwater algae is optimum at pH 8.0, which is an ideal condition to grow in the industrial effluents (Kong et al., 2010). The factors that decide the pH of the algal ponds are the productivity of algae, algal/bacterial respiration, the alkalinity and ionic composition (García et al., 2000b; Craggs, 2005; Heubeck et al., 2007; Park and Craggs, 2010). Experiments on species like *Chlorella* have shown a decrease in productivity by 22 % when the pH was raised from 8 to 9 (Weissman and Goebel, 1988). Moreover, the aerobic heterotrophic bacteria maintain their metabolic activities optimum at pH 8.3. Increase in pH can inhibit their growth (Craggs, 2005). Therefore, it is very important to maintain the optimum pH levels in HRAPs.

CO_2 enhances the algal productivity in experimental scale wastewater treatment HRAPs (Azov et al., 1982; Benemann, 2003; Park and Craggs, in press-a). But higher CO_2 and HCO^{-3} consumption during the photosynthetic activities of algae often increases the pH (Craggs, 2005; Heubeck et al., 2007; Park and Craggs, 2010).

The main nutrients affecting the growth of algae are Nitrogen and Phosphorus. The ratio of N: P is very crucial for the growth and metabolism of algae, which can vary from about to 4:1 to almost 40:1 depending on algal species and nutrient availability in algal culture (Craggs et al., in press). Therefore, high productivity may be achieved even at relatively low N: P ratios in wastewater treatment HRAPs. The biotic factors like pathogenic bacteria / predatory zooplankton may hamper the growth of algae (Lau et al., 1995).

2.4. *Effect of other factors:*

Rotifers and cladocerans at high densities (>105/L) reduced the algal concentrations by 90% within couple of days (Oswald, 1980). While a 99% reduction in algal chlorophyll-a was observed by Cauchie et al as a result of grazing daphnia for several days (Cauchie et al., 1995). Besides, the fungal and viral infections can also significantly reduce the pond algal population

(Kagamiet et al., 2007). The starting density of microalgae in the wastewater is also likely to be a critical factor for the growth of the whole population (Lau et al., 1995).

2.5. *Harvesting of microalgae*

Harvesting of microalgae can be done by filtration, centrifugation, foam fractionation and coagulation-flocculation (Poelman et al., 1997; Price et al., 1974; Gsordas and Wang, 2004; Rossingol et al., 1997). Microalgae like *Arthrospira sps* can be separated by simple filtration method as the cells are large in size. Chlorella sp have a cell size of about 3-5 microns and cannot be easily filtered out. Centrifugation can be carried out but the high gravitational and shear force can damage the cells. The process is time and energy consuming and demands costlier equipment. Flocculation involves the clumping of algal cells into flocs due to electrostatic and intermolecular or vanderwaals attraction caused by the addition of flocculants (Knuckey et al., 2006). Aluminum Sulphate is found to be the most efficient flocculants followed by ferric and zinc salts and some cationic polyelectrolytes (Papazi, Makridis and Divanach, 2009). Apart from the flocculants, flocculation mainly depends on the cellular surface, pH of the growth medium, ionic concentration, concentration of flocculants and number of cells per unit volume (Bilanovic et al., 1998; Lee et al., 1998). The algal biomass yields 20-40 g of dry matter per square metre per day (Dodd, 1980).

2.6. *Extraction of Biodiesel*

The extraction of the biodiesel is done from the harvested algae that contains fatty acids, on supercritical methanol Trans esterification (SCMT) reaction (Fig: 5) they react with methanol at elevated temperature and pressure. In this process both algal lipids and FFAs are converted to biodiesel upon action of a catalyst. The methyl esters (Biodiesel) are the product and the glycerol is the byproduct of this Trans esterification process (Merkle, 2009).

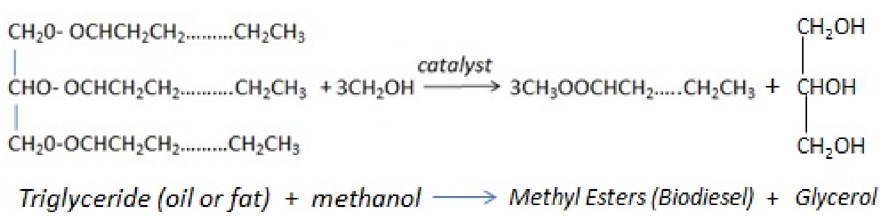


Fig 5: Reaction representing the biodiesel production

The algal biomass extracted is passed to the biodiesel production plant, where the SCMT method is applied and the biodiesel extraction is achieved. Apart from the biodiesel, glycerol is the main byproduct obtained. It was also found that the in an algae based wastewater treatment plant, the energy outputs are twice as compared to energy inputs (*www.oilgae.com*).

