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Effect of Sewage Wastewater on the Growth of Microalga-*Arthrospira platensis* (Nordstedt)

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ABSTRACT: This study presents the effect of sewage waste water on the growth of microalga *Arthrospira platensis* using appropriate standard methods. The results showed that different concentrations of sewage waste water affected the growth of *Arthrospira platensis*. A comparison of the growth responses of the different treatments revealed that there was significant difference ($p<0.05$) with the control (0%) and 5% treatments having the highest mean values for growth when compared with other treatments. Among the different treatments, 30% treatment had the highest inhibitive effect on the growth of the test microalga while growth was stimulated in control (0%) and 5% treatments. The highest growth inhibition was observed in 30% treatment. Total dissolved solids, conductivity and pH showed significant increase in all treatments while there was a decrease in temperature and biological oxygen demand. A reduction in chemical oxygen demand was also observed in all treatments.

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Water is one of the basic necessities of life that plants and animals require. In order to maintain the quality of life of living organisms, clean water is vital as humans and other living organisms cannot survive if the water present in their environments is loaded with harmful microorganisms. Increase in pollution, industrialization and rapid economic development impose severe risks to the availability and quality of water resources worldwide. The release of organic and inorganic substances into the environment as a result of domestic, agricultural and industrial water activities lead to organic and inorganic pollution (Mouchet, 1986; Lim *et al.,* 2010). The composition of wastewater is a reflection of life styles and technologies practiced in producing societies (Gray, 1989). Wastewater is a complex mixture of natural organic and inorganic materials as well as man-made compounds. Large quantities of organic compounds in sewage are present as carbohydrates, fats, proteins, amino acids and volatile acids. The inorganic constituents include large concentrations of calcium, sodium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals (Horan, 1990; Lim *et al.*, 2010). Ecotoxicology is the study of the effects of toxic chemicals on organisms at the population, community and ecosystem level. It is defined as the study of the fate and effects of toxic substances on an ecosystem and it is based on scientific research which employs both field and laboratory methods (Kendall, 1982; Kendall 1992; and Hoffman *et al*., 1995). Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state or to levels below concentration limits established by regulatory authorities (Mueller *et al.,* 1996). Bioremediation is an aspect of biotechnology that deals with the use of living organisms such as plants and microbes (bacteria and

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algae) to remove toxins and pollutants from soil and water. The contaminants may include agrochemicals, chlorinated compounds and nutrients such as phosphates, nitrates and chlorides. The techniques employed in bioremediation depend mostly on the degree of contamination of the affected area(s). Pollutant nature, depth, degree of pollution, cost and environmental policies are some of the selection criteria that are considered when choosing any bioremediation technique (Fructos *et al.,* 2012; Smith *et al.,* 2015).

These techniques are of two principal types, these are: in-situ and ex-situ bioremediation. Bioremediation performed by algae is referred to as phycoremediation (John, 2000). Phycoremediation accurately describes biological treatment of contaminants in a water body using algae (micro and macro algae). Phycoremediation is an invaluable method of boosting the physiochemical properties of sewage since harmful chemicals and a huge amount of chemicals are not involved as opposed to other convention methods (Brar *et al.,* 2017). The objective of this study is to ascertain the effect of sewage wastewater on the growth of the microalga- *Arthrospira platensis.*

MATERIALS AND METHODS

Test microalgae and source: The microalgae used for this experiment was a blue-green algae called *Arthrospira platensis*. It was imported from Suncoast Marine Aquaculture, located in Florida, United States of America.

Sewage wastewater collection: The sewage wastewater used for the experiment was obtained from Benin City, Edo State, Nigeria.

Culture medium: An artificial medium called modified Zarrouk medium was used in constituting the culture medium. The ingredients were mixed with clean water in a 250ml conifer flask. After mixing, sediment appeared at the bottom of the flask and was left to settle. Thereafter, the clear solution was poured into another clean container leaving the sediment at the bottom of the mixing container to be discarded. This sediment-free clear water was taken as the growth medium and was kept in a sealed container away from heat and light.

