Review

BIOFUEL: ALTERNATIVES AND SOLUTIONS

Ahed J Alkhatib, Mustafa A Al- Shehabat

Jordan University of Science and Technology, Jordan

Submitted on: 10.08.2015 Revised On: 16.08.2015 Accepted on: 18.08.2015

ABSTRACT:

Conventional fuels are subjecting to continuous loss due to increased population and increased consumption. At the same time, conventional fuels have been associated with environmental hazards. Due to the possibility of lacking conventional fuel sources, searching for other alternatives and solutions to cope with such deficiencies is a good approach.Scientists have demonstrated the production of ethanol and biodiesel from yeast and bacteria. In this review such options have been discussed.

KEYWORDS: Biofuel,bacteria, yeast, ethanol**.**

Corresponding Author: Ahed J Alkhatib

E mail: ajalkhatib@just.edu.jo

Indian Research Journal of Pharmacy and Science; 6(2015) 225-234;
Journal home page: https://www.irjps.in

INTRODUCTION

Biofuel

Biofuels are considered a potential alternative to the use of fossil fuels. They have various advantages including participation to the decreased greenhouse gas emission and they do not affect the agricultural land1.

According to Pamela et al2, advanced biofuels, can be produced by microorganisms, have similar properties to petroleum-based fuels, and can compete to the existing transportation infrastructure. Producing biofuels in large quantities needs to engineer the microorganism's metabolism.

It has been estimated that about 85% of global fuel reservoirs are used only for combustion purposes. Furthermore, the demand for energy is increasing, and the alternatives for renewable energy are varied and important o both global and local scale. Although appropriate technologies exist to cope with renewable electricity, but the prime challenge is the sustainability of fuel production3.

The conversion of biomass into transportation fuel is considered a good alternative compared to conventionalfuel. Such renewable fuels are called as biofuels. It has been shown that the increased production of biofuels and their use for transportation decrease the use of oil. If there are significant biofuel sources, then there will be a safe and rich future; in addition to having other significant roles indecreasing global warming4.

Studies have shown that increased growth of population has been associated with increased requirements for both food and energy production5-7.

The production of ethanol as a biofuel

The fuel crisis has attracted the attention to find out appropriate strategies to cope with oil shortage since 170s. Great attention was directed to biofuel sources, particularly, ethanol.Nowadays, the production of ethanol has become economically feasible to the degree it competes with standard diesel. Being a renewable resource, ethanol production becomes more attractive[8].

Ethanol is usually produced through two ways: synthetically from materials of petrochemical origin such as (crude oil, gas or coal); and/or biologically through sugar fermentation (bioethanol). It has been estimated that 93% of global ethanol was produced by microbial fermentation[9].

Yeast for ethanol production

Yeasts are widely used to produce ethanol, which can be up to 18% the product of the fermentation broth of S. cerevisiae. The same yeasts are the most preferred microorganisms for the production of ethyl alcohol fermentation[10].

Yeasts that accumulate ethanol, sometimes in the presence of oxygen, are called Crabtree-positive yeasts. Crabtree-negative yeasts, work in a contracting mode andbreakdown sugar to carbon dioxide aerobically[11].

Lactic acid bacteria (LAB)

LAB is usually involved in various fermentationprocesses at industrial level. LAB is associated by their commonphysiological and metabolic characteristics. They can be found in lactic acidcontaining products and in decomposing plants, producing lactic acid as themajor metabolic product of carbohydrate fermentation. Their specialcharacteristic to show high tolerance to low pH range makes them differentfrom other species of bacteria[13].

Production of ethanol by *Zymomonasmobilis*

Zymomonasmobilis is considered from a microbiology point of view as a Gram negative, facultatively anaerobic bacterium.It has been reported to be employed in ethanol production in several beverages[13].*Z. mobilis*can grow and fermentquickly[14].*Z. mobilis*has been involved in optimal production of ethanol from different carbon sources such as hexoses and pentoses includingglucose and xylose through the Entner-Doudoroff pathway[15, 16].

Z. mobilis has been considered as an organism of particular interest due to the consideration that oxidative phosphorylation mechanisms are not involved[17]. The process of ethanol fermentation implies the consumption of pentose and hexose sugars for the biosynthesis of ethanol under anaerobic conditions. It has been observed that the byproducts from aerobic growth including acetate andlactate accumulate in the growth medium decrease ethanol production as compared to anaerobic conditions[18].

