8-2014

Potential and Technological Advancement of Biofuels

Rajee Olaganathan
Embry-Riddle Aeronautical University, olaganar@erau.edu

Fabian Ko Qui Shen
University of Nevada, Las Vegas (Singapore Campus)

Lim Jun Shen
University of Nevada, Las Vegas (Singapore Campus)

Potential and Technological Advancement of Biofuels

Olaganathan Rajee[#1], Koh Qi Shen Fabian[#2], Lim Jun Shen[#3], Kwa Liang Hao[#4], Teo Cher Kiat Gabriel[#5], Tan Kok Ann[#6], Alistair Seetho[#7]

#1 University of Nevada, Las Vegas, William F. Harrah College of Hotel Administration, Singapore Campus, Singapore 188064 and School of Business (Bachelor of Business and Environmental Sciences) James Cook University, 600 Upper Thomson Road, Singapore Campus, Singapore 574421
Phone: +65 96513726.
#2 to 7 University of Nevada, Las Vegas, William F. Harrah College of HotelAdministration, Singapore Campus, Singapore 188064

ABSTRACT

This scientific paper examines the feasibility of biofuels as a solution to the world's energy crisis. It studies the development of the four different generations of biofuel that have been discerned over the years, determining the pros and cons of each. The paper further investigates the issues concerning each generation, and determines how their successors have solved and improved on those problems. In order to give the reader an unbiased perspective, the paper studies both general advantages and disadvantages that encompasses social, economic and environmental impacts. Research and development on the first two generations of biofuels have matured, and case studies have been used to allude to their current applications. The challenge of making third and fourth generation biofuels economically viable has also been highlighted due to their significant environmental and production benefits over the first two generations. The prospects of third and fourth generation biofuels have also been looked into to determine its outlook in the near future. If these next generation biofuels can garner enough support and become cost-competitive, mankind's quest for an alternative, renewable source of energy may finally be completed.

Key words: Biofuel, Energy, Sustainability, Biomass, Microalgae, Microbes, Renewable

***Corresponding Author:** Olaganathan Rajee[#1]

INTRODUCTION

Biofuels are energy sourcesobtained from renewable biological matter such ascorn kernels, stovers and ethanol,seasonal grasses, wooden biomass, algae, and diesel extracted from soy beans [1]. Since the early 2000s, a combination of surging energy demand and overexploitation of natural resources has resulted in a sharp, alarming depletion of fossil fuels. Moreover, experts have calculated that the earth's fossil fuel reserves will be entirely diminished in forty years' time if we continue to be over reliant on them [2]. Therefore, biofuels are widely lauded as a key solution to the world's energy crisis. Ironically, biofuels had long existed before the discovery of fossil fuels, however they were essentially disregarded due to the large reserves of gas, coal and crude oil available at the time; this in conjuncture the relatively low costs of fossil fuels made them highly desirable in the past, especially in developed countries. Today, the thinning supplies of these conventional fuels have sparked the search for an alternative, renewable answer to meeting our energy needs. Therefore, interest in the development of biofuels, and its uses have been reignited. Biofuels are viewed on a global scale to have immediate impact on plaguing concerns such as escalating oil prices, and emission of greenhouse gases.

While biofuels are commonly referred to as the 'fuel of the future', the idea was first conceptualized and developed by Rudolph Diesel in the late 19thcentury. Since its inception,biofuels have been used primarily in the automotive industry due to its potential to replace gasoline and diesel. However, advancements in the field have shown that aside from itsutility as a sustainable transport fuel, biofuel can also be used for manufacturing, cosmetic,pharmaceutical, heating and agricultural processes. Nevertheless, there have been on-going debates concerning the use of agro fuels over fossil fuels despite the former's obvious market potential and environmental benefits. The sceptics cited issues regarding the economic and environmental impacts of producing biofuel, raising questions over its feasibility and sustainability. In spite of this, continued research and development have been invested into the enhancement of biofuels, negating most of the abovementioned concerns. Additionally, governments, industry players and civil society have started several initiatives to develop criteria for sustainable production of biofuels [3].Through the combined efforts of meticulous administration, as well as the constant endeavor of improving biofuels, the merits of pursuing a future powered by biofuel will far outweigh any consequences eventually. This report aims to discuss the [I] impacts brought about by first and second generation biofuels and [II] the feasibility and practicability of third and fourth generation biofuel.

FIRST GENERATION BIOFUEL

It is important to note that the structure of biofuel does not change in between generations, but rather it is the source that varies. One of the key disadvantages of first generation biofuel is unsustainable production; it is mostly derived from agricultural resources such as starch, sugar, animal fats and vegetable oil, which in turn has an adverse effect on food production. Furthermore, it threatens our food supply and increases carbon emissions due to the intense growth requirements when planted outside traditional agricultural settings [4]. Oil is extracted from the crops in the form of biodiesel or bioethanol, which is obtained through fermentation [5].

Additionally there is an ongoing dispute over whether first generation biofuels actually reduce greenhouse gas and carbon emissions. This is because most of the biofuels that belong in this category release more carbon dioxideduring production as compared to theirfeedstocks, which results in a net energy loss. Nevertheless, the most contentious issue with regard tofirst generation biofuels has to be the matter of 'fuel vs food'. As demand for biofuels continue to rise, more and more food crops are being diverted away from the global food manufacturing industry, which has consequently resulted in soaring food prices across the globe [6]. Thegrowth and production of crops are affected by several factors which include agricultural prices, seasonal variation, market speculation, and extreme weather patterns. These issues pertaining to biofuel development indicate anunhealthy relationship between the use ofagriculture for food, and for energy, and is therefore considered and labelled as unfeasible in the long run and for the future.

SECOND GENERATION BIOFUEL

According to Ralph *et al*. (2009) [7], "Most of the problems associated with first generation biofuels can be addressed by the production of biofuels manufactured from agricultural and forest residues and from non-food crop feedstock." Second generation biofuels technologies have been developed due to the important limitations of first generation production. Unlike its first generation predecessor, this category of biofuels can be produced by using any component from the plant as feedstock, be it waste material or inedible parts. This helps to resolve the issue of food production, and reduces waste sent to landfills. In Singapore's context, the Westin Singapore has recently hopped onto the bandwagon by operating its fleet of limousinesusing second generation biodiesel. Using waste cooking oil from its kitchen, Westin Singapore has worked with local companies to refine waste matter into useful resources. As such, the vehicles are now fuelled with a blend of biodiesel and diesel, which is said to reduce carbon emissions by as much as 95 percent [8]. Similar examples can also be seen at the National University of Singapore, with two of its canteen operators joining efforts to recycle waste cooking oil for better use that will in turn benefit the environment [9]. In addition, Alpha Biofuels, is one of many companies in Singapore that tap into this knowledge and develop biofuels from second generation feedstock[10].They supply local companies with biofuels needed in transport operations.

