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STIMULATORY EFFECTS OF CASSAVA EFFLUENT ON THE GROWTH RESPONSES OF *Euglena viridis* (O.F. Müller) Ehrenberg AND *Chlorella vulgaris* Beijerinck

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Abstract
This study evaluated the effects of cassava mill effluent (CME) on the growth of the microalgae Chlorella vulgaris and Euglena viridis. The microalgae were cultured in seven CME concentrations (0, 5, 10, 25, 50, 75, and 100%), each in triplicate, and monitored over 14 days. Growth was determined at two-day intervals using a 721 visible spectrophotometer. Dry weight, percentage yield, percentage inhibition, and analysis of variance (ANOVA) were computed, while pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured using standard methods. Both species exhibited rapid growth between days 2 and 4, followed by fluctuating growth from days 6 to 12 and a decline by day 14, particularly at 75% and 100% CME. ANOVA showed significant differences in growth among effluent concentrations and exposure periods (p < 0.001). Growth responses demonstrated a concentration-dependent hormetic pattern, with significant stimulation at low CME concentrations (5–50%) and reduced growth or inhibition at higher concentrations (75–100%). Euglena viridis exhibited a stronger growth response and higher biomass yield than C. vulgaris. Physicochemical characteristics also varied with CME concentration. pH decreased with increasing effluent concentration, ranging from 8.36 to 3.58 in E. viridis *cultures and from 6.05 to 3.62 in C. vulgaris cultures. Conversely, EC increased from 417 to 3950 µS/cm and 437 to 3908 µS/cm, while TDS increased from 208 to 1975 mg/L and 218 to 1954 mg/L, respectively. These findings demonstrate that diluted cassava mill effluent can enhance microalgal growth, whereas higher concentrations create conditions that suppress growth, highlighting the importance of effluent dilution for potential phycoremediation and biomass production applications.*

Keywords: *Cassava mill effluent, Microalgae, Euglena viridis, Chlorella vulgaris, Phycoremediation*

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important staple food crops in tropical areas and is a source of carbohydrate for more than half a billion people globally (Cock and Connor, 2021). Nigeria is a major producer of cassava, from which a wide variety of products including “garri”, “fufu”, “lafun” and starch are produced (Sanni *et al*., 2024).

The disadvantages of cassava processing are however very high production of wastes such as cassava peelings and cassava mill effluent. Peels account for a large percentage of the tuber, whereas cassava effluent is the liquid waste that is generated in the washing, grating, fermentation and dewatering process (Achi *et al*., 2017; Izah *et al*., 2018). Such effluents are commonly discharged into the environment directly without undergoing any treatment in most of the developing countries, particularly in countries where the traditional processing methods are followed, resulting in significant environmental issues (Chinedu *et al*., 2023).

Cassava mill effluent (CME) has a high amount of organic matter, suspended solids, nutrients, and cyanogenic compounds. Its toxicity in the environment is due to the presence of cyanogenic glycosides like linamarin and lotaustralin (Egboduku *et al*., 2024), which when broken down release the toxic hydrogen cyanide. Cassava effluent discharges have been observed in various studies to change the physicochemical properties of the soil, decrease the amount of vegetation cover, pollute surface and ground water, and have detrimental effects on surface and subsurface water ecosystems (Izah *et al*., 2018). Additionally, during high rainfall, these pollutants can be washed into streams, ponds and rivers where they can cause eutrophication and harm aquatic organisms (Olaniyan *et al*., 2025). There have been many studies that documented the negative impact of cassava effluent on fish, plants, soil microorganisms or other biological system, thus revealing the need of proper management and treatment to minimize impacts (Howeler and Oates, 2018; Agoh *et al*., 2024).

Microalgae are minute photosynthetic organisms, which are essential to aquatic ecosystems. They play the important roles of primary productivity, carbon sequestration, oxygen production, and nutrient cycling in the world (Dolganyuk *et al*., 2020). Microalgae have tremendous economic value especially for biofuel, pharmaceuticals, nutraceuticals, cosmetics, environmental remediation, and aquaculture apart from their ecological significance (Peng *et al*., 2020). The biological genera that are especially noted are *Chlorella vulgaris* and *Euglena viridis* because they have rapid growth, adapt to fluctuations in environmental conditions, and have the ability to uptake and transform wastewater pollutants (Toyama *et al*., 2018).