According to the study of Seoet al[19], *Z. mobilis* has unique properties such as being tolerant to ethanol byproducts resulting from fermentation; it has a high sugar uptake rate, high ethanol production rate, and tolerance to high concentrations of ethanol (>16%, v/v).

It has been shown that the strains of*Z. mobilis*are involved in the fermentation processes helping in brewing and beer making. The bacteria can produce almost 1.9 mol of ethanol for every 1 mol of glucose present[20].*Z. mobilis* prefers a pathway in metabolism which serves both the overall yield of the microbial products as well as employing a dominant pathway for the production of ethanol by NADH-dependent activation of alcohol dehydrogenase (Figure 1), indicating that ATP hydrolysis does not have a central role in the production of ethanol[21].

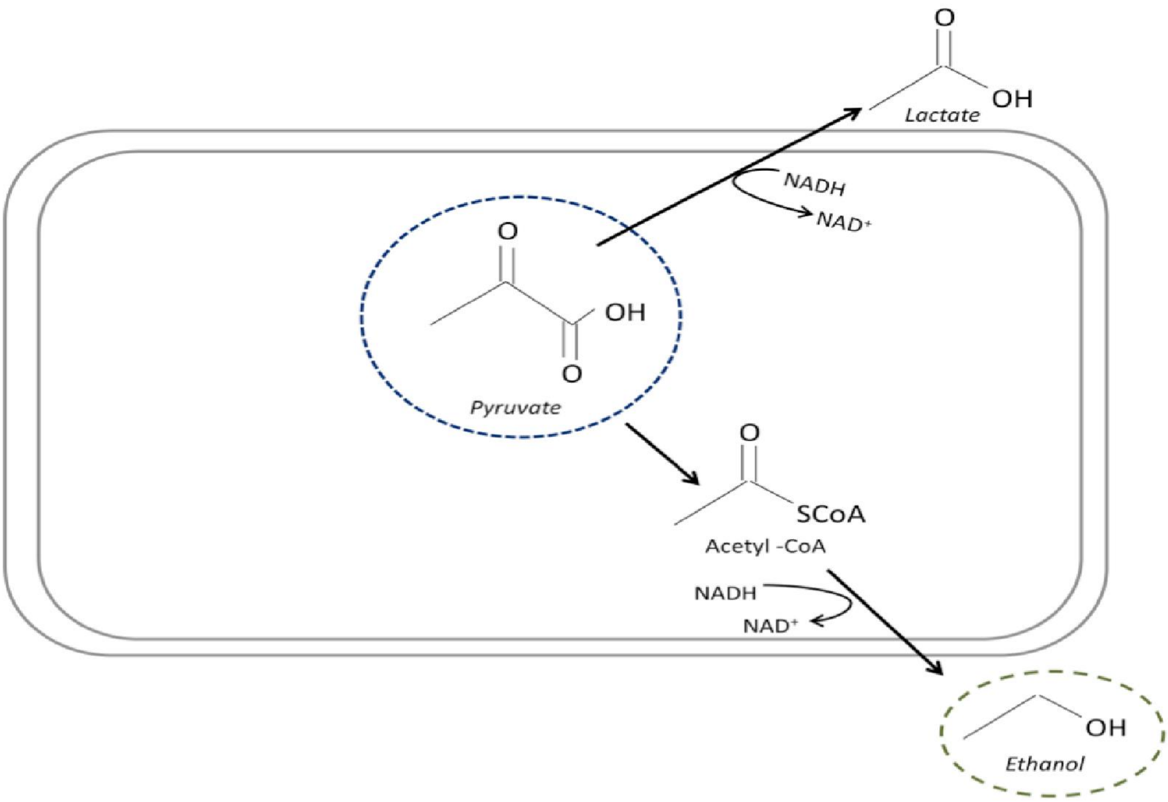

Figure 1: After initial glycolysis, at the branched metabolic path from pyruvate, Zymomonas. mobilis favors an NADH- dependent ethanol production cycle, Also, the biosynthesis of lactate, a by-product in Z. mobilis ethanol fermentation, is NADH-dependent.

