International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 4 Issue 9, September 2017

ISSN: 2394-4404

Decolourization Of Textile Dye Using Microalgae (Chlorella Vulgaris And Sphaerocystis Schroeteri)

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Abstract: ***The discharge of coloured effluents and dye waste water into water bodies is one of the major means of water pollution. Application of microalgae in the decolourization of textile dyes or effluents termed phycoremediation is a cost effective and an appropriate alternative measure in the treatment of textile effluents. This study deals with the application of two microalgae; Chlorella vulgaris and Sphaerocystis schroeteri in the decolourization of textile dyes. Search Tech 721GVisible spectrophotometer was used in reading absorbance. Microscopy was carried on the test microalgae before use in this study. Two dyes; blue and green coloured dyes at different concentrations of 1mg/l, 5mg/l, 10mg/l and 20mg/l were used for this study. Significant decolourization was recorded in a 14 day experiment. Maximum decolourization by both algae was obtained at 1mg/l for both dyes. In Chlorella vulgaris, maximum decolourization was 63.89% (1mg/l) in blue dye and 45.71% (1mg/l) in green dye while in Sphaerocystis schroeteri, maximum decolourization was 63.87% (1mg/l) in blue dye and 60.00% (1mg/l) in green dye. Comparatively, maximum cumulative percentage decolourization for the entire period of the experiment, ie 14days for Chlorella vulgaris was greater in blue dye while for Sphaerocystis schroeteri, it was greater in green dye. During the study, maximum dye decolourization was obtained on the final day. Comparatively, both maximum (63.89%) and minimum (-25%) decolourization were obtained in blue dye with both algae. Decolourization was dependent on dye concentration. Comparatively, there was no significant difference between the percentage decolourization achieved by the two microalgae in each dye. However, it has been found that Chlorella vulgaris and Sphaerocystis schroeteri can decolourize textile dye and can be used for decolourization of textile effluents.***

Keywords: Microalgae, Textile dye, Decolourization, Phycoremediation, Effluent.

I. INTRODUCTION

A dye is a coloured, ionizing and aromatic organic compound which shows an affinity towards the substrate to which is being applied (Booth, 2000) and dyes are generally applied in aqueous solution (Balter, 2009). The discharge of coloured effluents into water bodies is a major means of water pollution and degradation. The world is facing a number of environmental challenges. Wastewater from printing and dyeing units is often rich in color, containing residues of reactive dyes and chemicals and therefore, requires proper treatment before being released into the environment. (Wioletta, 2012).

The global consumption of textiles has currently risen to around 30 million tones with expected growth at 3% per annum and it is estimated that 10,000 different types of dyes and pigments are produced worldwide annually (Walker & Weatherly, 1997). There are nine classes of dyes according to their solubility and chemical properties and these are; basic dyes, acidic dyes, direct or substantive dyes, mordant dyes, vat dyes, reactive dyes, dispersed dyes, azoic dyes and sulfur dyes (Zollinger, 2000).

Environmental problem associated with the use of these dyes in industrial production is their lost during dyeing process since the fixation efficiency ranges from 60 to 90% (Sugiura *et al*., 1999). Fifteen percent of the total world's

utilization of dyes is lost during dyeing process into textile effluents. The release of coloured wastewaters into the aquatic ecosystem can cause aesthetic reduction, eutrophication and perturbations such as decrease in the photosynthetic activities, dissolved oxygen (DO). It can also cause alteration of the pH, increase in the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) in the aquatic ecosystem (Amin *et al.*, 2013). The discharge of textile effluents is toxic to aquatic life and even carcinogenic or mutagenic in nature (Puvanehware*et al.*, 2001). It causes damages to the quality of the receiving water bodies, the aquatic bionetwork and the environment at large (Pala *et al.*, 2003). The portability of the receiving streams, ponds and rivers are also affected. Pollution as a result of textile effluents released into the water can cause low productivity, biogenic inhibition, extinction of endengered species, introduction of poisonous benzene and other aromatic compounds

Treatment of industrial effluents is necessary prior to their final discharge into the environment and different chemical methods of doing this have been criticized to pose additional risk to the aquatic ecosystem. Convectional (chemical) dye effluent treatment methods can pose danger to the environment by resulting to production of some other toxic chemicals or compounds as by products (Joshi *et al.*, 2004).