Cassava effluent, containing potentially toxic substances and nutrients for algal growth, is a fascinating subject that has received a lot of scientific attention. High levels of pollutants can influence cell functions, whereas lower levels can act as nutrients to boost cell biomass. Soluble cassava mill effluent has been shown previously to be suitable as a nutrient source for microalgal culture (Sorgatto *et al*., 2021), but research comparing species' responses to different concentrations of cassava mill effluent is limited. These responses are key in assessing the ecological risks of effluent discharge, and in investigating efforts to use microalgae as sustainable bioremediators and wastewater treatment agents. This study was therefore conducted to examine the effects of different concentrations of cassava mill effluents (CME) with growth response, percentage inhibition and to assess the bioremediation potential of *Chlorella vulgaris* and *Euglena viridis*.

Materials and Methods

Test Organisms and Culture Establishment

Two types of microalgae species, *Chlorella vulgaris* and *Euglena viridis*, which can be grown in freshwater, were selected as test algae. A sample of the mixed algal samples was taken from the fish pond in the Faculty of Agriculture University of Benin, and selected ponds/ditches in Benin City. Unialgal cultures were obtained by isolating the species of interest from mixed samples and by successively subculturing in modified Chu 10 medium until unialgal cultures were achieved. Prior to the use of the species for experiments, species identity was established microscopically.

Cassava Mill Effluent Collection

The cassava mill effluent was sampled from a local cassava processing mill in Uromi, Edo State, Nigeria. The effluent was transferred to the laboratory for making up experimental treatments.

Culture Medium

Chu10 medium was used as the growth medium for the microalgae with modifications. All media components were weighed and constituted, and comprised of macronutrient stock, iron stock, trace element stock and vitamin stock solutions. Distilled water was used to prepare all stock solutions which were sterilized by autoclave prior to use.

Experimental Design

The experiment consisted of seven cassava mill effluent concentrations: 0% (control), 5%, 10%, 25%, 50%, 75%, and 100% (v/v). Various treatment concentrations were obtained by successive dilutions of cassava mill effluent in modified Chu 10. There were three replications of each treatment for each microalgal species, with the design being completely randomized. An experimental vessel such as a sterile 250 mL culture bottle was used for these. The effluent-medium mixture to be used for a particular effluent was prepared in each bottle and 5 mL of actively growing unialgal culture was added. After the inoculation the flasks were covered, but not tightly, to allow gas exchange while preventing contamination.

Growth Assessment

The cultures were kept for 14 days in natural light conditions, under laboratory ambient conditions. A 721 visible spectrophotometer was used to monitor algae growth at 2-day intervals at a wave length of 750 nm. Not before making measurements, the instrument was calibrated to distilled water. Optical density readings were made for all of the treatments and for all replicates during the period of the exposure. Culture vessels positions were systematically rotated after each sampling interval, in order to achieve uniform illumination for all bottles.

Determination of Growth Inhibition and Yield

Percentage growth inhibition was calculated using:

$$Percentage\ inhibition = 100 - \left(\frac{Measured\ biomass}{Theoretical\ biomass}\right)\ x\ 100$$

Percentage yield at the end of the experiment (Day 14) was calculated as:

$$Percentage\ inhibition = 100 - \left(\frac{Actual\ yield}{Theoretical\ yield}\right)\ x\ 100$$

Physicochemical Analysis

The pH, electrical conductivity (EC), and total dissolved solids (TDS) of the raw cassava mill effluent and treatment media were determined before and after the experiment. pH was measured using a HANNA pH meter, while EC and TDS were measured using a HACH CO150 conductivity/TDS meter according to the manufacturers' instructions.

Data Analysis

Data were expressed as mean ± standard error (SE) of three replicates. Differences among treatments were evaluated using one-way analysis of variance (ANOVA) in Microsoft Excel. Statistical significance was accepted at $p < 0.05$.

Results and Discussion

Growth Response to Cassava Mill Effluent

The present study showed that cassava Mill Effluent (CME) had a concentration dependent effect on growth, inhibition response, yield and the physicochemical conditions of *Euglena viridis* and *Chlorella vulgaris*. Figure 1 presents the growth response of *Euglena viridis* to cassava mill effluent and it shows an increase in the growth of the algae upon addition of the effluent. There was a gradual upward trend in growth response from day 0 to day 4 for all the effluent treatments except for the control. Growth in the control (0%) did not increase much over the days of experiment, with the highest growth being 0.109 on day 14. Low effluent concentration (5% and 10%) significantly stimulated growth, with a peak of 0.943 on day 14 at the lower effluent concentration of 10%. Optimum growth was observed in moderate concentrations (25% and 50%), but with some variations, while higher concentrations (75% and 100%) led to an increase up to day 10 before declining. There were significant treatment effect, $p<0.001$, and temporal effect, $p<0.001$, observed in the ANOVA.

