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Phycoremediation of Laundry Wastewater Using *Chlorella vulgaris* (Beijerinck)

Mabel Amen Akhere* and Honour Chukwuka

Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria

*Corresponding author; Email: mabel.akhere@uniben.edu, Tel: +234 703 927 8053

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ABSTRACT: A study of the effect laundry wastewater on the microalga *Chlorella vulgaris* was carried out. The study was carried out using different treatments 0% (control), 5%, 10%, 20%, 30%, 40%, 50% of laundry wastewater. The microalgal growth responses were determined optically using absorbance on spectrophotometer at 750nm. Samples were analyzed for physicochemical parameters such as pH, temperature, total dissolved solids, conductivity, biochemical oxygen demand and chemical oxygen demand using standard methods. Descriptive statistics, inferential (One-way analysis of variance (ANOVA) and post-hoc test Duncan multiple range (DMR) were done using Microsoft excel 2010 and statistical package for social science SPSS 22. The results showed that different concentrations of laundry wastewater stimulated the growth of *Chlorella vulgaris* with 50% treatment having the highest yield (3.2), followed by 40% treatment (2.7) and the control (0%) had the least yield of 0.6. There was a significant increase in pH and temperature while there was a decrease in total dissolved solids, biochemical oxygen demand, chemical oxygen demand and conductivity. The level of reduction in COD was from 1340mg/L to 364mg/L, which indicated that *Chlorella vulgaris* has the potential to bioremediate laundry wastewater.

Keywords: Pollution, Phycoremediation, Bioremediation, Microalgae, Environment

Introduction

Laundry wastewater is produced from large laundry amenities that wash uniforms, towels, floor mats mops etc. Often, these items are washed on a daily basis via industrial or commercial laundry facilities. The laundry wastewater generated contains lint, oil, grease, sand, heavy metals and volatile organic compounds. This wastewater has in the past low biodegradability due to the presence of synthetic detergents; recently, biodegradable products are substantially used due to regulation and an accelerated environmental awareness Sterber and Wiebel, (2011). These new bio detergents preserve the surroundings and allow a worthwhile use of natural techniques for laundry wastewater treatment. This laundry wastewater needs to be cleaned and treated, which usually involves several phases. The most frequent technique is the coagulation with the aid of aluminium sulphate and lime, after neutralization response with sulphuric acid (Owodunni and Ismail 2021). Some laundry wastewater goes immediately into the environment, due to the defect of water infrastructure, while the majority goes to sewage treatment plant before flowing into the environment. Some chemicals remain in the water after treatment, which may additionally contaminate the water system.

Bioremediation is a technique used to remove environmental contaminants from the ecosystem. It utilizes the biological mechanisms inherent in microbes and plants to eradicate hazardous pollutants and restore the ecosystem to its original condition (Sewvandi and Adikary, 2011). It is more cost-effective than other technologies that are used for the cleanup of hazardous waste (Kshirsagar, 2013; Validi, 2001). Phycoremediation is a type of bioremediation that employs algae for improving water quality. Autotrophs play a crucial role in the remediation of waste products especially wastewater. The use of algae in treating these domestic wastes has been significant because of their chemical processing ability. Algae have been universally

acknowledged as they are important in the natural water purification process (Han *et al.*, 2000; Olguin, 2003; Kshirsagar, 2013). Thus, the use of microalgae for removal of nutrients from different wastes has been described by a number of authors (De-Bashan *et al.*, 2002; De La Noue *et al.*, 1992; Queiroz *et al.*, 2007; Rao *et al.*, 2011).

Microalgal technology has been used to reduce issues concerning sustainable management of both air and water (Hasan *et al.*, 2014). Algae can fix carbon dioxide by the process of photosynthesis and remove excess nutrients effectively at a minimal cost. Oxygen produced by photosynthesis can relieve biochemical oxygen demand (BOD) in the wastewater (Dominic *et al.*, 2009). The risk of accidental release of pollutants into the atmosphere, inflicting health safety and environmental issues are avoided once algae are utilized for remediation. Algae utilize organic and inorganic waste as nutritional sources and enzymatically degrade the pollutants. The choice of microalgae to be utilized in wastewater treatment is determined by their potency to grow in and to take up nutrients from waste matter (Olguin, 2003). Nowadays, there are techniques of wastewater purification using microalgae that can remove nutrients from domestic wastewater more efficiently than traditional methods (Wang *et al.*, 2009). Therefore, it is becoming a very promising process (Harun *et al.*, 2010).