Globally, DupontDanisco Solution is a company based in the United States (USA) that uses second generation biofuel technologies. DupontDanisco puts emphasis on natural synergy that rely on shared strengths and unravels cutting-edge techniques. In the last decade, DuPont and Danisco have invested an upward of $100 million towards further developing cellulosic ethanol technology [11]. Additionally, in Seattle, USA, Blue Marble Biomaterial, a biofuel company that uses AGATE (Acid, Gas, and Ammonia Targeted Extraction) as its proprietary poly-culture fermentation technology, whichinvolvesthe displacement of oil with completely sustainable, carbon-neutral substitutes. They cultivate and propagate thebiochemicals from assorted cellulosic biomass, which uses the thrivingbacteria ecosystemto produce an extensive range of targeted compounds. This method commences using organic matter (biomass) such as algae, agricultural silage, spent brewery grain, food co-products, coffee and tea, and wood chips amongst others[12].

All in all, second generation biofuels are by and large more efficient and environmentally friendly when compared to their first generation counterparts. They require less farmland to harvest the same amount of feedstock since the every part of the plant can be utilized[13].Thus, the inedible plant parts can be retained for biofuel production instead, eliminating the competition for food, which was the primary issue that was addressed earlier.

THIRD GENERATION BIOFUEL

Third generation biofuels concentrates on the production of sustainable biofuels produced from algae and aquatic biomasses; there are five different possible pathways for the algae-to-biofuel production: Open Pond System, Hybrid System, Modular Closed Photobioreactor, Heterotrophic Fermentation, and Integrated Cultivated System.

Environmental Benefits of Algae Cultivation

The cultivation of algae requires a small area (to none) of arable land, ergo competition with food crops is minimal. As its production is largely dependent on non-arable land, algae-based biofuels has an advantage over its first and second generation counterparts, leading to an increase in consumer food security. Furthermore, in comparison to its predecessors, land-based fuel feedstock, algae cultivation requires lesser water. Methods such as bio-fixation and bioremediation further improve water quality and provides for an economical treatment method of wastewater. In addition, the growth of algae potentially prevents or mitigates any possibility of eutrophication and dead zones. Algae are "responsible for more than 40% of the global carbon fixation", with the capacity to reduce and mitigate industrial greenhouse gases, such as carbon dioxide, contributing to carbon balance [14]. Moreover, due to the lessened dependency on arable lands (in comparison to the crop-based biofuels), this form of cultivation results in lesser deforestation, which is a major contributor to the release of carbon into the atmosphere [15].

Secondly, with regards to water usage in biomass harvesting, a high percentage of water can be recovered and recycled back to the cultivation of algae, thus reducing wastage. Algae has the ability to treat wastewater, thus water released downstream could in essence be at a comparable or better quality than that at the start of the process [14].In addition, the potential oil yields for algae are approximately 20 times higher than yields of oil seed crops [16].

Furthermore, gases and waste heat are reused in the oil and residue conversion process, as with the use of low-value co-products such as glycerol, selected acids, and recalcitrant biomass residue. The quantity, toxicity, and usability of such products will determine the environment impacts it will have. Moreover, gases such as methane can also be burned as a fuel to generate electricity [17].

Environmental Concerns of Algae Cultivation

There is a need to maintain sustainable water levels for surface waters as well as shallow and deep aquifers. Construction of algae cultivation infrastructures such as open ponds, could limit the viability and health of natural aquifers, resulting in lowered biological activity in the soil beneath. This leads to an interruption in the natural hydrologic cycle, the inability for aquifer recharge, and the decline in the natural purification of water. As with other cultivation systems, the amount of land required might increase competition with food crops, as well as altering native habitats and migratory patterns for wildlife[15]. In addition, different algal species (such as modified or invasive strains) could threaten the integrity of ecosystems and agriculture in the area. Furthermore, the quality of biomass is highly dependent on the quality of water. As such, with the low quality of biomasses, more processing and upgrading work is required, ensuing in increased energy inputs and undesirable by-products. The amount of electricity used in the cultivation may adversely affect the energy balance of this biofuel as well. Certain system designs may even engage the use of materials that are potentially harmful for the environment.

In biomass harvesting, there is a need to store and transport harvested biomass for downstream processing, as well as process wastewater used before reuse or release. By means of the use of different additives, the quality and toxicity of the process water will require treatment before it is useful again. Genetically modified algae in such process wastewater could potentially

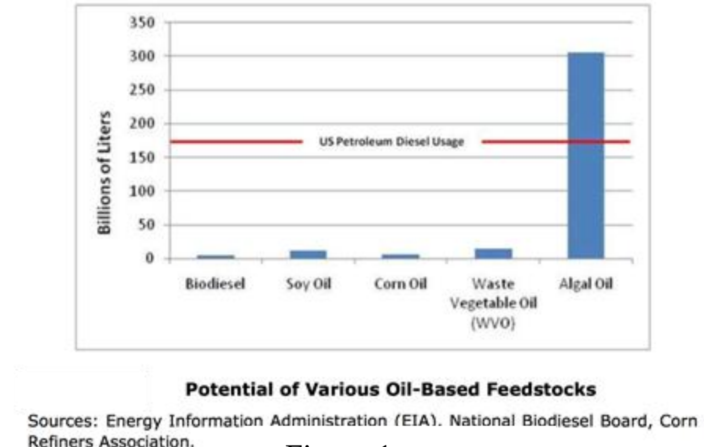

Figure 1.

threaten the natural ecosystem. Furthermore, biomass moisture content has a direct relation to the energy and chemical inputs in downstream processing. Energy-intensive harvesting processes may adversely affect the energy balance, giving rise to a need for more efficient harvesting mechanisms [15].

In algal extraction, the efficiency of algal oil extraction methods depend directly on the solid content of biomasses, leading to a need to have a higher energy input during biomass drying[18]. Furthermore, the chemical solvents used in such extraction processes have inherent health and safety implications. The toxicity of such solvents will restrict handling processes thus leading to higher costs. Environmental concerns at this stage mainly depend on the chemical and residue disposal methods, as well as the chemicals used.

Finally, the processes for oil and residue conversion are high in energy consumption and resource consumption. Hence, water used for these processes could potentially require treatment as wastewater [16].

Feasibility of Algae in Biofuel

In the early stages of algal technology development and cultivation, uncertainties include the direct and indirect demand for water, as well as the effect of these water demands on land, ecosystems, and the greater watershed. Furthermore, there is a risk of disrupting the natural ecosystem with the introduction of exotic algal species[19]. Many queries with regards to the rate of water recovery, chemical recovery, facility scalability, and the impact of energy-intensive drying systems, are still unanswered in the area of biomass harvesting.

