International Journal of Education, Science and Social Sciences

Original Research Article

Evaluation of the Growth Potential of Local Algal Genera (*Spirogyra*, *Zygnema*, and *Oedogonium*) in Wastewater from Kesses, Kenya

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Received: 18th March, 2026, Accepted: 15th May, 2026, Published: 23rd May, 2026

Abstract

The remediation of nutrient-rich wastewater using indigenous microalgae faces significant economic bottlenecks due to energy-intensive biomass harvesting methods. This study investigated the biological feasibility and growth dynamics of three locally isolated, easily harvestable freshwater filamentous green macroalgae genera (*Spirogyra*, *Zygnema*, and *Oedogonium*) cultivated in domestic sewage. Wild algal strains were harvested from the Kesses region of Uasin Gishu County, Kenya, and cultured under controlled laboratory conditions using secondary effluent collected from the First Maturity Pond of the Moi University Waste Stabilization system. Algal growth and metabolic vitality were monitored daily over a 7-day experimental period by quantifying chlorophyll a concentrations as a definitive proxy for biomass proliferation. A Friedman test was applied to evaluate the statistical significance of temporal variations in pigment accumulation. The empirical results demonstrated that all three genera underwent a distinct, statistically significant exponential growth phase driven by wastewater nutrient assimilation ($p<0.01$). Both *Spirogyra* and *Zygnema* exhibited peak chlorophyll a accumulation on day 4 (with median values of 37.84 mg m^{-3} and 34.14 mg m^{-3}, respectively), which directly coincided with their maximum internal nitrate sequestration capacity. Conversely, *Oedogonium* adapted more rapidly to the waste stream, mirroring the control growth kinetics of a synthetic medium to achieve its maximum chlorophyll a concentration by day 3 (median = 39.31 mg m^{-3}). Following these genus-specific peaks, ambient nutrient depletion triggered a sharp metabolic decline, with day 7 exhibiting the lowest pigment concentration across all treatments. These findings confirm that native Kenyan strains of *Spirogyra*, *Zygnema*, and *Oedogonium* can effectively exploit domestic sewage as a cost-free growth medium. Furthermore, this study establishes the first empirical baseline for the phycoremediation potential of the genus *Zygnema* in East African lagoon systems. Because these macroscopic, interlocking filaments can be recovered using simple mechanical screening or gravity settling, integrating them into decentralized waste stabilization ponds offers a low-cost, low-energy alternative for institutional wastewater polishing. This approach simultaneously mitigates downstream eutrophication in the Lake Victoria basin while generating scalable, non-crop biomass suitable for downstream bioenergy and bio-fertilizer valorization.

Keywords: Phycoremediation, filamentous algae, waste stabilization ponds; Chlorophyll *a*, *Zygnema*; Circular bioeconomy

Introduction

Background Information

Wastewater discharge from domestic, agricultural, and industrial sources remains a critical environmental challenge in sub-Saharan Africa, where treatment infrastructure is often inadequate or absent. In Kenya, rivers and lakes receive large nutrient loads from untreated sewage, agricultural runoff, and industrial effluent, leading to widespread eutrophication and degradation of aquatic ecosystems (Ondiek et al., 2025). Recent assessments of rivers feeding Lake Victoria have documented phosphate concentrations exceeding 0.5 mg/L and elevated nitrate levels, with visible algal blooms and episodic fish kills attributed to hypoxia following nutrient overloading and organic matter decay (Mrombo et al., 2025; Ondiek et al., 2025). The Kesses area in Uasin Gishu County, which hosts Moi University and surrounding agricultural enterprises including dairy processing facilities, contributes to this pollution burden through point-source discharges of domestic sewage and agro-industrial wastewater into the Kesses River, a tributary of the Yala River system that ultimately drains into Lake Victoria (Nile Basin Initiative, 2023). These nutrient-rich effluents create favorable conditions for excessive primary production, yet they also represent a potential resource if the nutrients can be captured and recycled through biological treatment systems.

Algae hold significant potential for large-scale application as a biofuel feedstock. While a number of microalgae-based products are already well established in high-value markets such as human dietary supplements and animal feed components the energy sector presents unique hurdles. Considerable advances in algal biology and substantial processing improvements are still

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required to achieve economic, environmental, and energetic sustainability in the commercial production of microalgae biofuels (Laurens et al., 2017).