Materials and methods

Test microalgae, collection of wastewater and culture medium: The freshwater test microalgae used in this work is *Chlorella vulgaris*. Pure cultures of these species were got after serial dilution of a wild culture using a ten-fold dilution factor.

The laundry wastewater samples used in this study were collected from a laundry company in Edo state, Nigeria 6°18'18"N 5° 38'18"E.

The freshwater test microalga was grown in an artificial medium, Chu 10 Modified medium (Chu, 1942). The microalgae species were grown in growth medium in 500ml culture vessels and were set up in triplicates for fourteen days (14) using seven concentrations (0 %, 5 %, 10 %, 20 %, 30 %, 40 %, 50 %). The culture vessels were each inoculated with microalgae (10 ml each) and were immediately covered with cotton plug so as to avoid contamination from the environment. The culture vessels were placed where there was no exposure to direct sunlight.

Growth measurement and monitoring: Monitoring of algae growth was done every two days for two weeks. A certain volume was collected from each culture vessel to determine the concentration based growth responses optically using absorbance 750 nm on a 721 visible spectrophotometer.

Data Analysis

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: Descriptive statistics, inferential (one-way analysis of variance (ANOVA), t-test) and post-hoc test was done using Duncan multiple range (DMR) test, Microsoft Excel 2010 and statistical package for social science (SPSS 22).

Physico-chemical analysis of sewage wastewater content in the culture vessels: The samples in the culture vessels were analyzed at the start and end of the study for the following parameters: pH, conductivity, total dissolved solids, temperature, biochemical oxygen demand and chemical oxygen demand.

Physico-chemical analysis of the laundry wastewater sample: Physico-chemical analysis of the laundry wastewater was carried out using standard methods for parameters such as temperature ($^{\circ}$ C), turbidity (NTU), total dissolved solids(mg/L),conductivity (μ S/cm), pH, total suspended solids (mg/L), chloride (mg/L), nitrate (mg/L), calcium (mg/L), magnesium (mg/L), sulphate (mg/L), sodium (mg/L), alkalinity (mg/L), phosphate (mg/L), biochemical oxygen demand (mg/L) and chemical oxygen demand (mg/L).

Results

The effect of laundry wastewater on the growth of *Chlorella vulgaris* was studied and the result is shown in Figure 1. The highest growth measured using absorbance was observed in 50% treatment, followed by 40%

treatment. The control (0%) treatment had the lowest growth. Statistically, One-Way ANOVA revealed that there was significant difference ($p < 0.05$) in growth. The mean difference was in the order $50\% \geq 40\% \geq 30\% \geq 20\% \geq 10\% \geq 5\% \geq 0\%$.

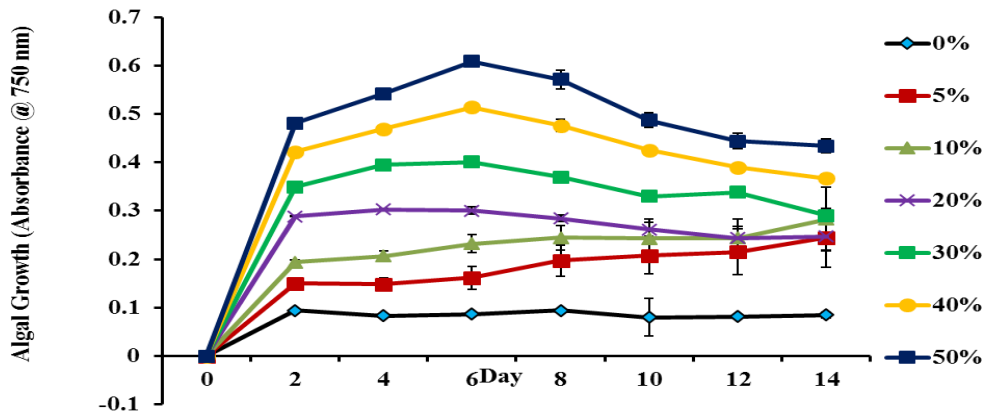


Figure 1: Effect of different concentration of laundry wastewater on the growth of *Chlorella vulgaris*.

