ASSESSING THE PRODUCTION OF ALGAL BIODIESEL AS A SUPPLEMENT TO FOSSIL FUEL

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ABSTRACT

The increase in dependence on fossil fuels in Uganda has been associated to the ever increasing population and is contributed to by rural-urban migration, as well as increased urbanization that causes increase in activities such as industrialization. The increasing strain on these fossil fuels, and the increase in their effects on the environment, infrastructure, as well as human beings has led to the need for renewable energy sources and the significant interest in algal bio-diesel. This study investigates the potential of three algae species,- Nannochloropsis, Chlorella Vulgaris and Botryococcus Braunii, for bio-diesel production. The algae was cultivated in similar conditions and the growth rates were monitored for each species using a manual stick measurement technique. Lipid extraction was performed using solvent extraction method to determine the lipid yield for each species with different solvent mixes and bio-diesel was synthesized through trans-esterification. The results showed that Nannochloropsis and Chlorella Vulgaris exhibited the highest growth rate while Nannochloropsis also had the highest lipid content and bio-diesel yield. Botryococcus Braunii had the lowest growth rate and lipid yield. A multi-criteria decision analysis using Analytical Hierarchy Process (AHP) was conducted to evaluate the best species based on growth performance, ease of culturing, lipid content and bio-diesel yield. The analysis identified Nannochloropsis as the most suitable option for bio-diesel production and the obtained bio-diesel was analyzed for key fuel properties, including cetane number, density, kinematic viscosity, flash point and copper strip corrosion following ASTM and EN standards. The study highlights the potential of algae as a sustainable feed-stock for bio-diesel production and emphasizes the need for further research on large scale cultivation and process optimization to enhance feasibility.

DECLARATION

I, NABAKKA PRISCA ANGEL hereby declare that this is my original work, has not been plagiarized and has not been submitted to any other institution for any award.

NABAKKA PRISCA ANGEL

SIGNATURE: ….…………………………………

DATE: ……………………………………………….

APPROVAL

This proposal has been submitted for examination with my approval as the University Supervisor.

MR. AZARIUS SOLOMON LUBAALE

SIGNATURE…………………………………………

DATE………………………………………………

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1.1 BACKGROUND AND INTRODUCTION

There is an increasing dependence on fossil fuels in Uganda which can be associated to the increasing population and human activities such as industrialization where it can be used to run generators and transportation where it is used to run engines.

According to ***(Atuhura, 2023)***, the constant increase in fossil fuel dependence has a number of effects on the environment, from human beings to infrastructure such as roads. Burning of fossil fuels like during transportation causes emission of greenhouse gases such as carbon dioxide, carbon monoxide whose increase in their concentrations has adverse effects such as causing respiratory issues to human beings because of ingestion of polluted air, destruction of the ozone layer when these gases react with ozone, global warming because of the ability of these gases to trap heat close to the earth's surface, ***(Terapass, 2022)***. The proposed algal bio-diesel also produces green house emissions when combusted but at a significantly lower percentage when compared to those from combustion: a study by USDA estimates the emissions from combustion of algal bio-diesel to be between 60-80% less than fossil fuel emissions, a study conducted by the National Renewable Energy Laboratory estimates emissions to be between 50-70% less than those of fossil fuels and a study by the international council on clean transportation estimates the emissions from bio-diesel to be between 70-90% less than those from fossil fuels. Infrastructure such as roads are prone to depletion during transportation and combustion of these fuels, and there is occurrence of increased environmental damage or degradation due to increased extraction of fossil fuels. Oil extraction and transportation can cause environmental degradation and decrease in biodiversity for example through oil spills.

According to ***(Robb & Amedolare, 2023),*** apart from the immense pollution caused by burning of fossil fuels during their use, another notable problem is that they are a non-renewable resource which therefore puts a species on them with increasing extraction and exploitation. Because of the increasing dependence on fossil fuels, it is becoming more difficult to extract the rapidly reducing resources and hence there is further increase in prices, as well as increasing possibility of less supply of these natural resources in comparison to the demand for fuels, ***(AFP, 2022)***. These concerns frame the need for sustainable alternatives, in this case, algal bio-diesel which is not only renewable but also a carbon neutral approach to energy production.

Uganda's fuel consumption increased by 7% between 2018 and 2019 and yet the production of oil within the country is currently low. Adopting renewable energy sources such as algal bio-diesel and aiming at increasing energy efficiency can minimize on the rate of depletion of fossil fuels, especially those extracted for petroleum production, ***(Andae, 2024)***. species of micro-algae that are rich in lipids can be processed and converted into bio-diesel which can be a viable supplement to these fossil fuels. When grown in optimum conditions like where there is sunlight, carbon dioxide, nutrients such as nitrogen and carbon dioxide and a favorable pH, algae grows better and fast enough for harvesting for bio-diesel and algal lipid content stands to increase which justifies the production of algal bio-diesel as a supplement to fossil fuels such as petroleum. This project aims at investigating the production of algal bio-diesel as a renewable energy source through various processes, tests and procedures.

However, the production of algal bio-diesel is also faced with a number of challenges involved in cultivation, extraction and processing, the need for efficient scaling methods and that there is lack of understanding of the optimal conditions for algal growth and oil yield.

1.2 PROBLEM STATEMENT

The increasing global dependence on fossil fuels has a number of effects including depletion of non-renewable resources, environmental and economic challenges such as air pollution, greenhouse gas emissions which increases their concentration in the atmosphere which contributes to global warming as these gases such as carbon dioxide trap air close to the earth's surface. Uganda heavily relies on fossil fuels for its energy needs and this has contributed to environmental degradation and increased vulnerability to global oil price fluctuations which negatively impact the country's economy and energy security.

Currently, Uganda's energy sector is dominated by fossil fuels with limited adoption of renewable energy sources. The ideal situation would involve a significant reduction in fossil fuel consumption through the integration of sustainable and environmentally friendly energy alternatives, such as algal bio-diesel. This is a promising renewable energy produced from algae that has the potential to lower gas emissions, minimize over exploitation of non-renewable fossil fuel resources, among other benefits.

The gap between the current energy conditions and the ideal situation lies in the limited research, development and adoption of this bio-diesel and addressing this requires targeted interventions such as this which would involve conducting comprehensive studies to evaluate the production of the suggested algal bio-diesel in Uganda. This includes consideration of factors such as availability of suitable algae species, optimized cultivation and harvesting methods, analysis of the economic and environmental benefits of algal bio-diesel production.

This research therefore aims to address these challenges, by developing strategies for cultivation and extraction of algal bio-diesel ultimately contributing to reduction in greenhouse gas emissions as well as a sustainable energy future.

1.3 OBJECTIVES OF STUDY

1.3.1 MAIN OBJECTIVE

To assess the production of algal bio-diesel as a supplement to fossil fuels.

1.3.2 SPECIFIC OBJECTIVES

1. To identify species of algae most suitable for lipid extraction for bio-diesel production.
2. To optimize the cultivation conditions for the identified algal species.
3. To analyze the physico-chemical properties of the obtained algal bio-diesel.
4. To develop a process design for the production of algal bio-diesel.

1.4 RESEARCH QUESTIONS

1. What are the most suitable species of algae for lipid extraction for production of bio-diesel?
2. What are the optimal parameters for cultivation of the selected algal species that would give maximum lipid productivity?

3. What are the physico-chemical properties of the algal bio-diesel produced from the selected species under the identified conditions and selected extraction methods?

1.5 JUSTIFICATION

As formerly highlighted, extraction, refining and consumption of fossil fuels greatly contribute to greenhouse emissions which are a high contributor to climate change. A study conducted by the Intergovernmental Panel on Climate Change (IPCC) highlights that there is a bid to limit global warming to 1.5℃which requires reduction in consumption of fossil fuels as they are responsible for approximately 70% of carbon dioxide emissions, ***(Dharmaprabhakaran, 2020)***.

Furthermore, oil extraction processes decrease biodiversity because they cause destruction of some animal habitats and thus ecological disruption. Oil drilling activities can result in degradation of sensitive ecosystems and contamination of soil and water sources**, *(WWF, 2020).*** The risks of oil spills during both extraction and transportation of fossil fuels have the potential to cause long lasting damage to marine and terrestrial environments.

Algal bio-diesel is a viable solution in mitigating these environmental impacts not only because it is renewable, but also because it can reduce emissions of greenhouse gases, especially carbon dioxide by more than 60% in comparison with petroleum diesel because algae absorbs it during growth. In addition, by-products as result of production of algal bio-diesel can be used as raw materials in manufacture of some pharmaceuticals and fragrances, they can be used as feeds for some aquatic animals and water plants, and all these mitigate the problems faced with disposal of by-products.

Furthermore, algae can grow in conditions such as wastewater and salty waters which mitigates the need for it to compete with aquatic life for freshwater for its growth. Production of bio-diesel from algae mitigates competition for food with human beings as is the case with production of bio-diesel from other raw materials such as cooking oils, food crops such as soybeans, sunflower, corn, rapeseed, among others. Algae yields higher lipid content upon extraction in comparison to most sources of lipids for bio-diesel production.

In conclusion, addressing the environmental destruction brought about by increasing dependence on fossil fuels is a necessity for sustainable development because technologies such as algal bio-diesel foster a sustainable energy future and protect the existing ecosystems.

