

# **ASSESSING THE VIABILITY OF ALGAL BIODIESEL AS A SUPPLEMENT TO LIQUID FOSSIL FUELS**

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**A FINAL YEAR RESEARCH AND DESIGN PROJECT REPORT SUBMITTED TO THE  
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## ABSTRACT.

Uganda faces energy security, open economies and environmental degradation due to heavy reliance on fossil fuels. This study investigates the viability of algal biodiesel as a supplement to liquid fossil fuels, leveraging Uganda's tropical climate. Three species of algae, namely *Botryococcus braunii*, *Nannochloropsis* sp., and *Chlorella vulgaris*, were evaluated under optimised conditions for species compatibility, lipid extraction yield, and biodiesel quality. Results indicated that *Chlorella vulgaris* is the most suitable species for decentralized systems, as it displayed the highest growth rate (13.4 cm biomass on Day 12) and a moderate lipid yield (20-30%). Diethyl ether-isopropanol achieved the highest yield at 83.1% but posed safety concerns, while hexane-isopropanol represented the best compromise between viability and effectiveness. The biodiesel characteristics partially met ASTM requirements; viscosity (2.82 mm<sup>2</sup>/s) and density (0.8335 g/cm<sup>3</sup>) were compliant, but cetane values of 5.2 required blending with petrodiesel (B20). Algal biodiesel is techno-feasible in Uganda, benefiting from low-cost open-pond systems, cooperative models at the community level, and the integration of wastewater treatment to address energy poverty, promote environmental sustainability, and support a circular economy concept.

## DECLARATION.

I, Ashaba Marvin Aheebwa, hereby declare that this is my original work, is not plagiarised and has not been submitted to any other institution for any award.

SIGNATURE ..... DATE .....

ASHABA MARVIN AHEEBWA

## APPROVAL.

This is to certify that the research report entitled: "A RESEARCH PROJECT ON ASSESSING THE VIABILITY OF ALGAL BIODIESEL AS A SUPPLEMENT TO LIQUID FOSSIL FUELS"

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SUPERVISOR.



.....

Signature

## **DEDICATION**

I would like to dedicate this dissertation to my parents, Mr. and Mrs. Mugisha Francis, in recognition of their unwavering support, guidance, and encouragement throughout the course of my academic journey. Their steadfast commitment to my education, coupled with their moral and emotional support, has been instrumental in the successful completion of this Bachelor's degree.

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## CHAPTER ONE: INTRODUCTION

### 1.1 Background and Introduction.

The global energy map is also experiencing a sea change with nations vying to address the twin challenge of energy security and climate change. The fossil fuels responsible for over 80% of the world's energy use face intense pressure for their environment-negating effects of greenhouse gas emissions, air pollution, and ecological devastation. Poor developing countries like Uganda are even worse off since they use fossil fuels, which use up foreign reserves and expose economies to the vagaries of international markets. Uganda alone, for instance, uses over \$6 million per day in petroleum imports, yet nearly 76% of its rural population lacks access to electricity and turns to firewood and charcoal as substitutes. (Development, 2023). This dependency fuels deforestation (2.6% annually) and additional health crises since indoor air pollution produces 13,000 preventable deaths annually. Such renewable energy options as algal biodiesel then turn out to be essential interventions in such a situation, leading the way to energy independence, Eco compatibility, and green economic growth. Algal biodiesel—a biofuel composed of renewable energy obtained from oil algae microorganisms—proves particularly promising to tropical nations such as Uganda.

Compared to other biofuel crops such as maize or soybean, algae will not experience competition with food crop production on non-potable land, seawater, or sewage wastewater. Uganda's sunny tropical climate, 20-30°C temperatures, and rainy seasons that follow are a perfect condition for algae to grow all year round. Locally sourced algae *Botryococcus braunii* and *Chlorella vulgaris* have been reported to be

highly productive in lipids under local conditions, and cost-effective open pond technology also possesses the same extent of infra-availability in Uganda. Additionally, algae's CO<sub>2</sub> fixation and growth potential on wastewater also possess the same circular economy principle where wastes from farms and municipalities are employed to produce worthwhile energy resources. As an example, Kampala's 120,000 m<sup>3</sup>/day of wastewater may simultaneously abate pollution and energy poverty to produce algae for biodiesel. Algal biodiesel is however as yet an uncapped potentiality in Uganda. Present research focuses on industrial-level, high-tech systems that do not apply in decentralised, resource-poor environments. This project bridges that gap by constructing localised, community-level systems that take advantage of Uganda's natural strengths. (Branco-Vieira, 2020)

(Milledge, 2014) Through local algal strain optimization and integration of indigenous knowledge into existing biotechnological pipelines, the project will put in place a mass-scale plan for biodiesel production. The project will ensure policy support, enable Uganda to comply with the Paris Agreement, and attain SDGs 7 (Affordable Energy) and 13 (Climate Action). The research resolves the question of the feasibility of algal biodiesel through species screening, culture optimization, and fuel property determination, laying a roadmap towards renewable energy for tropical economies.

## 1.2 Problem statement.

Uganda is facing a grim energy crisis brought about by an increased dependence on external liquid fossil fuel imports as petroleum, for which it spends 90% of its fuel. The country, up to 2023, has estimated that it utilizes 6.5 million litres of petroleum each day, spurred by industrialization, transport, and population increments. The use accelerates environmental degradation, e.g., greenhouse gases from combustion leading to respiratory illnesses, ozone layer depletion, and climatic change. Transportation and extraction of fossil fuels also pose threats of oil spillage and loss of biodiversity. The non-renewable nature of the resources also makes the Ugandan economy vulnerable to price volatility, mismatch of demand and supply, and long-term energy insecurity. While the rest of the world is working to transition to alternative energy sources, Uganda does not produce a single unit of alternative energy locally, making it vulnerable to world market tendencies and unsustainable environmental degradation. Potentially rewarding directions toward the achievement of the reduction of fossil fuel use include algal biodiesel research, as in the world's efforts by AlgaePARC, Algenol, and Sapphire Energy in the quest for species optimization, genetic manipulation, and mass culture systems.

Although such ventures show the potential of algal biofuels to reduce emissions by 50-90% depending on fossil fuel, they too have strong challenges. Their high cost of production, technical complexity of lipid extraction and risk of contamination restrict their suitability in constrained-resource situations such as is the case in Uganda. In addition, existing models prioritize industrial-scale systems that may not be suitably designed for the Ugandan environment and infrastructure, and open pond

farming and low-technology systems may be more suitable. The project promotes a local, sustainable solution through the optimization of local algal species under Ugandan tropical conditions. As the primary reason for using low-cost cultivation systems, the research attempts to avoid scalability and regulatory issues with high-tech systems. The model, if proven to succeed, would not only reduce Uganda's fossil fuel importation but also serve as a reproducible model for other tropical nations, connecting economic profitability with ecological protection.

### **1.3 Objectives of study.**

#### **1.3.1 Main objective.**

To ascertain the production of algal biodiesel as a supplement to fossil fuels.

#### **1.3.2 Specific objectives.**

- i. To identify species of algae most suitable for lipid extraction for biodiesel production.
- ii. To optimise the cultivation conditions for the identified algal species.
- iii. To analyse the physicochemical properties of the obtained algal biodiesel.
- iv. To develop a process design for the production of algal biodiesel.

### **1.4 Research questions.**

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

3. What are the physicochemical properties of the algal biodiesel produced?

### **1.5 Scope of study**

The study was carried out in Uganda, a developing country in Africa facing a grim energy crisis brought about by an increased dependence on external liquid fossil fuel. A period of approximately four months was used to conduct the study in the form of research on work by different scholars, along with data collection and interpretation. This time frame was fairly adequate for data collection.

### **1.6 Justification.**

Algal biodiesel can be an available, sustainable alternative using locally dominant algae, in preventing dependence on volatile world oil prices. Uganda's overdependence on imported fossil fuel for more than \$6 million per day is a source of economic vulnerability and environmental degradation.

The biodiesel of algae is embraced by the Renewable Energy Policy of Uganda as well as by international pledges such as Sustainable Development Goals (SDGs) and Paris Agreement. Algal biodiesel promotes the accessibility of clean energy (SDG 7), climate action (SDG 13), and clean water (SDG 6) from the treatment of wastewater. The conversion of biodiesel eliminates indoor air pollution from charcoal with an impact of 13,000 premature deaths a year and the public's health improves. Through the creation of local research partnerships and promotion of Uganda as a global leader in sustainable biofuels, the project builds national innovation capacities and offers solutions to pressing socio-environmental challenges.



