Journal of Nature Studies 11 (1&2): 103-124
ISSN: 1655-3179

The Promises of *Chlorella vulgaris* as the Best Alternative for Biodiesel: A Review

Lea C. Garcia
University of the Philippines Rural High School
UPLB, College, Laguna
Mobile #: (063) 9209143937
*Corresponding author: leureal@yahoo.com

ABSTRACT – This paper presents a review on *Chlorella vulgaris* as the best alternative for biodiesel production. The review starts with the discussion on the initial findings on *Cocos nucifera* and *Jatropha curcas L.* as other sources of biodiesel. Many studies were presented to show the usefulness of *C. vulgaris* as a source of biodiesel due to its high lipid productivity and fatty acid methyl esters (FAME), profile similar to conventional petroleum. *C. vulgaris* produces oils via photosynthesis, similar to plants, but does it more efficiently than oil crops. The oil from *C. vulgaris* and many microalgae greatly exceed the oil content of the best producing oil crops. The pros and cons of using open and closed systems of cultivating *C. vulgaris* were presented. Issues in terms of oil production and extractions were likewise discussed.

INTRODUCTION

There is the need to fulfill the ever-increasing global energy demand that causes the intensive use of fossil fuels like coal, petroleum and natural gas. These fossil fuels represent more than 80% of the energetic resources. Due to their exhaustibility and unsustainable environmental impact, both generated by their fossil origin, growing attention has emerged on renewable energy. The aim is to diversify the energy resources, reduce the utilization of fossil fuels, and thus limit their negative effect.

The diesel engines of trucks and buses are not only highly efficient power plants but also are also very versatile in the fuels they can use. Biofuels, such as biodiesel and bioethanol, are good for the environment because they emit fewer emissions to the atmosphere than petroleum-based fuels. Biodiesel is produced from triglycerides by trans esterification reactions (Li et al., 2008).

The use and production of biodiesel creates 78% less carbon dioxide emissions than conventional diesel fuel. As a greenhouse gas, carbon dioxide contributes to global warming by preventing some of the sun's radiation from escaping the Earth. Burning biodiesel fuel also effectively eliminates sulfur oxide and sulfate emissions, which are major contributors to acid rain. Unlike petroleum-based diesel fuel, biodiesel is free of sulfur impurities. Combustion of biodiesel additionally provides a 56% reduction in hydrocarbon emissions and significant reductions in carbon monoxide, smog-causing pollutants and soot particles compared to petroleum-based diesel fuel. Also, biodiesel can reduce the carcinogenic properties of diesel fuel by 94% (US Department of Energy, 2003).

Biodiesel is a biodegradable and nontoxic diesel fuel substitute that can be used without any need to modify the engines. It is also good for diesel engines because biodiesel lubricates better than petroleum-based diesel fuel and has excellent solvent properties. Conventional diesel fuel can leave deposits inside fuel lines, storage tanks, and fuel delivery systems over time. Biodiesel dissolves these sediments, and does not leave any deposits, resulting in cleaner, more trouble-free fuel handling systems. Use of 100% biodiesel fuel does reduce the fuel economy and power of diesel engines by 10%, which means that 1.1 gallons of biodiesel are equivalent to one gallon of conventional diesel fuel. Although both biodiesel and conventional diesel fuel tend to gel or freeze in cold weather, biodiesel switches from the liquid

To cite this paper: Garcia, L. 2012. The Promises of *Chlorella vulgaris* as the Best Alternative for Biodiesel: A Review. *Journal of Nature Studies*. 11 (1&2): 102-123

state at higher temperatures than petroleum-based diesel fuel.

The advantages of using biodiesel have instigated scientists to propose alternative sources of biodiesel.

This paper aimed to review the importance of *Chlorella vulgaris* as the best alternative for biodiesel. Different studies on the alternative sources of biodiesel are presented and the problems associated with them. Many studies on *Chlorella vulgaris* are also presented to better understand its usefulness as best feedstock for biodiesel production. Specifically, it answers the following questions:

1. What are the alternative sources of biodiesel? What are the problems associated with these sources?
2. Why is *Chlorella vulgaris* considered the best alternative for biodiesel?
3. What are the methods of cultivating *C. vulgaris*? Which is the best method for large scale production of *C. vulgaris?*
4. What are the issues about *C. vulgaris* in terms of oil production and extraction?
5. What are the prospects for the future *using C. vulgaris* as source of biodiesel?

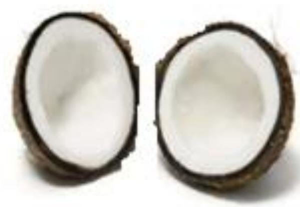
Alternative Sources of Biodiesel

Cocos nucifera as source of biodiesel

Different sectors worldwide have exerted efforts to explore alternative sources of energy as the demand for oil continually increases. Biofuels, as the renewable energy sources, have gained wider interest.

As a part of this initiative, the Philippines enacted the Biofuels Act of 2006 mandating the use of biofuel and providing policy support to its development. This provision calls for mandatory blending of bioethanol and biodiesel to gasoline and diesel, respectively. The blending of 2% biodiesel in all diesel fuels sold in the country was effective 6 February 2009. Not everyone may be aware of it, but the diesel sold in gasoline stations all over the country today is 2% biodiesel. Prof. Demafelis, of the University of the Philippines Los Banos, said that the demand last year reached 107 million liters as per required 2% blending of biodiesel with all diesel fuel sold (Gumapac, 2011). Projecting a 10% biodiesel blending requirement by 2015, studies showed that 663 million liters annually is required, thus increasing the demand for biodiesel.

Initially, the Philippines developed Coconut Methyl Ester (CME) as a source of biodiesel from coconuts (Fig.1). The Philippines



(a) (b)

Fig. 1. (a) The coconut fruit and (b) the open coconut fruit

is the first nation to produce and use coconut-derived biodiesel. Many studies on the research and development of the application of Coconut Methyl Ester (CME) as fuel, from 1983 to 1995, had been conducted by government and private institutions such as the Department of Science and Technology (DOST), Industrial Technology Development Institute (ITDI), Philippine Coconut Authority (PCA), National Power Corporation (NPC), Philippine National Oil Company-Energy Research Development Center (PNOC-ERDC) and Philippine Coconut Research and Development Foundation (PCRDF). Why coconut? Dr. Bruce Fife (September, 2004) in his book "The Coconut Oil Miracle" points at the medium carbon chain especially lauric acid (C_{12}) as the central point of its excellence. Compared to soybean, rapeseed, palm and *Jatropha*, only coconut has caproic acid, caprylic acid, capric acid and lauric acid as components of the plant's fatty acid. This is the central point of the uniqueness of coconut oil. Coconut-derived biodiesel is produced in the Philippines. One of the main reasons the nation is trying to promote its use.

> "The main interest in the Philippines for biodiesel is energy security at this point," said Teresa Alleman, a National Renewable Energy Laboratory (NREL) engineer and one of the coordinators of the project. "The industry is starting with coconut biodiesel and hoping to expand." (Bryan, 2004)

Some foreign initiatives were also done to test the biodiesel produced from coconut oil (*Cocos nucifera* L.). Araujo et al. (2009) conducted a study on biodiesel production from coconut using acid catalysis (with H_2SO4), followed by basic catalysis (with NaOH). Using a 1L jacketed pyrex glass reactor with 3 outlets, a mechanical agitator, thermocouple and *bath* for thermostat-regulated refrigeration were introduced. The analysis of oil composition and identification of the ester compounds were carried out by gas chromatography. The effect of oil/alcohol molar ratio, reaction time, and temperature on conversion was assessed by Experimental planning with a central point, in triplicate, for the route analyzed. The molar ratio variable had the greatest effect according to statistical planning analysis. The maximum conversion reached was 85.3% for a molar ratio of 1:6, temperature of 60ºC and reaction time of 90 minutes. The coconut oil was characterized by their physical and chemical properties and key constituents. The lauric acid was its main component and even showed high acidity. The biodiesel produced was characterized by its main physicochemical properties that had very satisfactory results when compared with the standard values from the National Petroleum Agency.

Engr. Roberto C. Ables (2001) established the technical and economic viabilities of replacing/substituting petroleum diesel with CME by directly feeding 100% of CME to diesel transport vehicles. However, the result is not economically viable due to high cost of coconut oil (CNO). Moreover, when the price of coconut oil increased, or when the price of petroleum diesel fuel (PDF) decreased to a level much lower than that of CNO, this proposal is discontinued because of economic viability issues that failed to attract local and foreign investors.

Jatropha curcas L. as source of biodiesel

In 2007, Goldman Sachs cited *Jatropha curcas* as one of the best candidates for future biodiesel production. Despite its abundance and use as an oil and reclamation plant, none of the *Jatropha* species has been properly domesticated. As a result, its productivity is variable, and the long-term impact of its large-scale use on soil quality and the environment is unknown.