2.0 CHAPTER TWO: LITERATURE

2.1 CRITICAL REVIEW

2.1.1 Selection of algal species

Micro-algae is isolated, identified and screened in preparation for the process of bio-diesel production. For the identification process, water samples from various sources are collected and micro-algae are isolated using algae culturing techniques. Each of the identified species is screened for lipid production using the volumetric lipid productivity test, ***(Akinola, 2021)***. Furthermore, the lipid productivity of the algal species can be determined through lipid extraction, for example by use of a mechanical oil press to extract the oils from the different algal species, and then use of gravimetric methods to determine the concentration of biomass, as well as quantification of the lipids from each species. The algal species or species with the higher lipid content is considered to be the most effective for algal bio-diesel production because of expectation of higher yields. The best species or algal species for bio-diesel production in this case are **scenedesmus** and **chlorella** because of their high lipid productivity, ***(Azeez & Oyelami, 2021)***

However, when looking at the concept of culturing algae, a variety of species is considered so as to create a wider sample space for comparison of the algal species to assess their suitability for algal bio-diesel production. In cases of culturing, this suitability is mainly determined by lipid yield of each species, as well as factors such as cultivation time, which looks at how much of that algae has grown in a specified amount of time. Following cultivation times and lipid yield, the most suitable algal species are **botryococcus braunii**, **nannochloropsis** and **chlorella vulgaris**. Botryococcus braunii is expected to grow within 10-14 days and it has an expected lipid yield of 50-70%; Nannochloropsis is expected to grow within 5-10 days and it has an expected lipid yield of 30-50%; and Chlorella vulgaris is expected to grow within 1-2 days and it has an expected lipid yield of 20-50%, ***(Moradi & Saidi, 2022)***.

Regarding determining the biomass productivity and lipid content and /or productivity, ***(Andrew & Misson, 2022)*** highlight mathematical formulas that can be used to calculate each of these following cultivation, harvesting and lipid extraction of and from the algae.

1. For biomass productivity, the biomass dry weight concentrations are determined at the starting point or time of cultivation and at the end point of cultivation. Biomass productivity, which is measured in gram per liter per day (g/L/day) is then calculated from;

$$\textbf{Biomass productivity (g/L/day)} = (X_2 - X_1) / (t_2 - t_1)$$

(Andrew, 2022)

Where X_1 an X_2 are the biomass dry weight concentrations at t_1 which is the starting point and t_2 which is the end point of cultivation respectively.

2. For lipid content and/ or productivity, the cultured algae is harvested and lipid extracted using the preferred extraction technique for example use of methanol liquid lipid extraction or any other solvent extractor. The extraction solvent is then removed through evaporation for about 20 minutes after extraction, ***(Andrew & Misson, 2022)***.

The lipid content in % is then calculated from;

$$\textbf{Lipid content (\%)} = [\text{volume of lipid extract (g)} / \text{dry biomass (g)}] \times 100\%$$

(Mission, 2022)

The lipid productivity, measured in milligrams per liter per day (mg/L/day), can then be determined by multiplying the lipid value value by the previously determined biomass productivity value for each species of algae.

2.1.2 Cultivation process

Cultivation of algae is more likely to give the most productive yields when it is done under the most possibly achievable optimal conditions. The conditions that are most vital for the growth of algae are:

- Presence of sunlight. This is important because algae, similar to green plants, are phototropic in nature. This means that they use sunlight to manufacture their own food and it is therefore important in their growth because it aids their metabolism.
- Presence of carbon dioxide. Again, similar to green plants, algae take in carbon dioxide during respiratory processes and it is therefore an important factor towards their growth.
- Nutrients. The main nutrients necessary of the growth of algae are nitrogen and phosphorus. According to ***(Chen, 2023),*** this is because nitrogen is aids the organisms in synthesis of proteins, amino acids and nucleic acids, while phosphorus a major component of nucleic acid and phospholipids found in cells of algae.
- pH. This is important for algal growth because it has the ability to affect the rate of photosynthesis of algae. The pH range most suitable for algal growth varies depending on the species for example pH range between 7.0-9.0 is most suitable for growth of species such as cyanobacteria, eukaryotic algae

flourish in pH ranging between 4-5, Chlorella Vulgaris grows better at optimum pH of 7.0.

According to ***(Yin & Mo, 2020),*** production of algae is still being done on a small scale, with a transitional development from cultivation in open ponds to use of techniques such as use of modern photo-bioreactors in the laboratory. The photo-bioreactor provides the ability to control conditions for growth of algae and limits contamination during the whole process of production. Furthermore, photo-bioreactors are convenient for high biomass productivity.

A study conducted by ***(Falfushynska, 2024)*** provides insights on the current methods of cultivation and harvesting for algae in regards to production of bio-fuel while emphasizing sustainability. It includes recent findings, technological advancements, as well as practical implementations to provide room for viability of algal bio-diesel in terms of productivity and in the economic aspect. This is done by highlighting the potential of these fuels as both a sustainable and renewable source of energy.

The study highlights technologies that are being utilized to convert lipids from algae into high quality bio-fuels. According to ***(Falfushynska, 2024),*** such technologies being used include the oil-to-jet process and the Fischer-Tropsch synthesis.

Oil-to-jet process

This generally refers to the process of converting oil to jet fuel specifically. In this case, this is the process by which lipids extracted from algae are converted into jet fuel; sustainable aviation fuel to be used to power jet engines. The process comprises of:

- Cultivation, which involves growing the algae biomass.
- Harvesting, which involves collecting or gathering the grown biomass to be used in production of bio-diesel.
- Oil extraction, which involves separating the oil or lipids within the algae from the solid part of the biomass.
- Chemical treatment. This is done through the trans-esterification process to convert the extracted lipids into bio-diesel.
- Separation and purification. This involves refining the bio-diesel. Processes such as distillation can be used.

In the oil-to-jet process, jet fuel production can be also be done following the steps of hydro-heating, where the fatty acid methyl esters (FAMEs) obtained after trans-esterification are converted into jet fuel by removal of oxygen and saturation of double bonds. This is then followed by isomerization of the obtained fuel so as to improve its cold flow properties. The obtained fuel after all this can be blended with the conventional jet fuel to meet the necessary specifications, ***(Journal of Renewable and Sustainable Energy, 2020)***.

The Fischer-Tropsch synthesis

This is a catalytic process in which a synthetic gas such as carbon monoxide or hydrogen gas is transformed into a mixture of hydro carbons, ***(Mazurova, 2023)***. Fuel obtained from this process has properties better than those of the conventional fuel such as high cetane number, low aromatic content and considerable absence of sulphur and nitrogen impurities. This study highlights that development of selective catalysts is necessary to obtain a high yield of diesel from the Fischer-Tropsch synthesis process.

Findings from this research

- The study highlights that there are a couple of methods that can be used for cultivation for example, use of open ponds, use of photo-bioreactors, each with its own unique advantages and challenges that can inform the decision for the better approach. For example, photo-bioreactors are usually costly but offer more controlled conditions for algae growth while open ponds are usually cost-effective but are susceptible to environmental variables.
- In order to enhance lipid productivity in algae, different manipulation techniques can be adopted, for example according to this study, cultivation of algae in fish farm wastewater (both modified and unmodified) revealed that the nutrients present in that wastewater promote accumulation of the desired fatty acids in regards to bio-diesel production from algae. Furthermore, the author highlights that bio-diesel obtained from algal species cultivated in fish farm wastewater exhibited properties desirable for fuel, such as those associated with cetane number, kinematic viscosity, density, higher heating value among others.

The research therefore concludes that micro-algae significantly demonstrate potential as a sustainable and renewable resource in terms of algal bio-diesel production. This is majorly associated to their rapid growth rates and their high lipid content. Furthermore, it concludes that algal bio-diesel is a good venture because it not only reduces competition for biomass but also addresses issues affecting ecology such as deforestation and loss in bio-diversity. Therefore, future research and efforts towards development should focus on further optimization of cultivation methods, enhancement of genetic engineering techniques, as well as development of cost-effective harvesting and processing methods, ***(Falfushynska, 2024)***.

2.1.3 Mode of operation of photo-bioreactors

Photo-bioreactors favor algal growth because they allow for penetration of sunlight which is very essential because algal operate similarly to green plants which use carbon dioxide and sunlight to manufacture their own food which in turn supports their growth. Therefore, it is important for photo-bioreactors to be manufactured out of sunlight penetrating materials for example glass. Artificial lighting can also be an option but it would be more costly, therefore outdoor photo-bioreactors are preferred; where they can receive light directly from the solar energy of the sun, ***(Delavar, 2022)***.

According to ***(Wang, 2022)***, photo-bioreactors operate continuously within a temperature range of 25-40℃. Using airlift pumps the algal culture can be cycled through solar receivers and when this is done, it is important that the algae cells circulate continuously without settling. Carbon dioxide is then introduced into the photo-bioreactor to provide the necessary aeration. During presence of sunlight, the algal species, similar to green plants, absorb carbon dioxide and release oxygen. By means of a heat exchanger, the tubes in the machine are cooled to prevent increase in heat, ***(Delavar, 2022)***. With more accumulation of oxygen, there can be inhibition of growth of algae therefore the excess oxygen amount is ejected through a de-passing port. Photo-bioreactors are also a potential tool for carbon dioxide conversion and bio-fuel synthesis and in this system, algal organisms grow while the products are produced during in the night, ***(Wang, 2022)***.

