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Mini Review
Low-cost Biodiesel Production

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Abstract

Use of fossil fuel after about 200 years of its dominance the world's primary source of energy has caused many environmental problems of which increase in air pollution and climate change are the major ones. Biodiesel production from microalgae is a renewable and bio-friendly source of energy recognized worldwide than fossil fuel. Unfortunately, the major challenge hindering biodiesel production at pilot scale is the high cost of production. This study, therefore, discusses some important steps taken to lower the cost of biodiesel production which includes a choice of lipid extraction methods, biomass drying methods, biodiesel conversion methods and choice of cultivating media. The cheap and effective method of lipid extraction, biomass drying and biodiesel conversion was identified and discussed. The use of natural media such as Palm Oil Mill Effluent (POME) for the cultivation of microalgae to produce biomass, which can be used as feedstock for biodiesel production was highlighted. The identification of the major strategies for up-scaling biodiesel production is a step forward in making biodiesel production economically viable.

Key words: Fossil fuel, biodiesel, microalgae, economically viable

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INTRODUCTION

In recent years, problems such as the combination of the energy crisis, environmental pollution and climate change are receiving a great deal of attention across the globe[1,2]. Due to the negative impact of the above-mentioned interconnected problems, the need for less polluting and more sustainable solution is therefore, necessary. Provision of sustainable solution can go a long way in eradicating the fear of escalating petroleum prices, shortage of fuel feed stocks and increase in atmospheric temperature will be reduced to the barest minimum. One such sustainable approach is the use of algae-based biofuel. A biofuel is a renewable form of bio-energy that fulfilled the promise of providing an alternative source of energy and future reduction of environmental pollution. The feed stocks used for biodiesel production are canola oil, sunflower, reap oil, maize oil and soy bean which are classified as first generation feedstock[1]. The disadvantage of the first generation feed stocks is that they pose a serious danger to food availability and supply because of their potential usage as human sources of food[3]. Sustainable production of biofuel from non-food based feedstock (microalgae) will provide an alternative solution to the current biofuel and global warming crisis. Microalgae are one of the non-food based plants that are at the forefront of renewable energy and provide a perpetual solution to environmental and climate problems. Apart from eradicating food scarcity and competition with other terrestrial crops, algae can produce value added product such as bio-fertilizer, bio-protein and non-toxic biodiesel without emitting carbon dioxide (CO_2) into the atmosphere[4].

Compared with fossil fuel, the rate of commercialization of biofuel is very low due to the high cost of production in biomass harvesting, lipid extraction and transesterification to produce biodiesel. The concept of increasing viability of this technology has received greater attention among researchers. One such viable option of reducing the high cost of algae-based biofuel is by substituting synthetic medium with natural media such as wastewater. Cultivation of microalgae in wastewater directly eliminates the need for growth medium. Wastewater, be it agricultural, industrial or municipal has been known to be composed of high nutrient (mainly nitrogen and phosphorus)[5]. In terms of nutrient strength, industrial wastewater contains higher nutrient than agricultural followed by the least which is municipal wastewater. The nitrogen and phosphorus content of wastewater are necessary source of food for algae growth[6]. In that case, when microalgae are cultivated in wastewater, it will perform the dual role of nutrient removal and production of biomass for energy generation, implying that biodiesel production from wastewater-grown algae is a viable option for the alleviation of global energy demand.

Therefore, considering the problem faced by biofuel industry, this review briefly discusses some of the major steps taken to lower the cost of biofuel production. This includes a selection of algae species with high lipid composition, challenges of harvesting, drying, lipid extraction and biodiesel conversion methods. The use of wastewater as a substitute for algae growth medium is on the increase. This review, therefore, proposed a schematic representation of utilizing Palm Oil Mill Effluent (POME) as an algal growth medium for biodiesel production.

FEEDSTOCK USED IN BIODIESEL PRODUCTION

High cost is one of the major problems with biodiesel production at commercial scale[2]. Studies on the various feedstock used for biodiesel production have shown that selection of low-cost feedstock can boost biodiesel production. These feed stocks are basically categorized into six (6) classes. These include (1) Virgin oil[7], (2) Waste vegetable oil, (3) Used frying fat such as yellow grease and brown grease[8], (4) Algae biomass which can grow in wastewater[9] and (5) Soap stock. Virgin oil is further categorized into edible and non-edible feedstock. Mostly, non-edible feedstock such as Jatropha and Castor oil are less discussed because of their rare application for biodiesel production. Waste oil, on the other hand, refers to the oil obtained from ready made cooking materials. It can also be sourced from animal fat. The advantage of waste oil is that it is cheaply available at commercial centers; however, if waste oil were utilized for transesterification purpose instead of producing biodiesel, the process leads to saponification (soap formation) as a result of the presence of impurities. Furthermore, to obtain good biodiesel using same feedstock, a pre-treatment step such as washing must be carried out for removal of impurities. In this study, only used frying oil, yellow grease, brown grease, soapstock and algae are discussed in detail due to their low cost.