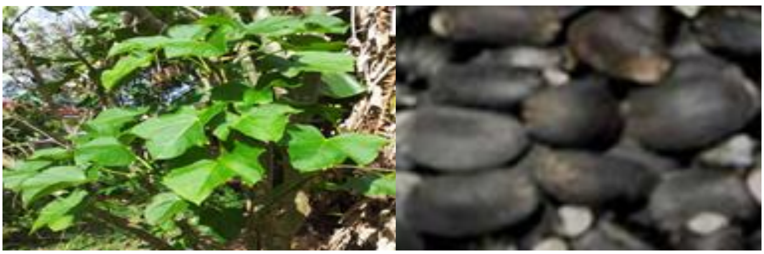
With the Philippines' initial findings on the use of Coconut Methyl Ester (CME) as a source of biodiesel having high cost which limited its sustainable use, an alternative non-food biofuel feedstock like *Jatropha curcas* L. was considered as an important alternative source of biodiesel (Mendoza et al. 2007; Demafelis 2008; Escobar et al; 2008). *Jatropha*, which is usually utilized as a medicinal and fence crop, has become popular due to its ability to grow in lands with marginal soil condition requiring an average annual rainfall of 300 mm to thrive in marginal soils, and 600 mm to bear fruits (PCARRD-DOST, 2009).

There are many benefits in planting this shrub. For one, this crop will not compete against other food crops because it is harmful to humans and animals, therefore, its plant parts, especially the seeds, can be solely used for oil production. *Jatropha* can also revitalize degraded lands. This crop has a four-lateral root system that serves as a water storage unit for the plant providing anchorage in times of heavy rainfall and preventing erosion. Several countries have already expressed their interest to promote the planting of this crop to combat the increase of energy prices and demands and mitigate environmental degradation because of pollutants. Its other uses include biogas production, soap production, animal feeds and as pesticide.

There have been experiments conducted on the usefulness of *Jatropha* as a source of biodiesel. By harvesting fruits in different stages of maturity based on color, number of seeds per fruit and average seed weight, Mendoza et al. (2007) showed that *Jatropha* becomes a viable source of biodiesel oil at PhP40L of crude oil under high fruit yields (36,000 kg/ha), high rates of oil extraction (34% and 38%) and by-products included as added income. At low fruit yields, (12,000 kg/ha), it will become profitable for farmers if the current diesel oil price increases to about PhP90L crude oil at 30% rate of oil extraction of which the estimates exclude processing and marketing costs. This also implies that the inherently low *Jatropha* seed yield explains the low revenue. The Philippine Forest Corporation analyzed the different parts of Jatropha in 2006. As reported, using bomb calorimetry, *Jatropha* seed kernel was found to have a gross calorific value of 11,928 BTU/lb while its seed hull was found to be 7,423 BTU/lb. The plant's biomass (seedcake) was found to be 8,188 BTU/lb. Using other methods such as solvent extraction, Dumas combustion network, atomic absorption and spectrophotometry, *Jatropha*'s other properties such as oil content, crude protein, copper, manganese and zinc were analyzed.

It has been reported that the *Jatropha* yield in fresh weight basis ranged from 4 kg to 12 kg. The yield for the first 2 years was 0.4 t/ha; 2–3 t/ha in 3–4 years; and 5–6 t/ha in 5–50 years. The dry seed weight accounted for 15% of the fruit's fresh weight. Also, the dry seed contained about 32% meal, 30–38% crude oil, and 38% seed coat (Mendoza et al., 2007).

On related studies, *Jatropha* Indian varieties have been observed to contain 30% oil content in air-dried seeds. Other literatures support this finding, presenting research results of having no more than 40% of oil content is observed (Henning, 1998).



(a) (b)

Fig. 2. (a) *Jatropha* shrub; (b) Dry Seeds of *Jatropha*

For *Jatropha* to be used as an alternative fuel, there should be researches and experiments on exploring the possibilities of adopting a mass production scheme for this plant species. One scheme as reported by Chitra et al. (2005) looked into the yield performance of the *J. curcas* oil in different experimental conditions such as temperature, time reaction, methanol, and sodium concentration. Results showed a wide variation of 30–98 % oil yield from the extracted perennial shrub. Maheshwari and Naik (2007) reported that the oil extracted from *Jatropha* ranged from 22-39% using such methods as the use of n-hexene, hydraulic press, mechanical expeller, boiling water and screw press oil. Parametric studies by Demafelis et al. (2008) were also done to determine the effects of varying oil-to-alcohol-to-catalyst molar ratio on reaction time and purity.

The estimated volume of *Jatropha* biodiesel in a 1000-L-per-day village scale *Jatropha* biodiesel plant using *Jatropha* seeds containing 30–35% oil ranges from 250 L/t to 292 L/t of seed. The establishment of *Jatropha* plantation is targeted at unproductive areas such as marginal grasslands and abandoned mine sites. Albano (2007) conducted a study on the growth and survival of *Jatropha curcas* L. in marginal and mine soils as affected by mycorrhizal inoculation. This study also determined the contribution of mycorrhizal inoculation on heavy metal accumulation by *Jatropha*. Results showed that nonmycorrhizal *Jatropha* seedlings planted in Caliraya soil died 1 month after transplanting. In Mogpog mine soil, all seedlings died 3 months after transplanting. Those inoculated with Mykovam survived longer (2 months) than the other treatments (1 month). On the other hand, all cuttings survived in both Caliraya and Paracale soils. In Mogpog mine soil, cuttings died 2 months after transplanting. Mycorrhizal fungi indigenous to mine sites (MineVAM) promoted 348% higher seedling height than Mykovam. Likewise, MineVAM promoted higher height of cuttings grown in Caliraya and Paracale soils. These indicated that *Jatropha* can be used to rehabilitate unproductive areas such as abandoned marginal grasslands and mine sites. They noted that mycorrhizal inoculation plays a significant role. It is expected that more fruits will be harvested from mycorrhiza-inoculated *Jatropha* where oil will be extracted for biodiesel production. In terms of heavy metal (HM) accumulation, mycorrhizal plants took more Cu, Fe, Zn and Mn than the nonmycorrhizal counterpart. Non-mycorrhizal cuttings took more HMs than the uninoculated seedlings. HMs were confined mostly in the roots and least in the leaves.

The economics of the industry depends significantly on production yields that should not only rely on application of expensive, imported chemical fertilizers. Zarate et al. (2007) studied the effect of mycorrhizal inoculant Mykovam and plant growth promoting bacteria (PGPB) on the growth of *Jatropha* seedlings grown in unsterilized field soil. Results showed that inoculation with Mykovam and PGPB at transplanting time, either singly or combined, significantly promoted taller height, wider stem diameter, larger leaf area, longer root length and heavier biomass of *Jatropha* seedlings after 3 months in the nursery. Root length and total leaf area were significantly longer and larger when both biofertilizers are present, as compared to when inoculated singly. Similarly, nitrogen and phosphorus uptake of seedlings was higher with both biofertilizers present. The phosphorus and nitrogen nutrition of the seedlings was greatly enhanced, due to the synergistic interaction of the *Rhizobacteria* and mycorrhizal fungi. Survival and growth of seedlings in the field are being monitored to determine if this good growth in the nursery will translate to early plant maturity and increased seed yield. It was observed that as *Jatropha* plant population increased, the concentration of the foliage nutrient, N and P decreased. On the other hand, plant spacing did not affect the concentration of the nutrients. However, a low nutrient content observed at closer spaced plants can be because of the competition in nutrient absorption (Chaudhary et al., 2009).

Jatropha is a light sensitive plant. Optimal spacing or plant density is recommended to avoid light and nutrient competition and reduce the vulnerability to pest and disease. Total tree canopy, leaf surface area, orientation, and spacing (plants/ha) are all key variables determining the maximum yield potential through photosynthesis (de Vries, 2007).

As an economic source of fuel, planting *Jatropha* has some drawbacks. For one, the crop has a long gestation period. The viability of its production is five years after planting, as fruit bearing starts after two years. Second, successful plantation requires superior plant stocks and efficient plantation management. Having an excellent fruiting behavior, prolific branches, and high resistance to pest and diseases will lead to a superior plant stock. Third, there is a need for efficient plantation techniques including standardized agro-techniques, land type, and strategic location (accessibility to market) (Argamosa 2007). At this point, commercialization of *Jatropha*, which entails its mass production, implies probably a big budget or cost.

Chlorella vulgaris- The Choice for Biodiesel Production

A great variety of biomasses have been investigated as feedstock for biodiesel production. Examples are vegetable oils derived from soybean and rapeseed, corn, cottonseed, peanut, sunflower, safflower, coconut or palm. However, these feedstocks derived from superior plants show many disadvantages, which include the competition between cultivar for energetic and agricultural goals and deforestation for the creation of new cultivable lands. Also, terrestrial cultures are not so efficient in capturing solar energy – they grow slowly, have low biomass yield and need fertilizers that produce N_2O emissions, reducing the greenhouse gasses savings.

Unicellular algae are one of the most promising feedstocks for biodiesel production. When compared with superior plants, microalgae show higher photosynthetic efficiency, higher biomass productivities and faster growth rates. This aspect, together with a high intracellular lipid content, can potentially make a number of unicellular algae species among the most efficient lipid producers of the planet.

How do carbon dioxide emissions from power stations, for example, be put to good use? One way of consuming CO_2 naturally and thereby producing oxygen is photosynthesis. Algae, one of those with the highest conversion rates , is still "responsible" today for half of all the oxygen molecules that we breathe.

Although microalgae cannot immobilize carbon for long periods like the trees in a forest, for example, their biomass can be cultivated together with power-plants that generate CO_2 excess (Cenciani, et al., 2011). This group of authors also added that microalgae cells use the CO_2, from industrial processes, to carry out the photosynthesis. It is interesting to note that microalgae culture is fertilized with CO_2, instead of it being released into the atmosphere. Microalgae require large quantities of CO_2 as nutrient with potential to function as carbon sink.

