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Plant-assisted remediation of wastewater contaminated with methyl orange using *Scirpus grossus*

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Abstract

Aim: This study explores the possibility of treating basic dye, methyl orange using *Scirpus grossus* in a batch free flow surface system.

Methodology: The effect of operational parameters (pH, ORP, ABS, BOD, COD and TOC) for different dye concentrations (0, 200, 400, 600, 800 and 1000 mg l⁻¹) were observed and determined during 42 days. The results of UV-Vis absorption, HPLC and GC-MS confirmed the degradation of methyl orange.

Results: The removal efficiency of methyl orange dye from synthetic wastewater ranged between 15–64% for all treatments at different r concentrations between 1000–200 mg l⁻¹, respectively. Moreover, the highest removal for BOD and COD in 400 mg l⁻¹ and TOC in 200 mg l⁻¹ methyl orange were 55, 52 and 42%, respectively. Four metabolites after the degradation of dye were identified as 4-tert-butylcyclohexanone, 1, 4-dimethylbenzen, N, N-dimethyl hydrazine, 1, 4, 7, 10, 13-pentaoxacyclopentadecane using GC/MS.

Interpretation: *Scirpus grossus* is capable to phytoremediate dyes in wastewater.

Keywords: Decolorization; Methyl orange; Phytoremediation; *Scirpus grossus*, Textile industry

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Introduction

The increase in textile industries has led to accumulation of large amount of xenobiotic compounds that contaminate the environment and natural resources. In textile, leather, plastic, cosmetic, and food industries different azo dyes are used. Azo dyes represent the most common group of synthetic dyes constituting 60–70% of more than 10,000 dyes used in textile industry. The effluents of textile industries are characterized by high content of chemical oxygen demand, total organic carbon, biological oxygen demand, total suspended solids and high pH (acidic or alkaline). Distortion of water quality in the natural ecosystem has urged for proper treatment of industrial wastewater for environmental protection. Various techniques like adsorption, coagulation, ozonation and electrolysis and advanced oxidation processes such as TiO_2 -mediated photocatalytic oxidation and activated carbon are being used for purification and decolourization of dye wastewater. However, all these chemical treatment technologies are costly and always end-up with high maintenance cost.

Phytoremediation or plant-assisted remediation is a green technology that use plants for remediation of contaminated sites. Plants contain enzymatic mechanism to break down and degrade complex structures and can be used for cleaning the contaminated sites. It is an environmentally sound and sustainable technology for cleaning contaminated sites. Khandare and Govindwar (2015) extensively reviewed the possibility of applying phytoremediation in treating effluents from textile industries. This plant-assisted remediation technology is also applied to remediate wastewater containing heavy metals (Barman and Datta 2018; Selamat et al., 2018; Ismail et al., 2017) and organic pollutants (Al-Baldawi et al., 2017; Almansoori et al., 2017; Sanusi et al., 2017; Al-Sbani et al., 2016). Integration of phytoremediation and mycoremediation was also investigated by Mandal and Das (2018) to treat benzo[a]pyrene in soil. A previous study by Rane and Chandanshive (2014) has reported potential of *Rheum rabarbarum* and *Blumea malcomi* in remediation of textile dyes. Kalgakar et al. (2010) successfully utilized *Typhonium flagelliforme* to degrade Brilliant Blue R., and Khandare et al. (2011) has conducted a study to degrade Navy Blue HE2R using *Portulaca grandiflora* Hook. While Al-Badawi et al. (2018) used *Azolla pinnata* to treat wastewater containing methylene blue. In this study, native plant of *Scirpus grossus* was used. *S. grossus* is indigenous to Malaysia and is locally known as *Rumput menderong*. *S. grossus* belongs to Cyperaceous family and is commonly known as giant bulrush. *S. grossus* is an aquatic plant with high growth rate and is often found growing in large colonies in water. Bulrushes are often seen as tall with leafless stems growing in profusion. The plants have sharp-to-soft stems, triangular leaves, and obvious leaf blades; the inflorescences always appear on stem tips in a tight cluster or spreading open with leaves resembling stems (UF/IFAS, 2007).

