CHEMICAL ENGINEERING TRANSACTIONS

VOL. 45, 2015

A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet

Guest Editors: Petar Sabev Varbanov, Jiří Jaromír Klemeš, Sharifah Rafidah Wan Alwi, Jun Yow Yong, Xia Liu

ISBN 978-88-95608-36-5; **ISSN** 2283-9216 DOI:10.3303/CET1545233

Algae Cultivation in Wastewater for Biodiesel – A Review

Chung Hong Tan[a], Wai Yan Cheah[b], Tau Chuan Ling[b], Pau Loke Show*[,a,c], Joon Ching Juan[d,e], Jo-Shu Chang[f,g,h]

[a] Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia.
[b] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[c] Manufacturing and Industrial Processes Division, Faculty of Engineering, Centre for Food and Bioproduct Processing, University of Nottingham Malaysia Campus, Malaysia.
[d] Laboratory of Advanced Catalysis and Environmental Technology, School of Science, Malaysia.
[e] Nanotechnology& Catalysis Research Centre (NANOCAT), University of Malaya, 50603 Kuala Lumpur, Malaysia.
[f] University Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan 701, Taiwan.
[g] Department of Chemical Engineering, National Cheng Kung University, Tainan 701, Taiwan
[h] Research Center for Energy Technology and Strategy, National Cheng Kung University, Tainan 701, Taiwan.
PauLoke.Show@nottingham.edu.my

Global concern for energy security and environmental sustainability has put a great prominence towards alternative energy resources, substituting the rapidly-depleting fossil fuels. Fossil fuels have been a major contributing factor for greenhouse gas production, leading to global warming. Hence, biofuels have been greatly researched in hopes to replace fossil fuels. One remarkable biofuel producer is microalgae, due to their high biomass productions, high cellular lipid accumulation, as well as the ability to sequester carbon dioxide from waste gas and remove pollutants from wastewater. Integration of wastewater as the medium for algal cultivation offers a green and cost-effective way for sustainable biofuel production. The zero-cost palm oil mill effluent (POME) in Malaysia will be an option for microalgae cultivation due to the high concentrations of nitrogen and phosphorus. Microalgae are able to survive in wastewater by utilizing the nutrients for growth. This has the potential to achieve economical microalgae production for bioenergy, while promoting environmental sustainability.

1. Introduction

The global primary energy consumption has increased by 2.3 % in 2013, compared to 1.8 % in 2012. Energy consumption rose far more rapidly than its production in 2013, despite a stagnant global economic. World-wide energy consumption is predicted to increase by 49 % from 2007 to 2035 due to the demands from the expanding world population, economic growth and social pressures. It is expected that this may exhaust natural resources, such as petroleum, natural gas and coal. The sustained use of fossil fuels has been implicated as a major contributing factor for the greenhouse gas production. Alternative energy sources, such as biofuels, have to be in place to reduce the dependence on fossil fuels. Microalgae will be the next remarkable biofuel source, due to their rapid growth rate and low nutrient requirement, compared to other oil crops such as oil palm, soybean and rapeseed. Moreover, microalgae are capable of producing 15 – 300 times more oil than traditional crops on per unit area of land. Microalgae have been observed as suitable for biodiesel production due to their high growth rate coupled with a considerable amount of lipid accumulation. Most microalgae exhibit oil contents of 20 – 50 % on dry-cell weight basis (Chisti, 2007). Table 1 shows the comparison between all three generations of biofuels.