Moreover, unlike traditional oilseed crops, microalgae cultures do not need herbicides or pesticides and can be done on non-arable lands including marginal areas unsuitable for agricultural purposes, minimizing damages caused to eco and food-chain systems (Chisti, 2007) and without compromising the production of food, fodder and other products derived from crops.

Algae have been studied extensively (Lardon et al., 2009) for its potentials in biotechnology particularly in biodiesel production. With the use of such microorganisms, there will be alternative cheap energy sources that will lessen dependency on oil as the major power provider of the century.

Third generation biofuel is synonymous with algal fuel or oilgae, basically any biofuel derived from algae. Algae are considered superior to other fuel feedstocks.

> "Algae grow rapidly, are rich in vegetable oil, and can be cultivated in ponds of seawater, minimizing the use of fertile land and fresh water. They can double their mass several times a day and produce at least 15 times more oil per hectare than alternatives such as rapeseed, palm soya or *Jatropha*…" (Chisti, 2007).

As photosynthetic organisms, microalgae require solar energy, carbon dioxide, water and nutrients. The provision and the efficient control of these parameters will define the final production of the system (Van Hermelen et al., 2006).

Solar energy determines the geometry and the materials of the reactor. In order to improve collection efficiency in reactors, transparent materials such as glass and plexiglas have been used. In experimental studies performed indoors using fluorescent lamps, strip lights and halogen lamps (Pulz, 2007), solar light has been replaced by artificial light with an optimum range between 100 and 200 ⌈gE sec^{-1} m^{-2}, which corresponds to about 5/10% of full daylight (200 ⌈gE sec^{-1} m^{-2}) (Scott et al., 2010).

The production of carbon dioxide is from flue gases of either fossil fuel combustion or coal and natural gas power plants. In power plants CO_2 concentrations usually range between 10% and 20%, with an average of 13%. (Sheehan et al., 1998; Green Fuels, 2007). Moreover, nitric oxide from flue gases can be a source of nitrogen for microalgae growth (Nagase et al., 2001).

Fresh or marine water medium is used for algal growth Examples are municipal wastewaters and wastewaters coming from power plants. These waters usually contain the inorganic nutrients such as nitrogen, phosphorus, nitrates, phosphates and metals like chromium, lead, iron, zinc, which can be used as microalgal nutrients. At the same time microalgae help to purify wastewaters, reducing the concentrations of these elements. For example, one microalga *has* been used as a single organism and in combination with bacteria to remove ammonium and soluble phosphate ions from municipal wastewater. Metals like iron, zinc, manganese, chromium, nickel, cadmium and cobalt have been removed by the same alga and *Scenedesmus* in a range of 64-100% in a continuous system (De-Bashan et al., 2004).

The minimal nutritional requirements can be estimated using the approximate molecular formula of the microalgal biomass, that is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$. Phosphorus must be supplied in significant excess because the phosphates formed complex with metal ions. Thus, not all the added P is available. Other micronutrients and sea water supplemented with commercial nitrate and phosphate fertilizers are commonly used for growing marine microalgae using a growth medium that is generally inexpensive.

Scragg *et al.* (2003) reported that the green alga, *C.vulgaris* has been proven to be one of the leading candidates and potential source for biodiesel production. Although there are a number of microalgae species capable of producing oils and lipids necessary for biodiesel processing, *C. vulgaris* was chosen since it is readily available and abundant in our country's natural environment. They are robust (Arroyo et al., 2011), have faster growth rate and high survivorship (Mohan et al., 2009). Research has shown that the potential oil yield of this microalgae species could range from 58,700 L/ha to 136,900 L/ha for microalgae with 30 and 70 percent oil by weight in biomass, respectively. Lipid content of microalgae, on the other hand, could be as high as 50 percent (Bilawan, 2010).

Chlorella vulgaris is a genus of single-celled green algae, belonging to the phylum Chlorophyta. It is spherical in shape, about 2 to 10 µm in diameter, and without flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll-a and -b in its chloroplast. Through photosynthesis, it multiplies rapidly, requiring only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce.

Compared to other groups of algae, *C. vulgaris* is easily grown because of its nature and simple nutrient requirements. Likewise, it is one of the fastest growing microalgae (Kim and Lee, 2011) with a doubling time of 19 h (Lv et al., 2010) thus, can be harvested daily. This supports the findings of Elumalai et al. (2011) who reported that microalgae are an economical choice for biofuel production because of availability and low cost. Their studies proved that biodiesel can be produced from freshwater (Temple tanks, Forest lagoons, Inland lakes, Rock ponds) microalgae (*Chlorella vulgaris*, *Senedesmis* sp). The Nile Red fluorescence method provided a rapid, easily manipulated and reliable way for *in vivo* quantification of neutral lipids in these microalgae. Results showed that the optimum drying period of microalgae biomass was determined to be 120 min. while the percentage of lipid content during n-Hexane soaking period for *Chlorella vulgaris* was 49% during 210 min. Extraction of lipid fragments was done by solvent extraction method from biomass. The fractions were analyzed for biodiesel under Fourier Transform Infra-Red Spectrometry (FTIR) and Gas Chromatography & Mass Spectroscopy (GC-MS).

Similarly, the lipid fractions from the biomass were extracted through solvent extractions and the fractions were analyzed for biodiesel under GC-MS (Elumalai et al., 2011). The percentage of lipids synthesized from *C. vulgaris* under light and dark conditions were analyzed and compared. As abiotic factors that might influence growth of *C. vulgaris*, the algae from dark sample were found to be rich in saturated fatty acid (capric acid, lauric acid & myristic acid) and considerable amount of PUFA (hexadecatrienoic acid, stearidonic acid, eicosapenaenoic acid, docosahexaenoic acid) compared to algae grown under light, implying that the algae grown in dark condition is an excellent source for high yield of saturated fatty acids (FA). Therefore, light can affect the composition of lipids.

The properties of biodiesel are largely determined by the structure of its component fatty acid esters. The most important characteristics include ignition quality (*i.e.* cetane number), cold flow properties and oxidative stability (Mata et al., 2010). Proper percentage of saturated FAs and unsaturated FAs is very important. They can make an excellent biodiesel. Unsaturated fats are excellent for cold weather biodiesel use because of lower gel point. (Deng et al., 2009).

Chlorella vulgaris is typically 5-10m in diameter and circular. The responsive ability of this microalga is largely due to its less complex structure, fast growth rate, and high oil content (Elumalai et al., 2011). Its small size also allows for a range of cost-effective processing options. It is easily studied under laboratory conditions. Once grown, the harvesting and transportation costs are lower than those of conventional crops. With such promising characteristics, *C. vulgaris* appears to be the only feasible future solution for replacing petrodiesel completely.

Production of *Chlorella vulgaris*

The biotechnology of *C. vulgaris* has gained considerable importance ranging from simple biomass production to valuable products for ecological applications. Its biomass production is useful in commercial level because, aside from lipids, it contains macromolecules such as proteins and nutrients. Biomass covers about 10% of the world's primary energy demand. It has the only potential to replace the supply of an energy hungry civilization.

Microalgal biomass contains approximately 50% carbon (derived from carbon dioxide) by dry weight (Sanchez et al., 2003). The production of 100 tonnes of algal biomass fixes roughly 183 tonnes of carbon dioxide, which must be fed continually during daylight hours. This feeding mechanism control, in response to signals from pH sensors, minimizes loss of carbon dioxide and pH variations. Biodiesel production can potentially use some of the carbon dioxide that is released in power plants by burning fossil fuels. This carbon dioxide is often available at little or no cost.

Microalgal biodiesel would be carbon neutral, as all the power needed for producing and processing the algae would come from biodiesel itself and from methane produced by anaerobic digestion of biomass residue left behind after the oil extraction. Although carbon neutral, microalgal biodiesel will not result in any net reduction in carbon dioxide that is accumulating as a consequence of burning of fossil fuels.

Large quantities of biomass that is harvested, and pre-treatments are used to reduce water content and increase the energy density in the algae paste. This is the requirement for the integrated production of microalgal biodiesel. Separation of the oil from the paste follows whether by a chemical process or by pressing in a high-pressure device such as a screw press. Algal oil finished product is in a form that is then suitable for use in the transesterification reaction to make biodiesel fuel. Microalgae can provide renewable biofuels from different sources and processes, such as: methane produced by anaerobic digestion of the algal biomass; biodiesel derived from microalgal oil, and photobiologically produced biohydrogen (Hossain et al. , 2008).

Large-scale production of microalgal biomass generally uses continuous culture during daylight in which fresh culture medium is fed at a constant rate and the same quantity of microalgal broth is withdrawn continuously (Molina et al., 1999). End of the feeding is during nighttime, but there should be continuous mixing of broth to prevent settling of the biomass. As much as 25% of the biomass produced, during daylight, may be lost at night because of respiration. The extent of this loss depends on the light level under which the biomass was grown, the growth temperature, and the temperature at night.

Not many studies have been done with regard to mass cultivation of *C. vulgaris* in the Philippines. However, many foreign studies have been documented.

