Assessing the impact of dyes accumulation on the growth of Lemna minor L. using image processing technique

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Abstract—Textile effluents have adverse effect on the receiving water courses due to the presence of dyes. Pond systems planted with Lemna minor are recommended as a cheap and effective alternative for dye removal. The aim of this study is to assess the impact dye accumulation on the plant growth rate using image processing technique. The ponds experiment operated under controlled conditions by preparing simulated wastewater. The growth rate of L. minor monitored depending on the relative growth rate, coverage area and colour of the plants using Aletheia Lemna Edition software and Munsell colour chart. Results clearly indicate that the dye basic red 46 treated significantly comparing with other dyes. In addition, the presence of dyes negatively impacted on the plants. Furthermore, the software was able to quantify the growth parameters providing more understanding about the plant in long term treatment system.

Keywords—colour; coverage area; frond removal; textile dye; wastewater

I. INTRODUCTION

The release of textile wastewater to the environment is unacceptable due to the presence of dyes, which are either toxic themselves or often produce toxic breakdown intermediates. Ponds system successfully operated for the treatment of diverse effluents using L. minor [1]. L. minor is a tiny free floating macrophyte, able to acclimatize easily and grow rapidly in diverse wastewater. It is widely used for textile dyes removal [2] and as a toxicity indicator in wastewater [3] by evaluated the impact of untreated wastewater on the plant growth in the treatment system. The growth rate of L. minor required specific monitoring to prevent over-crowding, which inhibit the ideal growth of these plants [2]. The impact of different light sources and nutrient concentrations [4], plant biomass [5], wastewater toxicity [3] and the dye accumulation [6, 7] on the growth of L. minor have been studied depending on counting the fronds number manually or based on the weight of fresh biomass. Note that counting the number of fronds will be very tricky in cases of the large area covered by a high number of plants. Therefore, dealing with another technique such as image processing is necessary for more accurate and ease. Reference [8] have been developed a method for determined the growth rate of L. minor and the coverage area using digital image processing by UTHSCSA Image Tools (IT Version 2.0) software, which providing promising results about the growth although the dead plants and the colour were not applicable. Digital image processing also used for evaluating the impact of light intensity [9] and the nutrients [10] on the growth rate of L. minor. The aim of this study is to assess the impact of dye accumulation on the L. minor growth rate. The objectives are to evaluate the removal efficiency of four textile dyes (acid blue 113, reactive blue 198, basic red 46 and direct orange 46) using ponds system planted with L. minor, and compare the impact of these dyes on the growth rate of L. minor using image processing technique. The contribution of this study is providing better understanding about the impact of dyes on the growth rate, frond number, coverage area, live and dead plant as well as their colour in long term study.

II. MATERIALS AND METHODS

A. Dyes and Nutrients

Four commercial dyes were used in this study: Reactive Blue 198 (RB198), Basic Red 46 (BR46) and Direct Orange 46 (DO46) which were supplied by Dystar UK Limited, and Acid Blue 113 (AB113), which was obtained from the Sigma-Aldrich Company UK Limited. The chemical formula and the molecular weights (g/mol) for RB198, BR46, DO46 and AB113, C₁₈H₁₈Ca₂Na₁₂O₂₃S₂, C₁₈H₁₈Na₁₂N₄O₁₄S₂, C₁₈H₂₁N₄Na₂O₂S and C₁₂H₁₂N₃Na₂O₄S₂, and 1304.8, 321.4, 299.2 and 681.6, respectively. Dye stock solutions were prepared for each dye by dissolving 5 g of a dye in one litre of distilled water, which was stored in the dark at 4°C. The TNC Complete (aquatic plant nutrient supplied by TNC Limited) was applied weekly to simulate dye wastewater rich in nutrients and trace elements to support plant growth (1 ml per 10 l dechlorinated tap water). The content was as follows: nitrogen (1.5%), phosphorus

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(0.2%), potassium (5%), magnesium (0.8%), iron (0.08%), manganese (0.018%), copper (0.002%), zinc (0.01%), boron (0.01%), molybdenum (0.001%) and Ethylenediaminetetra-acetic acid.

B. Plant Collection

L. minor used in this study were collected from local ponds near to Cowpe Reservoir (Cowpe, Rossendale, UK). The plant was not impacted by textile wastewater in the past. L. minor transfer in plastic tanks containing the same pond water to the project location (The University of Salford, United Kingdom). The plants were washed carefully with dechlorinated tap water before use in the experiment.