The percentage algal biomass of *Chlorella vulgaris* was extrapolated and the result is shown in Figure 2. There was a consistent increase in the algal growth with 50% treatment having the highest percentage yield. This was followed by 40% treatment. The control (0%) treatment had the lowest percentage yield.

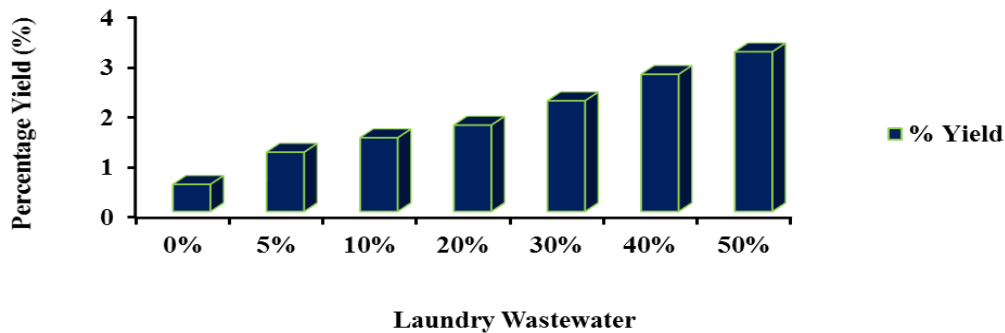


Figure 2: Percentage algal biomass of *Chlorella vulgaris*

The results of pH values at the beginning and end of the period of study are shown in Figure 3, with a decrease in the pH values of the control treatment (0%) at the end of study. While there were subsequent increases, in 5%, 10%, 20%, 30% and 40% and 50% treatment at the end of the study.

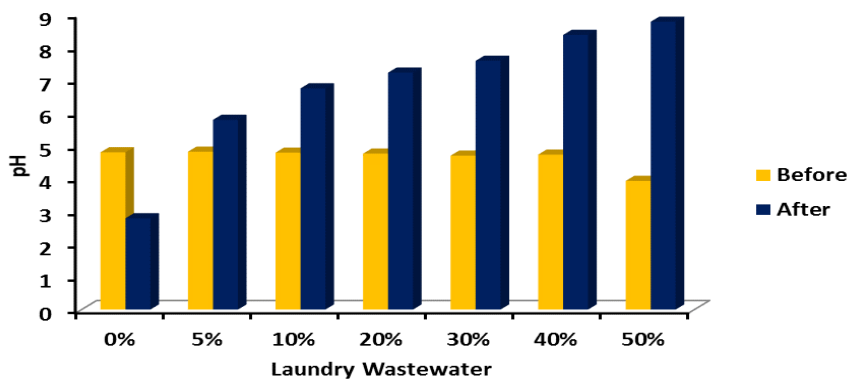


Figure 3: pH at the start and end of the study period

The results of temperature variations of the different treatments at the beginning and end of the period of study are shown in Figure 4. From Figure 4, it can be observed that temperature values for control (0%) increased at the end of the study while 5%, 10%, 20%, 30%, 40% and 50% treatment had decrease in temperature.

