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Biodiesel production from freshwater microalgae of Ghana

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Abstract. *Ruth OA, Ameka GK, Annag T. 2020. Biodiesel production from freshwater microalgae of Ghana. Bioteknologi 17: 37-45.* Due to rising oil prices and concerns about global warming, biodiesel has gained prominence as an alternative energy source. Biodiesel produced from microalgae is a potential carbon–neutral and renewable alternative to petroleum fuels. This study aimed to investigate the viability of manufacturing biodiesel from microalgae harvested from freshwater bodies in Ghana as an environmentally sustainable alternative to fossil diesel. In the study, water samples with algal blooms were taken from the Weija reservoir, wastewater ponds in and around the main campus of the University of Ghana in Prampram, and a freshwater pond in Accra's Teshie neighborhood. Four (4) microalgae were isolated and identified based on their morphology and cultivation ease: *Oedogonium* sp., *Chroococcus* sp., *Spirogyra* sp., and *Closterium* sp. After being grown in a 2-liter reagent bottle Photobioreactor (improvised) utilizing sunlight as the energy source, these isolates were collected, filtered to remove excess water, and centrifuged to form pastes. Extracting the oil from the algae required drying and grinding the pastes into powder. And then, the oils were transesterified into biodiesel. In terms of percentage dry weight, *Oedogonium* sp. exhibited a considerable increase (55.8%) and *Closterium* sp. exhibited a decrease (40.1%), while *Chroococcus* sp. and Spirogyra sp. ranged between 50.2 and 50.0%, respectively. Significant volumes (20- 38%) of microalgal oil were successfully extracted with hexane and diethyl ether and converted into biodiesel. Results also suggested that these species may grow abundantly under ambient temperature, air, natural source of light, and suitable substrate. 94% of biodiesel was produced from *Closterium* sp., compared to 80% from *Chroococcus* sp. The highest biodiesel yield was 49% for *Oedogonium* sp., and the lowest was 33% for *Spirogyra* sp. This study isolated and identified four freshwater microalgae that are seldom employed in biodiesel manufacturing studies. Conclusion: *Oedogonium* sp., *Closterium* sp., *Spirogyra* sp., and *Chroococcus* sp. are viable species capable of producing a large amount of algal oil for biodiesel synthesis. Consequently, efforts must be made to create a more effective and economically viable technology for the large-scale growth of algae for biodiesel production in Ghana. In addition, a comprehensive economic study must be conducted on every component of the algae-to-biodiesel production process. It can minimize the cost of biodiesel production, hence making it a more economical alternative to fossil fuel in Ghana.

Keywords: Biodiesel, microalgae, Ghana, sustainable bioenergy

INTRODUCTION

An estimated 27% of the world's primary energy supply goes toward the transportation industry, making it one of the fastest-growing industries (Antoni et al. 2007). However, the industry's lifeblood, crude oil, is becoming unsustainable due to dwindling reserves in a few geographically-favored areas (Ciubota-Rosie et al. 2008). As a result, fossil fuels meet about 80% of the world's energy needs today (Schenk et al. 2008). However, the biggest drawback of utilizing petroleum fuels is the pollution they cause to the atmosphere, especially diesel fuel, which produces a lot of carbon dioxide during burning (a greenhouse gas). In addition to this emission, petroleum diesel is a significant contributor to other air pollutants, including nitrogen oxides (NOx), sulfur oxides (SOx), carbon monoxide (CO), particulate matter (PM), and volatile organic compounds (VOC) (Hallenbeck and Benemann 2002). As a result, there is a worldwide push to create reliable renewable energy.

As an additional note, the cost of oil has been on the increase around the world. In 2010, rising demand from a recovering economy drove up global oil prices due to insufficient supply. Moreover, as social and political upheaval spread throughout numerous Middle Eastern and African economies, prices continued to rise through the end of 2010 and into 2011 (IEO 2011). Thus, non-oil alternatives have gained importance (Neltner 2008), and biodiesel's popularity as an alternative fuel source has skyrocketed in recent years (Koonin 2006; Durrett et al. 2008; Basha and Jebaraj 2009; Demirbas 2009).

In addition, a significant amount of effort has been invested into finding viable biomass feedstock from which biodiesel and gas fuels can be produced for power plants (Singh and Gu 2010). Soybean, rapeseed, sunflower, and safflower oil are some of the most studied oil sources since they are also edible (Altin et al. 2001; Lang et al. 2001; Twidell and Weir 2006; Parawira 2010). Other types of biomass, such as energy crops (both edible and non-edible oilseeds), bio-wastes, wood, and some types of aquatic plants like algae, have been identified as potential sources of bio-oil (Duku et al. 2011; Amirta et al. 2016).