Conventional physicochemical wastewater treatment technologies are often energy-intensive, chemically dependent, and economically unfeasible for small-to-medium-scale operations in low-resource settings. In contrast, phycoremediation the use of algae for nutrient recovery and pollutant removal offers a sustainable, low-cost alternative that simultaneously remediates wastewater and generates biomass for secondary applications (Wang et al., 2024). Filamentous green algae (Chlorophyta) have attracted particular attention for wastewater treatment because they form macroscopic, easily harvestable biomass, exhibit high nutrient uptake rates, and can tolerate the fluctuating environmental conditions typical of outdoor treatment systems (Lawton et al., 2024; Wang et al., 2024). Unlike unicellular microalgae, which require costly centrifugation or flocculation for harvesting, filamentous species can be removed from effluent by simple screening or settling, substantially reducing operational costs and improving the feasibility of biomass valorization (Zheng et al., 2025).

To optimize these systems, various cultivation strategies have been explored using unicellular models. For instance, when *Chlorella sorokiniana* was cultivated in a laboratory-scale photobioreactor under daily light-dark cycles, testing various timing strategies for adding acetate at waste-stream concentrations 1 – 2 g L-1. The results showed that the fastest growth occurred when adding the acetate at night (cyclic autotrophy/heterotrophy). However adding the acetate during the day (mixotrophty) also improved growth compared to autotrophic controls. Industrial wastewater was used as cultivation medium of *Chlorella sorokiniana*. The culture was able to grow at high rates upto a density of 4 g L-1. The deceleration-stat technique was used to create a series of pseudo-steady states to give information about the expected results of continuous cultivation of microalgae in the selected wastewater. At light intensities of 2100 and 200µmol photon m-2 s-1 the algae grew at a rate of over 5 and 1.67 g L-1day-1, re-spectively. The corresponding removal rates of nitrogen were 238 and 93 mgL-1day-1 and 40 and 19 mg L-1day-1 for phosphorous. Ammonium removal varied from below 40% to 99%, while phosphate removal was always nearly total (Ge & Champagne, 2017).

According to Shijian Ge and Pascale Champagne (2017), Queen's University, Kingston, Ontario, Canada, have used the macroalgae *Chaetomorpha linum* to recover nutrients from wastewater and convert them to useful biomass. The team used differently concentrated types of wastewater from a wastewater treatment plant to cultivate the macroalgae. The algae were grown in wastewater aerated by aquarium pumps and illuminated by commercially available aquarium LED lights. The algae were able to remove nitrogen and phosphorus from all types of wastewater with high efficiency. The larger size of the algae makes them

https://doi.org/10.69897

significantly easier to harvest than microalgae (Van der Weide, Schipperus & van Dijk, 2014).

Among filamentous green algae, the genus *Oedogonium* has been used for municipal and agricultural wastewater treatment. Recent outdoor mesocosm studies demonstrate that *Oedogonium* sp. achieves biomass productivities up to 7.3 g dry weight m^{-2} day^{-1} under summer conditions and maintains robust nitrate-N removal rates exceeding 90% in primary-treated effluent (Hariz et al., 2023; Lawton et al., 2024). *Oedogonium* also exhibits strong biological attachment to substrates, rapid establishment in floway systems, and tolerance to a wide range of temperatures, making it suitable for year-round cultivation in tropical and subtropical climates (Hariz et al., 2023; Liu et al., 2025). Beyond nutrient remediation, biomass harvested from wastewater-grown *Oedogonium* has been successfully processed into cellulose and biostimulant products, demonstrating a viable cascading biorefinery pathway that converts waste treatment into value creation (Zhang et al., 2024).

Spirogyra is another cosmopolitan filamentous genus with demonstrated capacity for nutrient sequestration in wastewater environments. Studies using algal turf scrubbers and constructed wetlands report that *Spirogyra* can achieve nitrogen absorptivity of 350–450 mg m^{-2} day^{-1} and phosphorus absorptivity of 25–40 mg m^{-2} day^{-1}, performance metrics that rival or exceed those of emergent macrophyte-dominated wetlands (Zheng et al., 2025). Comparative assessments of ammonia tolerance among filamentous genera indicate that *Spirogyra* maintains stable growth at total ammonia nitrogen concentrations up to 60 mg-N L^{-1} under moderate pH, though its productivity declines sharply at pH thresholds above 8.0 where free ammonia becomes toxic (Liu et al., 2023). These findings suggest that *Spirogyra* is well-suited to secondary or tertiary wastewater treatment where ammonium concentrations are substantial but pH is controlled.

Zygnema, by comparison, remains largely unexplored in the context of wastewater phycoremediation. Although this genus is commonly recorded in freshwater pools and polluted inland water bodies, current literature identifies no direct scientific evidence for its nutrient removal efficacy in wastewater systems (Dorgham et al., 2025). However, *Zygnema* possesses physiological traits that may confer adaptive advantages under nutrient stress, including the ability to form pre-akinetes and rearrange metabolic pathways during environmental fluctuations (Dorgham et al., 2025). Given its ubiquity in Kenyan freshwater ecosystems, evaluating *Zygnema* alongside better-characterized genera such as *Oedogonium* and *Spirogyra* is essential to determine whether locally adapted strains can contribute to integrated wastewater treatment schemes.

