Bioenergy: Biodiesel from Freshwater Green Microalgae and a Cyanobacterium Occurring in Ghana

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Abstract

Biodiesel from freshwater green microalgae, and cyanobacteria holds promise as an alternative to replace petroleum diesel to meet the energy demands for transportation, industry and domestic use. In this study, three green freshwater microalgae (*Closterium acerosum* Ehrenberg ex Ralfs, *Oedogonium ciliatum* Pringsheim ex Hirn, and *Spirogyra africana* (F.E.Fritsch) Czurda), and a freshwater cyanobacterium (*Chroococcus turgidus* (Kützing) Nägeli) occurring in Ghana, were assessed to verify their suitability for biodiesel production. Samples of the species were collected from the Weija Lake; and the green freshwater microalgae were cultured in Bold's Basal Medium while the cyanobacterium was cultured in BG-11 (Blue-Green Medium) medium, in photo-bioreactors for 14 days. Algal lipids were extracted from dry biomass of the species with hexane and diethyl ether, and biodiesel produced from the lipids by base-catalysed transesterification. The amount of lipid extracted from the species varied from 20.3 in *Spirogyra* to 38.2% dry wt. in *Oedogonium*. Biodiesel produced also varied from 6.7 in *Spirogyra* to 22.3% dry wt. in *Closterium*, and the values fell well within the range reported for such organisms around the world. We suggest that many more microalgae occurring in Ghana should be sampled for their lipid content in future work on biodiesel production and development in the country.

Introduction

The global economy, and for that matter the Ghanaian economy, literally depends on energy. An ever increasing global population which according to the United Nations (UN) in 2017 was estimated at 7.6 billion people, and which is expected to reach 8.6 billion in 2030 and 9.8 billion in 2050 (https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html), added to the desire for greater economic growth and human wellbeing have culminated in an ever increasing demand for energy worldwide. According to one estimate, with current trends and demands globally 60% more energy will be required in 2030 than today (Patil *et al*., 2008).

According to Antoni et al., (2007), and Patil *et al*., (2008) transportation is one of the fastest growing sectors in the world accounting for approximately 27% of primary energy. Apart from transportation, energy is also needed for industry, domestic and other miscellaneous uses. Transportation is dependent on crude oil which has limited reserves that are concentrated in only certain regions of the world (Ciubota-Rosie *et al*., 2008). In recent years nearly 80% of the world's energy needs is produced from fossil fuels (Schenk *et al*., 2008).

In Ghana, transportation and industry (particularly mining), are the fastest growing sectors of the economy (Onwona-Agyeman, 2013), which require a corresponding increase in demand for energy. Domestic use of energy derived from fossil fuel, is also growing steadily in the country. Demand for energy is projected

to continue to increase as the population of the country is also expected to increase. Based on UN (2017) projections Ghana's population is expected to reach 51 million in 2050 (https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html). Unexpectedly but fortunately, Ghana started producing crude oil (fossil fuel) in commercial quantities since October 2011. The production of crude oil and hence petroleum diesel is expected to meet the future energy needs of all sectors of the economy. However, the continued use of fossil fuels in Ghana and globally is not sustainable, simply because, they are finite resources. Furthermore, the combustion of fossil fuels results in pollution of the environment. It leads to emissions of greenhouse gases such as carbon dioxide (CO_2), sulphur dioxide (SO_2), nitrogen oxides (NO, NO_2), particulate matter and volatile organic compounds (VOC) (Hallenbeck & Benemann, 2002). Fortunately, there is a greater awareness about consequences of relying on fossil fuel to meet energy demands globally. Thus, there is a global desire, nowadays, to develop sustainable energy sources.

Energy to meet national and global needs and to reduce environmental problems must come from renewable and sustainable sources such as solar, wind, hydrogen, and biofuels. Biofuel is generally defined as gas, liquid, or solid fuel made up of, or derived from biomass. Biomass, according to Kulkarni & Dalai (2006), is one of the better sources of energy. Biodiesel obtained from biomass is an alternative to petroleum diesel. Biodiesel, mono-alkyl esters, is obtained from renewable sources, by the trans-esterification of triglyceride oil with monohydric alcohol. The transesterification process is not new, it had been demonstrated with vegetable oil by Duffy (1853); and Rudolf Diesel was the first to demonstrate the use of biodiesel from vegetable oil (peanut oil) to power his engine on 10th August 1893 in Augsbur, Germany (Biodiesel Day. Days Of The Year. https://www.daysoftheyear.com/days/biodiesel-day/; *Biodiesel Technical Information*, PDF, https://www.biodiesel.org). Biodiesel fuel can now be obtained from a variety of sources including: rice barn, corn oil, fish oil, algae (including blue-green algae), and waste cooking oil e.g., palm oil (Sharif *et al*., 2007).