This brings the inevitable need to subject these effluents through series of biological techniques of remediation and decolourization to promote environmental sustainability and ecosystem restoration. Biological techniques being easier and cheaper to operate have become the focus in recent studies of dye degradation and decolorization. Microbial, algal, fungal and enzymatic decolorization and degradation of textile dyes have significant advantage to conventional methods due to their environmental friendliness and sustainability, cost and low sludge formation (Saratale*et al.*, 2011). Phycoremediation is the term used to describe the application of microalgae in the decolourization and remediation of waste water.

II. MATERIALS AND METHODS

TEST ORGANISMS AND MICROCOPY

Two microalgae species; *Chlorella vulgaris* and *Sphaerocystics schroeteri* were used in this study. Two dye colours, four treatments and three replicates were used to demonstrate the ability of microalgae in dye decolourization. Monoculture of text microalgae were collected from the fish pond site of the Faculty of Agriculture, University of Benin, Nigeria. Proper microscopy was carried out in Limnology and Algology laboratory, Department of Plant Biology and Biotechnology, Faculty of life science, University of Benin. The algal samples were collected in pure culture from the source and used immediately for this study.

The ratio (size) of *Chlorella vulgaris* cells to *Sphaerocystics schroeteri* colony was determined using micrometry and was found to be 4:1. The concentrations of the algal biomass used were 1,882.5cells/ml for *Chlorella vulgaris* and 2145colonies/ml for *Sphaerocystis schroetrie.* 25ml (47,062.5cells) *Chlorella vulgaris* and 6.25ml (13,406.25colonies) for *Sphaerocystics schroeteri* samples were used for inoculation.

DYE SOURCE AND PREPARATION

Textile dyes (Azo dye; blue and green) were purchased in raw form from New Benin market, New Benin, Benin City, Edo State, Nigeria. Distilled water was used in preparing dye solutions in different concentrations; 1mg/l, 5mg/l, 10mg/l, and 20mg/l for each dye colour used for this study. In order to unify the inoculation, 6.25ml of *Sphaerocystis schroeteri* was used to inoculate each replicate of each treatment of each dye solution and 25ml of *Chlorella vulgaris* was also used in the inoculation in the same manner. Each experimental flask contained 300ml of dye solution and algal biomass. Sample flasks were arranged in replicates. Control was setup using distilled water and inoculated as mentioned earlier. It is to look at the effect of algal metabolic activities (respiration and excretion) on spectrophotometric reading (percentage decourization). The experimental flasks were cocked using cotton wool to avoid contamination.

DECOLOURIZATION STUDIES

The decolourization progress was read using Search Tech 721GVisible spectrophotometer by reading absorbance at maximum wavelength for each dye colour. Search Tech 721GVisible spectrophotometer showed that the maximum wavelength for Blue dye was 445Hz, Green was 380Hz. Reading was taken in the day the experiment was setup as the initial (Day 0). Thereafter, the readings were taking on Day 2, Day 4, Day 6, Day 8, Day 10, Day 12 and Day 14. Percentage decolourization was determined by the percent (%) ratio absorbance reduction using the formula below according to Gnanadoss and Jebapriya (2013).

$$\text{Decolourization \%} = \frac{\text{Initial Absorbance} - \text{final Absorbance}}{\text{Initial Absorbance}} \times 100$$

5ml of each sample was taken, filtered and read under spectrophotometer. Filtration was to avoid algal cells from contributing to the absorbance reading. Distilled water was used as blank. There was systematic randomization with the way the bottles were being arranged outside each day after reading. The replicate bottles were exchanged every two days to ensure equal exposure to environmental factors.

STATISTICAL ANALYSIS

Anova was used to determine the significance of concentration in dye decolourization. Unpaired T test was used to determine if there was significant difference between the amount of decolourization attained by the two microalgae. PAST Statistical software was used.

III. RESULTS

Significant decolourization of up to 63.89% was recorded in 14 days in blue dye with *Chlorella vulgaris* and 63.87% with *Sphaerocystis schroeteri.* Figure1 represents percentage decolourization in blue dye by *Chlorella vulgaris* and figure3

represents percentage decolourization in green dye by *Chlorella vulgaris.* In *Chlorella vulgaris*, the minimum and maximum decolourization respectively, were -25% and 63.89% for blue dye; -2% and 45.71% for green dye. Figure2 represents percentage decolourization in blue dye by *Sphaerocystis schroeteri* and figure4 represents percentage decolourization in green dye by *Sphaerocystis schroeteri.* In *Sphaerocystis schroeteri*, the minimum and maximum decolourization respectively, were 0% and 63.87% for blue dye; 10.00% and 60.00% for green dye.

