Use of *Chlorella vulgaris* for bioremediation of textile wastewater

Sing-Lai Lim, Wan-Loy Chu, Siew-Moi Phang

Abstract

The potential application of *Chlorella vulgaris* UMACC 001 for bioremediation of textile wastewater (TW) was investigated using four batches of cultures in high rate algae ponds (HRAP) containing textile dye (Supranol Red 3BW) or TW. The biomass attained ranged from 0.17 to 2.26 mg chlorophyll a/L while colour removal ranged from 41.8% to 50.0%. There was also reduction of NH4–N (44.4–45.1%), PO4–P (31.1–33.3%) and COD (38.3–62.3%) in the TW. Supplementation of the TW with nutrients of Bold’s Basal Medium (BBM) increased biomass production but did not improve colour removal or reduction of pollutants. The mechanism of colour removal by *C. vulgaris* is biosorption, in accordance with both the Langmuir and Freundlich models. The HRAP using *C. vulgaris* offers a good system for the polishing of TW before final discharge.

1. Introduction

The textile industry is the third largest foreign exchange earner after the electronic and palm oil industries in Malaysia, contributing total earnings of RM 18.0 million (US$ 5.4 million) from manufactured exports in 2007 (Malaysian Textile Manufacturers Association, 2008). There are about 1500 textile factories in Malaysia, many of which operate as backyard or cottage industries producing the local 'batik'. There is concern about the large volumes of effluent discharged from textile processing, which consumes large amounts of water. Colour has been included in the water quality standards for the discharge of industrial effluents in Malaysia. Under the Environmental Quality (Industrial Effluents) Regulations, 2009, the limits of colour for discharge of effluents according to standards A and B are 100 and 200 Platinum–Cobalt (PtCo) units, respectively (DOE (Department of Environment), 2010). It is difficult for most textile factories to adequately treat their wastewater. Textile wastewater is characterised by strong colour, high salinity, high temperature, variable pH and high chemical oxygen demand (COD) (Mantzavinos and Psillakis, 2004). The coloured wastewater affects aesthetics, water transparency and gas solubility in water bodies and can be toxic to aquatic flora and fauna, and this causes severe environmental problems worldwide (Vandevivere et al., 1998). Furthermore, most synthetic azo dyes and their metabolites are toxic, carcinogenic and mutagenic, posing a potential hazard to human health (Nilsson et al., 1993).

The treatment of textile effluent involves mainly physical and chemical methods, which are often very costly (Robinson et al., 2001). It is difficult to treat dye wastewater by chemical and physical processes because of the complex molecular structures. Furthermore, the disposal of the concentrated sludge creates another problem. There has been increased interest in using biological methods for remediation of textile wastewater, especially in colour removal. Most studies have concentrated on the use of fungi and bacteria to treat coloured wastewater (Tastan et al., 2010; McMullan et al., 2001). However, additional carbon sources are required for such systems. In recent years, the use of microalgae in bioremediation of coloured wastewater has attracted great interest due to their central role in carbon dioxide fixation. In addition, the algae biomass generated has great potential as feedstock for biofuel production (Huang et al., 2010).

Both living and non-viable algae have been used in colour removal from dyes and wastewater. The mechanisms involved include biosorption and bioconversion. For instance, non-viable biomass of *Spirogyra* has been shown to be a useful biosorbent for removal of reactive dye (Synazol) from textile wastewater (Khalaf, 2008). Living biomass of macroalgae such as *Caulerpa lentillifera* (Marungreau and Pavanet, 2006) and *Caulerpa scalpelliformis* (Aravindhan et al., 2007) are able to remove basic dyes by biosorption. Through bioconversion, some algae can break down the dyes and wastewater. The mechanisms involved include biosorption and bioconversion. For instance, *Chlorella vulgaris* can remove 63–69% of the colour from the mono-azo dye tectionil yellow 2G by converting it to aniline (Acuner and Dilek, 2004). The ability to remove colour by algae can be enhanced by stimulating their growth. For instance, the removal of reactive dye by the...
cyanobacteria *Synechocystis* and *Phormidium* is enhanced with the addition of the plant growth regulator triacontanol hormone (Karacakaya et al., 2009).

Recently, interest in using immobilised algae to remove colour from textile dyes has surfaced. For instance, Chu et al. (2009) showed that alginate-immobilised *C. vulgaris* can remove a higher percentage of colour from textile dyes than suspension cultures. Immobilised cultures of a thermophilic strain of *Phormidium* can remove 50–80% of textile dyes at high temperatures (Ertegun et al., 2008).