Patil et al (2008) said that the integrated production of microalgal biodiesel requires large quantities of biomass that is harvested, and pretreatments are used to reduce water content and increase the energy density in the algae paste. The oil is then separated from the paste wither by a chemical process or by pressing in a high-pressure device such as a screw press. The finished product is algae oil in a form that is then suitable for use in the transesterification reaction to make biodiesel fuel. According to Hossain et al. (2008) microalgae can provide renewable biofuels from different sources and processes, such as: methane produced by anaerobic digestion of the algal biomass; biodiesel derived from microalgal oil, and photobiologically produced biohydrogen.

Chlorella vulgaris has a great potential as a resource for biodiesel production due to faster growth and easier cultivation. However, lipid content in *Chlorella vulgaris* under general growth conditions is up to ~20% by weight of dry biomass (Illman et al., 2000; Spolaore et al., 2006), which cannot meet the standard industrial requirements. This is the reason why open pond system, closed system and vinasse are considered for the large-scale production of *C. vulgaris*.

Open Pond System

For microalgae cultivation , Aisyah (2009) identified the open pond system as one general method. This open system can be categorized into natural waters, such as lagoon, lake, or pond, and artificial ponds or container. The most commonly used artificial ponds are raceway ponds **(**Borowitzka, 1999). Large-scale cultivation of microalgae may be 10-20 times more productive on a per hectare basis than other biofuel crops and they are able to use a wide variety of water sources (Sharma et al., 2012).

The raceway pond is made of a closed loop circulation, oval shape recirculation channel that is typically about 20-50 cm deep. This is usually built in concrete, but compacted earth-lined ponds with white plastic. This consists in a racetrack with microalgae mixture, which is in a permanent circulation. The cover surface is usually between 1000 and 5000 m_2 (Travieso et al., 2001). The water is kept shallow (at around 30 cm deep) in order to give a proper exposition of the microalgae to sunlight.

To stabilize algae growth and productivity, mixing and circulation are produced by paddlewheel. In a symposium, Rengel (2008) reported that baffles placed in the flow channel guide the flow. During daylight, the culture is fed continuously in front of paddlewheel where the flow begins. Broth is harvested behind the paddlewheel, on completion of the circulation loop.

The paddlewheel operates all the time to prevent sedimentation. The microalgae CO_2's requirement is usually satisfied from the surface air, but submerged aerators may be installed to enhance CO_2 absorption. While the algae are flowing, nutrients like phosphorus and nitrogen are supplied into the system as either fertilizers or wastewaters (Sheehan et al., 1998; Van Harmelen and Oonk, 2006). Ponds can be designed as open or enclosed. With enclosed ponds, undesirable contamination with other microorganisms is avoided, however building costs are higher. Ponds are open in order to increase sunlight exposure. Carbon dioxide is injected as bubbles and in counter-current flow by diffusers and sumps (Van Harmelen and Oonk, 2006). Microalgae is harvested when the flow finishes the loop, just before the paddlewheel (Chisti, 2007).

One technology of cultivating *C. vulgaris,* as a group of Indian microbiologists (Mohan et.al 2009) suggested, involves the use of a suitable growth medium in a large-scale High Rate Algal (HRA) pond that was manually constructed to analyze the algae isolated from industrial effluents. The study shows that the pigments,ß-carotene levels increased with increase in number of days, and with the decrease in chlorophyll a levels. Moreover, the algae contained higher lipid than carbohydrates and proteins. *Chlorella vulgaris* cultivated outdoors had a very high growth rate of 0.16 while its algal biomass was harvested by settling using flocculants and auto-flocculation.

Wastewaters provide cost-effective and sustainable means of algal growth for biofuels (Pittmann, 2011). In his study, he reported that wastewaters derived from municipal, agricultural and industrial activities potentially provide the means of algal growth for cost-effective microalgal biofuel production. In addition, there is also potential for combining wastewater treatment by algae, such as nutrient removal, with biofuel production. Currently, the technology for algae biofuel production in connection with wastewater treatment makes use of facultative ponds. However, these ponds have low productivity (10 tonnes/ha/y), are not amenable to cultivating single algal species, require chemical flocculation or other expensive processes for algal harvest, and do not provide consistent nutrient removal. Instead, Craggs, et al. (2011) used shallow, paddlewheel-mixed high rate algal ponds (HRAPs). These ponds have much higher productivities (30 tones/ha/y) and promote bioflocculation settling which may provide low-cost algal harvest. The HRAP algae showed the potential to double production (to 60 tonnes/ha/y), improve bioflocculation algal harvest, and enhance wastewater nutrient removal. Moreover, algae biofuels (e.g. biogas, ethanol, biodiesel and crude bio-oil) were produced.

In a similar study by Park (2010), he investigated the influence of CO_2 addition on wastewater treatment performance and algal production of two pilot-scale HRAPs operated with different hydraulic retention times. The study shows that the algae biomass produced in the HRAPs was efficiently harvested by simple gravity settling units.

With BG11 as an expensive standard medium for algal growth, Ambrocio et al. (2010) studied the use of swine wastewater (SWW) as an alternative culture medium for *Chlorella vulgaris.* After the initial steps - collection of the SWW, media preparation and formulation using different concentrations of SWW and cultivation of *C. vulgaris,* biomass was weighed and finally, oil was then extracted by using the solvent extraction method with hexane as the extraction solvent. BG11 was the control being used as the standard medium that can effectively transfer and maintain nutrients required by the algae, but expensive. A comparative study on different ratios (full wastewater, ½ waste water to ½ tap water, ¼ wastewater to ¾ tapwater and 1/8 wastewater to 7/8 tapwater) of waste water to tap water was undertaken to analyze the potential growth and oil production of *Chlorella vulgaris* against algae grown in BG11. One-fourth wastewater concentration was then chosen and used for mass production because of results on optical density and cell count. Oil extracted from the algae in swine wastewater is less than that from BG11, however, the percentage of oil is statistically the same at $\alpha = 0.05$. This concludes that swine wastewater could support the growth and oil production of the *Chlorella vulgaris* comparable to BG11 although studies on incorporating essential nutrients to the SWW medium should be further conducted for a more efficient mass rearing of *C. vulgaris.*

Aisyah (2009) enumerated some of the advantages of using open pond to cultivate microalgae. It is a cheaper method of large-scale microalgae biomass cultivation. It involves a lower energy input. It can be implemented in areas with marginal crop production potential. Finally, using an open system involves an easier regular maintenance and cleaning. The biggest advantage of using the open ponds is its relative simplicity in structure, with biomass yields at low production costs and operational management.

However, the application of open pond to cultivate microalgae still has disadvantages. The efficiency and oil yield of the raceway ponds are significantly lower than the photobioreactors (Chisti, 2007). Not all species of algae are capable of growing in open ponds, due to easy contamination by other algae and bacteria. This is why there is still small number of microalgae species capable of growing in the open ponds. There is some difficulty to control the temperature and the amount of light in the ponds (Sheehan et al., 1998). Due to evaporation, temperature fluctuation in the growth media, CO_2 deficiencies, inefficient mixing, and light limitation, open pond systems are less efficient with respect to biomass productivity. Moreover, temperature fluctuation and seasonal variations are difficult to control.

Closed System

The closed system is another method of growing algae. It involves the use of a closed photobioreactor systemwhich houses and cultivates algae, providing them a suitable environment for growth, and supplying light, nutrients, air, and heat to the culture. A photobioreactor is an enclosed, illuminated culture vessel designed for controlled biomass production of phototrophic liquid cell suspension cultures. It can be in tubular, plastic bags, vertical column and annular forms

Installed in array, tubular photobioreactors consist of a set of transparent pipes made of glass, acrylic or plexiglas. The system works in a close-loop where the microalgae culture flows continuously. The microalgae, nutrients, CO_2 and water are fed to a vessel, from where these are pumped to the tubes. A harvesting system is installed at the end of the circuit, usually constituted by a filter, where algae are collected according to maturity.

Among the different types of photobioreactor available, the tubular type variety is among the most common because this has been used on a small scale for numerous laboratory assays (Gao, et.al., 2009). Christi (2007) reports that this design calls for a solar collector consisting of an array of tubes containing the cell suspension, each 0.1 m in diameter. During daylight hours, microalgae broth must be circulated throughout the system, and a high turbulence flow must be maintained at all times to prevent biomass sedimentation.

In a review made by Kunjapur and Eldridge (2010), they described design systems used to cultivate microalgae for biofuel production. The review addresses general design considerations pertaining to reactors that use natural light and photosynthetic growth mechanisms, with an emphasis on large-scale reactors. Important design aspects include lighting, mixing, water consumption, CO_2 consumption, O_2 removal, nutrient supply, temperature, and pH. One of the greatest challenges of closed photobioreactor design is how to increase reactor size in order to benefit from economy of scale and produce meaningful quantities of biofuel. Lassing (2008) reported that the closed photobioreactor system consists of a number of transparent reactors designed to maximize the absorption of the incoming light and to minimize negative effects such as oxygen oversaturation.

Mulumba and Farag (2005) designed photobioreactors for high yield algal biomass production. Suitable algal strain was selected and screened for fast growth and high oil content by observation of three weeks. One algal strain has been selected for the rest of the study. The tubular photo-bioreactor (TPBR), designed and mounted for algal biomass production consist of clear PVC tubing mounted in two spiral, main tank containing algal solution, fluorescence lamps as source of light, carbon dioxide and air sources, and pump which keeps the algal broth in motion in order to avoid microalgae biomass sticking on tubing hall. Growth requirements such as carbon dioxide, nutrients with water placed in main tank and source of photons were provided. Results show that the yield in microalgae biomass in this

TPBR is four times higher than the yield of microalgae biomass in a cylindrical batch reactor or an open pond. The TPBR is a closed system, thus it lessens risks of contamination.

