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# Green microalgae cultured in textile wastewater for biomass generation and biodetoxification of heavy metals and chromogenic substances

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ABSTRACT

Wastewater treatment is expensive and algae are increasingly tested for their usefulness in cleaning wastewater. Here, we evaluated six microalgae isolates for their ability to grow in textile wastewater. The sequences of the genes coding for the 18S, ITS 1 and ITS 2 regions placed all algae isolates in the Chlorellaceae family (green algae). Biomass was generated for each algae strain with textile wastewater diluted with deionized water and algal dry weight of 0.4–1.35 g $L^{-1}$ was obtained. Elemental analyses of the wastewaters were carried out before and after cultivation of the algae strains and dye colour removal was also estimated. Supernatant obtained after biomass harvest showed reduction/removal of heavy metals like Al, Cu, V, Pb and Se. Chromogenic substances present in the textile wastewater were also reduced by 47.10–70.03% at a lambda max of 558 nm.

## 1. Introduction

Microalgae are mostly unicellular photoautotrophic microorganisms capable of growth in a wide range of conditions – soil, freshwater and marine habitat, as well as industrial and domestic effluent dumping sites (Monteiro et al., 2012). The cultivation of microalgae requires a lot of fresh water which is scarce (Kummu et al., 2016); a good alternative is therefore to grow them in wastewaters to reduce fresh water consumption (Veyel et al., 2014). Their ability to take up nitrate and phosphate for growth and absorb heavy metals onto their cell surfaces have made microalgal treatment of wastewater an environmentally friendly alternative to remove nutrients and toxic pollutants from wastewater sources (Hu et al., 2008; Chan et al., 2014). Cultivation of microalgae in wastewaters to simultaneously generate biomass and clean up the wastewater has been successfully carried out with municipal effluent (Hiibel et al., 2015; Şirin and Sillanpää, 2015), pulp and paper mill effluent (Usha et al., 2016), carpet mill effluents mixed with municipal sewage (Chinnasamy et al., 2010), dairy effluent (Daneshvara et al., 2019), and even textile wastewaters (Kumar et al., 2018).

In Nigeria, the cottage textile (tie and dye) industries generate a lot of effluents which are released directly into sewage pipelines, surrounding soil, flowing streams and open water drainages without any form of treatment (Osuntoki et al., 2013). Based on previous reports, physicochemical analysis of the textile wastewater indicated that it is highly alkaline, high in biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (El-Kassas and Mohamed, 2014). It is also rich in total nitrogen (TN) and total phosphorus (TP) (Kumar et al., 2018), which after discharge are the major cause of eutrophication in water bodies receiving the wastewater. The textile wastewater also contains toxic heavy metals like cadmium, chromium, lead, and nickel (Fenta, 2014), as well as copper, iron, manganese, and zinc (El-Kassas and Mohamed, 2014). If not removed, these heavy metals pose a great health risk to humans, animals, plants, and the environment in general. Shanab et al. (2012) demonstrated some abilities of microalgae in the removal of heavy metals from wastewaters.

The few microalgae studies relevant to textile wastewater treatment addressed areas like carbon and nitrogen removal, colour removal and phytotoxicity of the treated textile wastewater (Dhaouefi et al., 2018). Others like Kumar et al. (2018) considered parameters like total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD), total solids (TS), volatile solids (VS), colour removal, and accumulation of protein and carbohydrate. Lowering of COD, colour removal, and biomass production of *Chlorella vulgaris* were the major foci in El-Kassas and Mohamed's (2014) research and occurred when the textile wastewater was diluted to 17.5%.

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The main focus of this research was to investigate the potentials of some microalgae species in the reduction/removal of heavy metals and colour as well as accumulation of biomass taking advantage of high nitrate and phosphate concentrations in wastewaters from a textile plant in Abeokuta, Nigeria.

## 2. Materials and methods

### 2.1. Strain isolation and purification

Isolation of green microalgae was achieved by serial dilutions and agar (BG-11 medium) plating method (Radha et al., 2013). Purification was in two phases – (1) isolation from other microalgae and cyanobacteria, and (2) purification from bacterial and fungal contaminants. Isolation from other microalgae and cyanobacteria was carried out by streak plate technique while purification from bacteria was achieved by using antibiotics cocktail treatment (Droop, 1967; Timmins et al., 2009).

