EFFECT OF FERMENTED CASSAVA AND MAIZE EFFLUENTS ON FRESHWATER MICROALGAE (*Scenedesmus ecornis and Chlamydomonas reinhardtii*).

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BENIN CITY

NOVEMBER, 2025

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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research titled "**EFFECT OF FERMENTED CASSAVA AND MAIZE EFFLUENTS ON FRESHWATER MICROALGAE (*Scenedesmus ecornis and Chlamydomonas reinhardtii*)"** was carried out by "**SULIHAT DESTINY YAKUBU"** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

DR. J. U. OGBEBOR **DATE**

PROJECT SUPERVISOR

PROF. (MRS). E. T. AISIEN **DATE**

HEAD OF DEPARTMENT

DECLARATION

I “**SULIHAT DESTINY YAKUBU”** declare that “**EFFECT OF FERMENTED CASSAVA AND MAIZE EFFLUENTS ON FRESHWATER MICROALGAE (*Scenedesmus ecornis and Chlamydomonas reinhardtii*)”** is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before any other degree at any other University.

SULIHAT DESTINY YAKUBU **DATE**

DEDICATION

This research work is dedicated to God Almighty for His unconditional love and mercy in my life and to those who have been a constant source of guidance, support, and inspiration in my academic journey, and to my lovely Mother, Mrs Yakubu .O. Afishat for her unending love and great support.

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ABSTRACT

This study was carried out to assess the effects of cassava and maize effluents, on the growth of two freshwater microalgae (*Scenedesmus ecornis* and *Chlamydomonas* reinhardtii). Cassava and maize effluent from agro-processing facilities produces large quantities of wastewater. The test algae were grown in seven concentrations (0%, 5%, 10%, 25%, 50%, 75% and 100%), which were set up in triplicates. Growth response of the test microalgae was monitored at two-day intervals for 14 days using a visible spectrophotometer (Model No. HV-721). The growth rate, percentage inhibition, and analysis of variance (ANOVA) were computed using Microsoft excel software. Physicochemical parameters such as hydrogen ion concentration (pH), electrical conductivity, and total dissolved solid (TDS) were also determined. The results indicated that the growth response of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* increased with the increasing concentrations of cassava and maize effluent. Statistical analysis using ANOVA showed that the growth response was statistically significant ($p < 0.05$) among the various concentrations for both species. Overall, *Scened*esmus *ecornis* recorded higher biomass relative to *Chlamydomonas reinhardtii.* Dry weight of *Scenedesmus ecornis* recorded the highest biomass of 398.53mg/L, 331.93mg/L, 306.86mg/L, 362.9mg/L, 175.55mg/L, 123.75mg/L and 85.28mg/L at 100%, 75%, 50%, 25%, 10%, 5% and 0% concentrations respectively. Percentage inhibition result revealed that both microalgae had a stimulatory response to the effluents. This stimulatory response was found to follow a concentration gradient with increased stimulation as effluent concentration increased. *Scenedesmus* reached -450% inhibition at the highest concentration (100%), while *Chlamydomonas* peaked at -330% at 100% concentration, this makes *Scenedesmus ecornis* a better candidate for a successful bioremediation. Results of the physicochemical parameters showed a concentration dependent relationship. pH was observed to decrease with increasing concentration across effluent medium. Based on the findings in this study, it can be deduced that both test microalgae can be utilized for the bioremediation of cassava and maize effluent polluted areas.

CHAPTER ONE

1.0 INTRODUCTION

Cassava (*Manihot esculenta*) belongs to the family *Euphorbiaceae*, and the tubers are quite rich in carbohydrates with a deficiency in vitamins and protein. The high carbohydrate content of cassava makes it a staple food item especially for the lower income earners in most countries, especially Africa and Asia. Cassava roots contain toxic substances such as; cyanogenic glucosides, primarily linamarin, and a small amount of methyl linamarin (Uguru *et al*., 2019). The growing demand for high-quality cassava products has encouraged the establishment of medium to large scale processing factories which are mostly located in rural and sub-urban communities due to accessibility to fresh cassava tubers. Similar to other agro-processing activities, cassava processing consumes enormous amount of freshwater (Lawal *et al*., 2019). In the past, the impacts of these factories are barely noticed by regulatory bodies due to the low volume of generated effluent with negligible environmental effects. In recent times, the enormous wastewater currently generated by these factories is currently alarming due to massive environmental pollution and contamination of freshwater sources. Cassava effluent is basically generated from root washing, soaking/fermentation, retting and decanting. Negative impacts from cassava mill effluent discharge include environmental degradation, reduction of the aesthetic value of the surrounding such as offensive odor, and contamination of soils and groundwater. The flow of generated effluent into streams and rivers affects aquatic plants and animals since the polluting constituents like cyanide and heavy metals exceed permissible limits (Lawal *et al*., 2019).

Before biogas production was developed, cassava pulp was mainly used for generating methane through the anaerobic process of methanogenesis. The concentrations of carbon, nitrogen, and phosphorous in this wastewater are relatively low after anaerobic digestion for biogas production. However, the effluent nutrient contents are still higher than the threshold for direct discharge to the environment. Most of the treatment methods nowadays rely on conditioning ponds employing the native organisms in the pond to treat the wastewater (Paddock *et al.,* 2019). A research by Ibiam (2025) discovered substantial volumes of cassava mill effluent of wastes and cassava peels are discarded annually in Nigeria when cassava is processed into garri. This waste ends up in open dumps or drainage systems, threatening surface water, ground water and soil quality. It is therefore necessary and expedient to convert this waste to bioethanol rather than allowing them to further degrade the environment. The main process variables which affect the hydrolysis and fermentation processes are concentration of enzymes, temperature, time, yeast concentration and pH. There is limited research on the optimization of these variables form maximum reducing sugar and bioethanol yield respectively. Treatment of cassava effluent with organic soap solution can significantly degrade the cyanide, pH, bacterial coliform count, and heavy metals content of cassava effluent. Wastewater from cassava processing mills can be effectively treated using a combination of hydrogen peroxide oxidation and sorption with ZnCl2 at activation levels of 2:3 and 1:1. Nigeria, like others developing countries, bioremediation of cassava effluent is still uncommon due to dearth of information (Uguru *et al*., 2019).

Maize (*Zea mays*) which belongs to the family: *Poaceae* is one of the most important Crops in the word and preferred staple food for more than 1 billion people in sub Saharan Africa and Latin America (Obi *et al*., 2022). Maize is one of the most important cereals which is a major source of nutrient for humans, feed and fodder for livestock, while maize silage is one of the most valuable forages for ruminants, maize is also used for different purposes; it serves as a basic raw material for the production Of starch, oil, protein, alcoholic beverages, food Sweeteners and, more recently, fuel (Olawuyi *et al*., 2018). Fermented maize is a widely utilized food in African countries and in fact cereals account for as much as 77% of total caloric consumption. Maize is rich in carbohydrates, including Potassium and minerals, Magnesium. It contains trace amounts of Lysine and tryptophan, contributing to the low content of protein, and trace amounts of B-Vitamins.

According to Obi (2022), Akamu, also known as “ogi” is a fermented Product usually from maize which is grown in all parts of Nigeria. Traditionally, akamu is produced after maize grains (usually White and Yellow maize) are steeped in water continuously for three days, in some cases the steep water is changed during the two days of fermentation. The effect of processing method (changing and not changing the steep water) on the nutrient status of the akamu so produced is investigated. After fermentation, the grains are rinsed in clean water, wet milled and sieved with a clean sterile muslin cloth. The filtrate is allowed to settle and the supernatant drained off. The resulting slurry paste is known traditionally as *Akamu* (Obi *et al*., 2022). Depending on the raw material processed, the sludge may be rich in carbohydrates, lipids or proteins. The production of maize produces a high volume of wastewater, with high chemical (COD) and biochemical oxygen demands (BOD). After mechanical wastewater treatment, the COD of the sludge may be more than 100kgm^{-3}, because of the high content of the corn starch. Ewida (2020) evaluated the organic content of corn processing sludge and found that COD and BOD concentrations were 70 and 58kgm^{-3}. Maize processing wastewater was enriched with a high content of nitrogenous and organic compounds. The indigenous yeast and bacteria, under ambient conditions with diffusion of excessive oxygen could be removed up to 85% of nitrogenous compounds and up to 95% of organic matters. The use of bioremediation technique for wastewater treatment before discharge to the sewage system is recommended. Akamu wastewater, which is ecologically friendly, can be used as an alternative to the conventional mineral acid known as sulfuric acid (H2SO4). To reduce the environmental impact of sulfuric acid, this serves as the electrolyte in the lead-acid accumulators and discharged onto the ecosystem (Ikunga *et al*., 2023).

Microalgae are photosynthetic eukaryotic microorganisms of colonial, unicellular or filamentous structure with sizes from 10µm to 200µm, these organisms can present an autotrophic (production of biomass from inorganic matter and light) or mixotrophic (production of biomass through photosynthesis and organic compounds simultaneously) metabolism. Microalgae has long been recognized as a critical microorganism in wastewater and sewage treatment (Paddock *et al.,* 2019). Three significant factors greatly influenced the development of microalgae wastewater treatment systems: fear of food insecurity, the housing boom, and the rise of environmentalism. Microalgae have been reported to be versatile and effective remediation agents in wastewater treatment (Padri *et al*., 2021). These factors led to making sizable investments in non-military projects as an effort to increase the production of resources (Paddock *et al*., 2019). This funding spurred Interest in new areas of engineering and science: one such area being the mass cultivation of microalgae, particularly for production of food and treatment of wastewater. Early studies of microalgae on wastewater were done to test their ability to aerate and treat wastewater. Most of this research were performed by William .J. Oswald and his fellow researchers at the University of California, Berkeley (Paddock *et al*., 2019). Since then, international efforts have backed in-depth research into the development of microalgae wastewater treatment. The use of microalgae for wastewater treatment has long been considered a viable method of treating waste streams while producing high-value microalgae biomass. According to Alalawy (2023) microalgae is a potential candidate for wastewater treatment due to its ability to remove Nitrogen and Phosphorus, inorganic or organic carbon, and other elements from wastewater. Microalgae cultivation combined with wastewater treatment has many advantages in the production of biofuels.

1.2 AIM AND OBJECTIVES

Aim

The aim of this study is to determine the effect of varying concentrations of cassava mill effluent and maize effluent on the microbial growth of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii.*

Objectives

The objectives of this study were to:

- determine the effect of varying concentrations of cassava mill effluent and maize effluent on the microbial growth of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii.*
- determine the percentage inhibition of cassava mill effluent and maize effluent on the growth of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii.*
- assess some of the physicochemical composition of the cassava mill effluent and maize effluent samples before and after experiment.
- assess the bioremediation potential of the selected microalgae.

CHAPTER TWO

2.0 LITERATURE REVIEW

Understanding the effects of cassava mill and maize effluents on microalgae is essential for assessing their ecological impacts and guiding sustainable wastewater management practices. Below are previous literature reviews on researches carried out as relates to the topic arranged in chronological order.

Budiyono and Kusoworo (2011) conducted a study to evaluate the integrated process for biogas production and purification from cassava starch effluent using microalgae as a biostabilizing and purification agent. Experiments were conducted in duplicate samples at ambient temperature for 30 days in 5L anaerobic biodigesters. The feed mixtures used various combinations of cassava starch effluent, yeast, ruminant bacteria, urea, and microalgae. The process included daily pH monitoring and correction with an Na_2CO_3 buffer when the pH fell below 6.8. A semi-continuous process involved regular feeding and fresh yeast addition of 0.08% concentration to stimulate production. It was observed in this study that yeast was highly effective, significantly accelerating and increasing biogas production. The highest total biogas yield (726.43 mg/L total dissolved solid) was achieved by the mixture without microalgae (Tank 1 having effluent + yeast + ruminant bacteria + urea). Adding microalgae resulted in a significantly lower yield in Tank 5: 189 mg/L TDS). A direct comparison of Tank 2 to Tank 4 showed that microalgae did not have a significant effect on biogas production when was used as a nitrogen source. The authors suggested that the main challenge is acid buildup and that microalgae are the “only method” to act as a pH biostabilizator and a CO_2 purifier to achieve high methane content. However, the experimental results contradicted this suggestion regarding production, the highest biogas output came from the mix without microalgae, and the microalgae-containing mixes showed significantly lower yields, indicating that microalgae did not influence biogas production exceedingly.

McConnell and Farag (2012) investigated the possibility of cultivating microalgae in wastewater offshore as a means of minimizing the use of land and freshwater resources and lowering the cost of biodiesel manufacturing. The goal of the study was to transition from terrestrial microalgae photobioreactors (PBRs) to offshore PBRs that float on the surface of the ocean and use wastewater as a growth medium. Since it can flourish in both freshwater and wastewater, the green algae species Chlorella sp. (C2) was employed. Three different kinds of PBRs were used to cultivate the algae: 2-liter glass flasks, vertical transparent plastic bags, and horizontal floating transparent plastic bags. The growth media was composed of freshwater (either distilled or reverse osmosis water) and ultraviolet (UV) treated municipal wastewater, with and without additional nutrients. The UV-treated municipal wastewater came from a plant located in Dover, New Hampshire. To guarantee its safety, the wastewater was treated to eliminate pathogens. For comparison, some experiments had a typical nutrient medium added. Daily monitoring of algal development was done using cell counts under a microscope with a hemocytometer and turbidity measurements using a spectrophotometer at a wavelength of 682 nm. The levels of nitrate, nitrite, and pH were also monitored every day. To avoid cell death from nutrient depletion, algae were collected during the stationary phase. The algal solution was dewatered using a centrifuge after harvesting, and the resulting dry algal biomass was freeze-dried. The oil was then extracted from the dried algae using hexane as a solvent. The freshwater medium with standard nutrients produced a higher microalgae concentration (1.76 g/L) in the horizontal floating PBRs than the treated wastewater medium (1.30 g/L). In wastewater without extra nutrients, algal production was much lower than in nutrient-rich media. The oil content of algae cultivated in the freshwater medium in the horizontal floating PBRs was 7.29 mass%, while the oil content of algae grown in the treated wastewater medium was lower, at 2.86 mass%. According to the study, the higher oil content in the algae was caused by nitrogen starvation in the nutrient-deficient wastewater. The maximum amount of oil, at a concentration of 128 mg per liter of medium, was produced by the freshwater medium in the horizontal floating plastic bag PBR. The wastewater medium in the same PBR type generated a considerably higher oil output (37.2 mg/L). The most oil (117 mg/L) was also produced in the freshwater with nutrients in the vertical hanging bag PBR. The least effective way to produce algae oil was using glass flasks on the ground. The study's employment of UV-treated wastewater to assure safety and prevent the proliferation of rival species is a significant factor for any wastewater source, including wastewater from fermented foods, which may harbor a wide range of bacteria.

Akinbami *et al.* (2014) carried out a study to investigate the viability of using anaerobic digestion to produce biogas from cassava starch wastewater. The study used cassava starch effluent (CSE) taken from a cassava processing plant in Ile-Ife, Nigeria. The physicochemical characteristics of the CSE were evaluated, including pH, total dissolved solids (TDS), total volatile solids (TVS), and total solids (TS). The experiments were carried out over a 40-day period in 2.0 L anaerobic batch bioreactors, where the CSE was inoculated with cow dung to supply the necessary methanogen microbes. Four different pH levels (5.5, 6.0, 6.5, and 7.0) and two temperatures (35°C and 55°C) were tested. The water displacement method was used to measure the volume of biogas produced by each reactor every day. The study demonstrated that biogas may be produced using cassava starch effluent as a viable feedstock. The greatest amount of biogas 1,480 milliliters was produced at a pH of 6.5 and a temperature of 35°C. The cumulative biogas output was 36.6 ml/g total volatile solids (TVS) under this ideal state, and the pH had a considerable impact on the biogas yield, with neutral to somewhat acidic conditions being the most conducive. The study further demonstrated that the biogas generated had a high concentration of methane, demonstrating its promise as a sustainable energy source. The researchers aimed to show that cassava starch wastewater, a significant environmental hazard, may be converted into a useful and sustainable energy source.

The purpose of the study by Gani *et al*. (2016) was to examine the use of freshwater microalgae, particularly *Botryococcus* sp., for the phytoremediation of food processing wastewater in outdoor environments. The purpose of this study was to see if wastewater from food processing might be a viable medium for growing microalgae without the use of synthetic fertilizers. Two concentrations of wastewater discharge from a Malaysian food processing plant were prepared for the experiment: a "pure" sample (100% wastewater) and a "diluted" sample (50% wastewater, mixed with sterile distilled water). At a starting concentration of 1.0 x 103 cells/mL, *Botryococcus* sp., a freshwater microalga, was introduced into these wastewater samples. The growth was done in flasks kept in sunshine for 15 days, with regular shaking to promote homogenization. The experiment tracked the development of microalgae by measuring cell concentration with a hemocytometer. The efficacy of pollutant removal was assessed at 0, 7, and 15 days by measuring important wastewater indicators, including nitrate, phosphate, Total Organic Carbon (TOC), Chemical Oxygen Demand (COD), and Biochemical Oxygen Demand (BOD). According to the study, Botryococcus sp. Successfully removed contaminants from food processing wastewater and grew well. The microalgae achieved its maximum cell density of around 3.72 x 106 cells/mL in the pure (100%) wastewater sample on day 12. In the diluted (50%) sample, the maximum growth was roughly 9.7 x 105 cells/mL on day 13. After 15 days of treatment, the pure wastewater sample showed the most effective removal of nutrients. Nitrate (86.62%), phosphate (78.23%), and TOC (76.66%) had the greatest removal percentages. In the unadulterated sample, there were also notable decreases in BOD (61.11%) and COD (70.68%). Because there were more nutrients accessible for microalgae development in the undiluted wastewater compared to the diluted sample, the research determined that it was superior for phycoremediation.

Using cassava processing wastewater as a growth platform, Neves *et al.* (2015) assessed the manufacture of third-generation biodiesel from the microalga *Phormidium autumnale*. The study examined the quality of the resulting biofuel while concentrating on evaluating various operational methods of a bioreactor (batch and fed-batch). The cyanobacterium *Phormidium autumnale*, which is renowned for its resilience and capacity to flourish in harsh conditions, was cultivated in axenic culture. The culture medium was cassava wastewater from a flour business in Brazil. The wastewater was collected every month and its physical and chemical characteristics, such as pH, chemical oxygen demand (COD), and total nitrogen, were examined. A bubble column bioreactor with a nominal working volume of 2.0 L was used for the experiments. The two tested operating modes were batch and fed-batch. The fed-batch method used pulses of cassava processing wastewater to keep the organic carbon level at its ideal level for growth. To assess the process's performance, cell biomass, organic carbon consumption, and total lipid concentration were measured. The fed-batch operational mode, according to the study, greatly enhanced the system's performance. This mode increased biomass production to 12.0 g L^{-1} and lipid production to 1.19 g L^{-1}, both of which were higher than what was attained in batch cultures. The organic carbon concentration was kept at an optimal level for the cells to continue their growth thanks to the continuous feeding method. Oleic acid was the major component of the microalga oil that was made, which were 60% saturated and 39% monounsaturated. This oil's biodiesel was determined to meet the requirements set by the United States, Europe, and Brazil. According to the study, this type of agro-industrial wastewater, which is high in nutrients and organic matter, can be a cost-effective medium for the cultivation of microalgae. The research makes a compelling argument for the economic and environmental advantages of this biotechnological method by successfully converting organic waste into a useful fuel.

Olawuyi *et al.* (2018) assessed the impact of liquid waste from a non-alcoholic beverage business on the development of maize genotypes. The purpose of the research was to ascertain whether this industrial wastewater might be used in a positive way in agriculture, particularly for irrigation farming. A complete randomized design was used in the experiment, which was conducted in a screenhouse. The International Institute of Tropical Agriculture (IITA) in Ibadan provided four maize genotypes: TZM 1439, TZM-29, TZM 1288, and TZM-1165. Liquid effluent was collected from a bottling business and diluted with distilled water to four concentrations: 0%, 80%, 90%, and 100%. Beginning ten days after planting, the soil in each container received 200 ml of the effluent every day. Height, stem length, and leaf count were among the plant growth characteristics that were assessed every three days for fifteen days. The effluent treatment of maize plants, particularly at the maximum concentration of 100%, yielded substantially superior growth results as compared to the control group, according to the results obtained. For example, the 100% effluent concentration led to greater plant height, stem length, and leaf count. Notably, some maize genotypes, such as TZM-1439, TZM-29, and TZM-1288, had the greatest favorable impact on all of the growth traits that were assessed. It was determined that the effluent from the non-alcoholic beverage business could be a potential source for irrigation agriculture.

The indigenous innovations in wet fufu paste manufacturing were evaluated by Lawal *et al.* (2019), with a focus on the potential, limitations, and processing risk implications. With the ultimate goal of recommending long-term solutions to lessen identified environmental and health hazards, the research sought to assess the quality of the produced wastewater by comparing it to national and international acceptable limits. Administering a structured questionnaire to 50 processing clusters in Ifo, Ogun

State, Nigeria. Oral interviews were also conducted with certain target audiences in order to corroborate the survey results and collect additional, specific data. This covered topics such as wastewater treatment methods, the machinery utilized, and processing procedures. Using accepted methods, wastewater samples from 15 processing clusters were taken and examined for a variety of physicochemical characteristics, including biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, heavy metals (cadmium, lead, and chromium), and total coliform count, in order to assess the environmental impact. The study discovered that around 47% of the wastewater parameters examined were over the allowable thresholds established by the National Environmental Standards and Regulations Enforcement Agency (NESREA) and the World Health Organization (WHO). In particular, the researchers discovered that the levels of heavy metals like cadmium, lead, and chromium were higher than the acceptable levels. The wastewater was further distinguished by a high COD (32,000 mg/L) and a low pH (3.73 to 3.86). The soaking and fermentation stage, which made up half of the total effluent, and the washing stage, which produced 39% of the total wastewater, were found to be the primary sources of wastewater. Open land disposal (50%), open drains (37%), and adjacent streams/rivers (13%) were the main methods of garbage disposal, according to research. The soaking and fermentation process, according to this study, produces a large amount of wastewater with a high organic content. Although some nutrients may be present in trace amounts when compared to other factors, this high organic load could be a potential resource for growing microalgae.

In a study conducted by Uguru *et al.* (2019) a bioremediation strategy for breaking down cassava effluent before it is released into the environment was assessed. The study looked at the potential of employing an organic soap solution to break down the cassava waste from processing mills. The study included a regulated trial with two groups: a treated group and a control group. The effluent from a processing mill was collected in a black container and stored at roughly 6°C. The organic soap solution was made using waste from palm fruit bunches. The trash was initially sun-dried, then burned to ashes, and finally dissolved in distilled water. The filtrate was then evaporated to dryness to produce crystals, which were used to manufacture the soap after the solution had been filtered. The last organic soap solution had a pH of 9.8 and an electrical conductivity of 1330µS/cm. The organic soap solution was applied at a concentration of 200g/25L to four dark plastic containers containing the cassava effluent. Over a six-day treatment period, eight parameters; pH, cyanide (Cn), chromium (Cr), zinc (Zn), nickel (Ni), phosphorus (P), potassium (K), and total coliform counts were monitored at 48-hour intervals in both the treated and control samples, which were kept in a dark chamber. It was found that the organic soap solution was very effective in breaking down the cassava waste. Over the course of six days, the cyanide concentration dropped from 3.33 mg/l to 0.08 mg/l representing a 97% reduction. Additionally, the overall coliform colonies in the effluent were greatly decreased by the organic soap solution which pointed to a decrease in pollution overtime. The pH of the samples was substantially raised by the treatment with organic soap solution from 4.93 to 7.20. In addition, the treatment raised the concentrations of potassium (from 28.2mg/l to 54.66mg/l) and phosphorus (from 15.39mg/l to 22.43mg/l) significantly. The author concluded that the treatment successfully broke down cyanide, heavy metals, and bacterial coliforms in the cassava wastewater. The quantities of potassium and phosphate, however, remained elevated following treatment, while the majority of the pollutant parameters were brought down to close to acceptable levels.