Biomass has been the focus of many studies as an alternative energy source (Sharif *et al*., 2008). There is considerable amount of information indicating that algae are an alternative non-food biodiesel feedstock due to their high oil content and rapid biomass generation. According to Shay (1993), macro- and micro-algae generally have a higher photosynthetic efficiency than other plant-based biomass (e.g., *Glycine max* (L.) Merr. - soybeans, and *Jatropha curcas* L. - Jatropha) and are thus considered as one of the best sources of biodiesel. For example, according to Nielsen *et al.*, (1996) the maximum, specific growth rate of microalgal species is approximately 1 day^{-1} whereas for higher plants it is 0.1 day^{-1} or less. Indeed, according to one estimate the yield per acre, of oil from algae is over 200 times the yield from the best-performing land plant (Sheehan *et al*., 1998); and another estimate indicated that algae can produce 250 times the amount of oil per acre as soybeans (Murphy *et al*., 2013). Furthermore, as algae are very productive completing their entire life cycle in a few days (Demirbas, 2009) they do not compete for arable land and are thus preferred to other plant-based biomass feedstock (Mata *et al*., 2010). There is enough

evidence to indicate that algae are the most promising source of alternative energy source (Chisti, 1980; Kapdan & Kargi, 2006; Mata *et al*., 2010).

There has been a revival in the use of biodiesel worldwide in recent years largely because of the escalating price of petroleum, and more importantly the realisation that global warming is associated with the burning of fossil fuel (Sawayama *et al*., 1995). Bioenergy and in particular, biodiesel from algae is a better source of energy to replace fossil fuels (Mata *et al*., 2010). Many projects to provide bioenergy from algae are progressing in the Americas and Eurasia (Reijuders, 2009; Chisti, 2010; Frac *et al*., 2010; Wijffels & Barbosa, 2010; Khan *et al*., 2017). Yet, very little is being done in this regard in Ghana and the rest of West Africa. In Ghana, as far as we know, apart from a preliminary investigation to produce biodiesel from green marine macroalgae by Ameka *et al*., (2019), virtually no work has been done on the use of algae for biofuel production. There is, therefore, the need to initiate studies to select the most promising algal species from Ghana, and to carryout the needed research and development for the commercial production of biodiesel from microalgae in the future. The purpose of this study was, therefore, to verify the suitability of three freshwater green microalgae and a freshwater cyanobacterium from Ghana for biodiesel production.

Materials and Methods

Three species of freshwater green microalgae, *Closterium acerosum* Ehrenberg ex Ralfs, *Oedogonium ciliatum* Pringsheim ex Hirn, and *Spirogyra africana* (F.E.Fritsch) Czurda, and the freshwater cyanobacterium *Chroococcus turgidus* (Kützing) Nägeli, occurring in Ghana were used in this study. The microalgae were collected from the Weija Lake, near Accra (Fig. 1), using a 20 µm plankton net. Preliminary field identifications were made by the third author who is an experienced phycologist. The samples collected were observed and the preliminary identifications confirmed in the laboratory with a Leica 3300 light microscope, and publications by Cronberg & Annadotter (2006), Janse van Vuuren *et al*., (2006), Brieley *et al*., (2007) and Bellinger & Sigee (2010).

The microalgae were grown on agar slants, and later transferred aseptically to culture media.