3. Wastewater treatment model

The actual functioning of the proposed model is detailed in Fig: 7. The industrial effluents (containing both solid and liquid) are initially passed through the primary settling tank/ primary clarifiers. In this tank, the sludge settles while the grease and oils rise to the surface. The sludge is collected by the mechanical scrapers and is separated from the clarified effluent (*Unit Operation and Processes of Wastewater Treatment*). The solids are then passed to the biogas digester. The sludge is digested and dried, after which it can be used as fertilizer. Grease and oil from the floating material can sometimes be recovered for saponification. The clarified effluent is passed for bacterial decomposition, where these bacteria consume biodegradable soluble organic contaminants (viz sugars, fats, organic short-chain carbon molecules, etc.) and bind much of the less soluble fractions into floc (*http://www.thewatertreatments.com/waste-water-treatment-filtration-purify-sepration-sewage/secondary-treatment*). In an algae based waste water treatment, algae provide oxygen for aerobic bacteria, thus reducing the high costs for mechanical aeration seen in traditional waste water treatments. The process of aeration generally accounts for 45% to 75% of a wastewater treatment plant's total energy costs (Rosso and Michael, 2006). The effluents are then passed through the different sections of the HRAP. The paddlewheel system is setup where the agitation takes place and the mixing of the medium occurs. As described, this system provides aeration for algae also helping them to form flocs and settle down thus helping in biomass extraction for biodiesel production. The extraction of biodiesel is done by SCMT method.

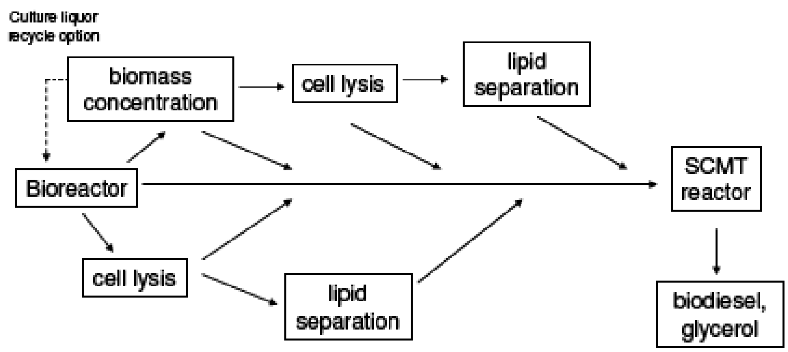


Fig 6: SCMT method for Biodiesel extraction

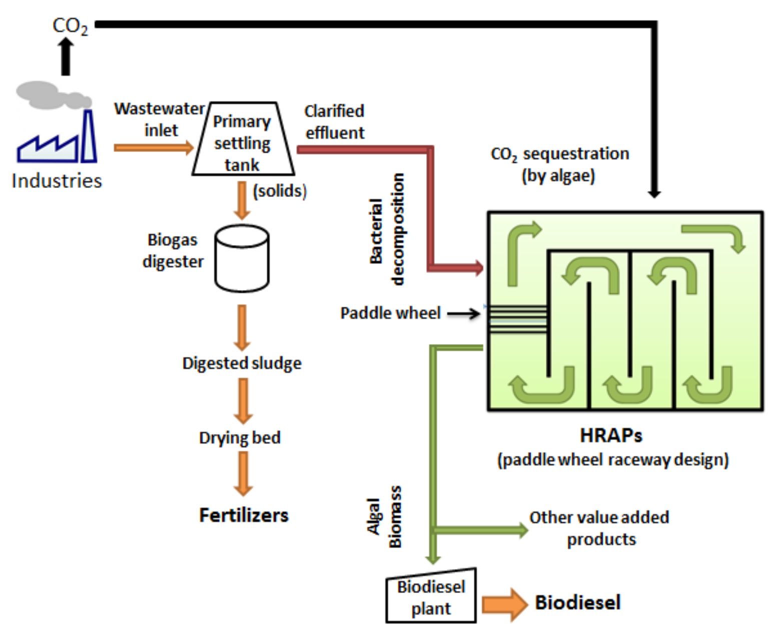


Fig 7: Schematic representation of the proposed model

4. Other Non-fuel Applications of Wastewater Grown Algae

Wastewater grown algae have many other potential uses other than the biodiesel produced from the neutral lipids (Fig: 8). They can be briefed as animal & fish feed, chemicals & fertilizer, biopolymers & bio plastics, paints, dyes, colorants, lubricants, uranium/plutonium sequestration, fertilizer runoff reclamation (*www.oilage.com*). Besides, business opportunities for both technologists and the common man exist in new fields.