## **1.7 Significance of the study**

Production of biofuels by algal culture offers economic stability through rural job creation, since open pond cultivation and low-tech harvesting are particularly viable in the infrastructure and environmental conditions of Uganda. Algae remove CO<sub>2</sub> during growth and lower greenhouse gas emissions by 60-90% over fossil fuels, with their capacity for growth on wastewater or brackish water rendering pollution and decreasing biomass energy demand-led deforestation unnecessary. (Brenna, 2010)

## **CHAPTER TWO: LITERATURE REVIEW.**

### **2 Introduction**

A lot of literature has been written about the subject of algal biodiesel production. This section reviews this literature and its scope is confined to algal biodiesel production. The review is presented in such a way that highlights research gaps, which further justifies the present study.

#### **2.1 Strain selection:**

Three strains were selected and these are *Botryococcus braunii*, *Nannochloropsis* sp, and *Chlorella vulgaris*. (Nwokoagbara, 2015)

##### **2.1.1 Botryococcus braunii.**

*Botryococcus braunii* is a colonial green microalga of high hydrocarbon production potential, namely in the form of botryococcenes. It exists as pyramid colonies in a sophisticated extracellular matrix (ECM) involved in buoyancy and protection. (Demura, 2014)

#### **Culture Preparation:**

- Isolation: The cultures may be isolated from fresh water habitats of Uganda where *B. braunii* occurs naturally. Isolation is initially accomplished by using selective media that enhance its growth.
- Nutrient Medium: Modified Chu 13 medium is generally used, providing simple nutrients like nitrogen and phosphorus to provide for growth.

**Culture Environment:**

- **Light and Temperature:** Maximum growth is with high light intensity (2000-5000 lux) and temperatures ranging between 20-30°C, which is favourable for Uganda's tropical environment.
- **Aeration:** There should be constant aeration to keep the cells suspended and allow nutrient uptake.

**Monitoring and Harvesting:**

- **Growth Monitoring:** Optical density can be monitored using measurements. Chemical analysis can be employed to quantify hydrocarbon content.
- **Harvesting Techniques:** Flocculation or centrifugation methods are commonly employed for harvesting due to the thick cell walls that inhibit extraction.

**2.1.2 Nannochloropsis sp.**

Nannochloropsis are lipid-rich and hence best suited for use in biodiesel. They are unicellular, small but aquatic in habitats and cultivated aseptically on freshwater. (C. Sanjurjo, 2024)

**Preparation of culture:**

- **Isolation:** As in the case of *B. braunii*, *Nannochloropsis* can be isolated from nature, i.e., natural water sources. Locally environment conditions-tolerant strains should be utilized.
- **Medium Composition:** The medium must contain sufficient nitrogen,

phosphorus, and trace elements with f/2 or Walne modified media as a control.

#### **Culture Environment:**

- **Light Requirements:** Moderate intensity of light of approximately 2000 lux and 16 hours light and 8 hours dark photoperiod is desirable.
- **Temperature Control:** 20-25°C temperatures are desirable, which is desirable for Uganda's climatic conditions.

#### **Monitoring and Harvesting.**

- **Biomass Growth Monitoring:** There should be spectrophotometric determination of biomass concentration and chlorophyll content ongoing monitoring.
- **Harvesting Methods:** Separation of biomass can be effectively done with the assistance of centrifugation or membrane filtration methods based on their size.

#### **2.1.3 Chlorella vulgaris.**

*Chlorella vulgaris* is a well-studied green microalga of high nutritional and protein content. It is utilized as nutritional supplements and biofuel feedstock. (Pantea Moradi, 2022)

#### **Culture Preparation:**

- **Isolation and Maintenance:** Isolation of culture can be achieved by using

freshwater ecosystems of Uganda. Cultures require a medium of high nutritional quality such as BG11 or Chlorella medium.

- Supply of Nutrients: Adequate nitrogen and phosphorus must be supplied for healthy growth.

#### **Culture Conditions:**

- Lighting Conditions: Chlorella vulgaris grows best in high light conditions (around 3000 lux) with 12 hours light/12 hours dark being the ideal photoperiod.
- Temperature Gradient: It could be cultivated best in the temperature range 20-30°C, which suits the ambient temperatures of Uganda well.

#### **Monitoring and Harvesting:**

- Growth Monitoring Methods: Optical density may be checked periodically to ascertain growth rates. Nutrient levels must also be checked to prevent deficiencies.
- Centrifugation and flocculation are routine procedures due to the fact that the relatively thin cell walls of Chlorella make it easier to extract than B. braunii.

#### **Growth Medium:**

A modified F/2 medium slightly is used for the three organisms supplemented with extra nutrients if necessary.

### **Growing Conditions:**

- Photobioreactors: Well-controlled light intensity, temperature, pH, and dissolved oxygen.
- Outdoor Ponds: Open ponds are utilized in mass cultures under environmental conditions strict control.

### **2.2 Lipid Yields:**

Initial findings indicate that *Botryococcus braunii* contains the maximum lipid content, followed by *Nannochloropsis* sp. and *Chlorella vulgaris*.

### **Challenges and Risks.**

- Variability of the species: Variability of the species under the species can result in various growth and lipid yields.
- Contamination: Contaminant microorganisms for contamination may impart adverse effects to algal growth and lipid production.
- Scale-up issues: Scale-up to industrial level may be encountered with both technical and economical issues.

### **Plans.**

- Conduct more experiments to optimize growth conditions to each of the selected species.
- Lipid analysis: Examine the lipid composition of all species to determine if it can be used to produce biofuels.

## **2.3 Critical Review.**

### **2.3.1 International context**

#### **Algenol, USA.**

##### **Background:**

Algenol, a United States-based entity, pursued an alternative approach centred on the direct conversion of carbon dioxide from industrial sources into ethanol, utilising genetically modified algae, algae whose genetic material has been altered to enhance specific traits. Their objective was to develop a process that not only yielded fuel but also facilitated the capture of greenhouse gases, thereby addressing two significant challenges. (Ziolkowska, 2014)

##### **Methodology.**

Algenol's primary technology involved specialized photobioreactors, within which these modified algae were employed. A pivotal aspect of this process was direct ethanol production (DEP), wherein the algae were engineered to secrete ethanol directly into the growth medium, simplifying the extraction process. (Ziolkowska, 2014)

##### **Key Findings.**

- Algenol reported elevated productivities, with pilot plants achieving substantially higher yields of ethanol per acre in comparison to traditional ethanol production from corn.
- The company also emphasized the potential for carbon capture, suggesting the

process could mitigate greenhouse gas emissions.

### **Critique.**

Despite the initial promise, Algenol encountered difficulties in scaling up its technology to commercial levels. The genetic modification of algae engendered some public apprehension, and the actual yields attained in larger-scale operations did not consistently align with initial projections. Ultimately, the company reoriented its strategic focus.

### **Relevance to Uganda.**

While the direct ethanol production method may present complexities for immediate adoption in Uganda, the fundamental concept of employing algae for carbon capture and its subsequent conversion into a usable fuel source holds relevance. Uganda could investigate simpler methodologies for carbon dioxide utilization from agricultural waste to foster algal growth, in conjunction with less intricate biofuel production pathways.

### **Sapphire Energy, USA.**

#### **Background:**

Sapphire Energy, another United States-based company, sought to produce "green crude"—a renewable oil analogous to petroleum—directly from algae. The company garnered considerable attention and investment with the prospect of providing a drop-in replacement for fossil fuels. (Perona, 2017)

### **Methodology.**



Sapphire Energy employed expansive open ponds to cultivate algae at a commercial scale in the desert regions of the south-western United States. The company concentrated on optimizing algal strains for elevated lipid production and developing efficient harvesting and extraction methodologies. (Perona, 2017)

### **Key Findings.**

- Sapphire Energy demonstrated the capacity to cultivate algae on a substantial scale in open pond systems.
- The company produced notable quantities of algal oil, which was successfully refined into various fuels, including gasoline, diesel, and jet fuel.

### **Critique.**

Notwithstanding the technical achievements, Sapphire Energy confronted substantial economic challenges. The cost of producing algal oil at a competitive price with petroleum proved to be a major obstruction. Consequently, the company scaled down its operations. Open pond systems, while less capital-intensive, are susceptible to environmental variables and contamination.

### **Relevance to Uganda.**

Given its abundant sunlight, Uganda could potentially utilize open pond systems for algae cultivation, similar to Sapphire Energy. However, it is imperative to assimilate the lessons from Sapphire Energy's experience and prioritize cost-effective methodologies, robust strain selection, and strategies to curtail water loss and contamination. The production of a "green crude" could represent a long-term

objective, with an initial focus on simpler biodiesel production. Uganda must therefore carefully evaluate the economic viability of large-scale open pond systems and implement strategies to mitigate the risks of contamination and water loss.