Due to depleting fossil fuel sources, the world's economy is in danger of experiencing an energy crisis. In addition, countries face challenges, such as environmental pollution and health issues due to carbon dioxide, pollutant emissions, and the accompanying economic consequences. Significant effects on aquatic life are also caused by the ocean dissolving around a third of the carbon dioxide created by the combustion of fossil fuels (Mata et al. 2010).

Biodiesel has been suggested as a solution to many of these issues. However, difficulties in sourcing the correct feedstock have slowed the growth of the biodiesel production business worldwide. Recent research shows that using biomass to create biodiesel is a promising way to help the planet (Hossain et al. 2008). Soybean, corn, sugarcane, canola, Jatropha, and other crops are viable biomass sources, but they are controversial due to global issues of food-fuel conflict and competition for land. Microalgae are being used more frequently in biodiesel production due to these issues. However, there is almost no data on the topic in Ghana. This study aims to identify viable algae species from Ghanaian freshwater bodies for use in biodiesel manufacturing.

MATERIALS AND METHODS

Collection of samples

The Weija reservoir, wastewater ponds in and around the University of Ghana campus in Prampram, and a freshwater pond in Teshie, Ghana, all provided water samples for isolating microalgae. Figure 1 is a map showing where the samples were taken. The research laboratory of the Botany Department at the University of Ghana in Legon-Accra, Ghana, was used for all of the experiments.

According to this research, the following procedures are required to convert algae into oil: Step 1: harvesting and identifying algae; Step 2: cultivating algae in a medium; Step 3: dewatering and drying the algae; Step 5: solvent extraction of oil from the algae; Step 6: transesterification into biodiesel (fatty acid methyl esters).

Media compositions and preparation

To isolate and cultivate the microalgae, the researchers employed Bold's basal medium, Sach's solution, and BG-11 as their medium formula. These combinations represent a versatile freshwater algal growth medium. In the formulas, NaNO3 is used as the nitrogen source, $K_2HPO_4.3H_2O$ is the phosphorus source, and metal mixes are used as the micronutrients. Components of Bold's basal medium and Sach's solution developed for isolating microalgal species are listed in Tables 1 and 2, respectively. In addition, Table 3 displays the BG-11 developed methodology for cyanobacteria culture (isolated in the study).

The seventh stock solution (Table 1) was made by dissolving EDTA and KOH in 1 liter of distilled water. The eighth stock solution (Table 1) was made by dissolving the same amount of $FeSO_4.7H_2O$ into sterile water. The $FeSO_4.7H_2O$ solution was adjusted to 1 liter by adding H_2SO_4, followed by distillate water.

Micronutrients (stock solution 10) were mixed into 800 mL of distilled water, one component at a time until fully dissolved between additions. The volume was then brought up to 1 liter (Table 1).

10 mL of each stock solution (1-6) was added to 940 milliliters of distilled water to make one liter of Bold's basal medium. The final volume was brought up to l liter by adding one (1) mL of stock solution (7-10). Before being kept for later use in the experiments, the medium was autoclaved at 121°C for 15 minutes.

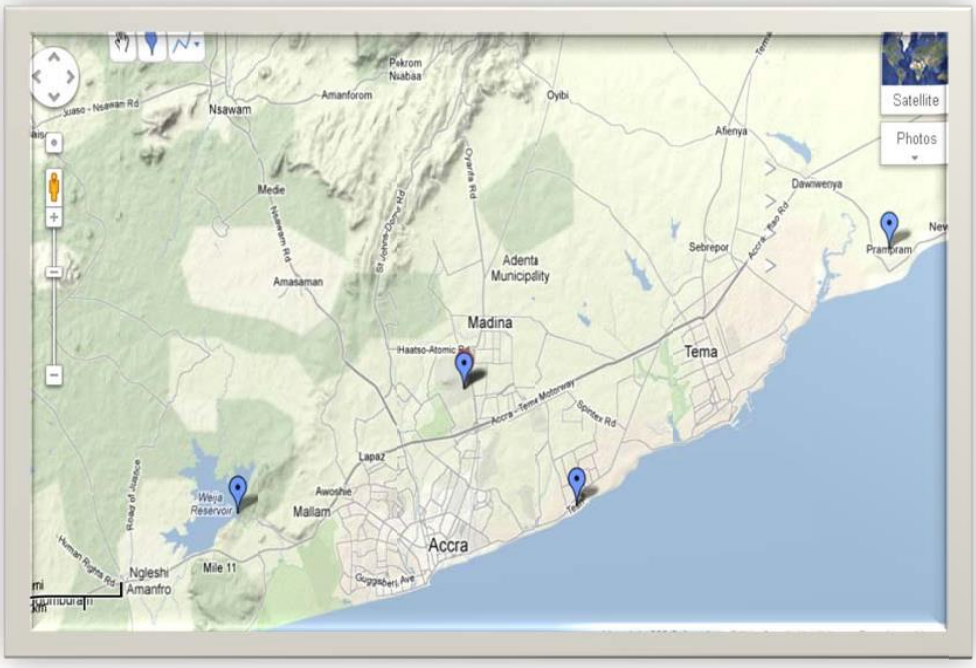

Figure 1. Map showing the location of collection site (source: Google Earth, retrieved April 10, 2013)