Ethanol production by Klebsiella

Klebsiellaoxytoca was successfully engineered for industrial productionof ethanol with yields around 45 g/ L after 2 days fromthe initiation of fermentation mix exposure. Byintegrating an operon termed PET (production ofethanol) into the genome. This operon consists of theaforementioned adh II and pdc genes, under thecontrol of the Plac promoter, and has since been testedin the metabolizing of paper wastes and cellobiose toethanol, while also demonstrating hyper-resistancetowards the fermentation product, ethanol[22].

Production of biodiesel by bacteria

The present work was aiming to exploit bacteria for biodiesel production, twentytwo bacterial isolates were screened for lipid content by Sudan black B stainingmethod, among those three isolates is lipid rich viz, F. oryzihabitans, P. aeruginosaand

Morococcus sp. which have been taken for lipid extraction by Bligh and dyermethod and thin layer chromatography for determination of fatty acid, whichrevealed that the amount of lipid was highest in Morococcus sp. (0.68mL/100mL)of Lauric acid (C12). Optimization of C/N ratio the amount of lipid was rangedhighest in Morococcus sp. i.e. carbon (0.70mL/100mL) of palmitic acid (C18),nitrogen (0.65mL/100mL) of arachidonic acid (C20) and at pH 7.0(1.96mL/100mL) of oleic acid (C18) and in strain improvement by mutagenesis F.oryzihabitans got improved and accumulate high lipid at 10 min (1.50mL/100mL)of linoleic acid (C16)[23].Biofuels are of rapidly growing interest for reasons of energy security, diversity and sustainability as well as for greenhouse gas mitigation[23]. Recently, with global shortage of fossil fuels, excessive increases in price of crude oil and increased environmental concerns have resulted in rapid growth in biodiesel production. In biodiesel production, the transesterification reaction which could be catalyzed either chemically or enzymatically, this novel approach might pave way for industrial production of biodiesel equivalents from renewable resources by employing engineered micro-organisms[23]. Biodiesel refers to a vegetable oil or animal fat based diesel fuel consisting of long chain alkyl (methyl, ethyl or propyl) esters which is typically made by chemically reacting lipids with an alcohol producing fatty acid ester[24]. Not all microorganisms can be considered as abundant sources of oils and fat, those microorganisms that do produce a high content of lipid are termed as oleaginous. The lipid which accumulates in oleaginous microorganisms is mainly triacylglycerol[25] and these microorganisms have been considered as an alternative to agricultural and animal oil and fat sources[26]. Biodiesel production using microbial lipids which is named as single cell oils (SCO) has attracted great attention in the whole world; there are various kinds of microorganisms that accumulate lipids such as Bacteria, Fungi and Algae[27], the regulation mechanism of oil accumulation in these microorganism and approach of making microbial diesel is economically competitive[26]. The genus like Lipomyces, Yarrowia, Cryptococcus, Rhodosporidium[28] are known to accumulate between 40% to 70% of their biomass as lipids, the moulds of Zygomycetes like Mortierella and Cunninghamella also possess the ability of lipid accumulation[29] and the major fatty acids present in single cell oil are oleic acid, linoleic acid30, palmitoleic acid, arachidonic acid, palmitic acid and stearic acid31.

Microbial fuel cells

Rabaeyet al32conducted a study taking into consideration that the possibility of using microbial fuel cells has been shown to have great hope as a sustainable biotechnological solution to cope with energy requirements. Due to deficient knowledge of microbial ecology of these systems, the efficacy improvement of fuel cells is limited. The study purposed to clarifyif a bacterial community can be involved in a microbial fuel cell to bring about higher power output, and to recognize species responsible for the electricity generation. Results indicated that bacterial fuel cell was able to increase the output from an initial level of 0.6 W m−2 of electrode surface to a maximal level of 4.31 W m−2 (664 mV, 30.9 mA) when plain graphite electrodes were used. Bacterial community was made of facultative anaerobic bacteria, such as *Alcaligenesfaecalis* and *Enterococcus gallinarum*, which can produce hydrogen.*Pseudomonas aeruginosa* and other *Pseudomonas* species were also included. Electrochemicalactivity was due to excreted redox mediators such aspyocyanin excreted by *P. aeruginosa*.