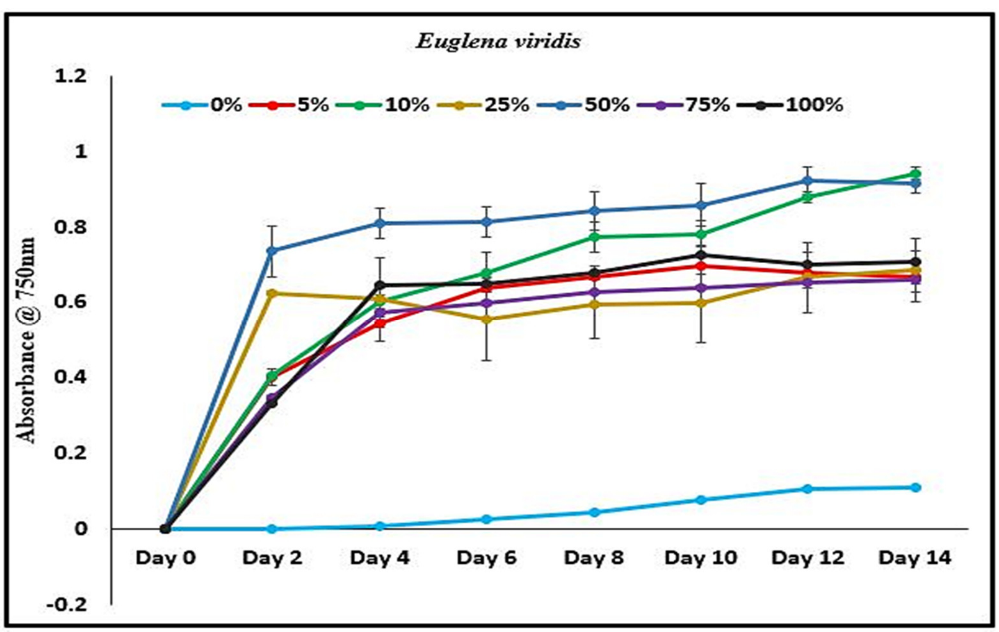

Fig. 1: Growth response of *Euglena viridis* to cassava mill effluent

The growth of *Chlorella vulgaris* followed different growth relationships with the different concentrations of cassava mill effluents. The growth in control was markedly less compared to the treatment concentrations. At 5%, a logarithmic phase was observed from day 0 to day 8, with a stabilizing stationary phase from day 12 to day 14, and a drop was observed at day 14. Growth remained steady, peaking at 0.773 on day 14 in the 10% CME culture. At 25% and 50%, growth showed a log phase (days 0–4), dips (days 4–8), then recovery toward day 14. At 75% and 100%, log growth occurred (days 0–4), slight dips (days 4–6), then steady growth to day 14. The differences between the treatment and the experimental days were found to be statistically significant (p<0.001) using ANOVA.