The use of three bubble column photobioreactors showed an increase of biomass production of *Chlorella vulgaris* Buitenzorg up to 1.20 times and a decrease of the ability of CO_2 fixation compared to single reactor at a periodic sun illumination cycle (Wijanarko, et al., 2008). Other research parameters such as microbial carbon dioxide transferred rate (qco2), CO_2 transferred rate (CTR), energy consumption for cellular formation (Ex), and cultural bicarbonate species concentration [HCO_3] also give better results on series of reactor.

According to Lassing (2008), photobioreactors provide high productivity per area of land and per volume. High algae content per volume makes separation easier and cheaper, because less water per kg dry biomass has to be removed. Likewise, contamination is easier to prevent from other microalgae since the system is closed to the environment. Moreover, there are small evaporative losses of water compared to open systems. Photobioreactors require much smaller surface area than the raceway ponds, which helps minimize the concerns on the use of lands to produce fuel, instead of food. The production of biomass and oil are also significantly higher than in the open ponds. Chisti (2007) calculates that for an annual production of 100 tonnes biomass and consuming the same amount of CO_2 for cultivating microalga with high oil yield (70% by weight oil in biomass), photobioreactors provide higher oil yield per hectare ((136.9 m^3 ha^-l) than the raceway ponds (99.4 m^3ha^-1).

However, there are some disadvantages in the use of this system. Cooling is needed to prevent the system from overheating. There are also problems due to oversaturation of oxygen. Cleaning is also a problem due to bio-adhesion on the inside of transparent surfaces. Finally, the system entails an expensive construction that is complex to build.

Alternative treatment for vinasse and cultivation of Chrlorella vulgaris

During distillation of fermented sugarcane juice for ethanol production, vinasse is a byproduct generated at approximate rates of 13 L vinasse / L alcohol. Vinasse's composition is well-varied, due to the components of the juice used in the alcoholic fermentation. During the fermentation process, the residues are water, organic matter, potassium (K) and sulfur (as sulfate).

There are GHG emissions at all stages of the sugar cane cycle. These gases are CO_2, CH_4 and N_2O, which show different global warming potentials (CO_2 = 1, $_{CH4}$ = 23 and N_2O = 296). The treatment of vinasse in anaerobic digesters to produce CH_4 can prevent the emissions of GHGs to the atmosphere, and consequently reduce the "carbon footprint" of ethanol production from sugar cane. Biological treatments can be used to clean the vinasse previously treated in anaerobic digesters, with potential of reusing it safely.

Vinasse digestion is an alternative method not so widely used and studied. Vinasse reduces its biological oxygen demand (BOD) and chemical oxygen demand (COD), through anaerobic reactors. The main residue is biogas, a mixture of CH_4 and CO_2 gases (55 and 45%, respectively) produced through oxidation of organic matter in the absence of oxygen, by the methanogenic bacteria. Vinasse is an alternative method to show low power consumption, small scale production of sludge for disposal, high efficiency in reducing the organic load and lower pollution potential, since the biogas produced can be used for co-generation power. The digested vinasse is used for fertirrigation of the sugar cane crop, since methanogenic bacteria remove only part of the organic carbon load during the fermentation.

Based on these characteristics, vinasse is a potential culture medium for many microorganisms. An important alternative is cultivating microalgae in the digested vinasse. However, to use vinasse as a complete culture medium for microalgal growth, it is very important to know its chemical composition, which can be compared with the nutritional requirements in terms of N, P and S to the metabolism of microalgae. Thus, vinasse is a potential cultivation medium and source of nutrients to the microalgae. Since CO_2 is needed to carry out photosynthesis, microalgae biomass is "fertilized" with CO_2, which does not spread quickly in liquid medium. The injection of CO_2, produced by burning of the CH_4 that is released in the anaerobic digestion of the vinasse, can be used as carbon source to the photoautotrophic microalgae, instead of being emitted into the atmosphere.

Oil Extracted from *C. vulgaris*

There are no other feedstocks that possess the high oil yield as algae, making them the ultimate energy source of the future especially in the transport sector. On top of this, algae are biodegradable and the oil is relatively harmless to the environment. The biodiesel produced will help reduce the carbon emission in the atmosphere (carbon footprint) because biodiesel does not emit much of the greenhouse gases compared to commercial petroleum.

Jacobs and Venter (2010) reported that algae, appropriately termed as microalgae, really are advantageous to use. They produce 2000 gallons of biofuel per acre in a year leading the other plant crops. The biofuel yield per acre per year of these crops are: , 650 gallons for palm, , sugar cane with 450 gallons, corn with 250 gallons and 50 gallons from soy. The total yield per year of these four crops is 1400 gallons per four acres, which is 600 gallons less than the microalgae yield. Compared to corn, soybean, canola, *Jatropha*, coconut and oil palm, microalgae (for b and c groups, 70% & 30% oil by weight) were found to have the highest oil yield at 136,900 and 58,700 L/ha, respectively. From the list of the different plants' oil yield, next to microalgae is oil palm at a very low oil yield of 5950 L/ha. This implies that microalgae appear to be the only source of biodiesel with the potential to completely displace fossil diesel. In a review made by Christi (2007), he reported that unlike other oil crops, microalgae are exceedingly rich in lipids and commonly double their biomass within 24 h. He further said that biomass doubling times during exponential growth are commonly as short as 3.5 h. As he presented in his review, oil content in microalgae can exceed 80% by weight of dry biomass and oil levels of 20–50% are quite common. The top 3 of these microalgae with high oil content are *Botryococcus braunii* (with 50-77 oil), *Nannochloropsis* sp. (with 31-68 oil) and *Neochloris oleoabundans* (with 35-54 oil).

The mass of oil produced per unit volume of the microalgal broth per day, otherwise known as oil productivity, depends on the algal growth rate and the oil content of the biomass. Thus, microalgae with high oil productivities are desired for producing biodiesel. The production of many different kinds of lipids, hydrocarbons and other complex oils is dependent on species. Not all algal oils are satisfactory for making biodiesel, but suitable oils occur commonly. The use of microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops. Potentially, instead of microalgae, oil producing heterotrophic microorganisms (Ratledge, 1993; Ratledge and Wynn, 2002) grown on a natural organic carbon source such as sugar, can be used for making biodiesel. However, the production of heterotrophic organisms is not as efficient as using photosynthetic microalgae. The renewable organic carbon sources required for growing heterotrophic microorganisms are produced ultimately by photosynthesis, usually in crop plants.

Microalgae contain two types of lipids, neutral and polar lipids (Dejoye, et al., 2011). The polar lipids are phospholipids and glycolipids; phospholipids are concentrated in the membrane structure while glycolipids are predominant in the membranes of photosynthetic organisms. Neutral lipids, which are essentially mono, di and triacylglycerol, are stored in cell organelles such as chloroplasts following a deficiency. Polyunsaturated fatty acids are rarely free in the cell, but are mainly located in lipid reserves (triglycerides). Microalgae use photosynthesis to fix their carbon dioxide by the enzyme Rubisco.

Various solvents are used to extract these lipids. More and more organic solvents used in industries were forbidden because of their toxicity and reduction of the ozone layer. In particular, the Volatile Organic Compounds (VOCs), usually composed of carbon and hydrogen, can easily be in gaseous form in the atmosphere reaching the troposphere, and thus reduce the ozone layer. On the other hand, VOCs have direct health effects, even at trace level. The use of supercritical fluids as extraction solvents appears as a promising way of replacement, particularly in the prospect of biofuel production to improve environmental impact. The supercritical fluid mostly used is carbon dioxide; it is abundant, incombustible, sluggish chemically, non-toxic for the operators, inexpensive and it presents an accessible critical point (31 °C/7.4 MPa). The supercritical carbon dioxide can dissolve and extract polar and organic fatty acids. Manipulating the temperature and pressure above the critical points affects the properties of supercritical carbon dioxide and enhances its ability to penetrate and extract targeted molecules. There are several major drawbacks in the use of conventional methods to achieve separation and fractionation of different polyunsaturated fatty acids. The solvent extraction, possibly coupled with HPLC chromatography resin, leave solvent residues in the final product.

Dejoye, et al. (2011) also added that in order to improve the efficiency of lipid extraction, thermal pretreatment could be used. Microwave could be an alternative way because of its ability to deeply penetrate through the cell wall structure. Microwave irradiation has been used to extract the oil from the biomass. The rapid heating leads to assured high internal temperature, and pressure gradient acting on the cell wall may enhance mass transfer rates without inducing thermal degradation of lipids.

There are also a few well-documented procedures for extracting oil from microalgae, such as mechanical pressing, homogenization, milling, solvent extraction, supercritical fluid extraction, enzymatic extraction, ultrasonic-assisted extraction and osmotic shock (Mercer and Armenta, 2011).