C. Experiment Setup and conditions

The setup consists of Twenty plastic container (length, 33 cm; width, 25.5 cm; depth, 14 cm) as a simulated pond, four replicate for each dye and four without dyes (control). Around (2.600±0.0292 gram) of initial fresh plant biomass (equal to 200 colonies contained four fronds) was added to each container in 5 l of tap water mixed with nutrient. After acclimatization period, the dose of the dyes mixed with tap water and nutrient was added to the pond systems in concentrations of 5 mg/l. However, for the control ponds without dyes, only tap water with nutrient was added. The solution was topped-up weekly to the same level (equal to five litres) as required to compensate for water loss due to evaporation and transpiration.

The experiment operated under controlled condition at 27°C and 2215 lux, light intensity, using OSRAM HQL (MBF-U) High Pressure Mercury Lamp (400 W; Base E40) grow lights supplied by OSRAM and supported by a H4000 Gear Unit. The photoperiod controlled by a timer to simulate the daylight conditions in Salford, with the help of the website [11]. The laboratory conditions maintained for providing approximately the optimum condition for L. minor growth as reported by [12, 13].

D. Dye Removal Analysis

The water samples of 12-ml withdrawn weekly, which were filtered using a 0.45-μm diameter Whatman filter paper and then analysed with a UV-vis spectrophotometer (DR 2800 Hach Lange) at the maximum absorption wavelengths for each dye. The maximum absorption wavelength was 566, 625, 530 and 421 nm for AB113, RB43, BR46 and DO46, respectively. The concentrations of each dye were determined by standard curves, which were computed for each dye by plotting the linear correlation between known concentrations (0 to 20 mg/l) versus the absorbance at maximum absorption wavelength. The dye removals were calculated according to (1).

\[ R(\%) = \frac{(\text{Load influent} - \text{Load effluent}) \times 100}{\text{Load influent}} \tag{1} \]

where R is the dye removal efficiency.

E. Plant Growth Monitoring

The impact of dye accumulation on L. minor assessed by the monitor and harvest the plants to prevent overcrowding, which adversely affected their growth. The growth rate measured according to the fresh biomass weight, and image processing. The fresh weight was measured after putting the harvested plants on absorbent paper for 5 minutes. The fronds number and coverage areas were monitored and recorded using the Aletheia Lemma Edition software developed by the authors. The relative growth rate (RGR) according to the frond number and the fresh weight were calculated by (2) as a toxicity indicator [14]. The colour of the fronds was calculated with the help of the Munsell colour chart [15] using the Aletheia Lemma Edition software.

\[ \text{RGR (Per day)} = \ln (x \text{ at day } n - X \text{ at day } 0) / (\text{day } n - \text{ day } 0) \tag{2} \]

where X is the fronds number or the fresh weight, day 0 is the initial time, and day n is the final time.

Regarding the Aletheia Lemma Edition Software application, the digital images were taken using OLYMPUS VH 520, a compact digital camera with 14 megapixels on a full liquid crystal display screen. The flash was switched off and the picture was taken under natural light. For a good resolution, the camera was in the automatic option mode (denoted “auto”) and the distance between the camera and the water level was 60 cm. After taking pictures of the buckets containing L. minor from the top and of the relevant colours in the Munsell colour chart, the Aletheia Lemma Edition was applied using the steps below (see also Fig.1):

1. Pasting the relevant picture of the Munsell colour chart together with the picture of each bucket (pond) into Microsoft Paint.

2. Specifying the border of the water surface using a polygon tool.

3. Modifying the leaf section by setting three parameters: Hue (colour tone), saturation (greyness), and brightness/value (HSV colour model). Each point (pixel) of the image has a specific colour defined by those three values, which were set to 20 for each one. Then directly the software will calculate the total number of the leaves inside the border highlighted in step 2.

4. Clicking on the specific colour in the Munsell colour chart, and the Aletheia software will count the percentage of the coverage area in the pond, matching this colour and Repeating the last step until all leaves (except died/ white leaf) in the pond are highlighted by orange colour.

F. Analysis of Data

Microsoft Excel (www.microsoft.com) was used for all standard analysis of data unless stated otherwise. The IBM SPSS Statistics software (Version 23) was applied to determine the non-parametric Kruskal-Wallis tests.
III. RESULTS AND DISCUSSION

A. Dyes Removal

Overall, the dyes AB113, RB198 and DO46 showed very low mean removal efficiency comparing with the dye BR46 (see Fig. 2). The removal efficiencies ranged between 26% and 9%, 28% and 3%, 88% and 24%, and 24% and 9% for the dye AB113, RB198, BR46 and DO46, respectively. Statistical analysis showed that the dye BR46 was removed significantly (p < 0.05) higher than the other dyes and the removal ranked as follow: BR46 > RB198 > AB113 > DO46. The main reason for this high removal efficiency for the dye BR46 compared with other dyes was due to the simple chemical structure and small molecular weight which improve the biosorption process of the dye BR46. These outcomes resemble [16] findings.