Maximum dye decolourization was achieved in 1mg/I by both algae in both dyes and the minimum dye decolourization was recorded in 10mg/l by both algae in both dyes. Dye concentration significantly affected decolourzation. A consideration of the effect of dye colour in decolourization revealed no significance difference in both dyes.

Highest decolourization was attained on the last day (day 14). A similar trend was attained by both algae at the end of the experiment where the order of highest dye decolourization attained was in 1mg/l > 5mg/l > 20mg/l > 10mg/l. Dye absorption in all the treatments was continuous through the entire period of the experiment. Physical examination showed that there was highest decolourization in 1mg/l.

Unpaired T test analysis showed that there was no significance difference in percentage decolourization attained by the two algae used in both dye solutions. Comparatively, maximum cumulative percentage decolourization (entire period of the experiment, ie 14days) for *Chlorella vulgaris* was greater in blue dye while for *Sphaerocystis schroeteri*, it was greater in green dye.

In looking at control and the effect of metabolic activities (growth, respiration, excretion) on the spectrophotometric reading, the overall maximum increase was attained on day 6 in both dyes using *Chlorella vulgaris*. For *Sphaerocystis schroeteri*, the peak was attained in day 4. For *Chlorella vulgaris*, the reading dropped on day 8 and continued increasing again in day 10 while in *Sphaerocystis schroeteri*, it dropped on day 6, day 8 and continued increasing again on day 10.

