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Periphyton biofilms: A novel and natural biological system for the effective removal of sulphonated azo dye methyl orange by synergistic mechanism

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HIGHLIGHTS

- Three types of periphyton were used to treat an azo dye, methyl orange (MO).
- Dye removal was elucidated in relation to adsorption and biodegradation.
- MO adsorption followed pseudo-second order and Intraparticle diffusion models.
- MO was degraded into simpler non-toxic aliphatic hydrocarbons.
- Periphyton is a promising methodology to adsorb and degrade azo dyes.

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ABSTRACT

Due to their large scale use, azo dyes are adversely affecting aquatic fauna and flora as well as humans. The persistent nature of sulphonated azo dyes makes them potential ecotoxic hazards. The aim of the present study was to employ a proficient, locally available biomaterial, viz. periphyton (i.e. epiphyton, epilithon or metaphyton), for removal of the azo dye, methyl orange (MO). Results showed that the periphytic biofilms are capable of completely removing comparatively high concentrations (up to 500 mg L^{-1}) of MO from wastewater. The removal of MO occurs by a synergistic mechanism involving bioadsorption and biodegradation processes. The adsorption of MO by periphyton can be described by pseudo-second order kinetics. Elovich and intraparticle diffusion models as well as Langmuir equations fit well to the MO adsorption process. FTIR analysis of MO and its metabolites demonstrated biotransformation into simpler compounds within 72 h. GC-MS/MS analysis showed the conversion of MO into simpler compounds such as phenol, ethyl acetate and acetyl acetate. The results indicated that periphyton is a promising biomaterial for the complete removal of MO from wastewater and that the treatment process has the potential for *in situ* removal of MO at contaminated sites.

1. Introduction

Industrial effluents, such as dyes from industrial processing, pose serious threats to aquatic fauna and flora as well as humans (Gong et al., 2007). Amongst dyes, the azo dyes containing one or more nitrogen-nitrogen double bonds (–N=N–), are particularly recalcitrant (Naresh Kumar et al., 2015) and form more than 70% of industrial dyes (~9 million tons) (Rawat et al., 2016). Effluents from textile industries contribute to higher chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in water bodies (Wang et al., 2011). Furthermore, metabolites produced by degradation processes are often carcinogenic and mutagenic in nature (García-Montaño et al., 2008; Hameed et al., 2008). They affect not only the aesthetic value of water, but also negatively impact aquatic biota by reducing light penetration for photosynthetic bacteria and plants (Guo et al., 2014). Hence, due to their toxicity and visibility,

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Nomenclature

Abbreviation	*full term*
MO	methyl orange
C_0	initial MO concentration (mg L^{-1})
C_e	MO concentration at equilibrium (mg L^{-1})
C_t	MO concentration at time t (mg L^{-1})
C	Intraparticle diffusion model constant
k_1	Pseudo-first-order kinetic model rate constant (min^{-1})
k_2	Pseudo-second-order kinetic model rate constant (g mg^{-1} min^{-1})
K_L	Langmuir adsorption constant (L mg^{-1})
K_F	Freundlich adsorption constant (mg g^{-1})
k_{id}	Intraparticle rate constant (mg g^{-1} min 0.5)
m	Mass of adsorbent (g)
n	Freundlich constant
q_e	The amount of dye adsorbed onto the adsorbents at equilibrium (mg g^{-1})
q_t	The amount of dye adsorbed onto the adsorbents at time t (mg g^{-1})
q_m	The maximum adsorption capacity for adsorbent (mg g^{-1})
R^2	Correlation coefficient
t	Time (hr)
V	The volume of solutions (L)
R_L	Adsorption intensity

even small amounts of dye in industrial effluents present a potential risk and have attracted the attention of scientists for the last few decades.

Different technologies are used for the treatment of dyes including chemical oxidation, flocculation, ozonation, photolysis, ion exchange, irradiation, precipitation, electrochemical treatment and adsorption (Wang et al., 2011). Unfortunately, these conventional physico-chemical techniques are not efficacious in eliminating dye wastes from effluents and have certain techno-economical limitations including: excessive consumption of chemicals; production of concentrated sludge; higher operational costs; lower efficiency in dye removal; generation of a wide variety of toxic metabolites; dye specificity and secondary processing that is sensitive to variable dye concentrations (Jadhav et al., 2011). Government authorities in developed countries are concerned about the escalation of dye concentrations in industrial effluents and have encouraged the innovation of novel technologies that are not only feasible and cost-competitive but also ecofriendly (Kong et al., 2015).

