

Potential of Local Algae Genera in Removing Nitrates from Wastewater: A Case Study of Uasin Gishu County, Kenya

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Abstract

Increased population growth in urban areas has resulted in production of large volumes of wastewater. This has had an effect on the efficiency of treatment of the wastewater, leading to discharge of poor-quality effluent into the environment. The main objective of the study was to determine the use of selected algae to remove nitrates from secondary-treated wastewater. In this study, local filamentous algae genera (*Spirogyra*, *Zygnema* and *Oedogonium*) were collected from Kesses, Uasin Gishu County, Kenya and cultured in sewage under laboratory conditions at Moi University. Wastewater samples were obtained from Moi University's stabilization ponds, including anaerobic, facultative, and maturation ponds, which treat wastewater from university facilities. Systematic sampling was conducted at multiple points in the ponds following EPA protocols, using composite samples made up of grab samples collected at 30 cm subsurface depth. After initial analysis, the first maturation pond, with adequate nutrient levels, was selected for culturing algae. The algae were grown under laboratory conditions, and nitrate removal was monitored daily for seven days. The concentration of nitrates removed by the algae was quantified daily for seven days. The collected data was coded into SPSS version 26 and analyzed using descriptive and inferential statistics. The study found that wastewater characteristics varied across the ponds, with nitrate concentrations decreasing significantly in the maturation ponds. *Spirogyra*, *Zygnema*, and *Oedogonium* algae effectively removed nitrates from wastewater, with *Spirogyra* achieving the highest reduction (6.56 mg/L to 0.83 mg/L by day four). Friedman and Mann-Whitney tests confirmed significant differences in nitrate removal across the days and between algae genera, with *Spirogyra* showing the highest nitrate removal efficiency, particularly on day two (65.39%). The study recommends that local authorities and wastewater treatment facilities should consider integrating algae-based bioremediation techniques into existing treatment systems to improve effluent quality while reducing reliance on chemical treatments.

Keywords: Nitrates, waste water, *Spirogyra*, *Zygnema*, *Oedogonium*, efficiency, treatment

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Introduction

Background information

Across the world, nitrogen removal in suspended growth processes is attained through several mechanisms: nitrification, denitrification, assimilation, volatilization and sedimentation (Rahimi et al., 2020; Grady et al., 2011; Mancuso et al., 2021). Typical nitrogen composition of an algal cell is 8% N, consequently, indirect nitrogen removal is more prevalent than direct nitrogen removal in wastewater-fed High Rate Algal Ponds (HRAPs) (Rani et al., 2021). Indirect nitrogen removal occurs through stripping of $\text{NH}_4^+ - \text{N}$ when pH levels are raised. Direct nitrogen removal indicates assimilation of nitrogen constituents into algal biomass which would be further removed in separation processes (Kube et al., 2018). In aquatic environments, higher nitrogen removal results from increased retention times in two ways: by allowing sufficient time for microbial processes such as nitrification and denitrification to occur and by providing algae and aquatic plants with extended periods to assimilate nitrogen for their growth (Saeed & Sun, 2012).

A study in the United Kingdom investigated the potential of growing algae in sewage effluent to concentrate nutrients like nitrogen, phosphorus and trace elements while sequestering carbon. The research reported algae's ability to thrive in various stages of wastewater treatment, offering economic and sustainable benefits. Motivated by environmental management and biological processes, the study aimed to replicate natural systems for enhanced nutrient removal (Mikkola & Randall, 2016).

Another study conducted in South Africa by Abdel-Raouf et al. (2012) noted that microalgae culture offers an interesting step for wastewater treatments, because they provide a tertiary bio treatment coupled with the production of potentially valuable biomass, which can be used for several

purposes. Microalgae cultures offer an elegant solution to tertiary and quaternary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth. And also, for their capacity to remove heavy metals, as well as some toxic organic compounds, therefore, it does not lead to secondary pollution.

