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RESEARCH ARTICLE **AJHSE040110**

COMPARATIVE STUDY OF THE INFLUENCE OF BREWERY EFFLUENT ON THE GROWTH OF TWO MARINE MICROALGAE

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ABSTRACT

A growing global trend is the use of microalgae for the treatment of industrial effluents due to their capacity to consume contaminants, sequester carbon, and provide biomass for high-value products. *Thalassiosira* sp. and *Chaetoceros gracilis*, two marine microalgae, are the subjects of this study, which seeks to ascertain the effect of brewery effluent on their growth. The test microalgae were grown in seven culture vessels which were in triplicates and different concentrations; control (without effluent), 5, 10, 15, 20, 25, and 30% of brewery effluent. On a visible spectrophotometer, optical density at 750 nm was used to quantify growth responses, and several physicochemical variables were studied at the start and end using standard methods. Using Microsoft Excel 2010 and the social sciences statistical tool SPSS20, descriptive statistics, inferential (one way analysis of variance) ANOVA repeated measures, paired t-tests, and Tukey tests were performed. The findings revealed a significant difference in *Thalassiosira* sp. and *Chaetoceros gracilis* with growth response ($p < 0.05$). The growth of *Thalassiosira* sp. was stimulated by different concentrations of brewery effluent, Tukey test showed that 20, 25 and 30 % concentrations had higher mean differences, followed by 15 and 10% concentrations while, there was no mean difference between the control and 5% concentration. While the results for *Thalassiosira* sp. showed that, 25 and 30 % concentrations had higher mean differences, followed by 10, 15 and 20% concentrations. There was no mean difference between the control and 5% concentration. The results of physicochemical variables indicated that only TOC and COD of brewery effluent fell at lower concentrations and stayed constant at higher concentraions, a decrease in temperature was seen across all concentrations. *Thalassiosira* sp. had more stimulatory effect than *Chaetoceros gracilis* making it a better option in the bioremediation of brewery wastewater when applied in higher concentrations.

Keywords: Brewery effluent, *Chaetoceros gracilis*, Marine microalgae, Pollution, *Thalassiosira* sp.

INTRODUCTION

Brewery industries are one of the many production industries in developing countries of the world and during the course of their production, effluents are discharged into water bodies like streams, rivers, lakes, municipal sewers without prior treatment. These effluents may also be disposed after pre-treatment, into the brewery's wastewater treatment facility (Huige, 2006). When waste materials from beer production such as excess yeast, discarded grains, combine with wastewater, they causes environmental contamination due to the presence of high chemical oxygen demand (COD), biological oxygen demand (BOD), nitrogen, phosphorus, organic components (sugars, soluble starch, ethanol, volatile fatty acids) Dvořák*et al*., (2019).

Brewery industries must, therefore, properly manage and treat their effluent before releasing it into the environment. (Karlović *et al*., 2020). The problem of indiscriminate discharge of untreated brewery effluents can be resolved by adopting microalgae, an affordable and efficient water treatment method since microalgae can effectively remove organic loads from wastewater and generate a beneficial byproduct of biomass. (Liu *et al*., 2013). The majority of aquatic ecosystems' food webs are made up of photosynthetic organisms called microalgae species, which have chlorophyll and can use carbon dioxide to build biomass. (John *et al*., 2011).

The use of algal biomass in wastewater treatment is currently receiving more attention on a global level (Renuka *et al*. 2015). There are a variety of compounds that can be found in waste water in high concentrations that may impede or stimulate biological growth, including that of microalgae (Tyagi and Couillard, 1988). In a manner similar to this, the turbidity and pH of wastewater may inhibit the growth of microalgae (Das *et al*. 2018). The environment for microalgal growth could be improved and the strength of the wastewater could be decreased through physicochemical pretreatment (Chen *et al*., 2016). Microalgal biomass cultivation in such pretreated wastewater could also reduce residual nutrients and turbidity from the treated wastewater (Lin, *et al*, 2017).This study's goal is to compare the effects of brewery effluents on the growth of two marine microalgae.

MATERIALS AND METHODS

Test Microalgae and effluent collection

The test microalgae used in the experiment were *Thalassiosira* sp. and *Chaetoceros gracilis*. These marine microalgae samples were imported from Carolina Biological Supply Company, a science supply company located in North Carolina, United State of America. The wastewater was gathered from International Breweries PLC in Onitsha, Anambra State, Nigeria.

Culture Vessels:

Five hundred millilitres (500ml) flat bottomed glass bottles were used as the culture vessels for the study. The culture vessels were washed properly with detergent at first and further acid-washed with diluted Hydrochloric acid solution in order to remove any impurities and contaminants that were present. The laboratory work table was sterilized using cotton wool and acetone to wipe the surface before the vessels were placed on it. The culture vessels were covered with cotton wool in order to allow air passage, reduce evaporation and prevent contamination

Culture Medium:

The culture medium used in this experiment was F/2 medium which is suitable for marine algae as it contains enriched seawater medium for growing marine algae. The constituent of the F/2 medium was defined Guillard (1975).

Experimental Setup:

The marine microalgae species was grown in F/2 medium and setup in triplicates for fourteen (14) days using seven (7) concentrations (control (0), 5, 10, 15, 20, 25 and 30%). Each culture vessel was then inoculated with microalgae and their growth responses were measured optically using absorbance at 750nm on a GOYOJO 721 visible spectrophotometer.