Table 1. Protocol for formulating Bold's Basal medium (BBM) (Kanz and Bold 1969)

Stock solutions	**per litre distilled water (dH_2O)**
1. $NaNO_3$	25.0 g
2. $CaCl_2.2H_2O$	2.5 g
3. $MgSO_4.7H_2O$	7.5 g
4. K_2HPO_4	7.5 g
5. KH_2PO_4	17.5 g
6. NaCl	2.5 g
7. EDTA (a)	50.0 g
KOH (b)	31.0g
8. $FeSO_4.7H_2O$ (c)	4.98 g
H_2SO_4 (d)	1.0 mL
9. H_3BO_3	11.42g
10. Micronutrients	**$g.L^{-1}$**
$ZnSO_4.7H_2O$	8.82
$MnCl_2.4H_2O$	1.44
MoO_3	0.71
$CuSO_4.5H_2O$	1.57
$Co(NO_3)_2.6H_2O$	0.49

Table 2. Protocol for the formulation of Sach's Solution (Full Strength)

Components	**Concentration in solution (g/L)**
$CaSO_4$	0.5
$Ca(PO_3)_2$	0.5
$MgSO_4$	0.5
NaCl	0.16
KNO_3	1.04
$FeCl_2$	Trace
Distilled water	2,000(ml)

Table 3. Protocol for the formulation of BG-11 (Stanier et al. 1971)

Stock solutions	**Per liter distilled water (dH_2O)**
1. $NaNO_3$	15.0 g
2. $K_2HPO_4.3H_2O$	4.0 g
3. $MgSO_4.7H_2O$	7.5 g
4. $CaCl_2.2H_2O$	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_2CO_3	2.0 g
9. **Trace metal mixture**	**$g.L^{-1}$**
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.39
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H2O$	0.0494

For the experiments, each component from Table 2 was dissolved individually in a measuring cylinder, then filled with distilled water until it reached a volume of 2 liters.

Dissolving the required mass of each component into one liter of distilled water yielded eight different stock solutions (1-8) (Table 3). Nonetheless, stock solution 9 was prepared by dissolving the specified mass of the trace metal mixture's constituents. Then, each component of the trace metal mixture was added individually to 800 mL of distilled water. It was guaranteed that each component thoroughly dissolved between additions and that the total volume reached 1 liter.

The stock solutions (1-9) were then used to prepare one liter of BG-11 medium for microalgae growth. First, an initial volume of 829 mL of pure water in a measuring cylinder was added to 100 mL of stock solution 1 (Table 3). Next, each stock solution (2-8) was added at a volume of ten (10) milliliters. Finally, 1 mL of stock solution 9 was added to raise the volume to 1 liter. Before autoclaving at 121°C for 15 minutes, the pH of the medium was adjusted to about 7.4 using 1M NaOH. The medium was preserved for use in future research.

Isolation and identification of microalgae

Microalgae were isolated and identified in the following ways: Ten (10) mL of water from each site was placed in a 250 mL conical flask with 200 mL of sterilized Bold's Basal Medium (BBM) (Kanz and Bold 1969). Sach's solution was used to do the identical operation, and the results were cultured for two weeks. Both cultures were kept at 29°C, illuminated by a 20-watt white fluorescent light, and aerated by a rotary shaker rotating at 150 revolutions per minute throughout a 12-hour day/night cycle. The flasks were inspected under an optical microscope for signs of algal growth after two (2) weeks of incubation (Leica DM 500). Next, isolated strains were grown in a BG-11 medium with 1% agar from 0.1 mL of water taken from a flask displaying growth. Sach's solution was used for the second round of testing. The algae were cultured as single colonies in BG-11 liquid medium and then re-cultured as non-axenic batch cultures. These were kept in subcultures for further testing. Three types of green microalgae and one kind of cyanobacteria were found and isolated for four different types of microalgae.

Culture of microalgae

Isolated green microalgae were cultivated in BG-11 medium for cyanobacteria, and 20 mL of the microalgal suspension was added to 200 mL of Bold's baseline medium (Table 1) (Stanier et al. 1971). A 500 mL conical flask was used to house the subcultures. The primary cultures were developed in a well-lit room with sunlight coming in via a window using homemade photobioreactors made from 2-liter reagent bottles. To facilitate gaseous exchange, plastic tubes were put into the openings of the makeshift photobioreactors (Figure 2). In this investigation, a continuous culture system was used.