The selection of algae strain to be used in wastewater treatment is determined by their robustness against wastewater and by their ability to grow in and to assimilate nutrients from wastewater. *Chlorella*, *Scenedesmus*, *Neochloris* and *Spirulina* are the widely used algae species in experimental studies of wastewater treatment. The major advantages of using microalgae over conventional methods are: (a) nutrients can be removed more efficiently; (b) no generation of toxic by-product (sludge) (c) biofuels can be produced from biomass harvested (energy efficient); (d) cost-effective (Wang et al. 2016; Taziki et al., 2015).

Microalgae have a great potential to solve energy and environmental challenges around the world. Wastewater treatment with microalgae is a more environmental sound approach to reduce nitrogen and phosphorus and to remove heavy metals from wastewater. Microalgae can absorb significant amount of nutrients because they need large amounts of nitrogen and phosphorus for proteins (45-60% microalgae dry weight) and metals as micronutrients for their growth. William Oswald (1957) first developed the idea of treating wastewater in using microalgae and performed photosynthesis in sewage treatment. The bacteria take up the oxygen and degrade organic material in the wastewater, the same process which is used in activated sludge treatment (Wang et al., 2016).

Nutrient removal by algae involves various metabolic pathways of the algal cell. Carbon,

nitrogen, phosphorous and sulfur are the main components responsible for algal growth. Metals such as potassium, sodium, calcium, iron and magnesium serve as micronutrients for the growth of algae. Emphasis is usually put on nitrogen and phosphorus uptake but importance is also given to the technological aspects of heavy metal removal by algal biomass (Rawat et al., 2011).

Microalgae have an ability to convert inorganic nitrogen only in the forms of nitrite, nitrate and ammonium to organic nitrogen through a process called assimilation. Only eukaryotic algae can perform assimilation of inorganic nitrogen. Translocation of inorganic nitrogen takes place across the plasma membrane where reduction of nitrate takes place followed by the incorporation of ammonium into amino acids and glutamine. Initially nitrate is reduced to nitrite by a "NADH-dependent" nitrate reductase and the nitrite reduced to ammonium by "NADPH-linked" nitrite reductase present within the algae. The resulting ammonium is assimilated to form amino acids by glutamine and glutamate synthase within the intracellular fluid using adenosine triphosphate (ATP), glutamate (Glu) and glutamine synthase. Thus, all inorganic forms of nitrogen are finally reduced to organic form (amino acids) (Mook et al., 2012).

Nitrate pollution in Kenya, primarily from agricultural runoff and untreated wastewater, poses significant environmental challenges (Rono, 2021; Njue et al., 2022). Increasing population and urbanization have intensified the discharge of nitrogenous waste into water bodies, leading to eutrophication and the degradation of aquatic ecosystems (Masime, 2022). Urban expansion has made water and sewage system planning and development costly and difficult. Effluents from domestic and industrial activities contribute heavily to water pollution, creating a significant burden for wastewater management and leading to point-source pollution. This results in higher treatment costs and the introduction of various pollutants into water sources. Waste stabilization ponds (WSP) are widely used in Kenya for wastewater treatment due to their cost-effectiveness and efficiency. A study conducted on the Kibendera WSP in Ruiru from January 2021 to December 2022 highlighted their effectiveness in removing nitrogen compounds, including ammonia, nitrate, and organic nitrogen. The research employed mass balance models to

characterize nitrogen transformation, successfully calibrating and validating the models over two years (Kirumba et al., 2024).