Inoculation:

Each of the culture vessels was later inoculated with 5ml of the micro algal culture using a 5ml capacity syringe. After inoculation, the experimental vessels were covered immediately with cotton wool. Thereafter, the culture vessels were placed at the east-facing window. This position was chosen in order to prevent direct exposure of sunlight.

Growth Measurement and Monitoring:

The growth of the microalgae was observed every two days for two weeks. A specific amount (5ml) of the culture sample was taken during this time from each of the culture vessels in order to calculate the absorbance, which is a growth index. On a visible spectrophotometer, the absorbance was measured at 750 nm which is the maximum wavelength for the absorbance of the visible spectrum of light by microalgae. At the beginning and conclusion of the experiment, the samples in the culture vessels were examined using standard techniques for the following parameters: temperature (ºC), total dissolved solids (mg/L), conductivity (µS/cm), pH, dissolved oxygen (mg/L), chemical oxygen demand (mg/L), phosphate (mg/L), nitrate (mg/L), and salinity ‰.

Percentage Yield: The percentage yield is the difference between the theoretical yield and the standard yield. Percentage yield was measured using values for growth at the beginning and end of the experiment. The formula for calculating percentage yield is shown below:

$$Y = \frac{G_t \ - G_0}{T} \times 100$$

G_0 = Growth on the experiment's first day

G_1 = Growth at the experiment's conclusion

t = Time (day) at the experiment's conclusion

Statistical Analysis

Using Microsoft Excel 2010 and the Statistical program for Social Sciences (SPSS) 20 software, the data were subjected to analysis of variance (ANOVA), repeated measures, paired t-tests, and Tukey tests.

RESULTS AND DISCUSSION

Figure 1 shows the results of a one-way repeated-measures ANOVA, which indicated a statistically significant change across the repeated measurements, $F(7, 98) = 85.774$, $p < 0.05$. Tukey test indicated that the mean differences were in this order 30%≥20%≥25%≥15%≥10%. There were no mean differences between the control and 5%.

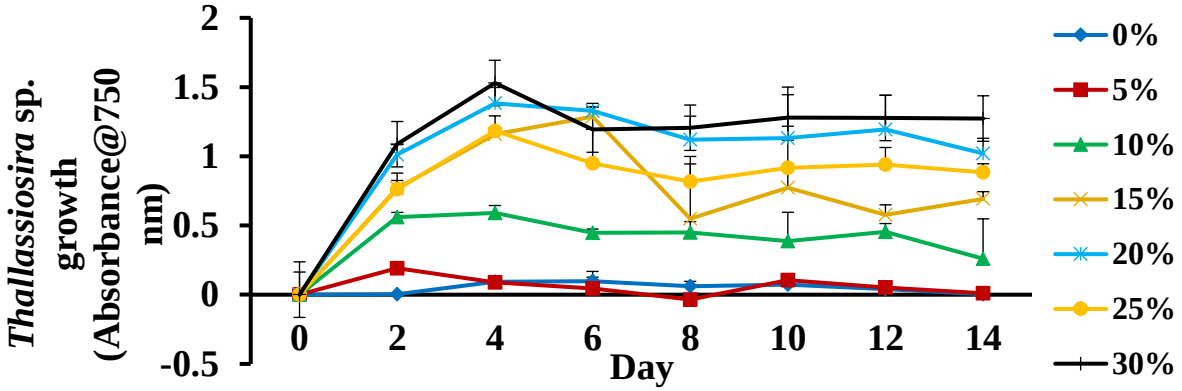

Figure 1: Effect of different treatments of brewery effluent the growth *Thallassiosira* sp.

As seen in Figure 1, *Thalassiosira* sp grew more in higher concentrations of 20, 25 and 30%. The highest growth recorded was seen in the concentration with 30% of brewery effluent, where there was a steady increase in growth from day zero (1st day of the experiment) to the 4th day with a little decline on the 6th and 8th days. Then the growth became stable from the 10th to the 14th day (last day of the experiment) of the experiment. Twenty percent (20%) concentration had the second highest growth which was observed on the 4th day followed by 25% concentration on the same day.

Figure 2 shows the results of a one-way repeated-measures ANOVA and indicated a statistically significant change across the repeated measurements, $F(7, 98) = 25.435$, $p < 0.05$. Tukey test indicated that the mean differences were in this order 30%≥25%≥20%≥15%≥10%. There were no mean differences between the control and 5% concentration.