In microalgal-bacterial granular sludge systems, the incorporation of filamentous algae has been shown to improve granule stability, enhance lipid production, and maintain nitrogen and phosphorus removal efficiencies above 93%

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and 64%, respectively, even under saline conditions (Cao et al., 2022). These results underscore the functional role of filamentous algae in strengthening microbial aggregate structures and sustaining treatment performance under environmental stress. Extending such integrated approaches to local wastewater streams in Kenya could address both pollution abatement and biomass generation objectives without the high capital expenditure associated with conventional activated sludge plants.
Algae are sunlight driven cell factories that convert carbon dioxide into potential biofuels, foods, feeds and fertilizers. The increase in world's population will create a demand for more food, feeds and greater agricultural production which can be provided by algae.

Statement of the Problem

Algae have potential to grow in wastewater thus can be cultivated for commercial purposes. Furthermore, algae have numerous benefits that make them ideal choice for creating a variety of products. They can be used to make biofuel such as biodiesel, biogas, bioethanol from their biomass. Algae can also be used as a food supplement for both livestock and human use because it contains essential amino acids, simple and complex carbohydrates, fatty acids, vitamins, minerals and trace elements. Algae can also be utilized as a fertilizer and soil conditioner due to their high nutrient content. Algae are also beneficial in wastewater treatment, reducing the need for toxic chemicals already in use. Algae can also capture fertilizers in runoff from farms. They are also used in power plants to capture carbon dioxide emissions. In spite of the numerous benefits, the cost of producing algae is prohibitively expensive. Significant cost reduction may be achieved if nutrients and water can be obtained at low cost. Sewage contains nutrients that can support plant growth as well as sufficient water, so this study attempted to find out if algal growth can occur in sewage.

Literature Review

Capacity of the Selected Algae Genera to Grow in Wastewater

Recent empirical research has established that filamentous green algae (Chlorophyta) are among the most promising photosynthetic organisms for coupled wastewater treatment and biomass production. Among the genera of interest, *Oedogonium* has received the most intensive experimental scrutiny. In a direct comparison of four algal systems treating secondary municipal effluent, Kube et al. (2022) demonstrated that the macroalgal system using *Oedogonium cardiacum* achieved the highest biomass production (102 ± 4 mg/L/day) and sustained operation longer than suspended, entrapped, or biofilm configurations of *Chlorella vulgaris*. The *Oedogonium* system also reduced total phosphorus to 1.3 mg/L and

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ammonium nitrogen to ≤0.5 mg/L, confirming that macroalgae can compete with or exceed microalgal counterparts in both productivity and effluent quality.

Outdoor mesocosm studies have further refined species-level rankings. Hariz et al. (2023) compared *Oedogonium* sp., *Spirogyra* sp., *Rhizoclonium* sp., and *Cladophora* sp. on floway-style attached algal systems (FANS) under ambient summer and winter conditions. *Oedogonium* sp. was the best-performing species overall, achieving mean biomass productivities of 4.5 g DW m^{-2} day^{-1} in summer and maintaining the strongest biological attachment to substrates, which minimized washout and non-target species invasion. *Spirogyra* sp. exhibited intermediate productivity but still formed dense, harvestable mats, while *Cladophora* and *Rhizoclonium* were less consistent across seasons.

Tolerance to nitrogen loading is a critical selection criterion for wastewater applications. Liu et al. (2023) examined ammonia tolerance across *Oedogonium*, *Spirogyra*, *Tribonema*, and *Cladophora* under controlled laboratory conditions. *Spirogyra* maintained stable growth at total ammonia nitrogen concentrations up to 60 mg-N L^{-1} at moderate pH, though its specific growth rate declined at pH > 8.0 where free ammonia becomes toxic. *Oedogonium* displayed similarly robust ammonia tolerance but with faster recovery after perturbation, suggesting superior resilience in variable wastewater streams.

In constructed wetland systems, filamentous algae have demonstrated nitrogen and phosphorus absorptivities that rival emergent macrophyte-dominated wetlands. Zheng et al. (2025) reported that *Spirogyra*-based turf scrubbers achieved nitrogen absorptivity of 350–450 mg m^{-2} day^{-1} and phosphorus absorptivity of 25–40 mg m^{-2} day^{-1}, metrics comparable to or exceeding those of conventional wetland systems (400–1200 mg N m^{-2} day^{-1} and 9–67 mg P m^{-2} day^{-1}). The study also noted that *Spirogyra* exhibited a slight preference for ammonium over nitrate, a metabolic trait that aligns well with the ammonium-rich chemistry of domestic and agricultural wastewater.