Biological fuel cells are recognized as a green energy technology with high potential32.In a microbial fuel cell, electrons produced by bacteria are attracted towardan electrode (anode). The electrons,then, will be conductedover a resistance into a cathode which implies the conversion of bacterial energy to electrical energy33.Twobacteria have been reported toexhibit high coulombic efficiency: Geobactersulfurreducens and Rhodoferaxferrireducens which have the capacity of transferring the majority of the electrons gained from the carbon sources acetate and glucose, respectively, to the electrode34, 35.These studies indicated that there was high coulombic efficiency, implying that there was high electron transfer efficiency.

Biofuel-Producing Environmental Bacteria

Escobar-Nin˜oetal36conducted their study taking into consideration the rapid consumption of conventional fuels. According to this context, there is a need to adopt other choices that are characterized by being cheap to create sustainable fuels including biodiesel. These choices depend on lipases that have several properties such as hydrolytic and esterification reactions; and transesterification reactions to be utilized in the production of biodiesel. The challenge involved is how to choose the lipases that have the ability to carry out transesterification reactions because there are very little biodiesel producing lipases. The research team reported the isolation of 1,016 lipolytic microorganisms through the use of a qualitative plate assay. In a second step, lipolytic bacteria were analyzed using a colorimetric assay to determine the transesterification activity. It has been found that biofuel production was estimated to be higher than 80% at benchtop scale. Furthermore, the bacterial-derived biofuel was shown to be of good quality and can be used directly in engines36.

Biofuel economic aspects

Several factors have been reported to participate for increased requirements for agricultural land among which are increased population, dietary changes and the use of biofuels37. In another study, it has been pointed out that increasing use of crop-based biofuel is considered an important factor putting great requirement on food production and its requisite resources38.

In a recent study by Medipallyet al39, emphasis has been put on alternatives for energy since there is an energy crisis on the global level as well as increased greenhouse gas emissions. In this context, researchers focused on renewable energy sources. Biofuel resulting from microalgae has been shown to be the main renewable energy source for sustainable development. Algae-based biofuels have several properties including technical and economic aspects such as viability, cost competitive, no additional lands are required, and minimal water is required.The researchers also pointed out a significant point in which microalgae biodiesel is not produced on commercial scale which may due to the low biomass concentration and costly downstream processes. Furthermore, it has been recommended various steps have to be taken to increase the production of microalgae biodiesel such as designing advanced photobioreactors, developing low cost technologies for biomass harvesting, drying, and oil extraction. It has also been recommended to enhance molecular strategies to overcome environmental stress conditions and to engineer metabolic pathways for high lipid production39.

The study of Tabatabaeiet al40 pointed to the increased usage of liquid biofuels including biodiesel, bioethanol, and jet fuel particularly in the transport industry. Furthermore, biodiesel is superior to fossil diesel from different points of view such as being biodegradable, nontoxic and it has lower emissions of greenhouse gases (GHG)41.

The importance of Microalgae biofuels come from the point that theyare parts of what is called the third generation type of biofuels in which biofuels have no disadvantages that were associated with the first and the second generations of biofuels[42]. It has been reported that the first generation biofuels were derived from variety of crop plants including soybean, corn, maize, and animal fats[42]. A debate about negative impacts on food security, global food markets, water scarcity, and deforestation was created[43, 44].

Furthermore, the second generation of biofuels was derived from nonedible oils such as Jatrophacurcas, Pongamiapinnata, andSimaroubaglauca, involvethe use of large areas of land that are no utilized for food production. It has also been shown that the production of biofuels in second generationis restricted by lacking appropriate technologies for commercial exploitation of wastes as source for biofuel generation[40].In the light of the above context, microalgae biofuel is considered a potential alternative source of energy to replace the fossil fuels.

It has been reported that microalgae has several species that are capable of producing hydrocarbons and lipids. These species include Botryococcusbraunii, Nannochloropsis sp., Dunaliellaprimolecta, Chlorella sp., and Crypthecodiniumcohnii. Furthermore, Botryococcusbraunii has been shown to produce a large number of hydrocarbons and other important compounds on commercial scale including carotenoids and polysaccharides[45-49].Other studies showed a high oil content up to 8% is produced by microalgae species[48, 50, 51].