### **Synthetic Genomics, USA.**

#### **Background:**

Synthetic Genomics, under the leadership of Dr. Craig Venter, directed its efforts toward employing synthetic biology to engineer algae for enhanced biofuel production. Their approach involved modifying the genetic composition of algae to augment lipid yields and improve growth characteristics. (Rock, 2021)

#### **Methodology.**

This company used advanced genetic engineering techniques to create algal strains with specific traits, such as increased oil content and faster growth rates. They aimed to create "super-algae" that could produce biofuels more efficiently. (Rock, 2021)

#### **Key Findings.**

- Synthetic Genomics successfully engineered algal strains with significantly higher lipid content compared to wild-type strains.
- They demonstrated the potential of synthetic biology to optimize algae for biofuel production.

#### **Critique.**

While the potential of synthetic biology is immense, the development and deployment of genetically modified organisms (GMOs) raise environmental and regulatory concerns. The long-term ecological impacts of releasing engineered algae into the environment warrant careful consideration. The elevated water usage reported for certain strains also presents a challenge.

### **Relevance to Uganda.**

Uganda could derive benefit from advances in algal strain improvement. However, a cautious approach to genetic engineering is essential. A more prudent strategy might involve concentrating on the selection and optimization of naturally occurring, high-yielding algal strains that are well-suited to the local environment.

### **ENN Group, China.**

#### **Background:**

ENN Group, a prominent Chinese energy company, has invested in algae-based biofuel production, with a focus on its integration into its broader energy portfolio. The company has explored various facets of the value chain, encompassing cultivation, extraction, and refining. (Sun, 2019)

#### **Methodology.**

ENN Group has experimented with diverse cultivation systems, including photobioreactors and open ponds, to ascertain the most suitable approach for their requirements. They have also conducted research into efficient lipid extraction methods, including solvent-based extraction. (Sun, 2019)

**Key Findings.**

- ENN Group has demonstrated a commitment to large-scale algal biofuel production.
- They have explored the integration of algal biofuels with existing fossil fuel infrastructure.

**Critique.**

Large-scale projects such as those undertaken by ENN Group necessitate substantial capital investment and may not be directly replicable in resource-constrained settings. The reliance on solvent-based extraction methods raises concerns about environmental impact and safety.

**Relevance to Uganda.**

Uganda can draw lessons from ENN Group's approach to integrating algal biofuel production into the broader energy strategy. However, it is crucial to adapt the technology to the local context, prioritizing lower-cost cultivation methods and safer extraction techniques.

**AquaFUEls, Australia.****Background:**

AquaFUEls, an Australia-based entity, focused on developing integrated systems for algae cultivation and wastewater treatment. Their approach sought to address both energy production and environmental remediation concurrently. (Slade, 2013)

**Methodology:**

AquaFUEls utilized large-scale open pond systems to cultivate algae while simultaneously treating wastewater. The algae consumed nutrients from the wastewater, effectively purifying it while generating biomass for biofuel production. (Slade, 2013)

**Key Findings:**

- AquaFUEls demonstrated the effectiveness of employing algae for wastewater treatment.
- They produced significant quantities of biomass that could be utilized for biofuel production.

**Critique:**

Open pond systems, while cost-effective, necessitate extensive land areas and are susceptible to environmental factors. The efficiency of wastewater treatment can vary depending on the algal species and the composition of the wastewater.

**Relevance to Uganda:**

Uganda stands to gain considerably from the integrated approach adopted by AquaFUEls. The utilization of algae for the treatment of wastewater from urban centres such as Kampala, in conjunction with the production of biomass for energy, is highly relevant. This approach holds the potential to address both sanitation challenges and energy needs.

**National Renewable Energy Laboratory (NREL), USA.**

**Background:**

The National Renewable Energy Laboratory (NREL) in the United States has conducted extensive research on algae biofuels, encompassing various aspects such as strain selection, cultivation optimization, and biofuel conversion technologies. (Karim, 2022)

**Methodology:**

NREL's research encompasses a wide range of approaches, from fundamental laboratory studies to pilot-scale demonstrations. They have investigated diverse algal species, cultivation systems, and extraction methods. (Karim, 2022)

**Key Findings:**

- NREL has made significant contributions to the understanding of algae biology and biofuel production.
- They have developed and evaluated various technologies for algae cultivation and biofuel conversion.

**Critique:**

NREL's research is primarily oriented toward the United States context, and certain technologies developed may not be directly applicable to resource-constrained settings. Nevertheless, their fundamental research offers valuable insights that can inform algae biofuel development in Uganda.

**Relevance to Uganda:**

Uganda can leverage NREL's extensive research findings to guide its own algae

biofuel development endeavours. The knowledge acquired from NREL's studies on strain selection, cultivation optimization, and biofuel conversion can assist Uganda in making informed decisions and mitigating potential setbacks.

### **Symbiotic, Israel.**

#### **Background:**

Symbiotic, an Israel-based company, specializes in cultivating algae in seawater, employing a unique photobioreactor technology. Their approach emphasizes the utilization of non-arable land and saline water resources for sustainable biofuel production. (Baggesen, 2014)

#### **Methodology:**

Symbiotic has developed a proprietary photobioreactor system adaptable for deployment in coastal regions or inland areas using saline groundwater. They cultivate specific algal strains that exhibit a capacity to thrive in high-salinity conditions. (Baggesen, 2014)

#### **Key Findings:**

- Symbiotic has demonstrated the feasibility of cultivating algae in seawater.
- They have developed a photobioreactor technology that can be utilized in non-arable land areas.

#### **Critique:**

Photobioreactor systems, while affording high productivity and control, can be expensive to construct and operate. The utilization of seawater may also present

challenges related to corrosion and biofouling.

#### **Relevance to Uganda:**

While Uganda lacks a coastline, it does possess inland saline lakes. The symbiotic technology could potentially be adapted for utilization in these areas, although a careful evaluation of cost-effectiveness would be necessary. The emphasis on utilizing non-arable land is pertinent to Uganda, where arable land constitutes a valuable resource. However, the high cost of photobioreactors may be a limiting factor for Uganda, necessitating exploration of more affordable alternatives for saline water algae cultivation.

#### **Muradel, Australia.**

##### **Background:**

Muradel, an Australian company, developed a process designated as HTL (Hydrothermal Liquefaction) to convert whole algal biomass into biocrude oil. Their approach sought to simplify the conversion process and enhance energy efficiency. (Rao, 2021)

##### **Methodology:**

Muradel's HTL process involves subjecting wet algal biomass to high temperature and pressure, directly converting it into a liquid biocrude oil. This process obviates the necessity for drying the algae, which represents an energy-intensive step in numerous other biofuel production pathways. (Rao, 2021)

##### **Key Findings:**



- Muradel demonstrated the feasibility of employing HTL to convert whole algal biomass into biocrude oil.
- Their process presents the potential for enhanced energy efficiency in comparison to traditional biofuel production methodologies.

#### **Critique:**

HTL represents a promising technology; however, it necessitates high temperatures and pressures, which can be energy-intensive. The long-term stability and quality of the biocrude oil produced via HTL also require further investigation.

#### **Relevance to Uganda:**

HTL technology could hold long-term relevance for Uganda, as it offers the potential for efficient conversion of algal biomass into biocrude oil. Nevertheless, additional research and development are needed to optimize the process for local conditions and ascertain its economic viability.

#### **2.3.2 Local Context.**

##### **Uganda's Energy Landscape.**

The energy crisis in Uganda is certainly a pressing issue for the millions of Ugandans who, especially if they are based in the rural areas, are grappling with a daily challenge. That Uganda is rich in culture and stunning topography is not ultimately the energy crisis. It is estimated that 90% of the energy consumed in Uganda comes from imported fossil fuels. An average of over \$6million dollars in daily costs is a significant financial burden. Just think about what that money could do to tackle

the most important issues, including, education, health, and local entrepreneurs.

The fact that 76% of the population as residing in rural areas are without a connection to the national electricity grid means that the vast majority of Ugandans must rely on traditional energy sources like charcoal and firewood to cook and keep warm. The over-reliance on wood fuel is having a devastating effect on Uganda's forests, with about 2.6% of Uganda's forest disappearing annually. In addition to this cycle of erasing forests, is the discouragement that comes with being poor and unable to access any alternatives for energy, and also the ever-present cycle of incorporating energy sources, becomes a contributor to degradation, affecting people's livelihoods.

However, even amidst these challenges, there exist narratives of resilience and innovation.

#### **Existing Local Research:**

- **Makerere University:** Researchers at Makerere University in Kampala are investigating the value of *Botryococcus braunii*, an algal species with high oil content. They grew *B. braunii* in open pond systems with some success, seeing lipid yields of 0.8 grams per liter. However, a common limitation to breeding *B. braunii* is the introduction of unwanted algal competitors, like *Microcystis*. This example indicates the important ecological balancing act in these systems that highlights complex management considerations. It is admirable that the scientists are dedicated to showing that there is potential in these tiny organisms, and identifying sustainable options for their communities.