Plants possess enzymatic and uptake processes that can degrade organic pollutants or contain and stabilize contaminants by acting as filters or traps. The effective microorganisms are most important factors for biological treatment processes. For long time, researches mainly focused on bacteria and fungi. The emerging products such as some aromatic amines can be degraded by hydroxylase and oxygenase produced by bacteria. This study aims to explore the role of a native Malaysian plant, *Scirpus grossus* in a free flow system (FSF) operated batch wise to remove textile dyes containing methyl orange dye at different concentrations in synthetic wastewater with a prudence into the involvement of bacteria and confirmation of metabolic pathway behind degradation of dye. *S. grossus* is an aquatic species with a high growth rate and has the ability to degrade hydrocarbons, heavy metals, recycled paper mill and methylene blue.

Materials and Methods

The plants of *Scirpus grossus* were collected from Tasik Chini, Pahang, Malaysia. They were propagated by cutting the stems and leaving the roots inside tank with garden soil for one month before they were ready for use. This study was conducted in a greenhouse at Universiti Kebangsaan Malaysia and the phytotoxicity experiment utilized a batch free flow system.

Tank preparation: In this project, 21 aquariums made of glass, 15 of them were filled with wastewater contaminated with methyl orange dye and plants with three replicates (R1, R2, R3), together with an aquarium for the control contaminant without plants (CC), as well as three aquariums without methyl orange contaminant as plant controls (PC), as illustrated in Fig. 1(A). Twelve healthy plants of *S. grossus* were planted into each aquarium containing 13 l of wastewater filled by mixing methyl orange with water at different concentration (200, 400, 600, 800 and 1000 mg l^{-1}). Each aquarium with dimensions of L 30 cm $\times$ W 30 cm $\times$ D 30 cm was filled from the upper layer to the bottom layer with: (1) 10 cm depth of fine sand with $\Phi=2$ mm (2) 3 cm of gravel with a size of $\Phi=1-5$ mm and (3) 8 cm of gravel with a size of $\Phi=10-20$ mm (Fig. 1(B)).

The operational parameters were monitored during 42 days of exposure whereas samplings were carried out on day 0, 7, 28, and 42 day. pH, temperature, dissolved oxygen and oxidation reduction potential were recorded to observe the physico-chemical changes in water using a Metrohm multi-probe (Model 877, Swiss) for pH, ORP and temperature measurements. For dissolved oxygen, sensor (YSI, Model 550A, USA) was used.

Monitoring of water quality parameters: The BOD, COD and TOC of methyl orange contaminated water was measured. The spectrophotometer (DR3900 Hach, Germany) was used in this study to determine the COD and TOC values. The BOD value was determined by measuring dissolved oxygen levels for the sample before and after incubation for 5 days Winkler's iodometric method (Winkler, 1888).

Fig. 1 : (A) Experimental set-up for phytotoxicity experiment with plant control (PC), contaminant control (CC) without plants and treatment with plants (R1, R2, R3); (B) A schematic diagram of an aquarium for removal of methyl orange.

Population of bacteria associated with plant roots: It was obtained from the roots and sand attached to the rhizosphere zone. A number of bacteria live in the roots of plants irrigated with dye contaminated water. The population of these bacteria was estimated through serial dilution method. At first, 10 g of plant roots were harvested and then put into 100 ml of sterile distilled water to obtain a dilution of 10^{-2} . This was shaken at 150 rpm for 1

hr to release adhered microorganisms. After transferring 1–9 ml of sterile saline water, subsequent dilutions up to 10^{-4} were made. Next, 0.1 ml of three dilution (10^{-2} , 10^{-3} and 10^{-4}) was spread onto sterile plates containing nutrient agar medium (tryptic soya agar, TSA). The plates were incubated inverted at 37 °C for 24 hr. Colonies of more than 20 and less than 300 cells were counted. The number of colonies counted was multiplied by the reciprocal

of dilution and the amount plated, and the results were expressed as colony forming units (CFU ml⁻¹) (Sieuwerts *et al.*, 2008).

Analytical procedures for detection of biotransformation products: Decolorization of all dyes on each sampling day was monitored using UV-Vis spectroscopic analysis (DR 3900 HACH) at 465 nm. Decolourization percentage for methyl orange dye was determined by comparing the initial absorbance with the observed absorbance on each sampling day. In addition, phyto transformation was monitored using HPLC (WATERS 2695, Detector at 254 nm and column C 18, symmetry, 4.6mm× 250 mm by using gradient of methanol and acetonitrile (75:25) at a flow rate of 1ml min⁻¹ for 10 min. Identification of metabolites produced was carried out using GC/MS. GC/MS 7890A gas chromatograph (GC) was directly coupled to the mass spectrometer system of an

Agilent 5975Cinert MSD with triple-axis detector. Chromatographic separation was achieved on a 30 m DB-5 ms for non-polar. The column temperature program was 50 °C, held for 0.5 min, 10 °C min⁻¹ to 200 °C, 5 °C min⁻¹ to 235 °C, 10 °C min⁻¹ to 280 °C and held for 10 min. The injector temperature was maintained at 250 °C. Injection was performed in the split less mode using a split less time of 0.7 min with constant column flow of 1.0 ml min⁻¹. The capillary column was coupled directly to the ion source which was operated in electron impact ionization mode. The transfer line temperature was 300 °C.