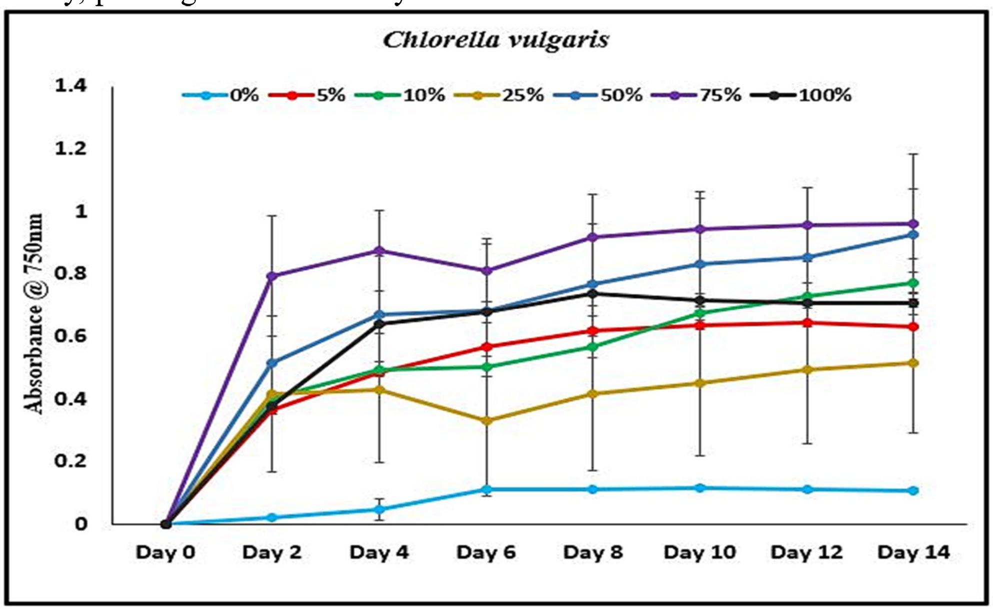

Fig. 2: Growth response of *Chlorella vulgaris* cassava mill effluent

Percentage Inhibition

Euglena viridis and *Chlorella vulgaris* were exposed to cassava effluent, and the percentage inhibition (stimulation is indicated with negative values) is shown in Fig. 3. Overall, both types of microalgae were stimulated at all concentrations, with lower concentrations (5% to 50%) resulting in higher degrees of stimulation compared to higher concentrations (75% to 100%). Less stimulation was found in *C. vulgaris* than in *E. viridis*. The best stimulation for *E. viridis* was 50% and the least was 75%. In the case of *C. vulgaris* it was found that 75% was the highest stimulation while 25% was the lowest.

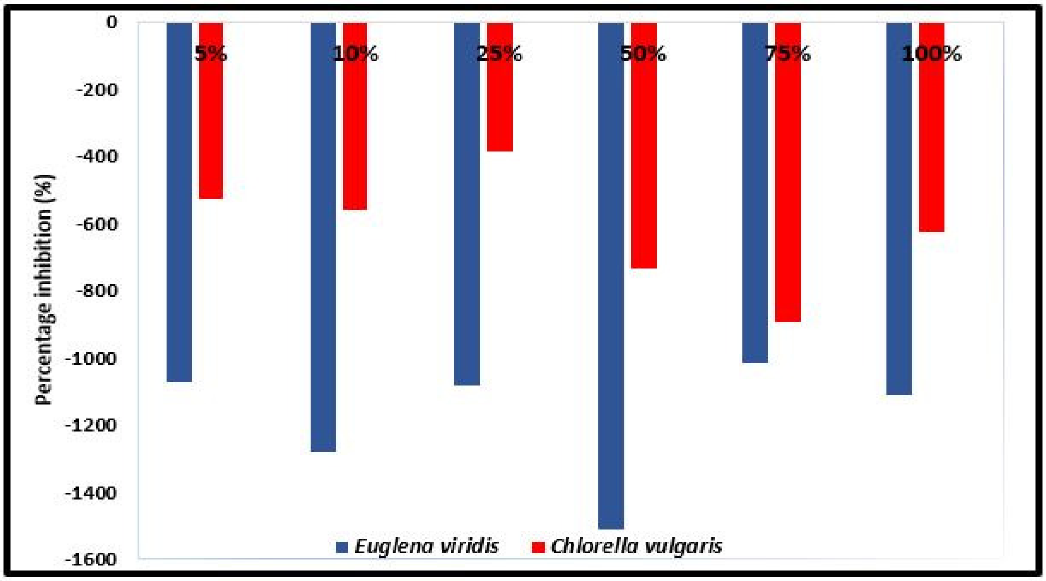

Fig. 3: Percentage inhibition of *Euglena viridis* and *Chlorella vulgaris* in cassava mill effluent

Low concentrations of CME (5–25%) stimulated growth and high concentrations of CME (75–100%) depressed growth performance, suggesting a concentration-dependent dual role of CME as a stressor and nutrient source in the system. This discovery is ecologically significant because microalgae play an important role in the biological community, in primary productivity and carbon fixation in aquatic ecosystems (Dismukes *et al*., 2018; Gigova and Marinova, 2016). Microalgae consume the minerals in the wastewater to optimize their growth process while creating biomass that can be employed for a variety of uses (Ezenweani *et al*., 2018). Akhere and Chukwuka (2023) reported optimal growth of *C. vulgaris* at a lower wastewater concentration while Nwankwo and Agwa (2019) found optimum development of algal growth in mixtures of cassava peels and wastewater. It can be inferred that the increased growth in early culture is probably due to the provision of various nutrients by a diluted CME that were needed for microalgal metabolic processes. Cassava processing residues (CPRs) contain nitrogen and phosphorus that aid in photosynthesis and biomass building (Agwa *et al*., 2014).