The use of microalgae have garnered noteworthy attention due to their high rate of photosynthesis that can be above 6.9 × 104 cells/ml/h, which is roughly 50 times morethan that of earthbound plants. It has been reported that microalgae accumulatesin excess of 70% lipid based on dry weight[20]. The triglyceride found within the lipid, is vital for producing biodiesel [21].

In Figure 1, the potential of algal oil accounts for 300 billion litres, in comparison with biodiesel, soy oil, corn oil, and waste vegetable oil (WVO), all of which has a production rate of below 50 billion litres. As the utilization of petroleum diesel in the U.S. is illustrated at approximately 175 billion litres, it exemplifies microalgae as an excellent source of oil as it can produce more than the country’s current demand and need for biodiesel.

FOURTH GENERATION BIOFUEL

According to Cornell University (n.d.) [22], fourth generation biofuels are made using specially created plants or biomass that have either smaller barriers to cellulosic breakdownor greater yields. Additionally, they can be developed on land and water bodies that are unfit for agriculture, thus no destruction of biomass is warranted [23]. It is generated using petroleum-like hydro processing[24].In order for an alternative fuel to be considered a suitable substitute for fossil fuel, it should possess greater environmental benefits over its displaced former, be cost competitive, and producible in sufficient amounts to have a meaningful impact on energy demands [25]. Most importantly, the net energy derived from the feedstock should exceed the amount that is required for production.

In recent years, scientists have made a breakthrough in this field by designing eucalyptus trees that are able to accumulate three times more carbon dioxide than usual, increasing optimism in mankind's bid to reduce greenhouse gases and salvage the current state of global warming [26]. Next, microbes, or microorganisms, are deemed as great alternatives to conventional feedstock for biofuels due to their short life cycle, lower labour requirements, reduced influence by location, season and climate, as well as the ease of scaling up production [27].

Oil content of some microorganisms

Microorganisms	Oil content (% dry wt)	Microorganisms	Oil content (% dry wt)
Microalgae		Yeast	
Botryococcus braunii	25–75	*Candida curvata*	58
Cylindrotheca sp.	16–37	*Cryptococcus albidus*	65
Nitzschia sp.	45–47	*Lipomyces starkeyi*	64
Schizochytrium sp.	50–77	*Rhodotorula glutinis*	72
Bacterium		Fungi	
Arthrobacter sp.	>40	*Aspergillus oryzae*	57
Acinetobacter calcoaceticus	27–38	*Mortierella isabellina*	86
Rhodococcus opacus	24–25	*Humicola lanuginosa*	75
Bacillus alcalophilus	18–24	*Mortierella vinacea*	66

Table 2. Source: Renewable Energy

Several examples of oleaginous microorganisms include microalgae, bacteria, fungi and yeast. However, not all of them are available for biofuel production as only certain species contain oil sufficient enough for the production of biofuels as seen in Table 2. Research efforts have been initiated to focus on improving the strain of harnessing the organism directly for production of biofuel [28].With the rising price of crude oil, and projected depletion of fossil fuel reserves according to [2], more intensive research have since been conducted to explore other viable alternatives to harvest such feedstock, which in the future may well become a potential source and solution for biofuel production and mankind's energy crisis.

The Use of Microorganisms in Fourth Generation Biofuel

Microalgae are cell factories powered by solar energy that convert carbon dioxide into potential biofuels [29]. Under favourable conditions, microalgae can grow rapidly and double in their biomass within 24 hours; most of them are exceedingly rich in oil. According to Table 2, species of microalgae such as *Botryococcusbraunii*and *Schizochytrium sp.* accumulate oil content as high as 75% and 77% respectively of its dry weight, which is favourable for biofuel production. However, larger acreages are needed in order to culture microalgae.

Microalgae can be divided to two categories, autotrophic and heterotrophic. The primary differences between the two are the cultivation processes and upscaling difficulties. Autotrophic microalgae produce microbial oil by relying on natural sunlight, regardless of daily variation in lighting levels [30]. Besides sunlight, other factors such as temperature, salinity, mineral, nitrogen sources and acidity also play a part in influencing oil accumulation [27]. Examples of such species includes *Botryococcusbraunii*, *Chlorella vulgaris*,*Crypthecodiniumcohnii*,*Dunaliellaprimolecta*, *Naviculapelliculosa*,*Neochlorisoleoabundans*, *Monallanthussalina*, *Phaeodactylumtricornutum*, *Scenedsmusacutus*, and *Tetraselmissueica*[31]. Heterotrophic microalgae, on the other hand, can be converted from autotrophic microalgae through genetic engineering modification or altering the environments during cultivation [27]. Such heterotrophic species can forgo sunlight and instead use natural carbon as its carbon supply point. Miao and Wu (2004) [32] illustrated how *Chlorella protothecoides*, which is originally an autotrophic microalgae, can tap on organic carbon sources and achieve about four times more oil production than before. The result can be yielded by the application of metabolic controlling technique through heterotrophic growth of *C. protothecoides* which allow the more cost efficient carbon source, corn powder hydrosate, to be used to achieve a higher biomass for biofuel production [33]. Therefore, the high photosynthetic efficiency, great biomass production and rapid growth of microalgae as compared to other energy crops have made it a leading contender for biofuel production.

In addition, bacteria having the highest growth rate and relative ease of cultivation have an advantage in the production of biodiesel. In comparison with microalgae, bacteria require shorter fermentation periods and can attain enormous biomass in just 12 to 24 hours [25]. Certain species such as *B. anthracis*and *B. subtilis*, housed under the *Bacillus* genus, are examples of bacteria capable of direct growth and are thus able to accumulate microbial oils [34]. However, nearly all bacteria accrue lower amounts of lipid as compared to microalgae, which are essential fatty acids for biofuel production; both the *Arthrobacter sp.* and *Acinetobactercalcoacetius* species records oil content of 40% and 38% respectively. Moreover, most bacteria only produces complex lipoid which are difficult to extract; and only some species can yield oils that can be usedfor biofuel production as feedstock[27]. As such, there is little industry significance to use oleaginous bacteria as raw materials and alternative sources.
Fungi can also amass oils for biofuel production under favourable cultivation conditions. Selected varieties of fungi are able to produce oil, however a large amount of them are mostly used for the exploration of certain specific lipids based on the report of Yi and Zheng (2006) [35]. Certain species such as the *Trichodermareesei*and*Aspergillus. A. niger*have undergone experiments to improve the strain of harnessing the organism directly for bioethanol production [28]. Other examples of potential species includes *Mortierella. ranmanianna*, *M. isabellina*, *M. mucedo*and *C ehinulata*. Despite the relative potential, researches and studies are still being conducted as there are few reports on the utilization of such as feedstock for the next generation biofuel.

