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INSTITUTE OF ENVIRONMENT AND SANITATION STUDIES

**SUSTAINABLE BIOENERGY: BIODIESEL PRODUCTION FROM
MICROALGAE OCCURRING IN GHANA**

By

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DECLARATION

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This thesis is the result of research work undertaken by Ruth Onwona-Agyeman in the school of Graduate Studies under the supervision of Professor G.K Ameka and Dr. Ted Annang. This thesis has never been presented either in part or in whole for a degree of any other university.

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DEDICATION

I dedicate this work to my parents Mr. and Mrs. Onwona-Agyeman who have supported me in my entire education and to my husband Mr. Abell Y. K. Owusu.



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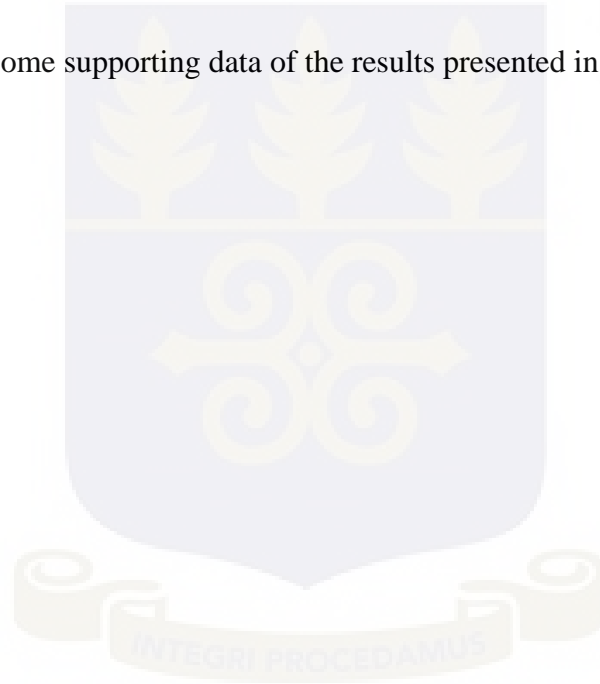
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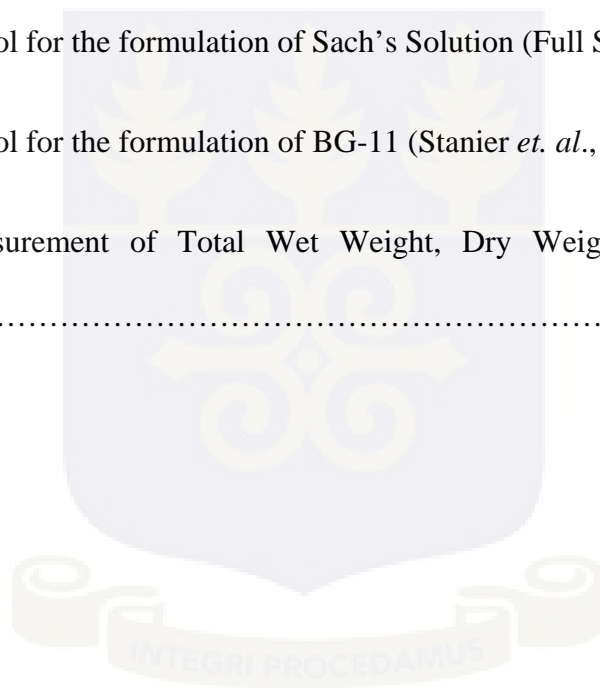
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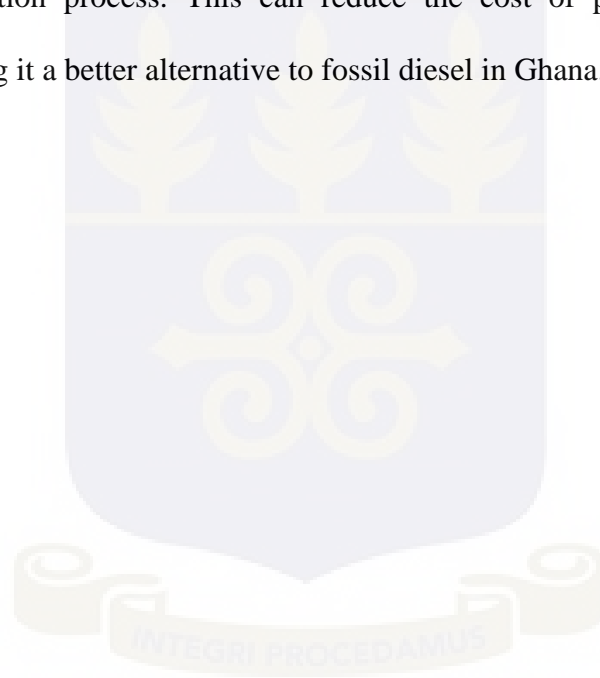
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ABSTRACT

Due to increasing oil prices and climate change concerns, biodiesel has gained attention as an alternative energy source. Biodiesel derived from microalgae is a potentially renewable and carbon-neutral alternative to petroleum fuels. This study was carried out to determine the potentials of producing biodiesel from microalgae obtained from freshwater bodies in Ghana to serve as an environmentally sustainable fuel and an alternative to the use of fossil diesel. In the study, water samples with algal blooms were collected from the Weija reservoir, wastewater ponds in and near University of Ghana, main campus, Prampram and a freshwater pond at Teshie in Accra. Based on their morphology and ease of cultivation, four (4) microalgae were isolated and identified as *Oedogonium* sp., *Chroococcus* sp., *Spirogyra* sp. and *Closterium* sp. These isolates were : (i) cultured in a 2 litre reagent bottle Photobioreactor (improvised) using sunlight as the energy source, (ii) harvested by filtration to reduce their water content and centrifuged into pastes, (iii) dried and ground into powder to extract algal oil and (iv) the algae oils were transesterified into biodiesel. Results indicated a significant growth in terms of percentage dry weight in *Oedogonium* sp. (55.8%) and a lower dry weight of 40.1% in *Closterium* sp., with *Chroococcus* sp. and *Spirogyra* sp. ranging between 50.2 and 50.0 % respectively. Results also indicated that under room temperature, air, natural source of light and appropriate medium these species can grow profusely. Significant amounts (20-38%) of microalgal oil were efficiently extracted with hexane and diethyl ether and transmuted into biodiesel. *Closterium* sp. yielded very significant amount of biodiesel of 94 %, while a yield of 80% was produced from *Chroococcus* sp. The percentage yield for *Oedogonium* sp. was 49% and the least percentage biodiesel yield was 33% for *Spirogyra*

sp. This work was able to isolate and identify four freshwater microalgae which are rarely used in research works for biodiesel production. In producing biodiesel, it can be concluded that, *Oedogonium* sp., *Closterium* sp., *Spirogyra* sp. and *Chroococcus* sp. are viable species which can provide substantial amount of algal oil for biodiesel production. Hence, investments must be made to develop a better and economically viable technology for large scale cultivation of algae for the production of biodiesel in Ghana. Also an in-depth economic analysis should be performed on each aspect of the algae to biodiesel production process. This can reduce the cost of producing biodiesel, and ultimately making it a better alternative to fossil diesel in Ghana.



CHAPTER ONE

INTRODUCTION

1.1 Background

Transportation is one of the fastest growing sectors in the world, consuming about 27% of primary energy (Antoni *et al.*, 2007). Crude oil which is the backbone of this industry is no longer sustainable because of limited reserves which are concentrated in only certain regions of the world (Ciubota-Rosie *et al.*, 2008). Today about 80% of the global energy demand is produced from fossil fuels (Schenk *et al.*, 2008). However, the major disadvantage of using petroleum fuels is their contribution to atmospheric pollution, created by the use of petroleum diesel whose combustion product is a major source of carbon dioxide (a greenhouse gas). Apart from this emission, petroleum diesel is also a major source of other air contaminants such as nitrogen oxides (NO_x), sulphur oxides (SO_x), carbon monoxide (CO), particulate matter and volatile organic compounds (VOC) (Hallenbeck and Benemann, 2002). Hence there is a global interest to develop new, clean, and sustainable energy sources.

Also, oil prices continue to rise globally. World oil prices rose in 2010 as a result of growing demand associated with signs of economic recovery and a lack of sufficient supply. Prices rose even higher at the end of 2010 and into 2011 as social and political unrest unfolded in several Middle Eastern and African economies (IEO, 2011). Consequently, alternative source of oil replacements have become significant (Neltner, 2008) and biodiesel as an alternative fuel source has gained immense popularity in recent years (Basha and Jebaraj, 2009; Demirbas, 2009; Durrett *et al.*, 2008; Koonin, 2006).

Moreover, a lot of emphasis has been put on the search for potential biomass feedstock from different sources, which can be converted to biodiesel and gas fuels for energy generation (Singh and Gu, 2010). The focus has been on oil sources like soybean, rapeseed, sunflower and safflower (Parawira, 2010; Twidell and Weir, 2006; Altin *et al.*, 2001; Lang *et al.*, 2001) which are essentially edible in nature. Other forms of biomass like energy crops (edible and non-edible oilseeds), bio-wastes and certain aquatic plants like algae have been identified as bio-oil sources (Duku *et al.*, 2011).

1.4 Problem Statement

Globally, economies are facing threatening energy crisis due to limited reserves of fossil fuels. Environmental pollution and health problems from carbon dioxide, pollutant emissions and its associated economic costs are among problems confronting nations. Moreover, the ocean also dissolves about one-third of carbon dioxide produced from fuel combustion which gradually reduces the pH of oceanic waters resulting in significant consequences on aquatic life (Mata *et al.*, 2010). Although biodiesel has been recommended in solving most of these problems, the right feedstock has impeded the expansion of the global biodiesel production industry. Studies have revealed that biomass for biodiesel production is one of the best sources of energy which could contribute to sustainable development on several fronts (Hosain *et al.*, 2008). However, with world issues of food-fuel conflict and the competition for land, some sources of biomass like soybean, corn, sugarcane, canola, *Jatropha* and other agricultural crops are highly contested. As a result of these problems, the use of microalgae has now become popular in the production of biodiesel but in Ghana, there is very little or no information on the use of algal biomass to produce biodiesel.

Consequently this study has been designed to provide answers to these questions:

1. Are microalgae from the Ghanaian freshwater bodies viable to produce oil for biodiesel production?
2. Do these microalgae contain high enough quantity of oil to produce maximum quantity of biodiesel?
3. Can microalgae be cultured under laboratory conditions or under room temperature with external source of light (sunlight) to produce optimum amount of algal oil?
4. Can the algal oil produced be converted into biodiesel?

1.5 Justification for the Study

The production of biodiesel from microalgae is considered as an ‘environmentally clean’ alternative to the use of fossil fuels, primarily as result of the phenomena which now threatens humankind i.e. climate change and global warming of the earth due to greenhouse effect. Growing concerns of the impact of conventional fossil fuels (diesel) therefore have made it imperative for the shift from fossil diesel to biologically derived fuels such as biodiesel. Moreover, greenhouse gases and other emissions such as nitrogen oxides (NO_x), sulphur oxides (SO_x), particulate matter, and its associated impacts will be greatly reduced when there is the change in the use of fossil fuels to the acceptance and use of algal fuels.

Secondly, studies have shown that culturing algae for biodiesel production requires large amounts of carbon dioxide (CO₂) (Frac *et al.*, 2010; Gavrilescu and Chisti, 2005), this has the potential of reducing effectively the amount of CO₂ in the atmosphere which has

already exceeded acceptable limits and mitigate global warming. Moreover, the global biodiesel industry is among the fastest growing markets (Scott and Bryner, 2006), and with the right research focus, Ghana can tap into this ever increasing market and become a major hub for development and advancement in biodiesel production. Thus, this study was conducted to demonstrate the potential production of biodiesel from suitable algal strains obtained from freshwater bodies in the country to serve as credible source of information and serve as a better alternative to the use of *Jatropha* as feedstock for biodiesel production which is being actively developed.

1.6 Objectives of the Study

The main objective of the study was to determine suitable algae from fresh water bodies in Ghana for biodiesel production.

The specific objectives were:

- i. To collect, isolate and identify suitable algae from fresh water bodies that can be used for biodiesel production in Ghana.
- ii. To culture isolated and identified microalgae to provide feedstock for the biodiesel process.
- iii. To extract algal oil, and undertake transesterification of the algal oil into biodiesel.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Algae – Basics and Composition.

Algae are a large and diverse group of photosynthetic eukaryotes with a simple cellular structure, ranging from unicellular to multicellular forms. They can be found anywhere water and sunlight occur and they grow in various environments such as soils, ice, lakes, rivers, hot springs and ocean (Parker *et al.*, 2008). Two categories of algae have been identified: macroalgae and microalgae. Macroalgae are the large multicellular algae often seen growing on rocky marine shores known as seaweeds and occasionally in freshwater bodies. Microalgae, on the other hand, are tiny, unicellular / multicellular algae that normally grow in suspension within water bodies. They represent a highly specialized group of micro-organisms that live in diverse ecological habitats such as freshwater, brackish, marine and hyper-saline, with a range of temperatures and pH, and unique nutrient availabilities. They are classified into major groupings including cyanobacteria, green algae, diatoms, yellow-green algae, golden algae and red algae. Algal biomass consists of three main components: carbohydrates, proteins, and lipids/natural oils. Many algal species have been found to grow rapidly and produce substantial amounts of triglycerides (TAGs) or oil, and are thus referred to as oleaginous algae (Falkowski, 1907; Nunoo and Ameka, 2005 as cited in Duku *et al.*, 2011). Microalgae cells can double every few hours during their exponential growth period. The fact that they grow so quickly makes them a promising crop for human use (Metting, 1996). During the peak growth phase, some microalgae can double every 3.5 hours

(Chisti, 2007). Oil contents of microalgae are usually between 20-50% (dry weight) while some strains can reach as high as 80% (Spolaore *et al.*, 2006; Metting, 1996).

Fatty acids are attached to triglyceride (TAG) within algal cells which may be short or long chain hydrocarbons. The shorter chain length acids are ideal for the creation of biodiesel and some of the longer ones can have beneficial uses such as the production of omega-3 fatty acids like docosahexanic acid (DHA 22:6) (Pyle *et al.*, 2008).