To show the economicimportance of biofuel produced by microalgae, Table 1 showed a comparison between the oil yield, production, and biodiesel productivity of microalgae with some other biofuel feedstock.

Table 1: Comparison of oil content, oil yield, and biodiesel productivity of microalgae with the first and the second generation biodiesel feedstock source (Medipally et al (2015).

Feedstock source	Oil content (% oil by wt. in biomass)	Oil yield (oil in litres/ha/year)	Biodiesel productivity (kg biodiesel/ha/year)
Oil palm	**36**	**5366**	**4747**
Maize	**44**	**172**	**152**
Physic nut	**41–59**	**741**	**656**
Caster	**48**	**1307**	**1156**
Microalgae with low oil content	**30**	**58, 700**	**51,927**
Microalgae with medium oil content	**50**	**97,800**	**86,515**
Microalgae with high oil content	**70**	**136,900**	**121,104**

According to Wanget al[52], the production of microalgae biofuel is considered to have commercially high potential because of its competitive cost compared with fossil based fuels, no need for extra lands, and it enhances the quality of air through the absorption of atmospheric CO2, and utilization of minimal water.

On the other hand, it has been reported that the production microalgae biofuels is still limited by several factors including low biomass production, low lipid content in the cells, and small size of the cells that makes harvesting process very costly. However, it is possible to overcome such limitations by technological of harvesting and drying, genetic engineering of metabolic pathways for high growth rate and increased lipid content. It has been shown that assessment of the production of microalgaebiofuel started since 1970, but due to technical and economic problems at that time, the idea was delayed[53]. The efforts of researchers in later studies indicated high potential in microalgae biofuel production[54].

REFERENCES

1- Thiago Bruce, Astria D. Ferrão-Gonzales, Yutaka Nakashimada, Yuta Matsumura, Fabiano Thompson, TomooSawabe (2015). Biofuel Innovation by Microbial Diversity.Springer Handbook of Marine Biotechnology, pp 1163-1180.

2- Pamela P. Peralta-Yahya, Fuzhong Zhang, Stephen B. delCardayre, Jay D. Keasling (2012). Microbial engineering for the production of advanced biofuels. Nature, 488, 320-329.

3- Patrik, R. Direct biological conversion of solar energy to volatile hydrocarbon fuels by engineered cyanobacteria, direct fuel, http://www.directfuel.eu/DFrenewFuel3.html(access on 5th October 2011).

4- Energy future coalition, energy future coalition, http://www.energyfuturecoalition.org/biofuels/index.html (access on 13th October 2011).

5- Ragauskas A. J., Williams C. K., Davison B. H., Britovsek G., Cairney J. , Eckert C. A., Frederick W. J. Jr., Hallett J. P., Leak D. J., Liotta C. L., Mielenz J. R., Murphy R., Templer R. and Tschaplinski T. (2006). The path forward for biofuels and biomaterials Science 311, pages 484-489.

6- Goldemberg J. (2007). Ethanol for a sustainable energy future Science 315, pages 808-810.

7- Nass L. L., Pereira P. A. A. and Ellis D. (2007). Biofuels in Brazil: An Overview Crop Science 47:6, page 2228-2237.

8- Hansen, A. C., Zhang, Q., and Lyne, P. W (2005). Ethanol-diesel fuel blends a review. Bioresource Technology. 96, 277-285.

9- Kádár Z. (2005) Biofuels from wastes and by-products: Production of hydrogen and ethanol by fermentation of paper sludge, corn stover and wood Doctoral Thesis, Budapest University of Technology and Economics, Hungary.

10- Lin, Y., and Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. Applied Microbiology Biotechnology .69, 627-642.

11- Piskur, J., Rozpedowska, E., Polakova, S., Merico, A., and Compagno, C. (2006) How did Saccharomyces evolve to become a good brewer? Trends Genet. 22, 183-186.

12- Gottshalk, G. (1986). Bacterial Metabolism. New york: Springer-Verlag, Edition 2. 214-224.

13- Nicholas Macedo , Christopher J. Brigham (2014). From beverages to biofuels: the journeys of ethanol-producing microorganisms.International journal of biotechnology for wellness industries, 3, 79-87.

14- Jeffries TW (2005). Ethanol fermentation on the move. Nat Biotechnol, 23: 40-1. http://dx.doi.org/10.1038/nbt0105-40.