B. Plants Monitoring

The fronds colour of L. minor which specified by [15] showed that the control ponds (without dye) achieved a high percentage of area (around 82.5%) covered by 7GY (mostly green colour) comparing with the ponds containing dyes, which ranged between 76.9% and 53.7% (Table I). However, small differences resulted regarding the area covered by 2.5GY (mostly yellow colour), which was 1% for the control ponds, and between 6.4% and 3.3% for the ponds containing dyes, total surface area of the pond is 841.5 cm². This may indicate that the impact of the dyes on the chlorophyll content in the plant was not significant as mentioned by [7].

Table II provided an overview of the mean total, life and dead of L. minor as a coverage area. The results clearly show that the total coverage area relevant the ponds without dyes was higher than the ponds contaminated with dyes which indicated that the presence of the dye in the system affected adversely in the coverage area, and the values ranked in the ponds as follow: control > AB113 > RB198 > BR46 > DO46. However, statistical analysis showed no significant difference (P > 0.05) founded among all the ponds.

RGR values in the ponds ranked as follow: Control > RB198 > AB113 > DO46 > BR46. These outcomes indicate that the presence of the dyes negatively impacts on the growth of L. minor, especially the dye BR46 due to the successful removal of this dye (Fig. 3), which may consequently exhaust the ability of plant for growth. Statistically, significant impact (p < 0.05) founded between the control ponds and only the ponds comprising BR46 and DO46. Resemble data regarding the adverse impact of the dye on the growth rate of L. minor observed previously by [7].

Literature documented that the growth rate according to the fresh weight, dry weight, and the frond area are more reliable than the growth rate based on counting the fronds number. This because the number of fronds will consider regardless of the biomass and the size of these fronds [14]. However, in this study, the growth values showed the same trend of sensitivity towards the dyes, although the values were not similar as mentioned above.

<table>
<thead>
<tr>
<th>Type of Dye</th>
<th>2.5 GY</th>
<th>5 GY</th>
<th>7 GY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid blue 113</td>
<td>3.6</td>
<td>6.2</td>
<td>73.1</td>
</tr>
<tr>
<td>Reactive blue 198</td>
<td>3.3</td>
<td>5.3</td>
<td>76.9</td>
</tr>
<tr>
<td>Basic red 46</td>
<td>4.7</td>
<td>12.5</td>
<td>60.1</td>
</tr>
<tr>
<td>Direct orange 46</td>
<td>6.4</td>
<td>15.4</td>
<td>53.7</td>
</tr>
<tr>
<td>Control (without dye)</td>
<td>1.0</td>
<td>5.4</td>
<td>82.5</td>
</tr>
</tbody>
</table>

*Hue (green-yellow)
TABLE II. OVERVIEW OF TOTAL, LIFE AND DEAD COVERAGE AREAS (%) FOR LEMNA MINOR L. FRONDS DURING THE STUDY PERIOD

<table>
<thead>
<tr>
<th>Type of dye</th>
<th>Total</th>
<th>Life</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid blue 113</td>
<td>89.9±4.34</td>
<td>74.7±5.72</td>
<td>7.05±4.51</td>
</tr>
<tr>
<td>Reactive blue 198</td>
<td>88.5±6.57</td>
<td>85.5±7.82</td>
<td>2.98±1.52</td>
</tr>
<tr>
<td>Basic red 46</td>
<td>82.8±8.46</td>
<td>77.3±10.79</td>
<td>5.23±4.36</td>
</tr>
<tr>
<td>Direct orange 46</td>
<td>80.3±5.91</td>
<td>75.5±8.61</td>
<td>4.81±3.89</td>
</tr>
<tr>
<td>Control (without dye)</td>
<td>91.3±4.23</td>
<td>88.9±5.63</td>
<td>2.40±1.69</td>
</tr>
</tbody>
</table>

Fig. 3. Overall mean values of relative growth rate based the fresh weight and the fronds number (mean of four replicate)

This may because the software counting the fronds number based on a predefined pond and leaf size in the setting tool. These results indicated that the Aletheia Lemna Edition software provided promising results regarding the growth rate, coverage area and the colour of the plant in toxic media.

IV. CONCLUSIONS

The ponds system planted with \textit{L. minor} was effective for treatment the dye BR46 significantly. Dye accumulation had inhibitor impact on the plant growth rate, especially with the dye BR46 which treated higher than other tested dyes. In addition, the presence of the dye contaminations affected the coverage area of the dead, life and the colour of the plants. However, the impact on the chlorophyll Content in plant tissue was very low. In conclusion, the Aletheia Lemna Edition software successfully quantified the growth parameters of \textit{L. minor} and provided a better understanding of the impact of untreated effluents containing textile dyes.

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