2.1.4 Optimal growth parameters for each species

As previously highlighted, the most suitable algal species for lipid extraction for algal bio-diesel production are Botryococcus Braunii, Nannochloropsis and Chlorella

Vulgaris and for cultivation, each of these has its own optimal conditions within which maximum lipid productivity is expected. These are;

- **Botryococcus Braunii** requires;

pH range of 6.5-8.5, with the most optimum pH being 7.5.

Temperature of 25-30℃ with the most optimum temperature being 28℃.

Light intensity of 50-150 μmol/ m^2s.

Aeration rate of 0.1-1.0 vvm.

Mixing speed of 50-200 rpm.

- **Nannochloropsis** requires;

pH range of 7.5-8.5, with the most optimum pH being 8.0.

Temperature of 15-25℃ with the most optimum temperature being 20-22℃.

Salinity of 20-40 ppt with the most optimum being 30ppt.

Light intensity of 50-150 μmol/ m^2s.

Aeration rate of 0.1-1.0 vvm.

Mixing speed of 50-200 rpm.

- **Chlorella Vulgaris** requires;

pH range of 6.5-8.5, with the most optimum pH being 7.5.

Temperature of 20-30℃ with the most optimum temperature being 25℃.

Salinity of 0-10g/L with the most optimum being 5g/L.

Light intensity of 100 μmol/ m^2s.

Aeration rate of 0.1-1.0 vvm.

Mixing speed of 50-200 rpm.

Each of these species require mixing by orbital shaking, nutrient requirements of nitrogen and phosphorus, with nitrogen in the measure of approximately 10-20mM and phosphorus in the approximate measure of 1-5 mM. They also all require a carbon dioxide flow rate of 0.1-1.0 L/min, as well as carbon dioxide concentrations ranging between 1-5% (v/v).

2.1.5 Harvesting of algae

Harvesting of algae and selecting a harvesting technique to use depends on the size and properties of the algal species up for collection.Harvesting of algae can be done by a number of methods and these include:

- Filtration. This is a simple method of algal harvesting in which filters are used to directly collect or capture and remove clumps of algae from where they have grown such as a pond. It can range from use of simple filters and screens, to use of micro speciesers depending on the size and characteristics of the algae being harvested.
- Flotation. Here, air bubbles are induced or introduced into the water so as to enable clumps of algae to rise to the surface of water and they are then collected.
- Gravitation or sedimentation. In this method, the algae is left to settle at the bottom as a result of gravity by its weight and the sediment clumps of algae are then collected.
- Direct picking. Here, hands with gloves or forceps are directly used to pick out the algae from the source of growth.
- Flocculation. This can be either naturally occurring or induced either chemically or biologically. In this case, the dispersed micro algal organisms aggregate and form larger particles or floccs which have a higher sedimentation rate and are collected as they settle.

- Centrifugation. This is basically an accelerated sedimentation process for algae harvesting. This employs a hydro-clone system in which the walls of the hydro-clone walls are fixed while the algal cells in the chamber move in spiral motion creating centripetal forces that cause denser p;articles to be spun out of the traversing liquid, ***(Wen, 2021)***.

Sedimentation and flotation are majorly convenient in case of open pond cultivation systems and filtration and centrifugation are most convenient for cultivation done using a photo-bioreactor, ***(Chen, 2021)***.

2.1.6 Lipid extraction of algae

Lipids are bio molecules that are soluble in organic solvents but insoluble in water, ***(Sati, 2019)***. Lipid extraction is basically the process through which oil contents within the cells of algae are extracted, in this case to be processed into algal bio-diesel. This occurs by disruption of the algae cells which causes them to release the lipids inside them. Lipid extraction from algae can be categorized into two major types of processes, that is chemical processes and mechanical processes of lipid extraction and these processes include;

1. **Acid base extraction**. This is an example of a chemical method of lipid extraction and it involves using an acid and a base solution to breakdown the cell membranes of algae to cause them to release lipids, and then separate them from other cellular components. Examples of acids used include hydrochloric acid, sulphuric acid and nitric acid. Examples of base solutions most commonly used include sodium hydroxide, potassium hydroxide and ammonium hydroxide.

2. **Continuous agitation method**. This is a mechanical method of lipid extraction. Agitation can be induced by stirring the algae in a tank reactor, use of a mechanical stirrer, or systems such as the ultrasonic homogenizer. Upon completion of agitation, the lipids are separated from the algal biomass and clarified through processes such as centrifugation and filtration.
3. **Solvent based extraction**. This is similar to acid base extraction because it also breaks down the cellular membrane of algal cells causing them to release lipids and then separate from other cellular component, but it differs because here organic solvents are used for this process. These solvents include hexane, chloroform and even methanol. They dissolve the lipids. Upon extraction, the desired lipids are separated from the extraction solvent by processes such as filtration.
4. **Use of an oil press.** For an oil press, the algae is fed into the device where it encounters a rotating shaft that compresses it. As the algae passes through the oil press, it experiences increasing pressure and friction that mechanically rupture the oil cells releasing the lipids that are then collected. Upon extraction, the lipids are separated from the solids such as through filtration and further processed into bio-diesel.

2.1.7 Trans-esterification

This is process by which lipid extracts from algae are converted into bio-diesel. This is mainly by breaking the triglycerides in the lipids to diglycerides and then to monoglycerides. The main necessities for this process are the extracted lipids, and alcohol, such as methanol, and a base catalyst solution such as sodium hydroxide.

- The algal oil is heated to a moderate temperature so as to improve its fluidity and increase the reaction rate of the process.
- The preheated lipids or oil is then mixed with the alcohol in presence of the catalyst to speed up the reaction. The mixture is stirred for proper mixing.
- The lipids then react with the alcohol in the presence of the catalyst to form fatty acid alkyl esters or bio-diesel, as well as glycerol by-products.
- After completion of the process, the mixture is allowed to settle so as to ease the separation process. The glycerol, because of its denser quality settles at the bottom and the bio-diesel floats on top making separation easy.
- The layer of bio-diesel is washed with water to remove any impurities or residue of the reactions before it goes through storage or further process.

2.1.8 Algal bio-diesel properties

These are properties used to assess the quality of the bio-diesel after it has been produced and process. They are measured against different standards, as well as petroleum fuels in use so as to determine whether the bio-diesel is suitable for use. Some of the properties looked at or considered for assessing the quality of bio-diesel and their desirable values are:

Table 1: Algal bio-diesel properties *(Source: (Karmakar.R, 2018)*

Fuel property	**Desirable value**
Viscosity	1.9 – 6.0 square millimeters per second
Density	0.86 – 0.90 grams per cubic centimeter
Cetane number	Above 50
FFA content	Less than 0.5%
Sulphur content	Less than 15 ppm
Flash point	Above 130 ℃
Cloud point	Below 0 ℃

3.0 CHAPTER THREE: METHODOLOGY

3.1 INTRODUCTION

This chapter is comprised of processes, activities and techniques that were implemented for each specific objective in a bid to achieve this project. The methodology serves as a plan to be followed during implementation of the project by achieving each of the set research objectives. This is through systematic collection of data, analysis of collected data, and finally interpreting the data. Different approaches are employed and the purpose of this approach is to ensure that there is an understanding of the research problem as well allowing for triangulation of results.

3.2 METHODOLOGY

1. **Identifying the species of algae most suitable for lipid extraction for bio-diesel production.**

Algal species selection: This involved screening a variety of algal species to select those with high lipid content and suitable growth characteristics for cultivation.

Algal species selected were determined by conducting comprehensive reviews of existing literature to identify species best known for factors such as high lipid content, regional availability as well as cultivation of these species.The literature review provided useful insight and knowledge regarding criteria for strain selection, as well as the implications of these on the productivity of algae in terms of growth and lipid production.

A multi-criteria decision analysis using Analytical Hierarchy Process (AHP) was conducted to evaluate the best species based on growth performance, ease of culturing, lipid content and bio-diesel yield.

Samples of the identified species were then collected so as to obtain pure cultures. The collected species were cultivated in a similar environments.

Lipid screening was carried out after a specified growth period. This involved harvesting the algal biomass after this growth period and performing lipid quantification using methods such as gravimetric analysis.

2. **Optimizing the cultivation parameters for the identified algal species and develop efficient lipid extraction methods.**

Cultivation experiments: This involved establishing algal cultivation systems using selected species to optimize growth conditions and lipid production. The biomass productivity and accumulation of the biomass of each species under similar conditions was measured.

Culturing was done under similar conditions in a bid to monitor the growth of each of the different identified species. The algae culturing was done unconventionally, that is, not under controlled or laboratory conditions. It was done in containers of the same size. Each container had a diameter of 24 cm and height of 27.7 cm.

An equal volume of 5 litres of water from the same source was poured into each of the containers and an equal volume of each sample species added to the water, each species in its own container.

Each algae species in each container was subjected to similar conditions specifically under the same light at the same time for the same duration. Light and temperature during this set up were provided by the sun. Other conditions provided for the culturing set up were nutrients, particularly nitrogen and phosphorus which were provided through a nutrient solution rich in these nutrients. Each of these conditions was necessary to facilitate absorption of nutrients, as well as photosynthesis for the algae to grow. The growth of each species was monitored on a daily basis to determine the most suitable species among the three selected species while culturing.