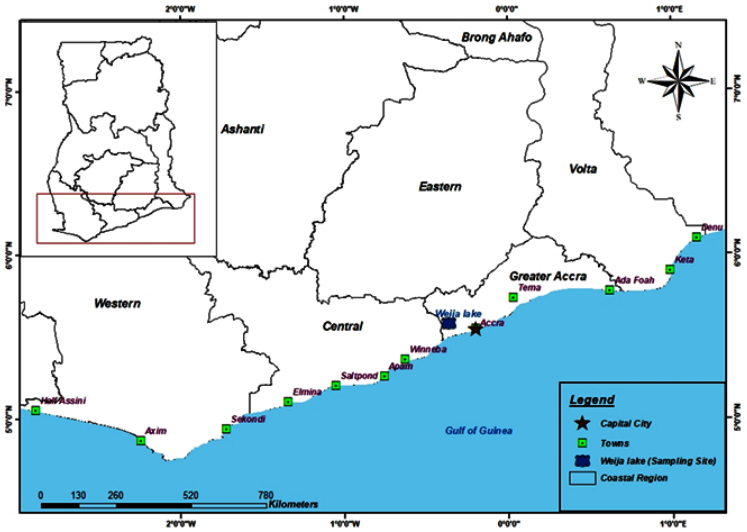

Fig. 1. Map of Ghana showing location of Weija Lake

The green microalgae were cultured in Bold's Basal Medium, and the cyanobacterium in BG-11 (Blue-Green Medium) medium following Stanier *et al*., (1971); in 2-litre reagent bottle photo-bioreactors (Singh & Sharma, 2012). The light source was provided by a 40 W fluorescent light under a light: dark cycle of 12: 12 h following Onwona-Agyeman (2013). The cultures were placed in an air-conditioned room with temperature set at 27.0 ± 2.0 ^{o}C. The microalgae were harvested every 14 days. *Oedogonium ciliatum* and *Spirogyra africana* cell suspensions were filtered to pass through a 2 mm sieve and the wet weight measured, in triplicate, with an analytical Mettler balance (Mettler AX 204); and oven dried at 80 ^{o}C for 6 hours according to Dejoye *et al.*, (2011). *Closterium acerosum* and *Chroococcus turgidus* were also filtered and their fresh weight determined in triplicate; and freeze dried overnight at -70 ^{o}C similar to the work of Belarbi et al., (2000) and Ziga *et al*., (2010). All dried samples were ground in a Kenwood electronic mill into a fine powder to pass through a 2 mm sieve.

Extraction of lipid from algal biomass and their trans-esterification to produce biodiesel followed closely the procedure by Demirbas (2009) and Raghuveer *et al*., (2015). Twenty grams each of dried pulverised green microalgae and cyanobacterium biomass were transferred into a 200 ml solvent mixture (1:1 v/v of hexane and diethyl ether), and stirred thoroughly on a magnetic stirrer for 24 hours at room temperature to extract lipids. For each sample the mixture was filtered to separate the biomass using a funnel and filter paper. The extracts were transferred into round bottom flasks and the solvents removed with a Cole-Palmer Rotary Evaporator under vacuum to release lipids. The weights of the lipids obtained were determined using a Mettler AX 204 analytical balance.

Sodium methoxide was produced by dissolving 1 g of sodium metal in 30 ml methanol in a 500 ml conical flask, and mixed thoroughly on a magnetic stirrer. Twenty millilitres of diethyl ether were used to wash the lipid produced into the sodium methoxide generated and stirred on a magnetic stirrer for 24 hours to produce biodiesel (fatty acid methyl esters) and sediment comprising mainly water, glycerol and pigments. The products of transesterification were transferred to a separating funnel and washed with 25 ml of distilled water. The biodiesel was separated from the sediment layer and dried with anhydrous magnesium sulphate ($MgSO_4$). The weight of the different components of sediment (glycerol and various pigments), and biodiesel were determined.

Statistical analyses were performed using R project software (www.r.org), on wet and dry weights, lipid and biodiesel yields, and sediment fraction data. One-way analysis of variance (ANOVA) was chosen for the comparison of each data set. Normality of the data were assessed using the Shapiro-Wilk normality test before ANOVA. Where the data were not normally distributed, they were log-transformed to achieve normality. When significant differences were identified following the ANOVA, Tukey HSD test was conducted to compare means. Statistical significance was reported at p-value of ≤ 0.05.

