



Evaluating the Energy Production Potential of Local Algae Genera: A Case Study of Uasin Gishu County, Kenya

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Abstract

The reliance on fossil fuels as an energy source has led to environmental degradation and a myriad of health problems. This has prompted the need for a renewable source of energy that is both economical and sustainable. The objective of this study was to determine the renewable energy potentials of the alga grown in sewage. In this study local genera of filamentous algae were grown in sewage and starch and lipids accumulated was quantified. *Spirogyra*, *Zygnema* and *Oedogonium* were collected from Kesses area of Uasin Gishu County, Kenya and cultured under laboratory conditions at Moi University. After seven days, the amount of starch was estimated and lipid content was extracted and quantified. Wilcoxon rank sum test was utilized to decide whether there was a statistical difference in starch and lipids produced by algae that grew in sewage and those that grew in growth medium. Results showed that the algae accumulated starch and lipids in the following order; *Spirogyra*, *Oedogonium* and *Zygnema*. It was also observed that growth in sewage favored lipid rather than starch accumulation ($r = -0.75$, $n = 18$, $p < 0.05$). The study recommends that fatty acid composition of the algae should also be analyzed to find their suitability in biodiesel production.

Keywords: Algae, Sewage, Biofuel, Renewable Energy, *Spirogyra*, *Zygnema*, *Oedogonium*



Introduction

Algae can be used to produce a wide range of biofuels including hydrogen, methane, bioethanol, biodiesel, and simple dried biomass (Chandrasekhar et al., 2023; Narayanan, 2024; Pittman et al., 2011). Algal biofuels offer many benefits compared to more traditional biofuel crops since algae grow exponentially, have higher biomass productivity per unit area, and do not directly compete with production of food crops (Jabłońska-Trypuć et al., 2023; Pittman et al. 2011). Microalgae are capable of producing many times more oil per acre than traditional oil-producing terrestrial crops, and can be grown in locations where they do not compete for land with food crops (Singh et al. 2011, Singh & Olsen, 2011). In fact, algae can be grown in sewage and seawater, meaning algal biofuels need not compete with food production for land or water (Sarwer et al., 2022; Singh & Olsen 2011). Current biofuel crops perform poorly in terms of CO₂ emissions and climate change, and when they replace natural ecosystems or food crops, they can release more CO₂ than they prevent (Rather et al., 2022). In contrast, algae's higher land use efficiency and faster growth make algal biofuels closer to carbon-neutral, with their carbon intensity expected to improve as more sustainable energy is used in cultivation, harvesting, and processing (Chew et al., 2021).

The processes for generating the various types of algal biofuels range from quite simple to very complex and energy intensive. Combustion of dried biomass simply requires harvesting and drying of algae using heat or the sun, but this form of energy is limited in terms of usefulness (Show et al., 2015). Though optimization of inputs can add complexity, anaerobic digestion is also a fairly simple technology and, in addition to methane, can produce useful co-products that can be utilized for fertilizer or compost, thus recycling key nutrients and offering greater benefits in terms of sustainability (Singh & Olsen 2011). Methane output, both total and relative to CO₂ production, can be maximized by careful balancing of lipid, carbohydrate, and protein composition of the material being digested, either by supplementing algal biomass with other feedstocks and lipid sources or by using algae with high lipid content (Sarwer et al., 2022). Biogas production can also be improved using a range of chemical and physical treatment techniques (Singh & Olsen 2011).

Algae offer many benefits over more traditional feedstocks for bioethanol production. They can require less pretreatment than popular plant inputs and some species directly produce ethanol (Veza et al., 2022; Jabłońska-Trypuć et al., 2023). Algal species with high starch and cellulose contents are particularly ideal for ethanol production (Varaprasad et al., 2021) Algae can also be used to generate hydrogen gas which can be used in fuel cells or combustion engines whose only emission product is water. Growing



certain species, like *Chlamydomonas reinhardtii*, and then depriving them of sulfur and O₂ can lead to the production of harvestable hydrogen (Yarkent et al., 2024; Singh & Olsen 2011; Sallam et al., 2022). Gasification of algae involves heating it to high temperature (800-1000 °C) to produce a burnable gas (Luo & Zhou, 2022). Due to the high heat requirements, the process typically has an energy balance near 1, but as with anaerobic digestion, the process generates products that can be reused to grow more algae (Singh & Olsen 2011).