2.2 Algae as a Feedstock for Biodiesel

Among the various life forms of algae, microalgae have particularly attracted considerable attention as an alternative non-food biodiesel feedstock owing to their high oil content and rapid biomass production (Deng *et al.*, 2009). Microalgae have several important advantages. First of all, they are easy to culture, able to convert solar energy to chemical energy in the process of photosynthesis increasing their biomass in a few days. Secondly, they are also able to grow in waters unsuitable for human consumption, with higher growth rate and productivity than the yielding of traditional crops, and they require significantly smaller area than other substrates of biofuel of agricultural origin (Frac *et al.*, 2010; Mata *et al.*, 2010). Moreover, algae are high productivity feedstock that do not compete for arable land and thus may be less greenhouse gas-intensive than biofuels based on plants. Different microalgae species can be adapted to live in a variety of environmental conditions. Thus, it is possible to find species best suited to local environments or with specific growth characteristics, which is not possible to do with other current biodiesel feedstocks e.g. soybean, rapeseed, sunflower and palm oil (Mata *et al.*, 2010). Microalgae can provide several different varieties of biofuel including

biomethane produced by anaerobic digestion of the algal biomass (Spolaore *et al.*, 2006), biodiesel from microalgae (Chisti, 2007; Banerjee 2002; Roessler *et al.*, 1994), photobiologically produced biohydrogen (Kapdan and Kargi, 2006; Fedorov *et al.*, 2005; Melis, 2002) and bioethanol (Mata *et al.*, 2010; Fortman *et al.*, 2008). In addition, microalgal oils are similar to those produced by crops such as soybean, and can be used directly to run existing diesel engines or as a mixture with fossil oil diesel. Algal oils used to produce biodiesel, are usually accumulated as membrane components, storage products, metabolites and sources of energy under some special conditions (Deng *et al.*, 2009).

The concept of using algae to produce fuels was discussed 50 years ago, but a concerted effort began with the 1970's oil crisis (Wijffels and Babosa, 2010). Large research programs in Japan and United States focused on developing microalgae energy production systems in 1978 to 1996 and 1999 (Wijffels and Babosa, 2010). Particularly during the past two or three decades, algal biotechnology has grown steadily into an important global industry with a diversified field of applications (Hallmann, 2007).

2.3 Growing Microalgae

2.3.1 Collection and Screening of Microalgae Strains for Biodiesel Production

The first step of the algae-to-biodiesel system is to choose suitable algae strain to grow (Chisti, 2007). According to Richmond (2004a), 500,000 species of microalgae are present worldwide which are not only aquatic but also terrestrial implying their widespread availability. So far a limited number of about 4,000 species have been identified (Deng *et al.*, 2009). These have been divided into several groups such as

cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae) and ‘pico-plankton’ (Prasinophyceae and Eustigmatophyceae) (Hu *et al.*, 2008). Among these, diatoms and green algae are relatively abundant (Khan *et al.*, 2009).

Much of current research works are focused on fast growing microalgae, which also accumulate high quantities of lipids (Scott *et al.*, 2010). Some common microalgae e.g., *Botryococcus*, *Chlamydomonas*, *Chlorella*, *Dunaliella* and *Neochloris* have appreciably high oil levels between 20 and 75% by weight of dry biomass (Table 2.1). These are all potential sources of biodiesel production.

Apart from oil contents of microalgae, biomass production is considered simultaneously in the selection of the most adequate species for biodiesel production (Deng *et al.*, 2009). Vasudevan and Briggs (2008) observed that a lower oil strain grows faster than high oil strains. Microalgae containing 30% oil grows 30 times faster than those containing 80% oil (Becker, 1994). One challenge is that microalgae accumulate oil under stressful conditions. Hu *et al.* (2008) reported that neutral lipids content, mainly triacylglycerol (TAG) double or triple when cells are subjected to unfavorable culture conditions. Nitrogen limitation could increase the oil content of *Neochloris oleoabundans* (Li *et al.*, 2008). Therefore the ability of microalgae to thrive in extreme conditions must be taken into account when selecting efficient strains for biodiesel production. For *Chlorella autotrophica*, high lipid content (by dry weight) was reported to be 38% under

autotrophic condition (Liang *et al.*, 2009) and 53% under mixotrophic and nitrogen limiting condition (Yeh and Chang, 2011).

Table 2.1. Lipid Contents of some Microalgal Species

Microalgae species	Lipid content (% dry weight biomass)	Microalgae species	Lipid content (% dry weight biomass)
<i>Ankistrodesmus</i> sp.	24–31	<i>Monodus subterraneus</i>	16
<i>Botryococcus braunii</i>	25–75	<i>Monallanthus salina</i>	20–22
<i>Chaetoceros muelleri</i>	33	<i>Nannochloris</i> sp.	20–56
<i>Chlamydomonas reinhardtii</i>	21	<i>Dunaliella salina</i>	6–25
<i>Chlorella emersonii</i>	25–63	<i>Dunaliella primolecta</i>	23
<i>Chlorella minutissima</i>	57	<i>Dunaliella</i> sp.	17–67
<i>Chlorella protothecoides</i>	14–57	<i>Neochloris oleoabundans</i>	29–65
<i>Chlorella sorokiniana</i>	19–22	<i>Pyrrosia laevis</i>	69.1
<i>Chlorella</i> sp.	10–48	<i>Prymnesium parvum</i>	22-39
<i>Chlorella vulgaris</i>	5–58	<i>Schizochytrium</i> sp.	
<i>Cryptocodinium cohnii</i>	20–51	<i>Scenedesmus dimorphus</i>	16-40
<i>Zitzschia</i> sp.	45-47	<i>Schizochytrium</i> sp.	50-77
<i>Isochrysis galbana</i>	7-40	<i>Prostanthera incisa</i>	62
<i>Euglena gracilis</i>	14-20	<i>Phaeodactylum tricornutum</i>	18–57
<i>Skeletonema costatum</i>	13–51	<i>Scenedesmus obliquus</i>	11-15

2.4 Algal culture conditions and inputs

Microalgae are adapted to scavenge their environments for resources. In general, for growth, algae require a carbon source, water, mineral salt and light to carry out photosynthesis. They can also adjust or change their internal structure (e.g., biochemical and physiological acclimation), whilst externally they excrete a variety of compounds to make nutrients available or limit the growth of competitors (Richmond, 2004a).

The main inputs required in the cultivation of algae are light (sunlight), water, carbon dioxide and nutrients. Temperature is also an important component which cannot be ignored. These components when provided in the right proportion lead to successful growth of microalgae.

2.4.1 Light

In both outdoor and indoor algal culture, the light source and intensity are critical factors affecting the performance of phototrophic growth (Mata *et al.*, 2010).

About 45% of the total light spectrum consists of photosynthetically active radiation, thus can be utilized by algal flora to capture carbon dioxide (Gao *et al.*, 2007). Algae absorb light such that the higher the algae concentration, the less deep light enters the broth. This suggests that, it is critical to optimize the shape and size (the design) of growth containers for reception of light by algal biomass, as much as possible.

Sunlight is available in different quantities in different geographical locations. It is more readily available in the tropics and for that matter Ghana. Sunlight collection can be improved through the use of solar collectors, solar concentrators and fiber optics (Chisti, 2007; Scott and Bryner, 2006). However, to reduce the economic cost of biodiesel production, algal biomass may be grown by utilizing the freely available sunlight.

On the other hand, the primary physical law that limits the production capabilities of algae is the first law of thermodynamics, which states conservation of energy for any system: $E_{in} \geq E_{stored}$. For a system of photosynthesizing algae, E_{in} is the rate of incident solar irradiance on the production area, and E_{stored} is the rate of chemical

energy storage by the algae as oil and other biomass. Thus, the amount of stored chemical energy is directly limited by the amount of solar irradiance available (Weyer *et al.*, 2010).

2.4.2 Carbon dioxide (CO₂)

Microalgae are ten times more efficient in biological fixation of atmospheric carbon dioxide (CO₂) than terrestrial plants (Usui and Ikenouchi, 1997) and this efficiency is highly variable with various species of the algae. In the cultivation of microalgae the only source of carbon is carbon dioxide (CO₂). Thus, the input cost of carbon dioxide (CO₂) plays an important role in the area of biodiesel production. In order to optimize the microalgal growth, carbon dioxide (CO₂) needs to be provided at much high concentration and this concentration can be attained under natural conditions (Pulz, 2007).

Areas of high CO₂ concentration than that of normal air include flue-gases from steel industries and thermal power plants and these sites can be used by both raceway ponds and photobioreactors for the cultivation of microalgae (Schneider, 2006).

2.4.3 Water and nutrients

Water is needed for various aspects of algae cultivation. Primarily, photosynthesis requires water for carbon fixation and microalgal cells growing in water gets essential nutrients from the water in which they grow. The source of water is usually occurring in treated or untreated livestock wastewater, but in experimental cultures water is obtained from formulated media.

Wastewater can be reprocessed to conserve resources as well as to reduce the culture medium cost. Algae need not compete with others for fresh water. Thus cultivating algal mass by utilizing wastewater such as those from waste water plants can be considered as a biological means of wastewater management. Extensive studies have been carried out by using wastewater for the cultivation of algal biomass, especially for the removal of nitrogen and phosphorus salts from effluents (Abdel-Hameed, 2007; Shi *et al.*, 2007; Hernandez *et al.*, 2006), which would otherwise result in eutrophication if dumped into lakes and rivers (Galvez-Cloutier *et al.*, 2006). In addition to nitrogen and phosphorus removal, microalgae are effective for the removal of heavy metals from wastewater (Priya *et al.*, 2007; Singh *et al.*, 2007; Munoz and Guieysse, 2006).

On the other hand, under experimental conditions, algal culture formulated media should contain mineral and vitamin components required for algal cells. These media should contain essential nutrients such as nitrogen, phosphorous, iron and in some cases silicon (Grobbelaar, 2004). Under appropriate environmental conditions and sufficient nutrients, microalgae can double their biomass within 24 hours or within 3.5hours during exponential growth phase (Chisti, 2007).

The effects associated with deficiency of major nutrients such as nitrogen, phosphorus and sulphur as well as some minor nutrients like iron and silicon has been determined as per lipid metabolism and fatty acid content among various species of algae (Verma *et al.*, 2010).

The consequence of nitrogen deprivation was investigated on lipid metabolism and signification alteration in lipid or fatty acid content is reported in various species

including *Anacystis nidulans*, *Microcystis aeruginosa*, *Oscillatoria rubescens* and *Spirulina platensis* (Basova, 2005). Generally, nitrogen deficiency greatly affects the synthesis and accumulation of lipids and fatty acid (Saha *et al.*, 2003). Phosphorus deficiency resulted in the enhancement of lipid in *Monodus subterraneus* (Khozin-Goldberg and Cohen, 2006).

Silicon deficiency resulted in increased neutral lipids content of *Cyclotella cryptica* and increased quantity of saturated and mono-unsaturated fatty acids than cells grown on silicon rich ambience (Roessler, 1988). Deprivation of sulphur was also investigated in *Chlamydomonas reinhardtii* (Sato *et al.*, 2000) and *Chlorella* species (Otsuka, 1961) and resulted in the enhancement of total lipid contents.

Not only organic carbon or a carbon source such as sugars, proteins and fats, vitamins, salts and other nutrient such as nitrogen and phosphorous are vital for algal growth, but also equilibrium between operational parameters like oxygen, carbon dioxide, pH, temperature, light intensity, product and by-product removal (Williams, 2002). Mata *et al.* (2010) observed that it is important to quantitatively define the influence of these operational parameters and their interrelation to be able to manipulate them. This way one may succeed in obtaining a certain control over the composition of microalgae populations, even on a large scale. Studies have shown that properly managed algal cultures are quite resistant and that infections are often an indication of poor culture conditions (De Pauw *et al.*, 1984). Generally algal cultures in the exponential growth phase contain more protein, while cultures in the stationary phase have more carbohydrates and glycogen.

2.4.4 Temperature

Various microalgae grow under different temperature ranges. Growth temperature must oscillate within 20 to 30°C (Frac *et al.*, 2010). One disadvantage of temperature on the growth of microalgae is that temperatures must be held constant in cultures, which can be difficult to maintain (Wagner, 2007). Moreover, temperature has been found to have a major effect on the fatty acid composition of algae. A general trend towards increasing fatty acid unsaturation with decreasing temperature and increasing saturated fatty acids with increasing temperature has been observed in many algae (Renaud *et al.*, 2002). The lipid content in *Ochromonas danica* (Aaronson, 1973) and *Nannochloropsis salina* (Boussiba *et al.*, 1987) increases with increasing temperature. But, no significant change was observed in the lipid content in *Chlorella sorokiniana* grown at various temperatures (Patterson, 1970). According to Verma *et al.* (2010), insight information about the effect of temperature on lipid synthesis and accumulation in microalgae needs to be explored.

2.4.5 Microalgae Species Growing Conditions with Different Energy Source

Microalgae may assume many types of metabolisms like autotrophic, heterotrophic, mixotrophic, photoheterotrophic and are capable of a metabolic shift as a response to changes in the environmental conditions. For example some organisms can grow photoautotrophically, meaning the use of light as a sole energy source that is converted to chemical energy through photosynthetic reactions. The photosynthetic reactions may be described as heterotrophic, i.e. utilizing only organic compounds as carbon and energy sources, mixotrophic, that is performing photosynthesis as the main energy source, though both organic compounds and carbon dioxide (CO₂) are essential,

Photoheterotrophic, which describes the metabolism in which light is required to use organic compounds as carbon source. The photoheterotrophic and mixotrophic metabolisms are not well distinguished, in particular they can be defined according to a difference of the energy source required to perform growth and specific metabolite production (Mata *et al.*, 2010).

2.4.6 Optimization of medium composition

The concentrations of nutrient in a growth medium have been found to have an influence on the concentration of dry weight cellular constituents such as lipids, carbohydrates and proteins content (Posthuma, 2009). This principle could be used to influence the quality of the biomass. In the case of biodiesel production this is especially important with regards to cellular oils or neutral lipids. Since lipids have very high energy content, ± 2 times higher as carbohydrates, fuels made from lipids, such as biodiesels therefore have more energy per liter and as such they are more convenient energy carriers (Johnson and Wen, 2009a).

Neutral lipids or triacylglycerides (TAG's) are stored in intracellular globules that are distributed over the cell (Danielo, 2005). The function of these lipids is to provide the cell with intracellular energy storage.

Although these lipids are always naturally present in cells, under stressful conditions they accumulate higher percentages of oils. This happens when cells are exposed to anaerobic conditions, low nutrient availability mainly nitrogen and phosphorus, extreme temperatures and / or light levels (Schlagermann *et al.*, 2012). In these events stressed cells are still capable of CO₂ fixation, but the starches produced are converted into lipids

to increase survival chances under suboptimal conditions. Based on such knowledge, it has been extensively shown that many algae species increase their total lipid content (% dry weight) by a factor of 2-3 times (Spolaore *et al.*, 2006; Chisti, 2007). However such nitrogen depleted cells are no longer able to divide, because of the requirement of nitrogen for DNA and protein synthesis. Overall increase of lipid yield may thus actually reduce overall biomass production if cells are grown under these suboptimal conditions (Sheehan *et al.*, 1998). Nevertheless, cultures can first be grown under optimal conditions and in a second stage depleted of nitrogen to allow the overall lipid content to increase just before harvesting.