Harvesting and drying of microalgae

Isolates of algae were grown in a petri dish for six weeks before being filtered and centrifuged every two weeks for harvesting. Cell suspensions of *Oedogonium* sp. and *Spirogyra* sp. were filtered before being air dried at room temperature (25°C) and then oven dried at 80°C. We also filtered and centrifuged at 500g for 10 minutes to remove *Chroococcus* sp. and *Closterium* sp. They were cooled to -70°C and freeze-dried for 12 hours.

Figure 2. Laboratory set up of some microalgal species (main cultures)

To begin the oil extraction process, all of the dried microalgae were ground into powder in an electronic mill.

Extraction of algal oil

For 24 hours at room temperature, twenty (20) g of each dried algal biomass was added to a 200 mL solvent mixture of 1:1 v/v hexane and diethyl ether. The mixture was agitated vigorously on a magnetic field stirrer (Figure 12). The extracted substance was filtered using a funnel and some filter paper to remove the biomass. To extract any remaining lipids, the biomass was washed three times using a combination of the solvents. After collecting the extracts (main and residual lipids), the solvents were evaporated using a rotary evaporator under a vacuum, resulting in the algal oil (Figure 12). Oil content in the biomass was calculated using the mass of extracted algal oil.

Transesterification of algal oil

Using a magnetic stirrer, one (1) gram of sodium metal was dissolved in 30 milliliters of methanol in a 500-milliliter conical flask. Hydrogen gas was released during the onsite production of sodium methoxide. Biodiesel (fatty acid methyl esters) and glycerol were obtained by washing algal oil with twenty (20) mL of diethyl ether and adding it to the sodium methoxide while stirring with a magnetic stirrer for twenty-four (24) hours.

Washing and drying of biodiesel produced

The transesterification by-products were collected in a separating funnel and rinsed three times with 25 mL of distilled water. The biodiesel was transferred from its original container into a volumetric flask after the aqueous layer had been collected in a conical flask and discarded. With the aid of anhydrous magnesium sulfate ($MgSO_4$), the biodiesel was dehydrated and filtered.

RESULTS AND DISCUSSION

Isolation and identification of microalgae

Using morphological analysis performed using a Leica DM 500 optical microscope, four (4) different types of microalgae were isolated and named for this study. *Chroococcus*, *Spirogyra*, *Oedogonium*, and *Closterium* were isolated (Figure 3-6). *Chroococcus* sp. is a cyanobacterium, while *Oedogonium* sp., *Spirogyra* sp., and *Closterium* sp. are all green algae. These species were chosen because they thrive in the designated growth medium combinations of Sach's solution and Bold's Basal Medium.

Productivity of microalgal species

Microalgae isolates cultured in photobioreactors were shown to have had tremendous cell multiplication, as depicted in Figures 7 and 8.

After harvesting microalgae, the total dry weight of microalgae was determined and used as the total biomass produced for cultures. Table 4 displays the total dry weight of biomass produced over six weeks.

The results show that *Oedogonium* sp. has the highest dry weight percentage (55.8%), followed by *Closterium* sp. at 40.1%, *Chroococcus* sp. at 50.2%, and *Spirogyra* sp. at 50%. (Table 4).