The results of the present study showed that *E. viridis* was more stimulated than *C. vulgaris* thereby having a better tolerance against the stress imposed by wastewater. This is consistent with Wang *et al*. (2018) and Gissibl *et al*. (2019), which described that *Euglena* spp. have a better resistance capacity to acidity, metal stress, and fluctuation in the environment compared to other microalgae. However, the diminished growth responses at the higher 75–100% CME may be due to toxicity caused by cyanogenic compounds and high organic loading; these interfere with the metabolic activities and photosynthesis of the organisms (Olorunfemi *et al*., 2024; Izah *et al*., 2018).

Percentage Yield

The results for test alga (*Euglena viridis*) percentage yield using various concentrations of cassava mill effluent are the same as in Figure 4. On the overall aspect, all concentrations had a gradual increase in yield between day 4 and day 12 and a slight drop and/or decrease by day 14. Statistically similar pattern of yield was observed between 5%, 25% and 75% concentrations. While lowest yield was observed at 50%, the rest were at their peak performance.

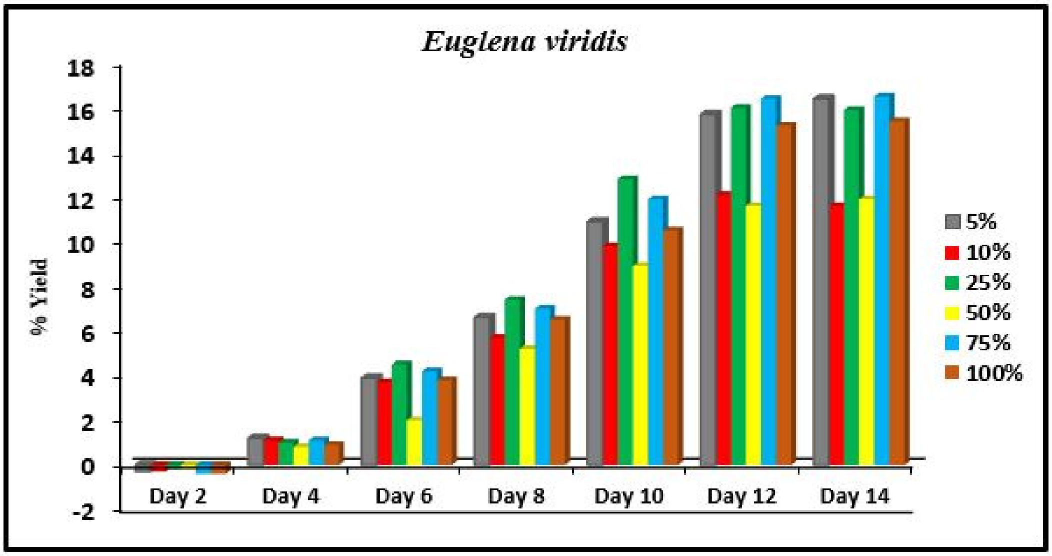

Fig. 4: Percentage yield for *Euglena viridis* in cassava mill effluent

The of percentage yield of *Chlorella vulgaris* at different concentration of cassava mill effluent is shown in figure 5. Lower yields were resulted between days 2 to 4 for all of the concentrations and the yields increased from day 6 until the end of the experiment (day 14). The maximum yields were seen at 25% Concentration and the minimum yields at 50% Concentration, 75% Concentration, and 100% Concentration respectively with the 75% concentration being the least.