A study on the historical summary of the application of microalgae to wastewater treatment was conducted by Paddock *et al.* (2019). The authors follows the evolution of this application from the beginning of the 20th century to the present, noting the elements that have shaped it, such as environmental issues, population increase, and energy shortages. This study reviewed historical scientific literature, publications, and government initiatives pertaining to wastewater treatment and microalgae. To back up its historical narrative, the study drew on a variety of academic sources, ranging from early publications from the 1800s to more recent studies from the 2000s. In the early 1900s, microalgae were discovered to improve the effectiveness of wastewater treatment by aerating the water and ingesting waste. Because of concerns about food insecurity, the housing boom, and the growth of environmentalism, there was a considerable investment in microalgae research in the post-war period. As a result, there was an investigation into the use of microalgae-based systems for the large-scale production of food and treatment of wastewater. Research on utilizing microalgae for life support systems, where they could treat human waste, generate air, and supply food in enclosed environments such as submarines and spaceships, was sparked by the space race in the 1960s. The Aquatic Species Program (ASP) gave a big push during the 1970s energy crises to use wastewater as a raw material for the cultivation of microalgae for biofuel production, which encouraged research into alternative fuels. Driven by renewed concerns about climate change and energy independence, the study concludes that current research is primarily focused on employing microalgae wastewater treatment to lower greenhouse gas emissions and generate biofuels in a sustainable manner.

Onuorah *et al.* (2019) examined the probiotic characteristics of lactic acid bacteria (LAB) obtained from Akamu, a traditional Nigerian fermented weaning food produced from grains such as sorghum and maize. The goal of the study was to determine if these LAB isolates had features that would make them useful in the prevention and treatment of gastrointestinal illnesses. The characteristics of the isolated lactic acid bacteria were examined in vitro using well-established analytical techniques. The fermentation of the cereal slurries was monitored for crucial indicators such as a drop in pH, an increase in titratable acidity, and an increase in the total LAB count. The research identified a number of strains, including *Lactobacillus plantarum, L. brevis, L. fermentum,* and *Lactobacillus delbrueckii*. Additional tests were conducted to evaluate the probiotic potential of these isolates by analyzing their ability to thrive in fresh bovine bile, their antibiotic resistance, and their optimum growth at a certain pH (4.0). Their capacity to stick to the intestinal mucosa was also determined by measuring the hydrophobicity of the cell surface. Numerous lactic acid bacteria strains with probiotic properties were successfully discovered in the study. The isolates thrived in fresh bovine bile and had ideal growth conditions at a pH of 4.0 and a salt concentration of 3.0%. With the exception of *Lactobacillus brevis*, all the isolates showed the capacity to stick to the lining of the intestines. The majority of the antibiotics used in the trial were found to be ineffective against the bacteria. The lactic acid bacteria recovered from akamu were found to have probiotic capabilities, suggesting that the cuisine may be helpful in the prevention and treatment of gastrointestinal illnesses.

The green microalga *Coelastrum morum* was used in a study by Adekanmi *et al.* (2020) to treat wastewater from fish ponds. The efficacy of the microalgae in eliminating pollutants and other wastes from the wastewater was assessed. Fresh fish pond effluent used in the study was taken from Agric, Oke Osun region, Osogbo, Nigeria. Using established procedures, the wastewater was tested for physicochemical characteristics, including pH, Total Dissolved Solids (TDS), Electrical Conductivity, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), ammonia, phosphate, and nitrate. Ten milliliters of *Coelastrum morum* were used to inoculate a 190-milliliter sample of the wastewater that had been sterilized. Under a cold fluorescent light, the inoculated sample was incubated for two weeks. To ascertain the removal efficiency, the physicochemical factors were then reanalyzed every seven days. The study discovered that *Coelastrum morum* was successful in eliminating contaminants from wastewater from fish ponds. The levels of numerous pollutants were greatly decreased by the microalgae. For nitrate, phosphate, ammonia, COD, BOD, and TDS, the removal efficiency was 75.77%, 80.14%, 85.96%, 82.86%, 79.80%, and 66.35%, respectively. Following treatment, the wastewater pH increased from 7.66 to 8.85. In contrast, the amount of dissolved oxygen fell by 68.35%. According to the study, treating fish pond wastewater biologically with *Coelastrum morum* might be a viable way to get rid of pollutants and other waste.

Ewida *et al.* (2020) employed native bacteria to lessen the pollutants in wastewater from maize processing in order to avert the environmental hazards that come with releasing untreated wastewater into bodies of water. The main goals of the study were to assess the wastewater's chemical and microbiological qualities and to track the breakdown of organic and nitrogenous substances. The experiment was carried out in triplicate *In-vitro*, using 5L flasks that were supplemented with 2L of wastewater from maize processing. For 30 days, the flasks were incubated in shaking ambient conditions at 120 rpm. The wastewater sample was also placed in a 2L flask with a control group, but it was kept in static incubation. The biodegradation process was tracked using a variety of indicators, such as pH, Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Total Kjeldahl Nitrogen (TKN). Counting viable bacterial and fungal cells allowed for monitoring the biomass growth rate. After the experiment the concentrations of COD, BOD, and TKN were found to have been significantly decreased. In particular, TKN was lowered from 2330 to 420 mg/L–1, COD from 12,000 to 430 mg/L–1, and BOD from 9000 to 220 mg/L–1. The pH of the wastewater rose from 6.9 to 9.3 during the course of the trial. The Dissolved Oxygen (DO) level ranged from 6 to 12 mg/L–1. The total number of bacterial cells increased from 2 x 106 to 4 x 1010 CFUmL-1, while the yeast count fell from 12 x 106 to 2.2 x 104 CFUmL-1. *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Saccharomyces cerevisiae* were the microbial strains determined to be responsible for the bio-treatment. The researcher concluded that utilizing native microorganisms is a viable and efficient biological approach for treating wastewater from maize processing industries.

Using cassava wastewater effluent as a synthetic ASM-1 medium Keilla *et al*. (2021) assessed the factors influencing the growth processes of *Scenedesmus sp*. Microalgae biomass and subsequently, the cultivation conditions, dry biomass, and lipid content through the study of cell lysis and extraction methods. The purpose of the study was to minimize the costs of a synthetic medium and utilize the available nutrients of the contaminant cassava effluent to produce microalgae biomass for biofuel applications. The growth of *Scenedesmus sp*. Was standardized in the synthetic ASM-1 medium to determine optimal concentrations of nitrogen, phosphorus, light exposure time, cultivation period, and biomass production. The microalgae was cultivated in an alternative culture medium formulated from cassava dilutions in ASM-1, from 5% to 100% effluent concentrations, with 0% concentration which served as the control. The cassava effluent was collected, sterilized by autoclaving, and filtered before use. Dry biomass concentration was determined daily using a spectrophotometer to read absorbance at 750 nm. Lipid extraction technique was conducted by combining pre-treatment methods and solvent systems using dry biomass. Extraction methods evaluated were the Bligh and Dyer method, modified with the use of ultrasound (for 30 minutes), and Soxhlet method. A pre-treatment of autoclaving for 15 minutes at 121°C and 1atm pressure was conducted. Chloroform-methanol and Chloroform-ethanol were evaluated by a 1:1 ratio. Biomass productivity and total lipids were compared with the synthetic ASM-1 medium and the diluted ASM-1 cassava effluent medium, and the lipid profile of the microalgae grown in both mediums was analyzed using quantitative gas chromatography. *Scenedesmus sp.* Microalgae showed better efficiency in dry biomass production in culture mediums supplemented with cassava (5%-10%) compared to the synthetic growth medium. Cultivation in cassava concentrations above 10% inhibited growth. All experiments using cassava-supplemented medium produced a higher percentage of lipids than the synthetic ASM-1 medium. The highest productivity of lipids (35.5%) was obtained with microalgae cultivated in the medium supplemented with 10% of cassava. The Bligh and Dyer method adapted with ultrasound and the chloroform-ethanol (CE) solvent system achieved the highest lipid yield of 19.27%. The same Bligh and Dyer with ultrasound method with the chloroform-methanol (CM) system yielded 19.94% using autoclave pre-treatment. The Soxhlet method with the chloroform-ethanol system resulted in a lower yield of 15.71%. The fatty acid composition was mostly composed of saturated fatty acids (SFAs) and mono-unsaturated fatty acids (MUFAs), which varied from 76% to 79% of total fatty acids in the cassava-supplemented cultures. *Scenedesmus* sp. Showed a low unsaturated content from 1.92 to 2.10, and low values of polyunsaturated fatty acids (PUFAs) of 19.28% to 23.96%.

In a study by Nayma *et al.* (2021) the comparative productivity of selected freshwater microalgae was evaluated. The study aimed to compare the growth rate and productivity of eight selected tropical freshwater microalgae species to screen for highly productive strains with fast growth and high lipid content for various commercial applications, including fishery and biofuel production. The researchers cultured eight species; *Nephrocytium* sp., *Nannochloropsis* sp., *Selenastrum* sp., *Sphaerocystis* sp., *Ankistrodesmus* sp., *Monoraphidium* sp., *Pectinodesmus* sp., and *Scenedesmus* sp. In a Bold Basal Medium (BBM). Cultures were grown in triplicates under controlled conditions: 24.0 ± 1.0°C and continuous light (150 $\mu Em^{-2}s^{-1}$), with gentle aeration, until the death phase. The cell density was measured daily using a Neubauer hemacytometer. This was used to calculate the specific growth rate (SGR), cell duplication time, and cell doublings per day. The biomass was determined by filtering 1mL of sample through pre-weighed filter paper, which was then dried and weighed to calculate volumetric and areal productivity. To calculate lipid productivity, the Bligh and Dyer method was employed. A one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to analyze the collected data. Significant differences were observed among the species: *Monoraphidium sp*. Performed best in growth and lipid accumulation, having the highest SGR in the lag phase (0.861±0.017 per day) and exponential phase (0.437 ± 0.016 per day). Also exhibited the highest cell doublings per day. *Scenedesmus sp.* Showed the highest biomass productivity with the volumetric productivity (47.738 ± 0.576 mg/L per day), highest areal productivity (4.774 ± 0.058 mg/cm^2/day). *Ankistrodesmus sp.* Had the highest SGR in the stationary phase. *Selenastrum sp.* Exhibited the lowest volumetric and areal productivity. *Monoraphidium* sp., *Scenedesmus* sp., and *Nannochloropsis* sp. Are superior indigenous tropical freshwater microalgae strains for commercial utilization, particularly as a potential source of biofuel. *Monoraphidium* sp. Was highlighted for its high lipid accumulation, making it a strong candidate for biodiesel production. The growth characteristics of *Scenedesmus sp.* (low cell duplication time) and *Monoraphidium* sp. (high cell doublings) indicate their high potential for mass production.

The study by Obi and Okoronkwo (2022) investigated the effect of steep water management (changed vs. unchanged) on the nutritional (proximate) and microbial profile of ibof (or ogi), a fermented maize product, using both White and Yellow maize varieties. After fermentation, the swollen grains were rinsed, wet milled, and sieved with a sterile muslin cloth. Samples of steep water were serially diluted and inoculated by spread plate method on suitable media (Nutrient, MacConkey, De Mann Rogosa Sharpe (MRS), and Sabourand Dextrose Agar). The plates for bacteria were incubated at 35°C for 24 hours, while fungal plates were incubated at 22°C for 5 days. Microbial isolates were characterized, and the microbial succession (change in microbial community over time) was determined every 24 hours. The researchers initially isolated bacteria including *Lactobacillus species, L. plantarum, Bacillus* *subtilis, Escherichia coli,* and *Staphylococcus aurues.* Fungi isolated included *Saccharomyces cerevisiae, Mucor alternaria,* and *Aspergillus flavus.* Maize fermentation successfully eliminated pathogenic and spoilage organisms (*S. aureus, E. coli, M. alternaria,* and *A. flavus*). The final ibof product contained only beneficial microbes: *Lactobacillus species, L. plantarum, Bacillus subtilis,* and the yeast *Saccharomyces cerevisiae.* The pH in the unchanged steep water dropped significantly from an initial 6.5 to as low as 3.5, driven by Lactic Acid Bacteria (LAB) activity. The population of microbes was higher in the unchanged water samples compared to the changed water samples, as changing the water reduced the starter culture. The study concluded that not changing the steep water during the 72-hour fermentation is the superior processing method, as it promotes a higher microbial load that successfully eliminates pathogens while simultaneously leading to an ibof product with significantly higher levels of protein, carbohydrate, and crude fibre.

Sedara *et al.* (2022) assessed the Impact of Cassava Processing Mill Effluent (CME) on Physical and Chemical Properties of Soil in Akure, Ondo State, Nigeria. The purpose of the research was evaluate the impact of heavy metal levels and chemical attributes from effluent discharge on the surrounding soil and to proffer solutions to improper disposal and analyze environmental hazards associated with CME. Soil samples were collected from three areas exposed to cassava mill effluent (labeled A, B, and C) in the Igbatoro community, Akure, Ondo State, Nigeria. Control samples (free from CME) were also obtained from two unique areas (labeled D and E). Samples were taken at depths of 15 cm, 30 cm, and 45 cm respectively for each area they were collected from. Samples were air dried for a week and were homogenized through a 2 mm sieve, and were analyzed in the laboratory. The quantitative chemical elements, including heavy metals (Na, Ca, K, Zn, Fe, Pb, and Cr) were determined through Atomic Absorption Spectrometry (AAS). Physicochemical parameters such as soil texture, soil porosity, particle size, Total Organic Carbon (TOC), pH, electrical conductivity (EC), Pb, Zn, Cr, Fe, K, Ca, and Na were taken note of. A one-way Analysis of Variance (ANOVA) was carried out to determine differences between the samples, and Duncan's Multiple Range Tests (DMRT) was used to isolate the mean of the samples. The analysis showed that soil samples with CME exceeded the WHO (World Health Organization), and FEPA (Federal Environmental Protection Agency) standards, indicating the cassava effluent has contaminated the soil and made it unsuitable for agricultural purposes. This also affected the environment and soil organic matter. Soil samples which received CME had a higher pH from 7.91 to 9.24, compared to control samples without the effluent (7.62 to 8.22), which exceeded the maximum neutral guideline of 7.00 recommended by FEPA and WHO. This alkalinity was due to the presence of hydrogen cyanide in the CME. The mean concentrations of Fe (Iron) recorded the highest values with Fe having the highest values at all sites, A, B, C, D and E (281.75±1.19, 254.99±17.97, 220.05±0.94, 217.20±5.76, and 192.15±1.43 respectively). The high range of Iron (Fe) suggested it's an important factor for chlorophyll synthesis, but the high levels due to pollution will massively reduce crop production. However, the metals exhibited hazardous concentrations based on WHO and FEPA standards. It was noted by the researchers an increase in Potassium and Iron, as well as the presence of Sodium and Calcium, in the effluent. CME had both negative and positive effects on the studied area and its surrounding environment.

The potential of utilizing freshwater microalgae for wastewater treatment under abiotic stressors, (notably salinity) was conducted by Alalawy *et al.* (2023). The researcher chose the freshwater microalgal strain *Desmodesmus communis* GEEL-12. The microalgae was grown in filtered municipal wastewater with NaCl concentrations ranging from 25 to 150 mM for 12 days. The experimental conditions were a regulated temperature of 25°C, a light intensity of 40 $\mu mol/m^2/s$, and a rotational speed of 150 rpm. A spectrophotometer was used to measure optical density at 680 nm in order to track the growth of microalgae during the course of the growth cycle. The specific growth rate was calculated, and the removal rates of total nitrogen (TN) and total phosphorus (TP) were measured. From the collected biomass, the projected biodiesel quality and fatty acid content were calculated. Results of the study showed that the microalgal development was greatest in the control group, which had no extra salt added, while high salinity levels (150 mM) significantly inhibited growth, indicating low tolerance. The removal efficiency of nitrogen and phosphorus decreased noticeably with higher salt concentrations, falling from 99% to 81% for nitrogen and from 5.9% to 5.0% for phosphorus when salt was added between 100 and 150 mM. Palmitic acid (C16:0) and stearic acid (C18:0) were the most prevalent fatty acids. Interestingly, under high NaCl concentrations (100–150 mM), the abundance of stearic acid (C18:0) increased from 49.37% to 56.87%.

The purpose of the study by Ikunga *et al.* (2023) was to test the viability of raw Akamu wastewater (rAWW), a biowaste effluent, as an environmentally friendly alternative electrolyte to conventional sulfuric acid in lead-acid accumulators. The rAWW was prepared by soaking and fermenting yellow maize, wet milling it, and pressing the resulting slurry to collect the wastewater. Initial pH of the rAWW was highly acidic with a pH of 1.50 ± 0.00. It contained multiple bacterial isolates, including *Staphylococcus aureus*, *E. coli*, *Bacillus sp.* And *Lactobacillus sp*. Chemical composition included organic acids, alcohols, and D-glucose 2, 3, 4, 5, 6-pentaacetate. A washed and disused 75 AH lead-acid accumulator was filled with the rAWW. The initial voltage of 11.75 ± 0.05 increased significantly to 12.20 ± 0.00 after 12 hours of charging. The accumulator was successfully used to drive a four-cylinder car for two years (24 months). The pH decreased further to 1.00 ± 0.00, with acidity attributed to the microbial fermentation of sugars into organic acids and alcohols. Only *Bacillus sp.* Was isolated post-usage, due to its high heat tolerance. The final chemical composition included organic acids, ketones, iboflavin, and a variety of other organic compounds. The study proposed that the organic acids in the rAWW reacted with lead (II) ions at the positive terminal to form soluble salts (e.g., lead lactate). This is significant because, unlike the insoluble $PbSO_4$ formed by sulfuric acid, these soluble salts would not have reduced the accumulator's efficiency. Furthermore, microbial catalytic activities are crucial for breaking down organic compounds, which released the electrons and protons necessary for current flow and power production.

Bellido-Pedraz *et al.* (2024) research was focused on the understanding the biotechnological potential of the microalgae *Chlamydomonas*. To achieve a comprehensive understanding of the potential of *Chlamydomonas* for bioremediation and bio-product production, the study highlighted the microalga's value in various fields, including the agricultural, medical, and industrial sectors. It was stated in this research that the specie *Chlamydomonas reinhardtii* is the most commonly used freshwater microalgae for the sustainable reduction of contaminants in wastewater due to its to thrive in heterotrophic and phototrophic conditions. It was observed in this study that *Chlamydomonas* exhibited a high capacity for fulfilling critical needs through its extensive metabolic diversity, rapid growth rates, and cost-effective production. The capability of *Chlamydomonas* for extracting a wide range of pollutants, was assessed, pollutants such as high removal efficiencies for Nitrogen, Phosphorus, and the Chemical Oxygen Demand (COD) in municipal, dairy, and industrial wastewater also the effective removal of heavy metals (including, Copper, Boron, Manganese, Arsenic, Nickel, Zinc, Cadmium, And Uranium) through biosorption and bioaccumulation. *Chlamydomonas* ability of biotransforming various compounds, including antibiotics, hormones (such as β-estradiol), organophosphorus pesticides, and microplastics. It was observed by the researchers that *C. reinhardtii* had the superior ability to fix CO_2, with 1g of biomass, sequestering 1.8g of CO_2. The study concludes that the microalga *Chlamydomonas* is a highly promising resource for a wide range of biotechnological applications. Bioremediation with

Chlamydomonas offers significant potential for sustainable contaminant reduction, resource recovery, and valorization of microalgal biomass, leading to important environmental and economic benefits.

Diaz *et al.* (2024) investigated the use of microalgae and cyanobacteria as a biological process for treating industrial sector effluents to reduce pollutant concentrations. The biological treatment, known as bioremediation, involved the degradation of organic matter by these microorganisms. The main advantage was the significant reduction of contaminant compounds due to the microorganisms' strong adaptation and tolerance. Positive outcomes included: high removal efficiency greater than 40% in removing pollutants like nitrogen, phosphorus, and Chemical Oxygen Demand (COD) from industrial and domestic effluents, over 50% reduction of nitrogen, phosphorus, and carbon compounds in specific effluents (e.g., brewery, dairy) was achieved, there was a reduction of heavy metals, emerging pollutants (via biosorption), and the elimination of color, odor, and fecal coliforms. Photobioreactors specifically reduced the color and nutrient concentration by 50%. The study highlights that microalgae not only treated the wastewater but also offered a path toward a sustainable, added-value technology. This dual-benefit approach (cleanup and product generation) makes microalgae-based treatment highly promising.

Salem *et al.* (2024) evaluated the effectiveness of selected microalgae in the phycoremediation (cleaning) of sewage effluent and then assessed the treated water's potential for safely irrigating ornamental plants to conserve fresh water. The study was conducted in two main phases using the microalgal strains *Chlorella vulgaris* and *Trichormus variabilis*. Sewage water samples were collected, and each microalgal strain was inoculated at a 5% concentration into 10-liter glass tanks containing 100% sewage water. The treatment process lasted for 30 days under illuminated and aerated conditions. The sewage water was analyzed after 10, 20, and 30 days to measure the reduction in parameters like pH, electrical conductivity (EC), Total Dissolved Solids

(TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), nutrients (N, P, K), and heavy metals. The ornamental plant *Tagetes erecta* (American marigold) was cultivated in a pot experiment. Plants were irrigated using six different water types, including domestic water (control), untreated sewage water, and sewage water treated with each of the two microalgal strains. After 45 days of planting, the plants and rhizosphere soil were analyzed for plant growth characteristics, pigments, NPK content, minerals, and soil microbial activity (dehydrogenase activity and CO2 levels). The microalgal strains were found to be very effective in the bioremediation of sewage water. Both microalgal strains significantly reduced the organic load, with reductions of over 91% for BOD and over 92% for COD. There was a significant decrease in essential nutrients. *C. vulgaris* reduced nitrate by 66%, ammonium by 74%, phosphate by 66.2%, and potassium by 70%. The treated sewage water also showed a decrease in pH, EC, and TDS. Irrigation with the treated water led to an increase in microbial activities (dehydrogenase activity and CO2 levels) in the rhizosphere soil. The phycoremediation process successfully converted raw sewage water into a reusable source. The wastewater treated by *C. vulgaris and T. variabilis* could be used effectively and safely for the irrigation of ornamental plants.

The study by Villaro-Cos *et al.* (2024) was conducted to determine how various water and nutrient sources impact the biochemical makeup of the microalga *Tetradesmus almeriensis.* The purpose of the study was to completely describe the microalga biomass produced using different media in order to forecast its possible commercial applications, and was carried out on a pilot scale as a continuation of a previous study. The four distinct culture media employed were diluted fresh pig slurry, primary urban wastewater, secondary urban wastewater, and freshwater with agricultural fertilizers. A dilution rate of 0.3 per day was used in a semi-continuous manner to generate the microalga biomass. After being centrifuged, freeze-dried, and stored for analysis, the biomass was harvested. Microalgae cultivated in wastewater and pig slurry had a higher protein content and were thought to have effective use in agricultural goods and animal feed. The biomass had the greatest protein content when it was made with primary urban wastewater (52.9 $g \cdot 100\ g^{-1}$) and secondary urban wastewater (48.0 $g \cdot 100\ g^{-1}$). Likewise, the lipid concentration was highest in the main wastewater sample (8.6 $g \cdot 100\ g^{-1}$). The biomass from diluted pig slurry had the greatest amount of monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). Compared to those cultivated in freshwater, all biomasses produced using treated wastewater had a greater concentration of all essential amino acids. The concentration of polyunsaturated fatty acids (PUFAs) in the four assessed samples ranged from 15.2 mg g^{-1} to 25.8 mg g^{-1}.