Used frying oil can be described as oil obtained as a by-product after animal fat has been burnt in food processing service. Soapstock is a by-product of edible oil with a market value of about one-fifth of crude vegetable oil prices making it less expensive than crude vegetable oil[10,11]. Yellow grease is the combination of animal fat and used frying oil with free fatty acid <15% but when the free fatty acid content goes higher than 15% it is therefore referred to as brown grease. The feedstocks have been considered attractive due to their low cost. However, the fact that they contained a high amount of free fatty acid, that limit their use for transesterification reaction to produce biodiesel. Biodiesel production with oil of high content of free fatty acid is quite difficult especially when it involved the use of sodium hydroxide as catalyst. The use of microalgae have for a long time proved less expensive and affordable feedstock with a low amount of free fatty acid that can be transesterified with either acid or base for biodiesel production.

OVERVIEW OF MICROALGAE STRAINS WITH REFERENCE TO DESIRABLE CHARACTERISTICS OF BIODIESEL PRODUCTION

Microalgae are unicellular microscopic algae typically found in freshwater and marine systems[12]. Lee defined microphytes as large and diverse group of eukaryotes or prokaryotes that are polyphyletic, meaning derived from different ancestral lineage[13]. The prokaryotic algae are the cyanobacteria that resemble typical bacteria. The three most important class of eukaryotic algae are basically diatoms (Bacillariophyceae), green algae (Chlorophyceae) and the golden algae (Chrysophyceae)[14]. For biodiesel production, green algae are usually the most exploited. This is because of their ability to perform photosynthesis to produce a large amount of biomass. The biomass of well-grown algae consists of about 50-75% oil content[15,16]. Depending on species, most species of microalgae contain oil content greater than 80% dry weight of biomass[17,18].

Selection of the suitable wild type of microalgae for biodiesel production presents the first line of action to be considered when choosing a strain. The major desirable features of microalgae for biodiesel production involved the rate of growth of algal strains as determined by the amount of biomass produced per unit time. In most cases, the quantity of biomass is determined by the amount of lipid produced. For example 3.97 mg L^{-1} dry biomass of *C. pyrenoidosa* produces about 183.71 mg L^{-1} day^{-1} of lipid[1]. Secondly, the ability to tolerate fluctuation of environmental factors such as temperature, light intensity, nutrient load and CO_2 consumption level. Thirdly, morphology, especially as the type of cell covering has an immense impact on shear tolerance which seems to be of high potential when grown in a closed photobioreactor system. *Nannochloropsis* and *Chlorella* spp. are typical examples of shear resistant algal strains[19]. Fourthly, the ability to compete actively with other contaminating microalgae and bacteria. Fifthly, the ease of harvesting process, because some species of microalgae are self-settling after growth leaving the biomass at the bottom for easy collection and finally, selection of algal species with little or no cell wall such as *Dunaliella* or motile cells of *Haematococcus* are easier to extract compared to thick cell-walled coccid spp. such as *Nannochloropsis*, *Chlorella*, *Scenedesmus* and *Haematococcus* where cell wall needs to be ruptured to achieved efficient lipid extraction. The balance of the above mentioned desirable features are therefore considered a great challenge to researchers in biodiesel industry and if it can be archive it will reduce the cost of biofuel production cycle. Today, the scope of reducing the cost of algae production has been broadened more than the previous scope.