Pressing and homogenization essentially involve using pressures to rupture the cell walls, in order to recover the oil from within the cells. Milling on the other hand, uses grinding media (consisting of small beads) and agitation to disrupt cells. These methods are usually used in combination with some kind of solvent extraction, which entails extracting oil from microalgae by repeated washing or percolation with an organic solvent. Hexane is a popular choice due to its relatively low cost and high extraction efficiency. Supercritical fluid extraction involves the use of substances that have properties of both liquids and gases (i.e. CO_2) when exposed to increased temperatures and pressures. This property allows them to act as an extracting solvent, leaving no residues behind when the system is brought back to atmospheric pressure and RT. Enzymes can also be used to facilitate the hydrolysis of cell walls to release oil into a suitable solvent. The use of enzymes alone, or in combination with a physical disruption method such as sonication, has the potential to make extractions faster and with higher yields. The use of sonnication alone can also enhance the extraction process immensely due to a process called cavitation. Ultrasonic waves create bubbles in the solvent, the bubbles burst near the cell walls of microalgae, which produce shock waves, causing the contents (i.e. lipids) to be released into the solvent. Osmotic shock, a less-employed procedure, makes use of an abrupt lowering of osmotic pressure that causes cells to burst and release their contents.

Mechanical disruption, which includes pressing, beadmilling and homogenization, is an approach that minimizes contamination from external sources, while maintaining the chemical integrity of the substance(s) originally contained within the cells. The pressing method involves subjecting the microalgal biomass (or more commonly, seeds or nuts) to high-pressure, which ruptures cell walls and releases the oil. Homogenization is the process of forcing biomass through an orifice, which results in a prompt pressure change as well as high shearing action. Bead milling entails vessels packed with very small beads that are agitated at high speeds. Biomass agitation within the grinding media (beads) results in damaged cells, where the degree of disruption depends mostly on contact between biomass and beads; also size, shape and composition of the beads, and strength of the microalgal cell walls (Doucha, and Lıvansky, 2008). Bead milling is generally used in conjunction with solvents to recover oil, and is most effective and economical when cell concentrations are significant and when extracted products are easily separated after disruption. Typically, this type of cell disruption is most effective and energy-wise when biomass concentrations of 100 to 200 g/L are used. In terms of finding an effective and efficient mode of disrupting cells, there are multiple options of using this kind of technology. Some options include the identification of biological features of the organism that make it possible to weaken the cell wall prior to mechanical disruption, such as pre-treatments (i.e. acid/alkali, and enzymatic), thus potentially minimizing the use of solvents.

Issues on Oil Production and Extraction of *C. vulgaris*

Chlorella has been evaluated as a source of algal oil (Christi, 2007). It is the best source of lipid for biodiesel production because of high productivity and growth rate. Moreover, it does not compete with agriculture for land and food resources. Although this microalga was chosen as one of the candidates for alternative fuel, scientists have encountered a major problem on the organism's cell wall. Because algal cells walls contain cellulose and phospholipids, they found it hard to find a means to split or dissolve the cell wall, so that the cytoplasm will be spilled along with the globules of lipids needed for the production of fuel or oil. This problem has been addressed in one discussion conducted by Dr. Ganti Murthy, a Professor, in Oregon State University. It was suggested that enzyme fermentation could dissolve the cell wall of the algae (Christi, 2007).

In a *Chlorella* community, there are noticeable parent and daughter cells. They have been categorized according to their sizes, 10 micrometers for the daughter cells and 10-25 micrometers for the parent cells. To be able to sustain and to increase the production of *Chlorella*, parent and daughter cells must be separated. The parent or the mature cells will be the one to undergo the process of fuel production while the daughter cells will be brought back to the place, often a pond where they were taken from, and be able to mature and reproduce. In order to separate the mature cells from the young ones, a two-step process is needed. The first step screens the mature cells and allows the juvenile cells to be transferred and returned to the pond. The second step involves the removal of water before the mature cells are ruptured. One thing recommended for this separation procedure is membrane technology. Cell wall of *Chlorella vulgaris* is a tough, fibrous material, which cannot easily be broken.

Another important thing to be considered is the enzymatic process involved in fuel production. This process is very important because it will greatly reduce the cost of degrading the algal cell walls and the refining of the algal oils. Enzymes are catalysts of chemical reactions. They tend to lower the activation energy needed for a certain chemical reaction, so the forward and backward processes will be hastened as possible. Though reactions are catalyzed, enzymes do not change the product; also the reaction is still in equilibrium.

It was discovered that a bacterium, *Paramecium bursaria* contains enzymes that act on *Chlorella*. Assuming that these enzymes operate on the cell walls of the algae, with the help of another enzyme, lipids along with carbohydrates catalyzed by a different enzyme will eventually change into nutrients. From this, it can be theorized that the second enzyme "snips" the long chain carbon esters from the glycerol molecule found in the algae. If these assumptions are true, then the enzymes needed for biofuel production from algae are already found.

Aside from these assumptions for fermenting *Chlorella*, there are more traditional ways of breaching the cell wall of the algae. First is low-pressure flash expansion where the mature cells are heated to the point when the water within the cells begins to form steam. Decreasing the pressure causes the steam to break through the cell walls, making the cell walls look like cups. Second is a high shear unit, which is used in industries to homogenize milk. Third is ultrasonic cavitation that is suitable for small samples.

Specially designed pumps can also create cavitations and vortexes necessary to rupture the cell walls.

After the cell walls have been broken, either by fermentation or high shear processing, the ruptured cell walls are immersed in a combination of water cells and cell walls. This is done to separate the solid particles from the liquid component. There are several filtration processes that can be used such as: mechanical, membrane and centrifugal technology. Membrane technology is considered the most promising among the three technologies. In this method, the broken cell walls are retained on the proximal side of the membrane while the liquid passes through the distal side of the membrane. If fermentation has been done (use of enzymes), a membrane filter is used to separate out the cell walls. The walls are pumped to fermentation process, ethanol will be produced, transesterification of the algal oil to biodiesel will be done.

Polishing process will be done after filtration. The filtrate, which includes crude algal oil, will be further processed until all the algal oil separates by the use of a membrane. Traces of water can still be observed. To be able to fully remove it, the liquid must be passed in an Amberlite medium, which absorbs water. This process is proven to remove .05% of water from the algal oil.

Processing *Chlorella* into algal oil is a multi-step process. Heater tanks will be used to further separate particulate matters from the fatty acid contents of the oil. The heated oil will then be passed to a three-stage filter with decreasing size meshes to be able to reach desired purity and refinement.

Unfortunately, lipid content of most microalgae is less than 20% of the dried biomass, which is low for industrial requirements. Hence, in 2010 a group of Italian researchers (Scarsella, Belotti, De Filippis and Bravi), (conducted a study using bubble column photobioreactors and varying conditions (autotrophic, mixotrophic and heterotrophic) in order to find the best condition to increase the lipid content of *Chlorella vulgaris*.

The *C. vulgaris* cultures were cultivated under different treatments in duplicates for 72 hours in several 500 mL bubbling tubes exposed to 13Klux of white light from two fluorescent lamps under 12:12 photoperiod. The BG11 culture medium was modified for mixotrophic and heterotrophic growth by adding 6 grams of glucose per liter of media. The results showed the highest lipid content in the mixotrophic cultivation at 16.6% followed by heterotrophic at 9% and by autotrophic at 6%. To further increase the lipid content, nitrogen and phosphorus concentrations were limited or deprived in the mixotrophic and heterotrophic cultivation. The highest lipid content was observed in the nitrogen limited and phosphorus deprived treatments at 39.4% (5.0 g/L) and 37.5% (0.05 g/L) for mixotrophic and heterotrophic cultivations, respectively. High glucose consumption was also observed in the treatments with both nitrogen and phosphorus limitation, compared to nitrogen limitation alone. More polar lipids (phospholipids in cell membranes), on the other hand, are produced in mixotrophic than heterotrophic cultivation due to the presence of high number of smaller cells with more membranes.

The results clearly show, considering both biomass and lipid productivity and lipid non-polar content, that for large-scale biodiesel production from *Chlorella vulgaris* cultures the best option appears to be mixotrophic nitrogen limited and phosphorus deprived growth conditions.

Ceciani et al. (2011) reported that the production of microalgae biomass in large-scale is one of the issues concerning the oil supply for use as biodiesel. Rich in lipids and fatty acids, the oil yield per hectare in some strains of microalgae is considerably higher than the most conventional crops such as oil palm, *Jatropha*, soybean and coconut. Oils found in microalgae cells show some physical and chemical properties similar to those of vegetable oils, and therefore, can be considered a potential raw material for the production of biodiesel (Chisti, 2007). Biodiesel from microalgae is a renewable energy source whose use does not contribute to the increase of GHG in the atmosphere, since its production and use represents a closed cycle of CO_2. Moreover, Wagner (2007) reported that it is biodegradable, non-toxic, can be safely handled, and contains no sulfur, benzene and other aromatic compounds. The cultivation of microalgae provides a number of other economic advantages such as relatively low costs for harvesting and transportation, lower cost of water, use of infertile soils as support for the system of cultivation, high efficiency of CO_2 photosynthetic fixation by area, and the ability to grow in saline simple media (Danielo, 2005).

Conclusion

The Philippines has initiated the use of *Cocos nucifera* and *Jatropha curcas* as sources of biodiesel production in order to solve the worldwide problem of petroleum shortage. As indicated in some of the studies presented, these two sources show some important disadvantages in terms of large-scale production of biodiesel. With such drawbacks, microalgae were used. As demonstrated here, microalgal biodiesel, specifically from *Chlorella vulgaris*, is technically feasible because it is the only renewable biodiesel that can potentially and completely displace liquid fuels derived from petroleum.