In a study on using a mixed culture of microalgae (*Chlorella vulgaris* and *Micrococcus luteus*) for wastewater bioremediation, Hamed and Abdelfetah (2024) assessed the effectiveness of this eco-friendly mixed microalgal culture in treating wastewater by monitoring the removal efficiencies of pollutants such as ammonium nitrogen NH3, phosphorus PO4, biochemical oxygen demand (BOD_5), and chemical oxygen demand (COD). The ten-day laboratory experiment investigated the impact of different wastewater concentrations of 40% to 70%, and the temperature ranges: 20°C to 35°C. The study found that both wastewater concentration and temperature significantly affected the microalgae's performance. The optimum conditions for overall maximum pollutant removal were found to be the lowest mixing concentration of 40% and a temperature of 30oC. Under these optimum conditions, the highest achieved removal efficiencies were Ammonium nitrogen NH_3 (93%), Phosphorus PO_4 (91%), BOD_5 of 87%, and COD having 82%. The results confirmed that the mixed culture of *Chlorella vulgaris* and *Micrococcus luteus* was highly effective at removing wastewater pollutants in a short time. This remarkable efficiency highlights the great potential for this mixed microalgae system as a promising, eco-friendly green technology for practical wastewater treatment applications.

The goal of the study by Ibiam *et al.* (2025) was to determine the best way to make bioethanol from cassava peels and cassava mill effluent, two different waste materials. Bioethanol was produced using a method that involved several steps. To break down complex carbohydrates, the cassava peel samples and cassava mill effluent were initially pretreated and hydrolyzed. The bacteria *Zymomonas mobilis* was used to ferment the resultant sugar solution. Using a classical optimization approach that varies one variable at a time to ascertain the best fermentation conditions. The impacts of temperature, agitation rate, reaction time, pH, and yeast concentration on the bioethanol yield were studied. Using ASTM methods, the manufactured bioethanol was then assessed for its fuel properties to make sure it met standard standards. Both cassava peel and cassava mill waste were effectively used to make bioethanol. A temperature of 80°C, an agitation speed of 400 rpm, a reaction duration of 4 hours, a pH of 6, and a yeast concentration of 2.5 (w/w) were determined to be the ideal conditions for bioethanol production from cassava mill effluent. At a yeast concentration of 2.5, the maximum yield from cassava mill effluent was 28.41 g/L. According to the study, cassava peels and cassava mill effluent are both excellent and sustainable raw materials for bioethanol production, and their utilization may help solve Nigeria's waste management and energy supply issues. In order to create a sustainable and environmentally sound alternative to fossil fuels, the study attempted to utilize the high carbohydrate content of these agricultural byproducts, which are plentiful in Nigeria.

Isimah *et al.* (2025) conducted a geospatial assessment of the environmental impact of Cassava Mill Effluent (CME) in the Ika North East Local Government Area of Delta State, Nigeria, focusing on its effects on soil quality and the environment. The purpose was to evaluate the after effects of CME on soil and human health, determine the spatial randomness of mill locations, and perform a geospatial analysis of the contamination spread. Various samples were collected (affected soil, unaffected control soil, and cassava mill effluent) from three communities (Owa-Ofie, Akumazi, Ute-Okpu). These samples were subjected to physicochemical analysis for parameters such as pH, EC, N, Cl, Cu, Fe, Zn, Pb, Ca, Mg, and K, using standard methods like Atomic Absorption Spectrophotometry (AAS), with results compared to WHO/FEPA safe limits. Student's t-test was used to determine the significance of the difference between the affected and unaffected soils. Geospatial analysis employed ArcGIS software, using Inverse Distance Weighting (IDW) for spatial interpolation to create a continuous surface of effluent concentration and buffer analysis to map the extent of effluent spread. The study highlighted significant environmental degradation, with the mean pH of the affected soil being 4.92 was found to be acidic and below WHO/FEPA standards, a significant change from the neutral unaffected soil (7.00). electrical conductivity (EC) in the wastewater and impacted soil achieved a maximum value of 2210s/cm, which significantly exceeded the WHO/FEPA standard of 1000s/cm. The Iron (Fe) content was high in the affected soil (116.2795 mg/L). Lead (Pb) concentrations in affected soil (0.1489 mg/L) and CME (0.5957 mg/L) were slightly higher than the WHO/FEPA guidelines. The distribution of cassava mills was determined to be random (P=0.000***), meaning their locations were neither clustered nor patterned. The researchers concluded that the effluent was found to hinder agricultural production, reduce soil fertility, and disrupt ecosystems. The low pH from cyanogenic glycosides in the effluent was found to be toxic and destroyed the microorganisms. The high concentration of soluble salt found in the impacted soil and effluent could lead to eutrophication, a process that involves the excessive growth of plant life, including microalgae, in water bodies.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the University of Benin, Benin City, Edo State, Nigeria. The experimental procedures were conducted at the Limnology and Phycology Laboratory of the Plant Biology and Biotechnology department.

3.2 Test microalgae

Two species of Microalgae (*Scenedesmus ecornis* and *Chlamydomonas reinhardtii*) were used for the study.

3.3 Collection of test microalgae

Samples from blooms of algae were collected from the Faculty of Agriculture fish pond University of Benin, Benin City and from several ponds and ditches within the city.

3.4 Isolation of pure culture of microalgae

Monoculture of the test microalgae were obtained by isolating desired algae from the mixed algal samples and inoculating into a growth medium (Chu 10). Microalga cultures were obtained after repeated series of subcultures till a microalgae culture was obtained. A small portion of the microalga portion was taken for microscopic examination to confirm the algal species prior to inoculation.

3.5 Botany test of Microalgae

3.5.1 *Scenedesmus ecornis*

Scenedesmus is a non-motile, colonial green microalga commonly found in freshwater. Its cells are usually arranged in colonies of 2, 4, 8, or more cells aligned side by side. It is widely studied in wastewater treatment and biofuel production because of its fast growth and ability to remove nutrients and pollutants. *Scenedesmus* is especially noted for its capacity in phytoremediation (The use of algae to remediate polluted water). It is widely used in wastewater treatment due to its ability to remove nitrogen and phosphorus efficiently, while also producing biomass that can be used for biofuel production

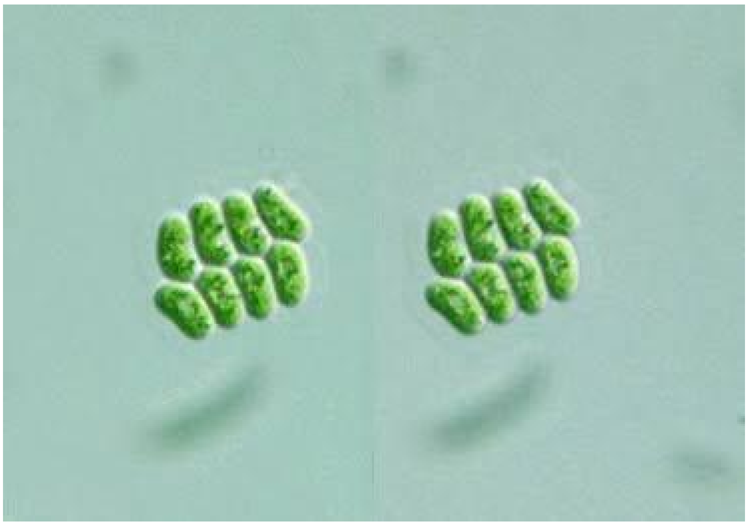

Plate 3.1: A photomicrograph of Scenedesmus ecornis

Taxonomic Classification of *Scenedesmus*

Kingdom: Plantae

Division: Chlorophyta

Class: Chlorophyceae

Order: Sphaeropleales

Family: Scenedesmaceae

Genus: *Scenedesmus*

Species: *Scenedesmus ecornis*

3.5.2 *Chlamydomonas reinhardtii*

Chlamydomonas is a unicellular, motile green microalga characterized by two anterior flagella, a cup-shaped chloroplast, and a red eyespot for light detection. It inhabits freshwater and soil environments. Chlamydomonas is widely used as a model organism in molecular biology, photosynthesis studies, and algal biotechnology. Members of this genus are cosmopolitan, occurring in freshwater, soil, and even snow habitats. One species, *Chlamydomonas reinhardtii*, is extensively studied as a model organism for photosynthesis, flagella function, and molecular genetics.

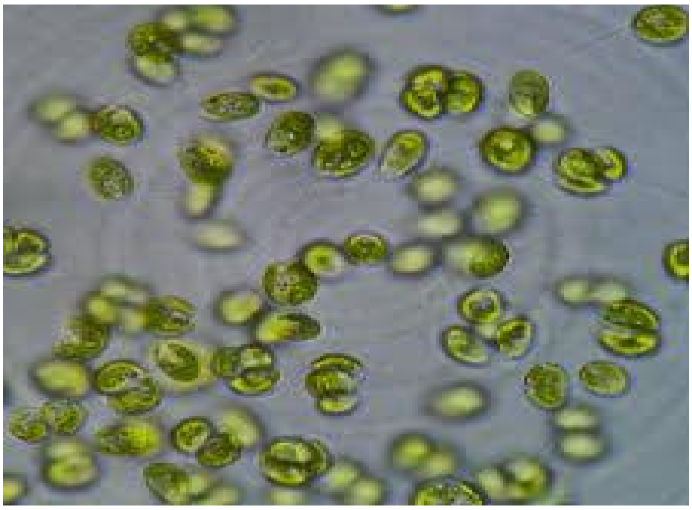

Plate 3.2: A photomicrograph of Chlamydomonas reinhardtii

Taxonomic Classification of *Chlamydomonas*

Kingdom: Plantae

Division: Chlorophyta

Class: Chlorophyceae

Order: Chlamydomonadales

Family: Chlamydomonadaceae

Genus: *Chlamydomonas*

Species: *Chlamydomonas reinhardtii*

3.6 Preparation of culture media

The microalgae species were grown in an artificial medium mixed with the cassava processing effluent and maize effluent separately. Chu modified number 10 was used in mixture with the cassava mill effluent, and maize effluent. The composition of the Chu10 modified medium is shown in table 3.1 below:

3.7 Composition of modified Chu10 culture medium

3.7.1 Macronutrient Stock Solution

A stock solution medium was made by dissolving the salts listed in the amount indicated (in grams) each in 100ml of distilled water as shown in table 3.1.

3.7.2 Trace Element Stock Solution

Trace element stock solution was made by dissolving the salts below in the amount (mg) indicated in 1L of distilled water.

3.7.3 Iron Stock Solution

Iron stock solution was prepared by adding 3.35g of citric acid ($C_6H_8O_7.H_2O$) and 50ml of distilled water, followed by the addition of 3.35g of ferric citrate ($FeC_6H_5O_3.5H_2O$). The volume was then brought up to 100ml final volume.

3.7.4 Vitamin Stock Solution

Vitamin stock solution is needed for essential micronutrients for optimal microalgae growth. It was prepared by calculating individual vitamin needs, dissolving them in distilled water using a stir plate, combining them in a volumetric flask and the volume adjusted.

Table 3.1: Preparation of Chu 10 medium

Macronutrient	**g/100ml**
$CaCl_2.\ 2H_2O$	3.67
$MgSO_4.7H_2O$	3.69
$NaHCO_3$	1.26
K_2HPO_4	0.84
$NaNO_3$	8.5
$NaSiO_3$	2.84
Trace Element Stock	**mg/L**
$CuSO_4.5H_2O$	19.6

$ZnSO_4.7H_2O$	44
$CaCl_2.6H_2O$	20
$MnCl_2.4H_2O$	36
$NaMO_4.2H_2O$	12.6
H_2BO_3	618.4
Iron Stock Solution	**g/100ml**
Citric acid ($C_6H_8O_7.H_2O$)	3.35
Ferric citrate ($FeC_6H_5O_3.5H_2O$)	3.35
Vitamin Stock Solution	**g/100ml**
Cyanocobalamin (B12)	0.004
Thiamine	0.004
Biotin	0.004

3.8 Culture Vessels

The study made use of bottles with a capacity of 250ml purchased brand new. The bottles were thoroughly cleaned using tap water and were rinsed severally with distilled water and left to air dry prior to utilization. They were then labeled to correspond with the test algae species and the specific cassava mill and maize effluent concentrations been studied.

3.9 Effluents Sources

The cassava mill effluent was gotten from a cassava grinding mill at Ekhenuan, Benin city, and maize effluent was gotten from a local Pap (maize) producer at Sapele road, Benin City, Edo State.

3.10 Proportionality of Volume of Cassava mill and Maize Effluent to Volume of Chu10 Medium

Effluents concentration of treatment samples was prepared by adding the volume stock solutions and growth medium as shown in table 3.2 below;

3.11 Experimental Setup

The experiment comprised of seven different treatments, with each one having three (3) replicates. The concentrations were 0% (which served as the control), 5%, 10%, 25%, 50%, 75% and 100%. The concentrations were prepared by mixing the effluents with the growth medium, Chu10, as indicated in table 3.2. The different treatments were obtained by serial dilution of the effluents with the growth medium.

3.12 Inoculation

Each culture vessel containing 150ml of the combined culture media/effluent or stock solutions were inoculated with 5ml of the unialgal culture obtained. After inoculation, the vessels were partially capped to enable effective respiration while preventing contamination. Thereafter, an aliquot of the mixture was read.

Table 3.2 Preparation of different concentrations of treatment

Concentration% Added	Volume of Chu10 added (ml)	Volume of effluent (ml)
0	900	0
5	855	45
10	810	90
25	675	225
50	450	450
75	225	675
100	0	900

3.13 Growth response of microalgae

The algae growth rate was measured using a V721 visible spectrophotometer and measurements taken at two-day intervals for 14 days. To start, the spectrophotometer was powered on, about 15 to 20 minutes before use, to make sure it was stabilized. The equipment was set to a wavelength of 750nm before any readings were conducted.

To prepare to take readings, distilled water was added and the cuvette's reset button was pressed to zero the equipment. Samples were introduced into a matching cuvette subsequently and with the lid closed, readings displayed were recorded in a lab notebook. This routine was uniformly performed across all concentrations and replicates of the cassava mill effluent and maize effluent. Upon completion of readings, the bottles were placed and arranged at a North facing window to enable sunlight exposure. Order of placement of the replicates was systematically changed after each set of readings to enable uniform sunlight exposure and the process was repeated every other reading day throughout the 14 days.

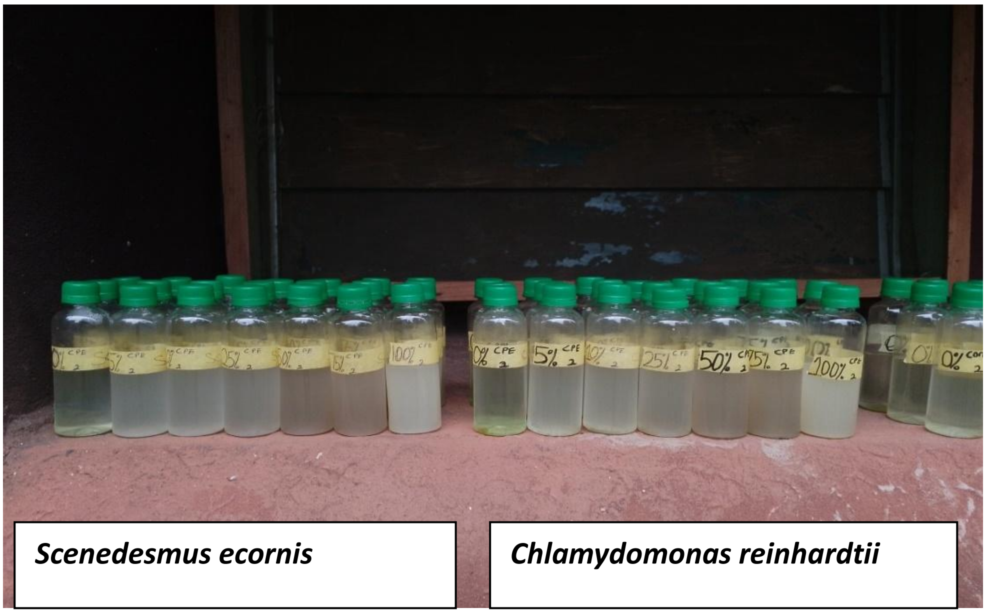

Plate 3.3a Scenedesmus ecornis and Chlamydomonas reinhardtii at day 0 in culture media of Cassava mill effluent

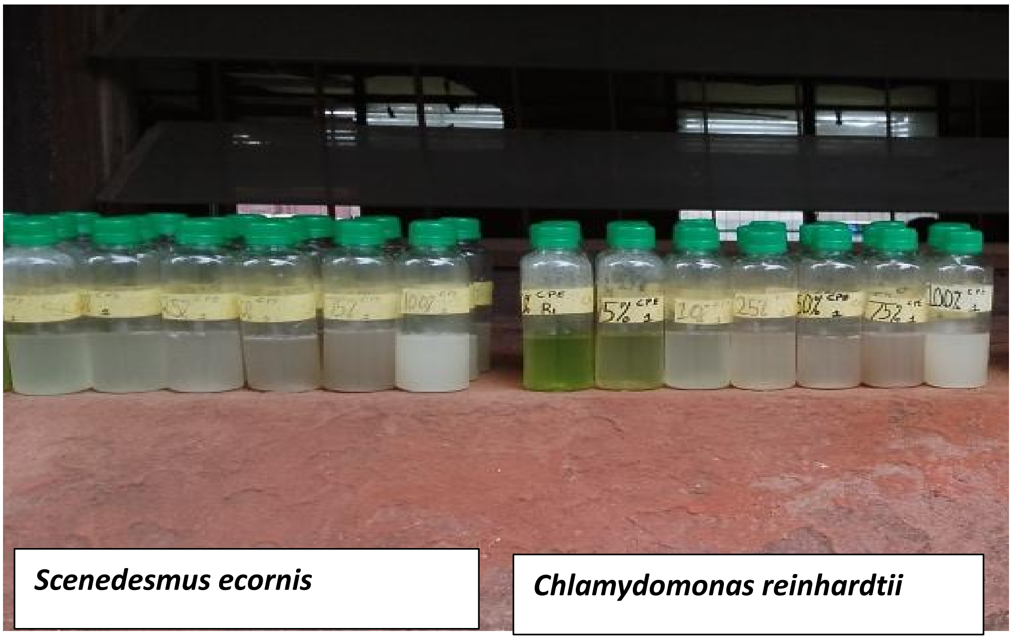

Plate 3.4a *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* at day 14 in culture media of Cassava mill effluent

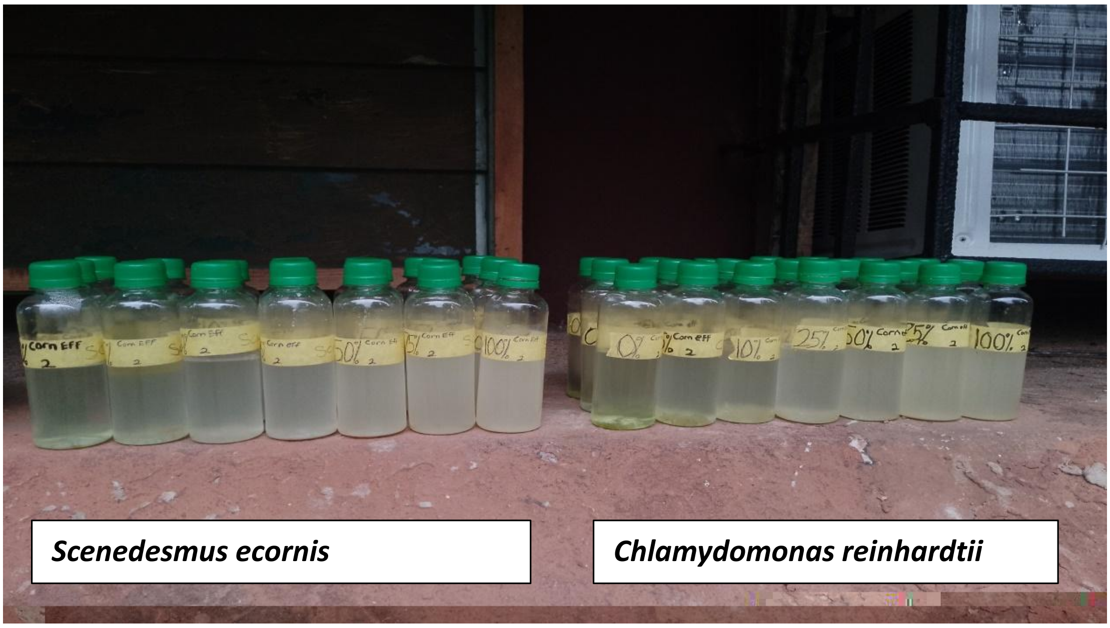

Plate 3.3b Scenedesmus ecornis and Chlamydomonas reinhardtii at day 0 in culture media of maize effluent

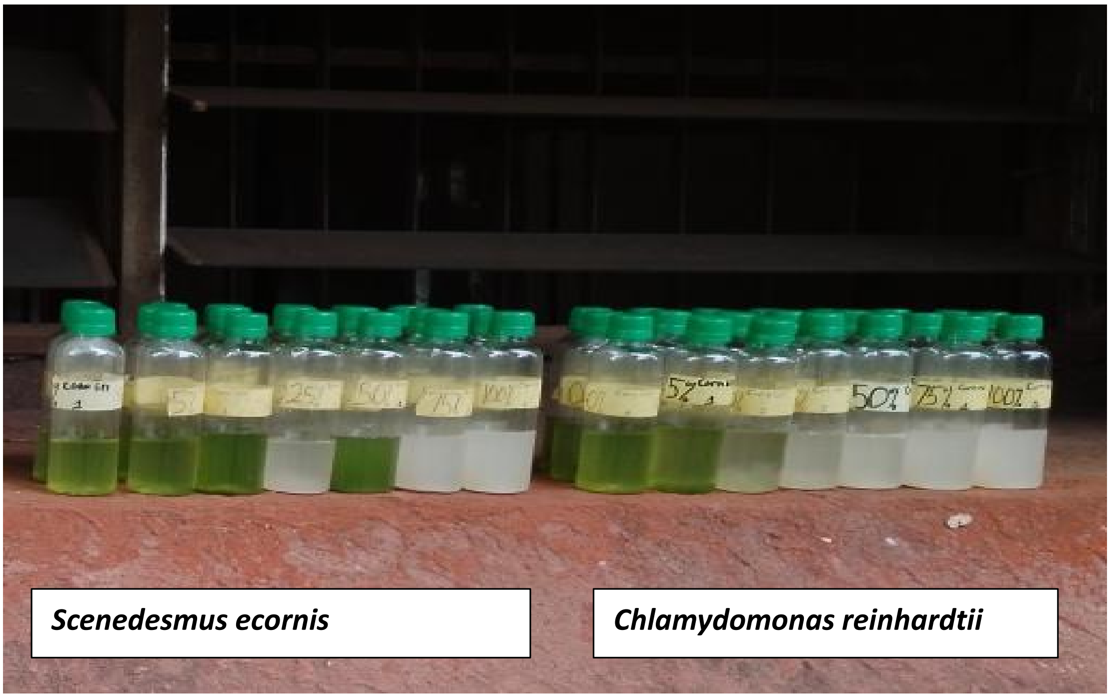

Plate 3.4b *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* at day 14 in culture media of maize effluent

3.14.1 Percentage Inhibition

The formula below was used for calculating the percentage inhibition.

$$Percentage \quad inhibition = 100 - \frac{Measured\ Biomass}{Theoretical\ Biomass} \times 100$$

eq 1.

3.14.2 Biomass - Dry Weight (mg/L)

The dry weight of the test algae estimated every two days by computation using the formula by Horvatic *et al.* (2003).

$$Dry\ Weight = 3.31 + 179.45 \times Absorbance\ at\ 750nm + 617.45(Absorbance\ at\ 750nm)^2$$

eq 2.

3.14.3 Statistical Analysis

Data statistical analyses were done using Microsoft Excel spreadsheet, to determine if there were statistically significant variations in the growth of the microalga cultures. Means and standard errors of the data were performed to identify statistically significant differences in microalgal growth in response to treatment concentrations.

3.15 Physicochemical Parameters

3.15.1 Hydrogen Ion Concentration (pH)

The pH values of the raw cassava mill and maize effluents, and each concentration of the effluents used were taken using a handheld pH meter.