Physiological acclimation to seasonal light and temperature regimes has been quantified for *Oedogonium*. Liu et al. (2025) measured photosynthesis and respiration responses of *Oedogonium* acclimated to averaged seasonal temperatures and light exposure levels. The genus showed high photosynthetic efficiency under moderate temperature and light conditions but experienced respiratory carbon losses under extreme high-light or low-temperature stress, explaining the seasonal die-off observed in temperate outdoor systems. These findings imply that tropical and subtropical climates where temperature and light remain within optimal ranges year-round may support more stable *Oedogonium* productivity than temperate zones.

According to Chen et al. (2011) study on Cultivation, photo-bioreactor design and harvesting of microalgae for biodiesel production; Results demonstrated that industrial wastewater can be a suitable replacement for algae cultivation

https://doi.org/10.69897

medium. The screening method developed will reduce the cost of identifying the best conditions to test at lab scale. The D-stat method offers a way to identify the best conditions for bio-mass production and nutrient removal. Various options for heterotrophic and mixotrophic utilization of waste organic carbon in effluents are identified. Further advances in microalgae cultivation and processing will be needed for the production of sustainable products from wastewater in the future.

Algae cultivation face one challenge that there are many potential combinations which must empirically screened. Tens of thousands of mi-croalgae species have been identified so far and there are numerous waste-streams that potentially could be of interest. A screening system was developed using the microplate as cultivation vessel and measurement cuvette. Flu-orescence was demonstrated to be an order of magnitude more sensitive than optical density for detecting biomass growth, which increased the length of time in which exponential growth was observable from hours to days. This enabled growth rate-light intensity (μ-I) curves to be measured in microplates which were found to be equivalent to those obtained in typical lab-scale photo-bioreactors. As μ-I curves are the key biological input to an already existing model, it was validated that low density microplate cultivations can be used to make predictions about industrially relevant autotrophic cultivation (Venteris et al. 2014).

When algae are grown within a wastewater treatment plant, the use of the chemical energy stored in the organic carbon dissolved in the wastewater could also be a useful option. Conventional aerobic sewage treatment ex-pends much energy in breaking down the biomass to CO2. However, various anaerobic treatment methods would result in effluent containing dissolved organic molecules suitable for algae species that have the ability to grow as mixo- or heterotrophs. *Chlorella sorokiniana* was cultivated in a lab scale photo bioreactor under daily light dark cycles and various timing strategies were tested for adding acetate at concentrations that can be obtained in waste streams of 1 – 2 g L-1. The results showed that the fastest growth occurred when adding the acetate at night (cyclic autotrophy/heterotrophy). However adding the acetate during the day (mixotrophty) also improved growth com-pared to autotrophic controls (Park et al., 2011).

The integration of filamentous algae into microbial aggregate systems has also advanced. Cao et al. (2022) investigated the effect of filamentous algae in microalgal-bacterial granular sludge treating saline wastewater. The presence of filaments improved granule stability, enhanced lipid production, and maintained nitrogen and phosphorus removal efficiencies above 93% and 64%, respectively, even under saline shock loading. This suggests that locally isolated filamentous genera could strengthen biological treatment systems in coastal or saline-influenced wastewater streams.

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Downstream valorization of wastewater-grown biomass has been demonstrated for *Oedogonium*. Zhang et al. (2024) cultivated *Oedogonium calcareum* during primary municipal wastewater treatment and extracted cellulose and biostimulant fractions. The biomass yielded high-quality cellulose suitable for material applications and biostimulant extracts that enhanced plant growth, confirming that nutrient remediation can be coupled to circular bioeconomy products.

Despite the progress with *Oedogonium* and *Spirogyra*, *Zygnema* remains conspicuously underrepresented in the global wastewater literature. A 2025 comparative review of phycoremediation potential noted that while *Zygnema* is commonly recorded in freshwater and polluted inland water bodies, no direct scientific evidence currently documents its nutrient removal efficacy in wastewater systems (Dorgham et al., 2025). This gap represents a significant opportunity for empirical studies in regions where *Zygnema* is naturally abundant.

Empirical research on filamentous algae in African wastewater systems is still in its infancy, with most recent work focusing on unicellular microalgae or cyanobacteria rather than the targeted filamentous genera. Shee et al. (2025) reviewed sustainable decentralized sewage treatment options for sub-Saharan Africa and identified microalgae-based systems as viable secondary and tertiary treatment technologies, particularly when combined with bacterial consortia. The authors noted that mixed microalgal cultures (including *Spirulina*, *Micractinium*, and *Chlorella*) achieved nitrogen removal efficiencies of 86–93% and phosphorus removal up to 83% in dairy wastewater, but they did not evaluate filamentous genera such as *Spirogyra*, *Zygnema*, or *Oedogonium*.