2.4.7 The Impact of photosynthetic Efficiency and theoretical maximization of algal yield

Photosynthetic efficiency (PE) is the fraction of light energy that is fixed as chemical energy during photoautotrophic growth (Brennan and Owende, 2010). Only photosynthetic active radiation (PAR) of wavelengths between 400 and 700 nm, representing 42.3% of the total energy from the light spectrum is captured. The captured energy is used in the Calvin cycle to produce carbohydrates by utilizing carbon dioxide (CO₂) and water (H₂O) molecules. Photosynthetic efficiency is only relevant for autotrophic algae; for heterotrophically cultivated algae, the utilization of sugars is more relevant.

Algae have simple structures that enable them to achieve substantially higher PE values compared to terrestrial plants. For example, some studies on *Chlorella* sp. have recorded PAR-based PE values of 7.05%, 6.48% and 6.5%. *Synechococcus* sp. was found to have a PE of between 2% and 4% indicating significantly higher values for algae compared to

terrestrial plants. Other studies have also suggested that even higher levels of PE can be attained by microalgae (Brennan and Owende, 2010).

2.5 Algae Mass Cultivation Systems

2.5.1 Open ponds verses closed ponds

Microalgae cultivation can be achieved in various ways. These include the open culture systems such as ponds, lakes, raceways, artificial ponds and containers, or in highly controlled environments or culture-systems known as photo-bioreactors (PBRs). Algae cultivation in open pond production systems has been used since the 1950s. Open-pond cultures are economically more favourable, but raise the issues of land use cost, water and appropriate climatic conditions. Also the problem of contamination by fungi, bacterial, protozoa and competition by other microalgae is prominent (Johnson and Wen, 2009a). Open-pond is a cheaper method of large-scale algal biomass production. Open - pond production does not necessarily compete for land with existing agricultural crops, since they can be implemented in areas with marginal crop production potential. They also have lower energy input requirement, and regular maintenance and cleaning are easier and therefore may have the potential to return large net energy production. Photobioreactors on the other hand offers a closed culture environment which is protected from direct fallout, relatively safe from invading microorganisms, where temperatures are controlled with an enhanced CO₂ fixation that is bubbled through the culture (Patil *et al.*, 2008). The biomass productivity of photobioreactors can be 13 times more than that of a traditional raceway pond on average (Chisti, 2007). Harvesting of biomass from photobioreactors is less expensive than that from a raceway pond, since the typical algal

biomass is about 30 times as concentrated as the biomass found in raceways (Chisti, 2007). Bioreactors are of different shapes and are classified into two broad categories: those that make use of natural light and those that make use of artificial illumination (Apt and Behrens, 1999).

According to Chisti (2007), both photobioreactors and open raceways have their own disadvantages. Although open ponds cost less to build and operate than closed photobioreactors, they are open air systems and experience a lot of water loss due to evaporation. Again open ponds do not allow microalgae to use carbon dioxide (CO₂) efficiently and biomass production is limited. In addition culture optimum conditions are difficult to maintain in open ponds and recovering the biomass from such a dilute cell yield is expensive (Molina-Grima *et al.*, 1999). In closed photobioreactors however, variation in light and temperature common in all photoautotrophic systems can cause suboptimal growth of the microalgae (Wen and Chen, 2003). Initial capital cost alone can be very high, due to their complexity, and differences in design and construction (Eriksen, 2008). This cost may be justified when producing a high value product such as a pharmaceutical, but a low value commodity, such as fuel, cannot recover the initial cost of construction in any reasonable time. One problem shared between open ponds and photobioreactors is the light penetration issue. Light penetration is inversely proportional to cell density (Chen, 1996) and decreases exponentially with penetration depth (Johnson and Wen, 2009a).

Another common problem with photobioreactors is that, the oxygen level will build up as the algae undergo respiration, and the toxicity with high oxygen contents can kill the

microalgae. To counteract this, modern photobioreactors employ an oxygen scrubber to remove the gas from the system and expel it to the atmosphere

2.5.2 Hybrid cultivation systems

Hybrid Systems are the advanced cultivation systems employed for commercial scale of cultivation of a wide variety of microalgae cultures. These systems include tubular photo bioreactor integrated with closed pond monitored and controlled by a Control Panel. Hybrid Systems reduce the disadvantages involved with Photo Bioreactors and open ponds cultivation, significantly improving economic viability. These systems are capable of maintaining higher algal densities for maximized algal biomass production within less cultivation area.

This option has already been successfully explored for large scale production of a high value product, astaxanthin, of which concentrations also increased upon nitrogen depletion (Huntley and Redalje, 2006). One advantage of a hybrid system is that, open ponds are less sensitive to invasion of foreign species when high inoculation volumes are used (Posthuma, 2009). Moreover using both bioreactors and open ponds could give advantages for other forms of control such as temperature. Bioreactors are known to heat up significantly more than open ponds due to the fact that there is no evaporation, a major form of heat loss. Due to this, closed systems are found to have high temperatures, whereas open ponds are relatively cooler. Heat transfer between these two different systems has therefore been proposed as method for temperature control and has been investigated. In a study, excess heat was transferred by means of channeling cooling

water from flat plate bioreactors to open ponds and as a result overall productivity was increased (Pushparaj *et al.*, 1997).

2.6 Harvesting, Drying and Biomass Conversion

2.6.1 Harvesting of Microalgae

To obtain algae biomass the cultivated algae will have to be harvested. This then comes down to separating the algae from the water. Algae harvesting is referred to as the concentration of diluted algae suspension to obtain a thick paste of algae.

The harvesting of algae is a crucial process in the biodiesel process. However, this is not easily achieved. Dewatering is an essential part which raises the cost of the feedstock (Uduman, 2009). Due to the dilute microalgae suspension, plus the negative charges they carry, the cells are highly dispersed (Danquah, 2009; de la Noüe, 1992; Benemann, 1989). The current technologies being studied in this process include: centrifugation, flocculation, filtration, flotation, and electrophoresis techniques (Xiang, 2012; Molina-Grima *et al.*, 2003).

There are three aspects to evaluating the performance of these harvesting methods: (i) the efficiency / yield of the technique, i.e. percentage of recovered microalgae from total processed microalgae, (ii) the solid content in the recovered microalgae and (iii) harvesting rate (the rate of water removal) (Uduman *et al.*, 2009).

2.6.2 Centrifugation

Centrifugation is a separation method which uses centrifugal forces to separate solids from liquids. The separation is based on the particle size and density difference of the

medium components. It is one of the most established methods to separate cells from the broth in biotechnology.

Even though effective harvesting can be accomplished by centrifugation, this method may be too difficult or costly to implement (Posthuma, 2009; de la Noüe, 1992; Benemann, 1989). This is because centrifuges consume a high level of energy (Heasman *et al.*, 2000).

Studies have proven high speed centrifugation to be reliable and effective recovery technique. Centrifugal recovery was evaluated in a review by Molina-Grima *et al.* (2003). This review compared different applications of centrifugation for the harvesting of microalgae and concluded that although centrifugal recovery is energy intensive, it is rapid and a preferred method for microalgal cell recovery. Cell viability was found to be significantly dependent on the microalgal species and the method of centrifugation. These observations have been confirmed by Moraine *et al.* (1980). Laboratory centrifugation tests were investigated on pond effluent at 500-1000 g (gravity force) and showed that 80-90% could be recovered in 2-5 minutes (Moraine *et al.*, 1980). Other studies have also determined that a nozzle disc type centrifuge with intermittent discharge is the best option for algae harvesting (Mohn, 1988). But Knuckey *et al.* (2006) have pointed out that the exposure of microalgal cells to high gravity and shear forces could damage the cell structure.

2.6.3 Filtration

In general, filtration methods require a pressure drop to be applied across the medium in order to force fluid to flow through. The required magnitude of the pressure drop is

dependent on one or more of the following driving force: vacuum, centrifugal, pressure or gravity (Xiang, 2012; Uduman *et al.*, 2009; Shelef *et al.*, 1984).

There are two main types of filtration: surface filters (where the solids are deposited on the filter medium in a thin film or cake) and depth filters / deep bed filters (where the solids are deposited within the filter medium). One of the major problems associated with filtration is that, media that are fine enough to retain the microalgae tend to bind and therefore require regular backwashes. This results in a decrease in the amount of microalgal concentrate (Uduman *et al.*, 2009).⁷

Molina-Grima *et al.* (2003) conducted a review on process options available for the recovery of microalgal biomass. They found that filtration methods that operate under pressure or vacuum are suitable for recovering microalgal species with large cell size but inadequate to recover microalgal species with sizes approaching bacterial dimensions (in the range of micrometers).

Also, to harvest microalgae, the filter pore size must be determined according to specific algae strains used (Moraine, 1980).

2.6.4 Flocculation, Floatation and Electrophoresis Techniques

Flocculation is the process whereby a solute particle in a solution forms aggregate called a floc. This process uses soluble particles to form aggregates with a chemically reactive cellular surface that has a net negative surface charge due to the ionization of functional groups, which facilitate microalgal cell suspension formation (Xiang, 2012; Tenny *et al.*, 1969). Two types of flocculants are mainly used in the flocculation process for harvesting algae. These include inorganic and polymer/polyelectrolyte flocculants. Inorganic

flocculants operates basically by charge neutralization, assuming the microalgae cell are small and approximately spherical (Bernhardt and Clasen, 1991), while the mechanism of microalgal flocculation by organic polymer flocculants are explained by a combination of both charge neutralization and particle bridging (Xiang, 2012). The polymer flocculant attaches itself to certain points on the microalgae leaving its tail trail out into the solvent. The trails then attach to similar parts of polymers thus forming a bridge between polymers resulting in a three dimensional matrix (Tenny, 1969). Examples of inorganic flocculants are aluminum sulfate and iron chloride. Some examples of polymer/polyelectrolyte flocculants are cationic polymers such as praestol, cationic polyacrylamides zetag 63 and zetag 92, and cationic polyamines (Oh, 2001; Bilanovic, 1988). Flocculation is a routine procedure in various separation technologies such as sedimentation (Mohn, 1980; Friedman *et al.*, 1977), flotation (Moraine *et al.*, 1980), filtration (Mohn, 1980; Mohn, 1978) and centrifugation (Moraine *et al.*, 1980; Golueke and Oswald, 1965).

Conversely, the use of chemicals such as flocculants to aid microalgae recovery involving the addition of chemicals into the system may result in an increase in total dissolved solids (Aragon *et al.*, 1992). This results in a risk of potential contamination of the medium which is usually recycled to the bioreactor.

Secondly, flotation is a gravity separation process based on the attachment of air or gas bubbles to solid particles, which then are carried to the liquid surface and accumulate as float which can be skimmed off. This process operates more efficiently and rapidly and achieves a higher solid fraction (up to 7 %) in a concentrate, but it can be more

expensive. Flotation is usually combined with other technologies, e.g. flocculation-flotation and electro-flotation (Svarovsky, 1979).

Lastly, electrophoresis technique is contrary to other methods which involve the use of chemicals. Electrophoresis is a technique which employs electrolysis in its approach. Microalgae are able to behave as colloid particles and can be separated from water-based medium solutions by movement in an electric field (Aragon *et al.*, 1992). The advantages of using electrochemical processes include environmental compatibility, versatility, energy efficiency, safety, selectivity, and cost effectiveness (Azarian *et al.*, 2007).

Electrophoresis techniques include electrolytic coagulation, electrolytic flotation and electrolytic flocculation. Electrolysis of water produces bubbles that can aid the microalgal flocs to rise to the surface (Poelman and Shelef, 1997).

Besides these technologies mentioned above, there is also gravity sedimentation method. Gravity sedimentation process usually takes a long time and has a poor performance, especially on low density samples (Uduman, 2009). For the purpose of this work, the emphasis is on centrifugation and filtration which were used in the research.

2.7 Drying of microalgae

This stage involves the drying of algae which is a further dewatering process after harvesting. Dehydration or drying of the biomass is commonly used to extend the shelf-life of the algal biomass especially if biomass is the final product. In biodiesel production, dry weight is important in some particular extraction processes (Johnson and Wen, 2009b) because drying enhances the extraction efficiency of lipids and other value-added compounds. However, drying may also help in the determination of the oil content

of a particular species. After harvesting microalgae, the wet biomass needs to be dried to reduce spoilage in hot climate (Richmond, 2004b). Just like harvesting, drying is very energy intensive (need for heat and high pressure) and it demands around 60% of the energy content of algae. It is therefore a major economic limitation to the production of low cost commodities such as food, feed and fuels. Drying of microalgae for the production of biodiesel is essential in some cases. For example, it is determined that a drying process combined with hexane extraction system very commonly used in oil extraction methods provide the desired results (Kumar *et al.*, 2011; Posthuma, 2009; Molina-Grima *et al.*, 2003; Ben-Amotz and Avron, 1987). Selection of a drying method depends on the scale of operation and the use for which the dried product is intended. Several drying methods have been used for microalgae such as *Chlorella*, *Scenedesmus* and *Spirulina*, the most common include spray drying, drum drying, freeze-drying, sun drying and fluidized bed drying (Posthuma, 2009; Richmond, 2004b; Molina-Grima *et al.*, 2003). Another method that some researchers have used is oven drying of algae (Kumar *et al.*, 2011). But this review is limited to discussions on oven-drying, freeze drying and solar-drying (sun drying) methods.

2.7.1 Freeze-Drying Method (Lyophilization)

Freeze-drying is a dehydration process which works by freezing the microalgae and reducing the surrounding pressure to allow the frozen water to sublime directly from the solid phase to the gas phase (Dejoye *et al.*, 2011). In its mechanism, the microalgae are first frozen at an appropriate temperature mostly between -50 to -80°C, depending on the type of microalgae used. The pressure is then lowered which dries the microalgae under vacuum (around 25 Pa). The ice becomes vapour and is recovered. After the

microalgae are dried, the temperature rises spontaneously once all free water has been sublimated. Temperatures between 20 and 70 °C for two to six hours can reduce the residual moisture (Dejoye *et al.*, 2011). Freeze-drying, or lyophilization, has been widely used for drying microalgae in research laboratories; however, freeze-drying is too expensive for use in large-scale commercial recovery of microalgal products. In some cases, solvent extraction of dry biomass has proved much more effective for recovery of intracellular metabolites than the extraction of wet biomass (Molina-Grima *et al.*, 2003). Intracellular products such as oils can be difficult to solvent-extract from wet biomass (Belarbi *et al.*, 2000) of undisrupted cells, but are extracted readily if the biomass has been freeze-dried. Lipids have been extracted directly from freeze-dried biomass of *Isochrysis galbana* (Molina- Grima *et al.*, 1994).