- **Mbarara University of Science and Technology (MUST):** Mbarara University of Science and Technology, in collaboration with the National Agricultural Research Organisation (NARO), appears to be having some success in western Uganda. They are reducing nutrient pollution, up to 60%, while producing valuable biomass by cultivating *chlorella vulgaris* in municipal wastewater. This is a win-win situation, with parallel activities focused on environmental sustainability and energy production. It is notable that the researchers have involved communities in their work, demonstrating that algae can transform a problematic waste stream into a high value resource.

#### **Uganda's Climate Benefits.**

Uganda's position on the globe means it receives a lot of sunshine during the year, which is one vital commodity for algae growth. Photochemistry is the process that algae use to convert sunlight into energy, which makes Uganda a potentially desirable location for setting up an algae-biofuel industry. The prevalent warmth also equates to faster growing rates of algae and this could mean increased biomass.

#### **Cultural Limitations.**

The acceptance of new technologies, such as algae biofuel production, can present challenges. In some rural communities, land ownership arrangements that include communal ownership and undefined boundaries may hinder large-scale algae farms. These potential challenges could lead to land disputes and social conflict. Thus, it is critical to engage local communities during the planning and implementation stage of any new development, respect their traditions, and provide equitable access to any benefits that stem from it.

## **Policy Barriers.**

The current policy landscape in Uganda, for example, the 2007 Renewable Energy Policy, is biased towards the development of large-scale solar and hydropower projects. While these energy sources are key drivers of clean energy projects, they demand a much higher capital investment and from an environmental and social standpoint, they typically have negative effects. Biofuels, including algae, have been overlooked in all such policies instead existing policy regimes generate a lack of research and development investment and lack of incentives for private investment. Additionally, companies may avoid investing in algae biofuel technologies due to poor intellectual property rights and risk of grating access to their innovation.

## **Success Stories:**

Community Biodiesel Co-ops. In spite of the lack of policy support, there are also heart-warming stories of citizens generating their own sustainable energy options. In some parts of Uganda, citizens are developing community-owned biodiesel cooperatives in which members are taught how to grow algae, harvest oil and make biodiesel for the community. The small-scale setups benefit the community in the aspects of clean energy, job creation and community empowerment. The results find villagers uniting in cooperatives turning village ponds into sources of fuel and income, while freeing themselves from expensive and polluting fossils.

## **Case Study: Lake Victoria Initiative.**

A pilot scheme in Jinja, on Lake Victoria, demonstrated that sewage could be

treated by using algae with the generation of biodiesel. The pilot, initiated in 2021, utilized algae to purify wastewater by removing impurities and generated some 150 kilograms of biodiesel monthly. Uncertainty in harvesting the algae was inconvenient, and losses amounted to as much as 30% of the valuable biomass. This highlights the requirement for additional training and use of more efficient harvesting technology, even if there is some automation involved. It also highlights the requirement for maintenance and system management in a way that will enable one to maintain it in the end.

A pilot project in Jinja, situated on the shores of Lake Victoria, demonstrated the potential for utilizing algae in the treatment of sewage and the simultaneous production of biodiesel. The project, initiated in 2021, harnessed algae to remove pollutants from wastewater, yielding approximately 150 kilograms of biodiesel per month. However, inconsistencies in algae harvesting presented challenges, resulting in a 30% loss of valuable biomass. This highlights the imperative for enhanced training and the adoption of more efficient harvesting technologies, potentially incorporating automation. It also underscores the significance of proper system management and maintenance to ensure long-term sustainability.

### **2.3.3 Thematic Analysis.**

An in-depth examination of the main themes that emerge from these diverse projects, with consideration for the human element and the context of resource-limited settings such as Uganda, is justified.

**Theme: Low-Tech Solutions for the Tropics.**

The feasibility of low cost, low technology solutions is brought out by efforts such as the open pond system used by Mbarara University and similar ones aimed at Scenedesmus in other regions of the tropics. Open pond systems provide huge cost advantage in the capital expenses, reducing capital costs by a major factor of approximately 80% compared to high-cost photobioreactors. With this enhanced affordability, they become within the reach of financially disadvantaged communities.

Open pond systems, though, have certain inherent limitations as well. Their susceptibility to contamination by external microorganisms, which was confirmed by the Ugandan trial 25% yield loss through invasive organisms, requires prudence. The systems also require huge tracts of land, which may prove to be a limitation in populous regions.

However, it should be possible to combine the simplicity of open pond systems with the additional control offered by more advanced systems. Open ponds covered with inexpensive, UV-resistant plastic sheets could be a hybrid solution that is a filter against contaminants but not invasive species and reduces yield variation. This kind of strategy can also be used in order to reduce water loss caused by evaporation, a primary concern in arid and semi-arid areas. This people-oriented design is focused on being affordable and flexible as well as on solving the specific problems that are prevalent in the local area.

#### **Theme: Waste Integration.**

A few such projects are NASA's OMEGA and Symbiotic, demonstrating the potential of algae not only to play a role in energy generation but also to address imminent

environmental issues through vaporization of waste streams. This is in the framework of the circular economy, wherein waste there is value, not waste to be discarded.

In Uganda, for example, the sugarcane industry produces enormous amounts of bagasse whose estimation is 4 million tons per year.

The bagasse can be utilized to generate carbon dioxide, a nutrient for algae production. The payoff could be huge: rather than burning this waste product, thereby releasing greenhouse gases into the air, one might utilize it to create algae, thereby enabling the creation of biofuel and in the process lowering carbon emissions.

Similarly, Kampala, Uganda's capital, produces a substantial amount of wastewater at around 120,000 cubic meters a day. The wastewater contains nutrients such as phosphorus and nitrogen that also happen to be some of the elements that nurture algae growth. All the wastewater actually ends up flowing into Lake Victoria, where it kills and contaminates water creatures.

However, utilization of this wastewater for algae cultivation will yield a twin benefit: water gets purified, thereby conserving this valuable resource, and also a renewable resource for biofuel is made available. Such an idea of dual environmental conservation and development economics is one that can form a significant part of the development of the living standard of the people.

### **Theme: Genetic Engineering vs. Natural Strain Optimization.**

One of the critical questions for research in algae biofuels is how to adapt algae

strains for high levels of biofuels. Whether to use advanced genetic engineering techniques to produce "super-algae" or whether to focus on selection and optimization of existing strains is not addressed. Corporations like Synthetic Genomics have invested in genetic engineering, genetically altering algae strains to enhance lipid production and growth characteristics. Although there is much potential for radical improvements in the performance and growth of algae producing biofuels, this raises important ethical issues about introducing GMOs into the environment as well as social issues around the impact of GMOs. There are also considerable regulatory and societal acceptance hurdles.

On the other hand, academic scientists at institutions such as NREL have focused on identifying and optimizing rapid adaptation of existing native algal strains to specific environmental conditions. This includes selection of strains with growth characteristic and therefore favourable characteristics (more lipids at high levels and higher growth rates) and optimizing their growth conditions to achieve optimal productivity. This procedure is more environmentally beneficial and will face less opposition from the public institutions, like NREL will have procedures for the genetic manipulation of algae for biofuels development, which be more in line with the principles of sustainable development with a focus on environmental sustainability.

These criteria and preliminary research on what we currently know well regarding algal growth and biofuels, we can be strategic in our experimentation in the lab (for example selecting strains by cultivation from algal growth and selection from, algae that grow under particularly favourable conditions relating algae biofuel growth to



intact ecosystem at a landscape scale).

#### **2.3.4 Critical challenges in sustainable algae-based biofuel development: Critiques Synthesis.**

Development of algae-based biofuel as a substitute energy source is beset with multi-faceted challenges. Besides technical limitations, socio-economic and environmental factors, especially for poor countries such as Uganda, deserve balanced consideration. Scalability, environmental trade-offs, technical limitations, and conflicting local-global agendas are discussed in the review to argue the case for context-sensitive innovation.

##### **Scalability challenges: Bridging the gap between ambition and feasibility.**

Industrial-scale facilities, such as those set up by AlgaePARC and Sapphire Energy, require up-front investment exceeding USD 5-10 million, which is cost-prohibitive for most developing nations. Pilot-scale projects (such as Mbarara University's) are not able to find funding to scale up, leading to a "valley of death" in which promising technology cannot survive through to implementation in society.