Lastly, yeasts are considered as favourable oleaginous microorganism as feedstock for biofuel production. Studies have recorded that several types of yeast can accumulate lipid as much as 70% of their biomass dry weight, in which some contain a similar extract as those of certain plant seed oils, hence making it a worthy candidate for biofuel feedstock [25]. Some examples are *Cryptococcus albidus,Lipomycesstarkeyi,Lipomyceslipofera, Rhodotorulaglutinis, Rhodosporidiumtoruloides*, *Trichosporonpullulan* and *Yarrowialipolytica*. Yeasts have also demonstrated high growth ratesin addition to havinglarge oil contents, thus proving its potential and remarkable potency.[27]

Production Systems

Efficient and effective biomass crops that remove carbon dioxide from the atmosphere and lock it up within itself are converted into fuel and gases by second generation techniques. Specially, carbon dioxide is captured by applying pre-combustion, oxyfuel or post-combustion processes, throughout the bioconversion processes. The greenhouse gas is then separated through storage in depleted oil and gas fields, coal seams that are not minable or in saline aquifers, where it stays sealed up for hundreds and thousands of years. The remaining fuels and gases then undergo fuel upgrading processes such as gas cleaning and liquefaction. The end product are fuels and gases that are not only renewable but also carbon negative [26].

Biomass crops aside, microbes are also chiefly used in the production of biofuel. Research had been carried out by the University of California in Los Angeles to refine the oils, carbohydrates or fats generated by microbes or plants into biofuel. In addition, microbes have been discovered to have the ability to produce fuels from proteins rather than using it for growth. This process is called protein utilization, scientists have tweaked the nitrogen metabolism and induced bio refining processes, as well as altering the metabolism of nitrogen at the cellular level. Benefits include decreasing greenhouse emissions and maintaining a neutral state of nitrogen during the fertilizer production [36].

Also, microorganism such as bacteria, yeast and fungi contain bio surfactants, which are surface active molecules produced by them. Bio surfactants had become a subject of interest due to its activity level and productivity characteristics that can be harvested for the production of biodiesel [37]. However, this production method is not widely commercialized due to its relatively low level of production yields and high cost involved in purification and use of feedstock [38]. Researchers have then begun to examine alternative feedstock or agricultural byproducts such as sludge palm oil, cassava wastewater, vegetable refinery waste, molasses and raw glycerol for bio surfactant production [39-43].

As raw glycerol is a byproduct derived from the process of trans esterification of fatty acids to produce biodiesel, it is estimated that the rising demand of biodiesel will result in a surge of raw glycerol and hence lowering its cost. Subsequently, test had been conducted using such raw glycerol as a carbon source for *Bacillus subtilis* strain which demonstrated the possibility of using raw glycerol as an efficient and renewable carbon source at an economical cost [44]. Hence, this production method has proven the effectiveness for efficient use of bio surfactants in microorganisms.

Studies have also been conducted on microbial production of fatty acid to reduce dependency on petroleum or petroleum-based products. Microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* are being considered as environmentally-friendly alternatives to bypass chemical transesterification to yield advanced biodiesel, fatty acid methyl esters

(FAME) or fatty acid ethyl esters (FAEE) [45-50]. In order to achieve high yields of microbial biodiesel, there must be sufficient fatty acids. Hence, *Escherichia coli* and *Saccharomyces cerevisiae* are ideal candidates since an existing field of research is being actively carried out on them already [51-53].

Results have shown that *Escherichia coli* could produce a significant increase in FAEE production by up-regulating the fatty acids biosynthetic pathway [50]. Likewise, *Saccharomyces cerevisiae* has been proven in the history of biotechnology as a feasible FAEE producer. However, both species are limited by the insufficient availability of free fatty acids to be extracted. In *Saccharomyces cerevisiae*, fatty acids are used to create phospholipids and storage neutral lipids. While phospholipids is essential to the cell as it constitute the cell membrane, the storage neutral lipids, such as triacylglycerols and steryl esters, are the main fatty acids reserves. These neutral lipids can constitute up to 97% of the storage lipid content of the cell which are not essential to the cell [54]. Although these syntheses of triacylglycerols and steryl esters are common among eukaryotic organisms, they do not occur in *Escherichia coli*. Therefore, blocking the storage lipid forming pathways would significantly help the accumulation of fatty acids, in turn increase the production of biodiesel [45].

Proterro, a bio-feedstock company, has created a licensed technique utilizing modified cyanobacteria, which could be utilized as feedstock for developing biofuels [55]. Specialists from the Biodesign Institute of Arizona State University are utilizing cyanobacteria to discharge any oil that might have gathered without causing damage to the cells. Microbial oil, also known as single cell oil, can be found in various microorganisms under specific conditions [56]. This system have the potential to improve microbial oil processing for modification into biofuel [57]. Cyanobacteria builds up lipids within its thylakoid membranes in relation to its high photosynthetic and rapid growth rate [58]. In addition, cyanobacteria, being prokaryotes, can be enhanced by genetic manipulations much more readily as compared to eukaryotic algae [59]. Researchers also studied specific fungi species that could further improve yield of microbial oil. *Trichodermakoningii*was used to ferment corn stalk to produce reducing sugar; subsequently, *Trichosporoncutaneum* was introduced to ferment the reducing sugar which further enhances microbial oil production and provided the technical basis for the technique [51].

Next, Aurora Algae, the premier provider of algae-derived solutions have established a pond system which cultivates and grow algae at an incredible rates. The executive chairman of Aurora Algae, Hinman (2010) [60] explains that their technique adds a photosynthetic approach to conventional algae cultivation in open pond farms. The results are lowered production costs that matches even the lowest cost producers while retaining their focus on the high-value market sectors. The design and technology leverages on carbon dioxide-rich conditions with optimal fluid dynamic characteristics that minimize energy throughout the entire growth and production process.

Researchers at the University of Michigan had a breakthrough the findings for a new type of 4^{th} generation biofuel. Researchers uses *TrichodermaReesei*, a fungi known to effectively decay non-edible parts of plants, and a bacteria, *Escherichia Coli* to a vat of dried cornhusks. The husks will be degraded into sugars by the fungi followed by bacteria finishing the job. This procedure would then yield a biofuel known as isobutanol, a colorless, flammable liquid [61]. The process of producing and optimizing bio-isobutanol is through fermentation as well as an integrated separation technology also known as the hydrocarbon processing technology. Isobutanol fermentation process is similar to the existing ethanol process and two modifications are needed to produce bio-isobutanol from existing ethanol plants. The two additions are modified biocatalyst and unique proprietary separation [62]. According to the University of Michigan (2013) [63], isobutanol emits 82 percent of the heat energy gasoline provides when burned, compared to ethanol's 67 percent. Isobutanol also does not mix easily with water whereas ethanol tend to absorb water, corrode pipelines and damage engines. With that, isobutanol may be a possible replacement for current gasoline pipelines.