In some cases, microalgae have been frozen dried for animals in laboratory captivity. For example, the concept of freeze drying algae has been explored as food for raising mollusc with good results (Kennedy *et al.*, 1993). The premise of this technique is that, phytoplankton raised in cultures under controlled conditions could be preserved for later use in laboratory and field experiments, while maintaining their original nutritional quality.

2.7.2 Oven Drying of Microalgae

This is the most common method of drying by supplying heat. This method is similar to the determination of the moisture content of a material. It is a method designed to remove moisture from microalgae using heat by contact. First of all, the oven is set at a particular temperature (between 30 to 80°C) depending on the type and size of the algae being

dried. Then the algae are dried in the oven for several hours and sometime even days. Algae paste or pellets is spread uniformly on an aluminum cap or in paper envelope at different thickness and allowed to dry. It is important to allow heat to penetrate the biomass by spreading algae as thin as possible. The caps or paper envelop are taken out of the oven and placed in a desiccator until it cools at room temperature. The weight of algae is then determined immediately by a scale. These processes are repeated until a constant weight of algae is obtained. This algal drying method was tested at CFRRI, Mysore, India by Becker *et al.*, (1982). The wet solids of *Spirulina*, containing 55 to 66% moisture, were dried at 62°C for 14hours in a compartment dryer. An approximately 2 to 3mm thick algae layer gave a good dried product with 4-8% moisture. Kumar *et al.* (2011) also dried *Pithophora*, *Spirogyra*, *Hydrodictyon*, *Rhizoclonium* and *Cladophora* in an oven at 40°C for 2-3 days to produce well dried algae for oil extraction used for the production of biodiesel.

2.8 Extraction of Algal Oil

One of the main challenges to fully taking advantage of lipid-producing microalgae is the ability to successfully extract oil from the algae. Concerns of extracting oil in the safest and most environmentally sustainable manner add to this dilemma. Table 2.2 shows some of the more recent extraction methods and their abilities to recover oil, more specifically, valuable fatty acids.

The most recently common methods of oil extraction are mechanical extraction via high pressure extraction (French press), chemical extraction with an organic solvent (Bligh and Dyer, 1959), Supercritical fluid extraction and ultrasonic assisted extraction. All of

these methods have their individual benefits and drawbacks. For most algae some kind of homogenization is necessary to disrupt the cell biomass prior to oil extraction. Pressing and homogenization essentially involve using pressures to rupture cell walls, in order to recover the oil from within the cells.

Table 2.2. Some common extraction methods explored in the last decade, and their effectiveness at recovering lipids and lipid products (modified from Mercer and Armenta, 2011).

Extraction	Organism	Recovery oil (%)	Fatty Acid (% in recovered oil)	References
Solvent/saponification	<i>Porphyridium cruentum</i>	59.5	EPA – 79.5 ARA – 73.2	Guil-Guerrero <i>et al.</i> (2000)
Bligh and Dyer (wet)	<i>Mortierella alpina</i>	27.6	N/A	
Bligh and Dyer (dry)		41.1	Oleic – 49.3 Palmitic– 15.3	
SC-CO ₂	<i>Arthrospira (spirulina) maxima</i>	2.1	GLA – 31.3	Mendes <i>et al.</i> (2005)
Bligh and Dyer		5.5	GLA – 73.0	
SC-CO ₂	<i>Nannochloropsis sp.</i>	25.	EPA – 32.1	Andrich <i>et al.</i> (2005)
Solvent	<i>Phaeodactylum tricornutum</i>	96.1	EPA – 23.7	
UEA	<i>Cryptocodinium cohnii</i>	21.9	DHA– 39.3 Palmitic –37.9	Cravotto <i>et al.</i> (2008)
Soxhlet	<i>Cryptocodinium cohnii</i>	4.8	DHA– 39.3 Palmitic–38.0	
Bligh and dyer (dry)	<i>Chlorella vulgaris</i>	52.2	N/A	
Solvent/transesterification	<i>Botryococcus braunii</i>	12.1	Oleic – 56.3 Linolenic– 19.0	
Frenchpress	<i>Chlorella protothecoides</i>	14.9		

SC-CO₂ = supercritical carbon dioxide extraction, UAE = ultrasonic assisted extraction, EPA = eicosapentaenoic acid, oleic = oleic acid (C18:1), palmitic = palmitic acid (C16:0), GLA = γ -linolenic acid.

The method of extraction for the recovery of oil from microalgae has some advantages and limitations. Pressing, a mechanical means of extracting oil is easy to use and does not involve the use of solvent. Also this method is a slow process of extraction and requires the use of large amount of samples (Popoola and Yangomodou, 2006). Solvent extraction is an extraction method which involves the use of relatively cheap solvents and the results are reproducible. However, it involves the use of large volume of highly flammable and/or toxic organic solvents; the solvent recovery is expensive and involves intensive energy (Herrero *et al.*, 2004; Galloway *et al.*, 2004). Supercritical fluid extraction is another method of extraction which makes use of non-toxic (no organic solvent residue in extract) ‘green solvent’ in a non-flammable and simple operation but involves high power consumption which is difficult to scale up (Macías-Sánchez *et al.*, 2005; Pawliszyn, 1993). Ultrasonic-assisted is an extraction method which involves reduced extraction, reduced solvent consumption, greater solvent penetration into cellular material and improved release of cell contents into bulk medium. It also has the limitation of high power consumption which is difficult to scale up (Martin, 2011; Lague-García and Castro, 2003).

2.8.1 Solvent Extraction

The following discussions give insight into the various extraction methods available. In this review emphasis is laid on solvent extraction because this method describes the main extraction method used in this work.

Solvent extraction involves extracting oil from microalgae by repeatedly washing or percolation with an organic solvent. Solvent extraction can successfully extract up to

95% of the lipid content of microalgae (Posthuma, 2009). Organic solvents such as benzene, cyclohexane, hexane, acetone and chloroform have proved to be effective when used on microalgal paste, they degrade cell walls and extract the oil because oil has a high solubility in organic solvent (Harun *et al.*, 2010). This is made possible when a microalga is one that secretes oil actively and is then recovered by a non-toxic biocompatible solvent (Mojaat *et al.*, 2008). Hejazi *et al.* (2004) described in their work the extraction of carotenoids using so-called biocompatible solvent with *Dunaliella salina*.

A solvent is considered suitable when it is insoluble in water but solubilize the compound of interest. It should have low boiling point to facilitate its removal after extraction, and have a considerable different density than water. Also for cost-effectiveness, it should be easily available as well as inexpensive and reusable (Banerjee *et al.*, 2002). For successful extraction, the solvent must fully penetrate the biomass and match the polarity of the target compound(s). For example, non-polar solvent such as hexane is used for extracting non-polar lipids. The solvent should have the ability to make physical contact with the lipid material and solvate it. One sure way of achieving this is to mechanically disrupt cells prior to exposing them to the solvent. Solvent extraction can be enhanced by using organic solvents at higher temperature and pressure above their boiling points; this is called accelerated solvent extraction (ASE). This is effective for solid and semi-solid matrices and requires samples to be dried before extraction (Shen *et al.*, 2009).

Traditionally, the Bligh and Dyer (1959) method has been used as the method of extracting biological matrices using a combination of chloroform, methanol and water. This method was originally designed to extract lipids from fish tissue and has been a

benchmark for comparison of solvent extraction methods. However, the disadvantage of using this method is that at large scale, significant quantities of waste solvents are generated making solvent recycling costly and also raising safety concerns due to handling of large amounts of organic solvents (Sahena *et al.*, 2009). Although Bligh and Dyer method have been proven effective, there are others that contradict it. Zhu *et al.* (2002) extracted significantly more oil from dried microalgal biomass compared to oil extraction yield from wet biomass, that is, 41.1 and 27% respectively.

Several researchers have used solvent extraction method with organic solvent in several works. Siddiqui *et al.* (1994) extracted four (4) sterols and nineteen (19) fatty acids using methanol and diethyl ether from green alga *Bryopsis pennata* Lamouraux. In another development, Guil-Guerrero *et al.* (2000) extracted with 96% v/v ethanol and simultaneous saponification yielded fatty acids which were similar to the fatty acid profile of the initial biomass. Miao and Wu (2006) also extracted lipids from *Chlorella protothecoides* using hexane and transesterified with methanol to produce biodiesel.

2.8.2 Mechanical Disruption (Extraction)

This method is an approach that uses mechanical means to disrupt cell wall of microalgae to enable oil extraction. This method minimizes contamination from external sources and maintains the chemical integrity of the substance(s) originally contained within the cell (Greenwell *et al.*, 2010). Examples include pressing, bead milling and homogenization.

Pressing involves subjecting the microalgal biomass to high pressure (this done for nut and seeds) to disrupt cell walls in order to release the oil. High pressure extraction usually yields around 75% of oil present in the microalgae (Posthuma, 2009).

On the other hand, homogenization involves forcing biomass through an orifice resulting in a prompt pressure change as well as high shearing action. Bead milling also entails vessels packed with very small beads that are agitated at high speed. Biomass agitation within the grinding media results in cell disruption. The degree of disruption depends mostly on contact between biomass and beads, size, shape, composition of bead and the strength of the microalgal cell wall (Doucha and Lívanský, 2008).

Bead milling is used in conjunction with solvent extraction. It is most efficient and economical when cell concentrations are significant and when extraction products are easily separated after disruption. Typically, this type of disruption is effective energy-wise when cell concentration of 100-200g/l is used (Greenwell *et al.*, 2010).

In terms of finding appropriate disruption methods, the above methods are among the numerous options. Others may include identifying biological features of the organism that makes it possible to weaken cell wall prior to mechanical disruption, that is, pre-treatment (acid/alkali and enzymatic), thus minimizing the use of solvents (Mercer and Armenta, 2011).

2.8.3 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) refers to the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. This method produces highly purified extracts that are free of potentially harmful solvent residues; extraction and separation are quick, as well as safe for thermally sensitive products (Sahena *et al.*, 2009; Mendiola *et al.*, 2007; Mendes *et al.*, 2003). In addition, fractionation of specific compounds is feasible, which may reduce separation

costs, as well as possibly counteracting greenhouse gas effects by using carbon dioxide waste from industry (Mendiola *et al.*, 2007; Herrero *et al.*, 2006; Mendes *et al.*, 2005; Mendes *et al.*, 2003). Moreover, in order to extract lipids, varied solvents are used. More and more organic solvents used in industries were forbidden because of their toxicity and effects on the 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 bio-fuel production to improve environmental impact (Dejoye *et al.*, 2011).

Supercritical fluid extraction takes advantage of the fact that some chemicals behave as both a liquid and a gas, and have increased solvating power when they are raised above their critical temperature and pressure points. For instance, carbon dioxide is one of such chemicals with relatively low critical temperature of 31.18°C and pressure of 72.9 atm (Cooney *et al.*, 2009). The supercritical carbon dioxide can solubilize 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 the ability of the supercritical carbon dioxide to penetrate and extract targeted molecules.

2.8.4 Ultrasound/sonication

Sonication is the method of applying sound energy to agitate algae particles leading to the damage of cells and thus release of cell content. Ultrasonic-assisted extractions can

recover oils from microalgal cell through the process of cavitation. Cavitation occurs when vapour bubbles of a liquid form in an area where pressure of the liquids is lower than its vapour pressure. These bubbles grow when pressure is negative and compress under positive pressure which causes a violent collapse of the bubbles. If bubbles collapse near cell walls, damage can occur and the cell contents are released (Harun *et al.*, 2010; Wei *et al.*, 2008). One company (Origin Oil) has taken advantage of this property of ultrasonics, by developing a process in which a combination of ultrasound and electromagnetic pulses are used to disrupt algal cell walls. Carbon dioxide is then injected into the resulted slurry of algae biomass to lower pH, which facilitates separation as cell debris sinks to the bottom, and oil rises to the top of the aqueous layer (Mercer and Armenta, 2011).

2.9 Biodiesel and Transesterification

Biodiesel refers to fatty acid methyl esters derived from vegetable oils or animal fat. Biodiesel has gained significant attention in recent years due to the following advantages: biodiesel is an alternative fuel to petroleum-based diesel fuel, it is renewable due to its primary feed stock sources being vegetable oils and animal fat (Gerpen, 2005), non-toxic fuel (Zhang *et al.*, 2003), also contributes no net carbon-dioxide or sulphur to the atmosphere and emits less gaseous pollutants than normal diesel (Lang *et al.*, 2001; Antolin *et al.*, 2002; Vicente *et al.*, 2004).

Basically, there are four ways of producing biodiesel. These are direct use and blending, microemulsions, thermal cracking (pyrolysis) and transesterification (Ma and Hanna, 1999). The commonest of these is transesterification as the biodiesel from

transesterification can be used directly or as blends with fossil diesel fuel in diesel engine (Peterson *et al.*, 1991; Zhang *et al.*, 2003).

Production of biodiesel can be done through direct transesterification of algal biomass or through a two-step procedure by which lipids are extracted, collected, and transesterified (Johnson and Wen, 2009b). Both processes require lipid extraction using combinations of solvents and alcohols, such as chloroform/methanol, hexane/isopropanol, or petroleum ethers and methanol (Johnson and Wen, 2009b; Mulbry *et al.*, 2009). The direct method has the advantage of combining lipid extraction and transesterification into one process, making it less time-consuming than extraction transesterification processes (Johnson and Wen, 2009b). However, studies show that biodiesel yield from wet biomass process using direct transesterification was significantly less than that obtained from dry biomass, indicating that drying of biomass is essential for this process (Johnson and Wen, 2009b). Contrarily, the two-step extraction transesterification process yielded similar results for both wet and dry biomass (Johnson and Wen, 2009b). This two-step procedure eliminates the need to dry the algal slurry (Johnson and Wen, 2009b). There is the potential of reducing costs and greenhouse gas (GHG) emissions associated with algal biodiesel production through solvent-free extraction and transesterification processes.