Please cite this article as: Tan C.H., Chech W.Y., Tau C.L., Show P.L., Juan J.C., Chang J.-S., 2015, Algae cultivation in wastewater for biodiesel – a review, Chemical Engineering Transactions, 45, 1393-1398 DOI:10.3303/CET1545233

Table 1: Advantages and disadvantages of 1st, 2nd and 3rd generation biofuels

Bioenergy Source	Main Bioenergy	Advantages	Disadvantages	Reference
1st Generation Biofuels				
Jatropha Oil Palm Rapeseed Soybean Corn Sugarcane	Biodiesel Bioethanol	• Cheap and abundant resource • Established technology for large scale production	• Less sustainable than 2nd and 3rd generation bioenergy due to the production stress placed on food crops • Driving up the price of food crops	(Brennan and Owende, 2010, Naik et al., 2010)
2nd Generation Biofuels				
Lignocellulosic biomass Agricultural residue	Bio-oil Bioethanol Biogas Syngas	• Cheap and abundant • Fully utilize organic wastes from agriculture (non-food) • Does not compete with food crops	• The bio-oil contains high amounts of nitrogen and oxygen, requiring expensive denitrogenation and deoxygenation process • Advanced technology is still being developed to reduce operating cost	(Jin et al., 2014, Naik et al., 2010)
3rd Generation Biofuels				
Microalgae	Biodiesel Bioethanol Biohydrogen Syngas Bioelectricity Bio-oil	• Rapid growth rate • High oil production per unit area of land • Does not compete for arable land • Can be cultured using wastewater • Microalgal bio-oil has higher quality than lignocellulosic biomass	• High capital cost due to expensive photobioreactors (higher yields) • Lower yields for open pond systems (lower cost) • Expensive oil transesterification and extraction process • The bio-oil requires expensive denitrogenation and deoxygenation process	(Brennan and Owende, 2010, Mata et al., 2010)

In order to achieve economical mass production of microalgal feedstock, the inputs of microalgae cultivation should be cost-effective. Light intensity, carbon dioxide concentration, available nutrients and water are the main factors determining cell growth and lipid accumulation. Wastewater from industries can become a nutrient source for microalgae cultivation due to its high concentration of organics, nitrogen and phosphorus. This includes wastewater from agriculture sector, fish-farming, landfills, as well as sewage treatment plants. Microalgae are often grown in tertiary treatment stage in wastewater treatment plants. Nutrients removal from the wastewater is reflected by the nutrients uptake of microalgae. Thus, microalgae cultivation by using wastewater could become the cheapest and most effective way to achieve maximum biomass production followed by high lipid excretion and environmental sustainability (Mata et al., 2014).
The Malaysian palm oil industry has grown rapidly over the past few decades. According to the Malaysian Palm Oil Board (MPOB), the export of oil palm has reached 17,306,247 Mt in year 2014 (MPOB, 2015). The industry was also identified as one of the agricultural industry that generates the highest pollution load into the receiving water bodies throughout the country. Most palm oil millers favour microalgae cultivation in wastewater treatment before palm oil mill effluent (POME) is discharged. The essential nutrients and minerals for microalgae growth, such as nitrate, zinc, iron, phosphorus and potassium, are present in POME. In fact, some researchers have reported on the utilization of POME as a medium for microalgae cultivation. An example of the research data was *Chlorella* sp. at 50% POME with urea showed high specific growth rate of 0.066 g/d. Therefore, microalgal culture using POME is a low-cost option for both biofuel production and wastewater treatment.

2. Current state of world CO_2 emission

Human activities have released large amounts of greenhouse gases to the atmosphere. The majority of greenhouse gases are generated from fossil burning to produce energy. These greenhouse gases act like a blanket covering the Earth, trapping heat from the sun. The Intergovernmental Panel on Climate Change (IPCC) has stated that our Earth's average temperature has risen more than 0.7 °C over the past century