3.15.2 Electrical Conductivity (µS/cm)

Conductivity values of the raw cassava mill and maize effluents, and each concentration used were obtained using a **HACH COI 50 TDS/Conductivity meter**. The cultures in the bottles were mixed thoroughly and the probe dipped immediately in the cultures and the displayed values were recorded.

3.15.3 Total Dissolved Solids (TDS) (mg/L)

Total dissolved solid values of the raw cassava mill and maize effluents, and each concentration used were obtained using a **HACH COI 50 TDS/Conductivity meter**. The samples were shaken vigorously to homogenize after which the probe was dipped into the cultures in the vessels and readings were recorded when the values steadied.

CHAPTER FOUR

4.0 RESULTS

4.1a Growth response of *Scenedesmus ecornis* in cassava mill effluent (0-100%)

Figure 4.1a illustrated the effect of varying concentrations of cassava mill effluent on the growth of the freshwater microalgae *Scenedesmus ecornis* after 14 days. Generally, most concentrations showed an exponential increase in absorbance from day 2 to day 8, with the rate of increase dropping around day 10 to day 14. Control (0%) showed the lowest growth response exhibiting a gradual increase, reaching 0.158 of peak absorbance on day 14. Lower concentrations (5% and 10%) showed an initial increase followed by a slight decrease after day 8. On day 10, 10% concentration reached a peak absorbance at 0.328 and remained stable. Medium to highest concentrations (25%, 50%, 75% and 100%) displayed the highest increase in absorbance. 100% consistently showed the highest absorbance throughout the period from day 8 to day 14 reaching a peak of 0.731. Using ANOVA, it was shown that there were significant differences (p<0.05) in growth response among the different concentrations throughout the experiment.

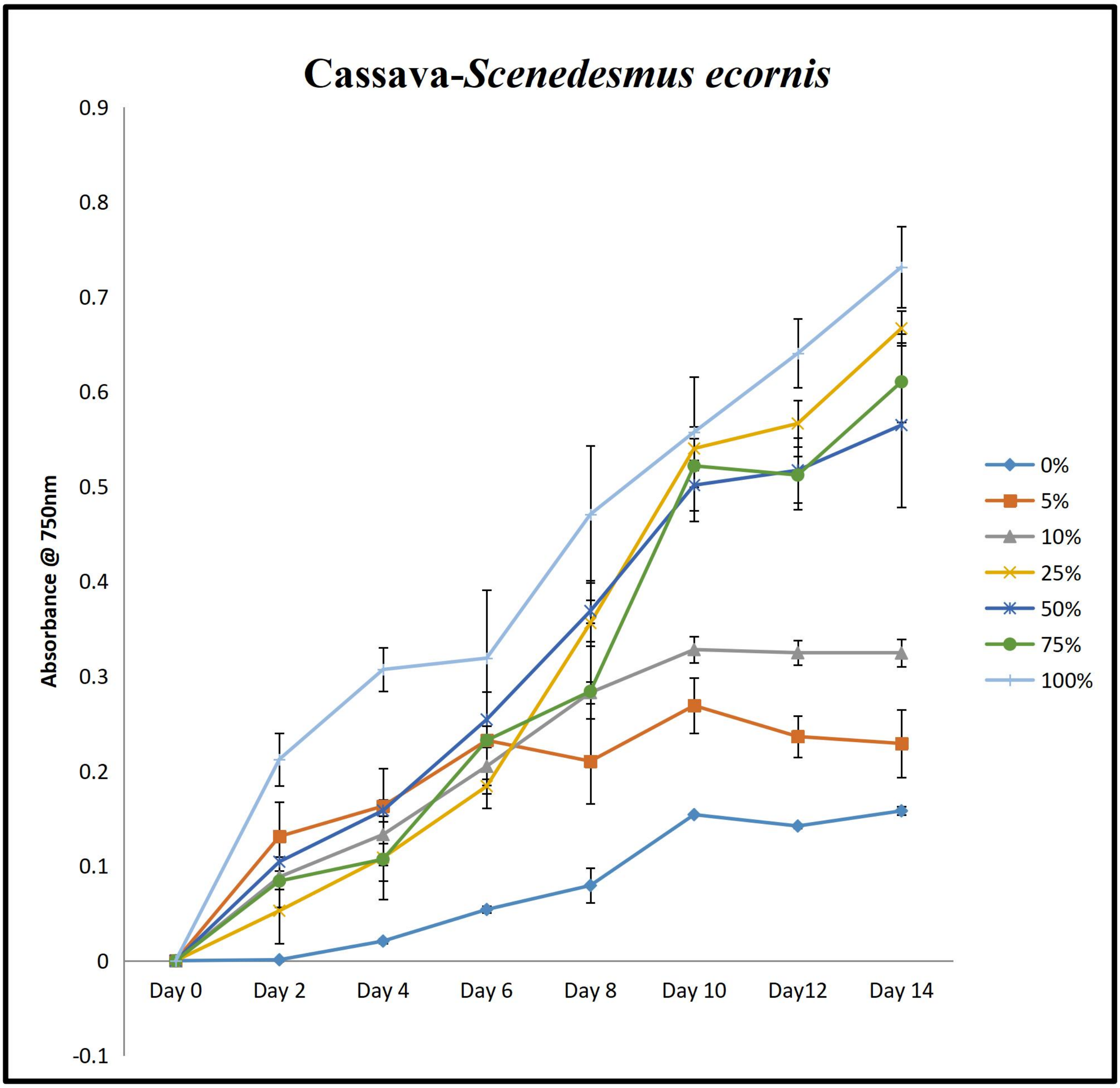

Figure 4.1a Growth response of *Scenedesmus ecornis* in cassava mill effluent (0-100%)

4.1b Growth response of *Chlamydomonas reinhardtii* in cassava mill effluent (0-100%)

Figure 4.1b illustrated the growth of *Chlamydomonas reinhardtii* cultured in varying concentrations of cassava mill effluent during a 14-day incubation period. Control (0%) showed minimal growth of 0.25 absorbance on day 10 before entering a decline. Reaching a peak absorbance of 0.17, 5% showed poor growth, between day 8-10. The 10% concentration performed better than 5% but declined early around day 8, maintaining a stagnant phase to about 0.319 by day 14. Medium concentrations (25% and 50%) displayed similar growth response, with absorbance reaching 0.59 by day 14, while high concentration (75%) showed a robust growth, reaching a peak absorbance of 0.569. The highest concentration (100%) achieved the highest growth response with peak absorbance of 0.68 at day 14, reaching a steep stationary phase around day 10 with absorbance 0.609, and maintained a high biomass until day 14. Using ANOVA, it was shown that there were significant differences (p<0.05) in growth response among the different concentrations throughout the experiment.

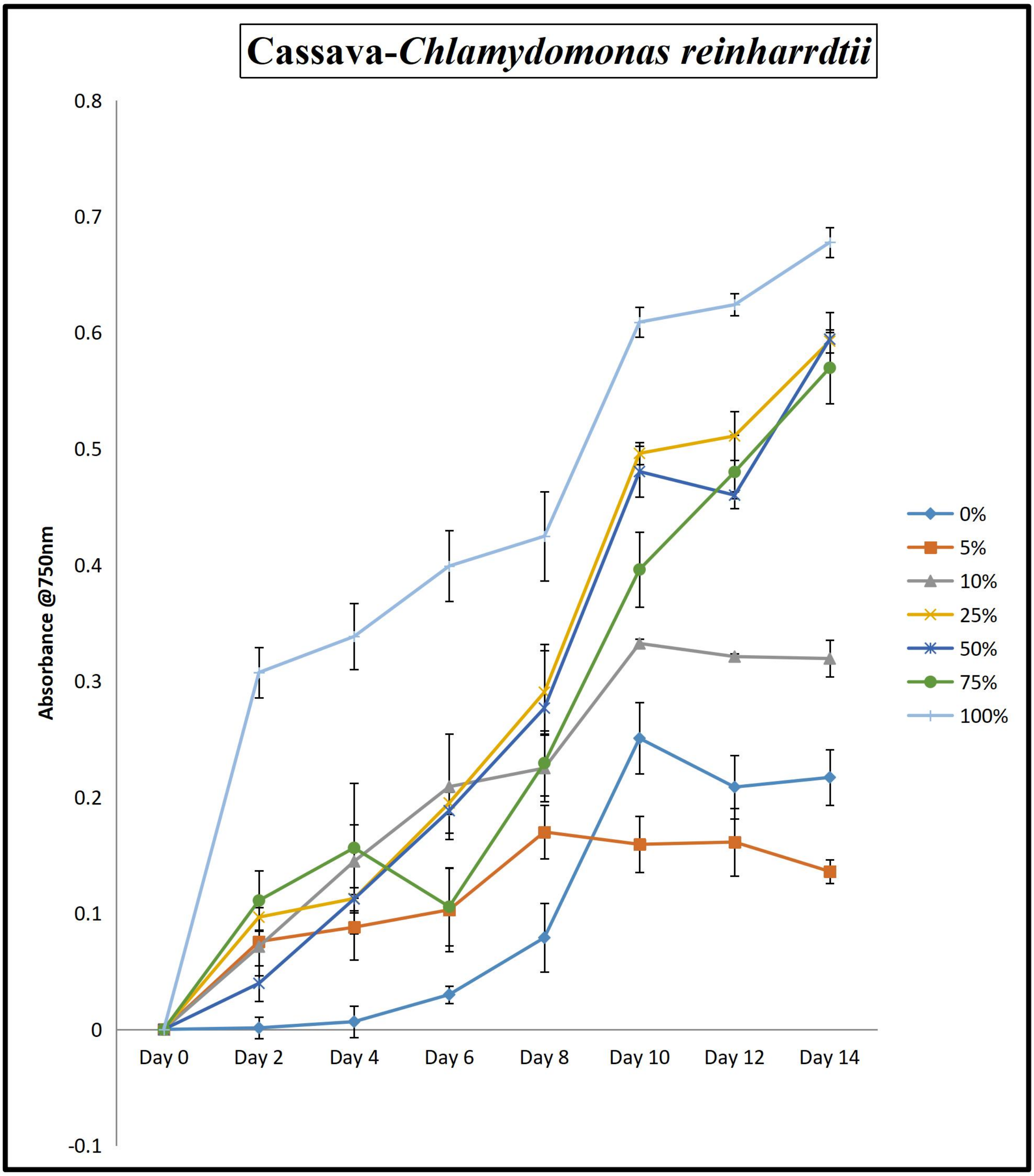

Figure 4.1b Growth response of *Chlamydomonas reinhardtii* in cassava mill effluent (0-100%)

4.2a Growth response of *Scenedesmus ecornis* in maize effluent (0-100%)

Figure 4.2a illustrated the growth of *Scenedesmus ecornis* cultured in various concentrations of maize effluent over a 14-day period. Control (0%) showed minimal growth response with peak absorbance of 0.165 around day 12-14. Low concentrations (5% and 10%) followed a similar pattern to the control, 5% experienced a peak absorbance of 0.15 on day 12, while 10% peaked at 0.247 on day 10, and both entered a decline phase until day 14. The 5% concentration ended with an absorbance of 0.140, and the 10% concentration slightly higher (0.158). There was a steady increase in biomass for 25% concentration, reaching an absorbance of 0.469 by day 14. Higher concentrations (50%, 75%, and 100%) exhibited the highest final biomass but showed unstable growth responses. The most successful of all was 50% concentration, reaching 0.711 absorbance of the growth response on day 14. The second-highest biomass was observed in 75% concentration, with absorbance of 0.733. Similar to the 75% concentration, 100% reached a final absorbance of 0.631; the overall growth rate after day 8 was lower than 50% and 75% concentrations. Using ANOVA, it was shown that there were significant differences ($p<0.05$) in growth response among the different concentrations throughout the experiment.

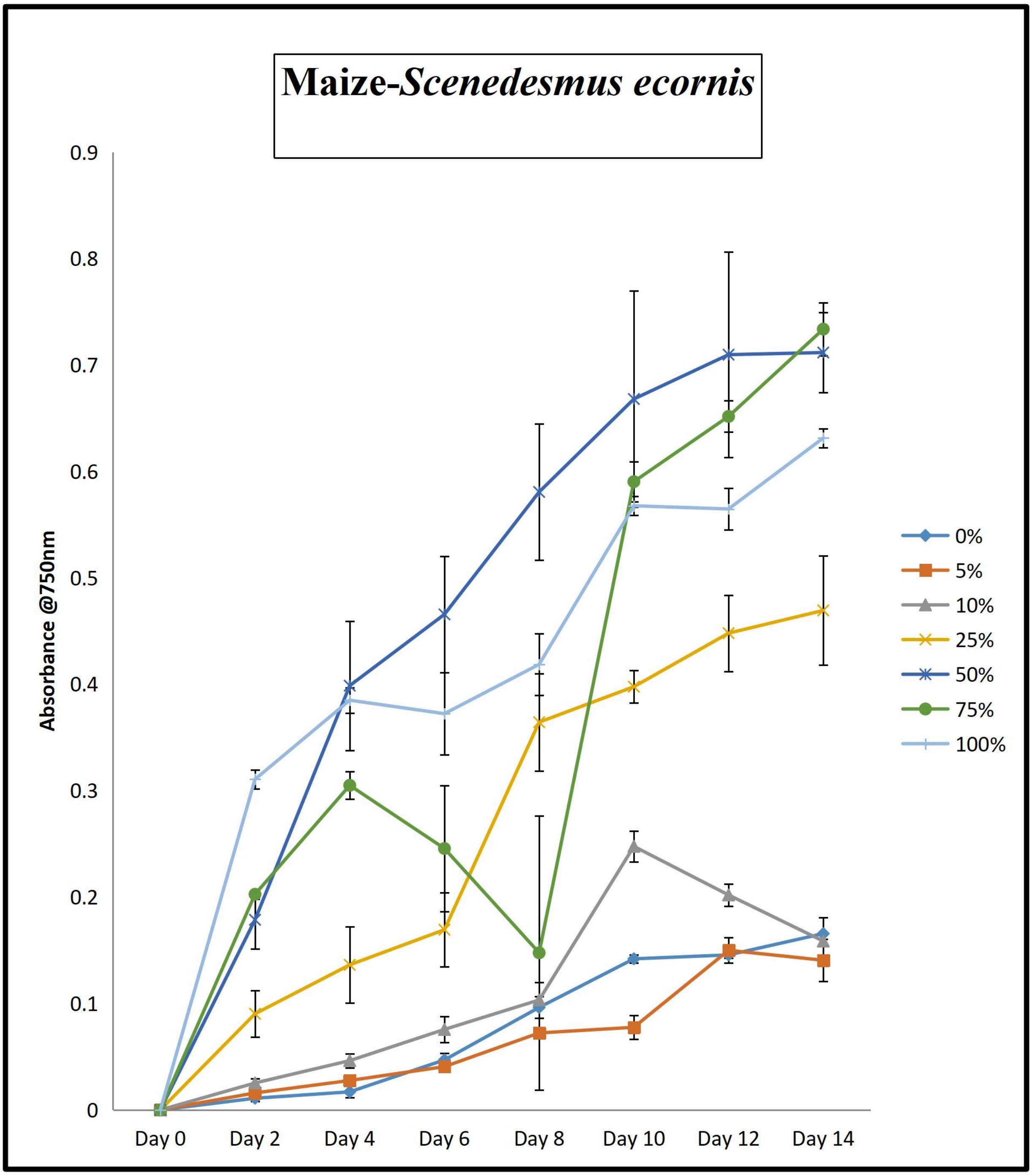

Figure 4.2a Growth response of *Scenedesmus ecornis* in maize effluent (0-100%)

4.2b Growth response of *Chlamydomonas reinhardtii* in maize effluent (0-100%)

Figure 4.2b illustrated the growth of *Chlamydomonas reinhardtii* exposed to varying concentrations of maize effluent over a 14-day period. The control (0%) exhibited very poor growth response with absorbance barely rising above 0.2 by day 14. Low concentrations (5% and 10%) showed relatively poor performance, 5% concentration unexpectedly showed an initial lag until day 6, then by slowed growth process, peaked at 0.31 by Day 14. The 10% treatment peaked by day 10 at absorbance growth response of 0.22, but showed a decline in biomass toward day 14. At day 2, 25% concentration experienced a significant lag phase until day 8, entering a rapid exponential growth phase between day 8 and day 10, reaching a plateau at an absorbance of 0.4, which it maintained until day 14. The highest final biomass were observed in the highest concentrations (50%, 75%, and 100%) showing highly effective growth response, the most effective treatments (75% and 100%), resulted in the maximum final biomass of absorbance 0.72 and 0.69 respectively by day 14. Using ANOVA, it was shown that there were significant differences ($p<0.05$) in growth response among the different concentrations throughout the experiment.

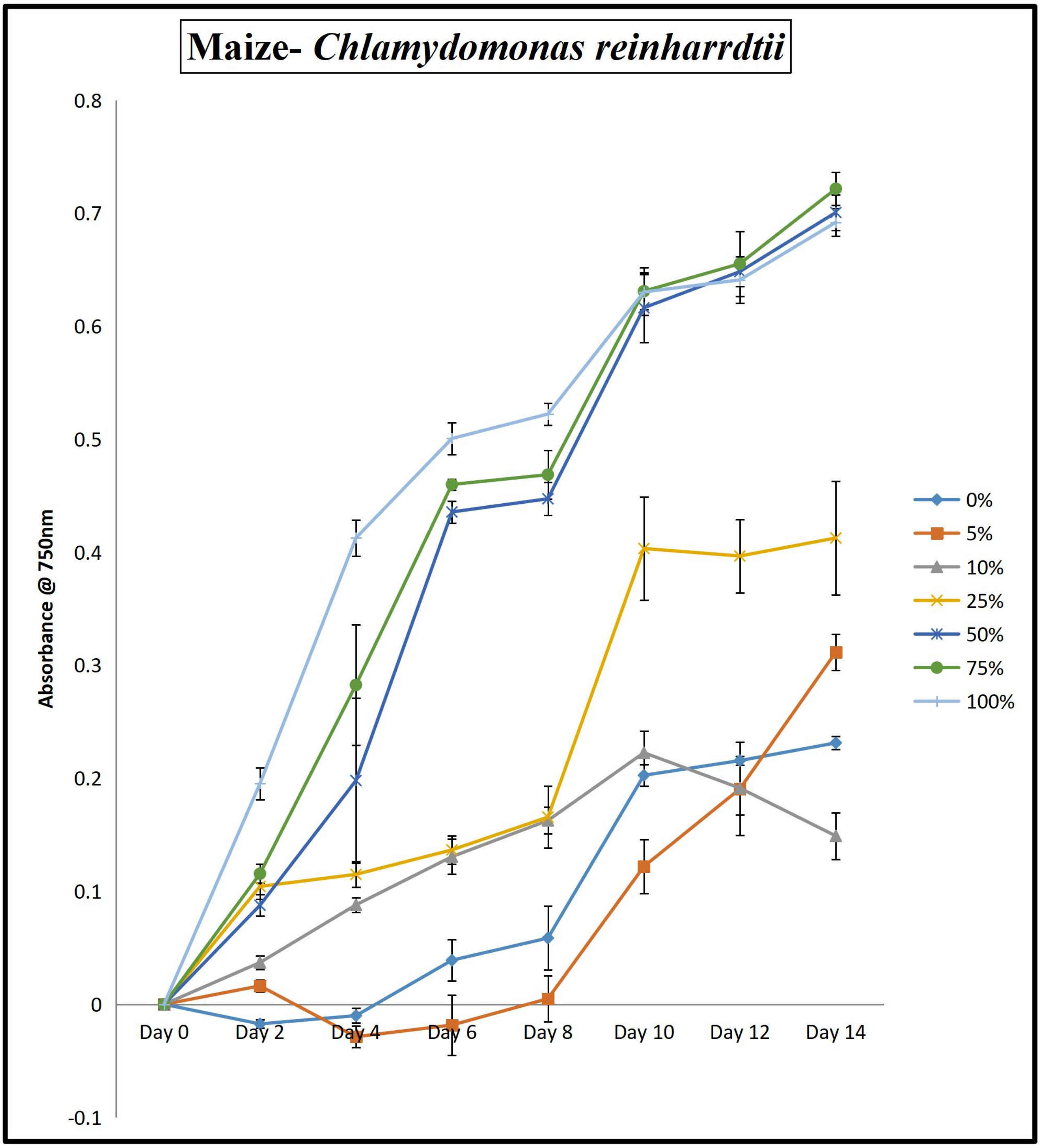

Figure 4.2b Growth response of *Chlamydomonas reinhardtii* in maize effluent (0-100%)

4.3a Dry weight of *Chlamydomonas reinhardtii* and *Scenedesmus ecornis* exposed to Cassava mill effluent.

The highest dry weights were observed at the higher concentrations (50%, 75% and 100%) treatments. The 100% treatment achieved the maximum dry weight on day 14 (398.53 mg/L). The 25% treatment showed a strong, near-maximum final dry weight of 362.9 mg/L, 0% (control) showed the lowest final dry weight (85.28 mg/L). Similar to *Scenedesmus, Chlamydomonas reinhardtii* had the highest dry weights in from medium to higher concentrations (25%, 50%, 75% and 100%). The 100% concentration achieved the maximum dry weight on day 14 (368.95 mg/L). The control (0%) showed a lower final dry weight (117.25 mg/L). The lower concentrations (5% and 10%) showed slightly lower final dry weight compared to the 25% to 100% concentrations, but still significantly higher than the control.

Table 4.1 Temporal variation in dry weight of *Scenedesmus ecornis* exposed to cassava mill effluent

TREATMENTS	**DRY WEIGHT (mg/L)**							
	DAY 0	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**	**DAY 12**	**DAY 14**

0%	**0**	**0.54**	**11.13**	**29.1**	**42.77**	**83.12**	**76.63**	**85.28**
5%	**0**	**70.68**	**87.99**	**125.56**	**113.63**	**145.47**	**127.73**	**123.75**
10%	**0**	**47.62**	**71.76**	**110.74**	**152.89**	**177.55**	**175.73**	**175.55**
25%	**0**	**28.38**	**58.61**	**99.36**	**192.79**	**293.33**	**307.77**	**362.9**
50%	**0**	**56.27**	**85.46**	**137.5**	**199.69**	**272.15**	**280.73**	**306.86**
75%	**0**	**45.28**	**57.71**	**125.56**	**153.62**	**283.29**	**277.99**	**331.93**
100%	**0**	**114.72**	**166.12**	**172.65**	**255.38**	**302.84**	**348.41**	**398.53**

Table 4.2 Temporal variation in dry weight of *Chlamydomonas reinhardtii* exposed to cassava mill effluent

TREATMENTS	**DRY WEIGHT (mg/L)**							
	DAY 0	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**	**DAY 12**	**DAY 14**
0%	**0**	**0.72**	**3.59**	**16.16**	**42.59**	**135.51**	**112.73**	**117.25**
5%	**0**	**40.79**	**47.44**	**55.55**	**91.78**	**86.01**	**87.09**	**73.38**

10%	**0**	**38.45**	**78.07**	**112.91**	**121.58**	**179.91**	**173.74**	**172.83**
25%	**0**	**52.12**	**60.77**	**105.32**	**157.06**	**269.24**	**277.45**	**322.22**
50%	**0**	**21.37**	**60.59**	**101.71**	**149.63**	**260.66**	**249.55**	**323.14**
75%	**0**	**59.87**	**84.38**	**56.99**	**123.93**	**214.6**	**260.48**	**309.6**
100%	**0**	**166.3**	**183.17**	**216.23**	**230.24**	**331.19**	**339.43**	**368.95**

4.3b Dry weight of *Chlamydomonas reinhardtii* and *Scenedesmus ecornis* exposed to maize effluent.