The review presents the two important methods for large-scale biodiesel production of *C. vulgaris*. Open pond systems, such as raceways, are less efficient in terms of biomass productivity. On the other hand, photobioreactors, particularly of the tubular type, have much greater productivity than raceways, and are likely to be used in producing much of the microalgal biomass required for making biodiesel. Likewise, photobioreactors provide a controlled environment that can be tailored to the specific demands of highly productive microalgae to attain a consistently good annual yield of oil. Vinasse, a byproduct generated during distillation of fermented sugarcane juice for ethanol production, has also been explored as a potential medium for the cultivation of *C. vulgaris*.

Some problems on oil production such as the nature of the cell wall, enzymatic process for fuel production and the amount of lipid content were also discussed.

In the Philippines, the prospect of research into the large-scale production of algal fuel is compelling and almost imperative. There is uncertainty with this prospect as well as the difficulty of the projection because the present national transport sector is very stringent. The National government has to work collaboratively with the University of the Philippines in perfecting a prototype model of the photobioreactor. Just like an algal culture living inside a closed vessel, the growth and development of the microalgal biofuel industry in the country will only progress given the right conditions and orientation (Bilowan, 2010).

Producing microalgal biodiesel economically has to improve substantially to make it competitive with petrodiesel, but the level of necessary improvement appears to be attainable. Genetic and metabolic engineering are important to improve algal biology in order to produce low-cost microalgal biodiesel. Moreover, use of the biorefinery concept and advances in photobioreactor engineering will further lower the cost of production.

Considering the photosynthetic organisms, microalgae are the most efficient in the absorption of CO_2 and their growth is directly related to the reduction of GHGs, since they require large quantities of CO_2 as carbon source. Given optimal conditions, microalgae can double its volume within hours. By comparing species of microalgae (up to 70% oil by dry weight) with the oleaginous crop of higher oil production – palm tree – they show a benefit of approximately 23 times in oil yield (Chisti, 2007). *Chlorella vulgaris*, a species of microalga , isdescribed as one of the most efficient producers of oil.

Microalgae are a very promising source of biodiesel, whose use and production represents a closed cycle of CO_2. Industrial or agricultural wastes can be reused and recycled through the cultivation of microalgal species. This would also qualify the cultivation of microalgae as a Clean Development Mechanism (CDM), or technological alternatives for development of clean energy sources which do not emit CO_2, or that may reduce the levels of the GHGs.

The fast-growing rates of microalgae favor the extraction of oil in large-scale although some barriers need to be overcome, which are: select the right algae species, create the photobiological formula for each species and build a low cost effective photobioreactor that can induce a highly efficient microalgal growth (Patil et al., 2008). The global interest in clean and sustainable technologies is ensured by a search of how to identify oil-rich algae and develop processes for extracting algae oil and other products economically.

LITERATURE CITED

Ables, R.C. (2001). Coconut Methyl Ester (CME) as Petrodiesel Quality Enhancer. Department of Agriculture, Philippine Coconut Authority.

Aisyah, R. (2009). Open Pond Production of Microalgae in Microalgae Cultivation. Retrieved 11 January 2012 from: http://www.researchalgae.com/cultivation/open-pond-production-of- microalgae.html.

Albano, A.B.; Kasahara, E.S.; Aggangan, N.S.; Ragragio, E.M.; Pampolina, N.M.; Torreta, N.K. (2007). "Growth and survival of *Jatropha curcas* L. in marginal and mine soils as affected by mycorrhizal inoculation".Transactions of the National Academy of Science and Technology Philippines 29(1):5.

Ambrocio, J. E., L. I. M. Angeles and E. Hernandez III. (2010). Swine Wastewater as an Alternative Culture Medium for Mass Production and Oil Production of *Chlorella vulgaris.* Unpublished manuscript.

Araujo, G. S.; R. H. R. Carvalho and E. M. B. D. de Sousa. (2009). Crude Coconut Oil for Biodiesel Synthesis. International Workshop Advances in Cleaner Production: Key Elements for a Sustainable World: Energy, Water and Climate Change.

Argamosa, F.R.M. (2007). *Jatropha curcas*: A strategic feedstock in the development of the Philippine biodiesel industry. In: Proceedings of the Symposium on Biofuels: Way to go Forward. National Academy of Science and Technology, pp.19-23.

Arroyo, T. H. W. Wei, R. Ruan, B. Huc. (2011). Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials. Biomass and Bioenergy 35: 2245-2253.

Benge, M. (2006). Assessment on the potential of *Jatropha curcas*, (biodiesel tree), for energy production and other uses in developing countries. ECHO Website. (CANNOT FIND IN THE ECHO website)

Bilowan, Jay. (2010). Microalgae: The ultimate source of biodiesel in the future?UPLB. Retrieved 20 January 2012 from: http://www.zambotimes.com/archives/25024-Microalgae-The-ultimate-source-of-biodiesel-in-the-future-UPLB.html

Bryan, Tom. (2004). U.S. helping Philippines develop coconut biodiesel. Biodiesel Magazine. Retrieved 14 January 2012 from: http://www.biodieselmagazine.com/articles/520/u.s.-helping-philippines-develop-coconut-biodiesel/

Borowitzka, M. (1999). Commercial production of microalgae: ponds, tanks, tubes and fermenters. Journal of Biotechnology70 (1-3):313-321.

Cenciani, K.; M.C. Bittencourt-Oliveira; B.J. Feigi and C. C. Cerri. (2011). Sustainable production of biodiesel by microalgae and its application in agriculture. Afr. J. Microbiol. Res 5(26): 4638-4645.

Chaudhary, D.R.; Patolia, J.S.; Ghosh, A.; Chikara, J.; Boricha and G.N.; Zala, A. Changes in soil characteristics and foliage nutrient content in Jatropha curcas plantation in relation to stand density in Indian wasteland, Gujarat, India: Discipline of Phytosalinity, Central Salt and Marine

Chemicals Research Institute. Retrieved 6 January 2011 from: www.fact-foundation.com/.../Patolia-Nutrient_analysis_in_soil_and_plant-spacing.pdf.

Chitra, P.; Venkatchalam, P.; Sampathrajan, A.(2005). "Optimisation of experimental conditions for biodiesel production from alkali-catalysed transesterification of *Jatropha curcas* oil". Energy for Sustainable Development 9(3): 13-18.

Christi, Y. (2007). Biodiesel from Microalgae. Biotechnology Advances 25:294-306.

Craggs, R.J., S. Heubeck, T. J .Lundquist and J. R. Benemann. (2011). Algal biofuels from wastewater treatment high rate algal ponds. Water Science and Technology 63 (4): 660-665.

Danielo O (2005). An algae-based fuel. Biofutur ,255: 1-4.

De-Bashan, L.E., A. Trejo, V.A.R.Huss, J.P.Hernandez, and Y. Bashan. (2004). Chlorella sorokiniana UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. Science Direct. Bioresource Technology 99:4980–4989.

Dejoye, C., M. A. Vian; G. Lumia; C. Bouscarle; F. Charton and F. Chemat. (2011). Combined Extraction Processes of Lipid from *Chlorella vulgaris* Microalgae: Microwave Prior to Supercritical Carbon Dioxide Extraction. Int. J. Mol. Sc. 12: 9332-9341.

Diaz, Rafael S. (2008). Biodiesel Featured Articles: Coconut Oil as Diesel Fuel vs Cocobiodiesel. Retrieved January 2012 from:http://www.thebioenergysite.com/articles/116/coconut-oil-as-diesel-fuel-vs-cocobiodiesel.

Demafelis, R. B. (2008). "Towards a Village-scale Biodiesel Processing of *Jatropha curcas* in the Philippines". Philippine Journal of Crop Science.33(1): 59-68.

Deng, X. Y. Li and X. Fei. (2009). Microalgae: A Promising Feedstock for Biodiesel. African Journal of Microbiology Research 3(13): 1008-1014.

de Vries, E. "Future of biodiesel? A look at the potential benefits of jatropha".(2007). Renewable Energy World.10(3). May/June Issue

Doucha, J., Lıvansky´, K. (2008). Influence of processing parameters on disintegration of Chlorella cells in various types of homogenizers. Appl. Microbiol. Biot 81, 431–440.

Elumalai, S, S. Baskaran, V. Prakasam, N. Senthil Kumar. (2011). Ultra Structural Analysis and Lipid Staining of Biodiesel Producing Microalgae - *Chlorella vulgaris* Collected from Various Ponds in Tamil Nadu, India. Journal of Ecobiotechnology 3(1): 05-07.

Elumalai, S., V. Prakasam and R.Selvarajan. (2011). Optimization of abiotic conditions suitable for the production of biodiesel from *Chlorella vulgaris*. Indian Journal of Science and Technology 4(2): 91-97.

Elumalai, S., R. Sakthivel and S. Ganesh Kumar. (2011). Ultra Structural and Analytical Studies of Biodiesel Producing Microalgae (*Chlorella vulgaris* and *Senedesmis* sp.) Collected from Tamil Nadu, India. Current Botany. 2(6): 19-25.