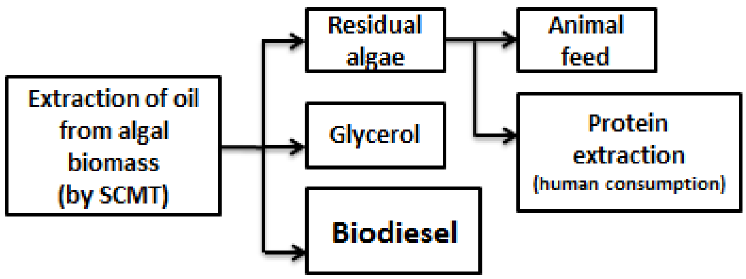


Fig 8: Various products from algal biomass

5. Conclusion

High algal biomass production from wastewater HRAPs is an efficient system for waste water management and biodiesel production. HRAPs coupled with biodiesel production are simple and cost effective. Developing countries, which have poor waste water management system, can adopt this process to combat the pollution and meet the fuel crises. HRAPs can also be implemented in local polluted water bodies to treat water besides providing a way for cheaper energy production. Establishment of the ponds can be initiated near the industrial areas where the industries can rely individually or as group for the production of algal biodiesel, accumulation of toxic compounds, sequestration of CO_2 and various other non-fuel applications contributing to an effective energy management system.

6. Acknowledgment

I dedicate this paper to my beloved parents, M. Radha Krishna and Susmitha for their constant support, motivation and blessings.

7. References

Abhay, B. Fulke., Mudliar, S.N., Raju, Yadav., Ajam, Shekh., Srinivasan. N., Rishiram , Ramanan., K, Krishnamurthi., S, Saravana Devi., T, Chakrabarti. 2010. Bio-mitigation of CO_2, calcite formation and simultaneous biodiesel precursor's production using Chlorella sp. *J.Biortech.*, **101:** 8473–8476

Aikateri ni, Papa zi., Pav los Mak ridis., Pascal, Divanach. 2010. Harvesting *Chlorella minutissima* using cell coagulants. *J Appl Phycol.*, **22:** 349 –355

An, J.Y., Sim, S.J., Lee, J.S., Kim, B.W. 2003. Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. *J. Appl. Phycol.*, **15:**185–191.

Anjana, Srivastava., Ram, Prasad. 2000. Triglycerides-based diesel fuels. *Renew Sust Energ Rev.*, **4:** 111-133

Anoop, Singh., Beatrice, M. Smyth., Jerry, D. Murphy. 2010. A biofuel strategy for Ireland with an emphasis on production of biomethane and minimization of land-take. *J. Rser.,* **14:** 277–288

Anoop, Singh., Deepak, Pant., Nicholas, E. Korres., Abdul-Sattar, Nizami., Shiv, Prasad., Jerry , D. Murphy. 2010. Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: Challenges and perspectives. *Bioresour Technol*., 101(13):5003-12

Aslan, S., Kapdan, I.K. 2006. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol. Eng*., **28:**64–70.

Azov, Y., Goldman, J.C.1982. Free ammonia inhibition of algal photosynthesis in intensive cultures. *Applied and Environmental Microbiology*., **43:**735–739.

Bajhaiya, A. K., S. K Mandotra., M.R, Suseela., Kiran, Toppo., S, Ranade., 2010. Algal Biodiesel: the next generation biofuel for India. *Asian J. Exp. Biol. Sci*., **1(4):** 728- 739

Bhatnagar, A., Bhatnagar, M., Chinnasamy, S., Das, K. 2010. *Chlorella minutissima* – a promising fuel alga for cultivation in municipal wastewaters. *Appl. Biochem. Biotechnol*., **161:**523–536.

Bilanovic, D., Shelef, G., Sukenik, A.1998.Flocculation of microalgae with cationic polymers: effects of medium salinity. *Biomass.,* **17:**65–76

Bouterfas, R., Belkoura, M., Dauta, A.2002. Light and temperature effects on the growth rate of three freshwater algae isolated from a eutrophic lake. *Hydrobiologia*., **489:**207–217.