The use of high rate algae ponds (HRAP) is an efficient approach in bioremediation of agro-industrial wastewaters. The system consists of shallow pond with dense algae cultures aerated with paddle wheels. Apart from removing the pollutants, the algae biomass generated is useful as high-quality animal feed. Microalgae such as *Chlorella* and *Spirulina* grown in HRAP have been shown to be useful in treating rubber effluent and sago starch factory wastewater, respectively (Phang et al., 2000, 2001). In HRAP treating rubber effluent, the productivity of *C. vulgaris* ranged from 25 to 61 g/m²/day, with high percentage removal of COD, NH₄–N and PO₄–P (Phang et al., 2001). High biomass productivity (18 g/m²/day) with efficient removal of COD, NH₄–N and PO₄–P (98.0%, 99.9% and 99.4%, respectively) was achieved using HRAP system treating digested sago starch factory wastewater with *Spirulina platensis* (Phang et al., 2000). In addition, HRAP treating piggery wastewater using mixed population of microalgae generate biomass productivities ranging from 21 to 28 g/m²/day, with COD and total Kjeldahl nitrogen (TKN) removal of 76% and 88%, respectively (De Godos et al., 2008). However, there has been no report on using HRAP for removal of colour from textile wastewater.

The primary objective of this study was to investigate the use of *C. vulgaris* UMACC 001 for bioremediation, especially in removing colour from textile wastewater. *Chlorella* was chosen as preliminary screening of 10 microalgae showed that it grew best in textile wastewater and attained the highest percentage of colour removal. The ability of *Chlorella* in removing colour from textile dye and textile wastewater using HRAP was investigated. The biosorption of textile dyes by the biomass of *Chlorella* was also studied.

2. Methods

2.1. Microalgae

Ten microalgae from the University of Malaya Algae Culture Collection (UMACC) were used in the preliminary screening for their ability to grow in textile wastewater and remove colour. The microalgae used were *C. vulgaris* UMACC 001, *Chlorella* UMACC 236, *Scenedesmus* UMACC 099, *Ankistrodesmus convolutus* UMACC 101, *Euglena* UMACC 058 and four strains of *S. platensis*, namely UMACC 159, 160, 161 and 162. All the cultures were maintained in Bold’s Basal Medium (BBM) (Nichols, 1973) except *S. platensis* which was grown in Kosaric Medium (Phang and Chu, 1999).

2.2. Textile wastewater

The textile wastewater (TW) was collected from a garment factory located at Senawang Industrial Estate, Negeri Sembilan. The wastewater discharge rate of the factory can be as high as 700 m³ daily, which is solely generated by the dye house. The factory employs a combination of physical, chemical and biological methods to treat the wastewater (Fig. 1). The TW is stored in the holding tank before discharge into the river. Despite the treatment processes, the TW still contains colour at the point of discharge. The source of TW for this study was from the holding tank. The physical and chemical parameters of each batch of TW collected were determined using standard methods (APHA (American Public Health Association), 1998), as described in Section 2.8.

2.3. Textile dyes

Three azo dyes were used in this study, namely Supranol Red 3BW, Lanaset Red 2GA, Levafox Navy Blue EBNA. The absorbance of the three dyes was determined using a spectrophotometer (Shimadzu UV/Vis 160) and scanned from 300 to 700 nm to determine the wavelength which gave the maximum absorbance. Supranol Red 3BW is an acidic dye with two azo groups and absorbance maximum (*λ*ₘₐₓ) at 528 nm while Lanaset Red 2GA is a monoazo metal complex dye with *λ*ₘₐₓ of 490 nm. Levafox Navy Blue EBNA is a vinyl sulfone reactive dye with *λ*ₘₐₓ of 603 nm.

2.4. Preliminary screening

Ten microalgae were screened for their ability to grow in TW and remove colour from the wastewater using flask cultures. Ten millilitres of exponential cultures, standardised at an optical density at 620 nm (OD₆₂₀) of 0.2, were inoculated into 90 mL of TW in 250 mL conical flasks in triplicate. The cultures were grown for 10 days in an incubator shaker (150 rpm) set at 25 °C, with an irradiance of 40–60 μmol/m²/s on a 12:12 h light–dark cycle. Initial pH of the TW was adjusted to 7.0 prior to inoculation. Chlorophyll a concentrations of the cultures were determined at the beginning and end of the experiment (Section 2.8). The specific growth rate (*µ*) was determined using the following formula:

\[ \mu \ (\text{day}^{-1}) = \frac{(\ln N₂ - \ln N₁)}{(t₂ - t₁)}, \]

where *N₁* and *N₂* represent the chlorophyll a concentrations at times *t₁* (day 0) and *t₂* (day 10), respectively.

The initial and final colour of the TW was measured and the percentage colour removal was determined (Section 2.8).