Statement of the problem

The occurrence of nutrients in water bodies and consequent eutrophication is a widespread environmental problem. The nutrients cause increased growth of microscopic plants, algae and dense mats of floating plants such as water hyacinths. When algae plants die, the process of bacterial decomposition uses up oxygen in the water leading to anoxia. This causes death of fish and invertebrates. Moreover, ammonia and hydrogen sulfide originating from bacterial activities can be released from sediments under conditions of anoxia, affecting plants and animals. High nitrate levels in water that exceeds 10 mg/L $\text{NO}_3\text{-N}$ can cause methemoglobinemia or blue baby syndrome, a condition found especially in infants less than six months. The stomach acid of an infant is not as strong as in older children and adults. This causes an increase in bacteria that can readily convert nitrate to nitrite. Nitrite is absorbed in the blood, and hemoglobin (the oxygen-carrying component of blood) is converted to Methemoglobin. Methemoglobin does not carry oxygen efficiently. This results in a reduced oxygen supply to vital tissues such as the brain. Methemoglobin in infant blood cannot change back to hemoglobin, which normally occurs in adults. Severe methemoglobinemia can result in brain damage and death. Improper treated domestic wastewater is a source of nitrate. This study addresses this issue by using algae to remove nitrates from domestic wastewater, thereby averting the problem of eutrophication and methemoglobinemia. Hence, the objective of this study was to assess and compare the nitrate removal efficiency of *Spirogyra*, *Zygnema* and *Oedogonium* algae species in wastewater over a four-day period. The study was guided by the following null hypothesis;

H₀₁: There are no significant differences in nitrate removal among the different algae genera on each day of the study.

Materials and Methods

Algae sampling site

Algae samples were collected from the Kesses area, specifically along the edges of Sambul River and nearby ponds, chosen for their proximity to Moi University. This area was selected to study local species of algae, with the sampling location situated at coordinates 00°16'N, 35°20'E, and an altitude of 2180 meters above sea level, south of Eldoret in Kesses constituency, Uasin Gishu County, Kenya. The region is characterized by shallow soils with murram close to the surface and poor drainage. The county experiences cooler temperatures ranging between 18°C to 21°C and receives an annual average rainfall of 1500 mm (Uasin Gishu County Government, 2023).

Sewage sampling site

The sewage samples were obtained from Moi University stabilization ponds. Waste Stabilization Ponds (WSP), often referred to as oxidation ponds or lagoons. They are shallow man-made basins used for wastewater treatment by natural processes involving pond algae and bacteria. The Moi University WSP comprises a series of anaerobic, facultative and maturation ponds.

Characteristics of the wastewater used in the study

The wastewater mainly comes from the student hostels, lecture halls, kitchens, offices and university clinic. It consists of ablution water, food preparation wastes, laundry wastes, and other waste products of normal living. It is transported through a sewer reticulation that is approximately 2 km to the treatment plant, which is found at the southern end of the university plot. The primary treatment consisted of a coarse screen, with spacing of 50 mm apart where all the un-degradable solids are trapped for removal. A fine screen spacing of 25 mm apart is also fitted downstream of the coarse screen.

Below the screenings, one meter of the channel is constricted. This is where flow rate measurement is recorded using Venturi Flume Measurement method. The volume of flow fluctuates from daily flow of 56.0 m³/day when students are in session to 0.28 m³/day when they are on holiday and away from the campus.

The sewage then flows into the twin anaerobic ponds. The anaerobic ponds are the smallest of the series, measuring 51m by 51m. There

are two anaerobic ponds in parallel which are about 4m deep and receive a high organic Biochemical Oxygen Demand loading (>100g BOD/m³/day) through an inlet. The retention time is one day. These high organic loads produce strict anaerobic conditions throughout the pond. The primary function of anaerobic ponds is to remove BOD. The suspended solids settle by gravity to the bottom of pond where they are degraded anaerobically. Once every year the ponds are desludged using the manually operated valves installed in the sludge draw-off chambers. During the exercise, care is taken not to draw off layer above the sludge which contains facultative bacteria that is necessary for the oxidation of the incoming organic matter.

Facultative ponds follow anaerobic ponds in the WSP system. They are about 1 m deep and are geometrically designed to have a big length-to-width ratio. There are two types of facultative pond: primary facultative pond that receives wastewater from the anaerobic ponds and measures of 220 m by 80 m. The secondary facultative pond receives settled wastewater effluent from the primary facultative pond and measures 220 m by 70 m. Aerobic conditions are maintained in the upper layers while anaerobic conditions exist towards the bottom of the pond. The lower anaerobic layer of a facultative pond is dominated by the same anaerobic mechanisms that occur in anaerobic ponds. These conditions are very effective in BOD removal.