Carlo, R Carere., Richard, Sparling., Nazim, Cicek., David B, Levin. 2008. Third Generation Biofuels via Direct Cellulose Fermentation. *Int. J. Mol. Sci.,* **9:** 1342-1360

Cauchie, H.M., Hoffmann, L., Jaspar-Versali, M.F., Salvia, M., Thomé, J.P.1995. Daphnia magna Straus living in an aerated sewage lagoon as a source of chitin: ecological aspects. *J. Zool*., **125:**67–78.

Chandralata, Raghukumar., 2000. Fungi from marine habitats: an application in bioremediation. *Mycol. Res*., 104 (10): 1222-1226

Daniel, Fishman., Rajita, Majumdar., Joanne, Morello., Ron, Pate., Joyce Yang. 2010. National Algal Biofuels Technology Roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program.

Dayananda C, Sarada R, Bhattacharya S, Ravishankar GA. 2005. Effect of media and culture conditions on growth and hydrocarbon production by *Botryococcus braunii*. *Pro. Biochem*., **40:**3125-3131.

De la Noue J, Laliberte G, Proulx D. 1992. Algae and wastewater. *J. Appl. Phycol*., **4:** 247-254.

De-Bashan, L.E., and Bashan, Y. 2010. Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresource Technology.,* **101:**1611–1627

Del Campo, J.A., Garcia-Gonzalez, M., Guerrero, M.G.2007. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Appl.Microbiol. Biotechnol*., **74:**1163–1174

Demirbaş, A. 2009. Production of Biodiesel from Algae Oils. *Energy Sources*, Part A: Recovery, Utilization, and Environmental Effects., **31:**163–168

Demirbas, AH., Demirbas, I. 2007. Importance of rural bioenergy for developing countries. *Energy Convers. Manag*., **48:** 2386-2398.

Diana Y. Hsueh., Nir Y. Krakauer., James T. Randerson., Xiaomei Xu., Susan E. Trumbore., John R. Southon. 2007. Regional patterns of radiocarbon and fossil fuel-derived CO_2 in surface air across North America. *Geophys Res Lett.,* **34:** L02816 (1-6)

Diego, Rosso., Michael, K Stenstrom., 2006. Surfactant effects on a-factors in aeration systems. *J.Watres*., **40:** 1397– 1404.

Dodd, J.C. 1980. Harvesting algae grown on pig wastes in Singapore. Paper presented at a workshop on high-rate algae ponds held in Singapore, 27-29 February 1980. Text included on microfiche iii IDRC-154e, International Development Research Centre, Canada.

Eduardo Jacob-Lopes, Lucy Mara Cacia Ferreira Lacerda, Telma Teixeira Franco. 2008. Biomass production and carbon dioxide fixation by *Aphanothece microscopica Nageli* in a bubble column photobioreactor. *J.Bej*., **40:** 27–34

García, J., Mujeriego, R., Hernandez-Marine, M.2000b. High rate algal pond operating strategies for urban wastewater nitrogen removal. *Journal of Applied Phycology*., **12:**331–339.

Goh. C. S and K.T. Lee. 2010. A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. *Renew. Sustain. Energy Rev.*, **14:**842–848.

Gonzalez, L.E., Canizares, R.O., Baena, S.1997. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. *Bioresour. Technol.,* **60:**259–262.

Green, F.B., Lundquist, T.J., Oswald, W.J. 1995. Energetics of advanced integrated wastewater pond systems, *Water Science and Technology*., **31(12):**9-20.

Gsordas A, Wang J-K.2004.An integrated photobioreactor and foam fractionation unit for the growth and harvest of *Chaetoceros sps*. in open systems. *Aquacult Eng*., **30:**15–30

Hanagata, N., Takeuchi, T., Fukuju, Y., Barnes, D. J., Karube, I. 1992. Tolerance of microalgae to high CO_2 and high temperature. *Phytochemistry*., **31(10):**3345-3348.

Heubeck, S., Craggs, R.J., Shilton, A.2007. Influence of CO_2 scrubbing from biogas on the treatment performance of a high rate algal pond. *Water Science and Technol*., **55:**193.

Hoffmann, J.P.1998. Wastewater treatment with suspended and nonsuspended algae. *J. Phycol*., **34:**757–763.

Hu. Q, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert and A. Darzins. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.*, **54:**621–639.

Huntley ME, Redalje DG. 2007. CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. *Mitigat. Adapt. Strat. Global Change*., **12:**573-608.