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Fig. 1. Flow chart showing the process of textile wastewater treatment adopted by the garment factory. The textile wastewater used in this study was collected from the holding tank.
2.5. Laboratory studies: Growth of C. vulgaris in different concentrations of textile wastewater and its effect on colour removal

C. vulgaris UMACC 001 was selected for further studies as the preliminary screening showed that it grew best in TW. Chlorella was grown at various dilutions of TW (20, 40, 60, 80 and 100%), with BBM as the control. The TW was diluted with distilled water and the pH was adjusted to 7.0 prior to inoculation. Shake flask cultures with 10% inoculum were used in this experiment as described above (Section 2.4). Growth was monitored every alternate day by cell count (improved double-Neubauer haemocytometer) and chlorophyll a determination. Colour was measured on the initial and final day. The specific growth rate (\( \mu \)) was determined using the following formula:

\[
\mu = \frac{\ln N_2 - \ln N_1}{(t_2 - t_1)},
\]

where \( N_1 \) and \( N_2 \) represent the cell number at times \( t_1 \) and \( t_2 \), respectively, within the exponential phase.

2.6. Batch cultures in High Rate Algae Ponds (HRAP)

The outdoor cultures of C. vulgaris were grown in two HRAP constructed on the rooftop of the Institute of Postgraduate Studies, University of Malaya. The HRAP were of single-loop raceway mixed with paddle wheels (15 rpm), with the dimensions of 1 m \( \times \) 0.5 m \( \times \) 0.3 m. Transparent corrugated acrylic roofs were fixed at a height of about 1.5 m above the ponds to shelter them from rain. A total volume of 40 L culture was used for each pond, with a surface area of 0.71 m\(^2\), culture depth of 0.15 m and mixing at a flow rate of 15 cm/s.

Four batches of Chlorella were grown in the two HRAP. The experimental design is shown in Table 1. The inoculum of Chlorella was from exponential phase cultures standardised at OD\(_{680}\) of 0.2. In Batch I, the control pond (control I) contained BBM (I-M) while the treated pond (I-R) contained Supranol Red 3BW. In Batch II, the first pond (II-C) contained Supranol Red 3BW without algal inoculum (control II) while the second pond (II-R) contained Supranol Red 3BW with 10% algal inoculum. In Batch III, the control pond (control III) contained BBM (III-M) while the treated pond (III-R) contained Supranol Red 3BW. In Batch IV, one pond contained TW (IV-TW) while the other (IV-TWM) contained TW supplemented with nutrients of BBM. The nutrients were weighed and the pH was adjusted to 7.0 before inoculation. The ponds were aerated overnight by the paddle wheels before inoculation.

Growth of Chlorella was monitored daily by cell count and chlorophyll a determination. The initial and final colour of all the batches were determined. Pollution parameters such as COD, NH\(_4\)-N and PO\(_4\)-P of TW were determined in Batch IV. Dry weight was determined at the end of the studies.

Physical parameters such as pH, temperature and solar irradiance were monitored. Semi-diurnal studies were conducted for Batch I, III and IV, where samples were taken every alternate hour from 0700 to 1900 on selected days during the exponential phase of growth. Both physical and chemical parameters as well as cell number and chlorophyll a content were determined during the studies.

2.7. Adsorption equilibrium studies

Adsorption equilibrium studies were conducted on living biomass of C. vulgaris exposed to Supranol Red 3BW, Lanaset Red 2GA and Levafix Navy Blue EBNA. The test solution of each dye was prepared by diluting the stock solution (1.0 g/L) to give a range of concentrations from 5 to 50 mg/L. The algal biomass was harvested by centrifugation (4000 g, 5 min), washed and resuspended in 100 mL distilled water. The suspension was homogenised and 1 mL aliquot was contacted with 100 mL of dye solution in a conical flask. The flasks were agitated on a shaker (150 rpm) for 24 h to ensure that the adsorption has reached equilibrium. Samples (3 mL) were then taken at time intervals of 0, 0.5, 1, 2, 4, 6, 8 and 24 h for the determination of residual dye concentration in the solution. The cells were removed by filtering through a glass-fibre filter (Whatman GF/C). Colour of the filtrate was measured at the wavelength that corresponds to the maximum absorbance of the dye tested. The dye concentration (mg/L) of the reaction mixture was extrapolated from a calibration curve. The dye bound to the biomass was calculated using the following formula:

\[
q_{eq} = \frac{(C_0 - C_{eq}) V}{W}
\]

where \( q_{eq} \) = amounts of dye adsorbed (mg/g biomass), \( C_0 \) = initial concentration of dye (mg/L), \( C_{eq} \) = equilibrium concentration of dye (mg/L), \( V \) = volume of the solution (L) and \( W \) = dry weight of the cells used (g).