### 2.2. Identification of isolates

Pure colonies from agar plates were used for the DNA extraction (Radha et al., 2013) and extraction was carried out with the PowerSoil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, US). Four different regions were amplified for the identification of the microalgae – 18 S, Rubisco, ITS 1 and ITS 2 regions. Amplification was carried out using the REDTaq ReadyMix PCR reaction mix (Sigma-Aldrich, St. Louis, MO, USA). The PCR products were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA) and the purified PCR products were sequenced based on Sanger's Dideoxy method (Gopinath et al., 2014) using BigDye V3.1 software and ran on ABI PRISMS 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) (Lee et al., 2015).

### 2.3. Physicochemical and elemental analysis of textile wastewater

Wastewaters from different local tie and dye industries were collected using the cluster sampling technique from Itoku and Asero areas in Abeokuta South Local Government of Ogun State in Nigeria. Physicochemical parameters like pH, conductivity, turbidity, chloride, total solids, biochemical oxygen demand, chemical oxygen demand, nitrate, phosphate, sulphate and total alkalinity were determined. Elemental analysis was carried out on both wastewaters and dyes (7 different colours). For the powdered dyes, 500 mg of each colour was weighed into a digestion vessel and 5.0 mL of 69% nitric acid and 2.0 mL 30% of $H_2O_2$ was added. The vessels were locked, arranged on the carousel and the vessels were then heated in a microwave system (MARS 5). The programme of mineralization was set at power – 1200 watts, Ramp time – 10 min, final temp – 180 °C, temperature hold time – 10 min and cool down time – 10 min (UWM, 2005). Filter papers were placed in glass funnels and washed three times with deionized water. The funnel with filter paper was placed into a 100 mL volumetric flask. The digested samples were diluted/washed from the vessels, poured into the filters and the filtrate was made up to 100 mL with Milli-Q water in the volumetric flask (Saunders et al., 2012). This was analyzed with Inductively Coupled Plasma – Optical Emission Spectrometer (ICP–OES, 4300 DV) (PerkinsElmer, Massachusetts, US).

### 2.4. Screening for Maximum Concentration for Growth (MCG) of textile wastewater

The microalgae isolates were cultured in different concentrations of the tie and dye wastewater (0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0%). This experiment was carried out in triplicates using the 96-well plate and into each well, different concentration of wastewater (200 µL in each well) was inoculated with 50 µL of actively growing microalgae culture (Fig. 1). Incubation was in an incubator under constant illumination at 60 µmol $m^{-2} s^{-1}$and agitation at 110 rpm on a rotary shaker in a chamber with $CO_2$ supply. The optical density (OD) at 680 nm and 750 nm, $OD_{680}$ and $OD_{750}$ was taken at intervals to monitor increase in chlorophyll and biomass (Lynch et al., 2015; Kumar et al., 2018).

### 2.5. Scale-up experiment and harvest of biomass

The set-up in wells which showed growth up till 2.0% in the initial DOE (Design of Experiment) on 96-well plate were scaled-up for six microalgae species in mini photo-bioreactors. Three different concentrations of the wastewater (0.5. 1.0 and 2.0%) was scaled up for each isolate and approximately 130 mL of the wastewaters was inoculated with 20 mL of actively growing microalgae culture in a 250 mL conical flask. This was incubated under constant illumination on a rotary shaker at 120 rev/min. Samples were withdrawn at intervals and placed in a cuvette to measure absorbance at 680 and 750 nm. This was centrifuged at 10,000 × *g* for 5 min; the supernatant was used for heavy metal and colour removal assay while the pellet was freeze-dried to obtain the dry weight per mL (Lee and Fiehn, 2008; Slocombe et al., 2013).

### 2.6. Assay for colour in supernatant

For estimation of the dye colour removal during cultivation, the initial and residual dye concentration in the supernatant was determined quantitatively by the absorbance measurements using a UV–Visible SpectraMax plus384 microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA, USA) (Khataee et al., 2013; Osuntoki et al., 2013). The absorption spectrum at wavelengths ranging between 250 and 700 nm of the wastewater sample run and maximum absorbance wavelength ($\lambda_{max}$) was determined. After culturing the microalgae in the wastewaters, the cells were harvested by centrifuging at 5000 × *g* for 10 min. The supernatant was collected and the disappearance of the absorbance peaks at the λmax was monitored and percentage colour removal was calculated as follows:

$$P = \frac{Ao–At \times 100\%}{Ao} \quad (1)$$

where

P = Percentage dye colour removal
Ao = Initial absorbance of wastewater
At = Absorbance of supernatant wastewater after microalgal cell removal