Because culturing was not done in a laboratory, measurement to determine growth of the algae was also done unconventionally. Each day, a ruler was immersed into each of the containers and the depth or length of the ruler covered by the biomass read off and noted in cm. The changes in depth indicated that there were changes taking place within each container occupied by each species. The changes in depth or length were observed and noted over the next 14 days. To more accurately determine the volumes and changes in volume for each day, the surface area of the containers, which was the same was calculated by;

$$\textbf{Surface Area} = \pi r (r + h)$$

Where diameter, D was 24 cm therefore radius, r was 12cm and height, h was 27.7 cm. Therefore area became;

$$A = \pi (12) [12 + 27.7] = 1496.6 \text{ cm}^2$$

$$A = 1497 \text{ cm}^2$$

With the surface area, the volume occupied by the algal biomass in each container was calculated by determining the differences between the new depths and the very first measured depth for each species and multiplying each of these differences by the surface area to get volume in cm^3. i.e. if the original depth was d_0, and the new depths were d_1, d_2, $d_3 \ldots d_n$, the differences in depths would be;

$(d_1 - d_0)$, $(d_2 - d_0)$, $(d_3 - d_0) \ldots (d_n - d_0)$.

MEASUREMENTS FROM CULTURING

Table 2: Depths for each day for each species of algae

Cultivation time (days)	Species 1 (cm)	Species 2 (cm)	Species 3 (cm)
1	2.1	2.5	2.2
2	3.2	4.6	2.5
3	4.7	7.5	2.6
4	5.3	8.1	2.6
5	7.5	10.3	2.7
6	9.6	10.8	2.8
7	10.0	11.5	2.8
8	10.0	12.2	3.2
9	10.2	12.7	3.2
10	10.5	13.0	3.2
11	11.0	13.2	2.9
12	11.9	13.4	2.6
13	12.0	13.4	2.6
14	12.0	13.4	2.6

3. **Determining the physico-chemical properties of the obtained algal bio-diesel.**

Lipid extraction: This involved extraction of lipids from algal biomass using various methods and optimize extraction efficiency. The methods commonly used for lipid extraction include solvent-based extraction methods, mechanical methods, enzymatic methods. The efficiency of each method based on lipid yield and process feasibility is then evaluated and the best method selected. Lipid extraction was done using the solvent extraction method.

Figure 1: Algae dried for lipid extraction

Figure 2: Shaking to properly mix solvents for lipid extraction

Figure 3: Filtration to separate lipids from algal biomass

Figure 4: Residual biomass after lipid extraction from algae

Bio-diesel production: This involved conversion of the extracted lipids into bio-diesel using a suitable trans-esterification process.

Figure 5: Filtration after trans-esterification to remove any solids

Figure 6: Resulting bio-diesel from trans-esterification after filtration

Characterization: This involved analysis of the physical and chemical properties of algal bio-diesel to assess its suitability as a fuel. The properties were tested using different laboratory tests and these properties included density, kinematic viscosity, flash point, cetane number and copper strip corrosion. Each of these properties can be tested using or through:

- Density can be measured using a hydrometer or densitometer.
- Viscosity can be determined using a viscometer.
- Flash point can be assessed using Pesky-Martens apparatus.
- Cetane number can be determined through standard calculations and specialized equipment.

PROCEDURES:

- Density was measured using a hydrometer. The specifications for diesel suggest that it lies between 0.80 and 0.90 g/cm^{3}. The sample was poured into a 250 ml measuring cylinder and a thermometer placed into the cylinder. The density is measured at any temperature above 15 ℃.and for this test, it was done at 17.4 ℃ and the density read off the hydrometer.

Figure 7: Measuring cylinder and hydrometer *(Source:(www.industrybuying.com)*

- Kinematic viscosity was measured using a viscometer and a kinematic viscosity bath. The bath comprises of silicone oil and this is used because it can adapt to various temperatures even very high ones, i.e. it can be heated to as high as 400 ℃.Kinematic viscosity is tested at two temperatures, 40 and 100 ℃. The viscometer was filled to half the bulb with the sample and then immersed into the bath set to 40 °C. A viscometer of size 50 was used and this as well as details such as the viscometer constant and serial number were filled into the machine. There are two marks on the viscometer one above the other and the flow of the sample diesel between these is monitored and the time taken, which is the falling time in seconds was recorded. The viscosity was also recorded and the procedure repeated with the bath at 100 °C.

***Figure 8: Viscometer bath machine (**Source: (www.matest.com)*

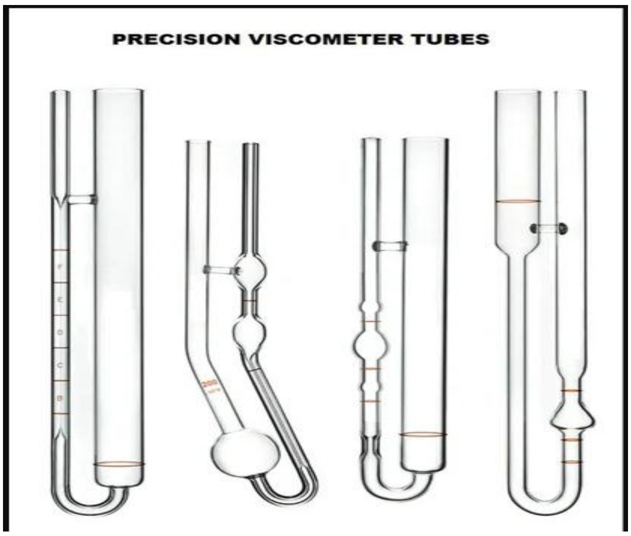

***Figure 9: Viscometer tubes** (Source: (www.indiamart.com)*

- Copper strip corrosion was tested using the Tamson Viscometer bath set to 50℃. The copper strip was polished by scrubbing then put in a test tube and submerged with the sample. The test tube was then put and sealed in a Tamson tube and the tube immersed in the bath. It was ensured that the tube be submerged for three hours and after this time to determine the state of the copper, the ASTM Copper strip corrosion standards, ASTM Method D 130/IP 154 were used.

Figure 10: Tamson bath, copper strips, copper strip corrosion standards and tamson tubes

(Source: (www.tradeindia.com)

- Flash point was tested using a Cleveland open cup, the sample poured into the cup and heated gradually at temperatures between 5 and 6… per minute while stirring continuously. A flame was then introduced to the surface of the sample at every 1 ℃ rise in temperature after reaching approximately 100 ℃. The flash point was then recorded as the temperature at which the vapor ignited for a moment.

The results obtained from each of these tests are then compared against bio-diesel standards for example ASTM D6751 or EN 14214. This is done so as to evaluate the quality of the produced algal bio-diesel.

4. Developing a process design for the production of algal bio-diesel

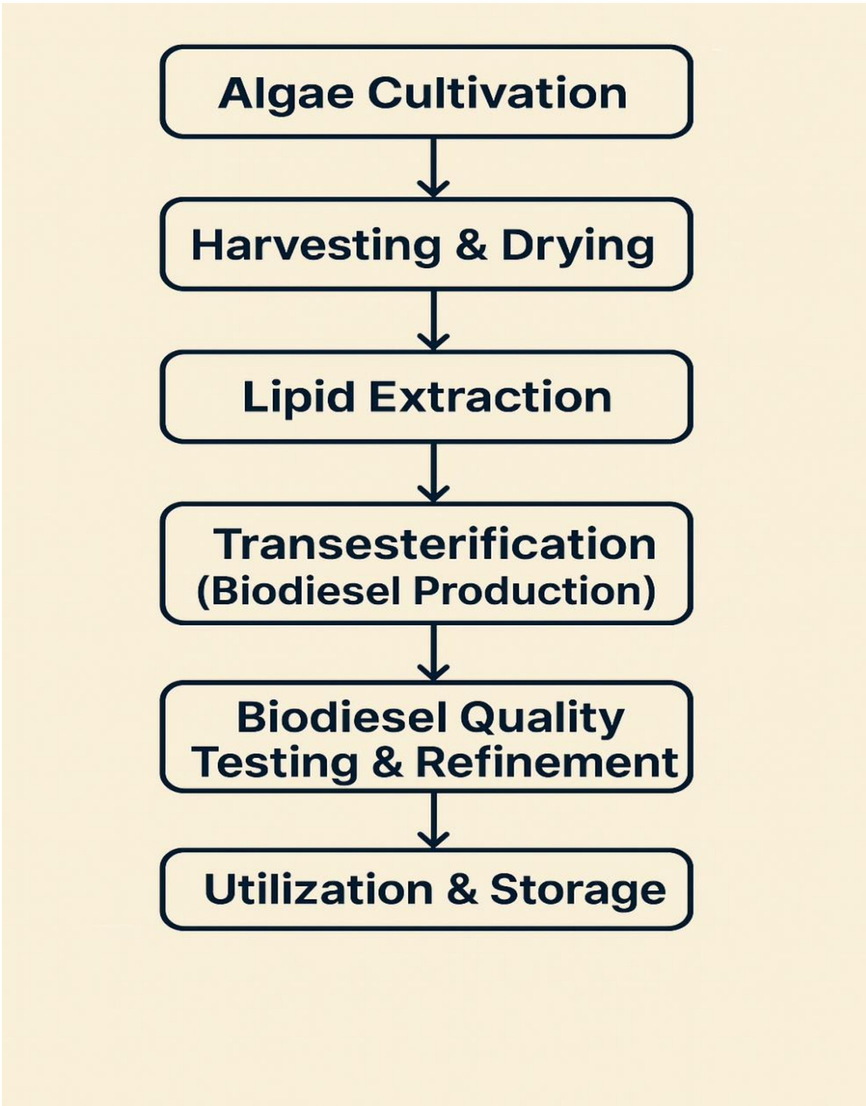

Figure 11: Process design for algal bio-diesel production

- Cultivation of algae

- speciess used: Chlorella Vulgaris, Nannochloropsis, Botryococcus Braunii
- Growth medium: Water rich in nutrients, mainly nitrogen and phosphorus, preferably wastewater, presence of carbon dioxide
- Cultivation method: Open ponds or open containers for exposure to sunlight
- Growth monitoring: Measurement of increase in volume of algal biomass

- Harvesting and drying

- Sedimentation: Allowing the algae to settle by virtue of its weight and the clumps formed collected.
- Direct picking: Using hands with gloves, or forceps to directly pick out the algae.