The adsorption isotherms based on the Langmuir (Langmuir, 1918) and Freundlich (Weber, 1972) models were tested. In mathematical form, the Langmuir's isotherm is expressed as:

\[
\frac{1}{q_{eq}} = \frac{1}{q_m} + \left(\frac{1}{K_f q_m}\right) \frac{1}{C_{eq}}
\]

where \( q_{eq} \) = amounts of dye adsorbed (mg/g biomass), \( q_m \) = maximum adsorption capacity, \( K_f \) = Langmuir's constant and \( C_{eq} \) = equilibrium dye concentration (mg/L).

A plot of \( 1/q_{eq} \) versus \( 1/C_{eq} \) will give a straight line. The Freundlich isotherm equation is written as follows:

\[
\log q_{eq} = \log K_f + (1/n) \log C_{eq}
\]

where \( n \) and \( K_f \) are Freundlich constants (Weber, 1972). A plot of \( K_f \) versus \( C_{eq} \) will give a straight line.

2.8. Analytical procedures

Pollution parameters such as COD, NH\(_4\)-N, PO\(_4\)-P and colour were determined using an Odyssey DR/2500 spectrophotometer (Hach Co.) based on standard methods (APHA (American Public
The determination of COD was based on the dichromate method. NH₄–N was determined using the modified phenate method while dissolved phosphate (PO₄–P) was determined by the ascorbic acid method. Colour was determined based on the APHA (1998) standard of 1 colour unit (PtCo) being equal to 1 mg/L platinum in the form of chloroplatinate ion. The percentage colour removal was calculated as follows:

\[
\text{Percentage colour removal} = \left(1 - \frac{C_\text{final}}{C_\text{initial}}\right) \times 100
\]

The contents of total solids (TS), total suspended solids (TSS), total dissolved solids (TDS) and total volatile solids (TVS) of TW were determined according to APHA (1998). The TS and TVS were determined after drying the samples (100 °C) on pre-weighed crucibles while TSS and TDS were determined from filtered samples after drying at 100 °C and 180 °C, respectively. The heavy metal contents of TW were determined using atomic absorption spectrophotometry (AAS).

Cells were harvested by filtration (Whatman GF/C) for chlorophyll a and dry weight determination. Chlorophyll a was extracted with acetone before being determined by spectrophotometry (Strickland and Parsons, 1968). Dry weight was determined after drying the pre-weighed filters at 105 °C for 24 h, using the following formula:

\[
\text{Dry weight (mg/L)} = \frac{(\text{Weight of filter with algal cells} - \text{Weight of blank filters}) \text{(mg)}}{\text{Volume of algal culture (L)}}
\]

2.9. Statistical analysis

Data from the flask culture studies were analysed by one-way ANOVA followed by Duncan’s multiple range test. The adsorption isotherms were tested using regression analysis.

3. Results and discussion

3.1. Characteristics of textile wastewater

The characteristics of the TW (Table 2) were highly variable but comparable to the range reported by a previous study (Rahman, 1993). In general, the TW was characterised by high colour, COD, NH₄–N and total solids. The colours of TW varied from orange, red, purple, blue to black. The TW contained much higher amounts of total dissolved solids (TDS) compared to total suspended solids.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.85–11.40</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>35.0–58.0</td>
</tr>
<tr>
<td>Conductivity (mS)</td>
<td>0.69–13.81</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>1.5–7.5</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>231.67–990.00</td>
</tr>
<tr>
<td>PO₄–P (mg/L)</td>
<td>0.07–4.01</td>
</tr>
<tr>
<td>NH₄–N (mg/L)</td>
<td>0.47–50.83</td>
</tr>
<tr>
<td>NO₃–N (mg/L)</td>
<td>1.23–5.60</td>
</tr>
<tr>
<td>Total solids (mg/L)</td>
<td>39.33–11689.33</td>
</tr>
<tr>
<td>Total suspended solids (mg/L)</td>
<td>22.67–150.00</td>
</tr>
<tr>
<td>Total dissolved solids (mg/L)</td>
<td>14.00–11564.00</td>
</tr>
<tr>
<td>Total volatile solids (mg/L)</td>
<td>54.46–531.00</td>
</tr>
<tr>
<td>Apparent colour (PtCo unit)</td>
<td>169.67–1937.33</td>
</tr>
<tr>
<td>True colour (PtCo unit)</td>
<td>76.00–1777.33</td>
</tr>
<tr>
<td>C:N:P ratio</td>
<td>88:2:1–8470:45:1</td>
</tr>
<tr>
<td>Heavy metals</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>0.01–2.93 mg/L</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.01–0.04 mg/L</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.01–0.05 mg/L</td>
</tr>
<tr>
<td>Copper</td>
<td>&lt;0.001–0.1 mg/L</td>
</tr>
<tr>
<td>Lead</td>
<td>0.08–0.09 mg/L</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.02–0.04 mg/L</td>
</tr>
<tr>
<td>Arsenic</td>
<td>&lt;0.001 mg/L</td>
</tr>
<tr>
<td>Iron</td>
<td>0.11–0.16 mg/L</td>
</tr>
</tbody>
</table>