The facultative ponds are colored dark green due to algae. Pond algae that predominate in turbid waters of facultative ponds are *Pediastrum*, *Eudorina*, *Anacystis* and *Gomphosphaeria*. The wind mixing mechanisms enable them to access the incident light, allowing them to photosynthesize. Maturation ponds are in series with facultative ponds. There are two maturation ponds; first and last maturation ponds in series. The first maturity pond receives the effluent from the secondary facultative pond. They are 1 m deep and are geometrically designed to have an equal length-to-width of 70 m by 70 m. The primary function of maturation pond is to remove excreted pathogens. The removal of BOD and nutrients also take place in maturation ponds.

The algal populations in maturation ponds are *Pediastrum*, *Eudorina*, *Anacystis*, *Gomphosphaeria*, *Scenesdus*, *Oocystis*, *Closterium* and *Urococcus*. From this pond, the wastewater is released into the Sambul River through a natural

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wetland that is mainly made up of plants of *Typha latifolia* species, the purpose being to polish the pre-treated wastewater. 90 % of the wastewater flows through the wetland in narrow channels. The approximate distance from the last pond to the river is 80 metres, of which 20-30 m is made up of the wetland that occurs along most of the length of the river.

Sewage sampling method

Composite samples made up of several grab samples were obtained from the different sampling points, following sampling protocol by EPA (2007). Sampling was systematic and three replicates were made at each point. Prior to sewage sampling, the containers to be used to sample and transport the sample to the laboratory were washed with a brush and phosphate-free detergent. They were then rinsed three times with cold tap water. Rinsing with 10 % hydrochloric acid followed before rinsing three times with de-ionized water. At the sampling site, the bottles were rinsed three times with portions of the sample before being filled. Sampling was directly into 250 ml plastic containers. A sampling rod made of stainless steel pole with a clamp on one end on which the sampling bottle was securely attached was used to collect the samples. Sampling was done on the subsurface, about 30 cm depth because the ponds were shallow. Samples were collected about one meter from the edge, as edge sewage is not typical of the majority of the wastewater. For each pond, there was a sampling point at the inlet area (A), another at the outlet section (B) and two other points at the two other opposite sections of the pond (C, D). After sampling from these four points, the wastewater was mixed and one liter from this composite sample was taken to the laboratory. This sampling was done in triplicate. Preliminary sampling involved all the ponds, but after the initial laboratory analyses on the nutrient status of wastewater, subsequent sampling was done on the first maturity pond because the nutrient levels of the wastewater from the pond was sufficient to culture algae.

Identification of algae in Kesses

Photosynthetic algae represent a large and diverse group of organisms that have only a limited history of characterization and exploitation. The application of resource exploitation from algae is relatively untapped, with the potential to produce fuel, food, fibers and pharmaceuticals on a large

scale. It is important to identify or characterize local species of algae as they may often offer significant but currently unknown benefits for bioresource production. This is because indigenous algae are adapted to prevailing abiotic and biotic factors thus are evolutionarily primed for local bioresource production.

Determination of nitrate concentration

Method used for analyzing Nitrate-N was automated hydrazine reduction method (APHA 2005). The nitrate concentration was then obtained using Jenway (UK) spectrophotometer with absorbance at 220nm.

Data analysis

The physicochemical parameters collected from the various wastewater samples were coded into SPSS version 26 and analyzed using descriptive statistics. To evaluate the effect of algae on nitrate reduction, a Friedman test was performed to assess the significant changes in nitrate concentrations across four days of algae growth. Further, Mann-Whitney tests were used to compare the nitrate removal efficacy between different algae genera. Further, the nitrate removal percentage by each algae species was calculated for each interval (Day 1-2, Day 2-3, Day 3-4) to compare their effectiveness in reducing nitrate levels in wastewater.