- Lipid extraction

- Solvent extraction: Use of solvent mixes such as hexane and isopropanol to disrupt cell walls and extract lipids.
- Separation: Evaporating solvents used to stay with pure algal oil.

- Trans-esterification

- Bio-diesel production from obtained algal oil by adding methanol and a base catalyst, in this case sodium hydroxide.
- This process is done at 60℃ for 2 to 4 hours and stirring is done continuously throughout the process. This allows for glycerol to settle at the bottom and the bio-diesel at the top.

- Purification such as washing and filtration is done to remove impurities.

➢ Bio-diesel quality testing

- Parameters tested included density, kinematic viscosity, copper strip corrosion, cetane number and flash point
- Refinement: Decanting, distillation or filtration can be done incase further purification is required.

➢ Use of bio-diesel

- Storage is to be done in sealed, air tight containers away from moisture and/ or oxidation.
- The algal bio-diesel is to be mixed or blended with conventional diesel for improved diesel qualities overall.

EXPERIMENTAL DESIGN OF ALGAL BIODIESEL PRODUCTION PROCESS

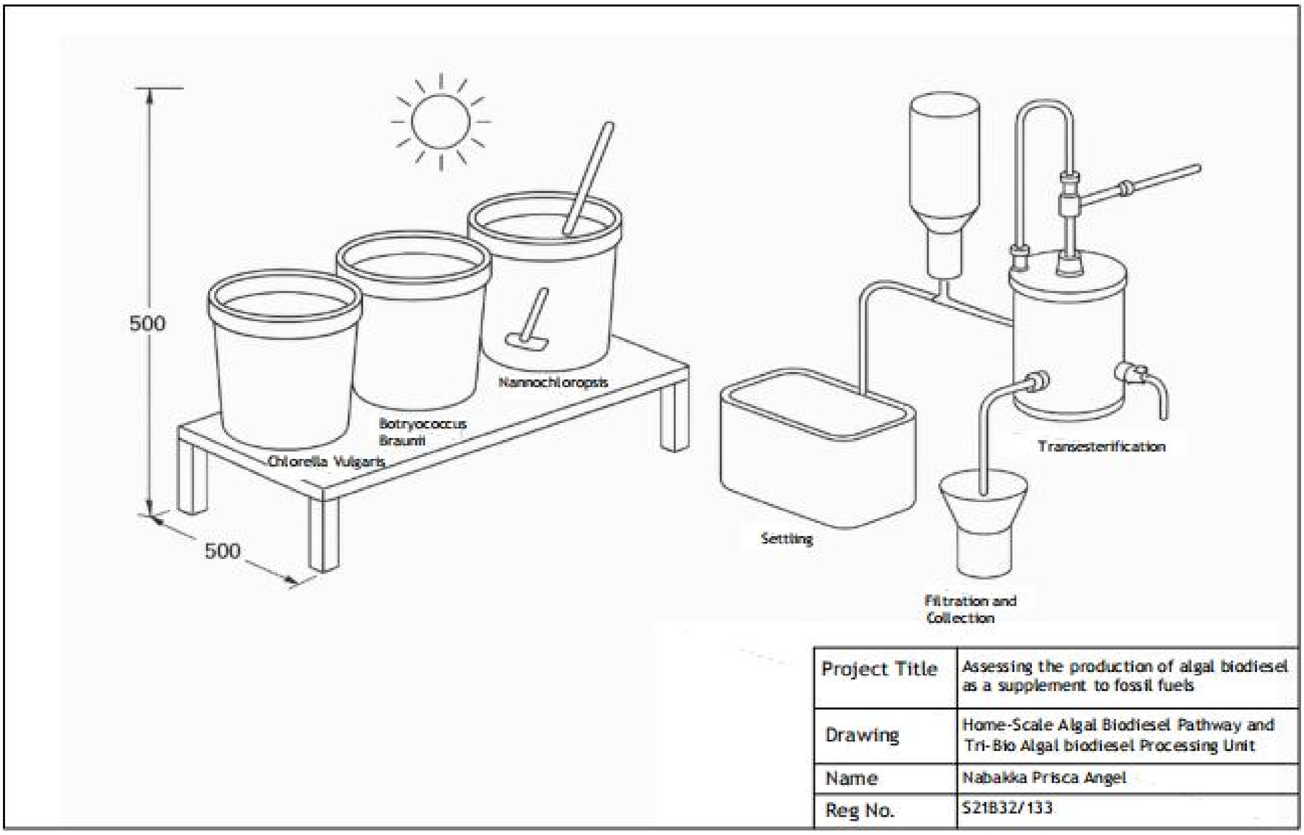

Figure 12: Experimental Design Drawing for algal bio-diesel production process

4.0 CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 INTRODUCTION

This chapter presents different findings from the investigations made while assessing the production of algal bio-diesel as a supplement to fossil fuels. These findings and results are organized according to the respective specific objectives set in a bid to fulfill the project. The main aim of this chapter is to provide insight into the progress made regarding identification of algal species most suitable for bio-diesel production, optimization of the cultivation conditions and evaluation of the physico-chemical properties of the obtained bio-diesel.

Furthermore, here in is a discussion on the the implications of the different data and results obtained, challenges faced and solutions to overcome them. The discussion in this chapter therefore aims to provide a detailed interpretation of the results as well as drawing meaningful conclusions from them.

4.2 PROGRESS OVERVIEW

4.2.1 Identification of algae species most suitable for bio-diesel production

Techniques and processes used:

Extensive literature review. The species most suitable for algal bio-diesel were identified by reading online resources written by various researchers. Such resources included journals, dessertations, papers and different publications. Furthermore, a Multi-criteria Decision Analysis was conducted to rank the identified species of algae.

MULTICRITERIA DECISION ANALYSIS FOR THE ALGAL SPECIES

Table 3: Multicriteria Decision Analysis for ranking the algae species

CRITERIA	**CHLORELLA VULGARIS**	**BOTRYOCOCCUS BRAUNII**	**NANNOCHLOROPSIS**	**CRITERIA WEIGHTS**
Growth rate	0.11 / 6.5	0.13 / 5	0.10 / 7	8
Lipid yield	0.52 / 8	0.51 / 7	0.69 / 9	10
Bio-diesel yield	0.56 / 5	0.49 / 3	0.69 / 6	7
Ease of culturing	4.5	2	5	6
TOTAL	12.18	7.69	16.05	
RANK	2	3	1	

- Multi criteria decision analysis (MCDA) was done to rank the species of algae most suitable for lipid extraction for algal bio-diesel production. The identified as most important parameters for influencing the choice of algal species chosen were growth rate, lipid yield, bio-diesel yield and ease of culturing.
- Weights were attached to the different parameters in order of importance. Lipid yield was given a weight of 10, growth rate a weight of 8, bio-diesel yield a weight of 7 and ease of culturing a weight of 6.
- For growth rate, the highest achieved volume for each species during culturing was expressed as a percentage by dividing it by the total volume for each species multiplied by 100. i.e.

For Chlorella Vulgaris, from Table 3 below, the highest achieved volume was 14820.6 cm^3 on day 13 of observation and totaling all the volumes gave 135628.8 cm^3. The percentage of these was therefore calculated giving;

$$\frac{14820.6}{135628.8} \times 100$$

= 10.927

= 0.11

A weight of 6.5 out of 8 was attached to this value considering the rate of growth of this species.

For Botryococcus Braunii, from Table 5 below, the highest achieved volume was 1497 cm^3 on day 8 of observation and totaling all the volumes gave 11526.9 cm^3. The percentage of these was calculated giving;

$$\frac{1497}{11526.9} \times 100$$

= 12.9870%

= 0.13

A weight of 5 out of 8 was attached to this value considering the rate of growth of this species.

For Nannochloropsis, from Table 4 below, the highest achieved volume was 16317.3 cm^3 on day 12 of observation and totaling all the volumes gave 167065.2 cm^3. The percentage of these was calculated giving;

$$\frac{16317.3}{167065.2} \times 100$$

= 9.7670%

= 0.10

A weight of 7 out 8 was attached to this value considering the rate of growth of this species.

- For lipid yield, the averages of the yield from each solvent mix was expressed as a percentage by dividing it by 100 and then weights attached to this i.e.

For Chlorella Vulgaris, from figure 13 below, the average yield from all four solvent mixes was 51.9105% which when divided by 100 gave approximately 0.52. A weight of 8 out of 10 was attached to this value basing on the lipid yield.