Results and Discussion

The wet weights of samples of three freshwater green microalgae (*Closterium acerosum*, *Oedogonium ciliatum* and *Spirogyra*

africana) and a freshwater cyanobacterium (*Chroococcus turgidus*) from which biodiesel was produced are shown in Table 1. The wet weights of the samples ranged from 65.1 g (*Oedogonium*) to 50.6 g (*Closterium*). The differences observed between wet weights of samples were significantly different. The wet weight of *C. turgidus* was significantly higher than that of *C. acerosum* ($p \leq 2.16 \times 10^{-5}$). Similarly, wet weight of *O. ciliatum* was significantly higher than that of *C. turgidus* ($p \leq 2.3 \times 10^{-2}$); and that of *C. turgidus* significantly higher than that of *S. africana* ($p \leq 2.48 \times 10^{-5}$); and that of *O. ciliatum* significantly higher than that of *C. acerosum* ($p \leq 2.3 \times 10^{-6}$); and that of *O. ciliatum* significantly higher than that of *S. africana* ($p \leq 2.5 \times 10^{-6}$). There was, however, no significant difference in wet weights of *S. africana* and *C. acerosum* ($p \leq 1.0$). The mean dry weight of samples of the species differed from each other (Table 1), and the mean value was highest in *Oedogonium* (36.40 g) and lowest in *Closterium* (20.19 g). The differences in dry weight values of samples were significantly different as follows: dry weight of *C. turgidus* was significantly higher than that of *C. acerosum* ($p \leq 9.0 \times 10^{-7}$). Similarly dry weight of *O. ciliatum* was significantly higher than that of *C. turgidus* ($p \leq 1.3 \times 10^{-4}$); and that of *C. turgidus* was significantly higher than that of *S. africana* ($p \leq 1.3 \times 10^{-4}$); and that of *O. ciliatum* was significantly higher than that of *C. acerosum* ($p \leq 0.0$); and that of *S. africana* significantly higher than that of *C. acerosum* ($p \leq 2.2 \times 10^{-4}$); and that of *O. ciliatum* significantly higher than that of *S. africana* ($p \leq 7.0 \times 10^{-7}$). The dry matter contents of the species were nearly the same (50 % dry wt.) except for *Closterium* and *Oedogonium* (Table 1). *Oedogonium* had the highest percent dry matter while *Closterium* was the lowest at 40% (Table 1). In their work, Sharif *et al*., (2008) showed that *Spirogyra* sp. and *Oedogonium* sp. had dry matter content of 33.0% and 34.8%, respectively. In the current study *Oedogonium ciliatum* and *S. africana* had much higher dry matter content than reported by Sharif *et al.* (2008); and in both cases *Oedogonium* dry matter was higher than that of *Spirogyra,* probably due to higher quantities of cellulose in cell walls of *Oedogonium*.

Algal lipids extracted from dried biomass of microalgae were used for biodiesel production, in this study, and not wet biomass. Johnson & Wen (2009) have demonstrated that biodiesel yield from microalgae after transesterification was much higher in dry biomass than from wet biomass. Sharif *et al*., (2008) and Kumar *et al*., (2011) used dried biomass in producing biodiesel from microalgae. In Table 2 are shown quantities of lipid (g) and biodiesel (g) produced by the three green algae and a cyanobacterium. *Closterium acerosum* (4.4 g) produced the highest quantity of lipid while *Spirogyra africana* (1.3 g) had the lowest.

The differences in the means of the lipid (g) produced by the microalgae and cyanobacterium were significant ($p \leq 2.99 \times 10^{-6}$). On dry weight basis the highest lipid content was obtained from *Oedogonium ciliatum* (38.2 %), while *Spirogyra africana* (20.3 %) was the lowest (Fig. 2). Again, the variation in the means of lipid yields (% dry wt.) were significant ($p \leq 4.25 \times 10^{-9}$). Similarly, with regard to quantity of biodiesel (g) produced, *Oedogonium ciliatum* 7.6 g was the highest, and *Sirogyra africana* 4.1 g had the lowest amount (Table 2).

The quantities of biodiesel (g) produced varied significantly ($p \leq 1.24 \times 10^{-5}$) among the species assessed. The amount of biodiesel

TABLE 1
Mean wet and dry weights (g) and percent dry matter (% dry wt.) content of three freshwater microalgae and a cyanobacterium from Ghana. [S.E. = Standard error]

Species	Wet wt. ± S.E.	Dry wt. ± S.E.	% dry wt.
Closterium acerosum	50.60 ± 1.25	20.19 ± 0.75	39.92
Chroococcus turgidus	61.37 ± 1.60	30.87 ± 0.10	50.30
Oedogonium ciliatum	65.10 ± 1.01	36.40 ± 1.23	55.91
Spirogyra africana	50.80 ± 0.87	25.33 ± 0.67	49.86

TABLE 2
Quantities of lipid (g), and biodiesel (g) produced from three freshwater green microalgae and a cyanobacterium from Ghana. [S.E = Standard error.]