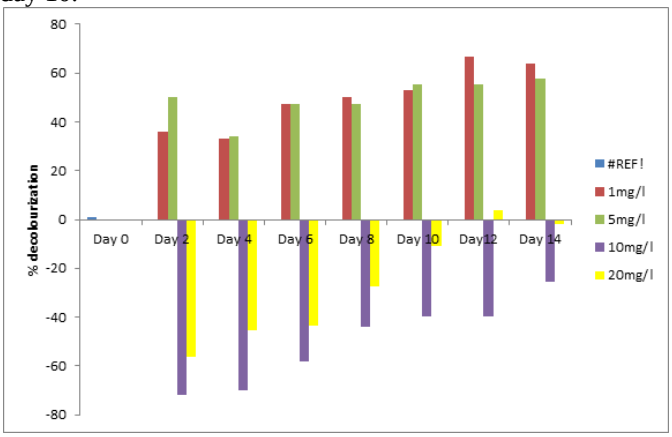

Figure 1: Percentage blue dye decolourization by Chlorella vulgaris

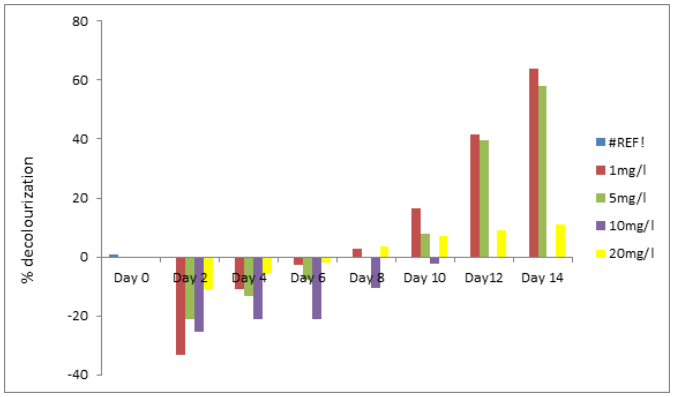

Figure 2: Percentage blue dye decolourization by Sphaerocystis schroeteri

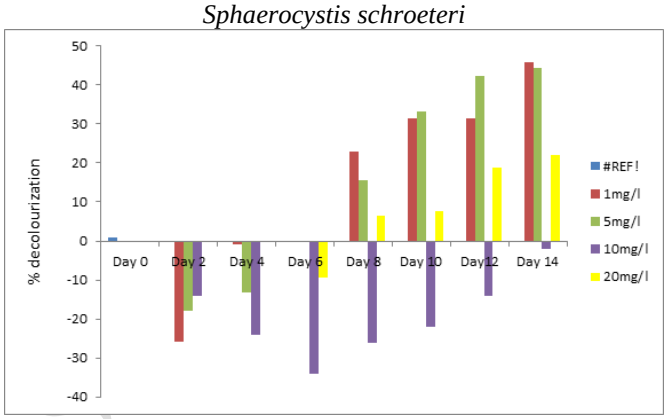

Figure 3: Percentage green dye decolourization by Chlorella vulgaris

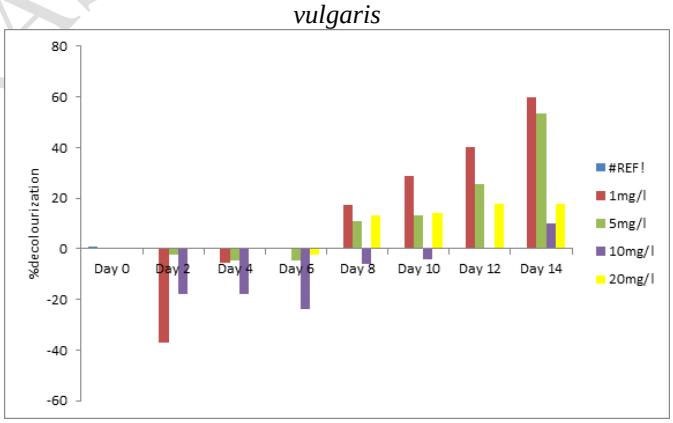

Figure 4: Percentage green dye decolourization by Sphaerocystis schroeteri

Table 1 and 2 shows the final percentage decolourization by *Chlorella vulgaris* and *Sphaerocystis schroeteri.* Both algae showed observable systematic sequence in decolourization. In using *Chlorella vulgaris*, the sequence was 1mg/l > 5mg/l > 20mg/l > 10mg/l in both dye and the same sequence was recorded in using *Sphaerocystis schroeteri.*

Concentration (Mg/l)	Blue dye	Green dye
1 Mg/l	63.89	45.71
5 Mg/l	57.89	44.44
10 Mg/l	-25.58	-2.00
20 Mg/l	-1.82	21.98

Table 1: The final percentage decolourization by Chlorella vulgaris

Concentration (Mg/l)	Blue dye	Green dye
1 Mg/l	63.87	60.00
5 Mg/l	57.88	53.33

10 Mg/l	0	10.00
20 Mg/l	10.98	17.58

Table 2: The final percentage decolourization by Sphaerocystis schroeteri

Table 3 shows percentage decolorization in control. It showed very high increase in absorbance reading and as a result, recorded high negative value for percentage decolourization with both algae. This shows high rate of biochemical activities in both organisms. Such biochemical activities that could increase spectrophotometric reading of the samples are respiration and excretion.

Concentration (Mg/l)	Control
Chlorella vulgaris	-650
Sphaerocystis schroeteri	-370

Table 3: The final percentage decolourization by both algae in control

Figure 5 shows the comparative analysis of dye decolourization by the two algae in blue dye at the end of the experiment and figure6 shows the Comparative analysis of dye decolourization by the two algae in green dye at the end of the experiment. A comparative assessment of the effectiveness of using the two microalgae in dye decolourization, using unpaired T text showed that there was no significant difference in the percentage decolourization attained by the two algae in each of the colours.

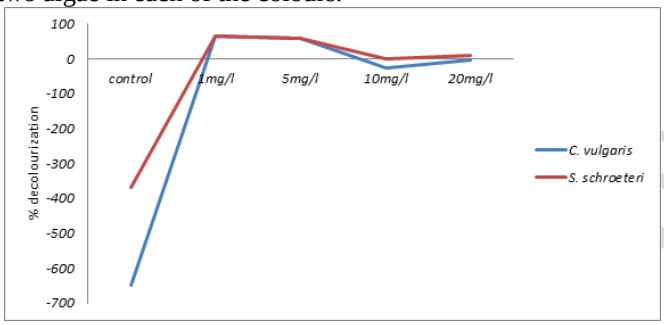

Figure 5: Comparative analysis of dye decolourization by the two algae in blue dye at the end of the experiment