The two strains of *Chlorella* were isolated from different sources. *C. vulgaris* UMACC 001 was isolated from a pond at the experimental farm of the University of Malaya while *Chlorella UMACC 236* was isolated from an oxidation pond treating run-off water from a landfill system. *C. vulgaris* UMACC 001 was shown to be a versatile alga that is able to grow under various harsh conditions (Phang and Chu, 2004). It can grow at high NaNO₃ or NH₄Cl levels and is also tolerant to high levels of heavy metals, especially Mn, Cr, Zn and Cd. It has great potential for bioremediation of agro-industrial wastewaters such as rubber effluent and palm oil mill effluent.

3.3. Growth of *C. vulgaris* in different concentrations of textile wastewater and its effect on colour removal

*Chlorella* grew in 100% TW although the final biomass attained was significantly lower (\( p < 0.05 \)) than in 20–80% TW (*Table 3*). Colour removal by *Chlorella* decreased with the increase in initial colour, especially in medium containing \( > 60 \% \) TW. This agrees with other studies using algae such as *Synecocystis* and *Phormidium* for removing colour from reactive dyes (Karacakaya et al., 2009). Without *Chlorella* (control), colour removal due to adsorption or biodegradation by the algae did not take place, hence, very low colour removal was observed.

3.4. High rate algae pond (HRAP) studies

The physical parameters recorded during the HRAP studies were dissolved oxygen (DO), pH, temperature and solar irradiance. The DO contents ranged from 6.7 to 11.4 mg/L while pH and temperatures ranged from 6.41 to 10.42 and 24.0 to 32.0 °C, respectively. Solar irradiance ranged from 135 to 1193 μmol/m²/s during the study. The semi-diurnal studies showed that maximum solar irradiance was recorded between 1400 and 1600 while highest temperatures (31.0–32.0 °C) were between 1300 and 1400. High pH (8.36–10.42) was recorded after noon (1200–1700).

The high solar irradiation of the tropics is particularly advantageous for the operation of HRAP where photosynthetic productivity of algae may be enhanced. The high DO and pH in the ponds...
resulting from the algal growth coupled with the high temperature and solar irradiation would have caused chemical transformation of the coloured compounds. Adsorption of the dyes onto the Chlorella cells probably accounts for the main reduction in colour. The TW consists of a complex mixture of dyes including other pollutants. To assess the potential use of Chlorella in bioremediation, especially in colour removal from TW, a single textile dye (Supranol Red 3BW) was used for comparison with the complex TW in the HRAP studies (Table 1). In the first three HRAP batch studies, the ponds contained Supranol Red 3BW while in Batch IV, the ponds contained TW.

In Batch I, there was a marked decrease in the final cell number and dry weight of Chlorella in Supranol Red 3BW (Pond I-R) compared with that in BBM (I-M) (Table 4). In Batch II, when both ponds contained Supranol Red 3BW, algal biomass was produced in the pond inoculated with Chlorella (Pond II-R) but not in Pond II-C, without inoculum. This showed that other algae from the ambient environment were not able to colonise such toxic medium. Results from Batch III showed that an increase in Chlorella inoculum (20%) improved the growth of Chlorella in Supranol Red 3BW, where the biomass and µ attained (Pond III-R) were comparable to that grown in BBM (Pond III-M).

In Batch IV, Supranol Red 3BW was replaced with treated textile wastewater (TW). Chlorella attained much lower biomass and µ than the first three batches in Supranol Red 3BW. However, the addition of a wide range of nutrients (IV-TWM) improved the biomass and µ of Chlorella. Nutrient deficiency was a limiting factor for Chlorella growth in TW.

Colour removal by Chlorella in the HRAP ranged from 41.8% to 50.0% (Table 4). This was higher than that attained by flask cultures (17.6–34.9%) of Chlorella in our previous study (Chu et al., 2009), but lower than that reported by Acuner and Dilek (2004), where 63–90% of the colour from the mono-azo dye tectilon yellow 2G was removed. In comparison, the white-rot fungus Bjerkandera adusta was reported to remove 57% of the colour from a textile wastewater in flask cultures (Anastasi et al., 2010).

There was no marked difference in terms of colour removal from Supranol Red 3BW and TW. The complexity of the TW with a whole range of dyes including other chemicals, especially heavy metals, did not seem to affect the ability of Chlorella to remove colour. There was low reduction in colour (4.0%) of the textile dye in the pond without algae (II-C) indicating again that colour removal is a result of active Chlorella growth. A doubling of algal inoculum in the ponds containing Supranol Red 3BW (II-C and III-R) increased final biomass but did not increase colour removal.