Species	Lipid (g) ±S.E	Biodiesel (g) ±S.E
Closterium acerosum	4.4 ± 0.35	6.7 ± 0.40
Chroococcus turgidus	4.0 ± 0.17	5.0 ± 0.31
Oedogonium ciliatum	3.7 ± 0.35	7.6 ± 0.44
Spirogyra africana	1.3 ± 0.15	4.1 ± 0.36

produced on percent dry weight basis was also determined, and the results are presented in Fig. 3. *Closterium* with 22.3% dry wt. was the highest and *Spirogyra,* 6.7% dry wt. was the lowest. There were significant variations ($p \leq 5.85 \times 10^{-5}$) between the yield of biodiesel on percent dry weight basis of the samples of microalgae and cyanobacterium studied.

The amount of sediment (glycerol and various pigments) derived from the transesterification of the lipids is also presented in Fig. 4. The sediment fraction varied from 1.5% dry wt. in *Closterium* to 19.6% dry wt. in *Oedogonium*. Quantities of sediment fraction (% dry weight) varied among the species, and the differences were significant ($p \leq 2.92 \times 10^{-11}$). In *Closterium* and *Chroococcus* sediment quantities were low (Fig. 4) and this resulted in 94 and 80%, respectively of lipids being converted into biodiesel. With *Oedogonium* and *Spirogyra*, about 50 and 34%, respectively of lipids were converted into biodiesel. In a similar work by Demirbas (2009), biodiesel produced on dry wt. basis, by *Cladophora fructa* and *Chlorella protothecoides* were 14.2% and 29.4%, respectively. Demirbas (2009) also provided

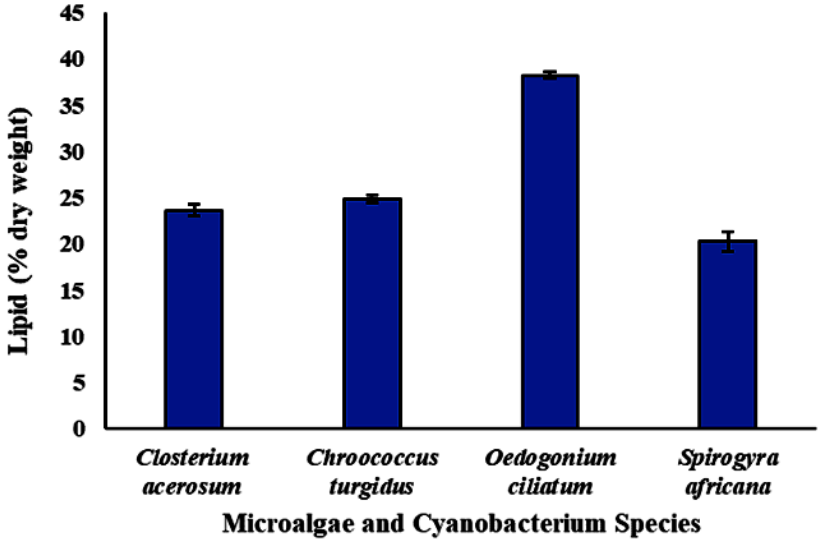

Fig. 2. Lipid content (% dry wt.) of three freshwater green microalgae and a cyanobacterium from Ghana. Error bar: Mean ± Standard error