At the continental scale, Omohwovo (2024) emphasized that African nations require innovative, low-energy biological treatment technologies to meet Sustainable Development Goal 6 targets. The review highlighted constructed wetlands, waste stabilization ponds, and algae-based systems as appropriate technologies, yet it also observed that most African wastewater treatment research has concentrated on conventional activated sludge or pond systems, with limited pilot-scale data on algal bioremediation performance under local climatic and wastewater composition conditions.

Githaiga et al. (2020) assessed nutrient removal by *Spirogyra* sp. and *Oedogonium* sp. in wastewater from Egerton University, Kenya, over a 90-day cultivation period. Both species significantly altered physicochemical parameters and nutrient concentrations, with significant differences in biomass weight gain between day 1 and day 90. The authors concluded that the two genera were effective in nutrient uptake but recommended further taxonomic and physiological characterization of the isolates.

ISSN: 3007-6781 https://doi.org/10.69897

Materials and Methods

Algae Sampling Area

The algae samples were obtained from Kesses area, mainly along the edges of Sambul River and in ponds. The locality was chosen because of the proximity to Moi University, where the laboratory is based. The study was also interested in local species of algae. The sampling area (0^0 16'N, 35^0 20'E, altitude 2180m above sea level) is situated south of Eldoret in Kesses division, Wareng district, Uasin Gishu County in Kenya. The mean annual rainfall is 1124mm, falling in one long season from March to September. The monthly temperature ranges from 26.1^0C in February to 8.4^0C in July (Uasin Gishu District Strategic Plan 2005-2010).

Sewage Sampling Area

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.

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/m^3/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

ISSN: 3007-6781 https://doi.org/10.69897

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 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.

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.

The characteristics of the wastewater at Moi University oxidation ponds were as follows:

https://doi.org/10.69897

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

Pond	Chloride	BOD	Nitrate	pH	Temp	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	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)

Procedure Determination of Chlorophyll *a*

Sampling

Sewage water was collected using a non-toxic water sampler or a pump in opaque plastic bottles. The appropriate sample volume will depend on the spectroscopic method chosen. Until filtration, samples were protected from warmth and light.

Pigment Extraction

Extraction was carried out by grinding the filters in a few millilitre of 90 % acetone in a glass homogenizer with a motor-driven Teflon pestle, for 1 minute, in an ice bath and under-subdued light.

After grinding, carefully transfer the extract to a stoppered and graduated centrifuge tube, rinse properly the glass homogenizer and the pestle with 90 % acetone and add rinsing volumes to the centrifuge tube.

Make up the extract volume in the centrifuge tube to exactly 10 ml 90 % acetone (i.e. 10 ml+ dead volume of filter) and stopper the tube.

Centrifugation

Immediately before measurement, specimen was mixed thoroughly and centrifuged the extracts for 10minutes at 500×g, where g is the gravitational acceleration. Assuming g to be 9.81 m s−2, then the centrifugation velocity (rpm) for a particular centrifuge can be estimated by668.8/R0.5 where R is the radius, the distance (in Metre Units) between the axis of the centrifuge head and the mid-point of the centrifuge tube.

Spectrophotometry: Trichromatic method

Use a spectrophotometer of 2 nm maximum bandwidth and stoppered cuvettes with path length up to 5 cm (such a path length is required in most instances for satisfactory measurements). Sample extracts was transferred from the centrifuge tubes to the cuvette by careful pipeting.

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Absorbance of the sample extract was measured at 750, 664, 647, and 630 nm against a 90 %acetone blank. Concentration of chlorophyll a, b and c, was calculated according to the equations of Jeffrey and Humphrey (1975):

Chlorophyll a = (11.85* (E664 − E750) − 1.54* (E647 − E750) − 0.08 (E630−E750))*Ve/L*Vf Chlorophyll b = (−5.43* (E664 − E750) + 21.03* (E647 − E750) − 2.66 (E630−E750))*Ve/L*Vf Chlorophyll c = (−1.67* (E664 − E750) − 7.60* (E647 − E750) + 24.52 (E630−E750))*Ve/L*Vf

Where: L = Cuvette light-path in centimetre. Ve = Extraction volume in millilitre. Vf = Filtered volume in litre. Concentrations are in unit mg m−3.

Spectrophotometry: Monochromatic method with acidification.

Using a spectrophotometer of 2 nm maximum bandwidth and stoppered cuvettes with path length up to 5 cm (such a path length is required in most instances for satisfactory measurements). The sample extracts was transferred from the centrifuge tubes to the cuvette by careful pipeting.