Fatty acid methyl esters (FAME), also known as biodiesel, are products of the transesterification (methanolysis) of vegetable oils and fats with methanol in the presence of a suitable catalyst yielding glycerol as a by-product. The biodiesel waste product of the reaction, glycerol is washed out of the biodiesel before the fuel is ready for use. The chemical reaction for the process is shown below:

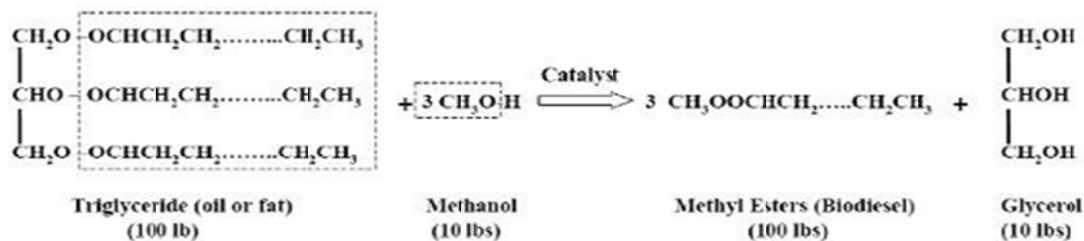


Figure 2.1: Transesterification Reaction to Produce Biodiesel.

The most commonly used catalysts are strong bases such as sodium hydroxide (NaOH) and potassium hydroxide (KOH) in large scale production of biodiesel. In the laboratory scales, acid-catalysed transesterification is often used due to its inability to produce soaps that occur when using a base as a catalyst. Biodiesel yield and FAME composition are significantly influenced by the transesterification conditions such as the methanol to oil ratio, the catalyst loading, reaction time, and temperature. Complete mixing of the reactants is another important parameter influencing the fuel quality (Johnson and Wen, 2009b).

The feedstocks of triglycerides are mainly from edible and non-edible oil sources. The edible oils, for example palm oil, canola oil, soy bean oil and animal fat sources are regarded as first generation biodiesel feedstocks while non-edible oils are the second generation biodiesel feedstocks. These are usually derived from feedstocks such as *Jatropha*, brassica and microalgae.

Although biodiesel is an acceptable alternative for petroleum diesel, it is unlikely that its production using terrestrial biomass feedstock will displace annual demand for petroleum diesel. Studies have revealed that 326% of available arable land in the United States would be required for soy-based biodiesel to meet 50% of the annual demand for

transportation fuel (Chisti, 2007). On the other hand, Chisti (2007) noted that algal biodiesel could satisfy the above mentioned demand with reduced land use effects.

Also, the issue of food for fuel with respect to feedstock from edible oils has become debatable. The increasing demand of feedstocks for biofuels have caused agricultural products price to go up resulting in fluctuate market price. Studies into the second generation biodiesel are far advanced to replace the feedstock for the first generation biodiesel (Xiang, 2012).

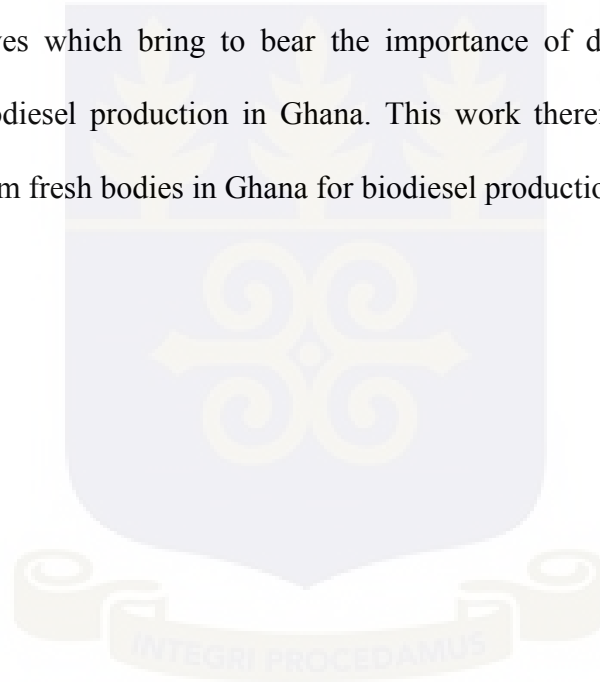
2.10 Ghana and biodiesel production

In Ghana, energy crops that have potential as feedstocks for biofuel production include sugarcane, sweet sorghum, maize and cassava for ethanol, and oil palm, coconut, sunflower, soy bean and *Jatropha* for biodiesel (Ahiataku-Togobo and Ofosu-Ahenkorah, 2009 as cited in Duku *et al.*, 2011). As a result, Ghana is among the few African countries that have tried the production of biodiesel with non-edible oil produced from *Jatropha curcus*.

For large scale production of biodiesel in Ghana, *Jatropha* was probably the most sustainable feedstock in 2009. A number of foreign companies acquired very large tracts of land to grow *Jatropha* to produce biodiesel in the country. For example, Gold Star Farms, a Ghanaian company, obtained about five million acres of land available to grow *Jatropha* for biodiesel production (Degbevi, 2012). Another Norwegian company, Scanfuel also acquired 400,000 hectares of land in the Asante-Akim North District of the Ashanti region to cultivate *Jatropha* for the production of biodiesel for export (Degbevi, 2012). All these companies have folded up due to possible competition for land.

However, it has also been established that the use of these feedstocks for energy could compromise the nation's food security and this could have socio-economic and environmental implications.

Apart from the rise in food prices, a UN report published in 2009 (Degbevi, 2012), warned that the production and use of biodiesel from palm oil on deforested peatlands in the tropics can lead to significant increases in greenhouse gas emissions-up to 2,000% or more when compared with fossil fuels (Degbevi, 2012). Hence there is therefore the need to find alternatives which bring to bear the importance of developing microalgae as feedstock for biodiesel production in Ghana. This work therefore aims at determining suitable algae from fresh bodies in Ghana for biodiesel production.



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Samples

Water samples for microalgae isolation were collected from the following places: the Weija reservoir, wastewater ponds in and near University of Ghana campus, Prampram and a freshwater pond at Teshie. Figure 3.1 shows a map of the collection sites while Appendix 1(Plates 1-5) also shows photographs of collection sites. All experiments were carried out in the research laboratory of the Botany Department, University of Ghana, Legon-Accra.

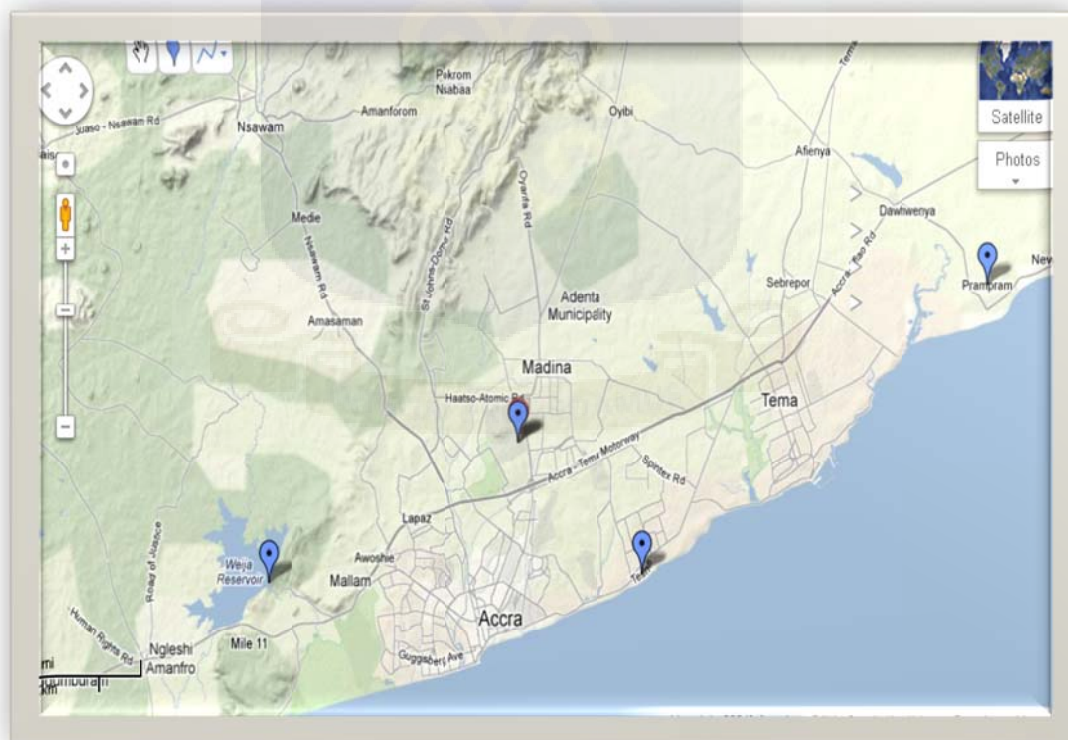


Figure 3.1. Map showing location of collection site (source: Google earth, retrieved April 10, 2013)

In this study, the algae to oil process involves the following steps: 1- collection of algae (for isolation and identification), 2- algae growth in medium (in the presence of nutrients, carbon dioxide, and water for photosynthesis), 3- algae harvesting, 4- algae dewatering and drying, 5- solvent extraction of oil from algae and 6- transesterification into biodiesel (fatty acid methyl esters) (Appendix 2).

3.2 Media Compositions and Preparation

The medium formula used for isolating and growing the microalgae in this study are Bold's basal medium, Sach's solution and BG-11. These formulae are general purpose freshwater medium for growing algae. The formulae contain NaNO_3 as nitrogen source, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ as phosphorus source and metal mixtures as micronutrients. Tables 3.1 and 3.2 show the composition of Bold's basal medium and Sach's solution formulated for isolating microalgal species. Similarly, Table 3.3 gives the protocol of BG-11 formulated for the culture of the cyanobacteria (isolated in the study).

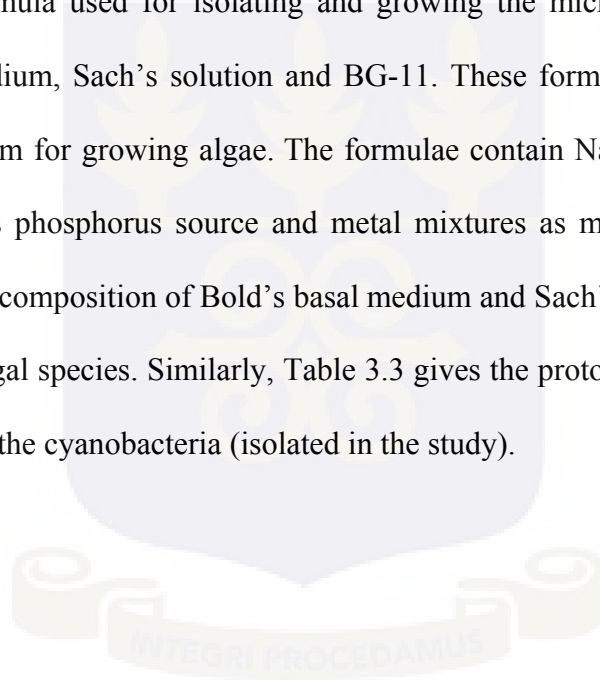


Table 3.1. Protocol for the formulation of Bold's Basal medium (BBM) (Kanz and Bold, 1969).

Stock Solutions	per Litre distilled water (dH₂O)
1. NaNO ₃	25.0 g
2. CaCl ₂ .2H ₂ O	2.5 g
3. MgSO ₄ .7H ₂ O	7.5 g
4. K ₂ HPO ₄	7.5 g
5. KH ₂ PO ₄	17.5 g
6. NaCl	2.5 g
7. EDTA (a) KOH (b)	50.0 g 31.0g
8. FeSO ₄ .7H ₂ O (c) H ₂ SO ₄ (d)	4.98 g 1.0 mL
9. H ₃ BO ₃	11.42g
10. Micronutrients	g.L⁻¹
ZnSO ₄ .7H ₂ O	8.82
MnCl ₂ .4H ₂ O	1.44
MoO ₃	0.71
CuSO ₄ .5H ₂ O	1.57
Co(NO ₃) ₂ .6H ₂ O	0.49

Stock solution 7 (Table 3.1) was prepared by adding EDTA and KOH, dissolving their respective masses and diluting with distilled water to 1 litre. Similarly, stock solution 8 (Table 3.1) was prepared by dissolving the mass of the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ with distilled water. H_2SO_4 was added to the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution and was then topped up with distilled water to 1 litre.

Each constituent of the micronutrients (stock solution 10) were added separately to 800 ml of distilled water and was completely dissolved between each addition and was then topped up to 1 litre (Table 3.1).

In order to prepare one litre of Bold's basal medium for use, 10 ml of each stock solutions (1-6) were added to 940 ml of distilled water. One (1) ml of stock solution (7-10) was also added to make up to 1 litre. The medium was then autoclaved at 121°C for 15 minutes and stored for the experiments.

Table 3.2. Protocol for the formulation of Sach's Solution (Full Strength)

Components	Concentration in solution (g/L)
CaSO_4	0.5
$\text{Ca}(\text{PO}_3)_2$	0.5
MgSO_4	0.5
NaCl	0.16
KNO_3	1.04
FeCl_2	Trace
Distilled water	2,000(ml)

Each of the components in Table 3.2 was dissolved separately into a measuring cylinder and topped up with distilled water to 2 liters for the experiments.

Table 3.3. Protocol for the formulation of BG-11 (Stanier *et al.*, 1971).

Stock Solutions	Per litre distilled water (dH ₂ O)
1. NaNO ₃	15.0 g
2. K ₂ HPO ₄ .3H ₂ O	4.0 g
3. MgSO ₄ .7H ₂ O	7.5 g
4. CaCl ₂ .2H ₂ O	3.6 g
5. Citric acid	0.6 g
6. Ferric ammonium citrate	0.6 g (Autoclave to dissolve)
7. EDTA	0.1 g
8. Na ₂ CO ₃	2.0 g
9. Trace metal mixture	g.L⁻¹
H ₃ BO ₃	2.86
MnCl ₂ .4H ₂ O	1.81
ZnSO ₄ .7H ₂ O	0.222
Na ₂ MoO ₄ .2H ₂ O	0.39
CuSO ₄ .5H ₂ O	0.079
Co(NO ₃) ₂ .6H ₂ O	0.0494

Each stock solution (1-8) was prepared by dissolving the specific mass of stock solution's component in 1 litre of distilled water (Table 3.3). However, the stock solution 9 was prepared by dissolving the specific mass of the components that forms the trace metal mixture. Each of the trace metal mixture's components was added separately to 800ml of distilled water. It was ensured that each of the added components completely dissolved between each addition and made up to 1 litre.