Figure 3. Micrograph of *Chroococcus* species

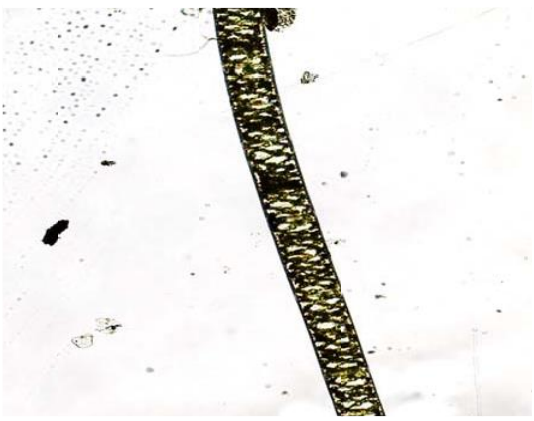

Figure 4. Micrograph of *Spirogyra* species

Figure 5. Micrograph of *Oedogonium* species

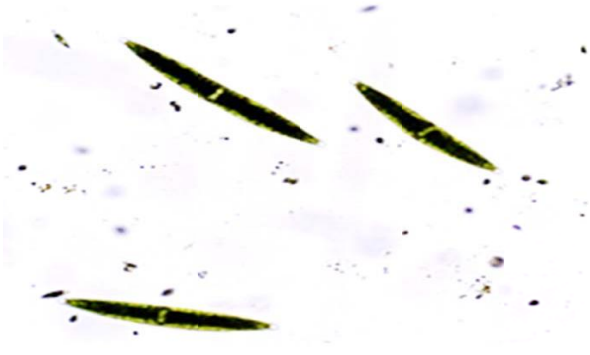

Figure 6. Micrograph of *Closterium* species

Figure 7. Photograph of growth observed after two weeks of culture

Figure 8. Photograph of growth observed after four weeks of culture

Table 4. 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)	Percentagedry weight (%)
	Week2	Week4	Week6		Week2	Week4	Week6		
Oedogonium sp.	21.6	21.6	21.8	65.0	12.0	12.1	12.2	36.3	55.8
Chroococcus sp.	20.0	20.1	20.4	61.5	10.0	10.4	10.5	30.9	50.2
Spirogyra sp.	16.7	16.8	17.3	50.8	8.1	8.4	8.9	25.4	50.0
Closterium sp.	16.7	16.9	16.9	50.5	6.5	6.8	7.0	20.3	40.1

Harvesting and drying of microalgae

The microalgae were collected every two weeks by filtration and centrifugation. Images of collected microalgae are shown in Figure 9. Photographs of dried microalgae are presented in Figure 10.

Algal oil production and transesterification

Biodiesel was created by transesterifying algal oil with sodium methoxide. Images of the transesterified algal oil are presented in Figure 11. This process involves the removal of organic solvents.

Algal oil was transesterified to create biodiesel, as seen in Figure 12. *Oedogonium* sp. was clear, *Spirogyra* sp. was brown, and *Closterium* sp. and *Chrooccocus* sp. were pale yellow as they produced the biodiesel collected in tubes.

The yield percentages of algal oil and biodiesel are displayed in Figure 13. According to the data, *Oedogonium* sp. produces the highest concentration of algal oil (38.1%). On the other hand, the oil yield from *Chroococcus* sp. and *Closterium* sp. was in the 20-25% range, while that of *Spirogyra* sp. was only around 21%.

The output of biodiesel was not proportionate to the yield of algal oil. Significant amounts of biodiesel oil (94%) were produced by the *Closterium* sp. strain, but the *Chroococcus* sp. strain only produced 80%. A 49% yield was achieved by cultivating *Oedogonium* sp., while *Spirogyra* sp. achieved the lowest biodiesel production at 33%.

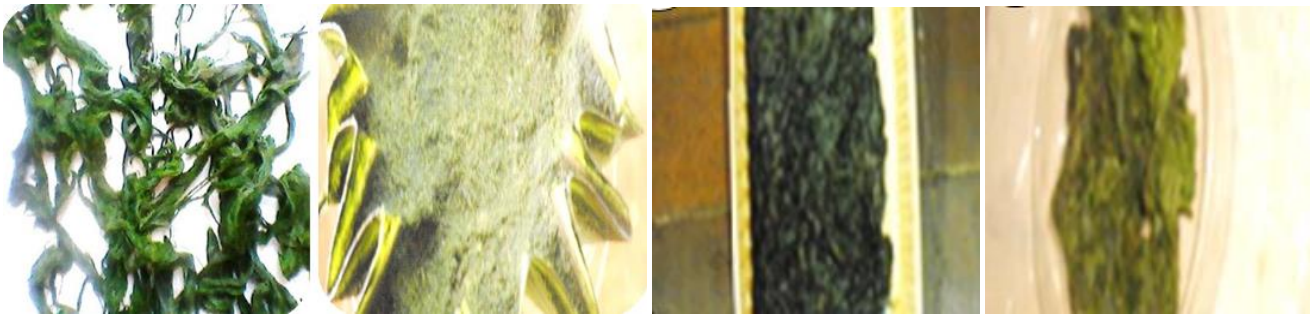

Figure 9. Photographs of harvested *Spirogyra* sp. (A) and *Chroococus* sp. (B), *Oedogonium* sp. (C), and *Closterium* sp. (D)

Figure 10. Photograph of dried *Oedogonium* species (A), *Chroococcus* species (B), *Spirogyra* species (C)

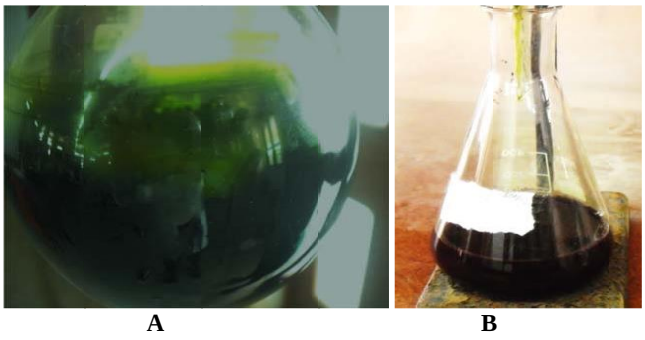

Figure 11. Photographs of algal oil and esterification (biodiesel and residue layers). (A) Algal oil, (B) Transesterification