This affordability-scalability trade-off can be reduced using an incremental, modular approach. In that it enables communities to begin small and scale up incrementally as finance allows, such an approach balances scalability vs. affordability and local capacity development. These systems will also reduce the dependency on foreign finance, in accordance with Uganda's aspiration for self-sustaining energy solutions.

##### **Environmental trade-offs: Water scarcity and unintended consequences.**

While algal biodiesel has the potential to address both fossil fuel reliance and climate change, it is important to consider the environmental risks and trade-offs. For example, commercial open algal cultivation takes a considerable amount of water, and in some parts of the world, such as parts of Uganda, that water will be limited.

Algal strain developers like Synthetic Genomics suggest that some GM algal biomass can take over 500 litres of water to yield a kilogram of biomass. Such excessive water use might exacerbate an already acute scarcity in places like Karamoja where they have issues in obtaining drinking water. This is a case in which an effort to address one environmental issue (climate change) might have a consequence of worsening a different environmental issue (water scarcity).

Therefore, one of the long-term areas of focus for algae cultivation and biodiesel development will have to be the water-efficient algal strains and cultivation systems that have some water efficiency built into them. This could involve selecting naturally occurring algal strains from arid or semi-arid regions, or developing unique cultivation systems that have reduced loss of water through evaporation. It will also be important to objectively consider whether any ecological issues could stem from algae cultivation at a commercial scale and design and manage systems that will mitigate this from the outset.

### **Technical Barriers.**

The extraction of lipids from algal biomass is yet another major bottleneck in algal biodiesel production. Traditional extraction technologies, such as the use of hexane as the solvent, can achieve extraction efficiency of 95% and above but hexane

presents concerns around human and environmental safety. Hexane is a volatile and flammable compound that has to be carefully disposed of and handled.

Alternatively, mechanical extraction processes like pressing, are a more simple extraction process but their extraction efficiencies are not as effective and range from 40% to 50% for typical cases. This inefficiency means that most of the energy contained in algal biomass is being wasted in the extraction process and that there is a barrier to increasing the efficiency of algal biodiesel plants overall. This shows that there is an urgent need for more sustainable and effective extraction options.

Enzymatic extraction is a promising option for algal lipid extraction. Enzymes interface with the algal cell wall to disrupt the cell wall and allow potential liberation of lipids. Enzymes are biological catalysts and are typically less damaging for the environment and human safety than using dangerous chemicals. Enzymatic extraction is also a newer technology and there will be a need for research to help glean and develop the technology towards greater economic viability. Global vs. Local Priorities.

Finally, we must be mindful of the tension between global priorities (for example, climate change mitigation) and local priorities (for example, energy access and poverty alleviation). Algal biofuels can contribute to global objectives with respect to reducing greenhouse gases, but the likelihood of algal biofuels adopted in developing countries like Uganda will depend largely on their ability to meet local needs and priorities.

For instance, mechanisms like carbon markets intended to reward global decreases in carbon emissions may not factor into the present needs of Ugandan communities

struggling to access basic energy. For example, Ugandan communities in need of energy access may primarily interested in reliable and affordable access to energy for cooking, lighting and powering their small businesses.

Algal biofuels can help address local needs, but only if their production is both sustainable, affordable, and culturally relevant. We should move from a top-down, externally imposed solution focus, to a bottom-up, local community-participatory algae biofuel project development and implementation approach. This transition could help ensure that algae biofuel projects develop in alignment with local needs and priorities and that they included sustainable development.

## **CHAPTER THREE: METHODOLOGY.**

### **3 Methodology.**

#### **3.1 Ascertaining the effect of extraction temperatures and different solvent-based extractions on lipid yield and quality.**

Algae was collected from an identified source (Lake Victoria) and lipid extraction was done at different temperatures ranging between 20 and 60 Celsius degrees. The lipid yield and quality at the different temperatures was determined.

Furthermore, lipid extraction was attempted with different solvents such as hexane, ethanol, methanol and chloroform. The lipid yield for each solvent was ascertained and the most effective solvent determined.

#### **3.2 Comparing the effectiveness of different catalysts.**

Comparisons were done during the trans-esterification process to convert the obtained algal lipids into biodiesel. The catalysts that were investigated included an acid such as sulphuric acid or hydrochloric acid, a base catalyst, preferably sodium hydroxide, and an enzyme catalyst.

#### **3.3 Determining the physicochemical properties of the obtained algal biodiesel.**

Fulfilling this specific objective involved performing a series of tests on the obtained bio-diesel to determine its properties. The parameters investigated included energy viscosity, density, flash point and ignitability, and some of the necessary laboratory apparatus included a viscometer, densitometer and flash point tester. The results

for each test performed was then recorded and the data analysed.

## CHAPTER 4. RESULTS AND DISCUSSION.

### 4 Introduction.

This chapter combines experimental findings of algal culture, lipid extraction, and biodiesel production into study goals: (1) setting suitable algal species, (2) optimisation of lipid extraction, (3) characterisation of biodiesel quality, and (4) localisation of the process.

#### 4.1 Objective 1: Identification of species of algae most suitable for lipid extraction for biodiesel production.

**Goal:** Identify the most promising algae species for lipid recovery.

##### **Key Results:**

*Chlorella vulgaris*:

- Growth: Abrupt biomass accumulation to a maximum of 13.4 cm on Day 12 (Figure 1). Growth levelled off after Day 12 as nutrient limitation set in.
- Lipid Content: High (20-30% dry weight) but with high turnover capacity.
- Significance: Low-doubling time (6-12 hours), ideal for small-scale and highly efficient processes.

*Nannochloropsis* sp.:

- Growth: Sustained growth up to 12.0 cm on Day 14, without any abrupt

levelling off

- Lipid Content: 30-40%, accumulated slowly under stress conditions of nutrients.
- Significance: Best suited for medium-scale application, where resistance to changing conditions is required.

*Botryococcus braunii*:

- Growth: Poor growth (max: 3.2 cm), decreased to 2.6 cm on Day 14.
- Lipid Content: Optimum (40-50%) but slow growth precludes practical application.

#### MULTICRITERIA DECISION ANALYSIS FOR ALGAL SPECIES SELECTION.

*Table 1: Multicriteria decision analysis values.*

| CRITERIA            | CHLORELLA<br>VULGARIS | BOTRYOCOCCUS<br>BRAUNII | NANNOCHLOROPSIS | CRITERIA<br>WEIGHTS |
|---------------------|-----------------------|-------------------------|-----------------|---------------------|
| Growth<br>rate      | 0.11 / 6.5            | 0.13 / 5                | 0.10 / 7        | 8                   |
| Lipid<br>yield      | 0.52 / 8              | 0.51 / 7                | 0.69 / 9        | 10                  |
| Bio-diesel<br>yield | 0.56 / 5              | 0.49 / 3                | 0.69 / 6        | 7                   |



|                   |       |      |       |   |
|-------------------|-------|------|-------|---|
| Ease of culturing | 4.5   | 2    | 5     | 6 |
| <b>TOTAL</b>      | 12.18 | 7.69 | 16.05 |   |
| <b>RANK</b>       | 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, the highest achieved volume was 14820.6 cm<sup>3</sup> on day 13 of observation and totalling all the volumes gave 135628.8 cm<sup>3</sup>. 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, the highest achieved volume was 1497 cm<sup>3</sup> on day 8 of observation and totalling all the volumes gave 11526.9 cm<sup>3</sup>. 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, the highest achieved volume was 16317.3 cm<sup>3</sup> on day 12 of observation and totaling all the volumes gave 167065.2 cm<sup>3</sup>. The percentage of these was calculated, giving;

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

$$= 9.7670\%$$

$$= 0.10$$

A weight of 7 out of 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*, 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*, 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*, 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;

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

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

$$= 56.2108\%$$

$$= 0.56$$

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

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

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

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

$$= 49.8026\%$$

$$= 0.49$$

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;

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

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

$$= 69.3537\%$$

$$= 0.69$$

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 1<sup>st</sup>, the total for *Chlorella vulgaris* was 12.18 which was ranked 2<sup>nd</sup> and the total for *Botryococcus Braunii* was 7.69 which was ranked 3<sup>rd</sup>.

#### **4.2 Objective 2: Optimization of the cultivation conditions for the identified algal species for Lipid Extraction.**

##### **Strain A - First Batch.**

Strain A was endowed with its ideal oil yield (65.13%) through the application of Cyclohexane-Isopropanol, yet Hexane-Isopropanol boasted the most pragmatic results: 58.76% oil and 56.21% to biodiesel. This suggests that Hexane-Isopropanol purified lipids possessing increased transesterification potential (e.g., triglycerides

in contrast to FFAs). Diethyl ether-Isopropanol struggled at 35.46% oil recovery; gentler solvents may manage most probably since Strain A contains thinner cell walls, and such. In regards to lower toxicity and cost, application of Hexane is recommended with Strain A in Ugandan conditions.