Johnny, Gasperi., Mathieu, Cladière., Vincent, Rocher., Régis, Moilleron. 2008. Combined sewer overflow quality and EU Water Framework Directive., 124-128

Johnson, M.B., Wen, Z.Y.2010. Development of an attached microalgal growth system for biofuel production. *Appl. Microbiol. Biotechnol*., **85:**525–534.

Jon, K. Pittman., Andrew, P. Dean., Olumayowa, Osundeko. 2011. The potential of sustainable algal biofuel production using wastewater resources. *J. Biortech.,* **102:** 17–25

Jorquera, O., Kiperstock, A., Sales, E.A., Embiruçu, M., Ghirardi, M.L.2009. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresour. Technol*., 101:1406–1413.

Joshua, S Yuan., Kelly, H Tiller., Hani Ahmad., Nathan R. Stewart., Neal, Stewart Jr C. 2008. Plants to power: bioenergy to fuel the future. doi:10.1016/j.tplants.2008.06.001. 1360-1385.

Kagami, M., de Bruin, A., Ibelings, B., Van Donk, E.2007. Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. *Hydrobiologia.,* **578:**113–129.

Knuckey, R., Brown, M., Robert, R., Frampton, D. 2006. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. *Aquacul Engin*., **35:**300–313

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

Lara, M.A., Rodríguez-Malaver, J., Rojas, O.J., Hoimquist, O., González, A.M., Bullón, J., Peñaloza, N., Araujo, E. 2002. Black liquor lignin biodegradation by *Trametes elegans*. *Int. Biodeter. Biodegr.,* **52:**167-173.

Lau, P.S., Tam, N.F.Y., Wong, Y.S.1995. Effect of algal density on nutrient removal from primary settled wastewater. *Environ. Pollut*., **89:**59–66.

Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N. 2008. Biofuels from microalgae. *Biotechnol. Prog*., **24:** 815-820.

Liam Brennan, Philip Owende. 2010. Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sustain. Energy Rev.,* **14(2):** 557-577.

Macedo, M.F., Duarte, P., Ferreira, J. 2002. The influence of incubation periods on photosynthesis–irradiance curves. *Journal of Experimental Marine Biology and Ecology.,* **274:**101–120.

Mallick, N. 2002. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *BioMetals*., **15:**377–390.

Mandal, S., Mallick, N.2009. Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. Appl. Microbiol. Biotechnol., **84 (2):**281–291.

Martinez, M.E., Sanchez, S., Jimenez, J.M., El Yousfi, F., Munoz, L.2000. Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresour. Technol*., **73:** 263–272.

Masseret, E., Amblard, C., Bourdier, G., Sargos, D., 2000. Effects of a waste stabilization lagoon discharge on bacterial and phytoplanktonic communities of a stream. *Water Environ. Res*., **72:**285–294.

Mehta, S.K., Gaur, J. P. 1999. Heavy metal induced proline accumulation and its role in ameliorating metal toxicity in *Chlorella Vulgaris*. *New Phytol.,* **143:** 253-259

Mulbry, W., Kondrad, S., Buyer, J. 2008. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. *J. Appl. Phycol*. **20:**1079–1085.

Mulbry, W.W., Wilkie, A.C.2001. Growth of benthic freshwater algae on dairy manures. *J. Appl. Phycol*. **13:**301–306.

Narendra, Mohan Verma., Shakti, Mehrotra., Amitesh, Shukla., Bhartendu, Nath Mishra. 2010. Prospective of biodiesel production utilizing microalgae as the cell factories: A comprehensive discussion. *Afr J Biotechnol.,* **9 (10):** 1402-1411

Oswald, William J. 2003. My sixty years in applied algology. *J Appl Phycol*., **15:** 99–106

Park, J.B.K., Craggs, R.J., Shilton, A.N. 2011. Wastewater treatment high rate algal ponds for biofuel production. *J.Biortech.,* **102:** 35–42

Park, J.B.K., Craggs, R.J.2010. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Science and Technology.,* **61:**633–639.

Pedroni, P., Davison, J., Beckert, H., Bergman, P., Benemann, J. 2001. A proposal to establish an international network on biofixation of CO_2 and greenhouse gas abatement with microalgae. *Journal of energy and environmental research.,* **1(1):**136-150.