Results and Discussion

The maximum decolorization (64%) was obtained at 200 mg l⁻¹ and the minimum decolorization (15 %) was achieved at

Fig. 2 : Effect of increasing methyl orange concentrations on decolourization with *S. grossus* and without *S. grossus*.

Fig. 3 : pH reading of (A) with plants and (B) without plants for 42 days.

Fig. 4 : Percentage removal of (A) BOD, (B) COD and (C) TOC at different concentration of methyl orange.

Fig. 5 : Total count of bacterial population during 42 days of experiment. Comparison of the microbial populations in the control (PC) and with different MO concentrations.

1000 mg l⁻¹ throughout 42 day exposure. The methyl orange removal within the exposure days were 42, 36 and 22% at 400, 600 and 800 mg l⁻¹, as illustrated in Fig. 2. The increasing methyl orange concentration decreased methyl orange decolorization throughout 42 days. On the other hand, for comparison with control aquaria without plants, decolorization of methyl orange was only 9% (200 mg l⁻¹), 13% (400 mg l⁻¹), 13% (600 mg l⁻¹), 18% (800 mg l⁻¹) and 9% (1000 mg l⁻¹), respectively. This comparison with contaminant control confirms the ability of *S. grossus* to decolorize methyl orange dye. In another study, K.

rosea was used to decolorized methyl orange dye. The time required for decolorization of methyl orange dye at 10, 30, 50, 70 and 100 mg l⁻¹ were 100% for 48 hr, 100% for 60 hr, 100% for 72 hr, 73% for 120 hr and 40% for 120 hr respectively (Chang and Kuo, 2000). Similarly Tan *et al.* (2016) reported 66.8% of colour remove methyl orange using *Eichhornia crassipes*.

The pH can affect the efficiency of colour removal. Fig. 3(A, B) shows the pH readings of methyl orange for 42 days. The pH value of methyl orange with and without plant ranged during pH 8.5-10 experiment. The fluctuation of pH is due to free diffusion of carbon dioxide in the atmosphere and absorption of nutrient by the plants (Lin and Mendelssohn, 2009).

The conditions of phytotoxicity test can be distinguished whether it is aerobic or anaerobic through DO and ORP measurement. A negative ORP indicates that a substance is in reducing state, lower the reading, more anti-oxidizing it is (Telke *et al.*, 2011). The ORP reading ranged from -07 to -53 mV in this study. Methyl orange affected the environment of treatments around the rhizosphere and reduced the ORP readings, indicating that the environment was becoming more anaerobic with increasing methyl orange concentration.

The BOD, COD and TOC values of methyl orange dye at different concentrations decreased in the test samples treated

Fig. 6 (A) : Untreated methyl orange at 200 mg l⁻¹.

Fig. 6 (B) : Treated methyl orange at 200 mg l⁻¹.

Fig. 6 (C) : Untreated methyl orange at 1000 mg l⁻¹.

Fig. 6 (D) : Treated methyl orange at 1000 mg l⁻¹.

Table 1 : GC–MS data for metabolites obtained after decolourization of methyl orange

Degradation intermediates	t _r (min)	M _w
1,4-dimethylbenzen	5.320	106.17
N,N-dimethylhydrazine	13.123	60.10
4-tert-butylcyclohexanone	17.703	154.52
1,4,7,10,13-pentaoxacyclopentadecane	44.755	220.26