Supplementation of TW with nutrients of BBM (Pond IV-TWM) did not improve colour removal, although the colour of both ponds was reduced throughout the study (Fig. 2). This observation is in contrast with other studies which showed that colour removal can be improved by enhancing algal growth. Stimulation of the growth of Synecocystis and Phormidium was reported to enhance the removal of colour from reactive dyes (Karacakaya et al., 2009).

Besides dyes, TW contains other pollutants such as COD, NH4–N and PO4–P (Table 5). Growth of Chlorella reduces both colour and the pollutants, resulting in bioremediation of the TW. In the present study, the reduction of the pollutants by Chlorella was lower

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### Table 3

Growth of and percentage colour removal by Chlorella vulgaris in medium containing different concentrations of textile wastewater. The duration of the culture period was 10 days. Data are presented as mean ± standard deviation (n = 3). Different alphabets within the same column indicate significant differences at p < 0.05.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Final cell number (×10^6 cells/mL)</th>
<th>Final chl a (mg/L)</th>
<th>Specific growth rate (µ, day⁻¹)</th>
<th>Cell number</th>
<th>Chl a</th>
<th>Colour (PtCo unit)</th>
<th>Percentage colour removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (BBM)</td>
<td>250.50 ± 6.02²</td>
<td>1.37 ± 0.17⁴</td>
<td>0.28</td>
<td>0.31</td>
<td>N/A</td>
<td>N/A</td>
<td>130.00 ± 7.04</td>
</tr>
<tr>
<td>Control (TW-without algae)</td>
<td>N/A⁴</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>309.67 ± 2.52</td>
</tr>
<tr>
<td>20% TW</td>
<td>128.17 ± 5.19⁶</td>
<td>0.38 ± 0.01⁶</td>
<td>0.15</td>
<td>0.13</td>
<td>93.33 ± 3.51</td>
<td>61.00 ± 4.00</td>
<td></td>
</tr>
<tr>
<td>40% TW</td>
<td>130.00 ± 7.04⁹</td>
<td>0.35 ± 0.09⁹</td>
<td>0.12</td>
<td>0.11</td>
<td>105.33 ± 6.63</td>
<td>10.73 ± 5.00</td>
<td></td>
</tr>
<tr>
<td>60% TW</td>
<td>111.33 ± 3.3³</td>
<td>0.54 ± 0.00³</td>
<td>0.14</td>
<td>0.17</td>
<td>183.33 ± 6.03</td>
<td>10.73 ± 5.00</td>
<td></td>
</tr>
<tr>
<td>80% TW</td>
<td>113.33 ± 5.28¹</td>
<td>0.48 ± 0.11¹</td>
<td>0.21</td>
<td>0.20</td>
<td>246.33 ± 7.57</td>
<td>219.67 ± 6.11</td>
<td></td>
</tr>
<tr>
<td>100% TW</td>
<td>84.00 ± 6.54¹²</td>
<td>0.54 ± 0.11¹</td>
<td>0.17</td>
<td>0.14</td>
<td>303.33 ± 1.53</td>
<td>272.33 ± 6.66</td>
<td></td>
</tr>
</tbody>
</table>

a N/A: non-applicable.

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### Table 4

Summary of the growth parameters based on specific growth rate (µ) and final cell number, dry weight and chlorophyll a concentrations and percentage colour removal of the different batches of Chlorella vulgaris grown in high rate algae ponds (HRAP). The cultures were grown for 10 days for the first three batches and 12 days for Batch IV.