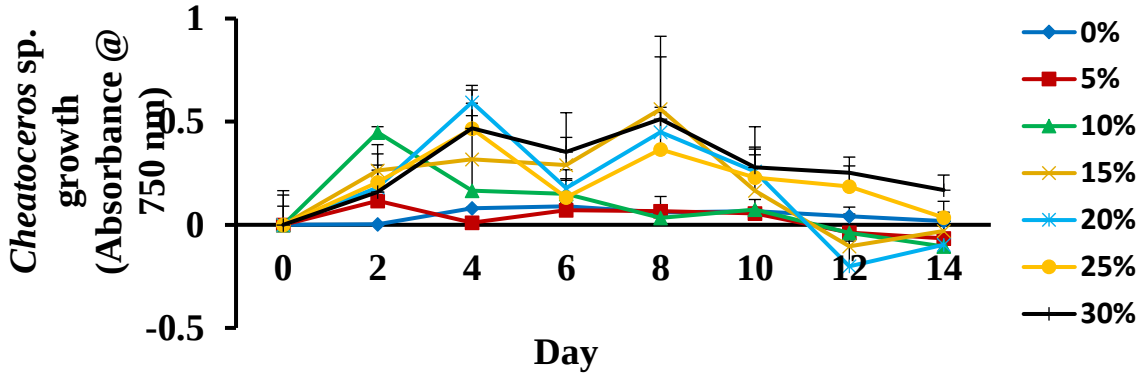

Figure 2: Effect of different treatments of brewery effluent the growth *Chaetoceros gracilis*.

Studying the impact of several brewery effluent concentrations on the development of *Chaetoceros gracilis*, the results revealed that 25 and 30% of the concentration had the highest mean differences, followed by 10, 15, and 20%. There were no mean differences between the control and 5% concentration of brewery effluent. From figure 2, *Chaetoceros*

gracilis grew best in lower concentrations (10, 20 and 15%). The highest growth was observed in the 20% concentration on the fourth day of the experiment, followed by the 10% on the second day, and the 15% concentration on the eighth day. Thereafter, all concentrations of brewery effluents experienced a drop in growth until the 14th day, while the 30% concentration had the least decline in growth. It was evident from the effects of various brewery effluent concentrations on *Thalassiosira* sp. and *Chaetoceros gracilis* that growth was stimulated in both instances. Due to the abundance of nutrients in the brewery effluent, the most pronounced growth was seen on the fourth day in all concentrations. Similar findings and observations were observed by Arbib and Garridope (2013) and Ansari *et al*. (2017).

There was significant difference between the percentage yield of *Thallassiosira* sp. and *Chaetoceros gracilis* ($p < .05$). As can be seen in Figure 3, *Thalassiosira* sp. had higher yield than *Chaetoceros gracilis* in all the concentrations with the exception of the control. Brewery effluent with 30% concentration had the highest yield followed by 25 and 20% (there was more yield in higher concentrations of the brewery effluent). Percentage yield of *Thalassiosira* sp. was higher than *Chaetoceros gracilis* throughout the duration of the study because of its ability to use different light intensity for photosynthesis which is different from that of *Chaetoceros gracilis*; this is comparable to the work of Zhang *et al*. (2017).

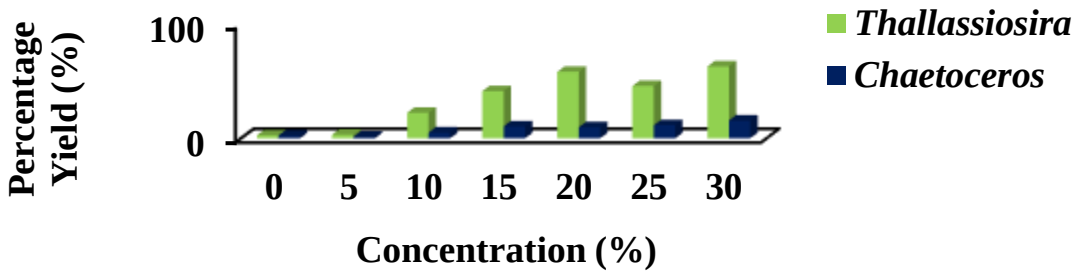

Figure 3: Percentage Yield of *Thallassiosira* sp. and *Chaetoceros gracilis*

Temperature of brewery effluent at the beginning and end of the experiment by *Thallassiosira sp* is shown in figure 4, where there was a decrease in temperature in all the concentrations with least decrease recorded for the control, 5, 10, 15 and 20% concentration. From figure 5, it was observe that there was a decrease in temperature in all the concentrations with a highest decrease recorded in treatments 30, 25 and 25%.

A comparison of the temperature of the brewery effluent using *Chaetoceros gracilis* and *Thalassiosira* sp. showed that, there was a decreased in temperature in all the concentrations. Dickinson *et al*. (2013) found that, a decrease in temperature had no impact on the growth of microalgae since the temperature was still quite near to the ideal temperature needed for microalgae to flourish.