Harvesting of algal biomass: One of the primary challenges faced by microalgae production is the separation of algae biomass from the growth medium. The process involved the selection of suitable method that conformed to the following characteristics which are energy efficiency, fast biomass recovery and cheap and cost effect method. Algae harvesting method is divided into two which are (1) Those that involve removing water directly from algal cell culture and (2) Those that require clustering of algal masses in order to facilitate water removal. The former consist of centrifugation and membrane filtration which are void of using chemicals, while the latter are flocculation, flotation and gravity sedimentation which are attributed to the cost of control and motor. The choice of suitable method depends on algal species, algal density and the potential product that will be produced[20]. In the case of biodiesel production, 90% of most researchers prefer centrifugation as suitable harvesting method due to its quick cell separation[21], high harvesting efficiency[22] and high applicability for high-value products[23]. For example, in a pilot scale research involving treatment of piggery wastewater, the dewatering methods tested such as membrane filtration, chemical flocculation and centrifugation was reported to be unsatisfactory[24]. Furthermore, the disadvantages associated with such methods, with the exception of centrifugation are specific in function (e.g., specific for large algae density), potential contamination due to the addition of flocculants, energy cost, time consuming[21] and partial separation of water from biomass. Furthermore, it was reported that the use of partially dried biomass for transesterification has presented some issues with arresting the release of lipid at the point of cell lyses[24].

Drying of algal biomass: Another way of improving energy efficiency is by drying algal biomass. Various techniques are employed for drying moisture content of algal biomass, including freeze drying, thermal drying and sun drying. The choice of suitable drying method is one of the problems confronting transesterification process[25]. The most applicable drying method is sun drying. Although, sun drying requires longer time duration, it does not affect the chemical composition of algal biomass[26]. Spray and thermal drying, despite their rapid water removal are attributed with the common disadvantages of affecting pigment composition of algal biomass due to high-temperature exposure of about 60°C and expensive to practice[27,28]. Freeze drying method provides efficient and rapid water removal with zero potential effect on the lipid composition of algal biomass. Thus when a better drying method is chosen it can go a long way in reducing the feedstock (fuel) cost of biofuel production.

LIPID EXTRACTION AND ITS MECHANISM IN MICROALGAE

Microalgae species consist of the cell wall of varying composition and strength. When this cell wall is properly ruptured, the maximum amount of lipid can be obtained. In order to improved efficiency of lipid extraction protocol, the choice of suitable lipid extraction method has to be considered. There are various methods of lipid extraction which are Bligh and Dyer method (Chloroform-methanol), supercritical fluid extraction, Ultrasonication or magnetic stirrer-assisted method and Soxhlet extraction method (chloroform, methanol, ethanol and hexane). Application of each method depends on the type of algal species, cultivating medium and lipid content. The D'Oca reported that three lipid extraction methods namely sonication, Soxhlet extraction and Bligh and Dyer methods were applied to lipid extraction from *Chlorella pyrenoidosa* and all the three extraction methods were efficient with sonication and magnetic stirrer as the best method[29]. In addition, different extraction solvent and time taken can change the efficiency of the extraction method[30]. In another separate study, it was confirmed that sonicated-assisted extracted methods were the most efficient, followed by microwave and osmotic shock assisted accounting for about 0.19 and 0.17 g, respectively, with autoclaving being the poorest method of all and therefore, recorded with the lowest efficiency of 0.12 g in *Chlorella* spp.[31]. The previous study carried out using beat-biting method was also reported to extract higher lipid content from *Botryococcus braunii* than sonicated-assisted method[32]. A similar scenario was also reported by Lee *et al.*[33]. When microwave oven method was applied and efficient lipid was obtained from biomass of *Chlorella*, *Scenedesmus* and *Botryococcus* spp[33].

The mechanism of lipid extraction is complex involving energy generation. In Fig. 1, the lipid is synthesized in chloroplast and pooled up in the cytoplasm. The procedure of lipid extraction begins with cell disruption using sonication or its related alternatives. The rupturing of algal cell wall gives solvent ability to penetrate and interact with lipid to form Solvent Lipid Complex (SLP). The SLP diffused across algal cytoplasm to the surrounding environment where solvent removal and lipid purification can be done with 5% distilled water[29]. Good and quality lipid extract can be obtained by improving the efficiency of the method via sonication at 4°C and adequate timing for lipid and solvent interaction.