<table>
<thead>
<tr>
<th>Pond</th>
<th>Specific growth rate (day⁻¹)</th>
<th>Cell number (×10^6 cells/mL)</th>
<th>Dry weight (DW) (mg/L)</th>
<th>Chl a content (mg/L)</th>
<th>Colour (PtCo unit)</th>
<th>Colour (PtCo unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-M⁴</td>
<td>0.40 ± 0.01</td>
<td>213.67 ± 4.97</td>
<td>613.33 ± 66.58</td>
<td>2.26 ± 0.17</td>
<td>0.37 ± 0.05</td>
<td>1.06 ± 0.05</td>
</tr>
<tr>
<td>I-R</td>
<td>0.34 ± 0.01</td>
<td>119.33 ± 2.16</td>
<td>496.67 ± 47.26</td>
<td>2.24 ± 0.12</td>
<td>0.45 ± 0.04</td>
<td>1.90 ± 0.13</td>
</tr>
<tr>
<td>II-C</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>II-R</td>
<td>0.55 ± 0.02</td>
<td>168.00 ± 9.58</td>
<td>303.33 ± 15.28</td>
<td>2.56 ± 0.19</td>
<td>0.84 ± 0.03</td>
<td>1.56 ± 0.12</td>
</tr>
<tr>
<td>III-M</td>
<td>0.33 ± 0.01</td>
<td>146.67 ± 3.50</td>
<td>183.33 ± 15.28</td>
<td>1.94 ± 0.06</td>
<td>1.06 ± 0.11</td>
<td>1.31 ± 0.37</td>
</tr>
<tr>
<td>III-R</td>
<td>0.30 ± 0.02</td>
<td>145.17 ± 3.11</td>
<td>190.00 ± 10.00</td>
<td>1.61 ± 0.09</td>
<td>0.85 ± 0.01</td>
<td>1.08 ± 0.08</td>
</tr>
<tr>
<td>IV-TW</td>
<td>0.05 ± 0.01</td>
<td>37.67 ± 1.08</td>
<td>106.67 ± 5.77</td>
<td>0.17 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>IV-TWM</td>
<td>0.39 ± 0.01</td>
<td>92.17 ± 2.48</td>
<td>203.33 ± 15.28</td>
<td>0.76 ± 0.09</td>
<td>0.38 ± 0.07</td>
<td>0.83 ± 0.08</td>
</tr>
</tbody>
</table>

a Batch I: I-M = Bold’s Basal Medium (BBM) alone with 10% algal inoculum (control); I-R = Supranol Red 3BW dye with 10% algal inoculum. Batch II: II-C = Supranol Red 3BW dye without algal inoculum; II-R = Supranol Red 3BW dye with 10% algal inoculum. Batch III: III-M = Bold’s Basal Medium (BBM) alone with 20% algal inoculum (control); III-R = Supranol Red 3BW dye with 20% algal inoculum. Batch IV: IV-TW = Textile wastewater with 10% algal inoculum; IV-TWM = Textile wastewater supplemented with BBM with 10% algal inoculum.

b N/A: non-applicable.
than that achieved with other agro-industrial wastewaters such as rubber effluent and palm oil mill effluent. In HRAP treating rubber effluent with *Chlorella*, the reduction of COD, NH$_4$–N and PO$_4$ ran-ged from 78% to 96%, 41% to 95% and 41% to 94%, respectively (Phang et al., 2001).

There was gradual decrease of COD throughout the study in both ponds treating TW (Fig. 3a). However, the COD reduction in Pond IV-TWM was more than Pond IV-TW, which could be due to the higher biomass in Pond IV-TWM, which also released organic acids at the stationary phase. In comparison, the NH$_4$–N contents of both ponds remained almost constant after an initial decrease on day 4 (Fig. 3b). High levels of NH$_4$–N (>670 mg/L) are known to inhibit the growth of *Chlorella pyrenoidosa* (Lin et al., 2007). However, in the present study the levels of NH$_4$–N (3.43–6.50 mg/L) are unlikely to inhibit the growth of *Chlorella*. Furthermore, *Chlorella* was reported to grow well in rubber effluent containing 70–980 mg/L NH$_4$–N (Phang et al., 2001). The PO$_4$–P content of Pond IV-TW was lower than Pond IV-TWM throughout the study (Fig. 3c). Excess PO$_4$–P in the nutrient-supplemented pond could account for the increased biomass attained compared with Pond IV-TW. However, due to the high initial supply, the reduction of PO$_4$–P in Pond IV-TWM was similar to Pond IV-TW (Table 5). Thus, in this situation, there is no necessity to have nutrient supplementation as the only advantage is to improve biomass of *Chlorella*.

The waste grown algae may not be suitable for use as animal feed due to the presence of toxic dyes and heavy metals. However, there has been increased interest in using algae for biodiesel production (Huang et al., 2010). The algal biomass from the HRAP can serve as a potential feedstock for such an application. This *Chlorella* produced lipids up to 40% dry weight in HRAP indicating its potential for biodiesel production (Wee, 2008).

Using the current treatment system employed by the factory, the coloured wastewater is not suitable for final discharge. The HRAP system will be useful to remove at least 50% of the colour from TW before discharge. A further enhancement of the system is to integrate the HRAP with a series of cartridges containing immobilised *Chlorella* to remove the remaining colour. Immobilised *Chlorella* in alginate has been shown to remove up to 49% of the colour from TW (Chu et al., 2009). This creates a cell-dense system with increased surface area for maximal adsorption of dye or any other bioconversion process that may be attributed to the algae viz. breakdown of the azo bond or the ring structure.

### Table 5

Reduction of pollutants by *Chlorella vulgaris* grown in HRAP containing textile wastewater. The cultures were grown for 12 days.