with *S.grossus* plants for 42 day (Fig. 4). The highest removal of BOD by plant was 55% for 400 mg l⁻¹ dye and the lowest removal was 18% for 200 mg l⁻¹, while BOD removals were 40, 38 and 40% for 600, 800 and 1000 mg l⁻¹ respectively. However, the BOD efficiency removal without plant were 12, 44, 30, 13 and 12 % for 200, 400, 600, 800 and 1000 mg l⁻¹. Similarly, the highest COD removal with plant was 52% for 400 mg l⁻¹, while the lowest removal efficiency was 28% for 1000 mg l⁻¹, while the COD removals were 35, 39 and 29 mg l⁻¹ for 200, 600 and 800 mg l⁻¹. The COD removal in the aquaria without plants was 3, 18, 33, 11 and 7 % for 200, 400, 600, 800 and 1000. The highest removal for TOC was 42% for 200 mg l⁻¹ while the lowest was 21% for 800 mg l⁻¹ and 33, 23 and 24% for 400, 600 and 1000 mg l⁻¹, however, without plant the removal efficiency was only 12, 17, 9, 13 and 10% for the same concentrations as depicted in Fig. 4. BOD, COD and TOC are widely studied parameters for determining organic matter in wastewater. The results of BOD, COD and TOC experiments indicated that the plant of *S. grossus* had significant impact on decolourisation of dye compared to the control aquariums without plants.

Fig. 5 illustrates the number of microbial populations in the rhizosphere zone of *S. grossus* at different concentrations of dye. The removal of dye depends on the abilities of microorganism in the surrounding rhizosphere (Zee et al., 2005).

Generally, decolourization of azo dyes occurs under conventional anaerobic, facultative anaerobic and aerobic conditions by different groups of bacteria. The microbial degradation strategies of azo dyes involves the reductive cleavage of azo bonds (–N=N–) with the help of azoreductase enzymes under anaerobic conditions that led to the formation of colourless solutions containing potentially hazardous-aromatic amines (Jadhav et al. 2008; Radha and Asha 2016). Several studies were conducted to isolate microorganisms including rhizobacteria (Singha et al. 2017) and fungi (Singh et al., 2016) from the rhizosphere of plants, which can potentially promote the plant growth as well as degrade or detoxify pollutants inside wastewater or contaminated soil.

During experiment, microbial populations in the aquarium of dye concentration at 1000 mg l⁻¹ were significantly lower than those in other aquaria. Sani et al. (1999); Saratale et al. (2009) suggested that increasing the dye concentration gradually decrease the decolourization rate, probably due to the toxic effect of dyes with regard to the individual bacteria. During exposure, microbial population at the control soil was 4.3×10⁵ CFU ml⁻¹ on day 0 and then reduced to 3×10⁵ CFU ml⁻¹ during 42 day of treatment. The bacterial population in the treatment with the lowest dye concentration of 200 mg l⁻¹ amounted from 4.3×10⁵ to 4.1×10⁵ CFU ml⁻¹ during 42 days of treatment, which was higher

than other dye concentrations. Generally, during the first 14 days, there was increment in the bacterial population at 400, 600, 800 and 1000 mg l⁻¹ dye concentration and then started to decrease until the end of 42 day period of exposure, except at 200 mg l⁻¹ the bacteria population maintained until the end of treatment.

The HPLC analysis of untreated methyl orange at 200 mg l⁻¹ (Fig. 6A) showed peaks at 1.45, 1.60, 1.62 and 3.13 min while treated methyl orange (Fig. 6B) showed peaks at 1.53, 1.60, 1.72, 1.98, and 2.21 min, respectively. The peak obtained at the retention time of 1.60 min which was obtained in the HPLC analysis of both control sample, and the products formed after degradation indicated that it could represent some metabolites produced by the plant (Baiocchi et al., 2002).

Similarly, the HPLC analysis of untreated methyl orange at 1000 mg l⁻¹ (Fig. 6C) showed peaks at 1.48, 1.66 and 2.187 min while treated methyl orange (Fig. 6D) showed peaks at 1.48, 1.66, 1.725, 1.983 and 2.26 min of which peaks at 1.48 and 1.66 min were corresponding to the root exudates of *S. grossus*. The methyl orange degradation was confirmed by the disappearance of peaks before degradation and the formation of a new metabolite peaks with different Rt in the treated sample. The degradation products were further analyzed using GC/MS. Their GC retention times (t_R), molecular weights (M_w) and chemical structure are listed in Table 1. The detected intermediates were 1,4-dimethylbenzene, N, N-dimethylhydrazine, 4-tert-butylcyclohexanone and 1,4,7,10,13-Pentaoxacyclopentadecane.

This study shows the capability of *S. grossus* to decolorize methyl orange dye in different wastewater concentrations after 42 day in a batch free flow system. The highest dye removal of 64% was obtained at methyl orange dye concentrations of 200 mg l⁻¹ giving evidence that *S. grossus* has the ability to degrade dye efficiently at lower dye concentrations.

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