Culture vessel: Culture bottles of 500 ml were used as the culture vessels for the experiment. They were washed thoroughly with detergent, rinsed with tap water and acid-washed using 1:1M solution of sulphuric acid in order to remove algal spores and thereafter rinsed with clean water.

Inoculation: The concentrations used were 0% (control), 5%, 10%, 20%, 30% 40% and 50% concentrations. The experiment was set up in triplicates. Each of the vessels was later inoculated with 10ml unialgal culture of *Arthrospira platensis*. A 10ml capacity syringe was used for inoculation. After inoculation, experimental vessels were immediately covered with cotton plug to reduce evaporation and prevent contamination. The cultures were placed at an east-facing window in order to avoid exposure to direct sunlight.

Growth measurement and monitoring: Monitoring of algae growth was carried out every two days for two weeks. During this period, a given volume of the culture sample was collected from each of the different treatments to test for absorbance which is a growth index. The absorbance was estimated at 750nm using a visible spectrophotometer.

The samples in the culture vessels were analyzed before and after the experiment for parameters such as temperature (^{0}C), dissolved solids (mg/L), turbidity (NTU), conductivity (µS/cm), pH, biological oxygen demand (mg/L) and chemical oxygen demand (mg/L).

Data Analysis

Percentage inhibition: Percentage inhibition was estimated at the end of the experiment on day 14. This was carried out using the formula put forward by Phatarpekar and Ansari (2000) as follows:

$$\%Inhibition = 100 - \frac{Measured\ Biomass}{Theoretical\ Biomass} \times 100$$

Where measured biomass = absorbance of test microalga in other treatment
Theoretical biomass = absorbance of test microalga in control

Percentage yield: Percentage yield was measured using values for growth before and after the experiment. The formula for calculating percentage yield is

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

Where G_t = growth at the end of the experiment;
G_0 = growth at the beginning of the experiment;
$_T$ = time (day) at the end of the experiment;

Statistical analysis: The data obtained were subjected to analysis of variance (ANOVA) and Duncan multiple range (DMR) test to detect significant differences between the levels of effects of various concentrations of treatment on *Arthrospira platensis*. The statistical software package used was SPSS version 22.

RESULTS AND DISCUSSION

The effects of different concentrations of sewage wastewater on the growth of *Arthrospira platensis* were studied and the results are shown in figure 1. There were significant differences of $p<0.05$ among the treatments and the highest growth was recorded in 0% and 5% treatments while the least growth was recorded in 30% treatment. The growth recorded in *Arthrospira platensis* is recognized as an important factor that plays a major role in determining the biochemical composition of the microalgae (Madkour *et al.*, 2012). The difference in specific growth rate in each treatment is caused by the ability of cells to absorb nutrients contained in the culture media as not all materials can be directly absorbed and used by the cells (Leksono *et al.*, 2017). In addition, differences in specific growth rates can also be attributed to the nutritional factors contained in the culture media (Wijayanti *et al.*, 2018).

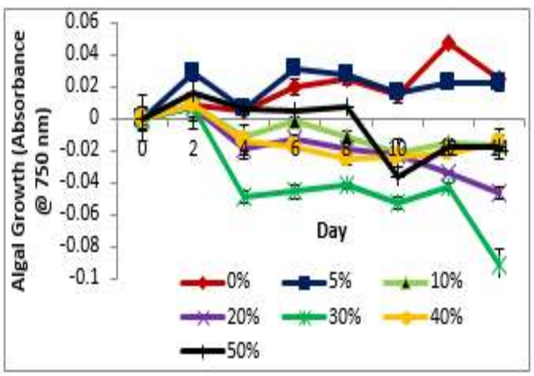

Fig 1: Effect of different concentration of sewage wastewater on the growth of *Arthrospira platensis*