For Botryococcus Braunii, from figure 13 below, the average yield from all four solvent mixes was 50.6020% which when divided by 100 gave approximately 0.51. A weight of 7 out of 10 was attached to this value basing on the lipid yield.

For Nannochloropsis, from figure 13 below, the average yield from all four solvent mixes was 69.2162% which when divided by 100 gave approximately 0.69. A weight of 9 out of 10 was attached to this value basing on the lipid yield.

- For bio-diesel yield, the percentage yield was calculated by dividing the mass of bio-diesel produced by the mass of the oil from which it was produced and multiplying the result by 100.

For Chlorella vulgaris, 50.0001g of lipids extracted from the algae produced 28.1055g of bio-diesel. So the percentage yield was calculated giving;

$$\frac{Mass\ of\ biodiesel}{Mass\ of\ lipids\ giving\ biodiesel} \times 100$$

$$= \frac{28.1055}{50.0001} \times 100$$

$$= 56.2108\%$$

$$= 0.56$$

A weight of 5 out of 7 was attached to this value basing on the bio-diesel yield.

For Botyrococcus Braunii, 50.0004g of lipids extracted from the algae produced 24.9015g of bio-diesel. So the percentage yield was calculated giving;

$$\frac{Mass\ of\ biodiesel}{Mass\ of\ lipids\ giving\ biodiesel} \times 100$$

$$= \frac{24.9015}{50.0004} \times 100$$

$$= 49.8026\%$$

$$= 0.49$$

A weight of 3 out of 7 was attached to this value basing on the bio-diesel yield.

For Nannochloropsis, 50.0002g of lipids extracted from the algae produced 34.6770g of bio-diesel. So the percentage yield was calculated giving;

$$\frac{Mass\ of\ biodiesel}{Mass\ of\ lipids\ giving\ biodiesel} \times 100$$

$$= \frac{34.6770}{50.0002} \times 100$$

$$= 69.3537\%$$

$$= 0.69$$

A weight of 6 out of 7 was attached to this value basing on the bio-diesel yield.

- For ease of culturing, weights were attached basing on the performance of each algae species during culturing. A weight of 4.5 out of 6 was attached to the Chlorella Vulgaris species, a weight of 2 out of 6 was attached to the Botryococcus Braunii species and a weight of 5 out of 6 was attached to the Nannochloropsis species.
- The totals for each criterion and the attached weights for each species was gotten and ranks were attached basing on the obtained totals.The total for Nannochloropsis was 16.05 which was ranked 1st, the total for Chlorella Vulgaris was 12.18 which was ranked 2nd and the total for Botryococcus Braunii was 7.69 which was ranked 3rd.

Summary of findings:

Following the extensive literature review, the species identified as most suitable for algal bio-diesel production were Chlorella Vulgaris, Nannochloropsis and Botryococcus Braunii. According to various papers and accessed resources, these species were selected basing mainly on their growth periods or how long it takes for each to grow in case of culturing, as well as the lipid content of each species.

4.2.2 Optimization of cultivation conditions

The table 2 under the chapter of methodology above shows the depths in cm that were recorded over the 14 day time span for each of the three species and how each changed. Species 1 was Nannochloropsis, species 2 was Chlorella Vulgaris and species 3 was Botryococcus Braunii. Using the formula explained previously for determining the volume occupied by the biomass for each species of algae, the volumes for each over the 14 day period were calculated and recorded.

From each of these tables of volumetric results, a graph was plotted showing the changes in volume over the cultivation period for each species as shown below.

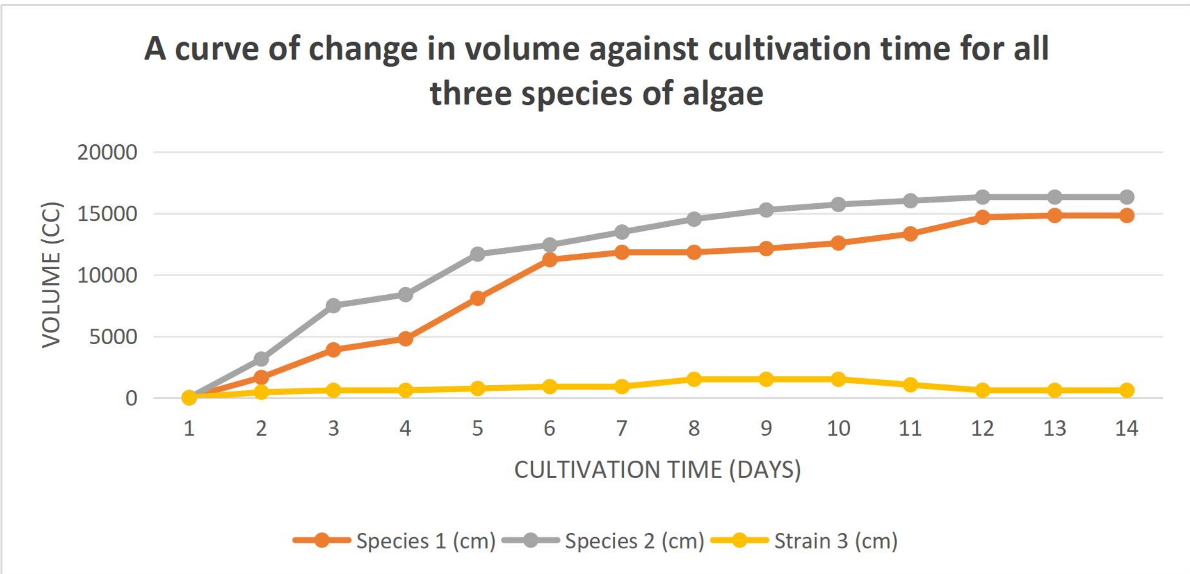

Figure 13: Comparison of volume increase for all three species

- The curve in figure 4 above shows an upward trend in the change in volume of algal biomass for Chlorella Vulgaris over the first seven days of culturing. This can be attributed to optimized nutrient levels at the start of the process. Between day 8 and day 12, there is a continued upward trend that is more subtle than in the beginning of culturing. This can be attributed to the fact that the algae was close to attaining its nutrient saturation point. This is a point at which no more nutrients can be taken in by the algae. From day 12 to day 14, the curve shows a stable trend and approximately no more increase in the volume of the algal biomass. This can be attributed to the algae having reached its nutrient saturation point and it could therefore not take in any more of what was necessary for further growth.
- The curve in figure 4 also shows an upward trend in the change in volume of algal biomass for Nannochloropsis. This trend is more stable compared to that of the Chlorella Vulgaris species and growth maintained this pattern for this species for most of the days of culturing, approximately until day 12. his can be attributed to optimized nutrient levels at the beginning of the process. Furthermore, the pattern demonstrated by the changes in volume of this species' biomass illustrated that it is more resistant to conditions that are not as accurately defined as would have been the case when culturing in the laboratory. Between day 12 and day 14, the curve shows an apparent halt in the growth of the algae or increase in volume, and this can be attributed to the algae reaching its nutrient saturation point therefore it was no longer able to consume enough to grow as at the start of the process.
- The curve in figure 4 also shows a fluctuating but upward trend in the change in volume of algal biomass for Botryococcus braunii and this can be attributed

to adaptation to the optimized nutrient conditions. However, the increase was significantly less compared to the previous two species and this can be attributed to the fact that this species requires more specific conditions to flourish and therefore is not as resistant to undefined conditions as Chlorella vulgaris and Nannochloropsis. Between day 8 and day 10, there is an approximately constant trend. This can be attributed to the algae reaching its nutrient saturation point and therefore being unable to take in any more nutrients. From day 10, there was a downward trend and in the last three days the algae exhibited a stable pattern after decreasing in volume. This decrease can be attributed to inability of this particular species to adapt to the conditions existing during the time of culturing. This is because this species requires controlled conditions for it to flourish for example, in case of insufficient light, there is a hindrance to the process of photosynthesis which is necessary for its growth while at the same time, excessive light can cause photo-inhibition for this species of algae more easily than is the case with other species.

From the results obtained and the discussion made, the trends of the curves suggest that species 2, which is Nannochloropsis is the most resilient among the three species and that it is the most efficient under the given conditions because of its adaptability. The patterns illustrated by the curves also suggest that species 3 which is Botryococcus Braunii exhibits fluctuating growth with significant dips at different time intervals.

Therefore, drawing a conclusion from the discussion above, Nannochloropsis is the most promising species of algae in terms of culturing for bio-diesel production basing on its growth period and ability to withstand and flourish in different conditions better than the other two species of algae.

4.2.3 Analyzing the physico-chemical properties of the obtained bio-diesel.

Table 4: Lipid yield for each species with the different solvent mixes

TABLE SHOWING RESULTS OF EXTRACTING OF OIL FROM ALGAE OF DIFFERENT SPECIES USING DIFFERENT SOLVENT MIXTURES.

	Percentage yield of Oil from different solvent mixtures (%).			
Species	Cyclohexane-Isopropanol	Diethyl ether-Isopropanol	Chloroform-Isopropanol	Hexane-Isopropanol
A	65.1265	35.4638	48.2873	58.7645
B	72.2337	45.6487	49.9834	34.5423
C	65.0981	83.1008	60.4535	68.2122

The initial step to making an analysis of the algal bio-diesel was lipid extraction from the algae, which was done using four different solvent mixes. This is because mixing solvents guarantees higher lipid extracts and makes it easier to break the cells the of algae to get the oil. The different species have different reactions to different solvents, for example Chlorella Vulgaris yields higher with hexane in the solvent mix compared to the other solvents. The table above shows percentage yield of oil from each solvent mix for each species.