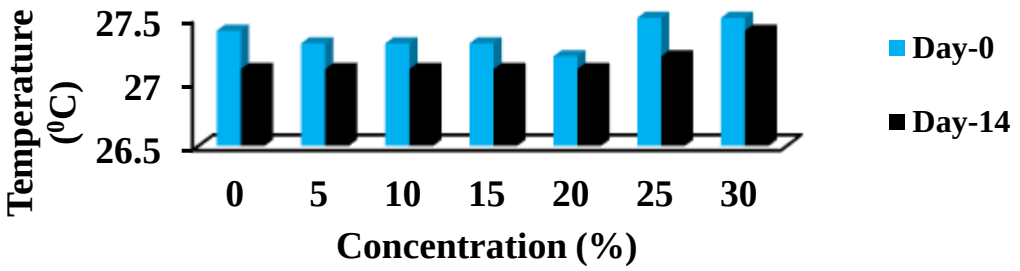

Figure 4: Temperature pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

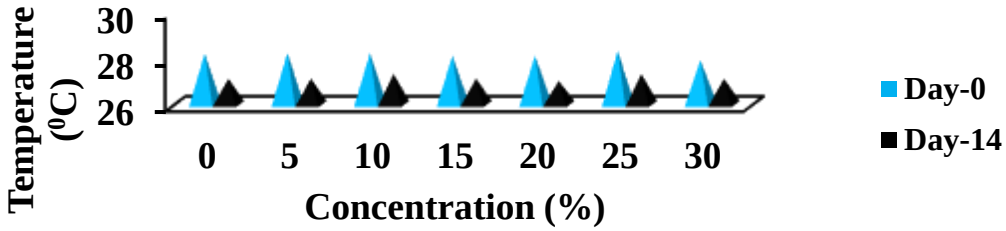

Figure 5: Temperature pre- and post-treatment of the brewery effluent by *Chaetoceros gracilis*

Figures 6 and 7 revealed a rise in pH in all concentrations from the beginning to the end of the study for *Thallassiosira* sp. and *Chaetoceros gracilis* in the brewery effluent culture medium. For *Thallassiosira* sp. there was an increase in pH in all the concentrations with the highest at 20% and the least in the control. While for *Chaetoceros gracilis*, there was also an increase in pH in all the concentrations as shown in figure 7 but there was a slight decrease in the control with the highest increase recorded in concentrations 20, 25 and 30%.

The pH of the medium for *Thalassiosira* sp. and *Chaetoceros gracilis* microalgae growth varied across all concentrations on the 1st day of the experiment. The optimum pH is the most favourable pH for the growth of microalgae, which was 6.19 and was observed on day 14 of the experiment. Due to the microalgae's absorption of inorganic carbon, the pH of microalgae cultures steadily increases throughout the day, Oiu *et al*. (2017). Higher pH limits the availability of CO2 thus, inhibiting cell growth (Chen and Durbin 1994).

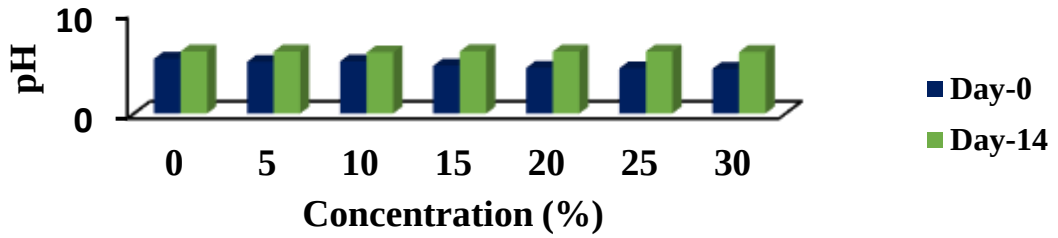

Figure 6: pH pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

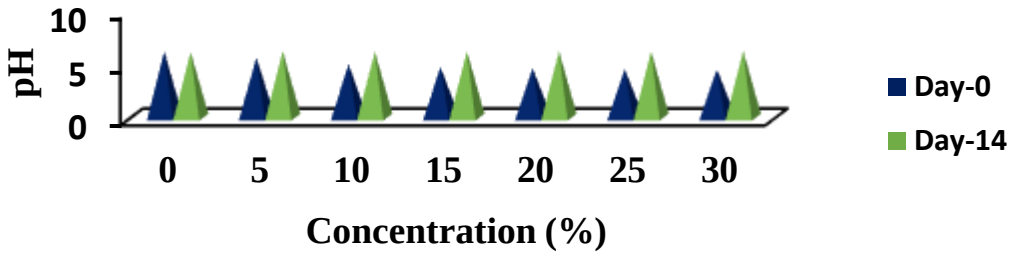

Figure 7: pH pre- and post-treatment of the brewery effluent by *Chaetoceros gracilis*.

From figure 8, it was observed that the TDS values were fluctuating. In 0, 5, 10, 25 and 30% concentration, there were increases in TDS, while in treatments 15 and 20%, TDS reduced.

There was an increase in TDS as shown in figure 9 in all the concentrations with the highest recorded in concentrations 30%, 15% and the control. The total dissolved solids for *Thalassiosira* sp. and *Chaetoceros gracilis* varied in all concentrations used. Similar findings were reported by Verma and Singh (2017); they discovered that the total dissolved solids of industrial dairy effluents were comparable to the brewery effluent employed in this investigation. According to Li *et al*., (2019), the majority of the total dissolved solids in wastewater are made up of Carbonates, Bicarbonates, Chlorides, Sulphate, Phosphate, Nitrate, Calcium, Magnesium, Sodium, Potassium, Manganese and organic materials (Verma and Singh 2017).

In the results obtained there was an increase in TDS at 30% concentration by *Thalassiosira* sp. and increase in all the concentration by *Chaetoceros gracilis*. The increase in TDS at the end of the experiment is due the declination of algal growth, as such adding up to the TDS content present in the brewery wastewater. This is in line with the findings of Xu *et al*. (2015) who claimed that a high algal density would result in the build-up of auto inhibitors and a decline in photosynthetic efficiency, both of which would hinder the proliferation of algae.