15- Dunn KL, Rao CV (2014). Expression of a xylose-specific transporter improves ethanol production by metabolically engineered Zymomonasmobilis. ApplMicrobiolBiotechnol, 98(15): 6897-905. http://dx.doi.org/10.1007/s00253-014-5812-6.

16- Lee KY, Park JM, Kim TY, Yun H, Lee SY (2010). The genomescale metabolic network analysis of Zymomonasmobilis ZM4 explains physiological features and suggests ethanol and succinic acid production strategies. Microb Cell Fact, 9: 1-12. http://dx.doi.org/10.1186/1475-2859-9-94.

17- Ingram LO, Conway T, Clark DP, Sewell GW, Preston JF (1987). Genetic engineering of ethanol production in Escherichia coli. Appl Environ Microbiol, 53(10): 2420-5.

18- Yang S, Tschaplinski TJ, Engle NL, et al (2009). Transcriptomic and metabolomic profiling of Zymomonasmobilis during aerobic and anaerobic fermentations. BMC Genomics, 10(34). doi:10.1186/1471-2164-10-34.

19- Seo JS, Chong H, Park HS, et al (2005). The genome sequence of the ethanologenic bacterium Zymomonasmobilis. Nat Biotechnol, 23(1): 63-8.

20- Swings J, De Ley J (1977). The Biology of Zymomonas. Bacteriol Rev, 41(1): 1-46.

21- Kalnenieks U, Galinina N, Toma MM, Marjutina U (2002). Ethanol cycle in an ethanologenic bacterium. FEBS Lett, 522: 6-8.

22- Dien BS, Cotta MA, Jeffries TW (2003). Bacteria engineered for fuel ethanol production: current status. ApplMicrobiolBiotechnol, 63: 258-66.

23- P.Shruthi, T.Rajeshwari, B.R.Mrunalini, V.Girish, and S.T.Girisha (2014). Evaluation of Oleaginous Bacteria for Potential Biofuel. Int.J.Curr.Microbiol.App.Sci, 3(9): 47-57.

24- A. Demirbas¸, "Production of biodiesel from algae oils," Energy Sources Part A, vol. 31, no. 2, pp. 163–168, 2009.

25- Hull., (2010). Patterns of Accumulation. J. Mol. Catal. B Enz., 62: 9 14.

26- Xin, M., Jianming, Y., Xin, X., Lie, Z., Qingjuan, N., Mo X., (2009). Biodiesel production fromoleaginous microorganisms. Renew. Energy., 34: 1 5.

27- Joseph Gonsalves, B., (2006). Anassessment of the biofuels industryin India. United Nations Conferenceon Trade and Development. UNCTAD/DITC/TED/2006/6.

28- Li, Y., Zhao, Z.K., Bai, F. (2007). Highdensity cultivation of oleaginousyeast Rhodosporidiumtoruloides Y in fed batch culture. Enz. Microb. Technol., 41: 312 317.

29- Papanikolaou, S., Galiotou-Panayotou, M., Fakas, S., Aggelis, G. (2007). Lipid production by oleaginous Mucorales cultivated on renewable carbon sources. Eur. J. Lipid Sci. Technol., 109: 1060 1070.

30- Chen, H.C., Liu, T.M., (1997). Inoculum effects on the production of gammalinolenic acid by the shake culture of Cunninghamellaechinualata CCRC 31840. Enz. Microb. Technol., 21: 137 142.

31- Peng, X., Chen, H. (2008). Rapid estimation of single cell oil content of solid-state fermented mass using near-infrared spectroscopy. Biores. Technol., 299: 8869 8872.

32- KorneelRabaey, Nico Boon, Steven D. Siciliano, Marc Verhaege, Willy Verstraete (2004). Biofuel Cells Select for Microbial Consortia That Self-Mediate Electron Transfer. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 70 (9): 5373–5382.

33- Rao, J. R., G. J. Richter, F. Vonsturm, and E. Weidlich (1976). Performance of glucose electrodes and characteristics of different biofuel cell constructions. Bioelectrochem.Bioenerg. 3:139–150.

34- Bond, D. R., and D. R. Lovley (2003). Electricity production by Geobactersulfurreducens attached to electrodes. Appl. Environ. Microbiol. 69:1548–1555.