The Inhibitive effect of different concentrations of sewage waste water on the growth of *Arthrospira platensis* is shown in figure 2. It was observed that 30% treatment had the highest inhibitive effect leading to decline in growth. This was closely followed by 20% and 40% treatments. However, growth was stimulated in 5% and 0% (control). In *Arthrospira platensis,* declining growth normally occurs in cultures where there is a specific requirement for mean division rate and in this phase, growth and biomass is often very high (Rajasekaran *et al.*, 2016). The Percentage yield of *Arthrospira platensis* is shown in figure 3, where it was observed that 5% treatment had the highest yield. The least yield was recorded in 30% treatment, while increase in algal biomass was recorded in the control (0%) and 5% treatment. This is as a result of increase in wastewater concentration in the medium which caused decreased growth rate (Ljubic *et. al.,* 2018).Temperature values before and after the experiment is shown in figure 4. The control (0% treatment) had the highest temperature before the experiment. After the experiment, all treatments showed significant decrease in temperature.

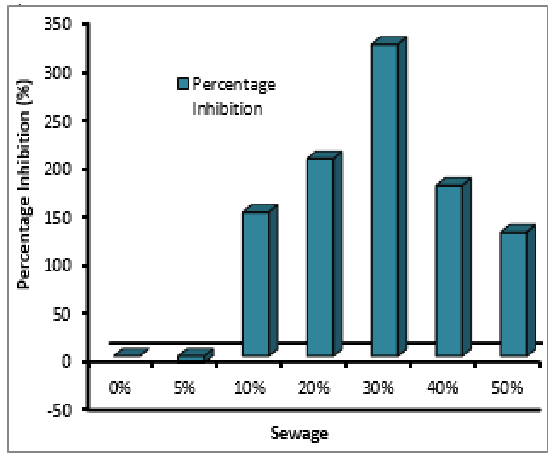

Fig 2: Inhibitive effect of different concentrations of sewage waste water on *Arthrospira platensis.*

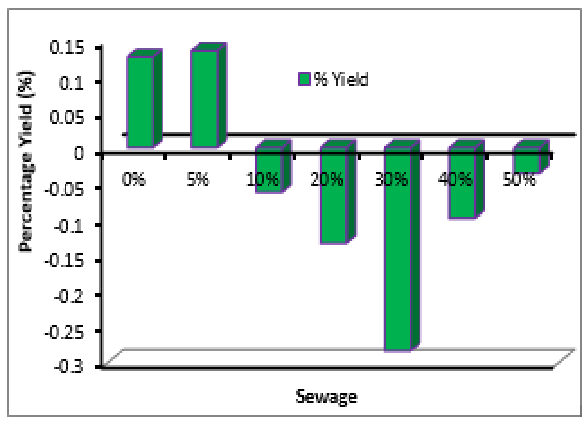

Fig 3: Percentage algal yield of *Arthrospira platensis*

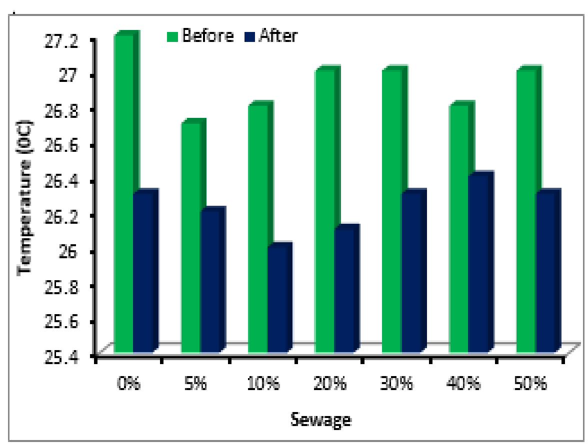

Fig 4: Temperature variation before and after the experiment

Temperature is the most important factor that affects all metabolic activities and nutrients uptake of microorganisms (Trabelsi *et al.*, 2009). Temperature can influence the growth of *Arthrospira platensis* and the composition of biomass produced by causing changes in metabolism (Cornet *et al.,* 1992). In this study, 0% treatment had the highest temperature before the experiment. After the experiment, there was a reduction in temperature from 27.2 to 26.3^{0}C. Temperatures above 30^{0}C could serve as a stress factor for cell growth (Trabelsi *et al.*, 2009). pH before and after the experiment is shown in figure 5 Control (0%) treatment had the lowest pH value before the experiment.