All treatments started with a dry weight of 0 mg/L on day 0. Higher concentrations of maize effluent, (50%, 75%, and 100 %) resulted in higher maximum dry weights. *S. ecornis* resulted in the highest final dry weight of 399.81 mg/L on day 14 at 75% concentration, followed by the 50% at 387.68 mg/L. The 50% concentration reached its peak biomass of 387.68 mg/L on day 14, while the 75% treatment showed a temporary drop on day 8 (79.69 mg/L) before increasing significantly again. For most concentrations (25%, 50%, 75%, and 100%), the dry weight showed a rapid increase between day 0 and day 14. Lower concentration (5%) showed continuous, steady growth but achieved the lowest final dry weight of 75.91 mg/L, compared to the higher concentrations. The highest concentration (100%) showed high dry weight from day 2 (168.12 mg/L), reaching 343.47 mg/L by day 14. *C. reinhardtii* yielded the highest final dry weight at 75% concentration reaching 393.2 mg/L on day 14, 50% concentration followed closely at 381.62 mg/L. The lowest concentrations (0% and 5%) showed negative dry weight values in the initial days (up to Day 4 and Day 6, respectively). At day 2, 0% reached -9.33 mg/L and by day 4 (-5.38 mg/L). At day 4, 5% reached -15.43 mg/L and -9.87 mg/L on day 6.

Table 4.3 Temporal variation in dry weight of *Scenedesmus ecornis* exposed to maize effluent

TREATMENTS	**DRY WEIGHT (mg/L)**							
	DAY 0	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**	**DAY 12**	**DAY 14**
0%	**0**	**5.92**	**9.15**	**25.32**	**51.94**	**76.63**	**78.61**	**89.43**
5%	**0**	**8.62**	**14.9**	**21.91**	**38.99**	**41.87**	**80.96**	**75.91**
10%	**0**	**13.64**	**24.96**	**40.79**	**55.73**	**133.88**	**109.11**	**85.64**
25%	**0**	**48.7**	**73.56**	**91.42**	**197.33**	**215.51**	**242.99**	**254.65**
50%	**0**	**96.47**	**216.05**	**252.64**	**315.64**	**363.63**	**386.58**	**387.68**
75%	**0**	**109.48**	**165.03**	**132.8**	**79.69**	**320.94**	**354.65**	**399.81**

100%	**0**	**168.12**	**208.6**	**201.69**	**226.97**	**308.5**	**306.86**	**343.47**

Table 4.4 Temporal variation in dry weight of *Chlamydomonas reinhardtii* exposed to maize effluent

TREATMENTS	**DRY WEIGHT (mg/L)**							
	DAY 0	**DAY 2**	**DAY 4**	**DAY 6**	**DAY 8**	**DAY 10**	**DAY 12**	**DAY 14**
0%	**0**	**-9.33**	**-5.38**	**21.01**	**31.61**	**109.48**	**116.52**	**125.02**
5%	**0**	**8.8**	**-15.43**	**-9.87**	**2.69**	**65.81**	**102.97**	**168.66**
10%	**0**	**19.93**	**47.44**	**70.5**	**87.81**	**120.32**	**103.33**	**80.41**
25%	**0**	**56.27**	**62.03**	**73.74**	**89.43**	**218.6**	**214.96**	**223.69**
50%	**0**	**47.26**	**106.95**	**236.25**	**242.62**	**335.22**	**352.81**	**381.62**
75%	**0**	**62.39**	**152.89**	**249.55**	**254.28**	**343.28**	**356.66**	**393.2**
100%	**0**	**105.5**	**223.69**	**271.79**	**283.65**	**342.92**	**348.78**	**376.85**

4.4a Percentage inhibition of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* in Cassava mill effluent

Figure 4.4a illustrated a comparative percentage inhibition of growth of the two algal species, *Scenedesmus ecornis* and *Chlamydomonas reinhardtii,* following exposure to cassava mill effluent after a 14-day period. At all tested concentrations, cassava effluent did not inhibit the growth of the microalgae, instead it stimulated their growth. For both species (*S. ecornis* and *C. reinhardtii*), as the concentration of cassava effluent increased from 5% to 100%, the magnitude of the negative inhibition (the stimulation of growth) also increased. For *Scenedesmus ecornis*, the stimulation was lowest at 5% (-140%) and highest at 100% (-450%). For *Chlamydomonas reinhardtii*, the stimulation was lowest at 5% at about -50% and highest at 100% (-330%). *Scenedesmus ecornis* showed significantly greater stimulation at every concentration compared to *Chlamydomonas*, its growth was stimulated at about -450% at 100% concentration. *Chlamydomonas reinhardtii* showed strong stimulation, but less obvious than *Scenedesmus ecornis*, its inhibition peaked at -330% at 100% concentration.

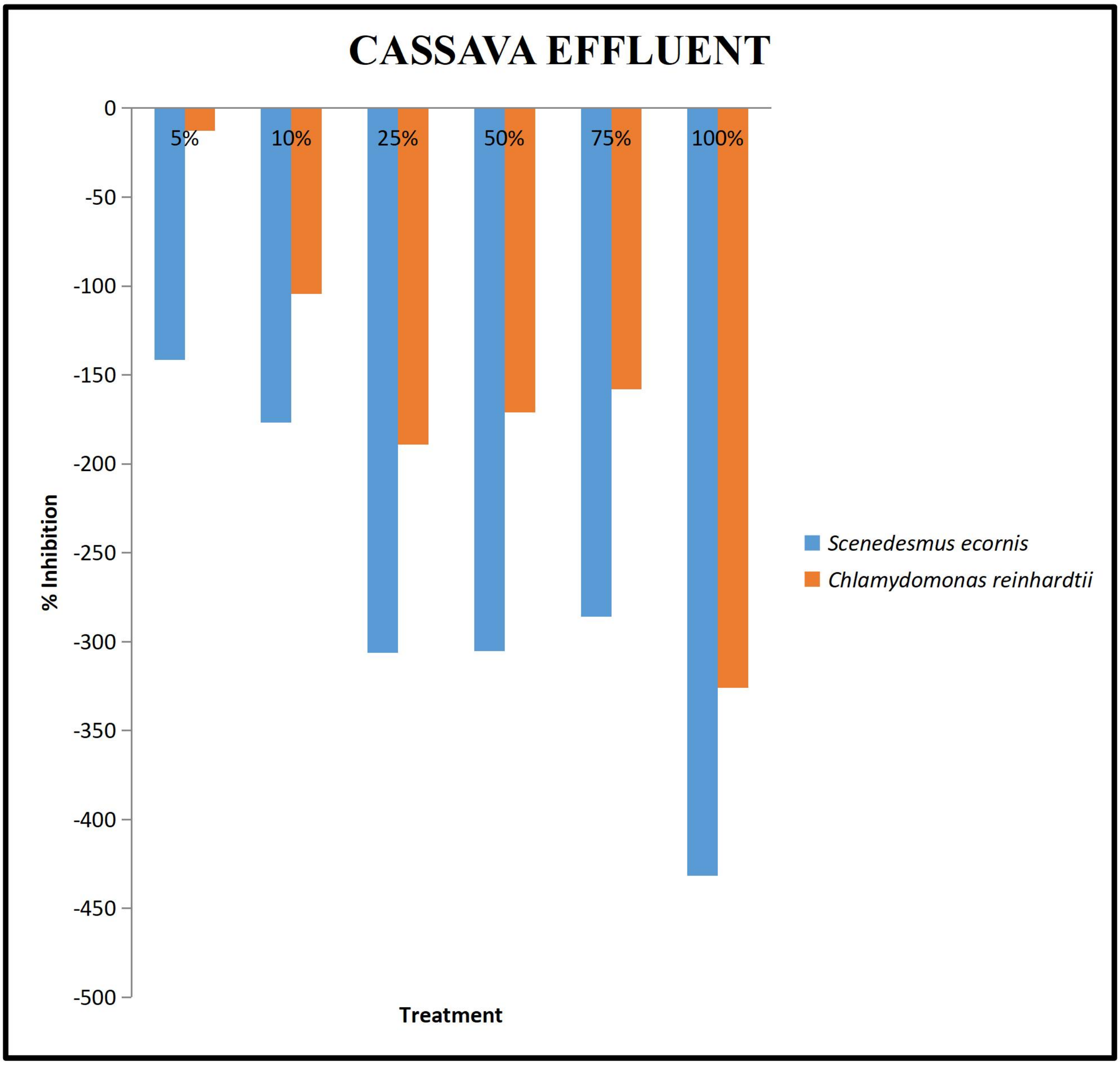

Figure 4.4a Percentage inhibition of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* in Cassava mill effluent

4.4b Percentage inhibition of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* in Maize effluent

Figure 4.4b showed a pattern of growth stimulation, especially at higher effluent concentrations of maize effluent on *Scenedesmus ecornis* and *Chlamydomonas reinhardtii*. At 5% Concentration: Both species showed a very slight positive percentage Inhibition at about +5% for *S. ecornis* and +10% for *Chlamydomonas*. From low to high concentrations (10% to 100%), the percentage inhibition was significantly negative, ranging from about -25% to -500%. The negative percentage inhibition signified increased growth compared to the control (0%). For both microalgae, increasing the maize effluent concentration from 10% to 100% resulted in a corresponding increase in the magnitude of growth stimulation. The medium concentration (50%), showed the highest growth inhibition of *Scenedesmus ecornis* at almost 500%, while the highest growth inhibition of Chlamydomonas reinhardtii was observed at the highest effluent concentration (100%) of about -400%.

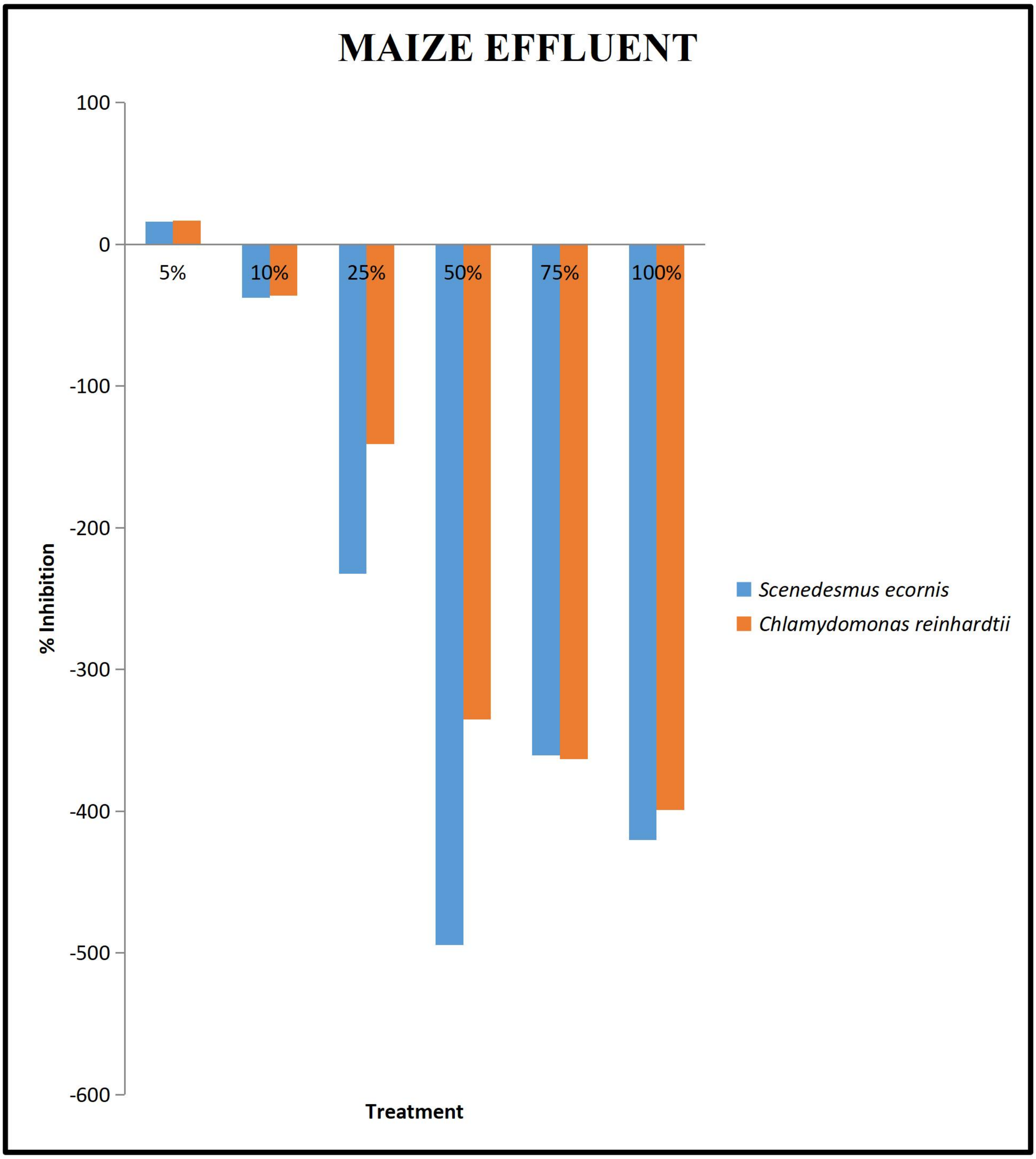

Figure 4.4b Percentage inhibition of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* after exposure to various concentrations of Maize effluent

4.5a Changes in pH of *Scenedesmus ecornis* exposed to cassava mill effluent over time

Figure 4.5a illustrated the fluctuation in pH of the culture media of *Scenedesmus ecornis* against various cassava effluent concentrations during the 14-day experiment. On day 0, the 0% concentration had the highest pH 6.5. As the concentration of cassava effluent increased from 5% to 100%, the initial pH gradually decreased to 4.9

for the 75% and 100% concentrations. Lower concentrations (0% and 5%) showed a significant increase in pH until day 14, 0% increased from 6.5 (day 0) to 10.1 by day 14. 5% increased from 5.6 (day 0) to 7.2. For concentrations from 10% up to 100% generally showed an exponential rise in pH in the early days (day 0 to day 8 or 10), followed by a slight decrease towards day 14. While 0% obtained alkalinity of 10.1 on day 14, 100% concentration showed slight acidity of 5.0.

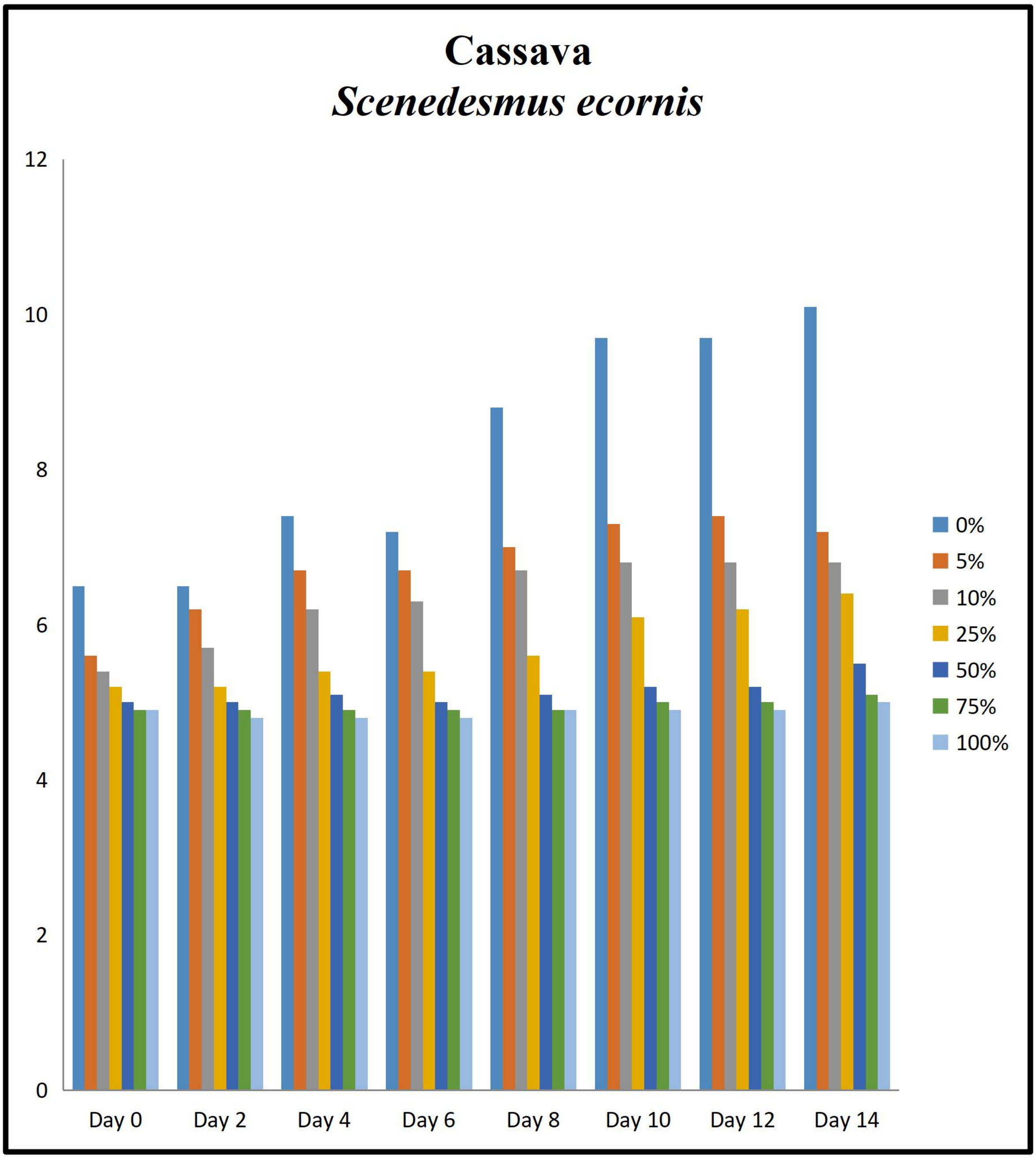

Figure 4.5a Changes in pH of *Scenedesmus ecornis* exposed to cassava mill effluent over time

4.5b Variation in Electrical Conductivity (EC) of *Scenedesmus ecornis* exposed to cassava mill effluent over time

Figure 4.5b displayed the changes in the electrical conductivity (EC) of the *Scenedesmus ecornis* culture medium at different concentrations of cassava effluent across 14 days. Control (0%) at day 0 showed the lowest EC of 186 µS/cm. The 100% concentration has the highest EC of about 1716 µS/cm. From medium to highest concentrations (25%, 50%, 75%, and 100%), at day 8 showed high conductivity from about 990 to 4110 µS/cm. the electrical conductivity confirmed that the raw cassava effluent at very high concentrations of *Scenedesmus ecornis* was ineffective at significantly lowering the electrical conductivity of the effluent over the 14-day period.

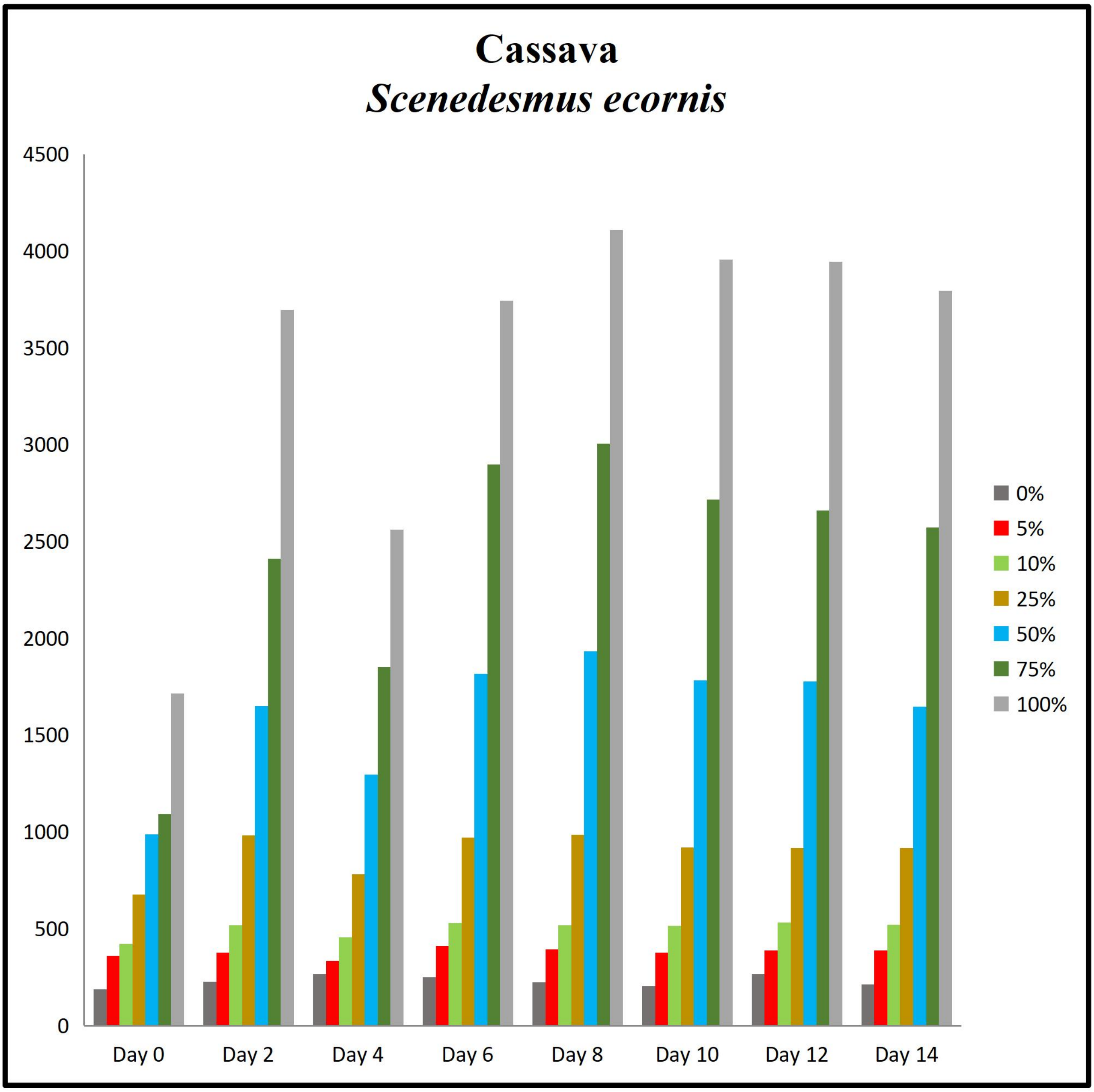

Figure 4.5b Variation in Electrical Conductivity (EC) of *Scenedesmus ecornis* exposed to cassava mill effluent over time

4.5c Variation in Total Dissolved Solids (TDS) of *Scenedesmus ecornis* exposed to cassava mill effluent over time

Figure 4.5c shows the variation of Total Dissolved Solids (TDS) of the culture medium of *Scenedesmus ecornis* across different concentrations of cassava effluent or 14 days. TDS is a measurable quantity of total weight of mobile charged ions in the form of metals, minerals, and salts dissolved in water. As measurements of TDS tend to be based on electrical conductivity (EC) readings (Figure 4.5b), trends shown here are primarily an indication of the EC values. At day 0, the 0% concentration (control) started at the lowest TDS, close to 0 mg/L indicating the dilute growth medium (Chu-10). The 100% concentration started at the highest TDS, about 853 mg/L. As the effluent concentration in the mixture increases, it introduces a proportionally larger amount of dissolved inorganic salts, organic matter, and other ions, leading to a higher initial TDS reading. At high concentrations of 75% and 100%, TDS remained high, with no visible sustained drop, while at low Concentrations (0%, 5%, 10%), TDS remained low and stable.