(IPCC, 2007). Rising global temperatures have led to changes in weather and climate. This has caused melting of ice caps, rising sea level, severe heat waves, intense floods and droughts, as well as El Niño and La Niña phenomena. Humans are largely responsible for these environmental impacts, leading to threat possession to public health.
Carbon dioxide is the main greenhouse gas in the atmosphere. It has increased in concentration from 280–390 ppm since pre-industrial revolution until present time. It is currently contributing approximately 52% in total global warming (Cogan et al., 2012). According to Malaysian Ministry of Natural Resources and Environment, total greenhouse gases emission was 223.1 Mt carbon dioxide equivalent (Mt CO_2 equivalent) in year 2000 and the removal was 249.78 Mt CO_2 eq. The net emission after removal is - 26.79 Mt CO_2 equivalent, indicating Malaysia as a net sink. However, Malaysia showed a significant increase in greenhouse gas emission, ultimately becoming a net emitter with 38.7 Mt CO_2 equivalent in year 2005 and 45.9 Mt CO_2 equivalent in year 2007. This showed that the greenhouse gas emissions increased by more than 100 %. The top three contributors were energy, transportation and manufacturing sectors, which require huge amounts of fuel burning. This has caused greenhouse effect and harmed the ecosystem and well-being of the people. Thus, biofuels will serve as a notable substitute for fossil fuels (Ministry of Natural Resources and Environment, 2011).
Unfortunately, the study on using POME for microalgae cultivation is still lacking in most oil palm exporters, such as Malaysia and Indonesia. Studies on culturing microalgae using municipal wastewater have been studied in European countries; however, in Malaysia, POME will be the most noteworthy waste medium because it is produced in large quantities. The lab-scale analysis has to be studied first to observe the adaptability of microalgae strains and determine the optimum conditions for rapid growth and lipid accumulation. Once results from the pilot scale are confirmed, the research can then be scaled up to industrial level, integrating flue gas as the carbon source. This method will definitely help in achieving cost-effective algae production for bioenergy, together with environmental preservation.

3. Microalgal Biofuel

Microalgae are unicellular organisms where most species are photosynthetic, utilizing nutrients, carbon dioxide, sunlight and water to produce their own food. In addition, they are able to grow in many habitats, including brackish water, saline water and even wastewater. For example, green microalgae, *Dunaliella salica* is a typical halophile which can grow very alkaline medium of pH 11 (Ahmad et al., 2012).
Chlorella sp. is a model green microalga for biodiesel synthesis, whereas *Chlamydomonas* sp. is common for hydrogen gas synthesis. Microalgae have high adaptability, enabling them to live in various environments such as damp soil, stagnant water, freshwater and sea water. They do not have structural components like stomata, stems or roots, enabling them to grow by floating on a medium. Apart from absorbing nutrients directly from the medium, microalgae do not need to divert energy for structural formation, thus allowing faster growth rate. The harvesting cycle of microalgae is typically 1–10 days, and short harvest cycle means biofuels can be produced more rapidly and efficiently compared to oil crops (Nakanishi et al., 2014).
Lipid productivity depends on the variability of microalgal growth. Slight changes in growth conditions may affect cell growth and their lipid content. Growth parameters such as nutrient sources, light intensity, pH, temperature and CO_2 concentrations, must be optimized as these affect the biomass productions. Microalgae are often cultivated in large open ponds and enclosed photobioreactors, supplied with carbon dioxide. Air-sparging is usually used to supply the culture with higher concentration of CO_2. An alternative way is to utilize CO_2 from flue gas, emitted from power plants. For example, *Chlorella* KR-1 was successfully grown using untreated flue gas from a boiler. With application of wastewater utilization and the recycling of carbon dioxide emission, microalgae cultivation can definitely be done in an economical and sustainable way (Kim et al., 2012).
Microalgae biodiesel is a clean-burning alternative fuel. It is biodegradable, user-friendly, non-toxic, and free from sulphur and odour. It can be easily used by blending with petroleum fuel to create biofuel blend, rather than fully dependent on petroleum fuel. The lipids inside the microalgal cells can be extracted from dry biomass through physical or chemical means. The simplest method of lipid extraction is by mechanical crushing. Dried microalgae can retain its oil content, which to be pressed out with oil press. Furthermore, other commonly used chemical lipid extraction methods are Bligh and Dyer, Folch method or hexane extraction. These extracted triglycerides, which are a combination of glycerol and fatty acids, can be converted to alkyl esters via transesterification. In the transesterification process, the reactants are alcohol (methanol or ethanol) and triglycerides. Under normal condition, the reactants are heated in the presence of a catalyst (acid or alkali) to speed up the reaction. Common acid catalysts used are sulphuric acid and

hydrochloric acid, whereas common alkali catalysts are sodium hydroxide and potassium hydroxide (Brennan and Owende, 2010).