In addition, the growing concern on global warming have sparked the search for a renewable source of energy and biologically-produced hydrogen or biohydrogen is considered a renewable, CO2- neutral energy form [64]. There are many methods of producing biohydrogen and it includes direct, and indirect biophotolysis, dark fermentation and photo-fermentation. Direct biophotolysis indicates sustained hydrogen evolution under light irradiation and examples include the use of cyanobacteria and *Chlamydomonasreinhardtii*, a type of microalgae in production[65]. Indirect biophotolysis refers to fixing the carbon dioxide into stored substrates before being converted to hydrogen. Dark fermentation, as its term suggest, uses anaerobic bacteria that is grown in the dark on carbohydrate-rich substrates while photo fermentation uses photosynthetic bacteria are capable of converting most organic acids or VFA to biohydrogen and carbon dioxide under anaerobic condition with the presence of light [66].

Prospects and Benefits

The various types of microorganisms illustrate the potential and opportunity for biofuels to be the chief energy source of the future. Although it is possible to create special cultivation conditions to promote growth of such microorganisms, there are still factors of consideration for each in terms of lipid coefficient and volumetric, as well as oil yield [27]. While bacteria are not commonly used for biofuel production due to its process complexity, and fungi still undergoing intensive research studies for future utilization, both microbes are nonetheless considered to be viable options for development of biofuels. On the other hand, present-day studies have established and proven that both microalgae and yeast have an extremely promising future in biofuel production.

One of the main advantages of using microalgae as a potential feedstock is its environmentally friendly production of lipids since it uses sunlight and carbon dioxide that are already in the atmosphere for oil accumulation. However, the challenge of needing a huge area for large-scale cultivation needs to be tackled[67]. There is also the issue of fashioning the means to effectively utilize and manipulate light energy for sustainable cultivation [27]. As the current methods adopted for extensive and comprehensive cultivation mainly involve cylindricalphotobioreactors and channeled ponds, there remains the problem of streamlining cultivation processes to reduce operating cost, so as to have a positive impact on the future prospects of this technology.

Conversely the primary advantages of using yeast are its fast growth rate and ability to store large amounts of oil. Current research convey how numerous factors such as carbon-to-nitrogen ratio, temperature, nitrogen resources, acidity value, oxygen, concentration of trace elements and inorganic salt can affect the level of oil accumulation [27]. In order to attain high levels of oil production, further developments have to be put in place to optimize the cultivation parameters. Consequently, more research should be conducted to look into the potential yield of oil from yeast to improve the abilities of using low-cost carbon sources to enhance biofuel production in the future. Studies have also revealed how growth in certain cells have had better effects using starch hydrolysate, an inexpensive carbon source, instead of glucose [68]. Similar to microalgae, the challenge is to keep operating cost low so as to ensure that future biofuel production will be sustainable.

The study of genetic and metabolic engineering of feedstock is also an imperative aspect to guarantee the prospects of biofuel becoming the main energy source of the future. Genetic modification is likely to have the greatest impact in the economics of production with regards to microbial processes. Most of the experiments have been conducted on microorganisms due to their ability and potential to accumulate high amounts of lipid which are essential in biofuel production [25]. With such engineering practices in place, the accumulation of oil in microorganisms is more than likely to see an improvement, which will result in higher yields.

Next, exploring the use of by-products from the production of biofuels can help lower the cost and final product prices considerably. Apart from lipid oils, oleaginous microorganisms also contain significant quantities of proteins, carbohydrates and other nutrient contents [25]. These by-products can be potentially used as animal feed, or be transformed into useful chemicals through other processes.

Finally, the growth of biomass crops such as the eucalyptus trees mentioned earlier will be useful for clearing up the release of carbon into the atmosphere. According to scientists, should the concept and theory of Bio-Energy with Carbon Storage (BECS) be practiced and executed globally, not only will it counter abrupt climate changes, it will also bring down carbon dioxide levels significantly, back to what it was during the pre-industrial era. Furthermore, it directly attacks the root of the problem, the combustion of fossil fuels, for they are replaced with biomass that is renewable and low at risk. The evolving global carbon and biomass market calls for the development of crops that sequester more carbon dioxide. Such crops will directly aid in the efforts of afforestation and reforestation that will eventually procure carbon credits. In essence, fourth generation biofuel can be produced wherever carbon dioxide and water are found in adequate concentration. It is less debated for biodiversity, and is backed by environmental activists and which the processes produce drop-in fuels [23].

Outlook of Biofuels and Beyond

In February 2014, the University of Greenwich [69] announced that it is investing €10m to research microalga *Dunaliella*, a bright pink-orange microalgae found in salt lakes and beachfront waters. They hope to convert it into a renewable and sustainable form of fuel that not only catches carbon dioxide, but can also be developed in the harsh conditions. Project leader Professor Pat Harvey, from the university's Faculty of Engineering & Science mentioned that this form of algae is able to produce up to 80 percent of its mass as fuel and should algae bio refineries be made commercially viable, the potential is vast and sustainable [69]. Presently, the cost of production remains as one of the most intriguing and troubling issue.

According toVogelpohl (2011)[70], from the Institute for Ecological Economy Research, there are many researches and development projects on third and fourth generation biofuels at the moment and as of yet, they are not remotely close to commercialization. The issues include the high costs, environmental balance and the yield amounts. With regards to the cost of biofuel production, it is reported to be multiplied tenfoldper generation. With the current state of technology, it is estimated that the cost of building a third generation biofuel plant of comparable capacity to a first generation one is approximately a hundred times greater[71]. In terms of production yields, the use of microbial organisms for fuel conversion may be a better option, but it is also extremely time consuming. These issues need to be addressed in order to make this category of biofuel cost-competitive [23]. Despite all the currentissues and upcoming challenges, the future of biofuels remains bright; biofuels are estimated to make up approximately eight percent ofoil volumes used for transportation worldwide by 2022. [24]