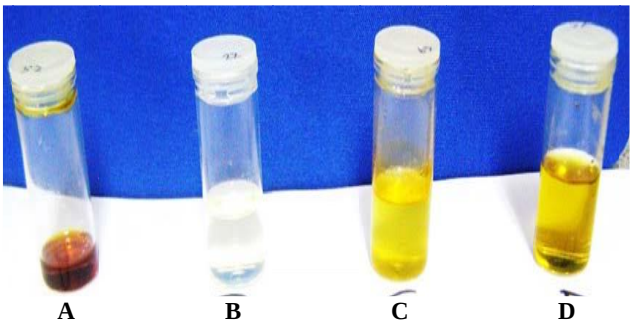

Figure 12. Photograph of Biodiesel Produced from Algal Oil. (A) *Spirogyra* sp. (B) *Oedogonium* sp. (C) *Chrooccocus* sp. (D) *Closterium* sp.

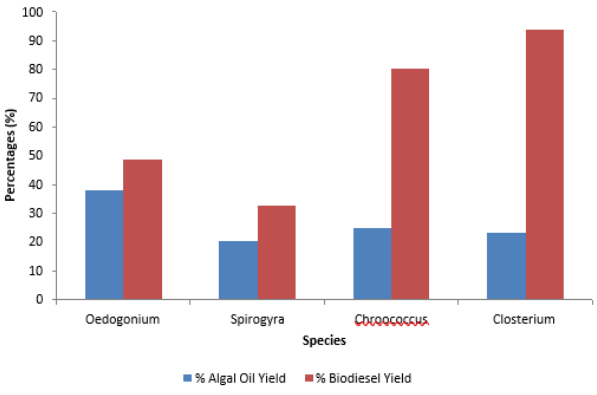

Figure 13. Percentage of oil and biodiesel yield

Discussion

Collection, isolation, and identification of microalgae

Microalgae are a heterogeneous group of organisms that can thrive in watery environments (Johnson and Wen 2009a). Though more than 50,000 species are thought to exist, only about 30,000 have been formally examined (Richmond 2004). This study gathered water samples from various freshwater sources, including wastewater ponds and reservoirs, to isolate microalgae. Three types of green algae and one type of cyanobacterium were found to have been isolated and identified from the samples (Figures 3-6). About 27 species of green algae, 20 species of cyanobacteria, and 8 species of diatoms were found in both treated and untreated wastewaters in a study by Chinnasamy et al. (2010), so these results are comparable.

Additionally, Reda et al. (2011) isolated 33 microalgal cultures, 18 from a reservoir and 15 from livestock effluent. Five green algae and three cyanobacteria were among the eight isolating organisms. Green algae and cyanobacteria were found in wastewater and reservoirs, indicating that these environments are a good place to collect microalgae isolates to grow into potential feedstock for biodiesel production.

Metzger and Largeau (2005) found that the morphology of algae might change with age and culture conditions, and it was true even within the same chemical race and strain. Because of their various shapes and sizes, algae can be challenging to study under a microscope. Because it is challenging to tell strains of the same genus apart under the microscope, the morphological examinations used in this work could only identify species to the genus level. It could lead to the misidentification of species. Hence DNA markers are preferable for determining which is which. In addition, if this restriction were lifted, more microalgae species could be discovered. However, some microalgae species were isolated and identified thanks to this study, which is a step in the right direction.

Two newly discovered species were found to have been isolated during the biodiesel production process. According to the results of this study, *Chroococcus* sp. and *Closterium* sp. both have promising applications as biodiesel feedstock. It is calculated using the percentage of algal oil and biodiesel they produce (Fig 4.1).