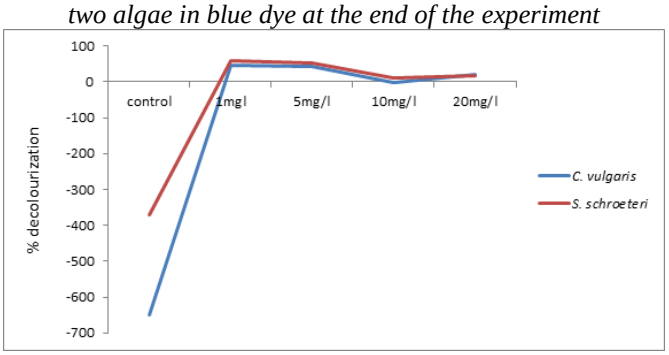

Figure 6: Comparative analysis of dye decolourization by the two algae in green dye at the end of the experiment

Figure 7 showsthe cumulative percentage decolorization by the two microalgae in blue dye at the end of the experiment and figure 8 shows the cumulative percentage decolorization by the two microalgae in green dye at the end of the experiment. The cumulative percentage decolourization was highest in 1mg/l and lowest in 10mg/l in using both algae in both dyes. The growth in control in both dyes was higher in *Chlorella vulgaris* than *Sphaerocystisschroeteri.*

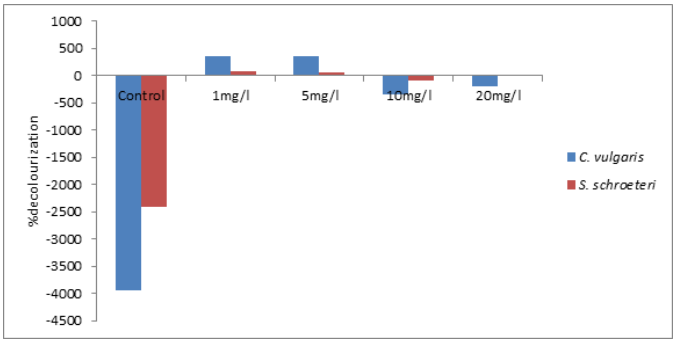

Figure 7: Cumulative percentage decolorization by the two microalgae in blue dye at the end of the experiment

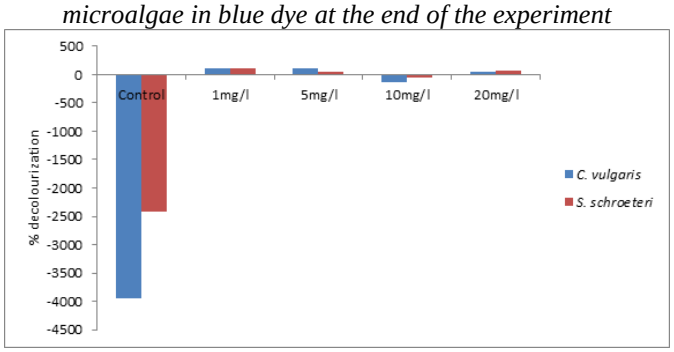

Figure 8: Cumulative percentage decolorization by the two microalgae in green dye at the end of the experiment

IV. DISCUSSION

So many microalgae which may include; *Senedesmus sp, Chlorella sp, Chlamydomonas, Chara sp, Oscillatoria sp, Dunaliella sp* etc, have been documented by many scientists to be good bioaccumulators and have been shown by researches that they can decolourize textile dyes and remove colour in effluents effectively. Chai *et al* (2014) recorded up to 100% decolourization in using immobilized *Scenedesmus quadricauda* to decolourize indigo blue dye. Al-Taee and Al-Ahmed (2012), using *Chlorella vulgaris* in removing malachite green from aqueous solution in concentrations (0.01, 0.05 and 0.1mg/l) by using batch culture experiment recorded up to 75% removal. Henciya *et al*., (2013) recorded high decolourization in using Lygbya *sp* to decolourize textile dye effluent. El-Kassas and Mohamed (2014) recorded up to 75% colour removal in using *Chlorella vulgaris* to remediate textile waste effluent.

The effect of dye concentration and colour in decolourization were looked into and it was shown that the highest decolourization was observed in 1mg/l in both dye solutions and in both algae. Change in concentration significantly affected the percentage decolourization but the effect was not a linear relationship with dye concentration as reported by Chia *et al.* (2014). Dye colour did not significantly affect percentage decolourization attained by both microalgae used and this could be an implication of both microalgae having similar light spectrum properties, reflection and their absorption being in the same colour spectrum as both microalgae are members of green algae. Both microalgae could have similar chromatic reaction in both blue and green dye.