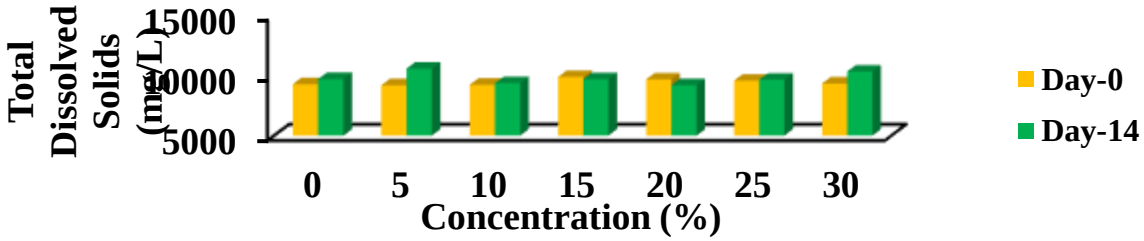

Figure 8: TDS pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

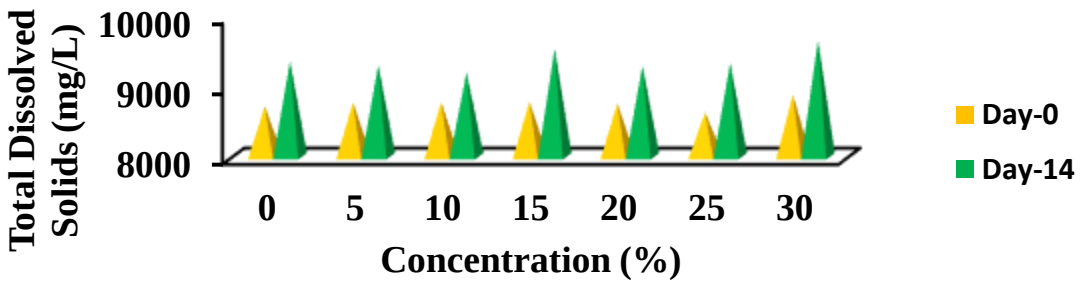

Figure 9: TDS pre- and post-treatment of the brewery effluent by Chaetoceros gracilis.

Conductivity values at the beginning and end of the experiment of brewery effluent for *Thallassiosira* sp. is shown in figure 10 with an increase in conductivity values of 0, 5, 10, 25 and 30% concentrations. There was a decrease in conductivity in 15 and 20% concentrations. Figure 11 shows the conductivity of brewery effluent for *Chaetoceros gracilis* at the beginning and end of the experiment with an increase in conductivity in all the concentrations. The highest increase was observed in concentration 5, 15 and 30% respectively. Conductivity was examined and results showed that an increase occurred in all concentrations of brewery effluent using *Thalassiosira* sp. and *Chaetoceros gracilis*. This is due to the increase in salinity and TDS, because salinity is the total concentration of all dissolved salts and the salts also produce ionic particles as they dissolve, salinity and TDS have an impact on conductivity (Rebello *et al*., 2020). The conductivity increases with the amount of ions present in the culture mrdium.

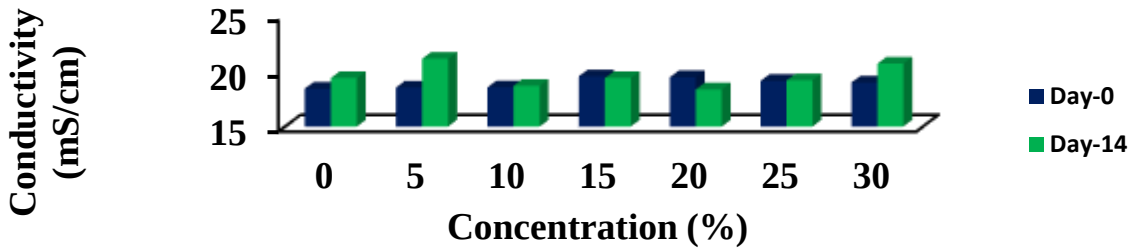

Figure 10: Conductivity pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

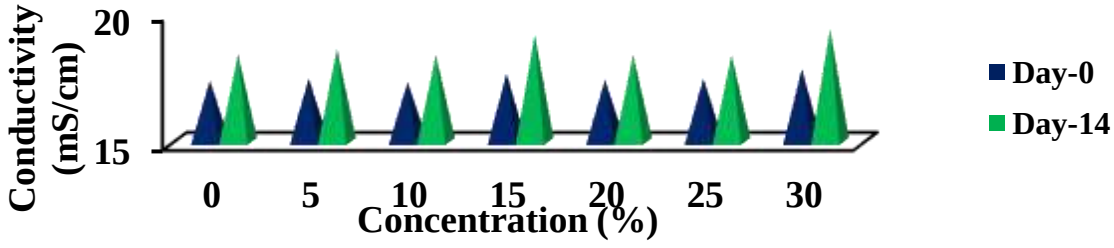

Figure 11: Conductivity pre- and post-treatment of the brewery effluent by *Chaetoceros gracilis*.

Salinity of brewery effluent at the beginning and end of the experiment using *Thallassiosira* sp. is shown in figure 12. The results show increase in salinity values in 0, 5, 10, 25 and 30% concentrations and a decrease in 15 and 20%. It was observed that 5% concentration had the highest increase while, 15% had the highest decrease; while that for *Chaetoceros gracilis* is shown in figure 13; the results indicated a statistically significant increase in salinity in all the concentrations from pre-treatment to post-treatment with 30% having the highest increase followed by 15% and control.