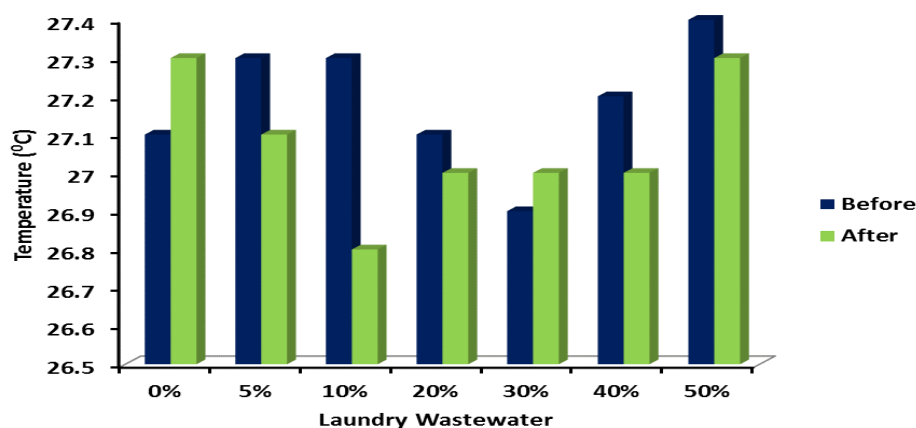


Figure 4: Temperature at the start and end of the study period

The total dissolved solids values of the different treatments at the start and end of the study period are shown in Figure 5. The control (0%) treatment had the highest TDS values at the start and end of the study; followed by 5% treatment. There were no much differences between the pre-treatments and post treatments of 10%, 20%, 30%, 40% and 50%.

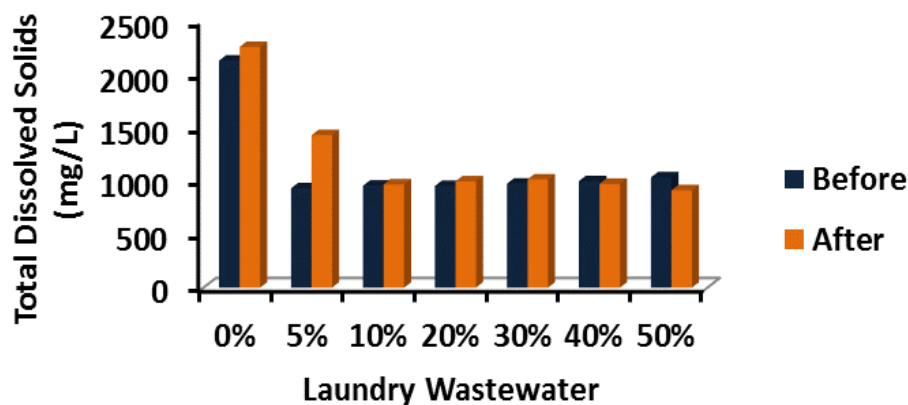


Figure 5: Total dissolved solids (TDS) at the start and end of the study period.

Conductivity values at the beginning and the end of the period of study are shown in Figure 6. Conductivity values at the start and end of the study for control (0%) were high compared to 5%, 10%, 20%, 30%, 40% and 50% treatments.

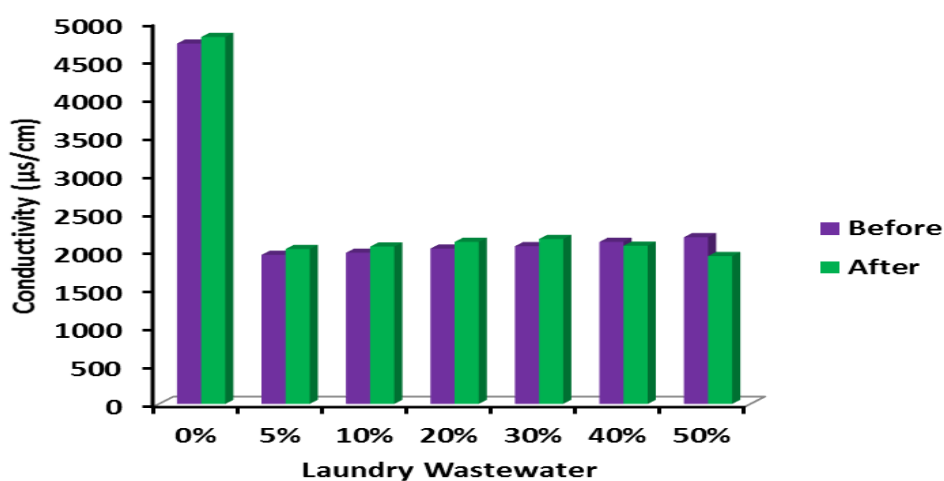


Figure 6: Conductivity at the start and end of the study period.