Peer, M. Schenk., Sky, e R. Thom as-Hall., Evan, Stephens., Ute, C. Marx., Jan, H. Mussgnug., Clemens, Posten., Ola, f Kruse., Ben, Hankamer. 2008. Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *Bioenerg. Res.,* **1:**20– 43

Peter, Merkle. 2009. Recovery of Biodiesel Precursors from Heterotrophic Microalga *Chlorella protothecoides*. US Department of Energy. *US Department of Energy Publications.,* 1-11

Prasad. S, A. Singh and H.C. Joshi. 2007. Ethanol as an alternative fuel from agricultural, industrial and urban residues. *Resour. Conserv. Recycl.*, **50:** 1–39.

Prasad. S, A. Singh, N. Jain and H.C. Joshi. 2007. Ethanol production from sweet sorghum syrup for utilization as automotive fuel in India. *Energy Fuels*., **21:** 2415–2420.

Pulz, O.2001. Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology.,* **57:**287–293.

Ragauskas, AJ., Williams, CK., Davison, BH., Britovsek, G., Cairney, J., Eckert, CA., Frederick , JrWJ., Hallett, JP., Leak, DJ., Liotta, CL., Mielenz, JR., Murphy, R., Templer, R., Tschaplinski, T.2006.The path forward for biofuels and biomaterials. *Science*., **31:** 484-489.

Richmond, A.2004. Principles for attaining maximal microalgal productivity in photobioreactors: an overview. *Hydrobiologia*., **512:**33–37.

Rossignol , N., Vandanjon, L., Jaouen, P., Quemeneur, F.1999.Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. *Aquacult Eng.,* **20:**191 – 208.

Ruiz-Marin, A., Mendoza-Espinosa, L.G., Stephenson, T. 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresour. Technol*., **101:**58–64.

Senthil, Chinnasamy., Ashish, Bhatnagar., Ryan, W. Hunt., Das, K.C. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *J.Biortech.,* **101:** 3097–3105

Senthil, Chinnasamy., Balasubramanian, Ramakrishnan., Ashish, Bhatnagar., Keshav, C. Das. 2009. Biomass Production Potential of a Wastewater Alga Chlorella vulgaris ARC 1 under Elevated Levels of CO_2 and Temperature *Int. J. Mol. Sci*., **10:** 518-532

Shi, J., Podola, B., Melkonian, M.2007. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *J. Appl. Phycol.*, **19:**417–423

Singh. A, B.M. Smyth and J.D. Murphy. 2010. A biofuel strategy for Ireland with an emphasis on production of biomethane and minimization of land-take. *Renew. Sustain. Energy Rev.*, **14:**277–288.

Singh. A, D. Pant, N.E. Korres, A.S. Nizami, S. Prasad and J.D. Murphy. 2010. Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: challenges and perspectives. *Bioresour. Technol.*, **101:** 5003–5012.

Soeder, C.J., Hegewald, E., Fiolitakis, E., Grobbelaar, J.U.1985. Temperature dependence of population growth in a green microalga: thermodynamic characteristics of growth intensity and the influence of cell concentration. *Zeitschrift fur Naturforschung.,* **40:**227–233.

Tan K, Lee K, Mohamed A, Bhatia S. 2009. Palm Oil: addressing issues and towards sustainable development. *Renew. Sust. Energy Rev.*, **13(2):** 420-427.

Torzillo, G., Pushparaj, B., Masojidek, J., Vonshak, A.2003. Biological constraints in algal biotechnology. *Biotechnology and Bioprocess Engineering.,* **8:**338–348.

Wang, L., Li, Y.C., Chen, P., Min, M., Chen, Y.F., Zhu, J., Ruan, R.R.2010. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella sp. Bioresour. Technol.,* **101:**2623–2628.

Weissman, J.C., Goebel, R.1988. Photobioreactor design: mixing, carbon, utilization and oxygen accumulation. *Biotechnology and Bioengineering.,* **31:**226–344.

Wilkie, A.C., Mulbry, W.W.2002. Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresour. Technol*. **84:**81–91.

Yantao, Li., Danxiang, Han., Milton, Sommerfeld., Qiang, Hu. 2011. Photosynthetic carbon partitioning and lipid production in the oleaginous microalga Pseudochlorococcum sp. (Chlorophyceae) under nitrogen-limited conditions. *J.Biortech.,* **102:** 123–129

Yusuf, Chisti., 2007. Biodiesel from microalgae. *J.Biotechadv.,* **25:** 294 – 306

Zhang, E.D., Wang, B., Wang, Q.H., Zhang, S.B., Zhao, B.D.2008. Ammonia-nitrogen and orthophosphate removal by immobilized Scenedesmus sp isolated from municipal wastewater for potential use in tertiary treatment. *Bioresour.Technol*., **99:**3787–3793.