35- Bond, D. R., D. E. Holmes, L. M. Tender, and D. R. Lovley (2002). Electrode reducing microorganisms that harvest energy from marine sediments. Science 295:483–485.

36- Escobar-Nin˜o A, Luna C, Luna D, Marcos AT, Ca´novas D, et al. (2014) Selection and Characterization of Biofuel-Producing Environmental Bacteria Isolated from Vegetable Oil-Rich Wastes.

PLoS ONE 9(8): e104063. doi:10.1371/journal.pone.0104063.

37- Kyle F. Davis, Paolo D'Odorico, Maria Cristina Rulli (2014).Land grabbing: a preliminary quantification of economic impacts on rural livelihoods.Popul Environ, 36:180–192.

38- Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., et al. (2010). Food security: The challenge of feeding 9 billion people. Science, 327, 812–818.

39- Srikanth Reddy Medipally, Fatimah Md. Yusoff, Sanjoy Banerjee, and M. Shariff (2015). Review Article Microalgae as Sustainable Renewable Energy Feedstock for Biofuel Production. BioMed Research International, Article ID 519513, 13 pages.

40- M. Tabatabaei, M. Tohidfar, G. S. Jouzani, M. Safarnejad, and M. Pazouki, "Biodiesel production from genetically engineered microalgae: future of bioenergy in Iran," Renewable and Sustainable Energy Reviews, vol. 15, no. 4, pp. 1918–1927, 2011.

41- A. Demirbas,, "Production of biodiesel from algae oils," Energy Sources Part A, vol. 31, no. 2, pp. 163–168, 2009.

42- L. Brennan and P. Owende, "Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products," Renewable and Sustainable Energy Reviews, vol. 14, no. 2, pp. 557–577, 2010.

43- FAO, Sustainable Bioenergy: A Framework for Decision Makers, United Nations Energy, 2007.

44- FAO, The State of Food and Agriculture, Food and Agriculture Organization, New York, NY, USA, 2008

45- Y. Li and J. G. Qin, "Comparison of growth and lipid content in three Botryococcusbrauniistrains," Journal of Applied Phycology, vol. 17, no. 6, pp. 551–556, 2005.

46- J. G. Qin and Y. Li, "Optimization of the growth environment of Botryococcusbraunii Strain CHN 357," Journal of Freshwater Ecology, vol. 21, no. 1, pp. 169–176, 2006.

47- J. Qin, Bio-Hydrocarbons from Algae: Impacts of Temperature, Light and Salinity on Algae Growth, Rural Industries Research and Development Corporation, Australian Government, 2005.

48- Y. Chisti, "Biodiesel from microalgae," Biotechnology Advances, vol. 25, no. 3, pp. 294–306, 2007.

49- A. R. Rao, R. Sarada, V. Baskaran, and G. A. Ravishankar, "Antioxidant activity of Botryococcusbraunii extract elucidated in vitro models," Journal of Agricultural and Food Chemistry, vol. 54, no. 13, pp. 4593–4599, 2006.

50- E. E. Powell and G. A. Hill, "Economic assessment of an integrated bioethanol-biodiesel-microbial fuel cell facility utilizing yeast and photosynthetic algae," Chemical Engineering Research and Design, vol. 87, no. 9, pp. 1340–1348, 2009.

51- T. M. Mata, A. A. Martins, and N. S. Caetano, "Microalgae for biodiesel production and other applications: a review," Renewable and Sustainable Energy Reviews, vol. 14, no. 1, pp. 217–232, 2010.

52- B. Wang, Y. Li, N. Wu, and C. Q. Lan, "CO2 bio-mitigation using microalgae," Applied Microbiology and Biotechnology, vol. 79, no. 5, pp. 707–718, 2008.

53- W. C. W. Barkley, R. A. Lewin, and L. Cheng, Development of Microalgal Systems for the Production of Liquid Fuels, Edited by T. Sadler, Elsevier Applied Science, Villeneuve d'Ascq, France, 1987.

54- Y. Li, M. Horsman, N. Wu, C. Q. Lan, and N. Dubois-Calero, "Biofuels from microalgae," Biotechnology Progress, vol. 24, no. 4, pp. 815–820, 2008.

Conflict of Interest Reported: Nil; Source of Funding: None Reported