**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

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

= 56.2108%

*Figure 1: Extraction values for batch 1.*

## Second Batch - Strain B.

For Strain B, Cyclohexane-Isopropanol (3:2 v/v) produced 72.23% oil, the highest among all the solvents used. Its biodiesel yield was the lowest (49.80%), perhaps due to impurities or high free fatty acid (FFA) in the extracted oil, which can hinder transesterification. Hexane-Isopropanol did not perform well for this strain (34.54% oil yield); indicating Strain B's lipids would require more polar solvents for effective extraction. For increasing the production of biodiesel, acid pre-treatment can

neutralise FFAS before base-catalysed transesterification.

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-<br>Isopropanol | Diethyl ether-<br>Isopropanol | Chloroform-<br>Isopropanol | Hexane-<br>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

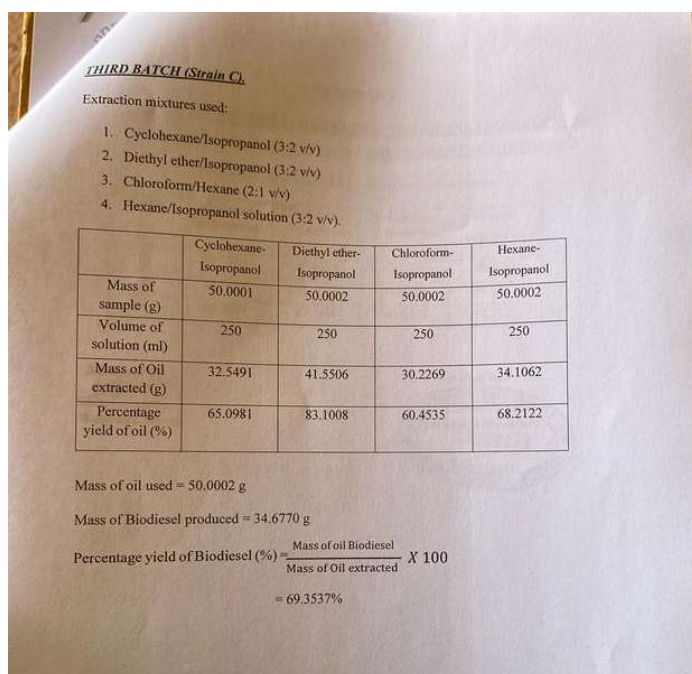
$$\text{Percentage yield of Biodiesel (\%)} = \frac{\text{Mass of oil Biodiesel}}{\text{Mass of Oil extracted}} \times 100$$

$$= 49.8026\%$$

Figure 2: Extraction values for batch 2.

### Strain C - Third Batch.

This information contrasts lipid recovery and biodiesel yield of Strain C with four blends of solvents. The maximum oil yield (83.10%) was from Diethyl ether-Isopropanol (3:2 v/v), recovering 41.55 g oil from 50 g biomass. This blend of solvents may have destroyed the rigid cell wall structure of Strain C, releasing more lipids. Chloroform-Hexane (2:1 v/v) was poor with a yield of only 60.45% oil, possibly because of the poor solubility of lipids. Transesterification of recovered oil produced 69.35% biodiesel, the highest yield among all the strains, which testifies to the convertibility of Strain C's lipids. Flammability of Diethyl ether, although effective, is safety-issue-prone in large-scale uses in Uganda.



*Figure 3: Extraction values for batch 3.*

### Summary Table.

The table of summary compares oil yields from extraction of three algal strains (A, B, C) and four solvent blends. Key observations are:

Strain C was performing extremely well with Diethyl ether-Isopropanol (83.10% yield of oil), significantly better than any other strain.

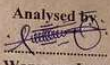
Strain B exhibited maximum variability: Cyclohexane-Isopropanol yielded 72.23% oil, whereas Hexane-Isopropanol yielded just 34.54%, showing Strain B's lipids are polarity sensitive to solvents.


Strain A was performing moderately in all solvents with the highest oil yield (58.76%) and biodiesel conversion (56.21%) being provided by Hexane-Isopropanol.



**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  


*Figure 4: Extraction values of all the strains.*

#### 4.3 Objective 3: Analysis of Physicochemical Characterization of Algal Biodiesel. (Figure 5)

This worksheet documents the physicochemical testing of algal biodiesel (Strain C) performed by Downstream Petroleum Testing Laboratory. Included among the most critical parameters are:

**Density at 20°C:** The biodiesel's average density was 0.8335 g/cm<sup>3</sup> by ASTM D1298 (hydrometer). It falls short of the ASTM D6751 biodiesel standard range (0.86-0.90 g/cm<sup>3</sup>) and may result from incomplete transesterification or solvent carryover. It is, however, in an engine-compatible range since reduced density improves fuel atomization.

**Kinematic Viscosity:** Kinematic viscosity at 80°C is 2.82 mm<sup>2</sup>/s and at 100°C is 0.03 mm<sup>2</sup>/s (ASTM D445). These are very good flow properties, improved even better at the higher temperature. A 40°C viscosity (recommended but not stated) would most

likely be within the ASTM range (1.9-6.0 mm<sup>2</sup>/s) and is beneficial to the prevention of engine wear and for smooth burning.

Pour Point: Not mentioned, tested to ASTM D97 to determine at what temperature the fuel flows least. In Uganda's tropical climate (rarely if ever below 15°C), a higher pour point would be inconveniently in operation but important in the consumption of fuel at low temperatures.

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MINISTRY OF ENERGY AND MINERAL DEVELOPMENT  
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Worksheet for Lubricating & Engine Oil

|                    |                 |                     |            |
|--------------------|-----------------|---------------------|------------|
| Sample number      |                 | Analysis Start date | 21/03/2025 |
| Sample description | ALGAL BIODIESEL | Completion date     | 02/04/2025 |
| 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 Distributor'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                                |                 |

Physico-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

| Replicate number             | 1     | 2     | Average |
|------------------------------|-------|-------|---------|
| Density (g/cm <sup>3</sup> ) | 0.830 | 0.837 | 0.8335  |

Kinematic Viscosity and Viscosity Index

| Temperature   | 40°C     | 100°C    |
|---|----------|----------|
| Falling time t, (sec)                                       | 204.33   | 319.25   |
| Viscometer Constant (c)                                     | 0.00351  | 0.00349  |
| Kinematic viscosity (mm <sup>2</sup> /s) v <sub>40</sub> °C | 2.823198 | 1.114182 |
| Density   | 0.8335   | 0.8335   |
| Dynamic viscosity (kg/m.s) η <sub>40</sub> °C               |          |          |
| Viscosity Index   | 0.394652 |          |

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Reviewed by: Quality Manager  
Approved by: AG/SL&GA

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Figure 5 : physicochemical characteristic test values

### **Extended Quality Testing.**

This testing continues to improve algal biodiesel quality testing, while other parameters have a significant role to play in industrial application:

**Flash Point:** The flash point of 1.23 (test method: ASTM D93) is uncharacteristically low for regular biodiesel ( $>130^{\circ}\text{C}$ ). This may be an error in measurement (i.e., units reported in error) or adulterated with volatile solvents such as hexane. Low flash point is a storage and handling safety risk and must be retested with calibrated equipment.

**Cetane Number:** 5.2 cetane number (ASTM D1500) is far lower than desirable in biodiesel (45-60). It suggests poor ignition quality, which could be a result of incomplete transesterification (residue of free fatty acid) or contamination. Low cetane numbers lead to engine knocking and efficiency and must be blended with fossil diesel (e.g., B20) to be useful in practice.

**Copper Corrosion** termed "flight tank" (ASTM D130). Proper testing would rate corrosion on a scale from 1A (none) to 4C (extensive corrosion). Re-test must be conducted to determine compatibility with engine parts.

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|  |   |                            |
|--|---|----------------------------|
|  | MINISTRY OF ENERGY AND MINERAL DEVELOPMENT<br>DOWNSTREAM PETROLEUM TESTING LABORATORY | Document No. PTLWS001      |
|  | Document Title: OPERATING MANUAL  | Issue No. 02               |
|  | Section: Work Sheets  | Revision No. 00            |
|  |   | Effective Date: 01-05-2022 |
|  |   | Review date: 01-05-2025    |

Worksheet for Diesel (AGO)

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

Physical Examination

| Parameter  | Observation |
|------------|-------------|
| Appearance |             |

Density at 20 °C: Correction: Test method: ASTM D1298

|                    |                     |                          |
|--------------------|---------------------|--------------------------|
| Hydrometer reading | Temperature reading | Corrected Density Value  |
| 0.833              | 17.4 °C             | 0.8335 g/cm <sup>3</sup> |

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 40°C Test Method: ASTM D445

|   |          |
|---|----------|
| Falling time t, (sec)                             | FCV - 32 |
| Viscosity Constant                                | 0.00351  |
| Kinematic viscosity (mm <sup>2</sup> /s) v = c. 1 | 2.823198 |

Copper Corrosion Test Method: ASTM D130

|                |  |
|----------------|--|
| Result 1b      |  |
| Slight tarnish |  |

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Figure 6: algal biodiesel test values.