In Egypt, a study done by Khan et al (2018) noted that microalgae are renewable, sustainable, and economical sources of biofuels, bioactive medicinal products, and food ingredients. Several microalgae species have been investigated for their potential as value-added products with remarkable pharmacological and biological qualities. As biofuels, they are a perfect substitute to liquid fossil fuels with respect to cost, renewability, and environmental concerns. Microalgae have a significant ability to convert atmospheric CO₂ to useful products such as carbohydrates, lipids, and other bioactive metabolites. Although microalgae are feasible sources for bioenergy and biopharmaceuticals in general, some limitations and challenges remain, which must be overcome to upgrade the technology from pilot-phase to industrial level.

In Kenya little research has been done on algae. Abubakar et al (2012) grew wild species of algae from Rift valley aquatic environments in the laboratory for biofuel. They obtained high lipid content of 1.5 – 10.5 % from five species, showing that algae from Kenya can be used for biofuel production.

Statement of the Problem

Fossil fuels are the primary source of energy used in several sectors of the economy such as transport, industry and electricity generation. However, burning of these fuels for energy releases a number of chemicals into the atmosphere including carbon dioxide and nitrous oxide, which are major contributors of global warming and ensuing climate change. Other by products includes sulfur oxides and nitrogen oxides, both of which contribute to acid rain formation that causes damage to plants and buildings. In addition to environmental harm, some by products can cause health problems to humans. Nitrogen oxides irritate the lungs while particulate matter contributes to respiratory illnesses and cardiac problem. This study contributes to the development of alternative sources of energy using local species of sewage-grown algae to produce lipids and starch that are feedstock for biodiesel and bioethanol respectively. Therefore, the study hypothesis is that;



H₀₁: There is no significant difference in the energy production potential of local algae genera found in Uasin Gishu County, Kenya.

Literature Review

Renewable Energy Potentials of the Alga Grown in Sewage

Microalgae have been proposed for a long time as a potential renewable fuel source as cited by Pittman (2011). Algae constitute an efficient system that utilizes solar energy to synthesize products of high value such as lipids and starch (Sun et al., 2018). Biofuel products from microalgae include biodiesel, bio-methane and bioethanol which can be obtained by Trans-esterification, anaerobic digestion and fermentation respectively (Kumar et al., 2023). Microalgae have the potential to generate greater quantities of oil suitable for biofuel production than most known plant-based feedstock; they require less cultivated land area and do not have direct impact on food supplies (Ogbonna & Nwoba, 2021). Over the last 50 years, there have been continuous efforts to examine the possibility of extracting important products of high economic value from microalgae. The use of microalgae for generating renewable energy provoked heightened interest during the energy crisis in the 1970s (Hallenbeck, 2011).

Algae as Feedstock for Biofuel

Algae fuel is a biofuel made from algae. "Algaculture" (farming of algae) can be a route to making biodiesel, bioethanol, biogas, and other biofuels (Devi et al., 2024). Algae are a diverse group of eukaryotic organisms that belong to the Phylum Protista. These organisms use light energy to convert carbon dioxide and water into carbohydrates and other cellular products. During this process oxygen is released. Algae contain chlorophyll a, which is required for photosynthesis. All algae are primarily made up of proteins, carbohydrates, fats, and nucleic acids in varying proportions. While the percentages can vary with the type of algae, some types of algae are made up of up to 40% fatty acids based on their overall mass. It is this fatty acid that can be extracted and converted into biofuel (Demirbas & Demirbas, 2011).

Unlike terrestrial crops such as corn and soybean, which require a full growing, season to yield crops, algae can be harvested day after day. Because algae consumes carbon dioxide and produces oxygen through photosynthesis, it is particularly attractive as a means to curtail carbon emissions along with producing fuel. Furthermore, algae can be used to clean up waste by processing nitrogen from wastewater and carbon dioxide from power plants. Unlike other biomass sources, algae do not compromise a food stock. It can be grown on marginal lands that are useless for ordinary crops.