The biochemical oxygen demand (BOD) values, for the various treatments at the start and end of the period of study are shown in Figure 6. Fifty percent (50%) treatment had the highest BOD value at the start and end of the period of study while the control (0%) treatment had the lowest BOD values. There was reduction in BOD values for 30%, 40% and 50% treatments.

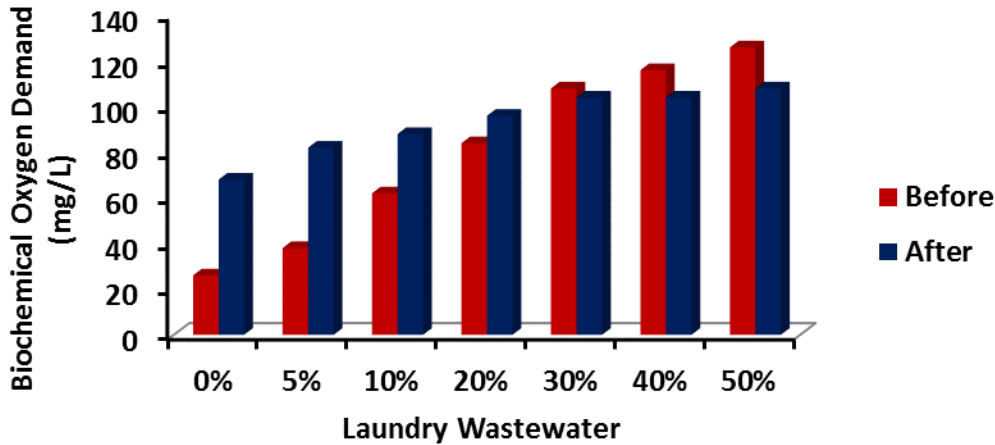


Figure 7: Biochemical oxygen demand (BOD) at the start and end of the study period.

Figure 8 shows the chemical oxygen demand at the start and end of the study period. Control (0%) treatment had the lowest COD value at the start and end of the study period. COD values of 20%, 30%, 40% and 50% treatments, reduced at the end of the study except for the control (0%), 5% and 10% treatments which increased.

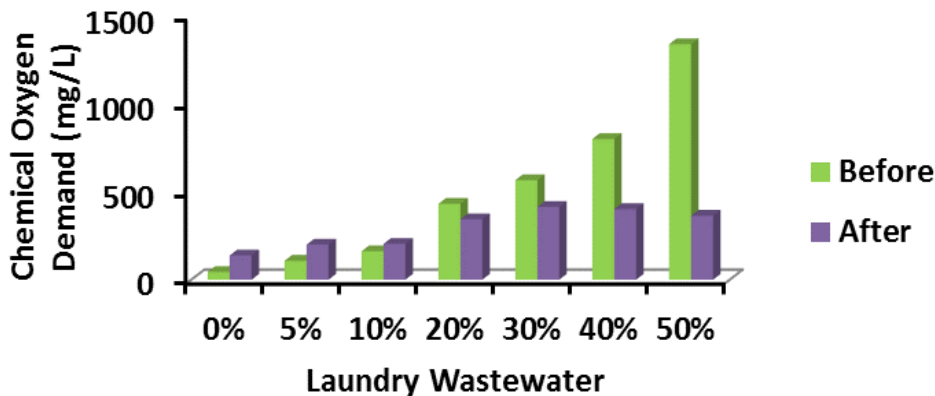


Figure 8: Chemical oxygen demand (COD) at the start and end of the study period.

The results obtained from physico-chemical analysis of the sewage waste water sample are given in Table 1. A comparative analysis of the physiochemical parameters of laundry wastewater with the permissible levels for drinking water by the World Health Organization (WHO) and freshwater shows that laundry wastewater exceeded WHO permissible levels in turbidity, alkalinity, magnesium, sodium and electrical conductivity. While the comparison of some physicochemical parameters of both laundry wastewater and freshwater showed that laundry wastewater exceeded freshwater in all the parameters except for pH.