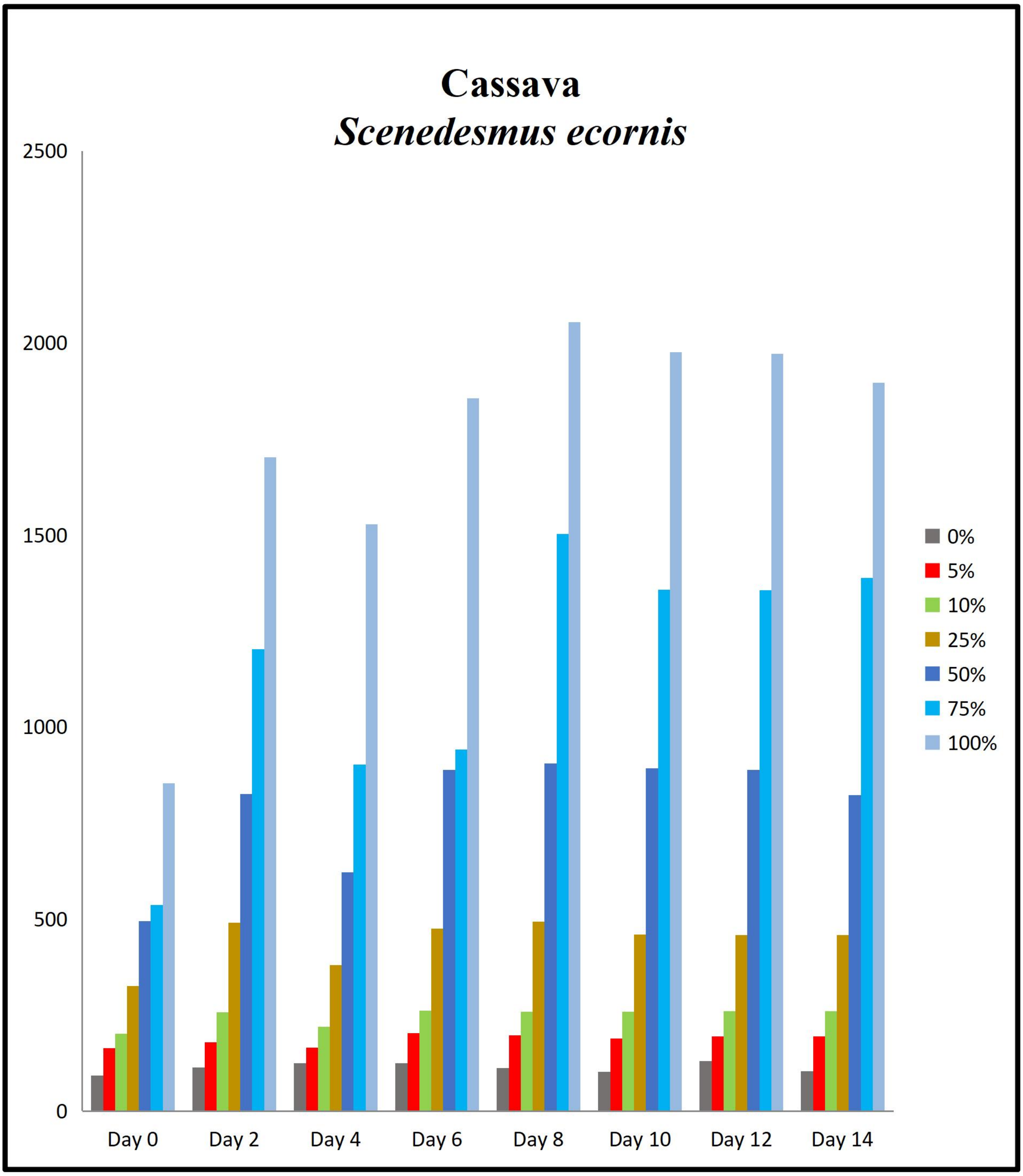

Figure 4.5c Variation in Total Dissolved Solids (TDS) of *Scenedesmus ecornis* exposed to cassava mill effluent over time

4.6a Changes in pH of *Chlamydomonas reinhardtii* exposed to Cassava mill effluent over time

Figure 4.6a illustrated variation in the *Chlamydomonas reinhardtii* culture medium pH readings under varying cassava effluent concentrations over a period of 14 days. At day 0, all concentrations show an initial pH below neutral (7.0), 0% (Control) began at the highest pH of 6.5. 100% (Undiluted effluent) started at the lowest pH level of 5.0. The 0% (Control) and 5% concentrations showed the most significant pH increase. With 0% rising sharply from 6.5 at day 0 to 10.0 by day 14. 5% rises significantly from 5.7 (Day 0) to 7.1 on Day 14. As the concentration increased from 10% to 100%, the pH increase becomes progressively suppressed. The 10% concentration peaked around 6.7 on day 10.

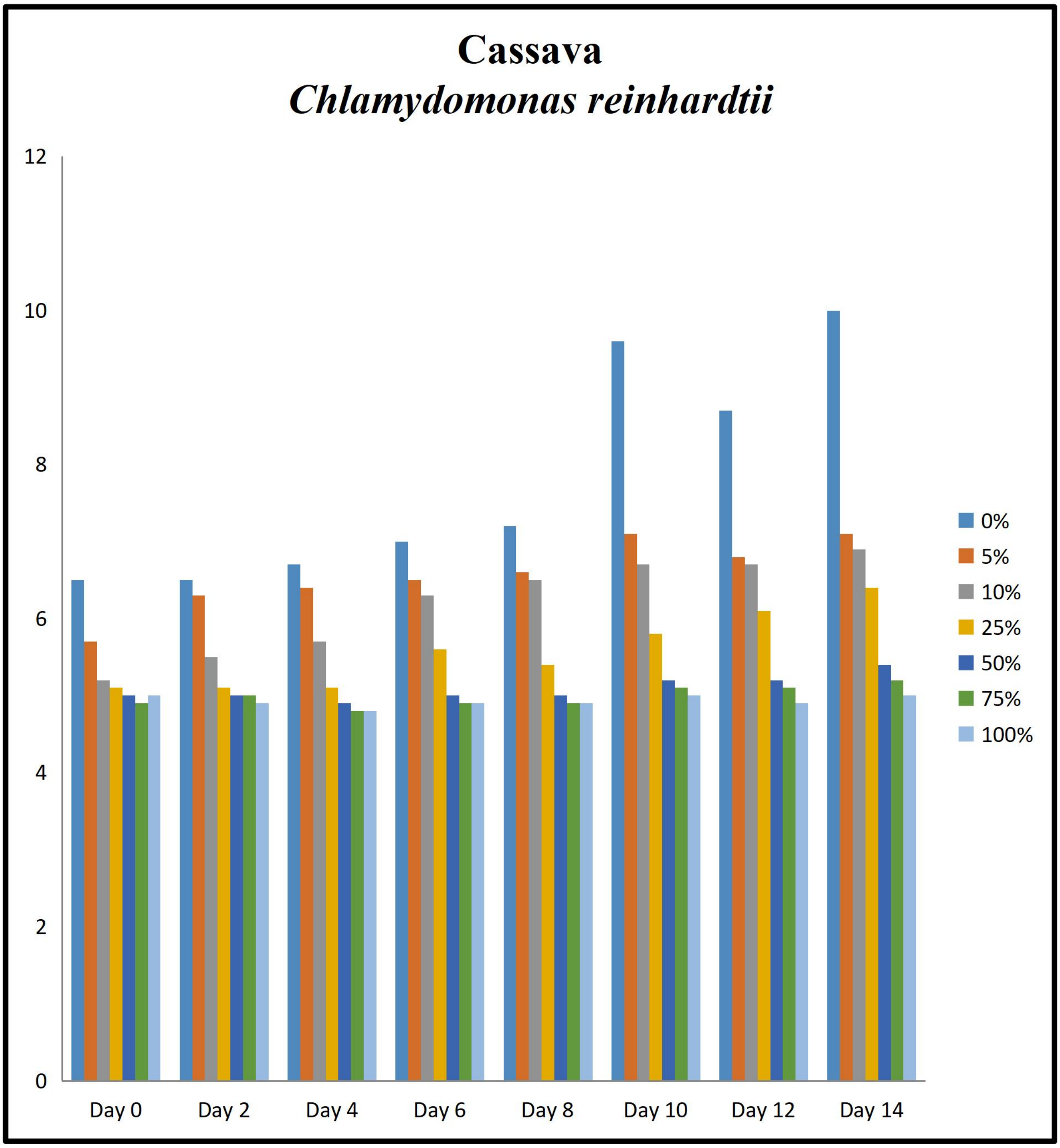

Figure 4.6a Changes in pH of *Chlamydomonas reinhardtii* exposed to Cassava mill effluent over time

4.6b Variation in Electrical Conductivity of *Chlamydomonas reinhardtii* exposed to cassava mill effluent over time

Figure 4.6b displays the changes in the electrical conductivity (EC) of the *Scenedesmus ecornis* culture medium at different concentrations of cassava effluent across 14 days. At day 0, 0% (Control) treatment started near 0µS/cm, while the 100% (Undiluted effluent) concentration began at the highest value, approximately 4000µS/cm. For all concentrations of Cassava effluent, the electrical conductivity readings demonstrated negligible change over the 14-day period. 5%, 10%, 25%, 50%, 75%, and 100% concentrations showed that the high initial EC values were maintained with minimal reduction throughout the experiment. The 100% concentration remained high, around 4000µS/cm, while the lower concentrations also remained stable at their respective starting points.

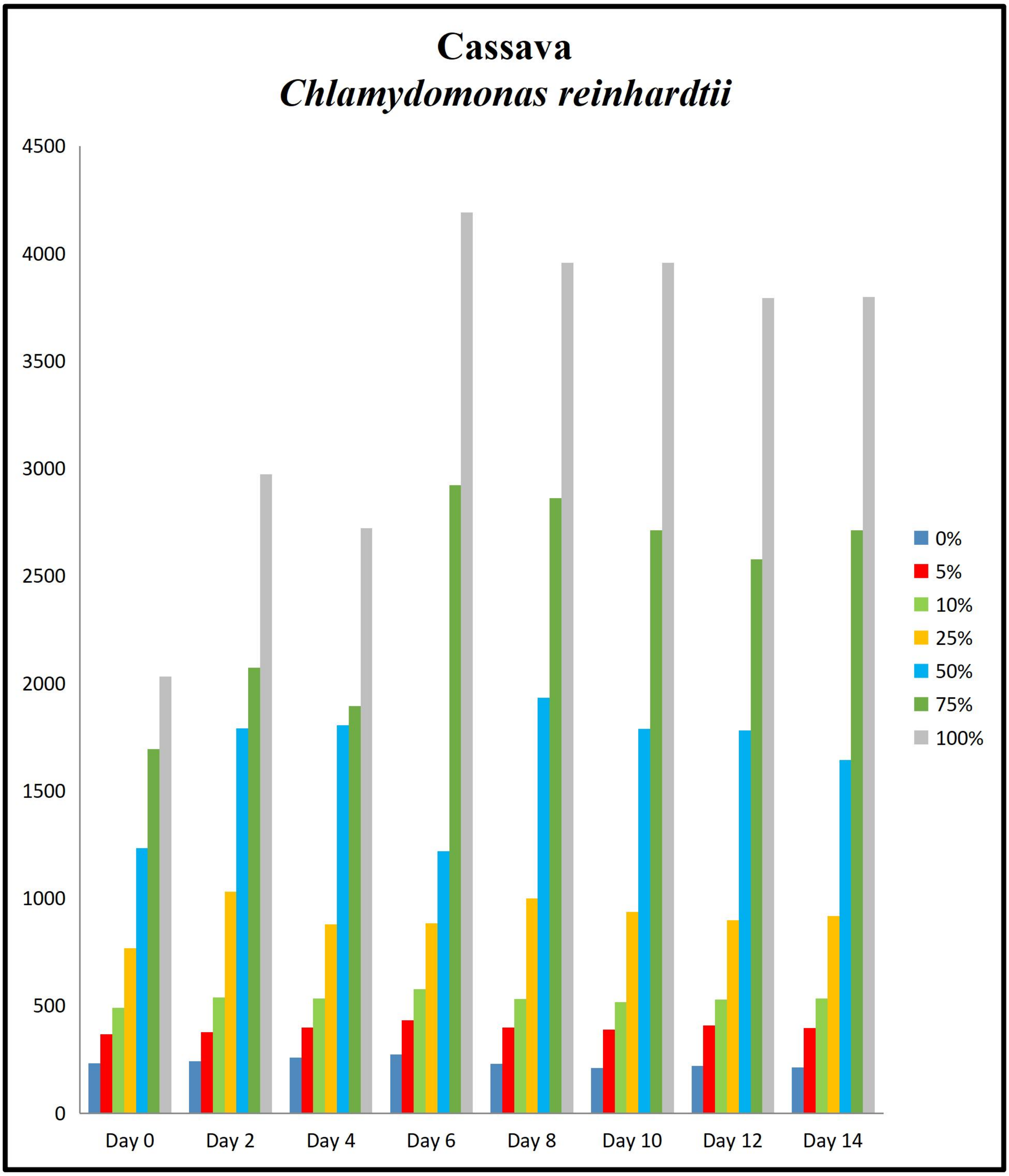

Figure 4.6b Variation in Electrical Conductivity of *Chlamydomonas reinhardtii* exposed to cassava mill effluent over time

4.6c Variations in Total Dissolved Solids (TDS) of *Chlamydomonas reinhardtii* exposed to Cassava mill effluent over time

Figure 4.6c showed the variation of Total Dissolved Solids (TDS) of the culture medium of *Chlamydomonas reinhardtii* across different concentrations of cassava effluent for 14 days. On day 0, the 0% concentration started at the lowest TDS, close to 0 mg/L. While the 100% concentration started at the highest TDS, around 2000 mg/L. For every concentration from 0% to 100%, the TDS values remain virtually constant over the 14-day duration, showing only minor, negligible changes. High concentrations (75% and 100%) remained high, around 1500–2000 mg/L, while low concentrations of 5% and 10% remained low, around 500 mg/L or less.

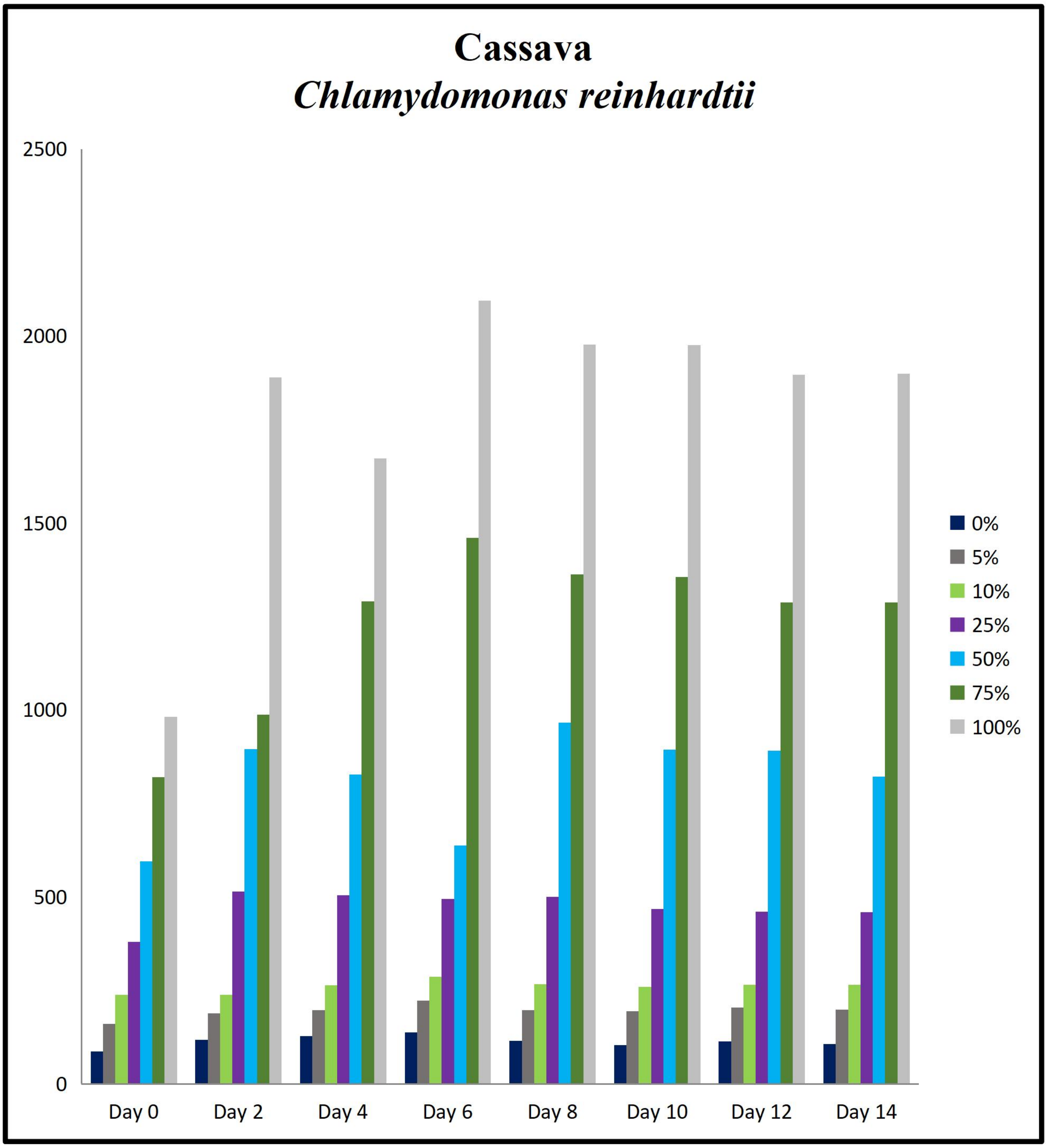

Figure 4.6c Variations in Total Dissolved Solids (TDS) of *Chlamydomonas reinhardtii* exposed to Cassava mill effluent over time

4.7a Changes in pH of *Scenedesmus ecornis* exposed to maize effluent over time

Figure 4.7a illustrated variation in the *Scenedesmus ecornis* culture medium pH readings under varying maize effluent concentrations over a period of 14 days. At day 0, 0% concentration started at the highest pH of 7.4. A major trend is the increase in pH for most concentrations as the experiment progresses from day 0 to day 14. The increase is most dramatic in the higher concentration treatments from 50% to 100%. The 0% treatment showed the largest absolute change, rising from 7.4 on day 0 to 9.5 by day 14. 100% concentration showed a decrease in pH from 5.0 on day 0 to 4.7 by day 14. Low to medium concentrations (5% to 50%) showed a slightly lower final pH than the 0% concentration which was highly alkaline (9.5) at day 14. For many concentrations, there is a significant drop in pH around day 2 to day 4 before the general upward trend takes over. The pH of 0% concentration dropped from 7.4 at day 0 to 6.7 by day 2.

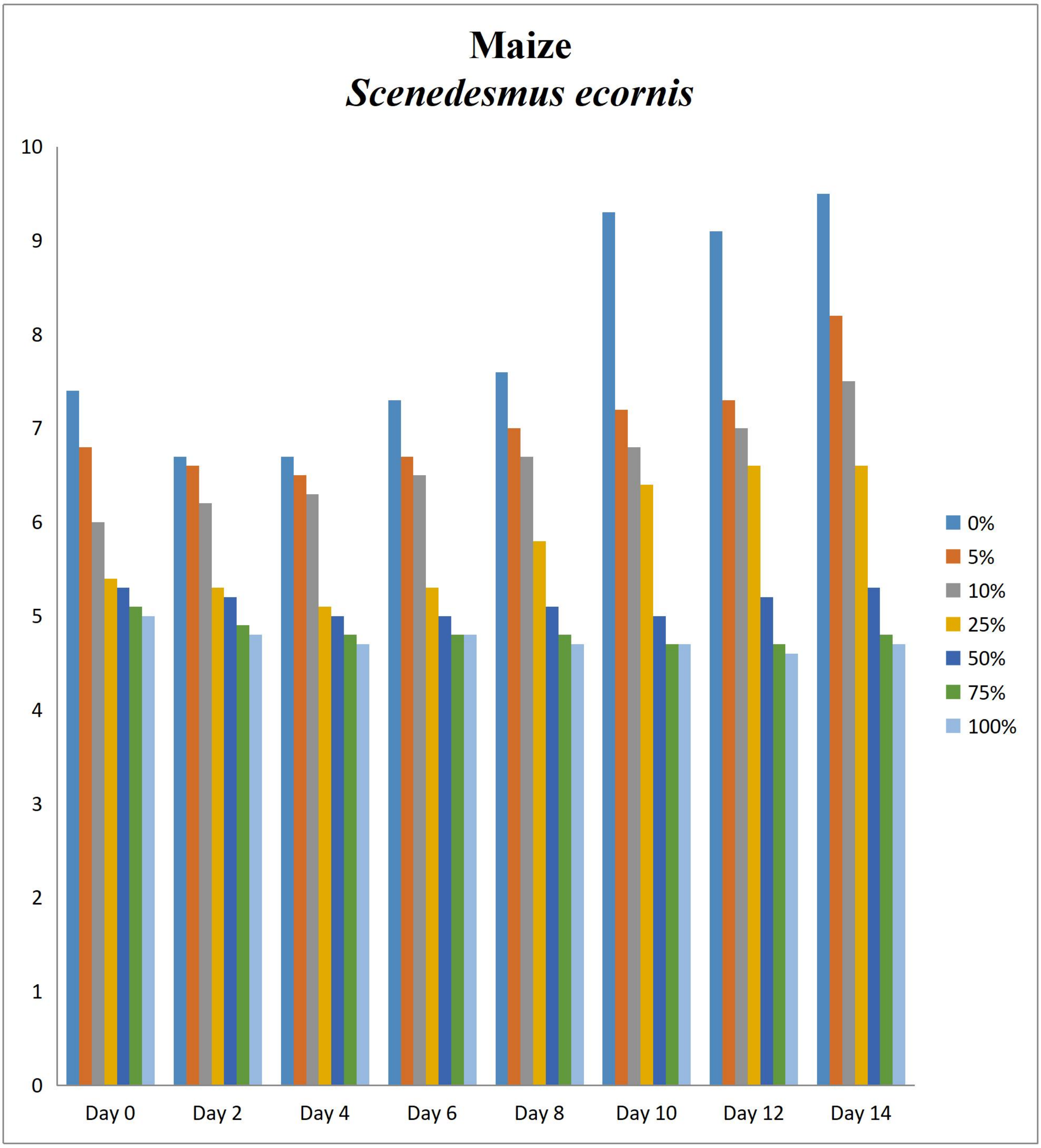

Figure 4.7a Changes in pH of *Scenedesmus ecornis* exposed to maize effluent over time

4.7b Variation in Electrical Conductivity of *Scenedesmus ecornis* exposed to Maize effluent over time

Figure 4.7b displayed the changes in the electrical conductivity (EC) of the *Scenedesmus ecornis* culture medium at different concentrations of maize effluent across 14 days. The 0% concentration has the lowest EC of approximately 200µS/cm on day 0. 100% concentration has the highest initial EC of approximately 350µS/cm. The rate of increase is highest in the initial phase (day 0 to day 4 or 6) and continues to rise until day 14. Control (0%) showed a modest increase, reaching a final EC of approximately 1000µS/cm. 75% and 100% concentrations also showed the most dramatic increase, both reaching the highest final conductivity value, approaching 1500µS/cm.