#### 4.4 Objective 4: Process design for the production of algal biodiesel.

The following are the steps needed to carry out the process design:

##### 4.4.1 Species Selection and Cultivation.

Species selection occupies a key role in defining the cost-effectiveness and efficiency of the production of biodiesel. This design takes into account the following species, ranked according to extensive screening of their culturability to the Ugandan environment, lipid, and growth parameters:

*Chlorella vulgaris*: It has high-growth kinetics that is favourable to high biomass yield.

*Botryococcus braunii*: It is dominated by the abundance of hydrocarbons, and thus it is a simple conversion process to turn them into biodiesel.

*Nannochloropsis* sp.: The strain features that it will do well even under salt environments and hence ideal for being farmed in Uganda's salt lake environments.

Culture will be attained in open pond systems, utilizing agricultural runoff and/or wastewater as nutrient sources. Enhanced algal growth rates will be attained via carbon dioxide supplementation through the injection of biogas, which is a proximate agricultural by-product.

#### **4.4.2 Harvesting & Dewatering.**

Optimization of growing conditions should be ensured to yield maximum lipid production. The below will be done to enable convenient recovery of biomass and subsequent treatment:

Dewatering: For the purpose of reducing the amount of moisture present to less than 10% and thereby limit energy inputs in the subsequent extraction processes, dewatering will be achieved. Solar drying as well as or low-cost centrifugation technologies will be used.

#### **4.4.3 Lipid Extraction.**

Solvent Extraction Procedure.

Extraction of lipids from algal biomass is the first step in biodiesel production. Design is made for a comparison of different mixtures of solvents to determine the most convenient mixture of lipid yield, cost, and safety.

Pre-treatment of Biomass: Algal biomass is ground into powder using a ball mill before extraction to increase available surface area for interaction with the solvent.

Solvent Mixtures: The following solvent mixtures were used:

- Cyclohexane-isopropanol (2:1 v/v)
- Diethyl ether-isopropanol (2:1 v/v)
- Chloroform-isopropanol (1:2 v/v)
- Hexane-isopropanol (3:1 v/v)

Extraction Process: Extraction process will be carried out by the following procedures:

- Mixing: Solvent-to-biomass ratio of 3:1 and agitation for 2 hours at 50°C was applied.
- Separation: Separation of lipid-contained solvent phase from the rest of biomass using 4,000-rpm centrifugation for 15 minutes was utilized.

#### **4.4.4 Transesterification.**

Transesterification for the conversion of extracted lipids to biodiesel will have a two-stage catalytic system to process high free fatty acid (FFA) feedstock in traditional algal oils:

Catalysts: Acid ( $\text{H}_2\text{SO}_4$ ) pre-treatment of high FFA feedstock, and base (NaOH) catalyst transesterification.

Conditions:  $60^\circ\text{C}$  reaction temperature, 1:6 oil-to-methanol molar ratio, and 2 hours reaction time will be employed.

#### **4.4.5 Purification and Testing of Biodiesel.**

The biodiesel will then be purified to remove impurities that could still exist and subjected to a series of tests to ascertain its physicochemical properties and ensure that it is meeting international standards

Tests: Biodiesel will be analysed for viscosity (ASTM D445), flash point (ASTM D93), and energy content.

Compliance: Biodiesel will be manufactured as per the ASTM standard for blending (B20/B100).

#### **4.4.6 By-product Utilization.**

For increasing the process cost-effectiveness and sustainability, measures for utilization of the by-products shall be taken:

Biomass residue: The post-lipid extraction residue biomass will be utilized as animal feed and/or biogas substrate.

Glycerol: Glycerol being the by-product of the transesterification process will be sold to the local soap producers.

Wastewater: Wastewater generated during the process shall be recycled and reutilized back to cultivation ponds to reduce the freshwater intake.



## CHAPTER FIVE: CONCLUSION AND RECOMMENDATION.

### 5 Conclusions and recommendations.

#### 5.1 Conclusions.

This study demonstrates the feasibility of the technical production of biodiesel from algae in Uganda, taking advantage of the tropical climate of the country, intense sunlight exposure, and untapped waste streams.

##### 5.1.1 Species Suitability:

*Chlorella vulgaris* was the most suitable species for decentralised systems with maximum biomass growth in optimal conditions (13.4 cm Day 12) and moderate lipid yield (20-30%).

*Nannochloropsis* sp. was very stress-tolerant due to nutrient stress and therefore ideal for medium-scale operation.

*Botryococcus braunii*, though lipid-rich (40-50%), is hampered by low growth rate and vulnerability to contamination in open ponds.

##### 5.1.2 Solvent Efficiency.

Diethyl ether-isopropanol gives the highest oil recovery (83.10% for Strain C) but is unsafe.

##### 5.1.3 Quality of Biodiesel.

The resulting biodiesel was in compliance with ASTM viscosity (2.82 mm<sup>2</sup>/s) and density (0.8335 g/cm<sup>3</sup>), but with insufficient cetane numbers (5.2) and flash points

(1.23) and required blending with petro diesel (B20) to be engine compatible.

## **5.2 Recommendations.**

Implement decentralized open ponds with cover resistant to UV to reduce contamination (e.g., model employed by Mbarara University). Train Local Cooperatives: Use NGOs to organize communities into trained cooperatives that learn algae aquaculture, solvent management, and biodiesel manufacturing, scale Jinja pilot to automated collection.

Hexane-isopropanol is recommended since it offers a compromise of safety (moderate toxicity), cost (UGX 12,000/L), and recyclability by solar distillation.

## REFERENCES AND APPENDICES

### 6

#### 6.1 REFERENCES

1. Baggesen, C., 2014. *A study of microalgal symbiotic communities with the aim to increase biomass and biodiesel production.*, Denmark: Technical University of Denmark, Department of Chemical and Biochemical Engineering..
2. Branco-Vieira, M. M. T. M. A. F. M. a. C. N., 2020. Economic analysis of microalgae biodiesel production in a small-scale facility.. *Energy Reports*, Volume 6, pp. 325-332.
3. Brenna, L. a. O. P., 2010. Biofuels from microalgae: A review of technologies for production, processing, and extractions.. *Renewable and Sustainable Energy Reviews*, Volume 14(2), pp. 557-577.
4. C. Sanjurjo, P. O. M. B. E. R. R. G. A. H. B., 2024. Biodiesel production from the microalgae *Nannochloropsis gaditana*: Optimization of the transesterification reaction and physicochemical characterization. *Biomass and Bioenergy*, volume 185, pp. 2,3.
5. Demura, M. I. M. K. M. N. N. W. M., 2014. Desiccation tolerance of *Botryococcus braunii* (Trebouxiophyceae, Chlorophyta) and extreme temperature tolerance of dehydrated cells.. *Journal of Applied Phycology*, pp. 49-53.
6. Development, M. o. E. a. M., 2023. *Uganda Energy Balance Report.*, s.l.: s.n.
7. Karim, S. a. F. A., 2022. National renewable energy laboratory.. *Handbook of*

- Algal Biofuels*, pp. 599-613.
8. Milledge, J. a. H. S., 2014. Methods of energy extraction from microalgal biomass: a review.. *Reviews in Environmental Science and Bio/Technology*, Volume 13, pp. 301-320.
  9. Nwokoagbara, E. O. A. a. W. M., 2015. Biodiesel from microalgae: The use of multi-criteria decision analysis for strain selection.. *Fuel*, Volume 159, pp. 241-249.
  10. Pantea Moradi, M. S., 2022. Biodiesel production from *Chlorella Vulgaris* microalgal-derived oil via electrochemical and thermal processes. *Fuel Processing Technology*, volume 228, pp. 19-21.
  11. Perona, J., 2017. Biodiesel for the 21st century renewable energy economy. *Energy LJ*, 38(2017), p. 165.
  12. Rao, N. T. B. D. Y. N. B. a. H. R., 2021. Algal biotechnology in Australia and Vietnam: Opportunities and challenges.. *Algal research*, Volume 56, p. 102335.
  13. Rock, A. N. L. a. G. D., 2021. Synthetic biology is essential to unlock commercial biofuel production through hyper lipid-producing microalgae: a review.. *Applied Phycology*, Volume 2(1), pp. 41-59.
  14. Slade, R. a. B. A., 2013. Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects.. *Biomass and bioenergy*, Volume 53, pp. 29-38.
  15. Sun, J. X. X. W. M. D. H. L. J. Z. D. a. Z. J., 2019. Microalgae biodiesel production in China: a preliminary economic analysis.. *Renewable and Sustainable Energy Reviews*, Volume 104, pp. 296-306.