Salinity for *Thalassiosira* sp. and *Chaetoceros gracilis* was examined at the beginning and end of the experiment. The findings revealed that both microalgae's growth rates increased. This might be due to both microalgae having poor salt tolerance levels, which leads to osmotic and ionic imbalance in the microalga cell (Miazek *et al*., 2015). Also, the growth and photosynthetic pigments tend to increase as salinity decreases (El. Din 2015).

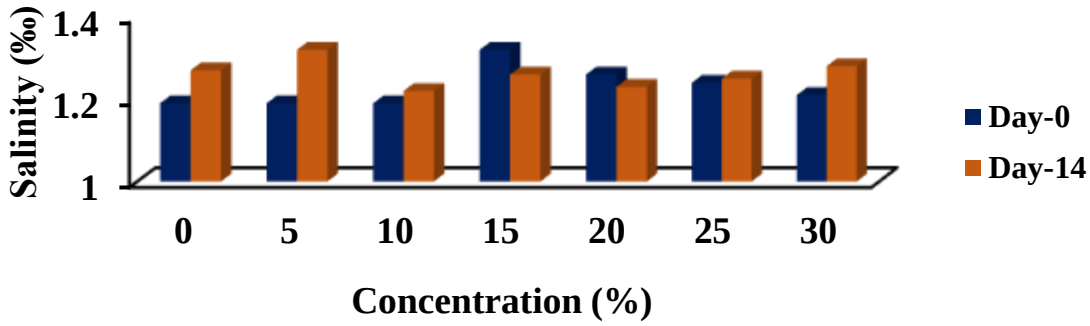

Figure 12: Salinity pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

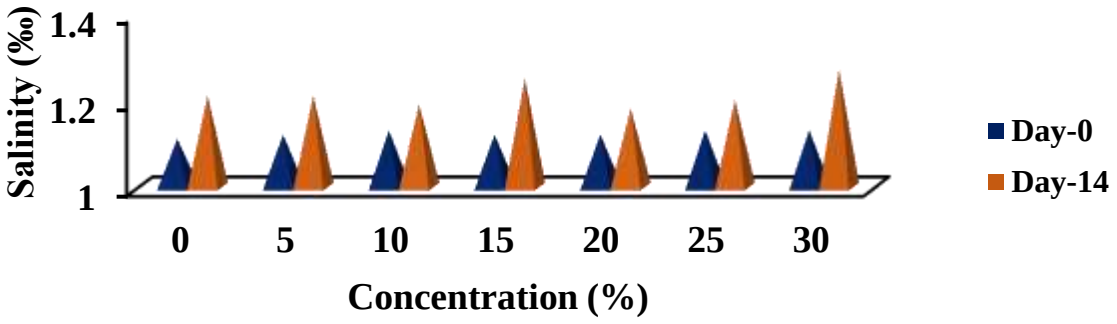

Figure 13: Salinity pre- and post-treatment of the brewery effluent by *Chaetoceros gracilis*.

The result of nitrate of brewery effluent at the beginning and the end of the experiment by *Thallassiosira sp* shows an increase in nitrate in all the concentrations. After the experiment, the control had the highest value followed by 10 and 15%. Thirty percent (30%) concentration had the least increase. While for *Chaetoceros gracilis*, there were increases in nitrate in all the concentrations from pre-treatment to post-treatment of brewery wastewater. Upon completion of the experiment, the control had great increase in microalgal growth, followed by 5 and 10% while 25% had the least growth.

Microalgae have the ability to take up nitrogen especially in inorganic forms such as nitrate, nitrite and ammonia ions (Darpito *et al*., 2014). However in the different concentration using *Thalassiosira* sp. and *Chaetoceros gracilis*, an increase was observed at the end of the experiment although this is not in line with the work of Darpito *et al*. (2014) where there was stimulation of microalgal growth at the end of the study.

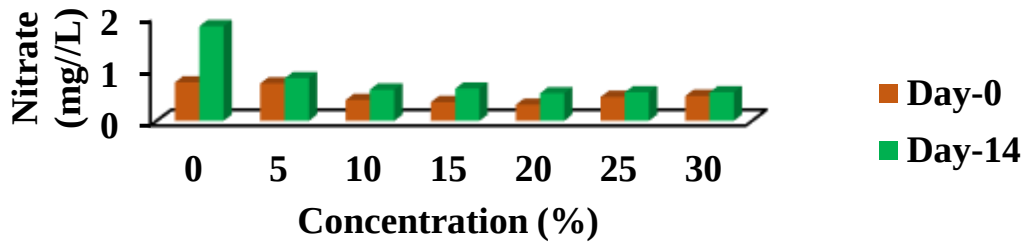

Figure 14: Nitrate pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

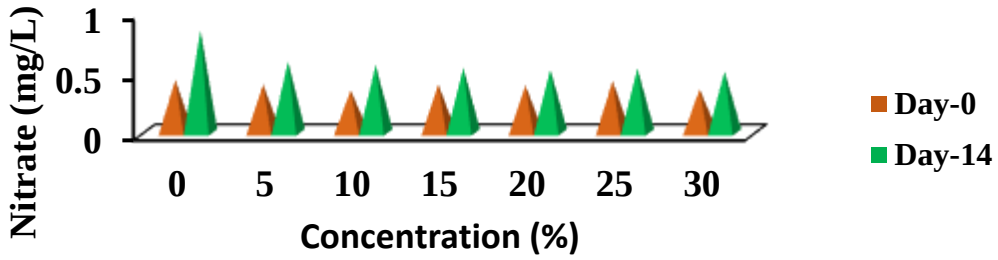

Figure 15: Nitrate pre- and post-treatment of the brewery effluent by *Chaetoceros gracilis***.**