Productivity of microalgal species

Microalgae can rapidly multiply if provided with the right conditions and enough nutrients. Therefore, the growth dynamics of increasing algal populations should be considered for maximum productivity. In the first stage of algae cultivation, the algal cells adapt to the new medium conditions for a relatively short time. It's important to remember this when transferring cells to a new medium or reactor vessel, as it may be some time before the cells begin reproducing in their new environment. *Oedogonium* sp., *Chroococcus* sp., *Spirogyra* sp., and *Closterium* sp. all had dry weights of 12.1g, 10.0g, 8.1g, and 6.5g, respectively, in week 2 of this investigation (Table 4). Each species' dry weight rose about the same between weeks 4 and 6. It indicates that the growth of the diverse microalgal species was exponential during weeks 2, 4, and 6. Small gains in dry weight were seen across all species between weeks 2 and 4 and again between weeks 6 and 8. It is because new nutrients are added to the medium after each harvest, causing a domino effect of growth. Thus, the small rise in dry weights observed between weeks 4 and 6 can be attributed to the higher nutritional levels observed between those two periods (Table 4). This rapid expansion during the cultivation periods agrees with research by Posthuma (2009), who described the exponential phase of algal growth, during which the number of cells in a given volume increases constantly. Since this experiment was constrained in acquiring counting equipment for cell counts, a common way of determining algae growth rate, dry weights were measured as an indicator of microalgae productivity (Table 4). However, Zuka et al. (2012) have determined that the dry weights of collected algae are a good predictor of biomass yield. The production of dry biomass was reported in terms of g dry algae/L^{-day}.

In addition, the results of this work revealed cell proliferation (Figures 7 and 8), which resulted in a harvest every two weeks within the continuously functioning culture system. Harvesting the microalgae every fourteen (14) days is in line with findings by Zuka et al. (2012), who determined that after twelve (12) to fourteen (14) days in culture, algae reach their stationary phase and must be harvested before the lyses stage (death stage). Furthermore, Mulumba and Farag have corroborated this (2012).

The composition of the medium and its concentration have been discovered to affect microalgal production and the concentration of dry-weight cellular constituents like lipids throughout cultivation (Posthuma 2009). The results showed that the microalgae dry weight ranged from 55.8 to 40.1% (Table 4). It may be because Bold's Basal Medium and BG-11 media have larger quantities of critical nutrients, including Nitrogen ($NaNO_3$), Phosphorous, and Potassium (KH_2PO_4 and K_2HPO_4), than other media do (Tables 1 and 3). These nutrients account for a sizable fraction of the product's dry weight and are thus crucial (Basova 2005; Chisti 2007; Posthuma 2009). Vitamins are just one example of a chemical that has been shown to improve growth rates, but there are many others (Croft et al. 2006). The high production of microalgae in this study may be attributed to the fact that the formulated media contained not only the basic nutrients but also vitamins and other metals such as iron (needed for photosynthesis), magnesium, copper, and zinc (Tables 1 and 3). It is in line with the recommendations of Grobbelaar and Bornman (2004), who noted the importance of including nitrogen, phosphorus, iron, and even silicon in specially prepared media for algae culture.

It is also confirmed in Table 4 that the productivity of *Oedogonium* sp., *Chroococcus* sp., and *Spirogyra* sp. is above average, whereas the productivity of *Closterium* sp. is slightly below average. The results of this study demonstrate the potential of these species as feedstock for biodiesel production, although they are typically overlooked in biodiesel research.

Harvesting and drying of algal biomass

Microalgae were filtered and centrifuged for harvesting within six weeks of cultivation. They were carried out to divide the medium from the biomass. The efficiency or yield may vary depending on the harvesting technique employed. The solid content of the recovered microalgae may also be influenced by the efficiency of the harvesting procedure. Aside from that, the efficiency of the harvesting technique can be gauged by the rate of water extraction (Uduman et al. 2009; Xiang 2012).

High-speed centrifugation, which was used to recover biomass from *Chroococcus* sp. and *Closterium* sp., is a tried and tested method. It's a fast and energy-intensive process, yet it is the most commonly used (Molina-Grima et al. 2003). Due to their diminutive cell sizes, centrifugation served as the method of choice for harvesting these microalgae in this investigation.

The level of pressure drop needed to drive fluid through a filtration medium can be affected by centrifugal force, pressure, or gravity, among others (Shelef et al. 1984; Uduman et al. 2009; Xiang 2012). Media that are fine enough to retain the microalgae tend to bond, making frequent backwashing a necessary part of the filtration process. As a result, the production of microalgal concentrations slowed (Uduman et al. 2009). The microalgal species (*Spirogyra* sp. and *Oedogonium* sp.), which have relatively high cell sizes, were harvested using pressure-driven surface filters in this investigation. Pressure filtering methods are suitable for collecting microalgal species with large cell sizes but ineffective for recovering microalgal species with sizes approaching bacterial dimensions (in the range of micrometers), as was observed in a study of process options undertaken by Molina-Grima et al. (2003).

In this experiment, three different drying techniques were applied. Drying microalgae initially degenerates and weakens the cell wall and plasma membrane, decreasing the cell's ability to hold oil and improving the effectiveness of the extraction procedure.