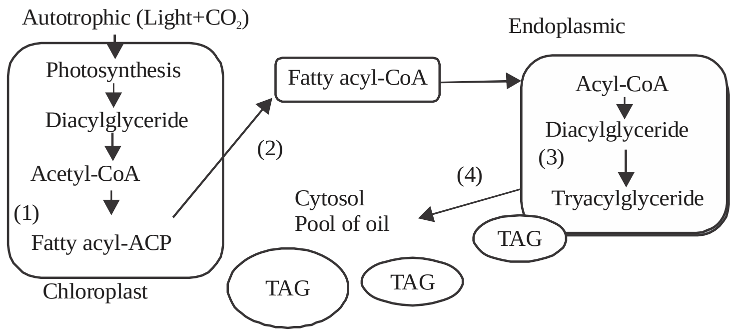

Fig. 1: Basic overview of the pathway of carbon capture and lipid biosynthesis. Precursor's fatty acids are synthesized *de novo* in the chloroplast, using carbon fixed during photosynthesis, the exact free fatty acids are exported from the chloroplast and then converted to TAGs in the Endoplasmic Reticulum (ER), where they formed a pool of oil bodies in the cytosol. (1) Acetyl-CoA carboxylase (ACCase), (2) Fatty acid thioesterases and acyl-CoA synthetases, (3) TAG biosynthesis enzymes, including acyl-CoA: diacylglycerol acyltransferase (DGAT)[35]

Table 1: Oil composition of different microalgae[17,34]

Species of microalgae	Oil composition (dry weight%)
Anabaena cylindrica	4-7
Botryococcus braunii	25-75
Chlorella spp.	28-32
Chlamydomonas reinhardtii	21
Crypthecodinium cohnii	20
Cylindrotheca spp.	16-37
Dunaliella spp.	6-23
Euglena gracilis	14-20
Isochrysis sp.	25-33
Monallanthus salina	>20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-64
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
Scenedesmus spp.	1.9-40
Schizochytrium sp.	50-77
Spirogyra sp.	11-28
Synechococcus sp.	11
Tetraselmis spp.	3-23

SURVEY OF MICROALGAE LIPID CONTENT AND MECHANISM GOVERNING LIPID PRODUCTION

Lipids are naturally occurring biochemical compounds that are insoluble in water due to hydrophobic hydrocarbon chain but soluble in organic solvent[16]. They are categorized into 7 respective classes which are glycerolipid, glycerophospholipid, saccharolipid, polyketide and fatty acid. Triacylglycerol (fatty acid), which is found in microalgae is the main source of fuel because when metabolized it generates a large amount of energy. Algae naturally produce lipid at a higher percentage than that of other arable crop plants such as sunflower and reap oil. However, the increase in the amount of lipid induction in microalgae can be achieved via cultivation in nitrate and light deficient condition[33]. Hu *et al.*[26] have reported that algae neutralized the effect of environmental constraint by producing lipid. It is currently acknowledged that algae produce lipid higher than engineered plants due to larger energy storage capacity. Becker explained that different species of microalgae account for different lipid content (Table 1)[34].

Mechanism of lipid biosynthesis is complex involving activities of several enzymes. Among the enzymes involved, acetyl-CoA carboxylase (ACCase) is the key enzyme catalyzing the ATP-dependent formation of malonyl-CoA from acetyl-CoA and bicarbonate. The ACCase is categorized into two, which are multi-subunit and multi-functional enzymes. The function of the former is that it catalyzed the bulk production of lipid more than the latter one[33]. Further biosynthesis of lipid in microalgae is highlighted in Fig. 1.

CHOICE OF SUITABLE METHOD FOR BIODIESEL CONVERSION

Biodiesel has emerged as an alternative source of renewable energy with efficiency equal to if not higher than the petrol fuel. It's non-toxic and biodegradable characteristic gave it competitive advantages over fossil-based fuel in the fuel market. Since the diesel is made from biological oil with low sulfur and carbon content, it has the potential of bridging the gap between two issues of environmental management and energy crisis[17]. Biodiesel can be used 100% in its pure form but may require certain modification to avoid engine performance problem. To some extent, it can be blended with petrol diesel and used as single diesel called BL[7]. The blends are coded with sign "B" which corresponds to the percentage of biodiesel blended with conventional petrol fuel. There are four different categories of biodiesel blend (B factor) which are B100, B20, B5, B2. Power and fuel economy using biodiesel is practically identical to petroleum diesel fuel and year-round operation can be achieved by blending with diesel fuel.

Biodiesel is produced by a single step chemical reaction known as Transesterification Reaction (TR)[36]. The process involved the reaction of three molecules of triglyceride with alcohol to produce methyl ester (biodiesel) and glycerol. During TR process, the common alcohols used are methanol, propanol and butanol. Methanol is seen as the best alcohol due to its less toxicity and low-cost application. The TR reactions are categorizing into three types which are basic transesterification (BTR), acid transesterification (ATR) and enzyme catalyzed transesterification (ETR)[37]. The BTR has a wider scope of application in biodiesel production because it is inexpensive and affordable in the market. The fact that some fatty acid from microalgae exists as Free Fatty Acid (FFA) limits the application of BTR. In order to improve the method, pre-treatment of the algal oil must be carried out. A Strong acid such as sulphuric acid and hydrochloric acid are often used in order to facilitate the reaction and prevent the potential of soap formation[38]. Due to the high rate of reaction efficiency, BTR is estimated to be about 400 times faster than ATR[38].