Table 1: Comparison of Laundry wastewater and freshwater with international standard permissible levels of measured parameters in drinking water

Parameters	Laundry wastewater	WHO	Freshwater
TDS (mg/L)	592	1200	35
Turbidity (NTU)	314.98	5.0	2.37
Temperature (°C)	26.8	NS	31.2
EC (µS/cm)	1259	1200	77.80
pH	3.82	6.5-9.5	6.4
BOD (mg/L)	360	10	3.87
COD (mg/L)	7680	2000	0

Parameters	Laundry wastewater	WHO	Freshwater
Phosphate (mg/L)	6.67	40	0.01
Calcium	22.44	200	1.28
Magnesium (mg/L)	3.89	0.2	0.39
Sodium (mg/L)	379.10	200	1.55
Alkalinity (mg/L)	140.30	120-600	3.05
Chloride (mg/L)	262.70	NS	7.09
Nitrate (mg/L)	15.46	50	0.03
Sulphate (mg/L)	15.58	500	0.76

Discussion

Duncan Multiple Range test showed in Figure 1 indicated a mean difference in the order $50\% \geq 40\% \geq 30\% \geq 20\% \geq 10\% \geq 5\% \geq 0\%$; all the treatments were significantly different from the control (0%). This was as a result of the presence of high nutrients as shown in Table 1, occasioned by the high concentration of laundry wastewater as well as *Chlorella vulgaris* possessing a small area to volume ratio which enhances the diffusion of nutrients into the cell and thus an increase in growth rate with increasing concentration. This is similar to the works of Sreekant *et al.* (2014), in which *Chlorella vulgaris* thrived very well in the presence of abundant nutrients in dairy wastewater; and also in agreement with the work of El-Kassa and Sallam (2014) whereby *Chlorella vulgaris* thrived very well in textile waste effluent at high concentrations.

Many factors that influenced the growth of *Chlorella vulgaris* were clarity of the water, time of day, effective range of sunlight, shape, color or transparency of the container, wall thickness and size of the container (Lavajoo and Dehghani, 2016). For production of high value microalgae products, light intensity and photoperiod are most important factors for growth rate and biomass composition (Khoeyi *et al.*, 2012).

pH is the degree of acidity or alkalinity of a water body. It is an important indicator of water quality and degree of pollution (Yisa and Jimoh, 2010). The pH of water affects the biological and chemical processes in the water (Jindal *et al.*, 2014b). The 50% treatment had the highest pH at the end of experiment; initially pH was 3.92 after treatment with *Chlorella vulgaris* it increased to 8.77 14 days after treatment. The values recorded in this study were similar to studies carried out by Makarevičienė *et al.* (2011) and Zhao *et al.* (2011) which revealed that *Chlorella vulgaris* maintained the maximum growth rate in a wide range of pH between 6.0 and 9.0. At the end of the experiment, the pH increased, this is comparable to the study carried out by Rajasulochana *et al.* (2009). According to Watson and Drapcho (2016), variation in pH values can affect metabolism and algal growth by altering the equilibrium of inorganic carbon, changing the availability of nutrients in addition to affecting the cell physiology. Some studies carried out by Arbib *et al.* (2014) and Zhao *et al.* (2016) revealed that pH increases during the growth of microalgae due to a shift in the chemical equilibrium system among carbon dioxide, carbonic, carbonate, and hydroxide.