There are also three energy production options from algae (i) production of methane gas from the whole biomass (ii) production of ethanol via fermentation from the carbohydrates and (iii) production of biodiesel from algal oil (Boechat and Giani, 2000). The economics of fuel production from algae demands that we utilize all the biomass as efficiently as possible (Pate et al., 2011).

Algae as Feedstock for Biodiesel

Oil rich algae can be found among diverse taxonomic groups, and the total lipid content may vary noticeably among individual species or strains within and between taxonomic groups. Oil rich green algae show an average total lipid content of 25.5% dry cell weight. The lipid content increases considerably (doubles or triples) when the cells are subjected to unfavorable culture conditions, such as photo-oxidative stress or nutrient starvation. The intrinsic ability to produce large quantities of lipid and oil is species/strain-specific, rather than genus-specific. According to Breuer et al. (2012), the increase in total lipids in aging algal cells or cells maintained under various stress conditions consisted primarily of neutral lipids, mainly triacylglycerols.

Triacylglycerol biosynthesis in algae has been proposed to occur via the direct glycerol pathway. Fatty acids produced in the chloroplast are sequentially transferred from CoA to positions 1 and 2 of glycerol-3-phosphate, resulting in formation of the central metabolite phosphatidic acid (PA). Dephosphorylation of PA catalyzed by a specific phosphatase releases diacylglycerol (DAG). In the final step of triacylglycerol synthesis, a third fatty acid is transferred to the vacant position 3 of DAG, and this reaction is catalyzed by diacylglycerol acyltransferase, an enzymatic reaction that is unique to triacylglycerol biosynthesis. PA and DAG can also be used directly as a substrate for synthesis of polar lipids, such as phosphatidylcholine (PC) and galactolipids. The acyltransferases involved in triacylglycerol synthesis may exhibit preferences for specific acyl CoA molecules, and thus may play an important role in determining the final acyl composition of triacylglycerol (Li *et al.*, 2013). Figure 1 show graph of triacylglycerol formation.

In most of the algal species/strains examined, triacylglycerols are composed primarily of C14–C18 fatty acids that are saturated or mono-unsaturated. As exceptions, very-long-chain (>C20) PUFA synthesis and partitioning of such fatty acids into triacylglycerols have been observed in the green alga *Parietochloris incise* (Trebouxiophyceae) and the freshwater red microalga *Porphyridium cruentum* among others. Lipid content and fatty acid composition are also subject to variability during the growth cycle. In many algal species in the literature, an increase in triacylglycerols is often observed during stationary phase (Breuer et al., 2012).



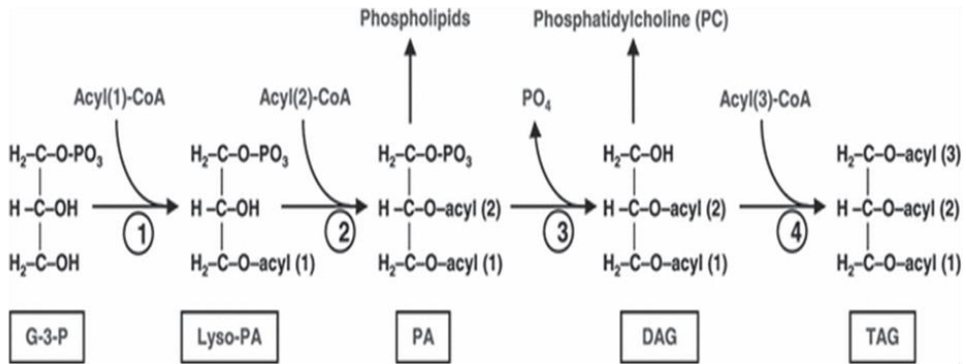


Figure 1: Triacylglycerol Formation

Algae as a Feedstock for Bioethanol

The absence of lignin production in most algae is a benefit because processing lignin is currently a major impediment for bioethanol production.