The application of ATR in the conversion of oil to biodiesel has attracted much attention due to its ability to catalyze FFA which is a function that could not be offered by BTR. In addition, ATR reaction provides a clear and easy avenue for separating oil from methanolic debris[39]. Some of the major challenges faced when using ATR are a slow reaction and contaminated the sample. The slow rate of reaction occurs as a result of high reaction temperature which decelerates the reaction process. Biodiesel obtained via ATR produced diesel that when used in cars cause corrosion in pipelines and other engine components[36].

Enzyme catalyzed transesterification reaction (ETR) on the other hand is regarded as a new technology with minimal literature report. The new technology is aimed at minimizing the constraint faced by application of ATR and BTR such as high temperature, the occurrence of saponification and purification of glycerol due to FFA. With the emergence of new technology, the economic feasibility of biodiesel production can be improved for the betterment of mankind worldwide[37]. Currently, one of the disadvantages of making ETR is that it is not cost-effective is due to issues with catalyst regeneration.

In most cases, the process of two-step lipid extraction and transesterification is associated with some issues such as low product yield, contaminated product due to many handling steps or even loss of the product. The two-step process can be coupled to form one step extraction/reaction process called Direct transesterification[40]. The major advantages of direct transesterification are that it is less time consuming[41], more efficient due to it essential one-step reaction process[42,43], production of high purity product (>99%) and high yield due to the use of an acid such as H_2SO_4 at 65°C. Direct transesterification has been shown to produce high amount of biodiesel (>60% FAME)[44].

POME AS AN EXCELLENT MEDIUM FOR CULTIVATING MICROALGAE

Global biodiesel production has faced challenges of high cost of production due to harvesting, lipid extraction and cultivation strategy. Cultivation protocol of microalgae forms an important point in upscaling biodiesel production. One problem that might interfere with upscaling the production is the source of nutrients for microalgae growth[45]. Nitrogen and phosphorus are the major nutrients required for algal growth. Jafari and his co-workers[46] has reported that, water pollution occur due to the presence of chemical substances channelled from agricultural, industrial and municipal site which severely alter the physical chemistry of the water. Based on recent study, it is however, noted that most industrial and municipal wastewater contains nitrate and phosphate at a certain proportion[47]. Since microalgae require nitrate and phosphate for growth, then they are suitable for cultivation in wastewater. Culturing microalgae in wastewater can actually not only reduce the cost of algal production but remove toxic pollutant from wastewater[48]. This is an important role that other conventional wastewater treatment systems could not offer, thus culturing microalgae in wastewater can serve the dual role of treating wastewater and using it as a source of nutrients for microalgae growth to produce biodiesel.

The POME was reported to provide sufficient nutrient necessary for algae growth[37,47,49]. Malaysia and Indonesia are the two countries faced with huge problems of POME generation from palm oil industry[50]. Despite the application of anaerobic digestion pond system by industries and related modifications such as the use of stirred tank reactor[51], single stage reactor[52], up-flow anaerobic sludge fixed film (UASFE)[53], modified anaerobic baffled bioreactor[54] and single stage stirred digester[55], nutrients are still not completely removed and for that reason, culturing microalgae in anaerobically digested effluent could provide next step treatment strategy. The characteristics of POME after anaerobic digestion method were summarized in Table 2. Heavy metals such as Cr, Cd, K and Mg are also available in POME and they form an important nutrient that can help microalgae photosynthesize[56]. Furthermore, dilution of wastewater before feeding microalgae inoculum can also offer a good method of reducing the intensity of dark colouration of POME for high photosynthetic activities[42]. The schematic representation of using POME as a medium for microalgae growth to produce biodiesel is shown in Fig. 2.