4. Potential of palm oil mill effluent (POME) in microalgal biodiesel production

4.1 POME

The Malaysian palm oil industry has grown rapidly over the last 30 y. Presently Malaysia has become the second largest oil palm producer in the world. The oil palm industry has served as the backbone to Malaysia's economy and generated an income of 9.8 % of the total national revenue in 2008. Yet, the sustainability and environmental standpoint of oil palm production are always being questioned. It was claimed that POME is the most polluting agro-industrial effluent and generates the highest pollution loads into the rivers. POME is mainly generated in fresh fruit bunches sterilisation and clarification process.

Approximately 0.9, 1.5 and 0.1 m^3 of POME are generated from steriliser condensate, sludge separator and hydrocyclone waste for every tonne of crude palm oil processed. For each tonne of crude palm oil produced, 5 – 7.5 t of water are required, and 50 % of water ends up in POME. POME contains 2 – 4 % suspended solids, 95 – 96 % water and 1 % oil. This large amount of water has resulted in massive loads of POME being generated per day. POME is a non-toxic liquid waste that smells unpleasant, acidic, a high discharge temperature of 80–90°C, as well as high concentrations of biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrogen and phosphorus (Lam and Lee, 2011). The typical POME characteristics and the POME discharge standards are shown in Table 2. Table 2 also contains carbohydrates, proteins, lipids and nitrogenous compounds that make up the high pollutant profile of POME.

Table 2: POME characteristics and discharge standards of POME in Malaysia

POME Characteristics			Discharge standards for POME	Major constituents of POME	Quantity (g/g) in dry sample
Parameter [a]	Mean	Range	Discharge standards [a]		
pH	4.2	3.4 - 5.2	5.0 – 9.0	Moisture	6.75
Temperature (°C)	85	80 - 90	45	Crude protein	9.07
BOD 3 days at 30°C [b]	25,000	10,250 - 43,750	100	Crude lipid	13.21
COD	51,000	15,000 -100,000	-	Ash	32.12
Total solids	40,000	11,500 -79,000	-	Carbohydrate	20.55
Suspended solids	18,000	5,000 - 54,000	400	Nitrogen-free extract	19.47
Volatile solids	34,000	9,000 - 72,000	-	Total carotene	20.07
Oil and grease	6,000	130 - 18,000	50		
Total Nitrogen	750	180 - 1400	200 [c]		
Ammoniacal Nitrogen	35	4 - 80	150 [c]		

[a] Units in mg/l except for pH and temperature
[b] The sample for BOD analysis is incubated at 30 °C for 3 days
[c] Value of filtered sample

4.2 Environmental Impacts

The prime reason for using biofuel is that it brings lesser negative impacts to the environment compared to fossil fuel. Burning of fossil fuel, undoubtedly, is causing a large amount of greenhouse gas emission to the atmosphere. In comparison, biofuel usage generally results in a decrease in pollutant emissions. Moreover, despite the fact that microalgal biofuel releases carbon dioxide when it is burnt, carbon dioxide is also removed from the atmosphere for microalgal growth. Microalgae are excellent bio-fixers, and they are able to remove carbon dioxide from the atmosphere and flue gas.