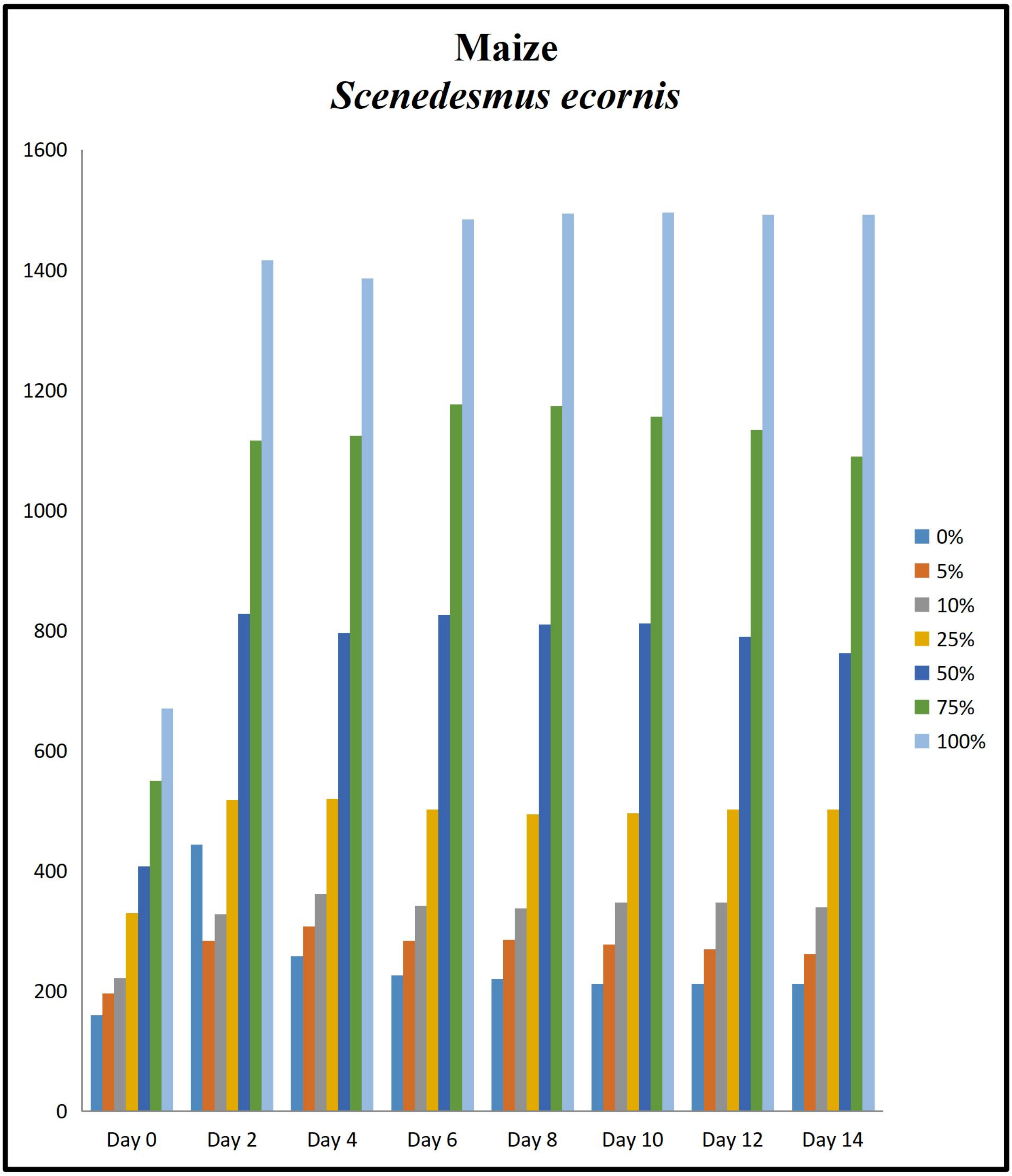

Figure 4.7b Variation in Electrical Conductivity of *Scenedesmus ecornis* exposed to Maize effluent over time

4.7c Variations in Total Dissolved Solids (TDS) of *Scenedesmus ecornis* exposed to maize effluent over time

Figure 4.7c showed the variation of Total Dissolved Solids (TDS) of the culture medium of *Scenedesmus ecornis* across different concentrations of maize effluent for

14 days. At day 0, 0% concentration (control medium), had the lowest TDS approximately 100 mg/L. TDS increases steadily with concentration, culminating in the 100% concentration treatment having the highest initial. TDS exhibited a significant increase across all treatments over the 14-day period. The increase was observed in the higher concentration treatments from 50% to 100%, compared to the lower treatments (0% to 25%). The highest concentrations (75% and 100%) resulted in having the highest final TDS of approximately 750-800mg/L. While that of 0% concentration (control) has the lowest final TDS of approximately 400mg/L.

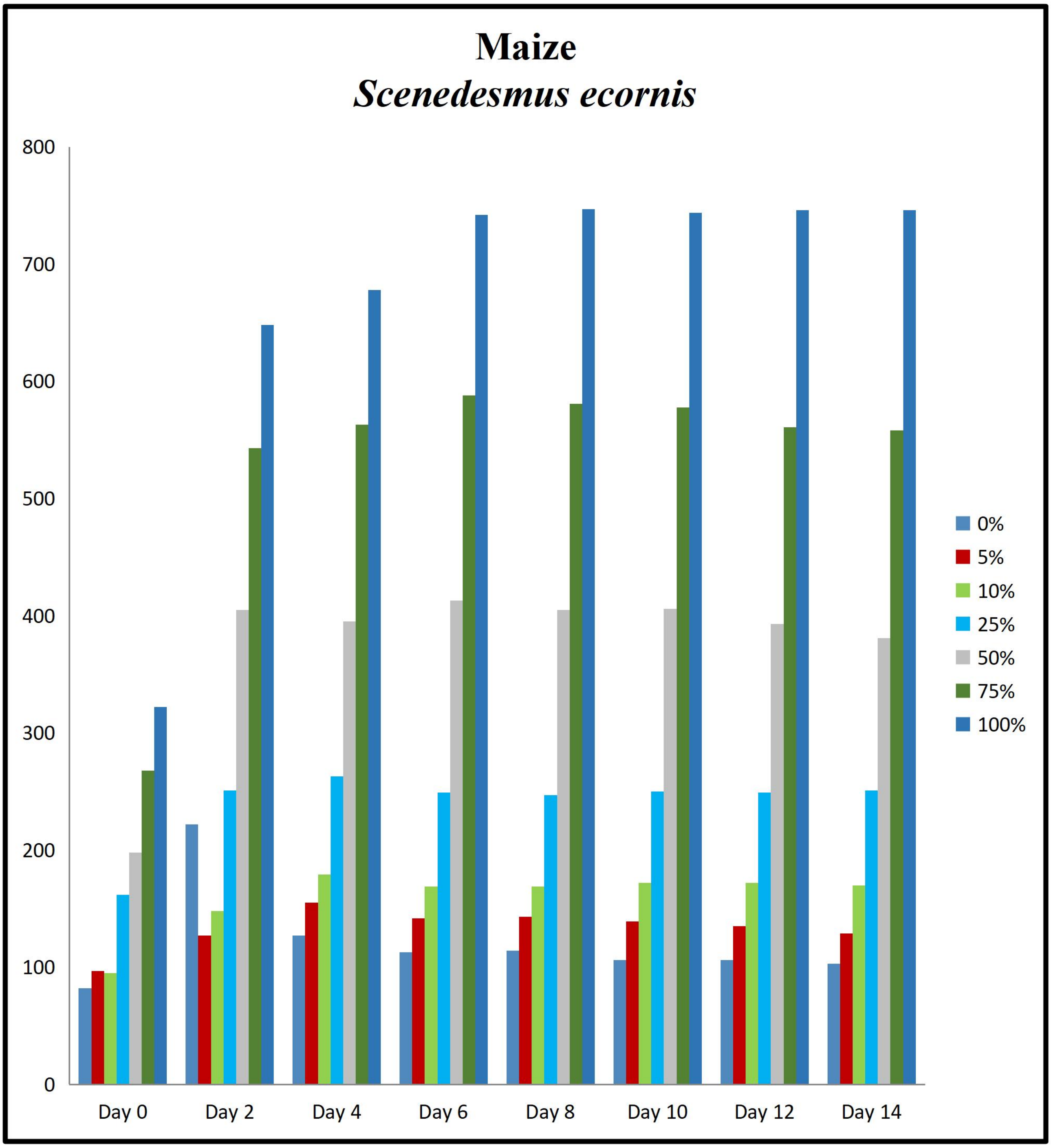

Figure 4.7c Variations in Total Dissolved Solids (TDS) of *Scenedesmus ecornis* exposed to maize effluent over time

4.8a Change in pH of *Chlamydomonas reinhardtii* exposed to maize effluent over time

Figure 4.8a illustrated the fluctuation in the pH of the culture medium of *Chlamydomonas reinhardtii* under exposure to varying concentrations of maize effluent for 14 days. The pH readings at day 0 indicated the effluent is neutral to slightly acidic. At 0% concentration the highest pH obtained was 6.5. The pH decreases as the maize effluent concentration increases, with 100% treatment having a low pH of 5.0, which is slightly higher than 75% concentration with pH of 4.9.. The 0% concentration (control) showed a distinct and significant alkalization trend increase in pH. With an increase from 6.5 on day 0 to a peak of 9.7 by day 14. Highest concentration (100%) exhibited a pH of 5.0 at day 0 and continued to decrease to a pH of 4.6 by day 14, while 5% to 25% concentrations showed an initial drop on day 0 to 4, followed by a partial recovery and increase.

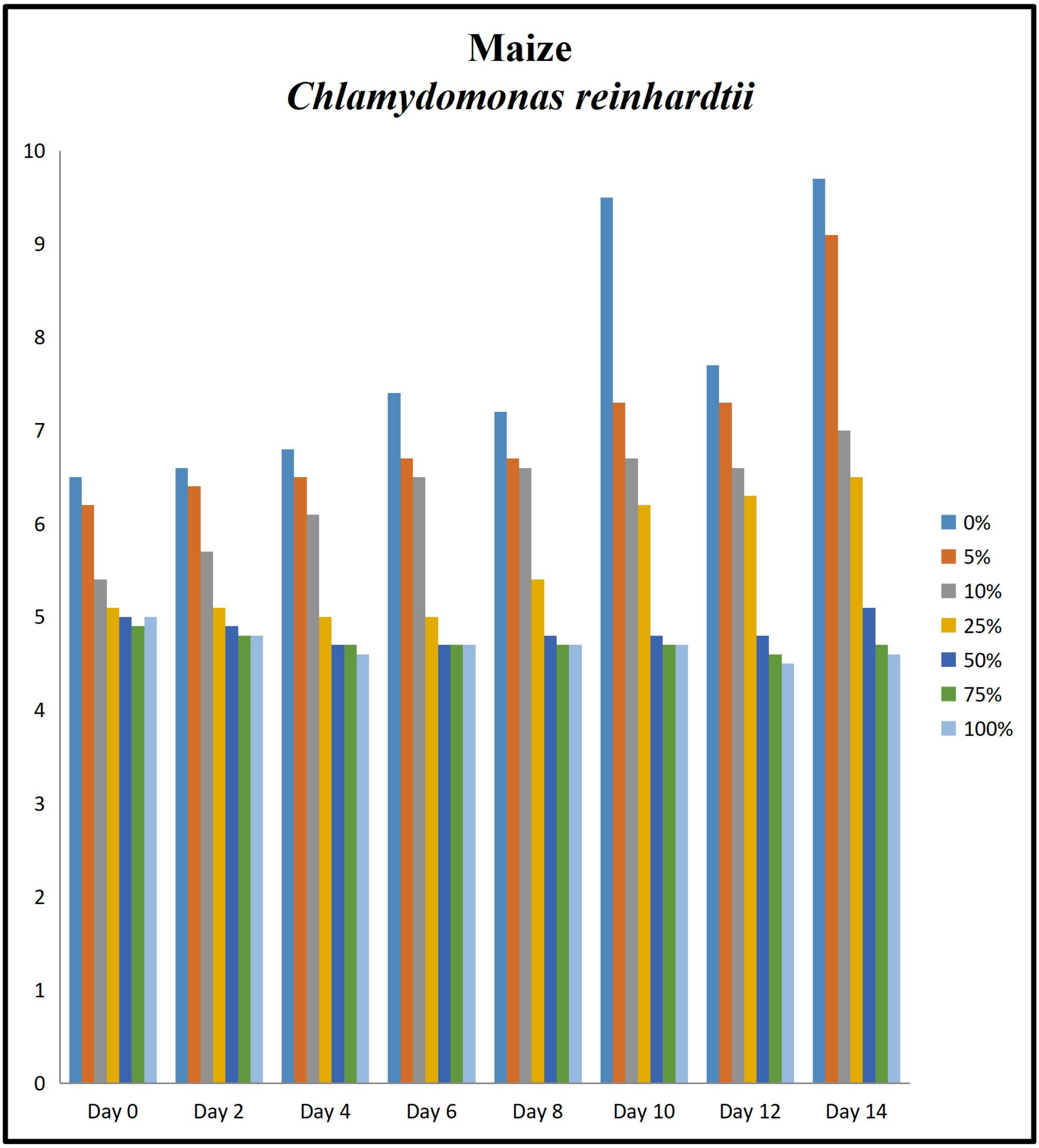

Figure 4.8a Change in pH of *Chlamydomonas reinhardtii* exposed to maize effluent over time

4.8b Variation in Electrical Conductivity (EC) of *Chlamydomonas reinhardtii* exposed to maize effluent over time

Figure 4.8b illustrated the changes in the electrical conductivity of the culture medium of *Chlamydomonas reinhardtii* upon exposure to various maize effluent concentrations for 14 days. Day 0 showed 0% concentration had the lowest EC approximately 200µS/cm. The highest treatment (100%) had the highest EC of approximately 800µS/cm. A clear increase in the conductivity for every concentration level from day 0 to day 14 was observed. The final EC on day 14 was strongly correlated with the maize concentration, exhibiting a dose-dependent increase. Reaching the highest conductivity value on day 14, 100% EC was 1900µS/cm, 75% concentration was slightly lower at about 1700µS/cm.

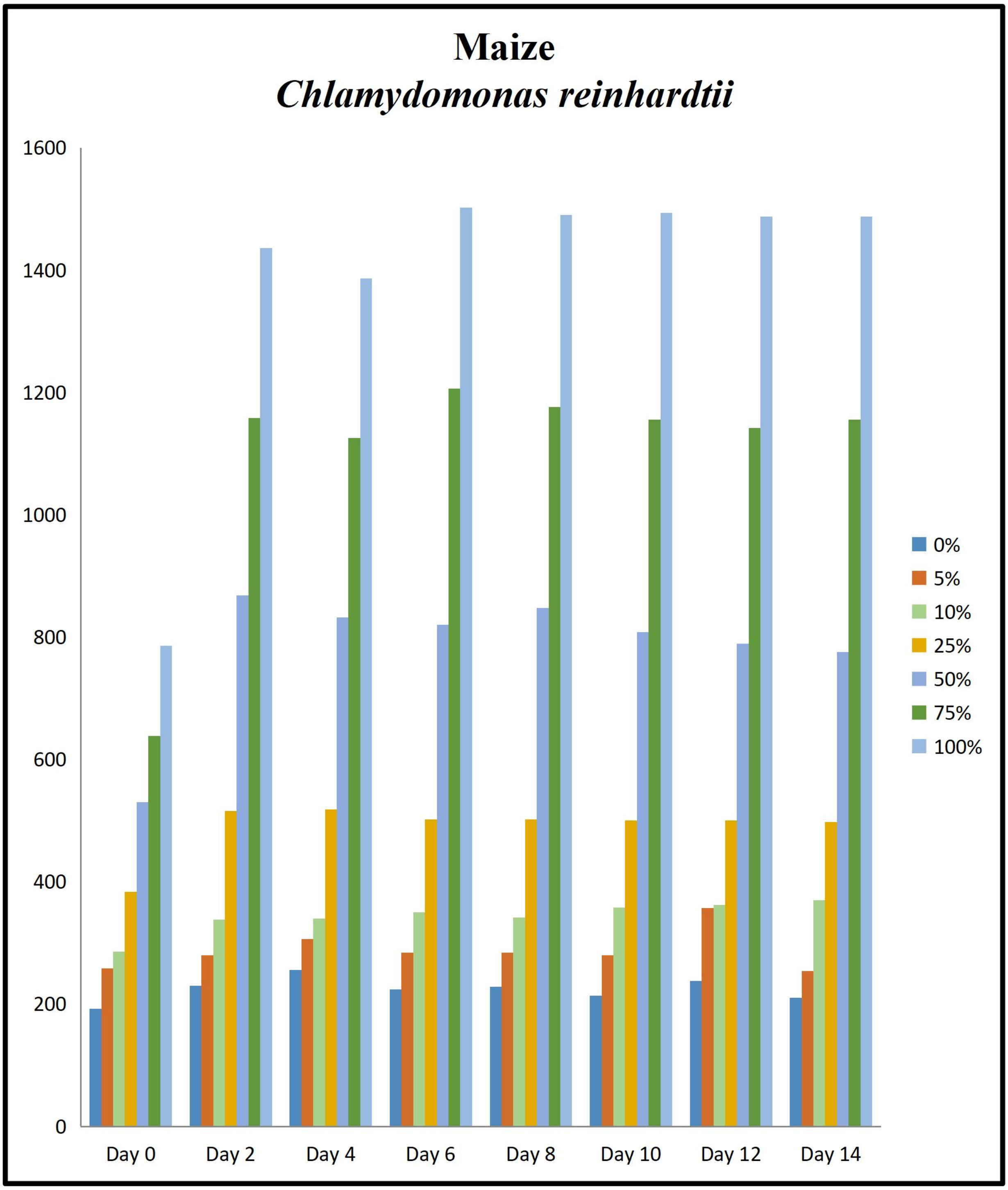

Figure 4.8b Variation in Electrical Conductivity (EC) of *Chlamydomonas reinhardtii* exposed to maize effluent over time

4.8c Variations in Total Dissolved Solids (TDS) of *Chlamydomonas reinhardtii* exposed to maize effluent over time

Figure 4.8c illustrated the manner in which the Total Dissolved Solids (TDS) of the culture medium of *Chlamydomonas reinhardtii* vary under the effect of different concentrations of maize effluent during the 14-day incubation period. At day 0, 0% treatment (control) had the lowest initial TDS of approximately 100mg/L. It was observed that there was a sustained increase in TDS for all concentration levels over the 14 days. The rate of increase is highest from day 0 to day 6 and continued to rise gradually toward day 14. The control (0%) concentration reached a final TDS of approximately 450mg/L. While 100% concentration reached the highest TDS value, approaching 800mg/L by day 14. The highest concentrations (50%, 75% and 100%) showed a visible increase in TDS on day 14 (about 388mg/L, 578mg/L and 758mg/L) respectively, indicating a dose-dependent effect.

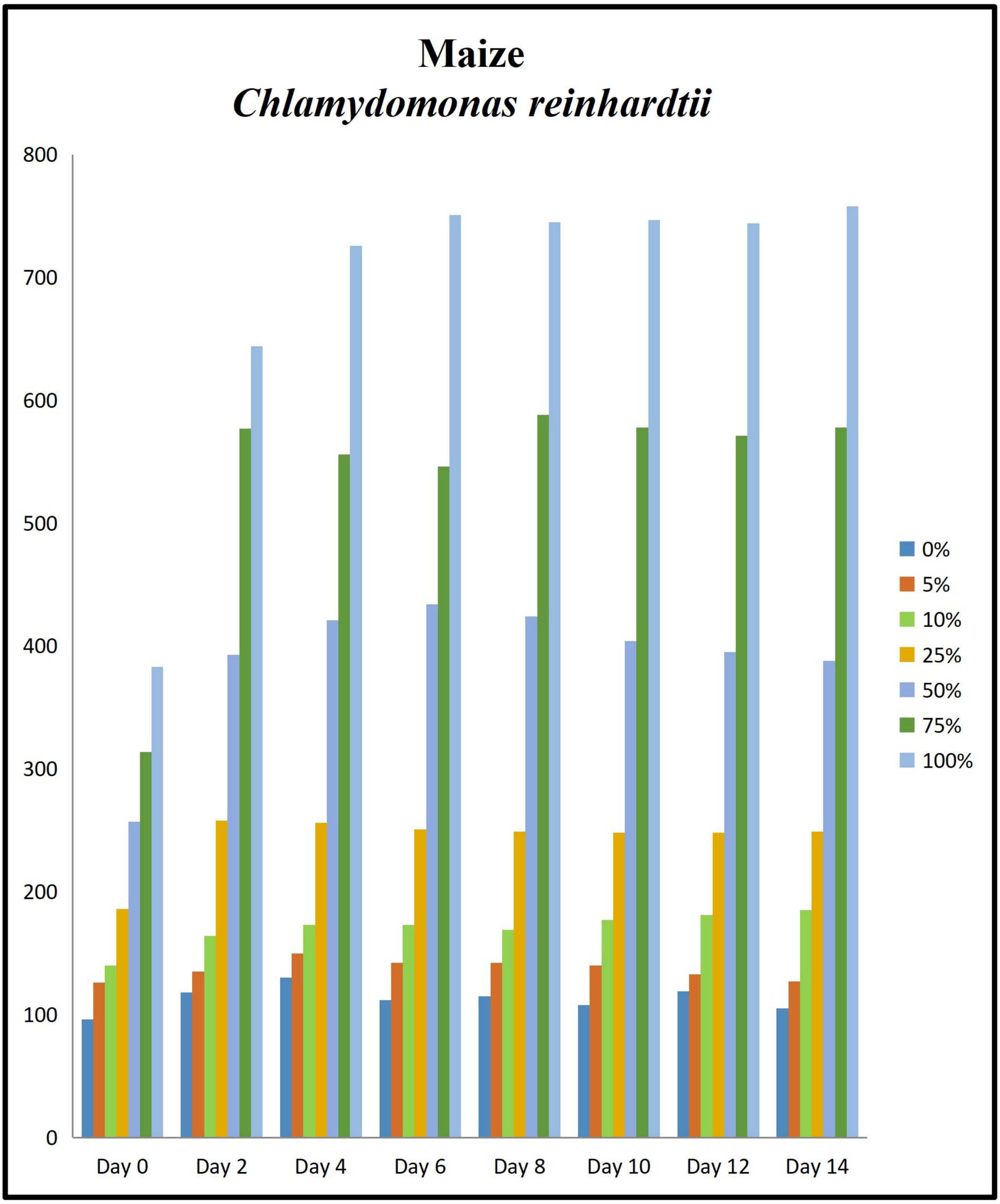

Figure 4.8c Variations in Total Dissolved Solids (TDS) of *Chlamydomonas reinhardtii* exposed to maize effluent over time

CHAPTER 5

5.1 DISCUSSION

Cassava contains toxic substances such as; cyanogenic glucosides, primarily linamarin, and a small amount of methyl linamarin (Uguru *et al*., 2019). Cassava effluent is basically generated from root washing, soaking/fermentation, retting and decanting. Negative impacts from cassava mill effluent discharge include environmental degradation, reduction of the aesthetic value of the surrounding such as offensive odor, and contamination of soils and groundwater. The flow of generated effluent into streams and rivers also affects aquatic plants and animals since the polluting constituents like cyanide and heavy metals exceed permissible limits (Lawal *et al*., 2019). Substantial volumes of cassava mill effluent are discarded annually in Nigeria when cassava is processed into garri. This waste ends up in open dumps or drainage systems, threatening surface water, ground water and soil quality (Ibiam *et al.,* 2025). Fermented maize is a widely utilized food in African countries and in fact cereals account for as much as 77% of total caloric consumption. Maize rich in carbohydrates, Including Potassium and Magnesium, contains trace amounts of Lysine and tryptophan, contributing to the low content of protein, and trace amounts of B-Vitamins (Olawuyi *et al*., 2018). After fermentation, the grains (maize) are rinsed in clean water, wet milled and sieved with a clean sterile muslin cloth. The filtrate is allowed to settle and the supernatant drained off. The resulting slurry paste is known traditionally as *Akamu* (Obi *et al*., 2022). Maize processing wastewater was enriched with a high content of nitrogenous and organic compounds was found to have COD and BOD concentrations were 70 and 58kgm^{-3} (Ewida *et al.,* 2020).

The effect of cassava mill and maize effluent on the growth of two freshwater microalgae (*Scenedesmus ecornis* and *Chlamydomonas reinhardtii*) was studied for a period of 14 days. In this study, the growth response of the test microalga; *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* to different effluent concentrations of cassava and maize were examined.