<table>
<thead>
<tr>
<th>Pollution parameter</th>
<th>Pond IV-TW$^a$</th>
<th>Pond IV-TWM$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>270.33 ± 2.52</td>
<td>102.00 ± 2.65</td>
</tr>
<tr>
<td>NH$_4$–N (mg/L)</td>
<td>6.50 ± 0.10</td>
<td>3.57 ± 0.06</td>
</tr>
<tr>
<td>PO$_4$–P (mg/L)</td>
<td>7.14 ± 0.04</td>
<td>4.76 ± 0.02</td>
</tr>
</tbody>
</table>

$a$ IV-TW – Textile wastewater with 10% algal inoculum.

$b$ IV-TWM – Textile wastewater supplemented with nutrients of BBM, with 10% algal inoculum.

![Fig. 2. Chlorophyll a concentrations of *Chlorella vulgaris* and colour reduction of the textile wastewater in the high rate algae ponds (HRAP). Each point represents mean value from three replicate measurements with standard deviation.](image-url)
Fig. 3. Changes in (a) COD, (b) NH₄-N and PO₄-P contents of the high rate algae ponds (HRAP) treating textile wastewater with *Chlorella vulgaris*. Each point represents mean value from three replicate determinations with standard deviation. ● Pond IV-TW (textile wastewater); ■ Pond IV-TWM (textile wastewater supplemented with nutrients of Bold’s Basal Medium).
3.5. Adsorption equilibrium studies

Our previous study showed that the mechanism of colour removal by Chlorella is biosorption (Chu et al., 2009). To further understand the mechanism of biosorption by Chlorella, adsorption equilibrium studies were conducted using three major dyes found in TW, namely Supranol Red 3BW, Lanaset Red 2GA and Levafix Navy Blue EBNA. The amounts of dye adsorbed to the algal biomass (qₘₐₓ) increased with increasing dye concentration. The qₑₒₐ values increased from 2.54 to 35.62 mg/g biomass with the increase of initial concentration (C₀) of Supranol Red 3BW from 2.77 to 45.37 mg/L. For Lanaset Red 2BW, qₑₒₐ increased from 2.88 to 44.98 mg/g biomass with the increase of C₀ from 3.12 to 45.92 mg/L. In comparison, qₑₒₐ of Levafix Navy Blue EBNA increased from 4.25 to 43.17 mg/g biomass with increasing C₀ from 4.68 to 47.61 mg/L.

The high values of regression coefficients (R²) according to the Langmuir and Freundlich models confirmed that the mechanism of colour removal by Chlorella is biosorption (Table 6). A similar trend was reported for the adsorption of a basic dye by the green seaweed C. lentillifera (Marungrueng and Pavsant, 2006). In another study, the adsorption of the reactive dyes Remazol Red and Remazol Golden Yellow by dried biomass of C. vulgaris followed the Langmuir model (Aksu and Tezer, 2005). The Langmuir model suggests that the dyes were absorbed on monolayer coverage on homogenous sites of the algal cell wall. On the other hand, conformation to the Freundlich model suggests the existence of a heterogenous surface with sorption sites of different affinities. The possibility of the dyes being absorbed into the cell and transformed through bioconversion is worthwhile for further investigation.

Based on the Langmuir isotherm, the maximum adsorption capacity (qₘₐₓ) can be ranked as follows: Lanaset Red 2GA > Supranol Red 3BW > Levafix Navy Blue EBNA. Based on the Freundlich isotherm, the adsorption intensity (1/n) was relatively low (1.04–1.28), suggesting the dyes were favourably adsorbed by the algal biomass. Therefore, this suggests that the removal of dyes from TW by Chlorella is through biosorption.

4. Conclusion

Of 10 microalgae, C. vulgaris UMACC 001 was able to grow in TW and remove colour from the textile wastewater. Colour removal by Chlorella decreased with increasing concentration of TW in the medium. The HRAP system using Chlorella offers a good system for bioremediation of TW as it could remove up to 50% of the colour besides reducing pollutants such as COD, NH₄–N and PO₄–P. The adsorption equilibrium studies showed that colour removal by Chlorella followed the Langmuir and Freundlich models. The algal biomass generated may be useful as feedstock for biofuel production.

Acknowledgements

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References


Table 6

<table>
<thead>
<tr>
<th>Dye</th>
<th>Langmuir isotherm</th>
<th>Freundlich isotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>qₘₐₓ (mg/g)</td>
<td>b (L/mg/g)</td>
</tr>
<tr>
<td>Supranol Red 3BW</td>
<td>256.4</td>
<td>0.0049</td>
</tr>
<tr>
<td>Lanaset Red 2GA</td>
<td>345</td>
<td>0.0038</td>
</tr>
<tr>
<td>Levafix Navy Blue EBNA</td>
<td>188.7</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

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