Starch production in green algae

Starch is the most widespread and abundant storage carbohydrate in plants. Humans depend upon starch for nutrition, exploit its unique properties in industry, and use it as a feedstock for bioethanol production (Keeling & Myers, 2010). Starch is only found in eukaryotes and serves as an important store of energy that is captured by plants using sunlight, water, carbon dioxide, and soil nutrients (Keeling & Myers, 2010).

Materials and Methods

Algae samples were collected from the Kesses area, near Sambul River and local ponds, chosen for its proximity to Moi University. Located at 00°16'N, 35°20'E, and 2180m above sea level in Uasin Gishu County, the region experiences shallow, poorly drained soils, with a mean annual rainfall of 1124mm and temperatures ranging from 26.1°C in February to 8.4°C in July. Sewage samples were obtained from Moi University's Waste Stabilization Ponds (WSP), which include anaerobic, facultative, and maturation ponds that use natural processes involving algae and bacteria for wastewater treatment.

The university's wastewater, sourced from hostels, lecture halls, kitchens, offices, and the clinic, is treated through a multi-stage system. After passing through coarse and fine screens, the water is measured for flow rate and flows into anaerobic ponds where high organic BOD loads are removed anaerobically. The treated water then moves to facultative ponds, where algae such as *Pediastrum*, *Eudorina*, *Anacystis*, and *Gomphosphaeria* help remove



BOD and nutrients through both aerobic and anaerobic processes. In the subsequent maturation ponds, additional algae like *Sceneddus*, *Oocystis*, *Closterium*, and *Urococcus* further treat the water, removing pathogens and remaining nutrients. Finally, the effluent is polished in a wetland made of *Typha latifolia* plants before being released into the Sambul River.

Composite samples were collected from various points according to the EPA sampling protocol (2007), with three replicates taken at each site. Prior to sampling, containers were thoroughly cleaned with phosphate-free detergent, rinsed with tap water, followed by 10% hydrochloric acid and de-ionized water. At the sampling site, bottles were rinsed three times with the sample before being filled. Samples were taken directly into 250 ml plastic containers using a stainless steel rod and clamp to collect subsurface samples at a depth of about 30 cm, one meter from the pond edge to avoid atypical edge water. The wastewater characteristics at Moi University's oxidation ponds varied, with raw sewage having a BOD of 121.5 mg/l and a pH of 7.11, while the final maturation pond showed a BOD of 14.2 mg/l and a pH of 9.74. Other parameters like chloride, nitrate, temperature, and color also varied across the ponds.

Green filamentous algae were sampled. Three genera of alga were chosen due to abundance: *Spirogyra* sp., *Oedogonium* sp. and *Zygnema* sp. The sewage samples were obtained from maturation pond of Moi University stabilization ponds. The algae were grown at 26⁰C for seven days in 100 ml sewage with the following characteristics; chloride 35.5 mg/l, BOD 14.2 mg/l, Nitrate 2.8 mg/l, pH 9.74 and temperature of 24.3⁰C. 0.05 mg of carbon dioxide was supplied once. After seven days, the algae were harvested and estimation of starch and lipid extraction was done.

Starch was extracted using the enzymatic-colorimetric method, a modified Bach-Knudsen assay. Lipids were extracted with a hexane-ethanol mixture in a 2:1 ratio using a Soxhlet apparatus and then weighed. Wilcoxon rank sum test was utilized to decide whether there was a statistical difference in starch and lipids produced by algae that grew in sewage and those that grew in growth medium.

Results and Discussion

Renewable Energy Potential of the Algae

Lipids in Algae

Harvesting was done after seven days. The algae had turned color from dark green to light yellow, probably as a result of lipid accumulation. Algae that had been grown on sewage produced more lipids than those grown on growth medium, as shown on Figure 1 below.