The absorbance of the sample extract was measured at 750 nm (E750o) and 665 nm (E665o) against a 90 % acetone blank. Add 0.2 ml 1 % v/v hydrochloric acid in the cuvette and mix. Wait 2-5 min (but not more). Measure again the absorbance at 750 nm (E750a) and 665 nm (E665a) against a 90 % acetone blank. Calculate the concentration of chlorophyll a and pheopigments a according to the equations of Lorenzen (1967):

Chlorophyll a = 11.4*K*((E665o − E750o) − (E665a − E750a))*Ve /L*VfPheopigments a = 11.4*K*((R*(E665a − E750a)) − (E665o − E750o))*Ve /L*VfWhere:L = Cuvette light-path in centimetre. Ve = Extraction volume in millilitre. Vf = Filtered volume in litre.R = Maximum absorbance ratio of E665o/E665a in the absence of pheopigments = 1.7.K = R/(R − 1) = 2.43. Concentrations are in unit mg m−3.

Results

Capacity of the Selected Algae Genera to Grow in Waste Water

In the three genera grown on sewage, exponential growth occurred during the first three days. This was then followed by decrease in growth, while those grown on BBM progressed in growth throughout the experiment, as shown in the graph below; Graph showing algal growth in sewage.

In *Spirogyra* the difference in chlorophyll *a* accumulated across the days day 1(median = 28.23), day 2 (median = 33.71), day 3 (median = 35.11) day 4 (median = 37.84), day 5 (median = 32.14), day 6 (median = 24.09) and day 7 (median = 17.06 was statistically significant (X^2 (N = 3) = 18, p = .006). Between day one and two there was a percentage increase in chlorophyll *a* of 19.41 %, 3.99 % increase between the second and third day and 7.76 % increase between the third and fourth day.