Both Oedogonium sp. and Spirogyra sp. underwent two days of air drying at room temperature (29°C), followed by six hours in an oven at 80°C to remove any remaining moisture. Similarly, Dejoye et al. (2011) dried algae between 20°C and 70°C for 2 to 6 hours to decrease the remaining moisture. Similarly to Ziga et al. (2010) and Belarbi et al. (2000), *Chroococcus* sp. and *Closterium* sp. were freeze-dried to eliminate moisture content. They found that once microalgae are freeze-dried, intercellular metabolites like oils can be easily recovered using solvent extraction.

Extraction of algal oil and transesterification

Microalgal biomass was dried and then used to extract algal oil for biodiesel production in this study. Similar results were found by Johnson and Wen (2009b), who demonstrated that the biodiesel production from microalgae following transesterification was greater in dry biomass than in biodiesel yield following transesterification utilizing wet biomass. The studies conducted by Kumar et al. (2011) are likewise comparable to this one. They extracted lipids from dried microalgal biomass to make biodiesel. *Tolypothrix*, *Pithophora*, *Spirogyra*, *Hydrodictyon*, *Rhizoclonium*, and *Cladophora*, all of which were utilized to create biodiesel, had their dry weights determined after being dried in an oven or by air. Prior to extracting lipids for biodiesel synthesis, Hossain et al. (2008) also determined the percentage of dry weights of *Oedogonium* and *Spirogyra*.

Algal oil yield is proportional to biomass production for *Oedogonium* spp., *Chrooccocus* spp., and *Closterium* spp., as shown in Table 4 and Figure 13. *Spirogyra* sp. had significantly larger biomass than *Closterium* sp., but it produced significantly less algal oil. These results show that *Spirogyra* sp. may produce significantly less algal oil than the other three species. Figure 13 further shows that the fatty acids content of the algal oil was easily converted to biodiesel in the following order: *Closterium* sp. > *Chrooccocus* sp. > *Oedogonium* sp. > and *Spirogyra* sp. Research conducted by Hossain et al. (2008) indicates that *Oedogonium* sp. produced more biodiesel than *Spirogyra* sp. The result shown in Figure 13 was congruent with their findings.

However, Johnson and Wen (2009b) argue that lipid loss during the extraction stage of the two-stage transesterification process (i.e., extracting algal oil and then transesterifying it) is a possibility, so that direct transesterification (methylation) may result in greater crude biodiesel production.

The results of this study demonstrated that the isolated algae are suitable for cultivation for biodiesel production. The algae *Chrooccocus* sp., *Oedogonium* sp., and *Spirogyra* sp. produce the least biodiesel, whereas *Closterium* sp. produces the most.

Algal cells have oil, which is sealed inside the cell wall and plasma membrane. These formations impede the cell's natural capacity to ship oil out of the body. In addition, plasma membrane degeneration occurs during the drying of algal cells, reducing the cells' capacity to hold oil. Hexane, an organic solvent, can enter the dry algae sample's cell wall and dissolve the oil. After the hexane has been extracted from the algae sample, the oil is taken from the cell. This oil is extracted from algae by removing the hexane solvent (Browne et al. 2010).

In conclusion this thesis contributes to a better comprehension of the feasibility of utilizing algal biomass as a biodiesel feedstock. It is accomplished through efforts in all steps of the process, from cultivating microalgae to making biodiesel. Four freshwater microalgae, which are rarely employed in research projects for biodiesel production, were isolated and identified in this study; it is one of the primary findings of this work. Green algae (*Oedogonium*, *Closterium*, and *Spirogyra* species) and Cyanobacteria (*Chroococcus* species) comprise these microalgae. The results showed that these species might flourish in a room temperature environment (29°C), with sufficient air, natural light source, and an appropriate medium. It serves as "proof of concept" that, under the right conditions, even relatively obscure species like the aforementioned algae can contribute significantly to biomass for use in biodiesel manufacturing. After that, cultural endeavors on a grand scale will be possible.

According to the results of this research, filtering is an effective harvesting method for dehydrating cultured *Oedogonium* sp. and *Spirogyra* sp., while centrifugation is an effective harvesting method for cultured *Closterium* sp. and *Chroococcus* sp. Air drying and oven drying are appropriate for *Oedogonium* sp. and *Spirogyra* sp.. In contrast, freeze-drying is appropriate for *Closterium* sp. and *Chroococcus* sp. It can be deduced from this study that *Oedogonium* sp., *Closterium* sp., *Spirogyra* sp., and *Chroococcus* sp. are viable species that can supply a significant amount of algal oil for biodiesel synthesis. Biodiesel production is highest in *Closterium* sp., next in *Chrooccocus* sp., then in *Oedogonium* sp., and finally in *Spirogyra* sp. It means that, when implemented on a big scale, biodiesel from these microalgae can serve as a renewable energy source that can replace petroleum fuel.

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