### 2.7. Assay for heavy metals in supernatant

Heavy metal analysis was carried out using Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) Perkin Elmer Optima 4300 DV (Dual View) coupled with an S10 auto sampler operating on Winlab32 V5.5 software using the EPA 200.7 method. To monitor heavy metal removal, the supernatant collected was acidified with 2% nitric acid and stored in the 4 °C refrigerator until it was analyzed with ICP-OES. Elemental analysis was run on the wastewater samples before the experiment to determine and quantify the metals present in the wastewaters (UWM, 2005). The heavy metal Removal Efficiency (RE) was calculated with equation below (Chan et al., 2014)

$$\text{Removal Efficiency (\%)} = \frac{\text{Initial concentration–Final concentration} \times 100}{\text{Initial concentration}} \quad (2)$$

### 2.8. Statistical analysis

We used STATISTICA (StatSoft 2013) for our statistical analyses. We

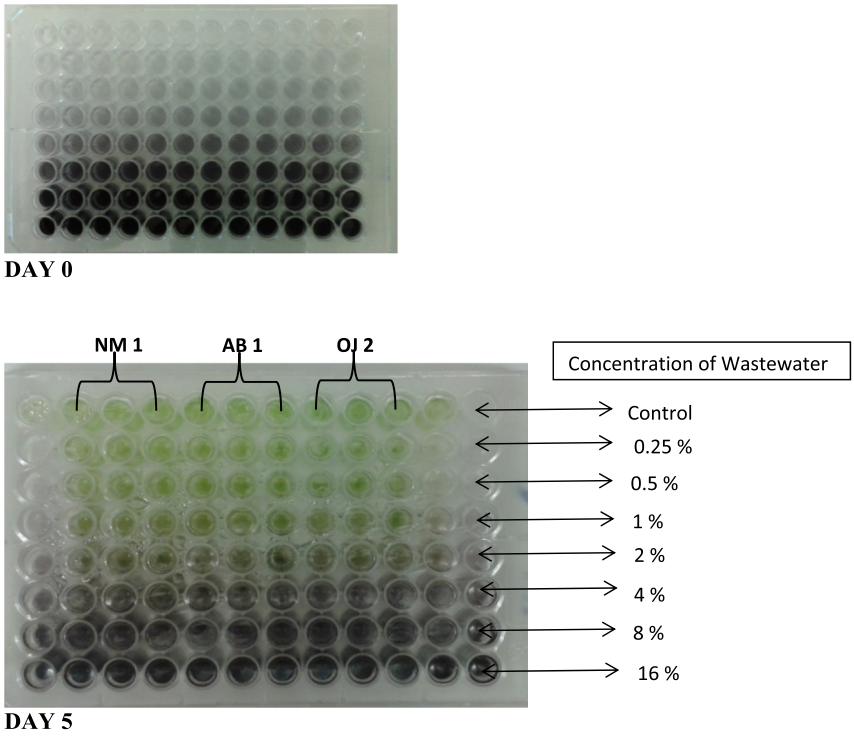


**Fig. 1.** Images of 96-well plate containing textile wastewaters of different concentration (0.25–16%) inoculated with the microalgae isolates. The image on top is from day zero and the image on the bottom 5 days after inoculation.

conducted 2-way ANOVAs using algae strain and concentration of wastewater as predictors for the response variables (algae dry weight or percent colour removal). For heavy metal removal, percent concentration was the variable responding to algae strain and type of heavy metal. Rejection level was set to 0.05 for all statistical analyses. Significant ANOVAs were followed up with a Tukey's post-hoc test to discern significant pairwise differences among treatments.

## 3. Results and discussion

### 3.1. Isolates obtained and phylogenetic analysis

A total of six pure microalgae isolates were obtained from three different fish ponds, a textile wastewater discharge site and the stream it discharges into in Nigeria. The electrophoresis gel image of 18S rDNA amplicons was discovered to be about 750 bp; ITS 1 - 250–500 bp; ITS 2 region - 400–500 bp. The 18S, rubisco, ITS 1 and ITS 2 blast results of the sequences were used in the identification process and the suggestion of closest relatives. All six isolates belong to the family Chlorellaceae (green algae): *Micractinium* sp. CCAP 211/92 (NM 1), *Chlorella sorokiniana* UTEX 1665 (NM 2), *Chlorella* sp. KU211a (IL 1) and *Chlorella* sp. KU211b (IL 3) were isolates from three different fish ponds in Nigeria. *Chlorella sorokiniana* UTEX 246 (AB 1) was the isolate from the textile wastewater discharge and *Chlorella* sp. CB4 (OJ 2) from the stream.