One litre of BG-11 medium was then prepared from the stock solutions (1-9) for the growth of micro algae. Initial volume of 829 ml distilled water was added to 100 ml stock

solution 1 (Table 3.3) in a measuring cylinder. Ten (10) ml each of stock solutions 2-8 were added to the solution. Then 1 ml of stock solution 9 was finally added to bring it up to 1 litre. The pH of the medium was adjusted to approximately 7.4 with 1M NaOH before autoclaving at 121°C for 15 minutes. The medium was stored for subsequent experiments.

3.3 Isolation and Identification of Microalgae

Isolation and identification of microalgae from the samples were carried out as follows: Ten (10) ml of the water sample collected from the various sites were transferred into 250 ml conical flask containing 200ml sterilized Bold's Basal Medium (BBM) (Kanz and Bold, 1969). The same procedure was repeated with Sach's solution and these were cultured for a period of two (2) weeks. Both cultures were incubated at room temperature (29°C) under a 20 Watt white florescent light on a rotary shaker set at 150 r.p.m with photoperiod of 12 hours (day/night) and aerated (Appendix 3. Plate 1). After two (2) weeks of the incubation, the flasks were examined for algal growth using an optical microscope (Leica DM 500). Strains were isolated by spreading 0.1ml of water samples from the flask showing growth into petri dishes containing BG-11 medium plus 1% agar (Appendix 3. Plate 2). This process was also repeated with Sach's solution. Single colonies of algae were then re-cultivated in BG-11 liquid medium as non-axenic batch cultures. Subcultures of these were maintained for subsequent experiments (Appendix 3. Plate 3). Four (4) species of microalgae were isolated and identified: three (3) species of green microalgae and one (1) species of cyanobacteria.

3.4 Culture of Microalgae

Twenty (20) ml of the isolated green microalgae were suspended in 200 ml of Bold's basal medium (Table 3.1) and the cyanobacteria were cultured in BG-11 medium (Stanier *et al.*, 1971). Subcultures were maintained in 500 ml conical flask. The main cultures were grown in 2 litre reagent bottle photobioreactors (improvised) in a well lit room with sunlight entering through a window. Plastic tubes were inserted at the opening of the improvised photobioreactors to ensure gaseous exchange (Plate 3.1). The culture system in this study was operated in a continuous mode.



Plate 3.1. Laboratory set up of some microalgal species (Main Cultures)

3.5 Harvesting and Drying of Microalgae

Algal isolates were cultured for six (6) weeks and harvested at two (2) weeks interval by filtration and centrifugation. For *Oedogonium* sp. and *Spirogyra* sp. cell suspensions were filtered, air dried at room temperature (25°C) and later oven dried at 80°C. *Chroococcus* sp. and *Closterium* sp. were also filtered and later centrifuged at 500g for 10 minutes. They were freeze dried overnight at a temperature of -70°C (Appendix 3. Plate 4)

All the dried microalgae were then ground into powder using an electronic mill for the commencement of the oil extraction.

3.6 Extraction of Algal Oil

Twenty (20) g of each dried algal biomass was taken in a 200 ml solvent mixture 1:1v/v of hexane and diethyl ether and stirred thoroughly on a magnetic stirrer for 24 hours at room temperature to extract oil (Appendix 3. Plate 5a). After the extraction, the content was filtered to separate the biomass using a funnel and a filter paper. The biomass was washed three times with a mixture of the solvents to extract residual lipids present. The extracts (main and residual lipids) obtained were poured into one round bottom flask and the solvents were then removed with a rotary evaporator under vacuum to release the algal oil (Appendix 3. Plate 5b). The weight of algal oil was taken to determine the oil content in the biomass.

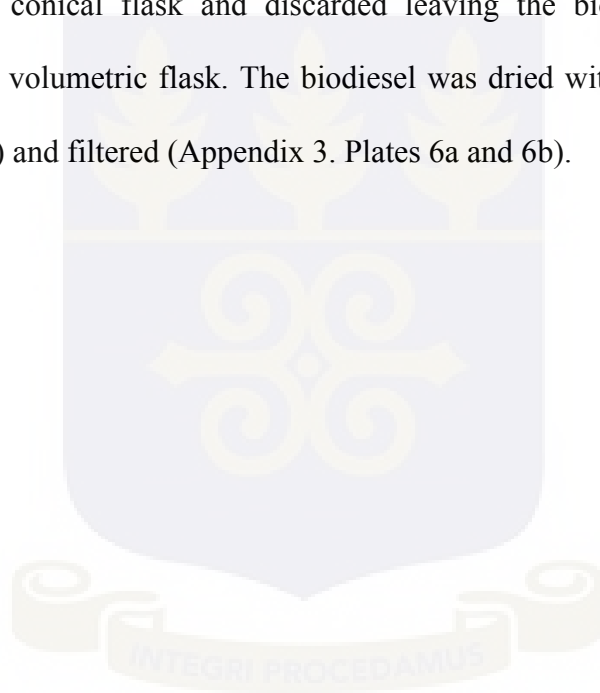
3.7 Transesterification of algal oil

One (1) g of sodium metal was dissolved in 30 ml of methanol in a 500 ml conical flask and thoroughly mixed on a magnetic stirrer. Sodium methoxide was produced *in-situ*

giving off hydrogen gas. Twenty (20) ml of diethyl ether was used to wash the algal oil into the sodium methoxide produced and stirred with a magnetic stirrer for 24 hours to produce biodiesel (fatty acid methyl esters) and glycerol.

3.7.1 Washing and Drying of Biodiesel Produced

The products of transesterification were transferred to a separating funnel and washed with 25 ml of distilled water three times (Appendix 3. Plate 6a). The aqueous layer was separated into a conical flask and discarded leaving the biodiesel which was then transferred into a volumetric flask. The biodiesel was dried with anhydrous magnesium sulphate (MgSO_4) and filtered (Appendix 3. Plates 6a and 6b).



CHAPTER FOUR

4.0 RESULTS

4.1 Isolation and Identification of Microalgae

In this study, four (4) microalgae were isolated and identified based on morphological examination observed under a Leica DM 500 optical microscope. Species identified were *Chroococcus* sp., *Spirogyra* sp., *Oedogonium* sp. and *Closterium* sp. respectively (Plate 4.1-4.4). *Chroococcus* sp. is cyanobacteria while *Oedogonium* sp., *Spirogyra* sp. and *Closterium* sp. are green algae. These species were selected based on their viability in the selected media, i.e. Sach's solution /Bold's Basal Medium.

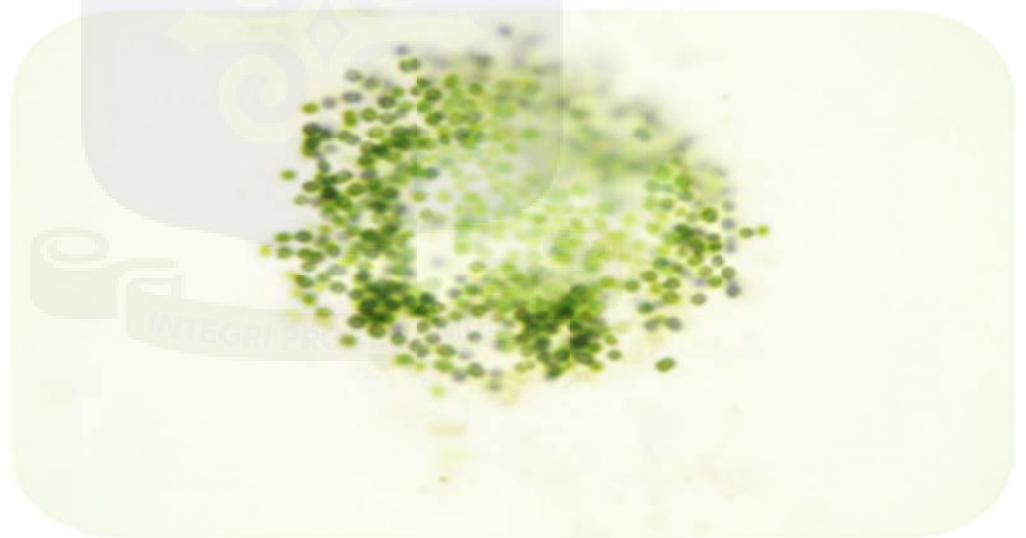


Plate 4.1. Micrograph of *Chroococcus* species

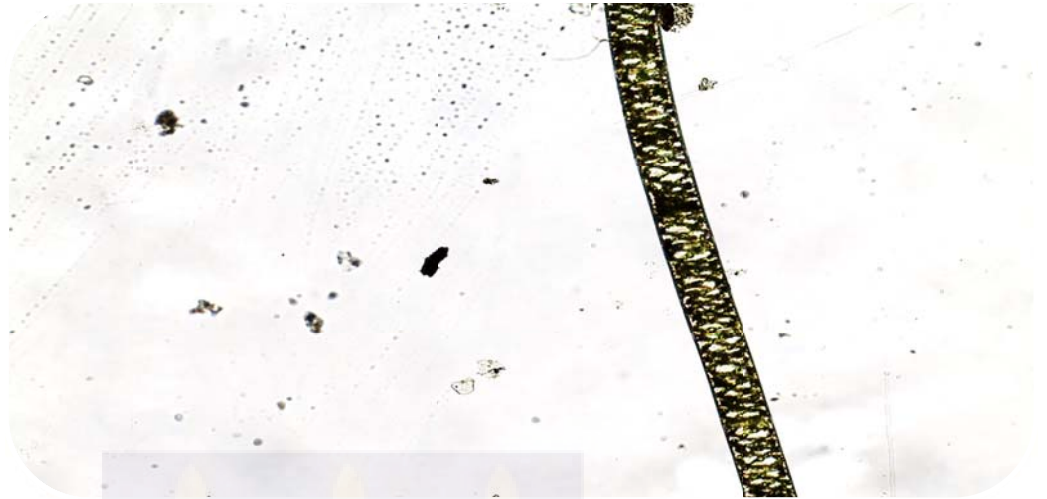


Plate 4.2. Micrograph of *Spirogyra* species



Plate 4.3. Micrograph of *Oedogonium* species

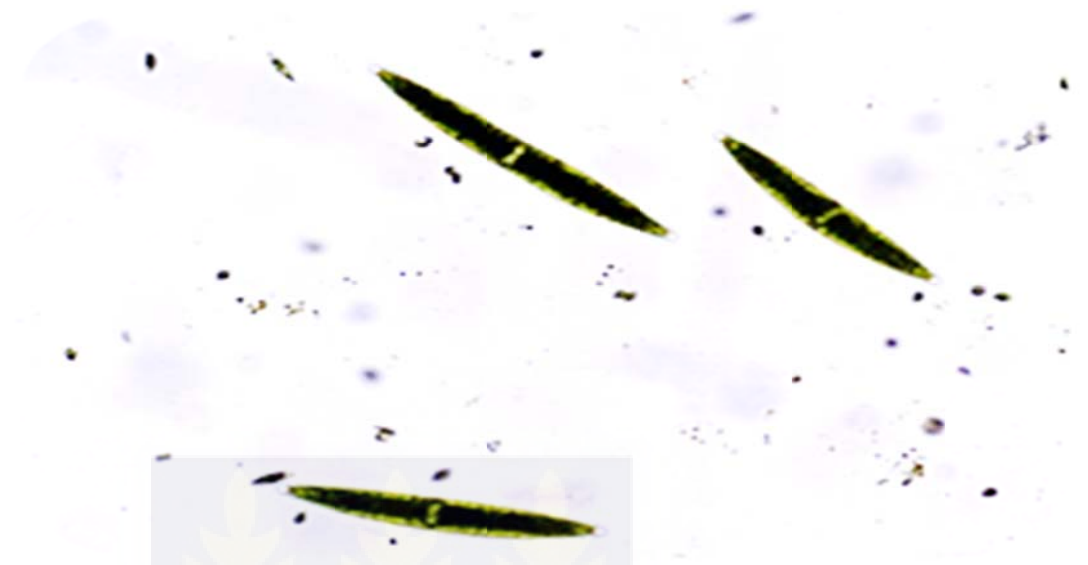


Plate 4.4. Micrograph of *Closterium* species

4.2 Productivity of Microalgal Species

During the culture of microalgae isolates in photobioreactors, cells were observed to have multiplied tremendously, these observations are shown in Plates 4.5 and 4.6 below.



Plate 4.5. Photograph of growth observed after two weeks of culture.



Plate 4.6. Photograph of growth observed after four weeks of culture.

The total dry weight of microalgae was measured after harvesting of microalgae and taken as the total weight of biomass produced for cultures. Table 4.1 below shows the total dry weight of biomass produced for a period of six weeks.

Results indicates a higher percentage dry weight in *Oedogonium* sp. (55.8%) and a lower dry weight of 40.1% in *Closterium* sp., with *Chroococcus* sp. and *Spirogyra* sp. ranging between 50.2 and 50.0 % respectively (Table 4.1).

Table 4.1. Measurement of Total Wet Weight, Dry Weight and Percentage Dry Weight

Microalgae species	Wet weight (g)			Total wet weight (g)	Dry weight (g)			Total dry weight (g)	Percentage dry weight (%)
	Week 2	Week 4	Week 6		Week 2	Week 4	Week 6		
<i>Oedogonium</i> sp.	21.6	21.6	21.8	65.0	12.0	12.1	12.2	36.3	55.8
<i>Chroococcus</i> sp.	20.0	20.1	20.4	61.5	10.0	10.4	10.5	30.9	50.2
<i>Spirogyra</i> sp.	16.7	16.8	17.3	50.8	8.1	8.4	8.9	25.4	50.0
<i>Closterium</i> sp.	16.7	16.9	16.9	50.5	6.5	6.8	7.0	20.3	40.1

4.3 Harvesting and Drying of Microalgae

In every two weeks of culture, the microalgae were harvested by filtration and centrifugation. Plate 4.7a and b show photographs of harvested microalgae whiles Plates 4.8a, 4.8b and 4.8c show photographs of some dried microalgae.