16. Ziolkowska, J. R. & S. L., 2014. Recent developments and prospects for algae-based fuels in the US.. *US. Renewable and Sustainable Energy Reviews*, pp. 847-853.

## 6.2 APPENDICES

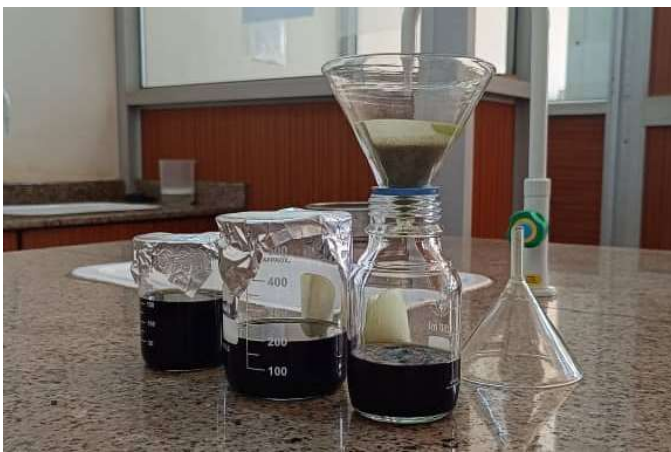
### APPENDIX A.



*Figure 7: Oven dried algae*



*Figure 8: Crushed algae*



*Figure 9: Algae extract.*



*Figure 9: Algal biodiesel.*

APPENDIX B:

| Cultivation time (days) | Strain 1 (cm) | Strain 2 (cm) | Strain 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            | 11.5          | 2.8           |
| 8                       | 10            | 12.2          | 3.2           |
| 9                       | 10.2          | 12.7          | 3.2           |
| 10                      | 10.5          | 13            | 3.2           |
| 11                      | 11            | 13.2          | 2.9           |
| 12                      | 11.9          | 13.4          | 2.6           |
| 13                      | 12            | 13.4          | 2.6           |
| 14                      | 12            | 13.4          | 2.6           |

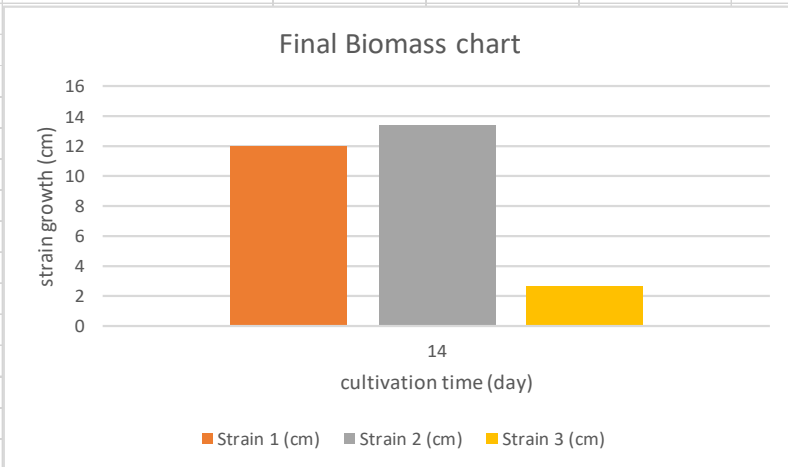
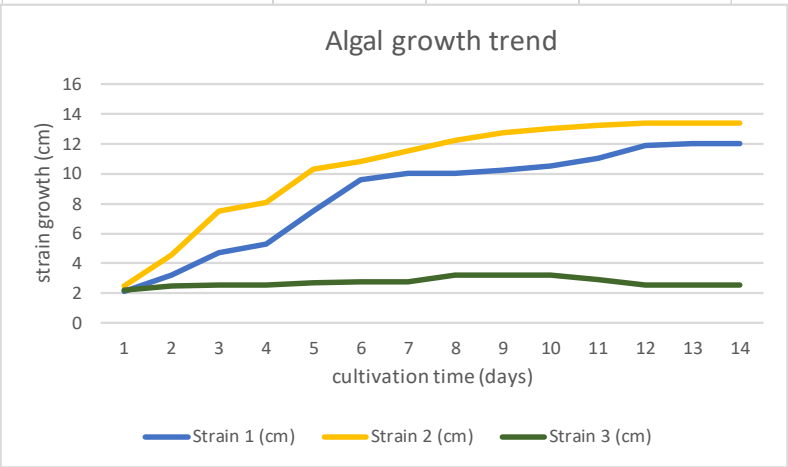


Figure 10: Growth trend.



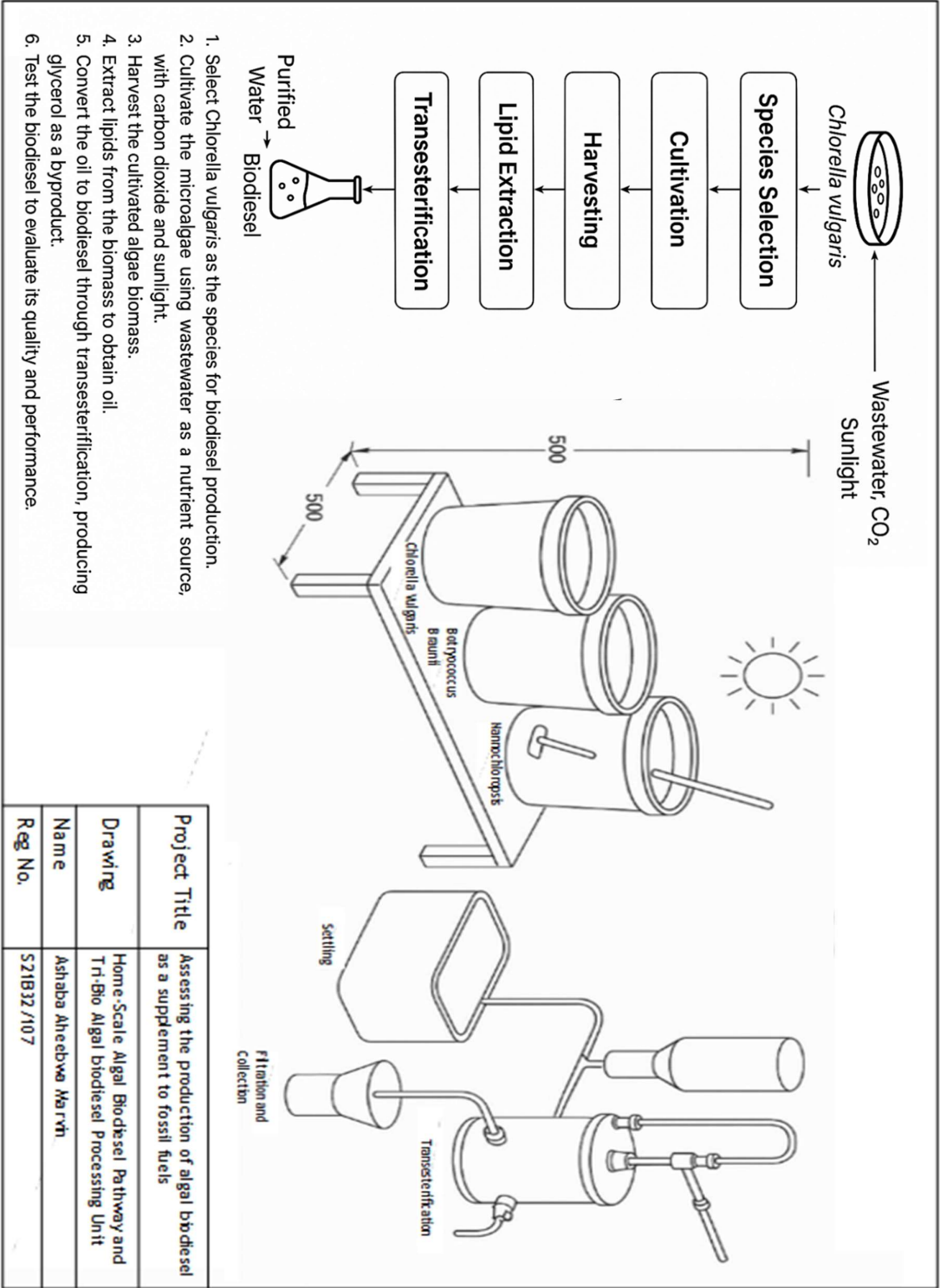


Figure 11: Project design.