### 3.2. Physico-chemical and elemental parameters of the wastewaters

The pH of the untreated textile wastewaters is highly basic (Table 1). The Biochemical Oxygen Demand (BOD) to Chemical Oxygen Demand (COD) ratio of 1:5 indicates presence of mostly inorganic matter in the wastewater (Lee and Nikraz, 2014) which can probably be attributed to the presence of excess concentration of soluble salt (Ghaly et al., 2014) and also signifies that a substantial part of the organic matter will be difficult to biodegrade (Henze et al., 2015). Also, the levels of nitrate and phosphate are much higher than allowable discharge standards set by the Federal Ministry of Environment, Nigeria (FME) and World Health Organisation (WHO) (Table 1). Since they are essential nutrients for photosynthetic organisms, their release into the environment typically causes eutrophication in natural water bodies.

The major elements that the textile wastewater contained were Aluminum, Calcium, Iron, Magnesium, Phosphorus, Potassium, Silicate, Sodium and Sulphur. Minor elements present in lower abundances like Cadmium, Chromium, Copper, Lead, Nickel, and Titanium, also included some heavy metals (Table 2). Some of the same heavy metals were also reported by Olguín and Sánchez-Galván (2012) that algae readily take up.

### 3.3. MCG determined on 96-well plate

The Maximum Concentration for Growth (MCG) of all microalgae isolates was determined. All the isolates were able to grow in concentrations ranging from 0.25 to 4.0% of the textile wastewaters (Fig. 1). *Chlorella sorokiniana* UTEX 246 and *Micractinium* sp. CCAP 211/92 showed the best growth in up to 4.0% of the textile wastewater. These experiments were scaled up to 150 mL in 250 mL Erlenmeyer flasks to make harvesting, centrifugation and further analysis possible.

### 3.4. Microalgae biomass generated in BG-11 medium and textile wastewaters

The dry weight was used to determine biomass production due to textile dye colour interference with absorbance. *Micractinium* sp. had the highest growth in the wastewater yielding 1.35 g $L^{-1}$ biomass in 1.0% concentration as against 1.1 g $L^{-1}$ harvested when the BG-11 medium was used. The six isolates yielded different quantity of biomass in the three different concentrations of textile dye (0.5, 1.0 and 2.0%). The biomass generated (g dry weight $L^{-1}$) by all six isolates was compared with the biomass produced in BG-11 media (Fig. 2). Except *Chlorella* sp. CB4, all others produced higher biomass in the 1.0% concentration of the wastewater than the UTEX recommended BG-11 medium, although that increase was only statistically significant for

**Table 1**
The physicochemical parameters of tie and dye wastewaters discharged into the environment in comparison with FME (Federal Ministry of Environment, Nigeria) and WHO (World Health Organisation) wastewaters discharge standards. Numbers represent the average of three measurements ± standard error.

| Parameters | Asero textile effluent | Itoku textile effluent | FME wastewater standard | WHO standard |
|---|---|---|---|---|
| BOD mg $L^{-1}$ | 8880 ± 340.2 | 9273 ± 243.6 | 50 | 50 |
| COD mg $L^{-1}$ | 42,442 ± 452.6 | 47,736 ± 438.5 | NA | 100 |
| Chloride mg $L^{-1}$ | 1327 ± 113.7 | 4834.3 ± 60.0 | 600 | 500 |
| Conductivity µS/cm | 56,155 ± 514.8 | 63,209 ± 398.4 | NA | NA |
| Nitrate mg $L^{-1}$ | 373.6 ± 11.8 | 464 ± 6.7 | 20 | 20 |
| Phosphate mg $L^{-1}$ | 78.7 ± 3.8 | 145.7 ± 7.4 | 10 | 5 |
| pH | 12.4 ± 0.5 | 14 ± 0.0 | 6.0–9.0 | 6.0–8.0 |
| TDS mg $L^{-1}$ | 40,149.67 ± 441.3 | 45,020.7 ± 143.7 | 2000 | 1200 |
| TS mg $L^{-1}$ | 60,372.33 ± 149.8 | 116,468 ± 622.1 | 2000 | |
| TSS mg $L^{-1}$ | 20,237.33 ± 449 | 71,095 ± 611.5 | 30 | 100 |
| Turbidity NTU | 2675 ± 88.4 | 9251 ± 141.5 | NA | 300 |