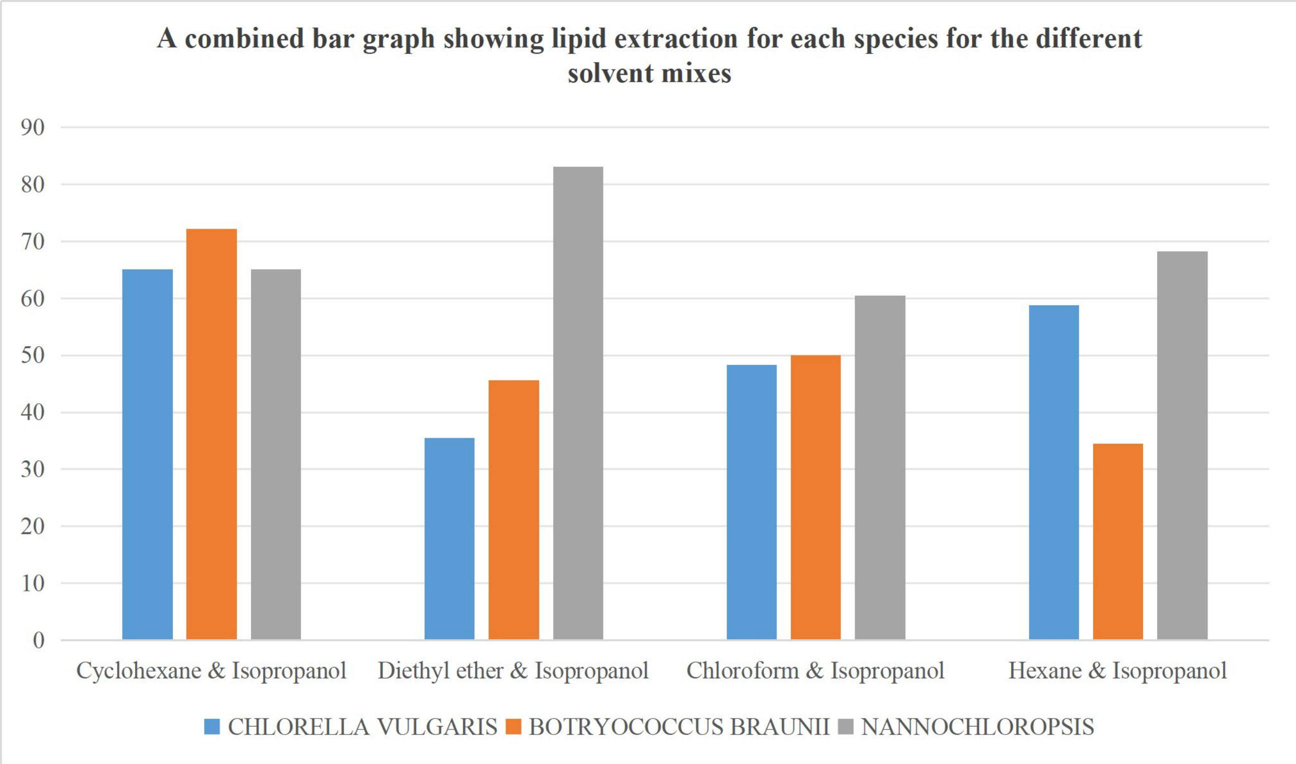

Figure 14: Graph comparing lipid yield for each species from each solvent mix

From the figure above, Nannochloropsis gave the highest lipid yield on average, followed by Chlorella vulgaris whose average lipid yield was not so far apart from the former and then Botryococcus Braunii gave the lowest lipid yield upon getting the average from all solvent mixes used for lipid extraction.

Table 5: Fuel property values for algal bio-diesel and acceptable standards

FUEL PROPERTIES	ALGAL BIODIESEL		STANDARDS	
Cetane Number (CN)	52		47 - 58	
Flash point (℃)	123		100 - 170	
Density (g/cm^3)	0.8335		0.820 - 0.860	
Kinematic viscosity (mm^2/s)	40℃	100℃	40℃	100℃
	2.823198	1.114182	2.6 - 3.5	1.9 - 4.1
Copper strip corrosion	1b		1a - 1b	

- The table 6 above comprises of results obtained from running different tests on the obtained algal bio-diesel to analyze different physico-chemical properties and these compared with standards to assess the usability of the diesel.
- The cetane number of the bio-diesel was 52 which was well within the standards of between 47 and 58, the flash point was 123 which was between the standard of 100 to 170℃, the density was 0.8335(g/cm^3) which was also within the acceptable standards. Copper strip corrosion was 1b which showed that the copper strip was slightly tarnished qualifying the diesel for use. Kinematic viscosity at 40℃ was within the standards however the viscosity at 100℃ was less than the required value. This can be attributed to residual and unreacted methanol from the conversion process, excessive breakdown of triglycerides to methyl esters during trans-esterification and a high degree of unsaturated fatty acid content.

4.3 CHALLENGES AND SOLUTIONS

4.3.1 Challenges faced

This chapter briefly highlights the challenges faced during carrying out the activities completed so far, which are conducting extensive reviews of literature as well as selecting the best algal species for bio-diesel production. These challenges included;

1. Limited access to comprehensive data. There was a challenge of restricted access to journals, papers and articles that were well equipped with up-to-date information about the different stages involved in algal bio-diesel production as well as about the process as a whole.
2. Diversity of algal species. Because of the various types of species of algae, there was difficulty in narrowing them down to the most suitable species for algal bio-diesel production. Furthermore, because of the various species, there was a lot of contradiction in results from different papers about which ones were best.
3. Limited regional relevance. Most studies focused more on species of algae that are only confined to locations that are not in Uganda or locally available.
4. Difficulty in evaluation of suitability of algal species. This was because of existence of multiple criteria for evaluating species basing on their lipid content, growth rates, their ability to adapt to environment, among others.
5. Inconsistencies in methodologies included in different published research. For example different studies highlighted different cultivation media, growth conditions, as well as lipid extraction techniques.

4.3.1 Possible solutions to the challenges faced

The ways in which the highlighted challenged could possibly be addressed include:

1. Provision of more access to published resources. This can be done through using institutional subscriptions, utilization of open access cites, as well as request of access from authors where possible.
2. Creation of a database entailing information about different properties as well as parameters that are necessary to be known for algal bio-diesel production such as lipid yield, growth rate of different species, among others.
3. Prioritizing algal species that are available within the local vicinity, as well as those that have the ability to not only thrive but also grow and accumulate within the local conditions.

5.0 CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 INTRODUCTION

This chapter entails key conclusions made from the research done on assessing the production of algal bio-diesel production, as well as potential recommendations basing on these. The conclusions made are based on the on the comparative performance of the algal species Nannochloropsis, Chlorella Vulgaris and Botryococcus Braunii. The study aimed to assess factors such as growth rate, lipid yield and bio-diesel potential of each of these species under controlled and similar conditions.

Based on experimental findings, methods such as multi-criteria decision analysis, conclusions are made regarding the most suitable species for bio-diesel production. Furthermore, recommendations are made basing on the conclusions drawn to guide future research and potential pursuit of up-scaled algal-bases bio-diesel systems in Uganda and even beyond.

5.2 CONCLUSIONS

Among the selected and studied algae species, Nannochloropsis and Chlorella vulgaris showed the highest growth rates under the conditions of culturing. Botryococcus braunii had the lowest growth rate under similar conditions.

Nannochloropsis gave the highest lipid and bio-diesel yield. Chlorella vulgaris have a high lipid yield but did not exceed Nannochloropsis.

Chlorella vulgaris is still a viable option for algal bio-diesel production but from this study, Nannochloropsis is the best species for algal biodiesel production.

Botryococcus braunii is not ideal for algal bio-diesel production because of its slow growth and lipid yield overall.

The viscosity of the bio-diesel at 100℃ was less than the required value which can be attributed to residual and unreacted methanol from the conversion process, excessive breakdown of triglycerides to methyl esters during trans-esterification and a high degree of unsaturated fatty acid content.

5.3 RECOMMENDATIONS

Nannochloropsis should be prioritized incase of large scale bio-diesel production.

Chlorella vulgaris could be considered for further reasearch, focusing on optimization of growth rate to increase its lipid content.

Further studies should be done to explore factors like optimization of nutrients, carbon dioxide enrichment, among others to enhance lipid accumulation especially in the more promising algae species.

The low kinematic viscosity of the bio-diesel at 100°C can be dealt with by blending with bio-diesel of a higher viscosity, using solely algae species with saturated or monosaturated fats such as Nannochloropsis and modifying the trans-esterification process to allow for checking for excess methanol, for example evaporating a small portion of the sample at about 105°C to monitor if there is change in weight.