Growth response of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* in cassava mill effluent

In the growth response of *Scenedesmus ecornis* in cassava effluent, all treatments including the control, showed a relatively small increase, suggesting a lag phase before the full effect of the treatment was realized. Most concentrations showed an exponential increase in absorbance from day 2 to day 8, and dropped around day 10 to day 14. 0% showed the lowest absorbance rate, indicating the lowest level of the substance being measured. Exhibiting a slow, gradual increase, this reached an absorbance of 0.158 by day 14. 10% concentrations peaked around day 10 at 0.328 and remained stable. 25%, 50%, 75% and 100% treatments displayed the highest and most sustained increase in absorbance. 100% consistently shows the highest absorbance throughout the period from day 8 to day 14, which reached a peak of 0.731. The observed initial lag and subsequent exponential increase in *Scenedesmus ecornis* at moderate to high cassava effluent concentrations reflects the patterns reported by Keilla *et al*. (2021), who cultivated *Scenedesmus* species in cassava dilutions and found growth stimulation at specific dilution ranges. Like Keilla *et al*., this study indicates that cassava effluent supplies usable organic substrates that support *Scenedesmus* growth once cells acclimate. This suggests that the highest concentration of the treatment of cassava effluent (100%) leads to the greatest accumulation of the microalgae being measured.

In the growth response of *Chlamydomonas reinhardtii* in cassava effluent, 0% exhibited minimal growth, reaching a peak of about 0.25 around day 10 before entering a decline or stationary phase. 5% showed poor growth, peaking at only about 0.17 around day 8-10. It quickly reaches the stationary phase, which indicated acute nutrient limitation or possibly growth inhibition from residual compounds at low dilution. The 10% performed better than the 5% but declined early by day 8, maintaining a stagnant phase to about 0.319 by day 14. 25% and 50% displayed similar and highly effective growth profiles, with absorbance reaching 0.59 by day 14. 25% and 50% concentrations exhibited a prolonged exponential phase up to day 10, indicating sufficient nutrients without toxic effects. 75% showed a robust growth, reaching a final absorbance rate of about 0.569. 100% concentration treatment displayed an exponential growth phase immediately and sustained a high growth rate, reaching a steep stationary phase around day 10 with absorbance 0.609, and maintaining a high biomass until day 14. This result is consistent with the findings of Adekanmi *et al.,* (2020), who reported that microalgae (*Coelastrum morum*) grew effectively in nutrient-rich wastewater from fish ponds, reducing pollutants while maintaining biomass productivity. Both studies highlight the capacity of green microalgae to utilize wastewater as a growth medium, thereby aiding environmental bioremediation.

Growth response of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* in maize effluent

In the growth response of *Scenedesmus ecornis* in maize effluent, 0% (Control) showed minimal, slow growth, with a peak absorbance of 0.165 around day 12-14. This confirmed that the standard basal medium (CHU-10) alone limited nutrients. 5% and 10% followed a similar pattern to the control, maintaining very low biomass throughout the experiment. The 5% concentration ended with an absorbance of 0.140, and the 10% concentration slightly higher (0.158). It indicates that the 10% maize effluent concentration does not provide a significantly greater growth advantage over the control medium for *Scenedesmus ecornis*. 25% showed a gradual, steady increase in biomass, reaching an absorbance of 0.469 by day 14. 50%, 75%, and 100% concentrations exhibited the highest final biomass but display unstable growth rates. The stimulation of *S. ecornis* growth at moderate maize effluent concentrations agrees with the study by Ewida *et al*. (2020), who found maize processing wastewater to be rich in biodegradable organics and nutrients that favor microbial proliferation. Similar to Ewida *et al*., this study indicated that increasing maize effluent concentration generally supplies more utilizable nutrients, leading to higher algal growth and biomass until other limiting factors arise.

In the growth response growth of *Chlamydomonas reinhardtii* in maize effluent, there was a lag phase up to day 6 for all concentrations followed by a steep exponential growth phase. 0% exhibited very poor growth, with absorbance barely rising above 0.2 by day 14. The 5% concentration unexpectedly showed an initial lag until day 6, then slow growth, peaking at 0.31 by day 14. 10% treatment peaked around day 10 at 0.22 but showed a decline in biomass by day 14, indicating that the initial, limited supply of nutrients has been exhausted, and the culture has entered the decline phase. 25% concentration experienced a significant lag phase until Day 8, entering a rapid exponential growth phase between day 8 and day 10, reaching a plateau at an absorbance of 0.4, which it maintains until day 14. This suggests that at the 25% concentration, a key limiting nutrient or substrate (likely a complex carbohydrate in the maize) takes longer to become bioavailable to *C. reinhardtii*, but once it is utilized, growth proceeds effectively. 50%, 75%, and 100% concentrations yield the highest final biomass, showing highly effective growth. 75% and 100% are the most effective treatments, resulting in the maximum final biomass of absorbance 0.72 and 0.69 respectively by day 14. The observed lag phase followed by rapid growth of *C. reinhardtii* at intermediate to high maize effluent concentrations parallels the dynamics described by Obi and Okoronkwo (2022) during maize fermentation studies, where microbial succession and changing chemical availability affected growth timing. Both studies suggest that complex maize-derived substrates require time or enzyme induction before becoming rapidly bioavailable, which explains the delayed but steep growth once hydrolysis proceeds.

Dry weight of *Chlamydomonas reinhardtii* and *Scenedesmus ecornis* in cassava mill effluent

In this study, the cumulative dry weight for both microalgae, *Scenedessmus ecornis* and *Chlamydomonas reinhardtii*, was observed the highest at the 100% cassava effluent concentration, 1758.64 mg/L and 1835.53 mg/L respectively. *Chlamydomonas reinhardtii* achieved a slightly higher maximum cumulative dry weight than *Scenedesmus ecornis.* In the 0% concentration, *C. reinhardtii* exhibited a higher cumulative dry weight of 428.54 mg than *S. ecornis* (328.56 mg). 5% effluent concentration *S. ecornis* showed an exponential increase and a significantly higher cumulative dry weight of 794.82 mg compared to *C. reinhardtii* (482.03 mg). 10%, 25%, 50%, and 75% concentrations illustrated that *S. ecornis* consistently maintained a higher cumulative dry weight than *C. reinhardtii* across this range of concentrations. *C. reinhardtii* achieved the highest overall dry weight in the 100% concentration of 1835.53 mg, surpassing *S. ecornis* (1758.64 mg) to a small degree. The high cumulative dry weight of *C. reinhardtii* at concentrated cassava effluent mirrors the research by Budiyono and Kusoworo (2011), who reported strong microbial activity and biomass production when cassava-derived waste streams were used in bioprocesses. While *C. reinhardtii* performed better in the control (0%) and 100% concentrations, *S. ecornis* showed greater cumulative growth across the intermediate concentrations (5% to 75%) of the cassava effluent. Both microalgae showed an increase in cumulative dry weight as the cassava effluent concentration increases, indicating the effluent acted as a nutrient source for promoting growth.

Dry weight of *Chlamydomonas reinhardtii* and *Scenedesmus ecornis* in maize effluent

In this study, the highest cumulative dry weight for both microalgae *Chlamydomonas reinhardtii* and *Scenedesmus ecornis* was observed at 50% concentration for *S. ecornis* (2018.7 mg) and 100% concentration for *C. reinhardtii* (1953.18 mg) with 75% concentration also yielding a very high result of 1812.25 mg. The high cumulative biomass observed for *S. ecornis* in maize effluent is comparable to the study by Olawuyi *et al.* (2018), which reports on the positive plant growth responses when maize-related effluents provided ample nutrients. The lowest cumulative dry weight for both microalgae was at 5% concentration, with *S. ecornis* having a cumulative dry weight of 283.14 mg, being lower than the control (0%) and the cumulative dry weight of *C. reinhardtii* (323.64 mg) also showed lower growth than the control. *C. reinhardtii* showed higher cumulative dry weight in the low-to-moderate (0%, 5%, 10%) and high (75%, 100%) concentrations. *S. ecornis* outperformed *C. reinhardtii* significantly at the 25% and 50% concentrations, its overall maximum growth achieved at the 50% treatment. The robust biomass yields of *C. reinhardtii* in concentrated maize effluent reflect McConnell and Farag (2012) findings that microalgae can achieve high productivity in wastewaters when nutrients are abundant. The growth response to maize effluent concentration is different for both microalgae. *S. ecornis* thrived at a moderate concentration of 50%, however *C. reinhardtii* demonstrated higher tolerance and growth at the high (75% and 100%) concentrations.

Percentage inhibition of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* in cassava mill effluent

The percentage inhibition of growth of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii,* exposed to cassava mill effluent showed a clear concentration dependent relationship. At all tested concentrations, cassava effluent did not inhibit the growth of the microalgae, instead its growth was stimulated. For both species (*S. ecornis* and *C. reinhardtii*), as the concentration of cassava effluent increased from 5% to 100%, the magnitude of the negative inhibition (the stimulation of growth) also increased. For *Scenedesmus ecornis*, the stimulation is lowest at 5% around -140% and highest at 100% (-450%). For *Chlamydomonas reinhardtii*, the stimulation is lowest at 5% at about -50% and highest at 100% (-330%). This suggests that the nutrients in the effluent are the limiting factor for the microalgae's growth, and providing a more concentrated source of the effluent maximizes this growth. *Scenedesmus ecornis* showed significantly greater stimulation at every concentration compared to *Chlamydomonas*. Its growth was stimulated at -450% at 100% effluent concentration, which means it is highly efficient at utilizing the resources in the raw cassava effluent. *Chlamydomonas reinhardtii* showed strong stimulation, but less obvious than *Scenedesmus ecornis*. Its inhibition peaked at -330% at 100% concentration. The marked inhibitory effects at certain cassava concentrations are consistent with Sedara *et al*. (2022), who documented adverse impacts of cassava processing effluent on soil and biota due to acidity and high soluble salts.

Percentage inhibition of *Scenedesmus ecornis* and *Chlamydomonas reinhardtii* in maize effluent

In the maize effluent, *Scenedesmus ecornis* and *Chlamydomonas* showed a very slight positive percentage Inhibition at 5% Concentration, about +5% for *S. ecornis* and +10% for *Chlamydomonas*. This indicated a very mild inhibitory or toxic effect at the lowest concentration. At 10% to 100% Concentrations, the percentage inhibition were significantly negative, ranging from about -25% to -500%. This confirmed that as the concentration of maize effluent increases, it shifts from being mildly inhibitory to strongly stimulatory (promoting growth). The negative percentage inhibition signified increased growth compared to the control (0%). The low inhibition at increasing maize effluent concentrations aligns with Onuorah *et al*. (2019), who showed that fermented maize media (akamu) are microbiologically active and can support high microbial loads and metabolite production. For both microalgae, increasing the maize effluent concentration from 10% to 100% resulted in a corresponding increase in the magnitude of growth stimulation, indicating that the effluent is a rich source of growth-promoting nutrients (such as nitrogen, phosphorus, and organic carbon from the maize processing waste), and increasing the concentration treatment supplied more of these limiting factors.

Physicochemical parameters of *Scenedesmus ecornis* in Cassava mill effluent

The physicochemical parameters used in this study were pH, Total Dissolved Solids (TDS) and Electrical conductivity EC).

In this study, the pH values for *Scenedesmus ecornis* exposed to cassava effluent showed a gradual increase with time and concentration. On day 0, the 0% concentration started with the highest pH 6.5. As the concentration of cassava effluent increased from 5% to 100%, the initial pH gradually decreased to 4.9 for the 75% and 100% concentrations. 0% Increased from 6.5 (day 0) to 10.1 by day 14. 5% increased from 5.6 (day 0) to 7.2 by day 14. The acidifying effect observed at higher cassava concentrations and the suppressed pH rise during algal growth concur with Isimah *et al*. (2025), who recorded low pH in cassava-impacted soils and waters due to cyanogenic glycosides and organic acids. While the photosynthetic activity of *S. ecornis* attempts to raise the pH, the high concentrations of cassava effluent contain toxic compounds like cyanide and various organic acids that can inhibit its growth and photosynthesis, thereby suppressing the algae's ability to raise the pH. The lowest concentrations (0% and 5%) allowed *S. ecornis* to thrive, leading to the significant increase in pH.

TDS is a measurable quantity of total weight of mobile charged ions in the form of metals, minerals, and salts dissolved in water. As measurements of TDS tend to be based on electrical conductivity (EC) readings, trends shown here are primarily an indication of the EC values. At day 0, the 0% concentration started at the lowest TDS, close to 0 mg/L indicating the dilute growth medium. As the effluent concentration in the mixture increases, it introduces a proportionally larger amount of dissolved inorganic salts, organic matter, and other ions, leading to a higher initial TDS reading. At high concentrations of 75% and 100%, TDS remained high, with no visible sustained drop, while at low Concentrations (0%, 5%, 10%), TDS remained low and stable. The consistently high TDS in cassava treatments mirrors Sedara *et al*. (2022)'s documentation of high dissolved solids in cassava-impacted sites.

0% concentration at day 0 starts at the lowest EC of 186µS/cm representing the background conductivity of the control medium (Chu-10). The 100% concentration had the highest EC of about 1716µS/cm. This confirmed that the cassava effluent contains a high concentration of dissolved electrolytes (ions and salts). Increasing the volume of effluent proportionally increases the number of free ions available to carry an electrical current, thus increasing the EC. The high electrical conductivity with the initial acidic pH and potential toxins in the effluent may have inhibited the growth of *Scenedesmus ecornis*. The electrical conductivity data confirmed that the raw cassava effluent contains a very high concentration of charged dissolved solids, and the *Scenedesmus ecornis* treatment was ineffective at significantly lowering the electrical conductivity of the effluent over the 14-day period. The high initial EC and limited EC reduction by *S. ecornis* in the highest cassava concentrations are consistent with Sedara *et al*. (2022), who documented elevated electrical conductivity in cassava-impacted soils and effluents.

Physicochemical parameters of *Chlamydomonas reinhardtii* in Cassava mill effluent

At day 0, all concentrations showed an initial pH below neutral (7.0), confirming the acidic nature of the cassava effluent. 0% (control) began at the highest pH of 6.5. 100% (undiluted effluent) started at the lowest pH level of 5.0. The 0% (control) and 5% concentrations showed the most significant pH increase. With 0% rising sharply from 6.5 at day 0 to 10.0 by day 14. 5% rises significantly from 5.7 (day 0) to 7.1 on day 14. This indicated that the low concentration of cassava effluent provided nutrients without being toxic to *Chlamydomonas* growth. As the concentration increased from 10% to 100%, the pH increase becomes progressively suppressed. The

10% concentration peaked around 6.7 on day 10. The high concentrations of cassava effluent introduce toxic compounds such as cyanide and heavy organic load that eventually inhibited the growth *Chlamydomonas*. The limited pH increase in high-cassava treatments and pronounced alkalization in controls reflect the dynamics Paddock *et al*. (2019) described for algal-mediated pH shifts: active photosynthetic growth raises pH unless counteracted by acidic effluent constituents.

On day 0, the 0% concentration started at the lowest TDS, close to 0 mg/L. While the 100% concentration started at the highest TDS, around 2000 mg/L. For every concentration from 0% to 100%, the TDS values remain virtually constant over the 14-day duration, showing only minor, negligible changes. High concentrations (75% and 100%) remained high, around 1500–2000 mg/L, while low concentrations of 5% and 10% remained low, around 500 mg/L or less. The majority of the dissolved solids in the cassava effluent consist of recalcitrant organic matter, salts, and ions that the algae *C. reinhardtii* could not easily assimilate or degrade within the 14-day period. For effective wastewater remediation, a pre-treatment step to reduce the overall TDS and toxicity, or a longer treatment time, would likely be necessary. The limited TDS decline over 14 days in the cassava treatments supports Paddock *et al*. (2019)'s point that certain effluent constituents are not rapidly assimilated by algae and require longer treatment.

At day 0, 0% (control) treatment started near 0µS/cm, while the 100% (undiluted effluent) concentration began at the highest value, approximately 4000µS/cm. For all concentrations of cassava effluent, the electrical conductivity readings demonstrated negligible change over the 14-day period. 5%, 10%, 25%, 50%, 75%, and 100% concentrations showed that the high initial EC values were maintained with minimal reduction throughout the experiment. The 100% concentration remained high, around 4000µS/cm, while the lower concentrations also remained stable at their respective starting points. The primary mechanism by which microalgae reduce electrical conductivity is by assimilating ionic nutrients for growth and converting them into non-conducting cellular biomass. The lack of a decrease in EC strongly indicated that *C. reinhardtii* growth was inhibited, the toxicity of the cassava effluent significantly suppressed the metabolic activity and growth *C. reinhardtii*.

Physicochemical parameters of *Scenedesmus ecornis* in Maize effluent

This study showed that on day 0, the pH values were generally neutral to slightly acidic across all concentrations. 0% concentration started at the highest pH of 7.4. As the concentration increases, the initial pH generally decreased, indicating that the maize effluent is slightly acidic. A major trend is the increase in pH for most concentrations as the experiment progressed from day 0 to day 14. The increase was most dramatic in the higher concentration treatments from 50% to 100%. The 0% treatment showed the largest absolute change, rising from 7.4 on day 0 to 9.5 by day 14. 100% concentration showed a decrease in pH from 5.0 on day 0 to 4.7 by day 14. Treatments from 5% to 50% indicated a slightly lower final pH than the 0% treatment. 0% concentration is also highly alkaline at day 14 approximately 9.5. For many concentrations, there is a significant drop in pH around day 2 to day 4 before the general upward trend takes over. For instance, the pH of 0% concentration drops from 7.4 at day 0 to 6.7 by day 2. The initial decrease suggests a possible initial fermentation or release of acidic metabolites before the primary pH-raising mechanism dominated.

At day 0, 0% concentration (control), had the lowest TDS approximately 100 mg/L. TDS increases steadily with concentration, culminating in the 100% concentration treatment having the highest initial. This confirmed that the maize component is the primary contributor of dissolved solids (nutrients, salts, organic molecules) to the culture medium. TDS exhibited a significant increase across all treatments over the 14-day period. The increase is much more pronounced in the higher concentration treatments from 50% to 100%, compared to the lower treatments (0% to 25%). Treatments with higher maize concentration (75% and 100% of approximately 750-800mg/L) likely supported significantly higher microalga biomass production. This rapid, high growth rate would lead to a corresponding higher release of dissolved organic metabolites, resulting in the dramatic TDS increase observed. The observed TDS increase in maize treatments particularly at higher concentrations reflects Adekanmi *et al.* (2020) observation that microbial metabolism may convert particulate organics into dissolved by-products, sometimes increasing TDS temporarily even as pollutants are transformed.

The 0% concentration has the lowest EC of approximately 200µS/cm on day 0. 100% concentration has the highest initial EC of approximately 350µS/cm. This confirmed that the maize effluent, by providing salts and nutrients, is the source of the initial dissolved ions. There was a clear and sustained increase in EC for every concentration level from day 0 to day 14. The rate of increase is highest in the initial phase (day 0 to day 4 or 6) and continues to rise until day 14. The control (0%) showed a modest increase, reaching a final EC of approximately 1000µS/cm. 75% and 100% concentrations also showed the most dramatic increase, both reaching the highest final conductivity value, approaching 1500µS/cm. The treatments increasing from 0% to 100%, indicates a dose-dependent effect of the maize component. The higher maize concentrations (75% and 100%) support the most exponential growth of *S. ecornis*. Higher algal activity means a faster and greater rate of organic matter consumption, leading to a massive release of metabolic by-products and breakdown products that significantly raise the electrical conductivity to nearly 1500µS/cm.

Physicochemical parameters of *Chlamydomonas reinhardtii* in Maize effluent

The pH readings at day 0 indicated the effluent is neutral to slightly acidic. At 0% concentration the highest pH obtained was 6.5. The pH decreases as the maize effluent concentration increases, with 100% treatment having a low pH of 5.0, which is slightly higher than 75% concentration with pH of 4.9. This concluded that the maize effluent was slightly acidic. The 0% concentration (control) showed a distinct and significant alkalization trend increase in pH. The pH increased from 6.5 on day 0 to a peak of 9.7 by day 14. This strong alkalization is typical of active photoautotrophic algal growth. 100% treatment exhibited a pH of 5.0 at day 0 and continued to decrease to a pH of 4.6 by day 14. 5% to 25% concentrations showed an initial drop on day 0 to 4, followed by a partial recovery and increase. The pH increase observed in control and some diluted maize treatments mirrored the study by Ewida *et al*. (2020), who recorded rising pH during biodegradation of maize wastewater as microbial oxidation and algal activity shift carbonate equilibria

0% treatment (control) had the lowest initial TDS (100mg/L) by day 0. It was observed that there was a sustained increase in TDS for all concentration levels over the 14 days. The rate of increase was highest from day 0 to day 6 and continued to increase gradually toward day 14. 0% concentration exhibited a final TDS of approximately 450mg/L. While 100% concentration reached the highest TDS value, approaching 800mg/L by day 14. 50%, 75% and 100% concentrations showed a visible increase in day 14, indicating a dose-dependent effect.

The control (0%) treatment showed the lowest EC on day 0 of approximately 200µS/cm.100% had the highest EC of approximately 800µS/cm. This confirmed that the maize effluent was the source of the initial ionic load, with higher concentrations having more dissolved salts and charged compounds. A clear increase in the conductivity for every concentration level from day 0 to day 14 was observed. The final EC on day 14 indicated it was strongly correlated with the maize concentration, exhibiting a dose-dependent increase. 100% concentration had the highest conductivity value on day 14; reaching 1900µS/cm. 75% concentration was slightly lower at about 1700µS/cm. The progressive rise in electrical conductivity paired with strong algal growth is similar to McConnell and Farag (2012) research that observed the microalgae grown in nutrient-rich wastewaters can be transformed to complex organics into dissolved metabolites that increase measured conductivity. This pattern indicated that increased maize effluent concentration led to a greater final accumulation of dissolved ions in the medium.

5.2 Conclusion

This study successfully demonstrated that both Cassava Mill Effluent (CME) and Maize Effluent (ME) are excellent, low-cost nutrient sources that significantly promote the growth and biomass accumulation of the microalgae *Scenedesmus ecornis* and *Chlamydomonas reinhardtii*. *S. ecornis* achieved the highest overall biomass at 50% Maize Effluent (approximately 2019 mg/L) identifying this combination as the optimal for biomass production. Both microalgae exhibited high tolerance to concentrated effluents, which stimulated growth by up to 500% compared to the control medium. However, the effluents presented distinct challenges; cassava had a much higher initial salinity EC and TDS, and was more acidic, severely inhibiting the microalgae's ability to reduce the overall ionic load in undiluted solutions. Conversely, maize effluent, despite having a lower initial ionic load, showed an increase in EC and TDS as the microalgae grew, indicating effective degradation of complex organic matter into charged by-products, rather than immediate full nutrient assimilation. The research validates the potential of using these effluents as microalga growth media for integrated bioremediation and biofuel feedstock production, but also highlights the necessity of pre-treatment for managing pH and high EC in cassava mill effluent, and the long-term management of organic degradation by-products in maize effluent.

5.3 Recommendations

Based on the study, the following recommendations are considered;

1. Both cassava and maize effluents should be used as excellent, low-cost nutrient sources for cultivating *S. ecornis* and *C. reinhardtii*. This study validates their potential for integrated bioremediation and biofuel feedstock production.

2. The microalgae are highly tolerant to concentrated effluents, with growth stimulated by up to 500% compared to the control medium, hence they can be utilized for bioremediation of wastewater.

3. The high ionic load in undiluted concentrations of cassava effluent severely inhibited the microalgae's ability to reduce the overall ionic load. Therefore, pre-treatment of cassava effluent is required to manage its high initial salinity (EC and TDS) and acidity (low pH).

4. *Scenedesmus ecornis* showed significantly greater growth stimulation than *Chlamydomonas reinhardtii* at every concentration, indicating it is more effective for any bioremediation process.

5. In order to observe the growth of the microalga in maize effluent, the lowest concentration (5%) should be avoided, as it showed in this study a very mild inhibitory and toxic effect on both species.

6. Subsequent studies should test a two-stage system: first, pre-treating the effluent (e.g., anaerobic digestion) to reduce recalcitrant organic load, followed by the microalga integration to remediate the effluents and maximize biomass yield.

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