Gupta *et al.,* (2006) and Ozer*et al.,* (2006) suggested that the dye removal may be attributed to the accumulation of dye ions on the surface of algal biopolymers and further to the diffusion of the dye molecule from aqueous phase onto solid phase of the biopolymer. Daneshvar*et al.* (2007) moreover stated that colour removal by algae was attributed to intrinsically different mechanisms of assimilative utilization of chromophores for the production of non-coloured molecules and adsorption of chromophores on algal biomass. Mohan *et al.,* (2002) opined that decolourization process was biosorption followed by bioconversion and bio-coagulation.

The percentage decolourization recorded by the two test organisms showed systematic sequence (1mg/l > 5mg/l > 20mg/l > 10mg/l). There was higher decolourization in 20mg/l dye concentraton than 10mg/l dye concentrention corroborating the result by El-Kassas and Mohamed (2014) in the bioremediation of the textile waste effluent by *Chlorella vulgaris* where they reported that higher colour and COD removal occurred with 17.5% textile waste effluent and was lesser with 5.0% textile waste effluent. The low and negative percentage decourization in 10mg/l and 20mg/l dye concentrations is as a result of the toxicity of the dye in that concentration (El-Kassas and Mohamed, 2014). High dye concentration will generally reduce decolourization capacity of the biological organisms (Wioletta *et al.,* 2012). Physical examination of decolorization showed more decolourization in 1mg/l dye.

There was no significant difference between the percentage decolourization attained by the two algae used in the various dye colours at the end of the experiment, indicating that any of the algae used can be adopted for use at a commercial level. Maximum decolourization attained by both algae was in 1mg/l in both dyes and this could indicate a faster decolourization as a result of low dye concentration but irregularities could occur in higher concentration as seen in 10mg/l and 20 mg/l dye concentrations where higher percentage decolourization was recorded in 20mg/l than in 10mg/l.

Algae grow in medium and absorb nutrient from the medium, use light to photosynthesize, metabolize products and excrete organic carbon into the medium (Nechama *at al* 1996). Growth, photosynthesis, algal metabolism and consequent excretion of dissolved organic carbon (glycolates) into the supernatant could be a major cause of increase in spectrophotometric reading making percentage decolourization negative (as expressed in in table 3). Biochemical activities that could increase spectrophotometric reading of the samples are respiration and excretion. Respiration results to the release of carbon iv oxide into the supernatant and excretion will result to input (glycolates) into the samples as mentioned earlier.

Table 3 reading is evident that there were high metabolic activities in both algae during the period of this study. These metabolic activities are also evidence of growth. In this case, statistics showed that *Chlorella vulgaris* showed higher growth than *Sphaerocystis schroeteri* in control. This could imply occurrence of higher rate of cell division in *Chlorella vulgaris* than *Sphaerocytis schroeteri*. However, Phang and Chu (2004) reported that *Chlorella vulgaris* was shown to be a versatile alga that is able to grow under various harsh and unfavorable conditions.

There was adaptation and growth of the algal species in dye solution. This was shown by physical examination of the samples at the end of experiment. The dye solution was absorbed and used as nutrient by the algae for growth as suggested by Daneshvar*et al.,* (2007). The application of these algae species are recommended in the decolourization of coloured industrial effluents before discharge into the environment. Commercially, algae can be employed to treat textile and other industrial coloured effluents where they can absorb dye molecule as source of nutrient, grow and bloom, forming enough biomass to be harvested and channeled into production of algae based products such as algae bio-diesel, feeds and supplements.

V. CONCLUSION

From the present investigation, *Chlorella vulgaris* and *Sphaerocystis schroeteri* are efficient decolourizer of dyes effluents but this generally could depends on; colour, concentration, time, algal biomass used in inoculation. Thus the use of biomaterial in decolourization of dye waste water will provide alternative to the conventional treatment method. Efforts are needed to commercialize this research through selection of suitable microalgae based on economic and market analysis. Using this current method in removing colour before discharge of textile and other industrial effluents, there can be achievement of environmental sustainability and management. Textile effluents and dye solutions should be treated before discharge into water bodies. To develop a low cost and environmentally friendly method of treatment of these effluents, a bioprocess of treatment should be adopted. In developing the bio-method for treating dye effluents in Nigeria, algal-technology involving the use of microalgae to decolourize these effluents should be employed for its efficiency, environmental friendliness, sustainability and protection.

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