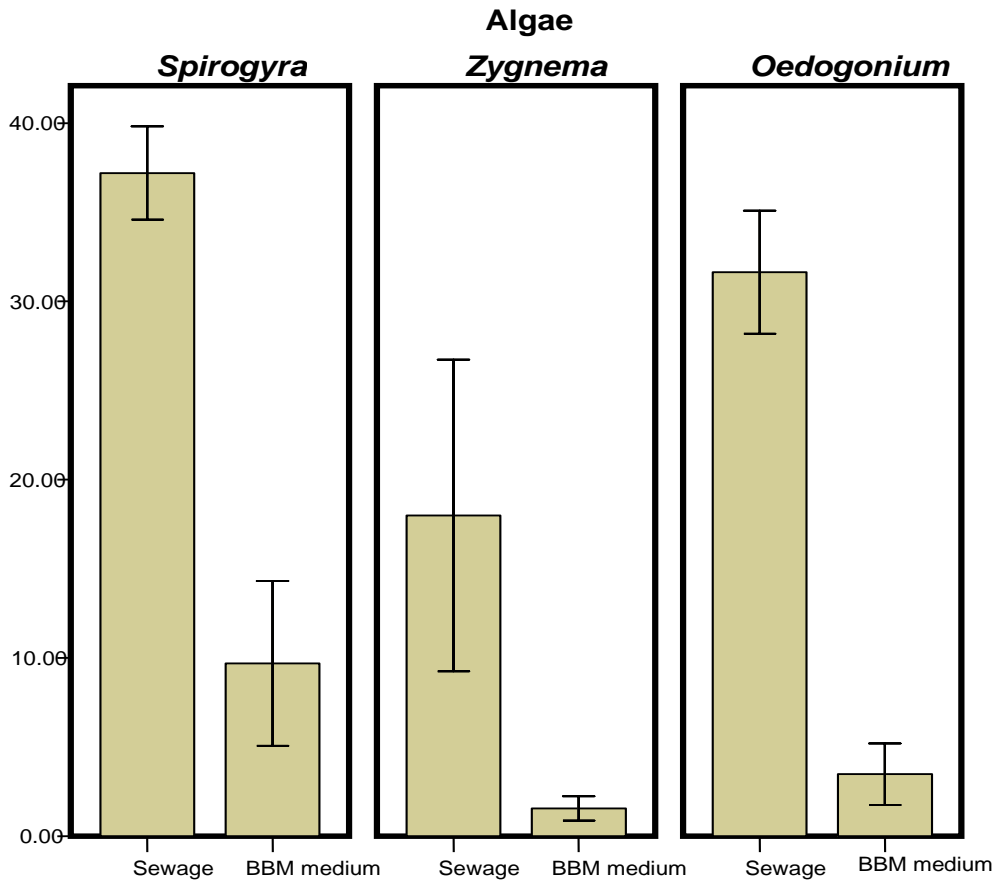


Figure 2: Lipid productions by algae

Wilcoxon (Mann-Whitney) rank sum test was used to determine whether there was difference in lipid accumulation between the algae that grew in sewage and those that grew in growth medium.

The study's findings indicate that lipid content varies among different algal species, with *Spirogyra* exhibiting the highest median lipid content at 23.86%, and *Zygnema* the lowest at 7.47%. When cultivated in a standard growth medium, all algae showed similar lipid levels, with a median of 1.5%. However, growth in sewage significantly enhanced lipid productivity across all genera. Notably, 20 grams of *Oedogonium* grown in 100 milliliters of sewage achieved a 91.38% increase in lipid accumulation, *Zygnema* exhibited an 89.04% increase, and *Spirogyra* showed a 73.99% increase. These results align with existing literature on algal lipid production. El-Sinawi and Shathele, (2014) study assessing the biomass and lipid content of *Oedogonium* and *Spirogyra* found that lipid levels varied with the cultivation medium, indicating that environmental conditions significantly influence lipid accumulation. Furthermore, research has demonstrated that microalgae grown in



wastewater can accumulate lipids ranging from low (10% dry weight) to moderate (25–30% dry weight), with some species achieving up to 80% dry weight. This suggests that nutrient-rich environments, such as sewage, can enhance lipid production in algae (El-Sheekh et al., 2023).