Phosphate ion results at the beginning and end of the experiment of brewery effluent by *Thallassiosira* sp. showed a decrease in phosphate in all the concentrations. Fifteen percent (15%) concentration had the highest decrease followed by 5% and 10% with 30% having the least decrease. While phosphate result for *Chaetoceros gracilis*, shows a decrease in phosphate in all the concentrations with an exception of the control and 25%, which had a slight increase. The highest decrease was observed in 20% concentration followed by 5% while 15% had the least decrease. The phosphate content of brewery effluent for *Thalassiosira sp* and *Chaetoceros gracilis* reduced in all concentrations due to the uptake of phosphorus which is one of the essential elements needed by microalgae for their growth. Singh *et al*., (2017) made a similar observation when they revealed that *Chlorella vulgaris* is capable of removing nutrients, with a nutrient removal efficiency of 87.9% for inorganic nitrogen and 98.4% for inorganic phosphorus.

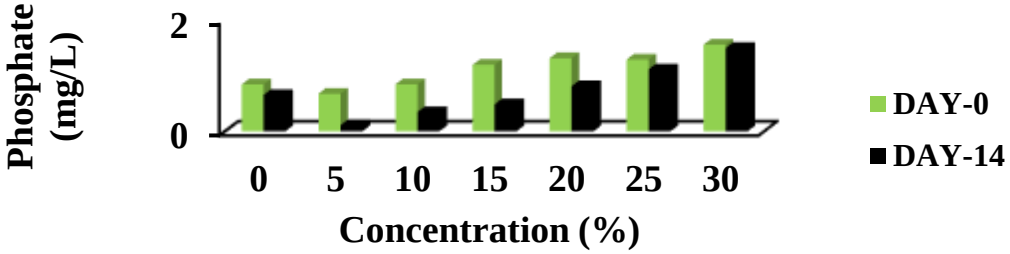

Figure 16: Phosphate pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

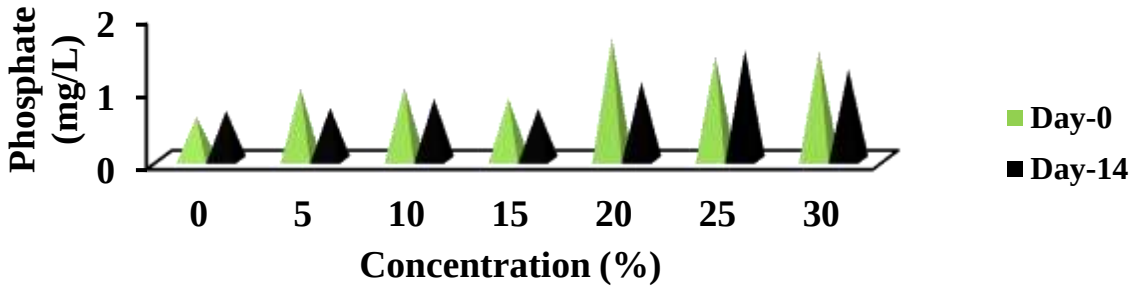

Figure 17: Phosphate pre- and post-treatment of the brewery effluent by *Chaetoceros gracilis*

The DO results of brewery effluent for *Thallassiosira sp* showed an increase in dissolved oxygen in the control, 5, 10 and 15% and a decrease in 20, 25 and 30% from the beginning to the end of the experiment. The increases were observed in lower concentrations (0, 5, 10 and 15%) while the decrease occurred in higher concentrations (20, 25 and 30%). The results of DO of brewery effluent at the beginning and end of the experiment for *Chaetoceros gracilis* indicated increases in dissolved oxygen in all the concentrations. Dissolved oxygen measured at the beginning and end of the experiment showed that the DO increased at lower concentrations and reduced at higher concentrations for *Thalassiosira* sp. This is because microalgae thrive more when dissolved oxygen is low than when it is high in microalgal culture system (Kazbar *et al*., 2019). The results of DO of different concentrations of the brewery effluent using *Chaetoceros gracilis* showed that an increase in DO was observed in all concentrations, due to the decline in growth of the microalgae on day 14 of the experiment.