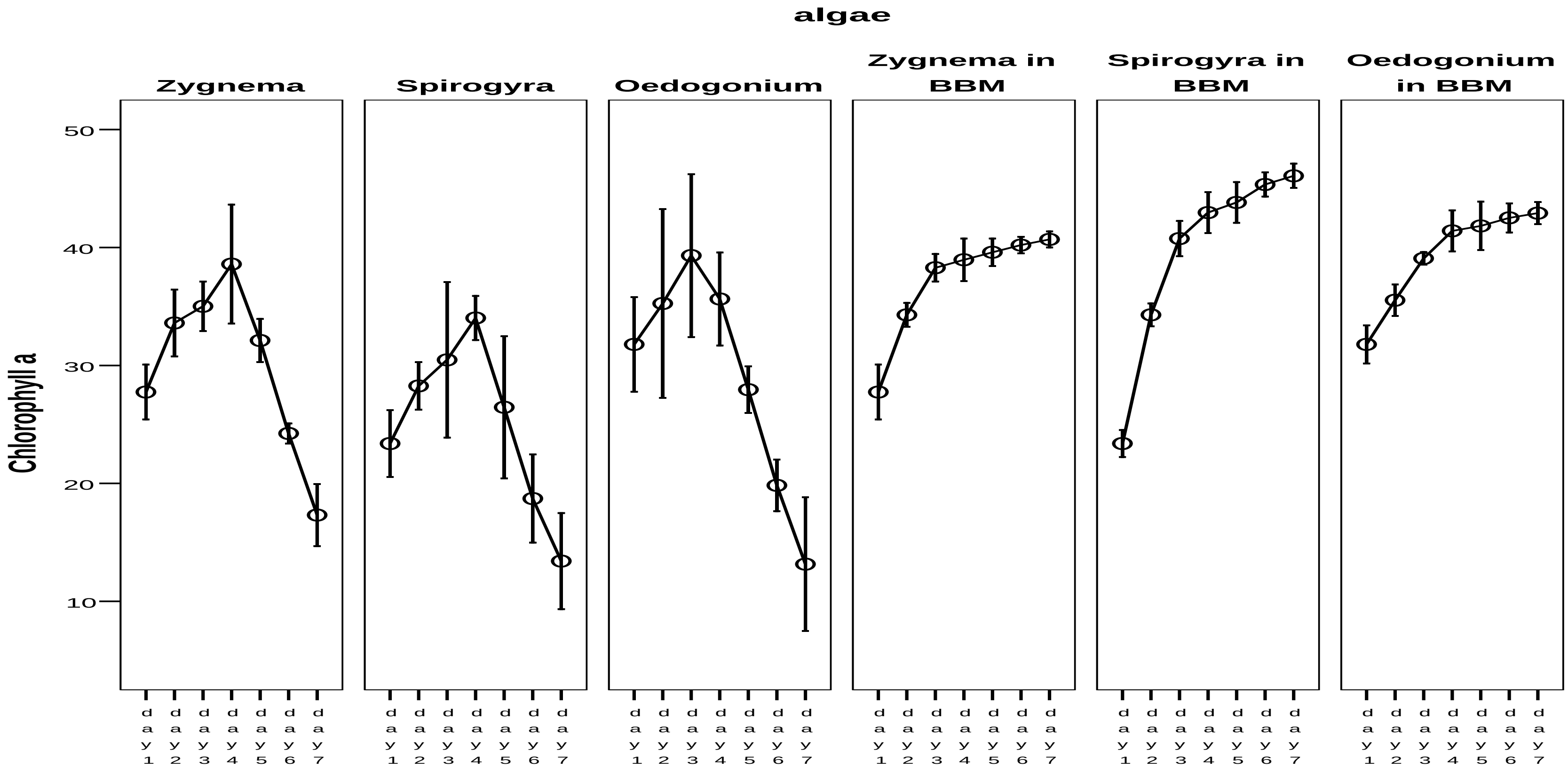

Figure 1: Growth of Algae in sewage

The fourth day had the highest amount of chlorophyll *a.* After day 4, the amount of chlorophyll *a* began to decline. There was a 15.06 % decrease in the pigment between days 4 and 5, 25.05 % decrease between days 5 and 6 and 29.18 % decrease between days 6 and 7. Day 7 had the least amount of chlorophyll *a.*

In *Zygnema*, the difference in chlorophyll *a* accumulation across the days day 1(median = 23.14), day 2 (median = 28.29), day 3 (median = 30.67), day 4 (median = 34.14), day 5 (26.85), day 6 (median = 18.59) and day 7 (median = 13.41) was significant (X^2 (N = 3) = 18, p = .006). There was a percentage increase in chlorophyll *a* of 22.26 % between day 1 and 2, 8.41 % increase between the second and third day and 11.31 % increase between the third and fourth day. The fourth day had the highest amount of chlorophyll *a.* After day 4, the amount of chlorophyll *a* began to decline. There was a 21.35 % decrease in the pigment between days 4 and 5, 30.76 % decrease between days 5 and 6 and 21.86 % decrease between days 6 and 7. Day 7 had the least amount of chlorophyll *a.*

In *Oedogonium*, the difference in chlorophyll *a* accumulation across the days day 1(median = 31.78), day 2 (median = 34.67), day 3 (median = 39.31), day 4 (median = 35.82), day 5 (27.79), day 6 (median = 19.88) and day 7 (median = 13.14) was significant (X^2 (N = 3) = 17.71, p = .007). Between day one and two there was a percentage increase in chlorophyll *a* of 9.44 %, 11.52 % increase between the second and third day. In this genus, the third day had the highest amount of chlorophyll *a.* After day 3, the amount of chlorophyll *a* began to decline. There was an 8.88 % decrease in the pigment between days 3 and 4, 22.41 % decrease between days 4 and 5, 28.46 % decrease between days 5 and 6, and 33.90 % decrease between day 6 and 7. Just like in the other genera, day 7 had the least amount of chlorophyll *a.*

Hypotheses Study results

In order to see if the selected genera could grow in domestic sewage, Spectrophotometry: Trichromatic method test was done. The study results showed that in *Spirogyra* there was a statistically significant growth (X^2 (N = 3) = 18, p = .006), in *Zygnema* accumulation of chlorophyll across the days day were significant (X^2 (N = 3) = 18, p = .006) and in *Oedogonium* chlorophyll accumulation was significant (X^2 (N = 3) = 17.71, p = .007) and the study thus rejected the null hypothesis, **H_{01}:** The algae genera are not able to grow in wastewater.

Discussion

The growth performance of *Spirogyra*, *Zygnema*, and *Oedogonium* cultured in the first maturity pond effluent of the Moi University Waste Stabilization Ponds (WSP) highlights the biological feasibility and metabolic boundaries of utilizing native Kenyan filamentous green algae for wastewater treatment. Tracking chlorophyll a content over a 7-day period provided a reliable proxy for cellular health, photosynthetic adaptation, and biomass proliferation. All three genera exhibited distinct exponential growth during the first three to four days of the experiment, a trend driven by the high availability of bioavailable nutrients specifically nitrates and phosphates characteristic of institutional effluents in the region. This rapid initial accumulation aligns with the findings of Kube et al. (2022) and Lawton et al. (2024), who observed that filamentous macroalgae thrive during initial exposure to secondary or tertiary municipal wastewater due to their high nutrient uptake rates and robust metabolic machinery. However, after reaching peak chlorophyll a concentrations, all three genera experienced a sharp, statistically significant decline in pigment content by the seventh day, which points to nutrient depletion or the onset of metabolic stress within the batch culture system.