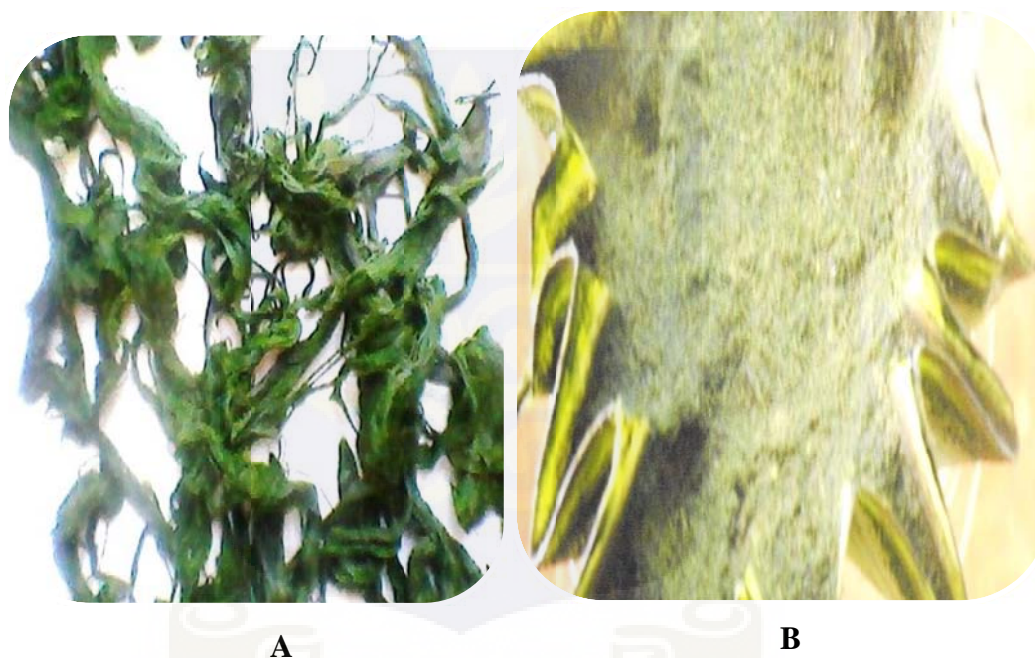


Plate 4.7a. Photographs of harvested *Spirogyra* sp. (A) and *Chroococcus* sp. (B)

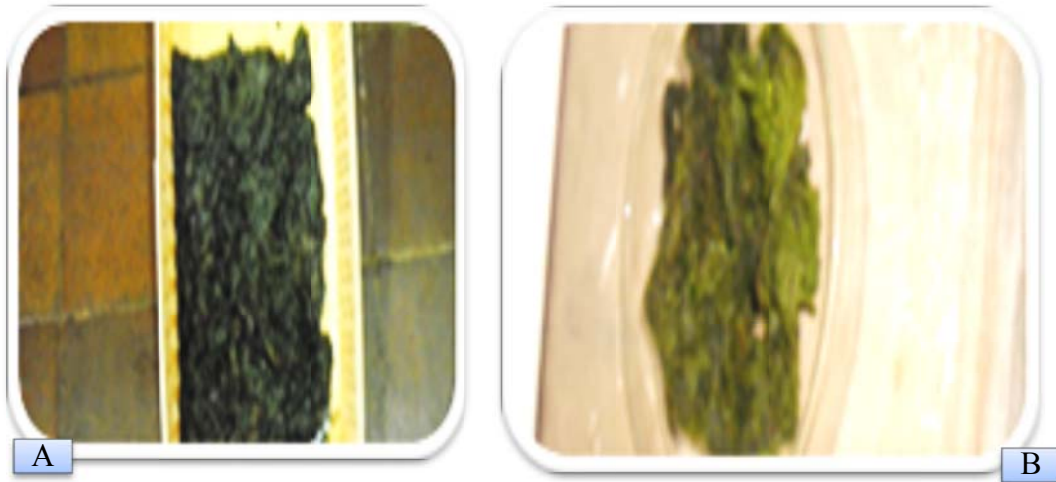


Plate 4.7b. Photographs of harvested *Oedogonium* sp. (A) and *Closterium* sp. (B)



Plate 4.8a. Photograph of dried *Oedogonium* species



Plate 4.8b. Photograph of dried *Chroococcus* species



Plate 4.8c. Photograph of dried *Spirogyra* species

4.4. Algal Oil Production and Transesterification

In the production of biodiesel, algal oil was extracted and transesterified with sodium methoxide to produce the biodiesel. Plate 4.9 shows the photographs of the algal oil produced after the organic solvents have been removed and transesterification carried out.



A

B

Plate 4.9. Photographs of algal oil and esterification (biodiesel and residue layers)

(A) Algal oil, (B) Transesterification.

Plate 5.0 indicates the biodiesel produced after the transesterification process from the algal oil obtained in the study. The biodiesel was collected into tubes with *Oedogonium* sp. having a transparent colour, *Spirogyra* sp. being brown, while *Closterium* sp. and *Chroococcus* sp. were pale yellow in colour.

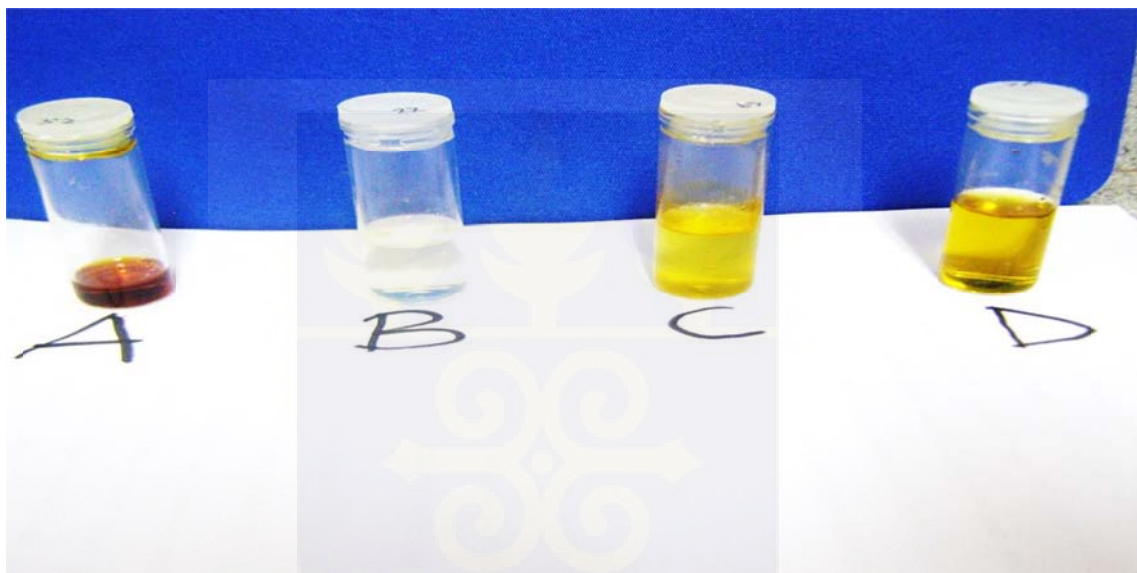


Plate 5.0. Photograph of Biodiesel Produced from Algal Oil. (A) *Spirogyra* sp. (B) *Oedogonium* sp. (C) *Chroococcus* sp. (D) *Closterium* sp.

Figure 4.1 shows the percentage algal oil and biodiesel yield obtained in the study. Results obtained show a high percentage of algal oil (38.1%) produced in *Oedogonium* sp. than all other species. *Chroococcus* sp. and *Closterium* sp. produced relatively moderate quantities ranging between 20-25% while *Spirogyra* sp. produced the least oil of about 21%.

However, the percentage biodiesel yield was not directly proportional to that of the algal oil yield. *Closterium* sp. yielded very significant amount of biodiesel oil of 94 %, while a yield of 80% was produced from *Chroococcus* sp. The percentage yield for *oedogonium* sp. was 49% and the least percentage biodiesel yield was 33% for *Spirogyra* sp.

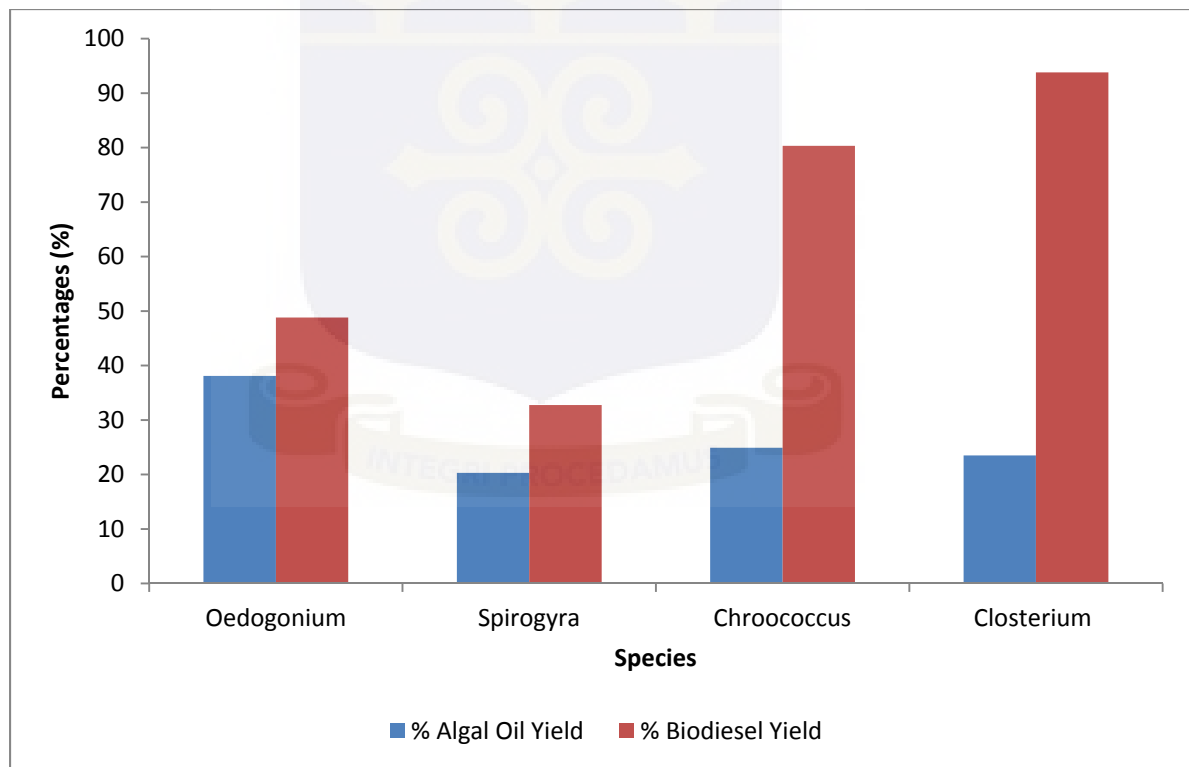


Figure 4.1: Percentage Oil and Biodiesel Yield

CHAPTER FIVE

5.0 DISCUSSION

5.1 Collection, Isolation and Identification of Microalgae

Microalgae are a diverse group of organisms that can grow in aquatic habitats (Johnson and Wen, 2009a). It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed (Richmond, 2004a). In this work, water samples for isolating microalgae were collected from various freshwater such as wastewater ponds and reservoirs based on their availability. Results indicated the isolation and identification of four microalgae species (Plate 4.1- 4.4); of which three (3) are green algae and one (1) is a cyanobacteria. This is comparable with the research of Chinnasamy *et al.* (2010) who found about 27 species of green algae, 20 species of cyanobacteria, and 8 species of diatoms in both treated and untreated wastewaters. Reda *et al.* (2011) also isolated a total of 33 microalgal cultures, 18 from a reservoir and 15 from a livestock wastewater. Eight (8) of the isolates were identified as 5 green algae and 3 cyanobacteria. These indicate the existence of both green algae and cyanobacteria in wastewater and reservoirs which can be a viable source of microalgae isolates for culture into potential feedstock for biodiesel production which was so in this work.

But Metzger and Largeau (2005) reported that, within each chemical race and for the same strain, the morphology of algae could vary in relation to age and culture conditions. The morphological heterogeneity of algae makes microscopic examination difficult. In this work, morphological examinations used in identification limited results to identifying species to the genus level due to difficulty in clearly distinguishing between strains of the

same genus under the microscope. This could possibly lead to wrongly identified species, thus DNA markers for species identification is more appropriate. Furthermore, more microalgae species could be identified if not because of this limitation. Nevertheless, this work was able to isolate and identify some microalgal species for the progress of the work.

Species isolated and identified indicated two new species which have not been used as feedstock in the production of biodiesel. This research indicates that *Chroococcus* sp. and *Closterium* sp. have potentials as feedstock for the production of biodiesel. This is based on their percentage yield of algal oil and biodiesel (Fig 4.1).

5.2 Productivity of Microalgal Species

Under suitable conditions and sufficient nutrients, microalgae can grow profusely. To optimize productivity, it is important to consider growth dynamics of growing algae populations. The first phase of culturing algae is a short period of time that requires algal cells to get used to the new medium conditions. This is particularly so if cells are transferred to a different medium or to a different reactor vessel and it will take some time before the cells start replicating. In week 2 of this study, the dry weights for *Oedogonium* sp., *Chroococcus* sp., *Spirogyra* sp. and *Closterium* sp. were 12.1g, 10.0g, 8.1g and 6.5g, respectively (Table 4.1). Similarly, in week 4 and 6, the dry weights for all the species increased nearly by the same weight. This suggests that, there was exponential growth of the various species of microalgal during the week 2, week 4 and week 6 of culture. It was observed that there was a marginal increase in dry weights of the various species between week 2, week 4 and week 6. This is attributed to supply of

fresh nutrients to the medium after each harvest. Thus, week 4 and week 6 had an increase nutrients level and this translated into the marginal increase in dry weights (Table 4.1). This exponential growth during the culture periods is consistent with the work of Posthuma (2009) who discussed the exponential phase of algae where cells grow regularly leading to an increase in cell density. Dry weights were measured as an indicator of microalgae productivity (Table 4.1), this approach was used to ascertain growth since this work was limited in acquiring counting equipment for cell counts which is a frequently used method of determining the growth rate of algae. Nevertheless, the research work of Zuka *et al.* (2012), has measured the dry weights of harvested algae as a determinant of biomass yield. The measured dry biomass yield was reported as g dry algae/L^{-day}.

Furthermore, observations made in this work suggested multiplication of cells (Plates 4.5 and 4.6) which culminated into a harvest every two weeks within the culture system operating in a continuous mode. Microalgae were harvested every fourteen (14) days which is consistent with the research done by Zuka *et al.* (2012) who showed that between twelve (12) to fourteen (14) days of culture, algae reach their stationary phase and must be harvested before the lyses stage (death stage). This has also been confirmed by Mulumba and Farag (2012).