CONCLUSION

In conclusion, biofuels remain a key solution to mankind's energy crisis, and there is an ongoing pursuit to find a balance between sustainability and cost. A multitude of issues pertaining to first generation biofuels such as competition with food crops have been addressed by its second generation successor. Subsequently, several companies both in Singapore and around the globe have utilized them in their day-to-day operations so as to reduce costs and lessen their ecological footprints. However, there is the sticking matter of plant-based fuels being space and time consuming. Furthermore, demand for these biofuels may exceed our ability to produce them. As such, further research and development have been invested third and fourth generation biofuels due to the belief that the best biofuels will cut out the middleman entirely and bypass crop plants. Instead, they will rely on algae and a few microorganisms that have the capacity todirectly and efficiently turn sunlight into energy through photosynthesis[72]. Research have shown these next generation biofuels have greater production and environmental benefits as opposed to their predecessors. Microalgae for instance only require a small amount of arable land, and its high photosynthetic rate gives it the ability toamass a considerable amount of lipid. Fourth generation biofuel on the other hand probes the limits of nature by experimenting with microbes. While results have been promising from an environmental perspective, both generations have yet to be proven on a large scale basis. The biggest challenge is finding a way to make these biofuels commercially viable while keeping it environmentally friendly. Nevertheless, should these issues be conquered, the entire energy landscape will be altered for the better. Moreover, it could even affect worldwide trade as a nearly identical version of petrol or diesel can be manufactured domestically rather than being imported from the Middle-East. For now, experts continue to invest time and money towards finding a way to make these next generation biofuels commercially viable;whoever manages to do so will make more than profits, they will be making history.

REFERENCE

[1] United States Environmental Protection Agency. (2013, October 28). *Basic Information | What Are Biofuels?* Retrieved February 15, 2014, from United States Environmental Protection Agency: http://www.epa.gov/ncea/biofuels/basicinfo.htm

[2] Shafiee, & Topal. (2009). Energy Policy. *When Will Fossil Fuel Reserves Be Diminished?, 37*(1), 181-189. Retrieved February 18, 2014, from http://www.sciencedirect.com/science/article/pii/S0301421508004126

[3] United Nations Environmental Programme. (2009). *Towards Sustainable Production and Use of Resources: Assessing Biofuels.* France: International Panel for Sustainable Resource Management. Retrieved February 4, 2014

[4] First Generation Biofuels. (2010). *FIrst Generation Biofuels*. Retrieved January 27, 2014, from First Generation Biofuels: http://biofuel.org.uk/first-generation-biofuel.html

[5] United Nations Report; Sustainable Bioenergy: A Framework for Decision Makers; April 2007

[6] Energy From Waste And Wood. (n.d.). *Generations of Biofuels*. Retrieved January 30, 2014, from Energy From Waste And Wood: http://energyfromwasteandwood.weebly.com/generations-of-biofuels.html

[7] Ralph, S., Michael, T., Jack, S., & Warren, M. (2009, March 9). *IEA's Report on 1st- to 2nd-Generation Biofuel Technologies*. Retrieved January 29, 2014, from Renewable Energy World: http://www.renewableenergyworld.com/rea/news/article/2009/03/ieas-report-on-1st-to-2nd-generation-biofuel-technologies

[8] Today Online. (2014, January 18). *Westin Hotel Uses Waste Cooking Oil From Kitchens To Power Limousines*. Retrieved February 1, 2014, from Today Online: http://www.todayonline.com/singapore/westin-hotel-uses-waste-cooking-oil-kitchens-power-limousines

[9] Gladwin, L. H. (2010, December). *Office of Environmental Sustainability | Used Cooking Oil*. Retrieved February 20, 2014, from National University Singapore: http://nus.edu.sg/oes/prog/waste/cooking_oil.html

[10] Alpha Biofuels. (n.d.). *Biodiesel Microrefinery System*. Retrieved January 18, 2014, from Alpha Biofuels: http://alphabiofuels.sg/pages/microref/micro_index.html

[11] Dupont Biofuels Solutions. (2012). *History*. Retrieved January 31, 2014, from Dupont Biofuels Solutions Web: http://biofuels.dupont.com/about-dupont-biofuel-solutions/history/

[12] Blue Marble Biomaterials. (2011). *Technology*. Retrieved February 22, 2014, from Blue Marble Biomaterials: http://bluemarblebio.com/technology/

[13] The Institute of Grocery Distribution. (2008, November 14). *Second Generation Biofuels*. Retrieved February 22, 2014, from The Institute of Grocery Distribution: http://www.igd.com/our-expertise/Sustainability/Energy/3700/Second-Generation-Biofuels/

[14] Michael, H., Javier, G., Miller, T., Beth, R., & Stephen, M. (2010). *Biofuels From Algae: Challenges And Potential*, 763-784. Retrieved February 9, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3152439/

[15] Catie, R. (2009, October). Cultivating Clean Energy. (H. Alice, Ed.) *The Promise of Algae Biofuels*, 1-65. Retrieved February 14, 2014, from http://www.ascension-publishing.com/BIZ/cultivating.pdf

[16] Darzins, A., Philip, P., & Les, E. (2010, August 6). *Current Status and Potential for Algal Biofuels Production*. Retrieved February 13, 2014, from http://www.globalbioenergy.org/uploads/media/1008_IEA_Bioenergy_-_Current_status_and_potential_for_algal_biofuels_production.pdf

[17] Liang, F. Y., Zhao, N., Marta, R., & Sara, S. (2012, April 23). *The Role of Natural Gas as A Primary Fuel in the Near Future, Including Comparisons of Acquisition, Transmission and Waste Handling Costs of as With Competitive Alternatives*. Retrieved January 11, 2014, from http://journal.chemistrycentral.com/content/6/S1/S4

[18] Song D.H., Fu J.J., & Shi D.J., (2008). Exploitation of Oil-bearing Microalgae for Biodiesel. *Chinese Journal of Biotechnology*, 24(3), 341–348

[19] Snow, A. A., Andow, D. A., Gepts, P., Hallerman, E. M., Power, A., & Tiedje, J. M. (2004, July 16). *Genetically Engineered Organisms and The Environment: Current Status and Recommendations*, 1-10. Retrieved February 28, 2014, from http://www.esa.org/esa/wp-content/uploads/2013/03/geo-positionPaper0405.pdf

[20] Lackner K.S. (2003). A guide to CO2 sequestration. *Science*. 300: 1677-1678.