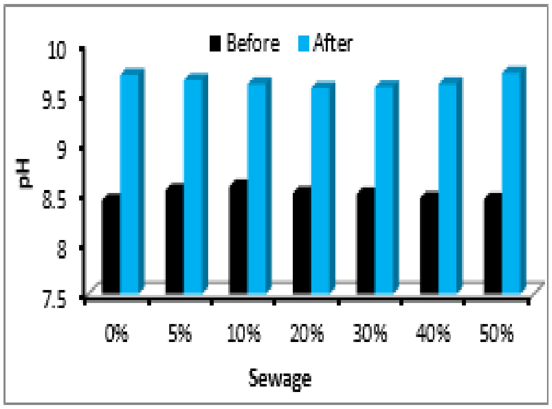

Fig 5: pH variation before and after the experiment

After the experiment, there was significant increase in all treatments.The pH serves as an indicator of acidity and alkalinity. In this study, 50% treatment had the highest pH after the experiment (pH = 9.71).This is close to the optimal pH of growth for this microalga (which is 10.5) as postulated by Richmond and Grobbelaar, (1986). Generally, all treatments showed significant increase in pH after the experiment. Increase in pH after the experiment was as a result of increased rate of photosynthesis of the microalgae (Kulkarni *et al.*, 2016). Wetzel *et al.* (1968) stated that increased photosynthetic rate leads to the consumption of large amounts of bicarbonates which ultimately results in increased pH. Assimilation of nitrate also tends to increase pH (Mezzomo *et al.*, 2010). pH is one of the limiting factors that affect the metabolic activities of the test microalgae (Richmond *et al.*, 1986; Rafiqual *et al.*, 2005; Abu *et al.*, 2007). It also affects physiological growth and biomass production (Celekli *et al.,* 2009). The values of TDS before and after the experiment are shown in figure 6. 40% treatment had the least TDS values before the experiment. After the experiment, all treatments recorded increase in TDS. TDS serves as an expression for the collective content of all organic and inorganic substances in a liquid which occur in molecular, ionized or micro-granular suspended forms. TDS is a good indicator of aesthetic characteristics of drinking water and as an aggregate indicator for the presence of different types of chemical contaminants (Dolatabadi *et al.,* 2016). Conductivity values before and after the experiment are shown in figure 7. 30% treatment had the least conductivity before the experiment, after the experiment, all treatments recorded increase in conductivity.