In comparison with terrestrial oil crops, microalgae production requires less land for growth due to their high productivity. The extra land area could be used to grow other food crops. In addition, microalgae can easily be grown on marginal lands, which cannot support most valuable crops. This is a significant reduction in land footprint. Moreover, pesticides, herbicides, fungicides and insecticides may need to be used to boost the growth of terrestrial crops. These chemicals may creep into nearby water bodies, causing underground water pollution or eutrophication. Microalgae, on the other hand, can grow well even without the use of herbicides or fungicides.

The depletion of freshwater occurs more prominently in Asia. However, freshwater is not necessary to grow microalgae. Microalgae are able to utilize saline water, run-offs and wastewater contaminated with fertilizers and nutrients, as their primary source of nutrients. In fact, microalgae are simultaneously treating the wastewater by utilizing the nutrients from wastewater for their growth, lowering the BOD of the wastewater. These positive benefits in land and water resources have made microalgal biofuel desirable and environmental-friendly.

4.3 POME nutrients removal

Growing microalgae in wastewater treatment helps to remove the pollutants present in the wastewater. The nutrient and pollutant removal from the wastewater is proportional to the nutrient uptake of microalgae. Microalgae can remove a significant amount of nutrients because they need N and P for protein, nucleic acid and phospholipid synthesis. Therefore, microalgae can serve as an agent for bioremediation of wastewater. Microalgae cultivation using wastewater could be the cheapest and most effective way to achieve maximum biomass production and high lipid accumulation together with environmental sustainability. The microalgae-treated wastewater can be discharged to the receiving water bodies, without posing any harm to the environment and public health.

POME has received considerable attention over the years, especially for palm oil exporters, due to the fact that it has created pollution to the rivers. In a study, the mixed culture of macroalgae and microalgae was isolated from an open pond system of POME treatment. These algae are efficient in removing COD in POME. The initial COD in 250 mg/L POME was 496 mg/L, and reduced to 143 mg/L after algae treatment. The total COD removal efficiency was 72 % (Kamyab et al., 2014). Another study found that *Chlorella sorokiniana* cultivated in 75 % POME (filtered and sterilised) was capable of removing 63 ± 3 % COD from the POME (Ogugua Nwuche et al., 2014). Moreover, Hadiyanto *et al.* has studied on phytoremediation of POME using aquatic plants followed by *Spirulina* sp. for COD and nutrients removal. He found out that the COD, N and P removal by aquatic plants are 50 %, 88 % and 64 % respectively, while *Spirulina* sp. removed 50.79 %, 96.5 % and 85.92 %. This clearly indicated that the latter is definitely effective in removing organics and nutrients from POME (Christwardana and Soetrisnanto, 2013).

5. Conclusion

Small scale research using microalgae to treat POME wastewater has already been proven successful. Common microalgal strains, such as *Chlorella* and *Spirulina*, reduced considerable amounts of BOD and COD from POME-amended culture medium. However, proper analysis of financial feasibility can only be accurately performed using large scale cultures. More research is needed in the large scale cultivation of microalgae using POME. Only then will the potential of culturing microalgae using POME be brought to light. Malaysia is a major exporter of oil palm; hence the issue of POME wastewater becomes very important. Research in this area will no doubt greatly benefit countries like Malaysia and offer alternative methods of dealing with POME wastewater.

Aknowledgements

This work is supported financially by SATU Joint Research Scheme (RU022E-2014) from University of Malaya, Malaysia; the Top University Project of NCKU; and Taiwan’s Ministry of Science and Technology under grant numbers MOST 104-3113-E-006-003, 103-2221-E-006-190-MY3; Fundamental Research Grant Scheme (Malaysia, FRGS/1/2013/SG05/UNIM/02/1); Ministry of Science, Technology, Innovation (Malaysia, MOSTI-02-02-12-SF0256) from the University of Nottingham in Malaysia.

References

Ahmad F., Khan A.U., Yasar A., 2012, Uptake of nutrients from municipal wastewater and biodiesel production by mixed algae culture, Pakistan Journal of Nutrition, 11, 648-652.