[21] May, Z. H., Lim, Y. L., Sek, H. Y., & Olaganathan, R. (2013, August 14). *Biofuel From Microalgae – A Review On The Current* , *4*(3), 329-341. Retrieved January 21, 2014, from http://researchonline.jcu.edu.au/28849/1/IJABR-V4I3-2013-08.pdf

[22] Cornell University. (n.d.). *What are biofuels?* Retrieved February 15, 2014, from Green Choices: http://www.greenchoices.cornell.edu/energy/biofuels/

[23] Biofuels Digest. (2010, May 18). *What Are – and Who's Making – 2G, 3G and 4G Biofuels?* Retrieved January 18, 2014, from Biofuels Digest: http://www.biofuelsdigest.com/bdigest/2010/05/18/3g-4g-a-taxonomy-for-far-out-%E2%80%94-but-not-far-away-%E2%80%94-biofuels/

[24] Nik, B. (2010, June 14). *What Are 3rd and 4th Generation Biofuels and When Are They Coming?* Retrieved January 29, 2014, from Autoblog Green: http://green.autoblog.com/2010/06/14/what-are-3rd-and-4th-generation-biofuels-and-when-are-they-comin/

[25] Meng, X., Yang, J., Xu, X., Zhang, L., Nie, Q., & Xian, M. (2009, January). Biodiesel Production from Oleaginous Microorganisms. *Renewable Energy, 34*(1), 1-5.

[26] Biopact. (2007, October 8). *A Quick Look At Fourth Generation Biofuels*. Retrieved January 9, 2014, from Biopact: http://news.mongabay.com/bioenergy/2007/10/quick-look-at-fourth-generation.html

[27] Li, Q., Du, W., & Liu, D. (2008). Perspective of Microbial Oils for Biodiesel Production. *Applied Microbiology and Biotechnology, 80*, 749-756.

[28] Rao, R. P., Dufour, N., & Swana, J. (2011). Using Microorganisms to Brew Biofuels. *In Vitro Cellular & Developmental Biology – Plant, 47*, 637-649.

[29] Patnayat, S., & Sree, A. (2006). Screen of Bacterial Associates of Marine Sponges for Single Cell Oil and PUFA. *Letters in Applied Biology, 40*, 358-363.

[30] Chisti, Y. (2007). Biodiesel from Microalgae. *Biotechnology Advances, 25*, 294-306.

[31] Liang, X. A., Dong, W. B., Miao, X. J., & Dai, C. J. (2006). Production Technology and Influencing Factors of Microorganism Grease. *Food Research Development, 27*(3), 133-136.

[32] Miao, X. L., & Wu, Q. Y. (2004). Bio-oil Fuel Production from Microalgae After Heterotrophic Growth. *Renewable Energy Resources*, *4*(116), 41-44. [14] Michael, H., Javier, G., Miller, T., Beth, R., & Stephen, M. (2010). *Biofuels From Algae: Challenges And Potential*, 763-784. Retrieved February 9, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3152439/

[33] Xu, H., Miao, X.L., & Wu, Q.Y. (2006). High quality biodiesel production from microalga Chlorella protothecoides by heterotorphic growth in fermenters. *Journal of Biotechnology, 126*, 499-507. Retrieved March 1, 2014, from https://www.palmbeachstate.edu/programs/biotechnology/Documents/Paper7.pdf

[34] Ramos, C. H., Hoffmann, T., Marino, M., Nedjari, H., Presecan-Siedel, E., Dreesen, O., Glaser, P., Jahn, D. (2000). Fermentative Metabolism of Bacillus Subtilis. *Journal of Bacteriology, 182*, 3072-3080. Retrieved February 17, 2014, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC94491/

[35] Yi, S. J., & Zheng, Y. P. (2006). Research and Application of Oleaginous Microorganism. *China Foreign Energy, 11*(2), 90-94.

[36] Alternative Energy. (2011, March 28). *The New Role of Microbes in Bio-Fuel Production*. Retrieved January 21, 2014, from Alternative Energy: http://www.alternative-energy-news.info/microbes-bio-fuel-production/

[37] Banat, I. M., Frazetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., & et al. (2010). Microbial biosurfactant production, applications and future potential. *Applied Microbial Biotechnology, 87*, 427–444.

[38] Makkar, R. S., &Cameotra, S. S. (1999) Biosurfactant production by microorganism on uncoventional carbon sources. *Journal of Surfactants and Detergents, 2*, 237–241.

[39] Nawawi, W. M. F. W., Jamal, P., &Alam, M. Z. (2010). Utilization of sludge palm oil as a novel substrate for biosurfactant production. *Bioresource Technology, 10*, 9241–9247.

[40] Barros, F. F. C., Ponezi, A. N., &Pastore, G. M. (2008). Production of biosurfactant by Bacillus subtilis LB5a on a pilot scale using cassava wastewater as substrate. *Journal of Industrial Microbiology Biotechnology, 35*, 1071–1078.

[41] Sobrinho, H. B. S., Rufino, R. D., Luna, J. M., Salgueiro, A. A., Campos-Takaki, G. M., Leite, L. F. C., & et al. (2008). Utilization of two agroindustrial by-products for the

production of a surfactant by Candida sphaerica UCP0995. *Process Biochemistry, 43,* 912–917.

[42] Reis, F. A. S. L., Servulo, E. F. C., & Franc, F. P. (2004). Lipopeptide surfactant production by Bacillus subtilis grown on low-cost raw materials. *Applied Biochemistry Biotechnology, 115,* 899–912.

[43] Morita, T., Konishi, M., Fukuoka, T., Imura, T., &Kitamoto, D. (2007). Microbial conversion of glycerol into glycolipid biosurfactants, mannosylerythitol lipids, by a basidiomycete yeast, Pseudozymaantarctica JCM 10317T. *Journal of Biosciences and Bioengineering,* 104, 78–81.

[44] Faria, A. F., Teodoro-Martinez, D. S., Oliveira Barbosa, G. N., Vaz, B. G., Silva, I. S., Garcia, J. S., Totola, M. R., Eberlin, M. N., Grossmand, M., Alves, O. L., &Durrant, L. R. (2011). Production and structural characterization of surfactin (C14/Leu7) produced by Bacillus subtilis isolate LSFM-05 grown on raw glycerol from the biodiesel Industry. *Process Biochemistry, 46,* 1951–1957.

[45] Kalscheuer, R., Luftmann, H., &Steinbüchel, A. (2004). Synthesis of novel lipids in Saccharomyces cerevisiae by heterologous expression of an unspecific bacterial acyltransferase. *Applied and Environmental Microbiology, 70,* 7119–7125.

[46] Kalscheuer, R., Stölting, T., &Steinbüchel, A. (2006). Microdiesel: Escherichia coli engineered for fuel production. *Microbiology, 152,* 2529–2536.

[47] Nawabi, P., Bauer, S., Kyrpides, N., &Lykidis, A. (2011). Engineering Escherichia coli for biodiesel production utilizing a bacterial fatty acid methyltransferase. Applied and Environmental *Microbiology, 77,* 8052–8061.

[48] Valle-Rodríguez, S. J., Siewers, V., & Nielsen, J. (2011). Prospects for microbial biodiesel production. *Biotechnology Journal, 6,* 277–285.