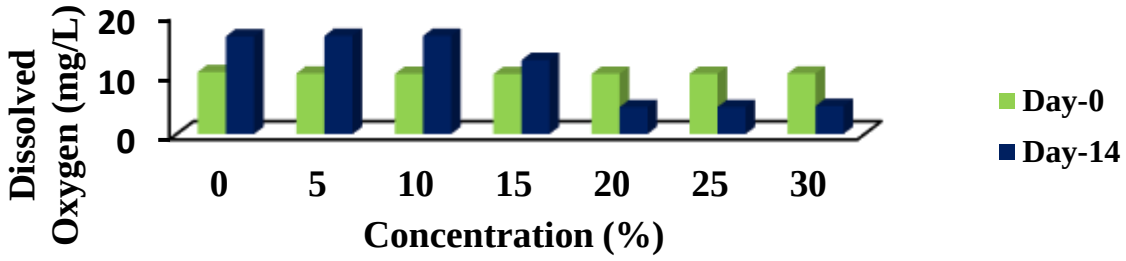

Figure 18: DO pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

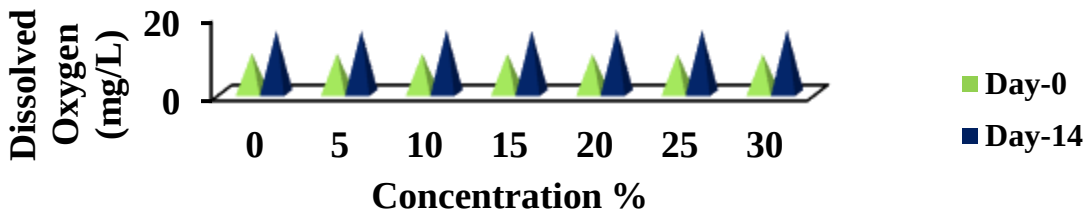

Figure 19: DO pre- and post-treatment of the brewery effluent by *Chaetoceros gracilis*.

The results of COD of brewery effluent at the beginning and end of the experiment for *Thallassiosira sp* showed a decrease in chemical oxygen demand in 0, 5, and 10% concentrations. At higher concentrations (20, 25 and 30%), the values became constant. While the results of COD of brewery effluent at the beginning and end of the experiment for *Chaetoceros gracilis* indicated a decrease in chemical oxygen demand in 5%, and 10% concentrations. From Figure 19, there was a slight increase in the control and a decrease in 5, 10, 15 and 20% concentration with the exception of 25 and 30% concentrations which had constant values. The chemical oxygen demand (COD) values during the study using *Thalassiosira* sp. and *Chaetoceros gracilis* showed that there was a decrease in lower concentrations and stable values occurred at higher concentrations. The number of organic compounds that were not impacted by the breakdown process of microalgae was the reason for the stable readings. According to Nagajaran *et al*. (2002) and Alvaraz-Bernal *et al*. (2006), COD rises in proportion to the amount of organic compounds present in the water that are not broken down by microalgae.

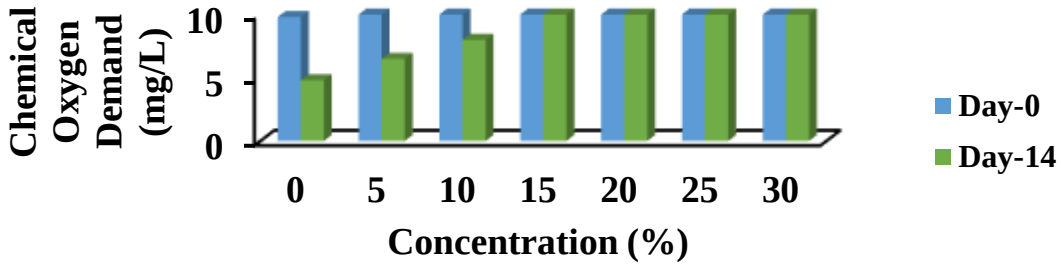

Figure 20: COD pre- and post-treatment of the brewery effluent by *Thallassiosira* sp.

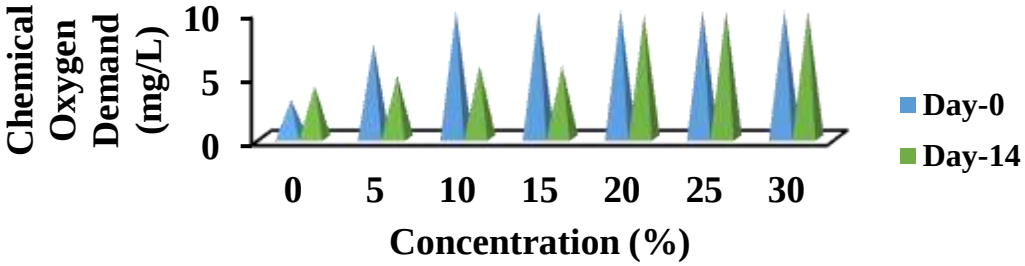

Figure 21: COD pre- and post-treatment of th e brewery effluent by *Chaetoceros gracilis*.

CONCLUSION

The growth of two marine microalgae: *Thalassiosira sp* and *Chaetoceros gracilis* was examined in this study using various brewery effluent concentrations. At the conclusion of the investigation, it was discovered that the various concentrations of brewery effluent employed had a stimulating effect on the growth of both microalgae, with the higher concentrations having this effect more so than the lower concentrations. *Thalassiosira* sp. had more stimulatory effect than *Chaetoceros gracilis* making it a better option in the bioremediation of brewery wastewater when applied in higher concentrations. In order to fully understand the impact of brewery effluent on *Thalassiosira* sp., *Chaetoceros gracilis*, and other microagal species, more extensive studies over longer time periods are advised. This will help determine the best method for microalgae bioremediation of brewery effluent.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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