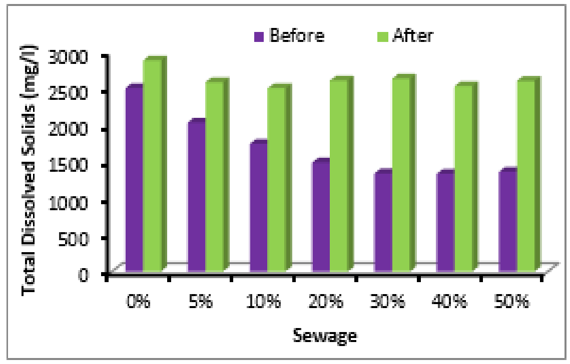

Fig 6: Total dissolved solids (TDS) before and after the experiment

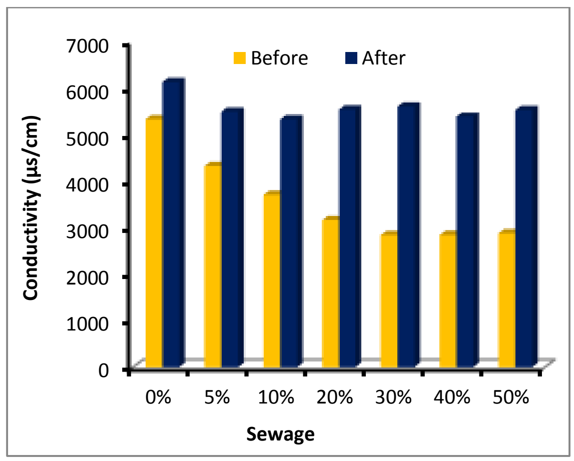

Fig 7: Conductivity before and after the experiment

In this study, both TDS and EC showed similar rates of increase after the experiment in all treatments. This suggests that there exists a relationship between the two parameters. To explain this relationship, Dolatabadi *et al.* (2016) stated that conductivity is one of the two foremost methods of measuring TDS since electrical conductivity of the culture sample is directly related to the concentration of dissolved ionized solids in the culture sample as ions from the dissolved solids present in the culture sample create the ability for the culture sample to conduct an electrical current which can be measured using a conductivity meter. Biological oxygen demand (BOD) values before and after the experiment are shown in figure 8 below and it was observed that 50% treatment had the highest BOD before and after the experiment, while 0% treatment had the least BOD before and after the experiment.

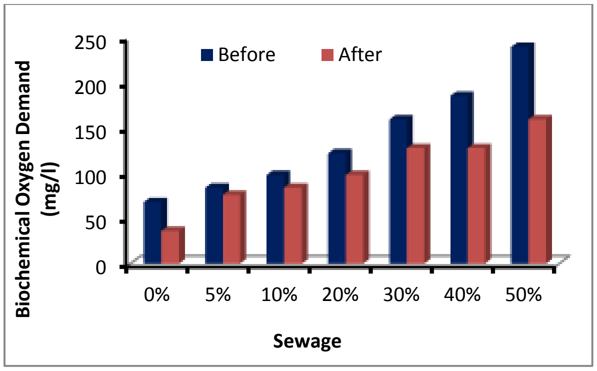

Fig 8: Biological oxygen demand (BOD) before and after the experiment

In measuring organic pollution, biological oxygen demand (BOD) serves as the most widely used parameter (Dolatabadi *et al.*, 2016). It is a measure of how microorganisms consume dissolved oxygen in a body of water. In this study, there were significant reductions of BOD after the experiment in all the treatments.

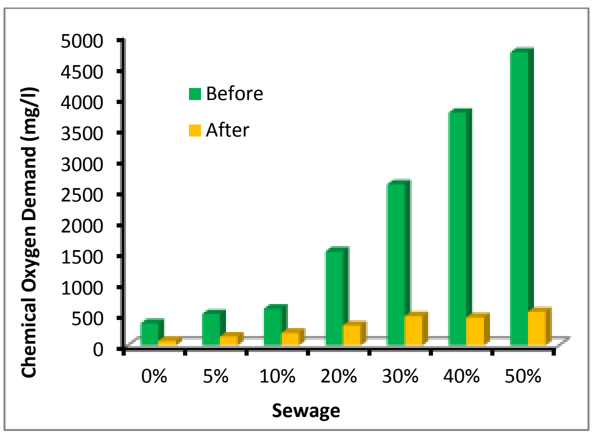

Fig 9: Chemical oxygen demand (COD) before and after the experiment

This is in line with the result of the study conducted by Agrawal, (2017) on domestic sewage water as he also reported a significant decrease in BOD. The removal of BOD is due to the ability of the microalga to oxidize organic material to carbon dioxide and water using molecular oxygen as an oxidizing agent. The removal of BOD is the primary goal of waste water treatment (Abdel-Raouf *et al.,* 2012). Chemical oxygen demand (COD) expresses the oxygen equivalents of organic matter that can be oxidized with the aid of strong chemical oxidizing agents. Figure 9 shows chemical oxygen demand (COD) before and after the experiment and it was observed that 50% treatment had the highest COD before the experiment while 0% treatment had the least COD before and after the experiment.

In this study, there were drastic reductions of COD in the various treatments. This is in line with the result of the study conducted by Zainab *et al.* (2012) as they also reported a drastic reduction in COD (90% reduction). This is because chemical oxidation caused by the aeration of the system can enhance reduction of COD (Meltcalf, 1983).

Conclusion: The study showed that the different concentrations of sewage wastewater used affected the growth of the microalga *Arthrospira platensis* by having inhibitory effects at higher concentrations and stimulatory effects at lower concentrations of sewage waste water and therefore, the microlga *Arthrospira platensis* can be used in the phycoremediation of sewage waste water.

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