APPENDIX

MULTICRITERIA DECISION ANALYSIS FOR ALGAL SPECIES SELECTION

CRITERIA	CHLORELLA VULGARIS	BOTRYOCOCCUS BRAUNII	NANNOCHLOROPSIS	CRITERIA WEIGHTS
Growth rate	0.11 / 6.5	0.13 / 5	0.10 / 7	8
Lipid yield	0.52 / 8	0.51 / 7	0.69 / 9	10
Bio-diesel yield	0.56 / 5	0.49 / 3	0.69 / 6	7
Ease of culturing	4.5	2	5	6
TOTAL	12.18	7.69	16.05	
RANK	2	3	1	

RESULTS FROM MONITORING INCREASE IN ALGAL BIOMASS DURING CULTURING

Cultivation time (days)	Species 1 (cm)	Species 2 (cm)	Species 3 (cm)
1	2.1	2.5	2.2
2	3.2	4.6	2.5
3	4.7	7.5	2.6
4	5.3	8.1	2.6
5	7.5	10.3	2.7
6	9.6	10.8	2.8
7	10.0	11.5	2.8
8	10.0	12.2	3.2
9	10.2	12.7	3.2
10	10.5	13.0	3.2
11	11.0	13.2	2.9
12	11.9	13.4	2.6
13	12.0	13.4	2.6
14	12.0	13.4	2.6

RESULTS FROM CONVERSION OF DEPTHS OCCUPIED TO VOLUME FROM MONITORING DURING CULTURING

Cultivation time (days)	Species 1 (cm^3)	Species 2 (cm^3)	Species 3 (cm^3)
1	0	0	0
2	1646.7	3143.7	449.1
3	3892.2	7485	598.8
4	4790.4	8383.2	598.8
5	8083.8	11676.6	748.5
6	11227.5	12425.1	898.2
7	11826.3	13473	898.2
8	11826.3	14520.9	1497
9	12125.7	15269.4	1497
10	12574.8	15718.5	1497
11	13323.3	16017.9	1047.9
12	14670.6	16317.3	598.8
13	14820.6	16317.3	598.8
14	14820.6	16317.3	598.8

UGANDA INDUSTRIAL RESEARCH INSTITUTE

"A Lead Agency in Industrialisation of Uganda"

Research Test Report

Students: Nabakka Prisca Angel and Ashaba Marvin Aheebwa

EXTRACTION OF OIL FROM ALGAE TO BE USED IN PRODUCTION OF BIODIESEL THROUGH TRANSESTERIFICATION.

FIRST BATCH (Strain A).

Extraction mixtures used:

1. Cyclohexane/Isopropanol (3:2 v/v)
2. Diethyl ether/Isopropanol (3:2 v/v)
3. Chloroform/Hexane (2:1 v/v)
4. Hexane/Isopropanol solution (3:2 v/v)

	Cyclohexane-Isopropanol	Diethyl ether-Isopropanol	Chloroform-Isopropanol	Hexane-Isopropanol
Mass of sample (g)	50.0003	50.0002	50.0003	50.0001
Volume of solution (ml)	250	250	250	250
Mass of Oil extracted (g)	32.5634	17.7320	24.1438	29.3823
Percentage yield of oil (%)	65.1265	35.4638	48.2873	58.7645

Mass of oil used = 50.0001 g.

Mass of Biodiesel produced = 28.1055 g

$$\text{Percentage yield of Biodiesel (\%)} = \frac{\text{Mass of oil Biodiesel}}{\text{Mass of Oil extracted}} X\ 100$$

$$= 56.2108\%$$

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Tel: +256-414-286 124/245, Fax: +256-414-286 695 Email: info@uiri.org Website: www.uiri.org

SECOND BATCH (Strain B).
Extraction mixtures used:

1. Cyclohexane/Isopropanol (3:2 v/v)
2. Diethyl ether/Isopropanol (3:2 v/v)
3. Chloroform/Hexane (2:1 v/v)
4. Hexane/Isopropanol solution (3:2 v/v).

	Cyclohexane-Isopropanol	Diethyl ether-Isopropanol	Chloroform-Isopropanol	Hexane-Isopropanol
Mass of sample (g)	60.0008	60.0008	60.0009	60.0010
Volume of solution (ml)	250	250	250	250
Mass of Oil extracted (g)	43.3408	27.3896	29.9905	20.7257
Percentage yield of oil (%)	72.2337	45.6487	49.9834	34.5423

Mass of oil used = 50.0004 g

Mass of Biodiesel produced = 24.9015 g

$$\text{Percentage yield of Biodiesel (\%)} = \frac{\text{Mass of oil Biodiesel}}{\text{Mass of Oil extracted}} X\ 100$$

= 49.8026%

THIRD BATCH (Strain C).

Extraction mixtures used:

1. Cyclohexane/Isopropanol (3:2 v/v)
2. Diethyl ether/Isopropanol (3:2 v/v)
3. Chloroform/Hexane (2:1 v/v)
4. Hexane/Isopropanol solution (3:2 v/v).

	Cyclohexane-Isopropanol	Diethyl ether-Isopropanol	Chloroform-Isopropanol	Hexane-Isopropanol
Mass of sample (g)	50.0001	50.0002	50.0002	50.0002
Volume of solution (ml)	250	250	250	250
Mass of Oil extracted (g)	32.5491	41.5506	30.2269	34.1062
Percentage yield of oil (%)	65.0981	83.1008	60.4535	68.2122

Mass of oil used = 50.0002 g

Mass of Biodiesel produced = 34.6770 g

Percentage yield of Biodiesel (%) = $\frac{\text{Mass of oil Biodiesel}}{\text{Mass of Oil extracted}} X\ 100$

= 69.3537%

SUMMARY.

TABLE SHOWING RESULTS OF EXTRACTING OF OIL FROM ALGAE OF DIFFERENT STRAINS USING DIFFERENT SOLVENT MIXTURES.

Strains	Percentage yield of Oil from different solvent mixtures (%).			
	Cyclohexane-Isopropanol	Diethyl ether-Isopropanol	Chloroform-Isopropanol	Hexane-Isopropanol
A	65.1265	35.4638	48.2873	58.7645
B	72.2337	45.6487	49.9834	34.5423
C	65.0981	83.1008	60.4535	68.2122

Analysed by.

Wanyana Jerom

Verified by

UGANDA INDUSTRIAL RESEARCH INSTITUTE
P.O. BOX 7086, KAMPALA
Technical Signatory
25 MAR 2025

NABAKKA PRISCA ANGEL S21B32/133

ASHABA MARVIN AHEEBWA S21B32/107

MINISTRY OF ENERGY AND MINERAL DEVELOPMENT DOWNSTREAM PETROLEUM TESTING LABORATORY		Document No.	PTL/WS/003
		Issue No.	02
		Revision No.	00
Document Tittle:	OPERATING MANUAL	Effective Date:	01-05-2022
Section:	Work Sheets	Review date:	01-05-2025

Worksheet for Lubricating & Engine Oil

Sample number		Analysis Start date	31/03/2025
Sample description	ALGAL BIODIESEL	Completion date	01/04/2025
Customer sample ID		Analysed by	
		Checked by	

General Requirements, Labelling and Classification

Parameter	Observation
Product Name and Application	ALGAL BIODIESEL
Type (Mineral or synthetic)	
Manufacturer's identification and/or Disributor's name	
Address of manufacturer and/or distributor	
Performance service classification (API, ACEA, ISO or JASO)	
API EOLCS Listing and quality mark	
Viscosity grade Classification (multigrade/monograde , SAE)	
Quantity/Net Content	
Origin of the product/Made in	
Date of Production and Batch identification number	
Expiry date	
Packaging type and condition	

Pysico-chemical Requirements

Parameter	Method	Observation
Appearance	Visual	
Presence of Suspended matter and sediments, grit, water or foreign matter and impurities	Visual	
Pour Point	ASTM D97	

Density of oil at 20 °C *Correction:* **Test Method: ASTM D1298**

Replicate number	1	2	Average
Density (g/cm3)	0.830	0.837	0.8335

Kinematic Viscosity and Viscosity Index **Test Method: ASTM D445**

Temperature	40°C	100°C
Falling time t, (sec)	804.33	319.25
Viscometer Constant (c)	0.00351	0.00349
Kinematic viscosity(mm²/s) $v = c*t$	2.823198	1.114182
Density	0.8335	0.8335
Dynamic viscosity, (kg/m.s) $\eta = \rho v$		
Viscosity Index	0.394652	

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MINISTRY OF ENERGY AND MINERAL DEVELOPMENT DOWNSTREAM PETROLEUM TESTING LABORATORY		Document No.	PTL/WS/001
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Document Tittle:	OPERATING MANUAL	Effective Date:	01-05-2022
Section:	Work Sheets	Review date:	01-05-2025

Worksheet for Diesel (AGO)

Sample number		**Analysis start date**	31/03/2025
Sample description		**Completion date**	01/04/2025
Customer sample ID		**Analysed by**	
		Checked by	

Physical Examination

Parameter	**Observation**
Appearance	

Density at 20 ⁰C. *Correction*: **Test method: ASTM D1298**

Hydrometer reading	Temperature reading	Corrected Density Value
0.833	17.4 °C	0.8335 g/cm³

Marker Concentration Measurement **Test method: GFI-XRF**

Replicate No	1	2	3	Average
Results:				

Flash point **Test Method: ASTM D93**

Replicate No	1	2	3	Average
Results				123

ASTM/Colour CETANE NUMBER **Test Method: ASTM D1500**

Replicate No	1	2	3	Average
Results				52

Viscosity at 40⁰C **Test Method: ASTM D445**

Falling time t, (sec)	804.33
Viscosity Constant	0.00351
Kinematic viscosity(mm²/s) ν= c. t	2.823198

Copper Corrosion **Test Method: ASTM D130**

Result 1b
Slight tarnish

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