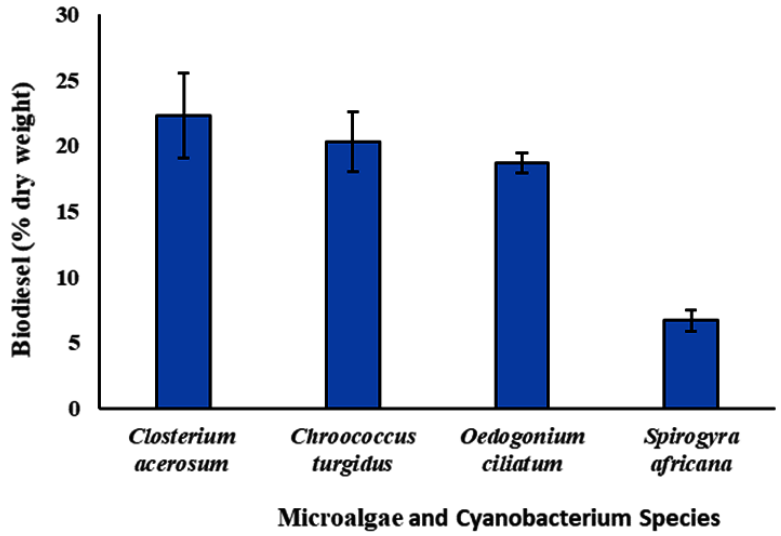

Fig. 3. Biodiesel content (% dry wt.) from three freshwater green microalgae and a cyanobacterium from Ghana. Error bar: Mean ± Standard error

data on the lipid content (% dry wt.), of some microalgae as follows:

Chlamydomonas rheinhandii 21; *Chlorella vulgaris* 14-22; *Euglena gracilis* 14-20; *Prymnesium parvum* 22-38; *Scenedesmus dimorphus* 16-40; *Spirogyra* 11-21; and for cyanobacteria as follows: *Anabaena cylindrica* 4-7; *Spirulina maxima* 6-7; *Spirulina platenisi* 4-9; and *Synechoccus* sp. 11. The lipid content (% dry wt.) of microalgae in the present study fell well within the range presented by Demirbas (2009). The lipid content of *Chroococcus*, the only cyanobacteria in the current study, was 24.9% dry wt. (Fig. 3) which is much higher than reported for some cyanobacteria by Demirbas (2009). It is important to indicate that the results from the different studies were based on different genera. Clearly different species of microalgae or cyanobacteria contain different amounts of lipid and produce different quantities of biodiesel.

Differences in amount of lipid accumulated by some algae depend on the availability of nitrogen in the culture medium. According to Griffiths & Harrison (2009), and Davey *et al*., (2014) high accumulation of lipids in some microalgae is influenced by nitrogen

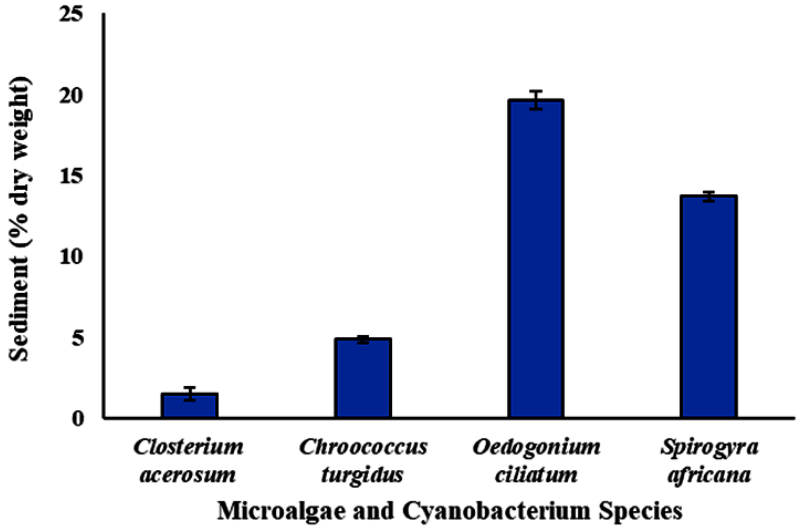

Fig. 4. Sediment fraction (% dry wt.) of three freshwater green microalgae and a cyanobacterium from Ghana. Error bar: Mean ± Standard error

starvation.

Ameka *et al*., (2019) in an earlier work produced biodiesel from marine macroalgae occurring in Ghana. However, the quantities of biodiesel obtained from five green marine macroalgae were much lower than obtained for the three freshwater microalgae and a cyanobacterium in the present study. With the green marine macroalgae biodiesel yield ranged from *Chaetomorpha antennina* 2.40 to *Chaetomorpha linum* 4.85% dry wt., while that for the freshwater microalgae was from *Spirogyra* 6.7 to *Closterium* 22.3% dry wt. Thus, the highest quantity of biodiesel on percent dry weight basis for the green marine macroalgae was lower than the lowest for the freshwater microalgae. Future studies for biodiesel production from algae in Ghana should, therefore, focus on the microalgae.