**Table 2**
The elemental component of the local tie and dye industries wastewaters (mg $L^{-1}$) simulated in the laboratory in comparison with both FME (Nigeria) and WHO elements discharge standards. Numbers represent the average of three measurements ± standard error. NA means Not Available (agencies have not established standards).

| Element | Wastewater discharge | FME standard | WHO standard |
|---|---|---|---|
| Al | 8.60 ± 0.06 | NA | 0.1 |
| Bi | 0.45 ± 0.003 | NA | NA |
| Ca | 88.56 ± 0.30 | NA | NA |
| Cu | 3.65 ± 0.27 | 0.01 | 0.05 |
| Fe | 31.43 ± 0.26 | 5 | 1 |
| K | 67.79 ± 0.46 | NA | NA |
| Li | 1.40 ± 0.02 | NA | NA |
| Mg | 28.85 ± 0.53 | NA | NA |
| Mn | 1.33 ± 0.01 | 0.05 | 0.05 |
| Na | 18,957.33 ± 97.29 | NA | NA |
| P | 17.56 ± 0.19 | NA | 5.0 |
| Pb | 0.28 ± 0.02 | 0.02 | 0.05 |
| S | 39,573.67 ± 310.05 | NA | NA |
| Se | 1.08 ± 0.01 | < 1 | 0.2 |
| $SiO_2$ | 138.66 ± 0.24 | NA | NA |
| Sr | 0.85 ± 0.01 | NA | NA |
| V | 1.38 ± 0.02 | NA | NA |
| Zn | 1.03 ± 0.01 | < 1 | < 1 |

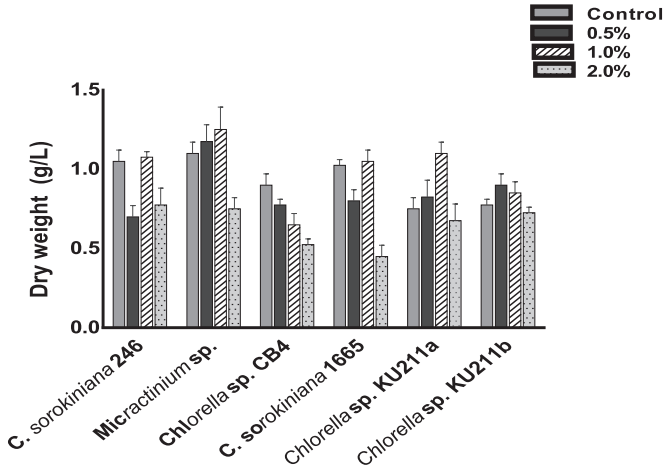


**Fig. 2.** Biomass (g dry weight $L^{-1}$) of microalgae in different concentrations of textile wastewater after two weeks of cultivation.

*Chlorella* sp. KU211a (Tukey's; $p < 0.05$). The positive growth response is probably due to nitrate and phosphate (approximately in the ratio of 4:1) present in the wastewater (Geider and La Roche, 2002; Wang et al., 2010; Martins et al., 2011). This was also the range in textile wastewater used by Kumar et al. (2018) to culture microalgae. Table 2 clearly showed that the average nitrate: phosphate ratio (372.62–466.67:78.45–145.26) was within this range (3.21–4.75:1). Another factor considered to be responsible for the good growth of some microalgae in the textile wastewaters is the ability of light to penetrate the concentration of textile wastewaters used. The concentration of 2% showed a significant reduction of growth in all algae strains and those concentrations with even darker colour (4–16%) did not show much/any algae growth or green colouration after the study period. Apart from the COD:BOD ratio of 5:1 that suggests highly inorganic wastewaters, the inability of the microalgae to grow in dark wastewaters shows that the only source of nutrient are inorganic salts for photoautotrophy.