In culturing microalgae, the composition of medium and its concentration have been found to have an influence on productivity and the concentration of dry weight cellular constituent such as lipids (Posthuma, 2009). Results showed 55.8- 40.1% dry weight of microalgae (Table 4.1) which is significant. This may be due to the presence of essential nutrient such as Nitrogen (NaNO₃), Phosphorous and Potassium (KH₂PO₄ and K₂HPO₄)

in higher concentrations in Bold's Basal Medium and BG-11 media (Tables 3.1 and 3.3) used in the formulated media of culture. These nutrients are all important components which make up a considerable part of total dry weight (Posthuma, 2009; Chisti, 2007; Basova, 2005; Grobblaar, 2004). There are also quite a few elements and even compounds such as vitamins that have been found to increase growth rates (Croft, 2006). In addition to essential nutrients, formulated media also had both vitamins and various metals such as iron (needed for photosynthesis) magnesium, copper and zinc (Tables 3.1 and 3.3) which could account for the significant productivity of microalgae in this work. This is consistent with Grobblaar (2004), who stated that formulated media for algae culture should contain essential nutrients such as nitrogen, phosphorus, iron and in some cases silicon.

Also Table 4.1 confirms significant productivity in *Oedogonium* sp., *Chroococcus* sp., *Spirogyra* sp. and a little below average productivity in *Closterium* sp. These species are all lesser used species in biodiesel research focus but results of this work show their capability in providing feedstock for biodiesel production.

5.3 Harvesting and Drying of Algal Biomass

Within six weeks of growing microalgae, harvesting was done by filtration and centrifugation. These were done to separate the medium from the biomass. The technique used in the harvesting method can determine the efficiency or yield. Also, the performance of the harvesting method can have effect on the solid content in the recovered microalgae. In addition, the rate of water removal can be used to evaluate the performance of the harvesting method used (Xiang, 2012; Uduman *et al.*, 2008).

High speed centrifugation is reliable and an effective technique for recovery of biomass and was done for *Chroococcus* sp. and *Closterium* sp. This method is energy intensive and rapid but a preferred method (Molina-Grima *et al.*, 2003). In this study, centrifugation was employed as the preferred harvesting method of these microalgae due to their small cell sizes.

In filtration, the required magnitude of the pressure drop (this forces fluid to flow through the medium) is dependent on one or more of the following forces: centrifugal, pressure or gravity (Xiang, 2012; Uduman *et al.*, 2009; Shelef *et al.*, 1984). A major challenge of filtration is that, media that are fine enough to retain the microalgae tend to bind and hence require regular backwashes. This result in a decrease in the amount of microalgal concentrates (Uduman *et al.*, 2009). In this study, the filtration method used was surface filters whose driving force was pressure to harvest the microalgal species (*Spirogyra* sp. and *Oedogonium* sp.) which are of large cell size. This is similar to the work of Molina-Grima *et al.* (2003) who conducted a review on process options and found that filtration methods that operate under pressure are suitable for recovering microalgal species with large cell size but inadequate to recover microalgal species with sizes approaching bacterial dimensions (in the range of micrometers).

Three drying methods were used in this study. The drying process helps to degenerate and weaken the cell wall and plasma membrane of microalgae so as to reduce the cell's ability to retain oil and increase the efficiency of the extraction method.

Air drying at room temperature (29°C) was employed for both *Oedogonium* sp. and *Spirogyra* sp. for a period of two days and both were later oven dried at 80°C for 6 hours

to reduce residual moisture. This is consistent with the work of Dejoye *et al.* (2011), who dried algae between temperatures of 20°C and 70° C for 2 to 6 hours to reduce residual moisture. Also, *Chroococcus* sp. and *Closterium* sp. were freeze dried to remove moisture content, similar to research works by Ziga *et al.* (2010) and Belarbi *et al.* (2000). In their work, they concluded that solvent extraction of intercellular metabolites such as oils are extracted readily after freeze drying of microalgae.

5.4 Extraction of Algal Oil and Transesterification

Dried biomass of the microalgae in this work was used for extracting algal oil for biodiesel production. This is comparable to a study by Johnson and Wen (2009b) who showed that biodiesel yield obtained from microalgae after transesterification was higher in dried biomass than biodiesel yield after transesterification using wet biomass. This work is also similar to the research done by Kumar *et al.* (2011) and Hossain *et al.* (2008). In their works, they used dried microalgal biomass for extracting lipids for biodiesel production. Kumar *et al.* (2011) measured the dry weights of *Tolypothrix*, *Pithophora*, *Spirogyra*, *Hydrodictyon*, *Rhizoclonium* and *Cladophora* after air and oven drying which were used for biodiesel production. Hossain *et al.* (2008) also measured the percentage dry weights of *Oedogonium* and *Spirogyra* before extracting lipid for biodiesel production.

From Table 4.1 and Fig. 4.1, it can be observed that the biomass produced for *Oedogonium* sp., *Chroococcus* sp. and *Closterium* sp. are directly proportional to the algal oil yield. However, in spite of the appreciable biomass of *Spirogyra* sp., it rather yielded less algal oil than *Closterium* sp. This may suggest that *Spirogyra* sp. may

produce less algal oil than the other three species. The results in Fig.4.1 further suggests that, the fatty acids content of the algal oil were readily converted to biodiesel in the following order; *Closterium* sp.> *Chroococcus* sp.> *Oedogonium* sp. > and *Spirogyra* sp. Research conducted by Hossain *et al.* (2008) suggests that *Oedogonium* sp. produced more biodiesel than *Spirogyra* sp. and the result in Figure 4.1 was found to be consistent with their research.

Contrary to this, Johnson and Wen (2009b) have suggested that in two-stage transesterification process i.e. involving extracting algal oil and subsequent transesterification, there is the potential of lipid loss during the extraction stage and as a result, direct transesterification (methylation) may lead to a higher crude biodiesel yield.

Results of this study showed that the isolated algae can be cultured to produce biodiesel. In addition, it can be observed that, *Closterium* sp. is a higher biodiesel producing algae followed closely by *Chroococcus* sp., *Oedogonium* sp. and *Spirogyra* sp. being the least.

Oil is present in the cells of algae which are trapped by the cell wall and plasma membrane. These structures inhibit the cells ability to easily export oil from the cell. When algae cells are dried, the plasma membrane are degenerated and weaken the cells ability to retain oil. When hexane, an organic solvent is introduced to the dry algae sample, the cell wall is penetrated by the hexane and the oil within the cell is dissolved. When the hexane is removed from the algae sample, the oil is transported through the cell wall and effectively removed from the algae cell. The collection of the oil is done by evaporating the hexane off which will leave the algae oil behind (Browne *et al.*, 2010).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The work presented in this thesis leads to a deeper understanding of the use of algal biomass as a biodiesel feedstock. This is achieved through areas related to the entire process from growth of microalgae to biodiesel production. From the results and discussion made, the major findings of this work include: the isolation and identification of four freshwater microalgae which are rarely used in research works for biodiesel production. These microalgae are green algae: *Oedogonium* species, *Closterium* species and *Spirogyra* species, and a cyanobacteria: *Chroococcus* species. Results indicated that under room temperature (29°C), air, natural source of light and appropriate medium these species can grow profusely. This provide a 'proof of concept' that, some lesser known species like the above mentioned algae can provide significant biomass as feedstock for biodiesel production when the right conditions are provided. Subsequently large scale culture may be undertaken.

In this study, it can be concluded that filtration is a suitable harvesting method for dewatering cultured *Oedogonium* sp. and *Spirogyra* sp. while centrifugation is a suitable method of harvesting for *Closterium* sp. and *Chroococcus* sp.

Also, it can be concluded that freeze drying is a suitable drying method for *Closterium* sp. and *Chroococcus* sp. while air drying and oven drying are suitable for *Oedogonium* sp. and *Spirogyra* sp.

In producing biodiesel, it can be concluded from this work that, *Oedogonium* sp., *Closterium* sp., *Spirogyra* sp. and *Chroococcus* sp. are viable species which can provide substantial amount of algal oil for biodiesel production. *Closterium* sp. is a higher biodiesel producing algae followed closely by *Chroococcus* sp., *Oedogonium* sp. and *Spirogyra* sp. being the least. Hence biodiesel from these microalgae can be a renewable source of energy that can substitute petroleum diesel when embarked upon on a large scale.

6.2 Recommendations

The following recommendations may be made from the study:

- (i) Investments must be made to develop a technology on algae cultures for the production of biodiesel that will be economically viable in Ghana to produce biodiesel on a large scale.
- (ii) An in-depth economic analysis should be performed on each aspect of the algae to biodiesel production process to reduce the cost of biodiesel production making it a better alternative to fossil diesel in Ghana.
- (iii) Future research should concentrate on manipulating media composition to aid isolation, identification, and culture of more algal species.
- (iv) Research work should be carried out to characterize biodiesel produced from microalgae in Ghana and compared to fossil diesel in the Ghanaian market.

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APPENDIX 1

Appendix 1 includes supporting photographs of collection sites in the study.



Plate 1. Collection site at Prampram



Plate 2. Researcher at collection site at Prampram



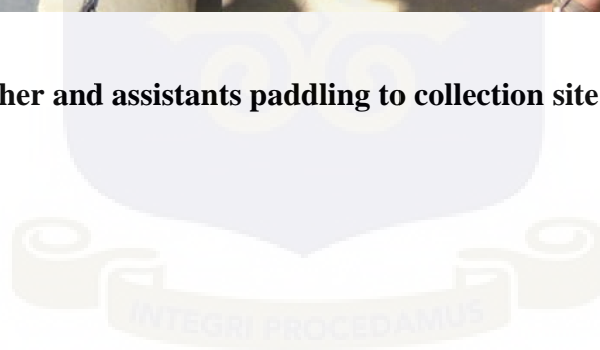
Plate 3. Collection site at Teshie



Plate 4. Collection site at the Weija Reservoir



Plate 5. Researcher and assistants paddling to collection site on the Weija Reservoir



APPENDIX 2

Appendix 2 is a supporting data of the methodology presented in chapter 3.

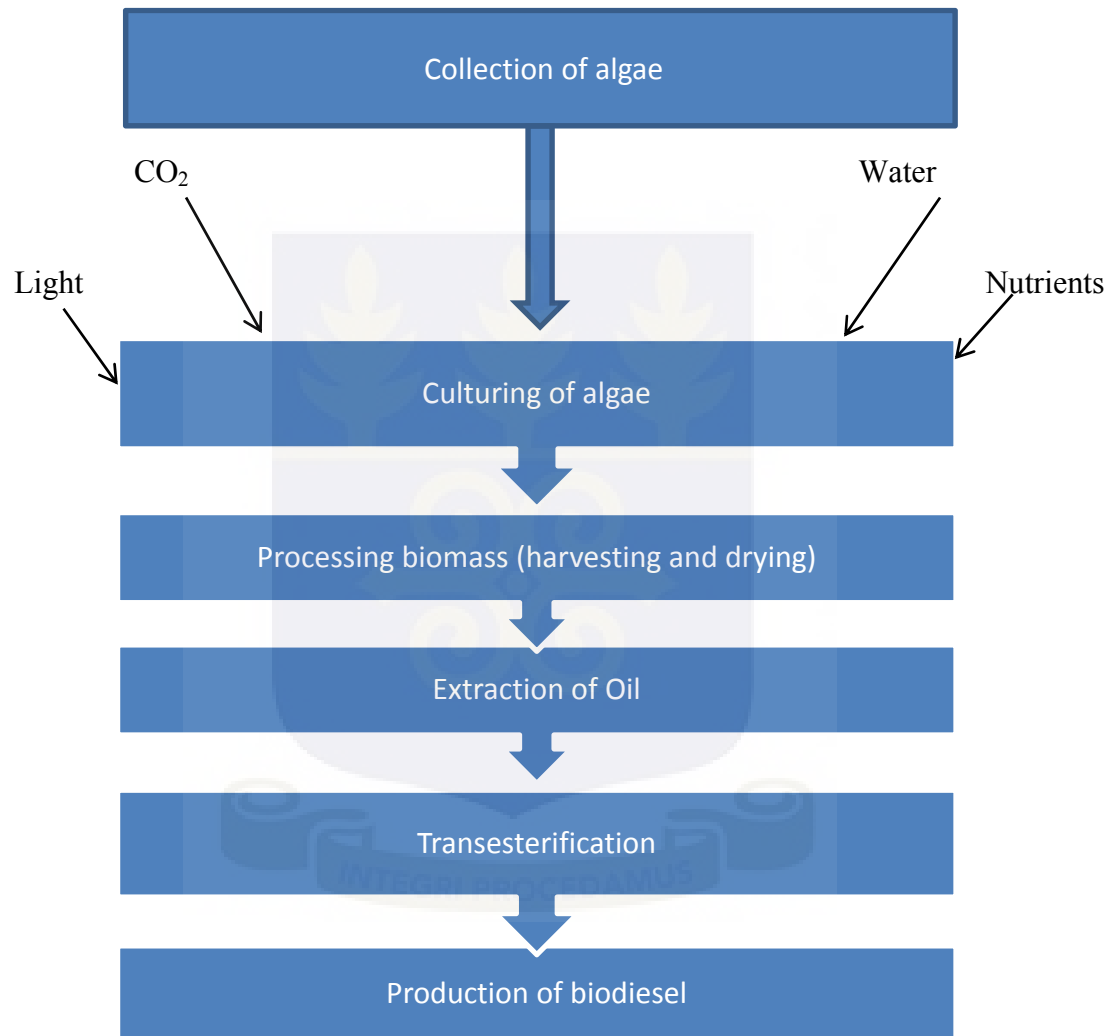


Fig. 1 Flow Chart showing the Stages of Biodiesel Production

APPENDIX 3



Plate 1. Initial culture of species in the laboratory for species acclimatization to formulated medium.



Plate 2. Agar prepared for the isolation of microalgae species



Plate 3. Some subcultures prepared for growing microalgae



Plate 4. Freeze drying of microalgae in the laboratory



Plate 5a. Extraction of oil from microalgae (from stirring to extract)



Plate 5b. Extraction of algal oil (solvent removal)



Plate 6a. Washing and separating biodiesel from other organic component of the algae



Plate 6b. Drying biodiesel with magnesium sulphate (MgSO_4)



Plate 6c. Separating the drying agent from the biodiesel

APPENDIX 4

Appendix 4 includes some of the supporting data of the results presented in chapter 4.

Percentage Weights of Algal Oil and Biodiesel Produced

Species	Algal Oil (%)	Biodiesel Produced (%)
<i>Oedogonium sp.</i>	38.1	48.81
<i>Spirogyra sp.</i>	20.3	32.76
<i>Chroococcus sp.</i>	24.9	80.32
<i>Closterium sp.</i>	23.5	93.81

Algal Oil and Biodiesel Produced from Species Used in the Study

Species	Algal oil (g)	Biodiesel produced (g)
<i>Oedogonium sp.</i>	7.62	3.72
<i>Spirogyra sp.</i>	4.06	1.33
<i>Chroococcus sp.</i>	4.98	4.00
<i>Closterium sp.</i>	4.69	4.40