Escobar, E.C.; R.B. Demafelis; L.J. Pham.; L.M. Florece and M.G. Borines. (2008). Biodiesel production from Jatropha curcas L. Oil by transesterification with hexane as cosolvent. Philippine Journal of Crop Science 33(3): 1-13.

Gao, Y., C. Gregor., Y. Liang, D. Tang and C. Tweed. (2009). Algae Biodiesel. A Feasibility Report.

Gerpen Jo Van, (2005). Biodiesel processing and production. Fuel processing technology 86(2005): 1097-1107.

Canadian Renewable Fuel Association (CRFA). (2011). Green Fuels Award. Retrieved 20 December 2011 from: http://www.greenfuels.org/en/industry-information/greenfuelsawards.aspx

Harun, R., Singh, M., Forde, G. M., (2010). Danquah, M. K., Bioprocess engineering of microalgae to produce a variety of consumer products. Renew. Sust. Energ. Rev. 14,1037–1047.

Henning, R. K. (1998). 'Use of *Jatropha curcas* L. (JCL): A household perspective and its contribution to rural employment creation. Experiences of the Jatropha Project in Mali, West Africa, 1987 to 1997'. http://www.jatropha.de/harare98.htm. (WHEN I CLICK ON THIS, this article does not appear)

Hossain ABMS, Salleh A, Boyce AN, Chowdhury P, Naqiuddin M (2008). Biodiesel fuel production from algae as renewable energy. Am. J. Biochem. Biotechnol 4(3): 250-254.

Jacobs, E. and J. C. Venter. (2010). Algae Biofuels. Retrieved 26 January 2011 from: http://www.exxonmobil.com/Corporate/energy_vehicle_algae.aspx.

Illman, A.M., A.H. Scragg and S.W. Shales (2000). Increase
in Chlorella strains calorific values when grown in low nitrogen medium. Enzyme Microb Tech. 27: 631–635.

Kim, J. and J.Y. Lee. (2009). Growth Kinetic Study of *Chlorella vulgaris*.Retrieved 27 December 2011 from: http://www.aicheproceedings.org/2009/Fall/data/papers/Paper154015.pdf.

Kunjapur, A.M. and R.B. Eldridge. (2010). Photobioreactor Design for Commercial Biofuel Production from Microalgae. Industrial & Engineering Chemistry Research (49)8: 3516-3526.

Lardon, L., A. Helias, B. Sialve, J.P. Steyer, and O. Bernard. (2009). "Life-Cycle Assessment of Biodiesel Production from Microalgae." Environ. Sci. and Tech 43(17): 6475-6481.

Lassing, M., P. Mårtensson, E. Olsson and M. Svensson. (2008). Final Report On Biodiesel Production from Microalgae- A Feasibility Study – Department of Chemical Engineering, Faculty of Engineering, LTH, Lund University Sweden.

Li Q., Du W., Liu D.H. (2008). Perspectives of microbial
oils for biodiesel production. Applied Microbiology and Biotechnology 80:749-756.

Lv, JM., L.H. Cheng, X.H. Xu, L.Zhang and H.L. Chen. (2010). Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions. Bioresource Technology 10:6796-6804.

Maheshwari, Prof. R.C., and Dr. S.N. Naik. "*Jatropha curcas* [sic.] (Ratan Jyot) and Karanja as a Renewable Source of Biofuel." Amity University. Centre for Rural Development and Technology, Indian Institute of Technology. Retrieved 6 January 2012 from: <http://www.amity.edu/asnrsd/R.C.Maheswari.ppt>

Mata, T. M., A. A. Martins and N. S. Caetano. (2010). Microalgae for Biodiesel Production and Other Applications: A Review. Renewable and Sustainable Energy Reviews 14: 217–232.

Mendoza, T.; Castillo, E.; Aquino, A. (2007). "Towards making *Jatropha curcas* (tubang bakod) a viable source of biodiesel oil in the Philippines". Phil J of Crop Sci 32(1): 29-44.

Mercer, P. and R. E. Arment. (2011). Developments in oil extraction from microalgae. Eur. J. Lipid Sci. Technol 1-9.

Mohan, N., P. H. Rao, R. R. Kumar, S. sivasankaran and V. Sivasubramanian. (2009). Studies on mass cultivation of *Chlorella vulgaris* and effective harvesting of bio-mass by low-cost methods. Phyco Spectrum Inc. Journal of Algal Biomass Utln.1(1):29-39.

Molina, G. E. (1999). Microalgae, mass culture methods. In: Flickinger MC, Drew SW, editors. Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation, vol. 3.Wiley; p. 1753–69.

Molina, G. E, A.F.G. Fernández, C.F. García, Y. Chisti. (1999). Photobioreactors: light regime, mass transfer, and scaleup. J Biotechnol. 70:231–47.

Mulumba, N. and I. Farag. (2005). Biodiesel Production from Microalgae. Chemical Engineering Department, University of New Hampshire. United States of America.

Nagase, H., K. Yoshihara, K. Eguchi, Y. Okamoto, S. Murasaki, R. Yamashita, K. Hirata and K. Miyamoto. (2001) Uptake pathway and continuous removal of nitric oxide from flue gas using microalgae. Biochem. Eng J. 7: 241-246.

Park, J.B.K. and R. J, Craggs. (2010). Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. Water Science and Technology.61(3): 633-639.

Patil V, Tran KH, Giselrød HR (2008). Towards sustainable production of biofuels from microalgae. Int. J. Mol. Sci9: 1188-1195.

Philippine Council for Agriculture, Forestry and Natural Resources Research And Development (PCARRD),Department of Science and Technology (DOST). (2009). Jatropha Compendium of Information & Technologies. Los Baños, Laguna. 84p.

Philippine Forest Corporation. (2006). Philippines (Bicol) Embarks on *Jatropha* Production. Retrieved 12 January 2012 from: http://asiacleantech.wordpress.com/2007/08/24/philippines-bicol-embarks-on-jatropha-production/.

Pittman, J. K., A.P. Dean and O. Olumayowa . (2011).The potential of sustainable algal biofuel production using wastewater resources. Bioresource Technology.102(1):17-25.

Rao, M.P.H., R. R. Kumar, S. Sivasankaran and V. Sivasubramanian. (2009). Phyco Spectrum Studies on Mass Cultivation of Chlorella vulgaris and Effective Harvesting of Biomass by Low-Cost Methods. J. Algal Biomass Utln. 1 (1): 29 – 39.

Ratledge, C. (1993). Single cell oils — have they a biotechnological future? Trends Biotechnol 11:278–84.

Ratledge, C, J. and P. Wynn. (2002). The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Adv Appl Microbiol 51:1–51.

Rengel, A. (2008). 8th European IFSA Symposium held 6 - 10 July 2008 Clermont-Ferrand (France).

Sacchs, Goldman. (2007). Goldman Sachs invests in Texas biodiesel company. Biodiesel Magazine.

Sánchez, M. A, C. García M-C, C. A. Gómez, C. F. García, E. M. Grima and S. Y. Chisti. (2003). Stress tolerance and biochemical characterization of *Phaeodactylum tricornutum* in quasi steady-state continuous culture in outdoor photobioreactors. Biochem Eng J 16:287–97.

Scarsella, M., G. Belotti, P. De Filippis and M. Bravi. (2010). Study on the optimal growing conditions of *Chlorella vulgaris* in bubble column photobioreactors. Dept. of Chemical Engineering Materials Environment, Sapienza University of RomaVia Eudossiana 18, I-00184 Roma, Italy.

Scott, S. A., M. P. Davey, J. S. Dennis, I. Horst, C. J. Howe, D. J. Lea-Smith and A. G. Smith. (2010). Biodiesel from Algae: Challenges and Prospects. Current Opinion in Biotechnology 21:277-286.

Scragg, A.H., J. Morrison, and S.W. Shales. (2003). "The use of a fuel containing *Chlorella vulgaris* in a diesel engine ." Enzyme and Microbial Technology. 33(7): 884-889.

Sharma K.K., H. Schuhmann and P. M. Schenk. (2012). High lipid induction in microalgae for biodiesel production. Energies 5: 1532-1553.

Sheehan, J., T. Dunahay, J. Benemann and P. Roessler. (1998). A look back at the U.S. Department of Energy's aquatic species program – biodiesel from algae. National Renewable Energy pp. 81-97.

Spolaore P., C. Joannis-Cassan, and D. E., Isambert A.
(2006). Commercial applications of microalgae. Journal of Bioscience and ioengineering. 1389-1723.

U.S. Department of Energy • Office of Energy Efficiency and Renewable Energy. (2003). Biodiesel. Retrieved 2 January 2012 from: http://www1.eere.energy.gov/vehiclesandfuels/pdfs/basics/jtb_biodiesel.pdf.

Van Harmelen, T.. and H. Oonk. (2006). Microalgae Biofixation Process: Applications and Potential Contributions to Greenhouse Gas Mitigation Options. Report, International Network on Biofixation of CO_2 and Greenhouse Gas Abatement, The Netherlands.

Wagner L. (2007). Biodiesel from algae oil. Research report. Mora Associates Ltda,

Zarate, J.T.; Fernando, L.M.; Paterno, E.S. (2007).“Mykovam and plant growth promoting bacteria for growth enhancement of *Jatropha curcas*”. Transactions of the National Academy of Science and Technology Philippines. 29(1):82-83.

JOURNAL OF NATURE STUDIES
(formerly Nature's Bulletin)
ISSN: 1655-3179