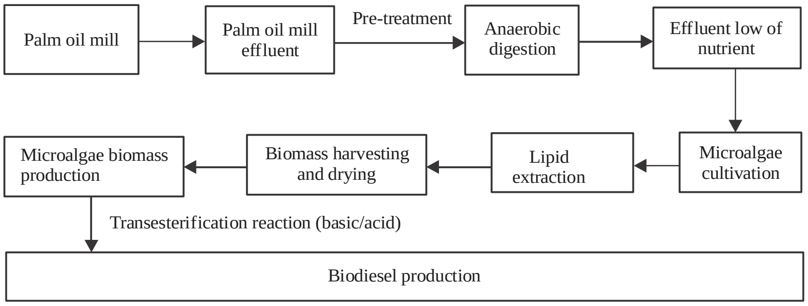

Fig. 2: Design of biodiesel production from POME grown microalgae

Table 2: Characteristic of POME after anaerobic pond digestion[35]

Parameters	Digested POME
pH	7-7.4
Oil and grease	10-430
BOD	110-1800
COD	340-19,680
Total solid	6090-18,400
Suspended solid	760-14,850
Volatile solid	2680-14,570
Total nitrogen	320-1070
Ammoniacal nitrogen	60-300

CONCLUSION

The continuous use of petroleum-based fuel has caused several environmental problems such as air pollution. The emission of carbon dioxide, nitrogen dioxide and carbon monoxide when burnt greatly affect habitat structure. Biofuel from microalgae is a renewable alternative source of energy that offers a better solution for a reduction in environmental pollution and meeting global energy crisis. This is due to its ability to utilize carbon dioxide from the environment while producing oxygen. Selection of a more efficient harvesting, drying, extraction and bioconversion methods and use of natural media present an important step in reducing the cost of biofuel production. In addition, for the economic and environmental benefit, the use of biofuel is proposed as the alternative source of energy far greater than fossil fuel.

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REFERENCES

1. Moghaddam, G., M. Amiri and J. Khodaei, 2012. Fuel energy saving and environmental benefits from a combined heat, cooling and power system driven by gas engine. Asian J. Applied Sci., 5: 266-278.
2. Pongpiachan, S., K.F. Ho and J. Cao, 2014. Effects of biomass and agricultural waste burnings on diurnal variation and vertical distribution of OC/EC in Hat-Yai city, Thailand. Asian J. Applied Sci., 7: 360-374.
3. Azad, A.K., M.G. Rasul, M.M.K. Khan and S.C. Sharma, 2014. Review of non-edible biofuel resources in Australia for second generation (2G) biofuel conversion. Proceedings of the 9th International Green Energy Conference, May 25-28, 2014, Tianjin, China, pp: 867-878.
4. Rawat, I., R.R. Kumar, T. Mutanda and F. Bux, 2011. Dual role of microalgae: Phytoremediation of domestic wastewater and biomass production for sustainable biofuels production. Applied Energy, 88: 3411-3424.
5. Hadi, N.S., 2010. The research on water quality of water area around drain outlets of Longwangzui sewage plant in China. Asian J. Applied Sci., 3: 166-185.
6. Chinnasamy, S., A. Bhatnagar, R.W. Hunt and K.C. Das, 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour. Technol., 101: 3097-3105.
7. Omidvarborna, H., A. Kumar and D.S. Kim, 2014. Characterization of particulate matter emitted from transit buses fueled with B20 in idle modes. J. Environ. Chem. Eng., 2: 2335-2342.
8. Leonard, C., 2007. Not a tiger, but maybe a chicken in your tank. Washington Post, January 3, 2007, http://www.washi ngtonpost.com/wp-dyn/content/article/2007/01/02/AR20070 10201057.html
9. Kiong, E., 2006. NZ firm makes bio-diesel from sewage in world first. NZ Herald, New Zealand, May 12, 2006, http://www.nzherald.co.nz/nz/news/article.cfm?c_id=1&objectid=10381404.
10. Haas, M.J., 2005. Improving the economics of biodiesel production through the use of low value lipids as feedstocks: Vegetable oil soapstock. Fuel Process. Technol., 86: 1087-1096.
11. Canakci, M. and H. Sanli, 2008. Biodiesel production from various feedstocks and their effects on the fuel properties. J. Ind. Microbiol. Biotechnol., 35: 431-441.
12. Thurman, H.V. and A.E. Burton, 1997. Introductory Oceanography. Prentice Hall, New Jersey.
13. Lee, R.E., 2008. Phycology. 4th Edn., Cambridge University Press, Cambridge, UK., ISBN-13: 9780521682770, Pages: 560.
14. Demirbas, A. and M.F. Demirbas, 2011. Importance of algae oil as a source of biodiesel. Energy Convers. Manage., 52: 163-170.
15. Widjaja, A., 2009. Lipid production from microalgae as a promising candidate for biodiesel production. Makara Teknologi, 13: 47-51.
16. Greenwell, H.C., L.M.L. Laurens, R.J. Shields, R.W. Lovitt and K.J. Flynn, 2010. Placing microalgae on the biofuels priority list: A review of the technological challenges. J. R. Soc. Interface, 7: 703-726.
17. Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv., 25: 294-306.
18. Ma, F. and M.A. Hanna, 1999. Biodiesel production: A review. Bioresour. Technol., 70: 1-15.
19. Mata, M.T., A.A. Martins and N.S. Caetano, 2010. Microalgae for biodiesel production and other applications: A review. Renewable Sustainable Energy Rev., 14: 217-232.