Temperature is an important water quality parameter that influences dissolved oxygen content of water and metabolic rates of aquatic organisms (Cahyonugroho *et al.*, 2022). An increase in temperature increases the algal growth until an optimum temperature is reached (Chevalier *et al.*, 2000; Yu *et al.*, 2014). An increase beyond optimum temperature leads to a rapid decline in growth rate. The optimum temperature range 15-25 °C is favourable for most microalgal species, even those which are adapted to growth at colder temperatures. Kessler *et al.* (1989) reported the upper limit of temperature for growth of 14 strains of *Chlorella vulgaris* to be within the temperature range of 26 to 30 °C which is in agreement with the data which had a range of 23 °C - 27.5 °C. A similar trend was reported by Cassidy (2011) with the optimal growth temperature for *Chlorella vulgaris* observed between 25 °C - 30 °C. The optimal temperature for *Chlorella vulgaris* is about 30 °C, in which the maximum biomass productivity is achieved (Chinnasamy *et al.*, 2009, Xu *et al.*, 2006). Converti *et al.* (2009) reported that *Chlorella vulgaris* growth rate at 35 °C decreases 17% compared to 30 °C. An excessive rise in temperature to 38 °C leads to an abrupt halt in microalgae growth and the cells die. With increasing temperature to 30 °C cell growth rate increases and then decreases with increasing temperature to 35 °C (Cassidy, 2011). There was minimal fluctuation in the temperature values of the various treatments on the other days, low temperature limits cell growth and therefore reduces biomass production (Ali *et al.*, 2021).

Total dissolved solids (TDS) comprise of inorganic salts and a small amount of inorganic matter dissolved in water Kataria *et al.*, 1996). In this study, the TDS value for 50% treatment reduced from 1035 mg/l to 914 mg/l. This is comparable to the study carried out by Ahmad *et al.* (2013) which stated that the reduction in TDS in the wastewater was attributed to the utilization of various inorganic nutrients by microalgae. The reduction in TDS was due to the consumption of dissolved solid from wastewater which is nutrient-rich for the growth of microalgae.

In this study, high conductivity values indicate the presence of a high amount of dissolved inorganic substances in ionized form. The fluctuations in conductivity in any particular location depend on the fluctuation in TDS and salinity (Pandey *et al.*, 2003).

BOD is the amount of oxygen required by microorganisms to aerobically decompose organic matter; it is an indicator of water pollution (Jindal *et al.*, 2014a). Before the experiment, BOD showed the highest value of 126 mg/L in the 50% treatment. At the end of the study period, the value was reduced to 108 mg/L. BOD value has been widely adopted as a measure of pollution in a particular environment. It is one of the most common measures of organic pollutants in water Mahananda *et al.* (2010).

COD is the determinant of the level of organic matter and carbon. At the beginning of the study, 50% treatment had the highest COD value of 1340 mg/L it was reduced to 364 mg/L at the end of the study. This is in synchrony with the study carried out by Colak and Kaya (1988) which showed that *Chlorella vulgaris* induced a progressive reduction in COD values and BOD values of the effluent and this could be attributed to the high algal growth rate and intense photosynthetic activity. Also, this is in consonance with the work of Sreekant *et al.*, (2014) and El-Kassa and Sallam (2014) whereby *Chlorella vulgaris* reduced COD in dairy wastewater cultures and textile waste effluents respectively.

A comparative analysis of some physiochemical parameters of laundry wastewater with the permissible levels for drinking water by the world health organization (WHO) and freshwater is shown in Table 1. Parameters in which laundry wastewater exceeded WHO permissible levels were turbidity, alkalinity, magnesium, sodium and conductivity. Also, laundry wastewater when compared with freshwater showed that, laundry wastewater exceeded freshwater in all the parameters except for pH which was low.

Conclusion

The study of phycoremediation of laundry effluents by *Chlorella vulgaris* is an effective method and can be a good substitute for conventional remediation processes. To meet the environmental regulation, all types of wastewater need to be treated before they are discharged to the river. Bioremediation is an important process to remove or minimize contaminant. *Chlorella vulgaris* has been widely used in wastewater treatment as they have fast growth rates and high nutrients removal capabilities. From this study, it was clear that when the growth rate of *Chlorella vulgaris* in the wastewater increases, the concentration of different pollutants or nutrients decreases. The results obtained in the experiment demonstrated the ability of *Chlorella vulgaris* to bioremediate laundry effluents achieving a significant reduction in TDS, BOD, COD, and conductivity value. Therefore, it can be concluded that bioremediation of wastewater using *Chlorella vulgaris* is an effective environmental option.

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