[49] Valle-Rodríguez, S. J., Khoomrung, S., Siewers, V., & Nielsen, J. (2012). Functional expression and characterization of five wax ester synthases in Saccharomyces cerevisiae and their utility for biodiesel production. *Biotechnology for Biofuels,* 5, 7–16.

[50] Zhang, F., Carothers, J. M., &Keasling, J. D. (2012). Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids. *Nature Biotechnology, 30,* 354–359.

[51] Li, X., Lu, B., Li, H., & Yin, T. (2012). Production of Microbial Oils fermented by two-step fermentation with corn stalk. *Procedia Environmental Sciences, 12,* 432–438

[52] Li, M., Zhang, X., Agrawal, A., & San, K. Y. (2012). Effect of acetate formation pathway and long chain fatty acid CoA-ligase on the free fatty acid production in E. coli expressing acy-ACP thioesterase from Ricinuscommunis. *Metabolic Engineering, 14,* 380–387.

[53] Lu, X., Vora, H., &Khosla, C. (2008). Overproduction of free fatty acids in E. coli: Implications for biodiesel production. *Metabolic Engineering, 10*, 333–339.

[54] Michinaka, Y., Shimauchi, T., Aki, T., Nakajima, T., Kawamoto, S., Shigeta, S., & et al. (2003). Extracellular secretion of free fatty acids by disruption of a fatty acyl-CoA synthetase gene in Saccharomyces cerevisiae. *Journal of Biosciences Bioengineering*, 95, 435–440.

[55] Kyriakos, M. (2010). *Algae, Cyanobacteria and Microbiological Production of Biofuels*. Retrieved February 07, 2014, from European Biofuels Technology Platform: http://www.biofuelstp.eu/algae.html

[56] Li, J., Liu, H., Zhang, J., & et al. (2007). Progress in and prospect of microbial lipid production by fermentation. *Modern Chemical Industry, 27(2),* 133-136.

[57] Richard, H. (2010, June 10). *Benchtop Biofuels: Fine-tuning Growth Conditions Helps Cyanobacteria Flourish*. Retrieved February 3, 2014, from Biodesign Institute: http://www.biodesign.asu.edu/news/benchtop-biofuels-fine-tuning-growth-conditions-helps-cyanobacteria-flourish

[58] Rittmann, B. E. (2008, March 7). *Opportunities for Renewable Bioenergy*. Retrieved February 10, 2014, from http://onlinelibrary.wiley.com/doi/10.1002/bit.21875/pdf

[59] Vermaas W. (1998). Gene Modifications and Mutation Mapping to Study the Function of Photosystem II. Meth Enzymol 297:293–310.

[60] Hinman, B. (2014). *Innovative End-to-End Production Process*. Retrieved March 1, 2014, from Aurora Algae: http://www.aurorainc.com/technology/production-process/

[61] Brodwin, E. (2013, August 19). *Bacteria and Fungi Together: A Biofuel Dream Team?* Retrieved March 23, 2014, from Scientific American: http://www.scientificamerican.com/article/bacteria-fungi-biofuel-dream-team/ [15] Catie, R. (2009, October). Cultivating Clean Energy. (H. Alice, Ed.) *The Promise of Algae Biofuels*, 1-65. Retrieved February 14, 2014, from http://www.ascension-publishing.com/BIZ/cultivating.pdf

[62] Kolodziej, R., & Scheib, J. (2014, Jaunuary). *Bio-based Isobutanol — a versatile*, . Retrieved March 23, 2014, from http://www.digitalrefining.com/data/articles/file/1236857922.pdf

[63] University of Michigan. (2013, August 19). *Microbial team turns corn stalks and leaves into better biofuel*. Retrieved March 23, 2014, from Michigan News, University of Michigan: http://www.ns.umich.edu/new/multimedia/videos/21643-microbial-team-turns-corn-stalks-and-leaves-into-better-biofuel

[64] Benemann. J.R. (1998). *The technology of biohydrogen. Edited, O.R. Zaborsky International Conference on Biological Hydrogen Prodution.* Oct. 15-19, Waikoloa, Hawaii, USA.

[65] Takahashi, P., & Yu, J. (2007). *Biophotolysis-based Hydrogen Production by Cyanobacteria*. Retrieved March 23, 2014, from http://www.formatex.org/microbio/pdf/Pages79-89.pdf

[66] Hay, J. X., Ta, Y. W., & Joon, C. J. (2013, February 7). *Biohydrogen Production Through Photo Fermentation Or Dark Fermentation Using Waste As A Substrate: Overview, Economics, And Future Prospects of Hydrogen Usage*. Retrieved March 23, 2014, from Biofuels, Bioproducts and Biorefining: http://onlinelibrary.wiley.com/doi/10.1002/bbb.1403/pdf

[67] Peng, L., & Gutterson, N. (2011). Energy Crop and Biotechnology for Biofuel Production. *Journal of Integrative Plant Biology, 53*(3), 253-256.

[68] Han, X., Miao, X. L., & Wu, Q. Y. (2006). High Quality Biodiesel Production from Heterotrophic Growth of Chlorella Protothecoides in Fermenters by Using Starch Hydrolysate as Organic Carbon. *Journal of Biotechnology*, *126*(4), 499-507.

[69] University of Greenwich. (2014, February 13). *Algae Research Gives Hope For Renewable Carbon-negative Source of Food and Medicines*. Retrieved February 28, 2014, from http://www2.gre.ac.uk/about/schools/science/about/news/sci/a2816-algae-research-gives-hope-for-renewable-carbon-negative-source-of-food-and-medicines

[70] Advanced Biofuels USA. (2014, February 20). *Algae Research Gives Hope for Renewable Carbon-Negative Source of Food and Medicines*. Retrieved February 26, 2014, from Advanced Biofuels USA: http://advancedbiofuelsusa.info/algae-research-gives-hope-for-renewable-carbon-negative-source-of-food-and-medicines

[71] Vogelpohl, T. (2011, September 28). *First and Next Generation Biofuels- An Overview of Potentials and Challenges for Sustainable Production* . Retrieved February 18, 2014, from http://www.ioew.de/uploads/tx_ukioewdb/Thomas_Vogelpohl_28.09.2011_CONTEC.pdf

[72] Brendel, P.-A. (2013, June 21). Let's Drive Bio! *What biofuels for What Uses Tomorrow*. Retrieved January 2, 2014, from http://www.michelinchallengebibendum.com/eng/publication/drive-bio

[73] Wenner, M. (2009, March 1). *The Next Generation of Biofuels*. Retrieved February 28, 2014, from Scientific American: http://www.scientificamerican.com/article/the-next-generation-of-biofuels/