Brennan L., Owende P., 2010, Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products, Renewable and Sustainable Energy Reviews, 14, 557-577.

Chisti Y., 2007, Biodiesel from microalgae, Biotechnology Advances, 25, 294-306.

Christwardana M., Soetrisnanto D., 2013, Phytoremediations of Palm Oil Mill Effluent (POME) by Using Aquatic Plants and Microalge for Biomass Production, Journal of Environmental Science & Technology, 6, 79-90.

Cogan A.J., Boesch H., Parker R.J., Feng L., Palmer P.I., Blavier J.F.L., Deutscher N.M., Macatangay R., Notholt J., Roehl C., 2012, Atmospheric carbon dioxide retrieved from the Greenhouse gases

Observing SATellite (GOSAT): Comparison with ground‐based TCCON observations and GEOS‐Chem model calculations, Journal of Geophysical Research: Atmospheres, 117, 1-17.
IPCC, 2007, Climate Change 2007: Synthesis Report, Intergovernmental Panel on Climate Change (IPCC), Valencia, Spain.
Jin B., Duan P., Zhang C., Xu Y., Zhang L., Wang F., 2014, Non-catalytic liquefaction of microalgae in sub- and supercritical acetone, Chemical Engineering Journal, 254, 384-392.
Kamyab H., Din M.F.M., Tin C.L., Ponraj M., Soltani M., Mohamad S.E., Roudi A.M., 2014, Micro-Macro Algal Mixture as a Promising Agent for Treating POME Discharge and its Potential Use as Animal Feed Stock Enhancer, Jurnal Teknologi, 68, 1-4.
Kim W., Park J.M., Gim G.H., Jeong S.-H., Kang C.M., Kim D.-J., Kim S.W., 2012, Optimization of culture conditions and comparison of biomass productivity of three green algae, Bioprocess and Biosystems Engineering, 35, 19-27.
Lam M.K., Lee K.T., 2011, Renewable and sustainable bioenergies production from palm oil mill effluent (POME): win–win strategies toward better environmental protection, Biotechnology Advances, 29, 124-141.
Malaysian Palm Oil Board (MPOB), 2015, Monthly Palm Oil Trade Statistics <www.mpoc.org.my/Monthly_Palm_Oil_Trade_Statistics.aspx>, accessed 21.01.2015
Mata T.M., Martins A.A., Caetano N.S., 2010, Microalgae for biodiesel production and other applications: a review, Renewable and Sustainable Energy Reviews, 14, 217-232.
Mata T.M., Santosa J., Mendesa A.M., Caetanoa N.S., Martinsc A.A., 2014, Sustainability Evaluation of Biodiesel Produced from Microalgae Chlamydomonas sp Grown in Brewery Wastewater, Chemical Engineering Transactions, 37, 823-828.
Ministry of Natural Resources and Environment, 2011, Malaysia: Second national communication to the United Nation Framework Convention on Climate Change (UNFCCC), Ministry of Natural Resources and Environment, Kuala Lumpur, Malaysia.
Naik S.N., Goud V.V., Rout P.K., Dalai A.K., 2010, Production of first and second generation biofuels: A comprehensive review, Renewable and Sustainable Energy Reviews, 14, 578-597.
Nakanishi A., Aikawa S., Ho S.-H., Chen C.-Y., Chang J.-S., Hasunuma T., Kondo A., 2014, Development of lipid productivities under different CO_2 conditions of marine microalgae Chlamydomonas sp. JSC4, Bioresource Technology, 152, 247-252.
Ogugua Nwuche C., Chidimma Ekpo D., Nwoye Eze C., Aoyagi H., Chukwuma Ogbonna J., 2014, Use of Palm Oil Mill Effluent as Medium for Cultivation of Chlorella sorokiniana, British Biotechnology Journal, 4, 2231–2927.