20. Olaizola, M., 2003. Commercial development of microalgal biotechnology: From the test tube to the marketplace. Biomol. Eng., 20: 459-466.
21. Grima, E.M., E.H. Belarbi, F.G.A. Fernandez, A.R. Medina and Y. Chisti, 2003. Recovery of microalgal biomass and metabolites: Process options and economics. Biotechnol. Adv., 20: 491-515.
22. Benemann, J.R. and W.J. Oswald, 1996. Systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass. Final Report to the U.S. Department of Energy, Department of Civil Engineering, California University Berkeley, CA.
23. Heasman, M., J. Diemar, W. O'Connor, T. Sushames and L. Foulkes, 2000. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs-a summary. Aquac. Res., 31: 637-659.
24. Naghdi, F.G., S.R. Thomas-Hall, R. Durairatnam, S. Pratt and P.M. Schenk, 2014. Comparative effects of biomass pre-treatments for direct and indirect transesterification to enhance microalgal lipid recovery. Front. Energy Res., Vol. 2. 10.3389/fenrg.2014.00057
25. Uduman, N., Y. Qi, M.K. Danquah, G.M. Forde and A. Hoadley, 2010. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. J. Renewable Sustainable Energy, Vol. 2. 10.1063/1.3294480
26. Hu, B., M. Min, W. Zhou, Z. Du and M. Mohr *et al*., 2012. Enhanced mixotrophic growth of microalgae *Chlorella* sp. on pretreated swine manure for simultaneous biofuel feedstock production and nutrient removal. Bioresour. Technol., 126: 71-79.
27. Yan, W., T.C. Acharjee, C.J. Coronella and V.R. Vasquez, 2009. Thermal pretreatment of lignocellulosic biomass. Environ. Progr. Sustain. Energy, 28: 435-440.
28. Rohloff, J., S. Dragland, R. Mordal and T.H. Iversen, 2005. Effect of harvest time and drying method on biomass production, essential oil yield and quality of peppermint (*Mentha× piperita* L.). J. Agric. Food Chem., 53: 4143-4148.
29. D'Oca, M.G.M., C.V. Viegas, J.S. Lemoes, E.K. Miyasaki, J.A. Moron-Villarreyes, E.G. Primel and P.C. Abreu, 2011. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. Biomass Bioenergy, 35: 1533-1538.
30. Halim, R., B. Gladman, M.K. Danquah and P.A. Webley, 2011. Oil extraction from microalgae for biodiesel production. Bioresour. Technol., 102: 178-185.
31. Prabakaran, P. and A.D. Ravindran, 2011. A comparative study on effective cell disruption methods for lipid extraction from microalgae. Lett. Applied Microbiol., 53: 150-154.
32. Lee, S.J., B.D. Yoon and H.M. Oh, 1998. Rapid method for the determination of lipid from the green alga *Botryococcus braunii*. Biotechnol. Tech., 12: 553-556.
33. Lee, J.Y., C. Yoo, S.Y. Jun, C.Y. Ahn and H.M. Oh, 2010. Comparison of several methods for effective lipid extraction from microalgae. Bioresour. Technol., 101: S75-S77.
34. Becker, E.W., 1994. Microalgae: Biotechnology and Microbiology. Cambridge University Press, Cambridge, UK., ISBN-13: 9780521350204, Pages: 293.
35. Li, Y., D. Han, K. Yoon, S. Zhou, M. Sommerfeld and Q. Hu, 2013. Molecular and Cellular Mechanisms for Lipid Synthesis and Accumulation in Microalgae: Biotechnological Implications. In: Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Richmond, A. and Q. Hu (Eds.). 2nd Edn., Wiley, New York, ISBN: 9781118567180, pp: 545-565.
36. Harwood, J.L., 1988. Fatty acid metabolism. Annu. Rev. Plant Physiol. Mol. Biol., 39: 101-138.
37. Lam, M.K. and K.T. Lee, 2011. Renewable and sustainable bioenergies production from Palm Oil Mill Effluent (POME): Win-win strategies toward better environmental protection. Biotechnol. Adv., 29: 124-141.
38. Fukuda, H., A. Kondo and H. Noda, 2001. Biodiesel fuel production by transesterification of oils. J. Biosci. Bioeng., 92: 405-416.
39. Teo, C.L., H. Jamaluddin, N.A.M. Zain and A. Idris, 2014. Biodiesel production via lipase catalysed transesterification of microalgae lipids from *Tetraselmis* sp. Renew. Energy, 68: 1-5.
40. Lewis, T., P.D. Nichols and T.A. McMeekin, 2000. Evaluation of extraction methods for recovery of fatty acids from lipid-producing micro heterotrophs. J. Microbiol. Methods, 43: 107-116.
41. Johnson, M.B. and Z. Wen, 2009. Production of biodiesel fuel from the microalgae *Schizochytrium limacinum* by direct transesterification of algal biomass. Energy Fuels, 23: 5179-5183.
42. Vicente, G., L.F. Bautista, R. Rodriguez, F.J. Gutierrez and I. Sadaba *et al*., 2009. Biodiesel production from biomass of an oleaginous fungus. Biochem. Eng. J., 48: 22-27.
43. Saputera, Muliansyah and T.A. Atikah, 2014. Extraction and transesterification of *Croton tiglium* oil seeds from central kalimantan, indonesia as an alternative biodiesel raw materials. Asian J. Appllied Sci., 7: 140-149.
44. Wang, L., Y. Li, P. Chen, M. Min, Y. Chen, J. Zhou and R.R. Ruan, 2010. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. Bioresour. Technol., 101: 2623-2628.
45. Clarens, A.F., E.P. Resurreccion, M.A. White and L.M. Colosi, 2010. Environmental life cycle comparison of algae to other bioenergy feedstocks. Environ. Sci. Technol., 44: 1813-1819.
46. Jafari, A., H. Mirhossaini, B. Kamareii and S. Dehestani, 2008. Physicochemical analysis of drinking water in kohdasht city lorestan, Iran. Asian J. Applied Sci., 1: 87-92.