Results

Physico-Chemical Characteristics of Wastewater in Moi University Oxidation Ponds

The physico-chemical characteristics of wastewater at Moi University stabilization ponds are summarized in Table 1. According to the findings, the physico-chemical characteristics of wastewater across different ponds at Moi University reveal significant variations in several parameters. Raw sewage had a high BOD of 121.5 mg/l, a moderate nitrate level of >0.1 mg/l, a pH of 7.11, and a temperature of 32.7°C, with a light brown color. In the anaerobic pond, BOD and nitrate levels decreased slightly to 119.3 mg/L and >0.3 mg/l, respectively, with a lower pH of 6.35 and a temperature of 25.8°C, presenting a grey color. The primary facultative pond showed a significant reduction in BOD to 53.6 mg/L and a slight increase in pH (8.03), with a green color. In the secondary facultative pond, BOD further

reduced to 30.8 mg/l, nitrate concentration reached 0.3 mg/l, and pH increased to 8.26. The first maturation pond saw a more pronounced reduction in BOD (17.4 mg/l) and a substantial increase in nitrate concentration to 6.7 mg/l, with pH rising to

8.83, while the last maturation pond had the lowest BOD (14.2 mg/l), a further decrease in nitrate concentration (2.8 mg/l), and the highest pH of 9.74, with green color maintained throughout.

Table 1: Physico-chemical characteristics of wastewater in the various ponds

Pond	Chloride	BOD	Nitrate	pH	Temp °C	Color
Raw sewage	35.5	121.5	>0.1	7.11	32.7	Light brown
Anaerobic	35.5	119.3	>0.3	6.35	25.8	Grey
Primary facultative	35.5	53.6	>0.3	8.03	24.2	Green
Secondary	35.5	30.8	0.3	8.26	24.4	Green
First maturity	35.5	17.4	6.7	8.83	24.5	Green
Last maturity	35.5	14.2	2.8	9.74	24.3	Green

(NB: Values are averages. All values are in mg l⁻¹ unless otherwise stated)

Potential of Algae to Remove Nitrates from Domestic Wastewater

The wastewater in which *Spirigyra* sp. was growing decreased in nitrate levels from 6.56 mg/L on the first day to 0.83 mg/L on day four. *Zygnema* sp. reduced the nitrate concentration in the

sewage from 6.56 mg/L on the first day to 1.07 mg/L on day four. Similarly, the nitrate content of the wastewater in which *Oedogonium* sp. was growing decreased from 6.56 mg/L on day one to 1.01 mg/L on day four. Figure 1 below shows a graph of nitrate removal from wastewater by different species of algae.