The experimental data revealed critical differences in the growth kinetics and physiological pacing of the three genera under identical wastewater conditions. *Oedogonium* reached its peak biomass and pigment concentrations earliest, on day three. Interestingly, during these first few days, the chlorophyll a values of *Oedogonium* in the wastewater medium closely mirrored those grown in the controlled Bold's Basal Medium (BBM). This indicates that the first maturity pond effluent provided an optimal chemical profile that fully satisfied the immediate metabolic demands of the genus. The subsequent steep decline in *Oedogonium* chlorophyll a after day three directly correlates with the period when it had accumulated its highest percentage of nitrates. This suggests a highly efficient, rapid luxury uptake mechanism where the algae swiftly sequestered available nitrogen from the wastewater, leading to an early exhaustion of ambient nutrients in the batch medium. This rapid adaptation and recovery after shifting into wastewater environments reinforces the conclusions of Liu et al. (2023) and Hariz et al. (2023), who identified *Oedogonium* as an exceptionally resilient genus capable of maintaining high biomass productivities due to its high photosynthetic efficiency and superior resistance to nutrient perturbations.

However, *Spirogyra* exhibited a slightly prolonged exponential phase, achieving its highest chlorophyll a concentration on day four. This peak exactly coincided with the day it accumulated its highest percentage of internal nitrates. This metabolic behavior suggests that *Spirogyra* possesses a steady, high-capacity nitrogen absorption rate, validating reports by Zheng et al. (2025) that *Spirogyra*-based systems can achieve exceptional nitrogen absorptivity metrics that outcompete conventional macrophyte wetlands. The steady increase up to day four also reflects its high tolerance to the substantial ammonium-nitrogen fractions typically found in domestic lagoons before high pH levels induce free ammonia toxicity (Liu et al., 2023).

The performance of *Zygnema*, which also peaked on day four in tandem with maximum nitrate accumulation, provides highly valuable baseline data for algal biotechnology. While better-studied genera like *Oedogonium* and *Spirogyra* dominate the literature, *Zygnema* has remained completely uncharacterized in African wastewater systems, with global literature noting an absolute absence of

https://doi.org/10.69897

direct empirical data regarding its nutrient recovery efficacy (Dorgham et al., 2025). The significant growth and pigment accumulation observed here prove that local *Zygnema* isolates are fully capable of exploiting domestic sewage for growth. Its ability to track the growth curve of *Spirogyra* may be attributed to its specialized survival traits, such as the cellular capacity to reorganize metabolic pathways and form resilient pre-akinetes under changing environmental conditions (Dorgham et al., 2025).

The capacity of these local isolates to rapidly assimilate nutrients and build biomass holds profound relevance for the management of aquatic ecosystems in Kenya. Currently, major river basins and lakes across the country receive massive, unsustainable nutrient loads from untreated municipal sewage, institutional discharges, and agricultural runoff. This has caused widespread, severe eutrophication and catastrophic ecological degradation (Nile Basin Initiative, 2023; Ondiek et al., 2025). In the Lake Victoria basin, for example, phosphate concentrations regularly exceed 0.5 mg L^{-1}, triggering massive algal blooms and large-scale fish kills due to episodic hypoxia when the organic matter decays (Mrombo et al., 2025; Ondiek et al., 2025). The Kesses region of Uasin Gishu County directly exacerbates this regional pollution crisis. Point-source effluents from local institutional complexes such as student hostels, lecture halls, kitchens, and clinics at Moi University along with nearby agro-industrial and dairy processing facilities drain directly into local ponds and the Sambul/Kesses River. This river acts as a key tributary to the Yala River system, which ultimately discharges straight into Lake Victoria (Nile Basin Initiative, 2023).

Through intercepting these effluents in the secondary and tertiary stages of Waste Stabilization Ponds, such as the primary maturity pond evaluated here, these nutrient-rich streams can be transformed from an environmental hazard into a valuable bioresource. Utilizing native macroalgae for phycoremediation offers an economically viable, low-cost, and low-energy treatment alternative tailored for resource-limited settings where conventional activated sludge plants are structurally and financially unfeasible (Omohwovo, 2024; Wang et al., 2024). Furthermore, because these filamentous species are macroscopic, they form floating, interlocking mats that can be harvested using simple mechanical screening or gravity settling. This completely bypasses the prohibitive capital and operational expenses associated with the centrifugation or chemical flocculation required by unicellular microalgae like *Chlorella sorokiniana* (Ge & Champagne, 2017; Zheng et al., 2025). Once harvested, this biomass can be fed into a cascading circular bioeconomy, serving as a clean feedstock for green energy or processed into high-value bio-fertilizers and agricultural biostimulants, successfully converting institutional waste streams into localized economic value (Laurens et al., 2017; Zhang et al., 2024).

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Conclusion

From the results of this study, it is concluded that the use of sewage as a culture medium to grow algae can significantly reduce the need for chemical fertilizers and use of scarce but valuable freshwater, thereby maximizing water use efficiency.

Recommendations

Wastewater generated from different sources should be tested for algae growth. The other possible sources that could be tested for algae cultivation are water from concentrated animal feed operations and industrial wastewaters

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