Conclusion

Petroleum fuels are not acceptable as energy sources for the future energy needs of humankind. This is due to the uncertainties concerning their availability and more importantly their contribution to global warming and, therefore, climate change. This has resulted in renewed interest in plant-based biomass derived oils for biofuel production. Currently, algae are the preferred plant-based biomass source for biofuels.

The current study has demonstrated that green microalgae and cyanobacteria from Ghana synthesized lipids that could be converted by transesterification to biodiesel to replace petroleum (fossil) fuels. Future studies should, therefore, focus on determining which species of microalgae in Ghana can produce high amounts of oil and therefore biodiesel.

References

Ameka, G. K., Doamekpor, L. K., Amadu, A. A. and Amamoo, P. A. (2019). Production of biodiesel from marine macroalgae occurring in the Gulf of Guinea off the coast of Ghana. *Ghana Journal Science*. **60**: 50-58.

Antoni, D., Zverlov, V. V. and Schwarz, W. H. (2007). Biofuels from microbes. *Appl.* Microbiol. *Biotechnol.* **77**: 23-35.

Belarbi, H., Molina, E. and Chisti, Y. (2000). A process for high and scalable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. *Enzyme Microb. Technol.* **26**: 516-529.

Bellinger, E. G. and Sigee, D. C. (2010). *Freshwater Algae: Identification and Use as Bioindicators.* John Wiley & Sons: London.

Briely, B., Carvalho L., Davies, S. and Krokowski, J. (2007). *Guidance on the quantitative analysis of Phytoplankton in freshwater samples.* Water Framework Directive, United Kingdom Technical Advisory Group (WFD UKTAG).

Chisti, Y. (2010). Fuels from microalgae. *Biofuels*. **2**: 233-235.

Chisti, Y. (1980). An unusual hydrocarbon. *J. Ramsay Soc.*, **81**: 27-28.

Ciubota-Rosie, C., Gavrilescu, M. and Macoveanu, M. (2008). Biomass – An important renewable source of energy in Romania. *Environmental Engineering and Management Journal.* **7(5)**: 559.

Cronberg, G. and Annadotter, H. (2006). *Manual of aquatic cyanobacteria. A photoguide and a synopsis of their toxicology*. ISBN 87-990827-0-5.

Davey, M. P., Horst, I., Duong, G-H., Tomsett, E. V., Litvinenko, A. C. P., Howe, C. J. and Smith, A. G. (2014). Triacylglyceride

Production and Autophagous Responses in Chlamydomonas reinhardtii Depend on Resource Allocation and Carbon Source. *Eukaryotic Cell*. **13(3)**: 392-400.

Dejoye, C., Vian, M, A., Lumia, G., Bouscarle, C., Charton, F. and Chemat, F. (2011). Combined extraction processes of lipid from Chlorella vulgaris microalgae: microwave prior to supercritical carbon dioxide extraction. *Int. J. Mol. Sci*. **12**: 9332-9341.

Demirbas, A. (2009). Production of Biodiesel from Algae oils. *Energy Source. Part A,* **31**: 163-168.

Duffy, P. (1853). XXV. On the constitution of stearine. *Quarterly Journal of the Chemical Society of London*, 5: 303. Doi: 1039/ QJ8530500303.

Frac, M., Jezierska-Tys, S. and Tys, J. (2010). Microalgae for biofuels production and environmental applications: A review. *African Journal of Biotechnology* **9(54)**: 9227-9236.

Griffiths, M. and Harrison, S. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol.* **21**: 493–507. http://dx.doi.org/ 10.1007 /s10811-008-9392-7.

Hallenbeck, P. C. and Benemann, J. R. (2002). Biological hydrogen production: fundamentals and limiting processes. *International Journal of Hydrogen Energy,* **27**: 1185-1193.

Janse van Vuuren, S., Taylor, J., Gerber, A. and van Ginkel, C. (2006). *Easy identification of the most common freshwater algae. A guide for the identification of microscopic algae in South African freshwaters.* ISBN 0-621-35471-6.

Johnson, M. and Wen, Z. (2009). Production of biodiesel fuel from the microalga Schizochytrium limacinum by direct transesterification of algal biomass. *Energy Fuels*. **23(10)**: 294-306.