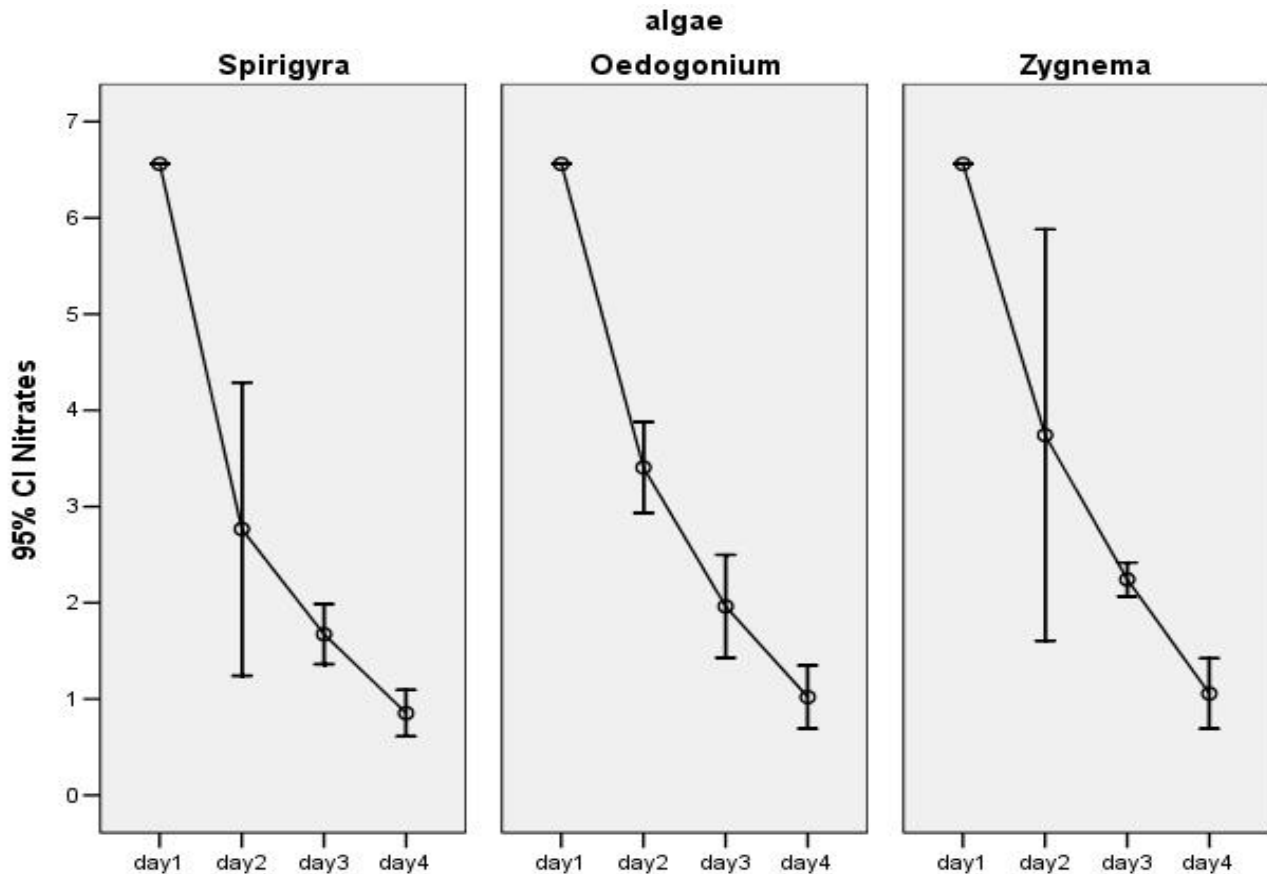


Figure 1: Nitrate Removal from Wastewater by Different Algae Species

Comparison of Nitrate Removal Efficiency Across Algae Genera

The data size in the study was small so the most appropriate statistical test was Friedman test. A Friedman test was conducted to determine if there was a significant change in levels of nitrate that was reduced by the algae between days 1, 2, 3 and 4. In *Spirogyra* the difference in nitrate removal across the days; day 1 (median = 6.56), day 2 (median = 2.96), day 3 (median = 1.69) and day 4 (median = 0.83) was statistically significant ($X^2 (N = 3) = 9, p = .02$). Kendall's coefficient of concordance of 1 indicated that there was a very strong difference in nitrate removal on each subsequent day.

In *Zygnema*, the difference in nitrate removal across the days was as follows; day 1 (median = 6.56), day 2 (median = 3.42), day 3 (median = 2.04) and day 4 (median = 1.07) was significant ($X^2 (N = 3) = 9, p = .02$). Kendall's coefficient of concordance of 1 indicated that there was a very strong difference in nitrate removal on each subsequent day.

In *Oedogonium*, the difference in nitrate removal across the days was as follows; day 1 (median = 6.56), day 2 (median = 3.41), day 3 (median = 2.23) and day 4 (median = 1.01) was significant ($X^2 (N = 3) = 9, p = .02$). Kendall's coefficient of concordance of 1 indicated that there was a very strong difference in nitrate removal on each subsequent day.

The percentage of nitrate reduced by the algae between the first and second day was as follows; *Spirogyra* at 65.39 %, *Oedogonium* at 48.02 % and *Zygnema* at 42.98 %. The percentage of nitrate reduced by the algae between the second and third days show that *Oedogonium* led at 42.51 %, *Spirogyra* was at 26.43 % while *Zygnema* decreased the nutrient by 40.11 %. Between the third and fourth day, *Zygnema* reduced nitrate by 52.67 %, *Oedogonium* by 47.95 % and *Spirogyra* by 49.1 %.