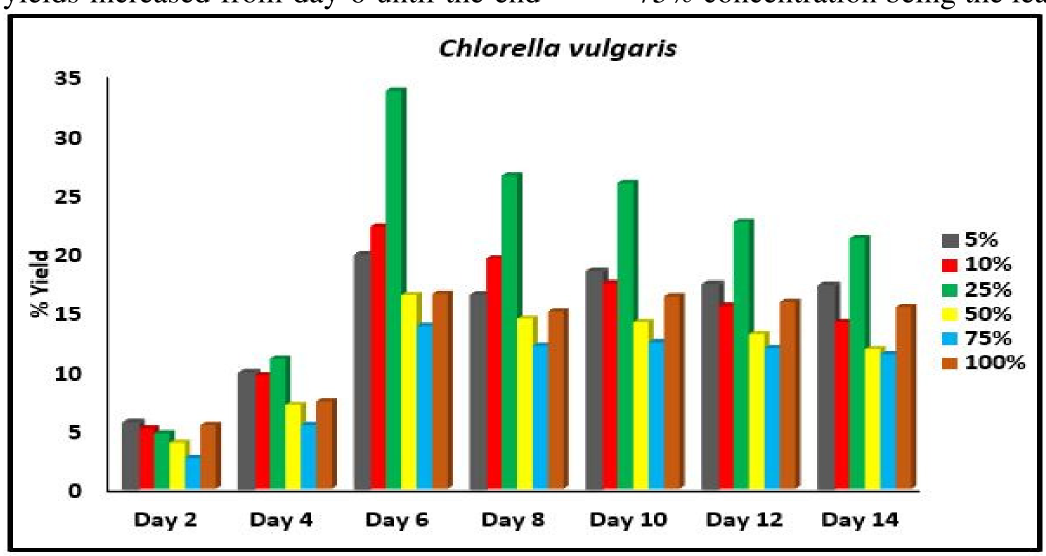

Fig. 5: Percentage yield for *Chlorella vulgaris* in Cassava mill effluent

The growth response observations in this study are corroborated by yield patterns. From this, it can be inferred that larger yields in the intermediate stage may be indicative of active biodegradation and utilization of nutrients by algae resulting from their enzymatic degradation of organic pollutants to less toxic metabolites (Touliabah *et al*., 2022). Pollution stress and changes in culture conditions caused by increasing concentrations presumably affected yield and caused negative responses in the higher concentrations (Ziganshina *et al*., 2022).

Physicochemical Properties

The physicochemical properties of raw cassava mill effluent and algae culture are presented in Table 1. The electrical conductivity and total dissolved solids of the raw effluent was high (4023.0 µS/cm and 2011.0 mg/L) with its low pH (4.07). For *Euglena viridis*, pH decreased from 8.36 (0%) to 3.53 (100%), with intermediate values of 5.51 (5%), 5.06 (10%), and 4.01 (25%). Conductivity increased from 417.0 to 3950.0 µS/cm across 0%–100%, while TDS rose from 208.0 to 1975.0 mg/L. For *Chlorella vulgaris*, pH also declined from 6.05 (0%) to 3.62 (100%). Conductivity increased from 437.0 to 3908.0 µS/cm, and TDS from 218.0 to 1954.0 mg/L across 0%, 5%, 10%, 25%, 50%, 75%, and 100%, showing consistent trends.

Table 1: Physicochemical properties of cassava mill effluent

	pH	Conductivity (µS/cm)	TDS (mg/L)
Raw Cassava Effluent	4.07	4023.0	2011.0
Euglena viridis			
0% (control) of EV	8.36	417.0	208.0
5%	5.51	844.0	421.0
10%	5.06	1396.0	698.0
25%	4.01	2337.0	1162.0
50%	3.98	3211.0	1605.0
75%	3.53	3642.0	1821.0
100%	3.53	3950.0	1975.0
Chlorella vulgaris			
0% (control) of CV	6.05	437.0	218.0
5%	5.60	920.0	460.0
10%	5.06	1420.0	714.0
25%	4.34	2348.0	1174.0
50%	4.01	3323.0	1661.0
75%	3.80	3642.0	1821.0
100%	3.62	3908.0	1954.0

These biological responses of the microalgae were confirmed by measurable changes in physicochemical parameters measured. The increasing electrical conductivities and total dissolved solids were accompanied by a decrease in pH with increasing CME concentration, which are related to ionic and organic inputs. These modifications can impact nutrient availability, cell regulation, algal productivity, and ecosystem health (Eze *et al*., 2015; Taikhao and Phunpruch, 2025).

Conclusion

Based on the result of this study, cassava mill effluent (CME) successfully causes the growth, yield change, inhibition response and the physicochemical environment of *Euglena viridis* and *Chlorella vulgaris*. The results demonstrated that the effluent concentration of 5–25% were found to enhance the algal growth and yield while increasing concentration of effluent (75–100 percent) were found to reduce algal growth and yield by increasing the effluent toxicity. The results of this study indicated species-specific responses of *Euglena viridis* and *Chlorella vulgaris* to effluent stress, with the former showing greater tolerance and higher stimulation values. The results of physicochemical analysis showed a decrease of pH and increase of electrical conductivity and TDS with increase in concentration of the effluent, indicating high pollutant load. Additionally, the results reveal that at controlled dilutions, cassava effluent could be successfully used for microalgal cultivation and biomass production.

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