47. Hadiyanto and M.M. Azimatun Nur, 2014. Lipid extraction of microalgae *Chlorella* sp. cultivated in Palm Oil Mill Effluent (POME) medium. World Applied Sci. J., 31: 959-967.
48. Hosseini, S.A., 2010. The effect of marine algae on radioactivity adsorption of iodine in drinking water. Asian J. Applied Sci., 3: 317-321.
49. Nwuche, C.O., D.C. Ekpo, C.N. Eze, H. Aoyagi and J.C. Ogbonna, 2014. Use of palm oil mill effluent as medium for cultivation of *Chlorella sorokiniana*. Br. Biotechnol. J., 4: 305-316.
50. Abdullah, N., A. Yuzir, T.P. Curtis, A. Yahya and Z. Ujang, 2013. Characterization of aerobic granular sludge treating high strength agro-based wastewater at different volumetric loadings. Bioresour. Technol., 127: 181-187.
51. Choorit, W. and P. Wisarnwan, 2007. Effect of temperature on the anaerobic digestion of palm oil mill effluent. Electron. J. Biotechnol., 10: 366-385.
52. Yacob, S., Y. Shirai, M.A. Hassan, M. Wakisaka and S. Subash, 2006. Start-up operation of semi-commercial closed anaerobic digester for palm oil mill effluent treatment. Process Biochem., 41: 962-964.
53. Najafpour, G.D., A.A.L. Zinatizadeh, A.R. Mohamed, M.H. Isa and H. Nasrollahzadeh, 2006. High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor. Process Biochem., 41: 370-379.
54. Faisal, M. and H. Unno, 2001. Kinetic analysis of palm oil mill wastewater treatment by a modified anaerobic baffled reactor. Biochem. Eng. J., 9: 25-31.
55. Borja, R. and C.J. Banks, 1994. Anaerobic digestion of palm oil mill effluent using an up-flow anaerobic sludge blanket reactor. Biomass Bioenergy, 6: 381-389.
56. Onalo, J.I., H.M.M. Peralta and N.M. Sunar, 2014. Growth of freshwater microalga, *Botryococcus* sp. in heavy metal contaminated industrial wastewater. J. Sci. Technol., 6: 29-40.