H_{01} stated that there are no significant differences in nitrate removal among the different algae genera on each day of the study. The Mann-Whitney test was conducted to determine if there were any differences in nitrate removal across the algae genera on each day. On day two, the results showed no statistically significant difference in nitrate removal between *Spirogyra* and *Zygnema* ($U=1, p=.127$). On day three, however, the test revealed significant differences in nitrate removal between *Zygnema* and *Oedogonium* ($U=.000, p=.05$), and between *Spirogyra* and *Oedogonium* ($U=.000,$

$p=.05$). No significant difference was found between *Spirogyra* and *Zygnema* ($U=1, p=.127$). Based on these results, the null hypothesis was rejected.

Discussion

The effectiveness of nutrient removal by the algae was accomplished through an experimental study whereby the algae were cultured in the laboratory using the sewage as a growth medium. Nitrate concentration of the sewage was recorded once daily for four days. The control had no algae. The results show that substantial reduction of nitrate in the growth media occurred as a result of algae. The length of contact time the algae grew on the wastewater was important as it affected the amount of nitrate removed by the algae genera. Whereas on the second day of study, *Spirogyra* decreased the highest amounts of nitrates, on the third day, *Oedogonium* decreased more nitrates than the other genera. On the fourth day, *Zygnema* reduced more nitrates than the other two genera. On further inspection of the results obtained in this study, it was noted that the percentage levels of nitrate reduction matched the percentage of chlorophyll *a* that was contained within that genus, but only after the third day of study. Between day 2 and 3 of study, the genus *Oedogonium* had the highest percentage increase in concentration of chlorophyll *a*. This genus also decreased the highest percentage of nitrates between these days. Likewise, on day four of study, *Zygnema* had the highest percentage increase of chlorophyll *a* and had also decreased the highest percentage of nitrates from wastewater.

The specific use of algae in the efficient removal of different forms of nitrogen as well as phosphorus from waste stream has been reported. Of the three genera used in this study, *Spirogyra* has been frequently used in the past compared to the other two species. This can be attributed to the fact that *Spirogyra* is an abundant and widespread genus worldwide. Similarly, Wen and Fan (2010) observed that *Spirogyra* removed 85.4% of total nitrogen from eutrophic waters after a 20-day treatment. These findings highlight the potential of *Spirogyra* and other algae in wastewater treatment, particularly in the removal of nitrogenous compounds. In Kenya, several studies have explored the potential of algae for treating wastewater. Swaleh et al. (2022) study explored the use of microalgae in wastewater for nutrient removal and biomass production. Three

microalgal species, *Oscillatoria sp.*, *Chlorella sp.*, and *Entomoneis sp.* were cultured in wastewater-seawater mixtures (0% to 60% v/v). The study found significant differences ($p < 0.05$) in the concentrations of nitrates before and after the experimental period. Nitrate removal ranged from 4.0% to 67.6%, with *Chlorella* again showing the most effective removal. These findings suggest that the effectiveness of wastewater treatment and biomass production depends on both the algal species and nutrient levels.

Conclusion

The study demonstrated that local algae genera *Spirogyra*, *Zygnema* and *Oedogonium* are effective in removing nitrates from wastewater, with *Spirogyra* exhibiting the highest nitrate removal efficiency. All three species significantly reduced nitrate concentrations over four days, with the highest removal percentages observed on specific days for each species. During algal growth in the sewage, the three local algae genera are capable of reducing nitrates contained in the wastewater, thus preventing eutrophication of receiving water bodies.

Recommendations

Based on the findings, the study made the following recommendations;

1. Uasin Gishu County Government sewage treatment plant and other waste treatment facilities should such as Moi University should consider integrating algae-based bioremediation techniques into existing treatment systems to improve effluent quality while reducing reliance on chemical treatments.
2. The fatty acid composition of the algae should also be analyzed to find their suitability in biodiesel production.

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