Kapdan, I. K. and Kargi, F. (2006). Bio-hydrogen production from waste materials. *Enzyme Microb. Technol*. **38**: 569-582.

Khan, S., Siddique, R., Sajjad, W., Nabi, G., Hayat, K. M., Duan, P. and Yao, L. (2017). Biodiesel production from algae to overcome the energy crisis. *HAYATI Journal of Biosciences*. **24**: 163-167.

Kulkarni, M. G. and Dalai, A. K. (2006). Waste cooking oil - an economical source for biodiesel: A review. *Ind. Eng. Chem. Res.* **45**: 2901-2913.

Kumar, P., Sueela, M. R. and Toppo, K. (2011). Physico-Chemical characterization of algal oil: a potential biofuel. *Asian J. Exp. Biol. Sci.* **2(3)**: 493-497.

Mata, T. M., Martins, A. A. and Ceatano, N. S. (2010). Microalgae for biodiesel production and Rather applications: A review. *Renew. Sustain. Energy Rev.* **14**: 217-232.

Murphy, F., Devlin, G., Deverell, R. and McDonnell, K. (2013). Biofuel production in Ireland – an approach to 2020 targets with a focus on algal biomass. *Energies.* **6**: 6391-6412.

Nielsen, S. L., Enriquez, S., Duarte, C. M. and Sand-Jensen, K. (1996). Scaling maximum growth rates across photosynthetic organisms. *Functional Ecology*. **10**: 1676-175.

Onwona-Agyeman, R. (2014). *Sustainable Bioenergy: Biodiesel production from microalgae occurring in Ghana.* MPhil. Thesis, University of Ghana, Legon.

Patil, V., Tran, K-Q. and Giselod, H. R. (2008). Towards sustainable production of

Biofuels from microalgae. *Int. J. Mol. Sci.* **9**: 1188-1195.

Raghuveer, D., Vijay, M., Sreeraulu, D., Suman, P. and Kumar, S. A. (2015). *Production of Biodiesel from renewable biological resources*. International Conference on Biotechnology, Nanotechnology & Environmental Engineering (ICBNE'15) April 22-23, 2015, Bangkok, Thailand.

Reijnders, L. (2009). Microalgal and terrestrial transport biofuels to displace fossil fuels. *Energies.* **2**: 45-56.

Sawayama, S., Inoue, S., Dote, Y. and Yokoyama, S. Y. (1995). CO_2 fixation and oil production through microalga. *Energy Convers Manage*. **36**: 729-731.

Schenk, P. M., Thomas-Hall, S. R., Stephens, E., Marc, U. C., Mussgung, J. H., Posten, C., Kruse, O. and Hankamer, B. (2008). Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenergy Res.* **1**: 20-43.

Sharif, Hossain, A. B. M., Nasrulhaq, A. B., Majid, H. A. M., Chandran, S. and Zuliana, R. (2007). *Biodiesel production from waste cooking oil as environmental benefits and recycling process. A review*. Asia Biofuel Conference Book. Dec. 11-13, 2007, Singapore.

Sharif Hossain, A. B. M., Salleh, A., Boyce, A. N., Chowdhury, P. and Naqiuddin, M. (2008). Biodiesel fuel from algae as renewable energy. *American Journal of Biochemistry and Biotechnology.* **4(3)**: 250-254.

Shay, E. G. (1993). Diesel fuel from vegetable oils: status and opportunities. *Biomass Bioenergy.* **4**: 227-242.

Sheehan, J., Dunahay, T., Benemann, J. and Roessler, P. (1998). *A Look Back at the U.S. Department of Energy's Aquatic Species* P*rogram-Biodiesel from Algae.* National Renewable Energy Laboratory: Golden, CO, USA.

Singh, R. N. and Sharma, S. (2012). Development of suitable photobioreactor for algae production – A review. *Renewable and Sustainable Energy Reviews* **16**: 2347-2353.

Stanier, R. Y., Kunisawa, R., Mandel, M. and Cohen Bazire, G. (1971). Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriol. Rev.* **35**: 171-205.

Wijffels, R. H. and Barbosa, M. J. (2010). An outlook on microalgal biofuels. *Science* **329**: 796-799.

Ziga, Z., McConnell, B. and Farag, I. (2010). *Algae Bioreactor.* Final Report. Rose-Hulman Institute of Technology, India.