Starch in Algae

Starch was estimated in algae that had been grown on sewage for seven days. The control had grown on BBM medium. Alpha-amylase and amyloglucosidase enzymes were used to hydrolyze and quantify the percentage of starch.

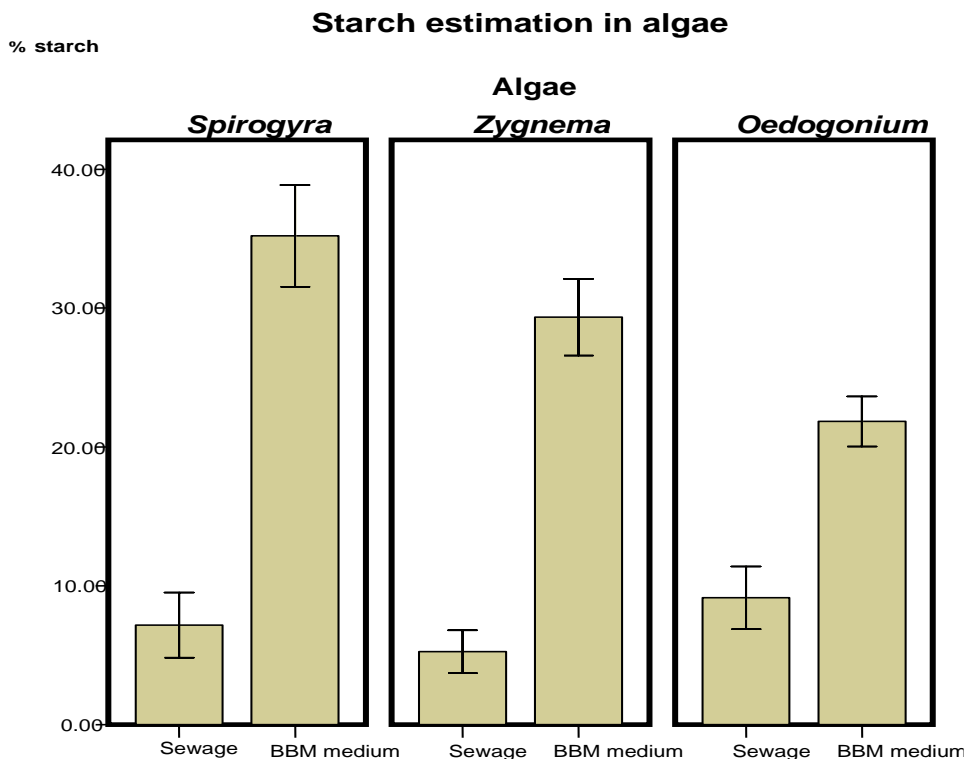


Figure 3: Starch Accumulation by algae

The study results showed that the growth of the algae on sewage decreased starch accumulation, probably due to nutrient unavailability. The highest decrease was observed in *Zygnema* where growth in sewage led to a decrease in starch by 82.11%. In *Spirogyra*, the decrease was at 79.63% and *Oedogonium* at 58.13%. Thus, *Oedogonium* had higher percentages of starch than both *Spirogyra* and *Zygnema*. This is probably because this species could grow well in sewage than the other two species. *Spirogyra* grown on the growth medium had the highest percentage of starch. *Spirogyra* grew better



in BBM than in sewage therefore was able to store up more starch while growing in this medium.

Hypothesis Testing

Pearson correlation showed that the accumulation of starch in the algae had a negative but significant correlation with lipid production ($r = .75$, $n = 18$, $p < .05$ two tails). The study thus rejected the null hypothesis that: H_{01} : The algae genera do not have potential for renewable energy. Thus, there was a statistically significant difference between the amounts of lipids and starch that were accumulated by algae that grew in sewage and those that grew in bold basal medium at the end of both treatments.

Conclusion and Recommendations

In conclusion *Spirogyra*, *Zygnema* and *Oedogonium* grown in sewage can accumulate oil and starch that can be converted into sources of clean energy.

The study recommends that fatty acid composition of the algae should also be analyzed to find their suitability in biodiesel production. Also, the starch should be fermented into fuel alcohol and the suitability of the fuel ascertained.

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