### 3.5. Heavy metals and colour removal percentage

The ICP - OES assay for elements/heavy metals showed the partial or total removal of five heavy metals detected in the tie and dye wastewaters – Aluminum, Copper, Lead, Selenium, and Vanadium (Fig. 3). Heavy metals like Al and Cu were reduced by 44–67% and Pb and Se completely removed by each of the six algae strains after two weeks of cultivation. Four algae strains reduced V by around 50% while two algae strains were also able to eliminate V from the water. The mechanism of heavy metal detoxification is attributed to class III metallothioneins (MtIII) in microalgae (Delrue et al., 2016). Heavy metals are removed by processes called biosorption (metabolism–independent) or bioaccumulation (metabolism-dependent) (Bilal et al., 2018). The treated wastewater (supernatant after culturing microalgae) showed colour removal in all the three concentrations of wastewater used. The UV–Visible spectrophotometer scan showed the lambda maximum to be 558 nm in the visible region. Percentage colour removal ranged from 47.10% for *Chlorella* sp. KU211b – 70.03% for *Chlorella sorokiniana* UTEX 246 (Fig. 4). The colour removal is attributed to two major mechanism – adsorption to the cell wall which was evident in the colour of the dry biomass obtained and suggested by Kumar et al. (2018) and the

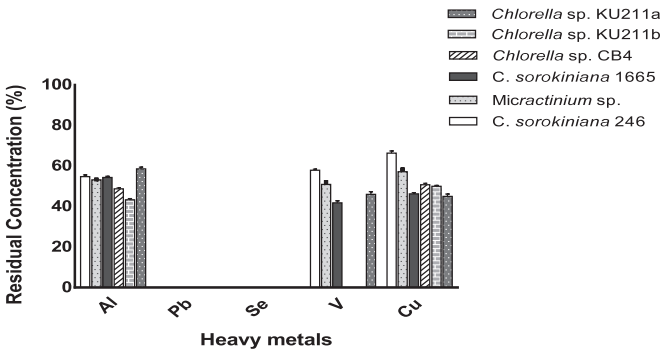


**Fig. 3.** Residual concentration of various heavy metals in the wastewater after 2 weeks of microalgal growth.

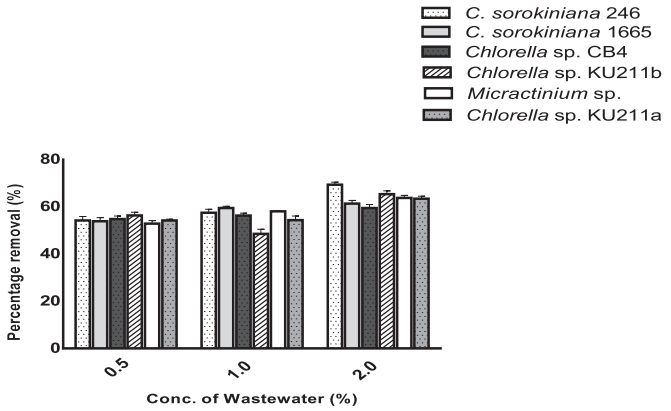


**Fig. 4.** Percentage colour removal by microalgae in different concentrations of the textile wastewater after algae cultivation for two weeks.

production of laccase enzyme, a phenol oxidase which catalyses the oxidation of many aromatic substrates (Otto and Schlosser, 2014).

Percentage colour removal (47.10–70.03%) obtained by UV–visible spectra scan of the supernatant was less than that obtained by El-Kassas and Mohamed (2014) who reported a range of 71.16–76.32% for *Chlorella* sp. but the physicochemical parameters of the textile wastewater used by these researchers had values that were lower than the values obtained in this study. The UV–visible spectra scan of the inoculated effluent showed the loss of the peaks at 558 nm after 48 h indicating that the chromophores in the visible range were degraded. The shift in $\lambda_{max}$ value of the dye or disappearance of the characteristic peaks in the visible spectrum has been reported to indicate the degradation of such dyes (Olukanni et al., 2009; Youssef et al., 2008). Colour removal by algae has been attributed to other reasons like: $CO_2$ and $H_2O$ transformation of coloured molecules to non-coloured molecules and accumulation of dye ions on the surface of algae biopolymer (Gupta et al., 2006), assimilative utilization of chromophores for the production of algal biomass (Daneshvar et al., 2007) and the use of algal azo dye reductase enzyme for degradation (El-Sheekh et al., 2009).

## 4. Conclusion

The textile wastewater was used by the chlorellaceae family to build biomass which indicates removal of nutrient (nitrate and phosphate) from the wastewater. So also, the removal of colour and heavy metals is a treatment process not inherent in the conventional wastewater treatment plants. Therefore a coupling of the wastewater treatment process with algae farming is a win – win situation with benefits of producing microalgae biomass and improving the quality of treated wastewater. Textile wastewater has been shown to produce microalgae biomass which can be exploited for biofuels while elimining the negative impact of nutrient laden wastewater on the environment.

## Declaration of Competing Interest

Authors declare none.

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