Microbiological measures of dye removal are a cost-effective and ecofriendly technology compared to the different physico-chemical approaches. They primarily involve dye metabolism in the presence of different oxidative and reductive enzymes (Wu et al., 2012, 2014) and reduced electron carriers (Feng et al., 2012). Theoretically, microbial decolorization consists of three phases: biosorption, bioaccumulation and biodegradation (Wu et al., 2012). The majority of current studies focus on the investigation of single process, i.e. adsorption (Feng et al., 2012; Arunarani et al., 2013), or degradation by microorganisms such as fungi (Patel and Suresh, 2008; El-Rahim et al., 2009), algae (Daneshvar et al., 2007; Khataee et al., 2011) and bacteria (Phugare et al., 2011; Shah, 2013). Most of these microbial entities are dye specific and cannot be easily up-scaled for industrial scale wastewater treatment (Dafale et al., 2008a). Furthermore, microbial techniques often produce toxic metabolites that are more toxic than the parent dye (van der Zee and Villaverde, 2005). There is subsequently a need to develop innovative and efficient technologies that can be easily used in natural conditions to remove azo dyes and degrade them to less harmful products.

Periphyton are multifaceted assemblages including cyanobacteria, algae, protozoa and organic debris, dominated by phototrophic microorganisms. Adhesion within this multilayered structure is facilitated by extracellular polymeric substance (EPS), secreted by the microbial periphyton community (Larned, 2010). The ubiquitous and native periphyton attaches to substrates in aquatic ecosystems, with short acclimation and adaptation times when employed in novel scenarios (Battin et al., 2003). Previous studies have shown high efficacy of these biofilms in the treatment of organic and inorganic phosphorus (Lu et al., 2014, 2014), microcystin-RR (Wu et al., 2010; Li et al., 2012), hormones (Writer et al., 2011), and toxic metals (Dong et al., 2003). Using microbial aggregates such as periphyton instead of a pure culture is beneficial for adsorption and the subsequent degradation of dyes, and can be up-scaled more easily (Wu et al., 2012). Furthermore, a combination of aerobic and anaerobic treatment within microbial aggregates might be more useful to simultaneously improve water quality by dye degradation and decreasing COD (Dafale et al., 2008b).

To our knowledge, the role the periphyton in the removal of dye by adsorption and degradation has never been investigated. The main objectives of this investigation were (i) to study the feasibility of different types of periphyton (epiphyton, metaphyton and epilithon) for the removal of MO, (ii) to evaluate the experimental data using different kinetic models to determine the fate of MO in the presence of periphyton and (iii) to determine whether the mechanism of decolorization is due to adsorption and/or biodegradation. The findings of this study will (i) enable us to evaluate a novel ecofriendly technology for the removal of MO from wastewater, and (ii) assist in understanding the bioadsorption process and biodegradation pathway of dye removal by periphyton or similar microbial aggregates.

2. Material and methods

2.1. Dyestuff and chemicals

Analytical grade methyl orange ($C_{14}H_{14}N_3NaO_3S$) and other chemicals were purchased from Sinopharm Chemical Reagents, Shanghai. All the chemicals used in the study were analytical grade.

2.2. Periphyton culture and characterization

The source of periphyton for this study was Xuanwu Lake, Nanjing, East China. Three different types of periphyton were collected *in situ* from plants (epiphyton), rocks (epilithon) and surface water (metaphyton). The harvested periphyton was placed in three indoor glass tanks (length × width × height = 100 cm × 100 cm × 60 cm) and allowed to grow on specific substrates - Industrial Soft Carriers (length × width × height = 9 cm × 1 cm × 1 cm) (Jineng Environmental Protection Company of Yixing, China). The tanks were first thoroughly washed with 95% alcohol and rinsed with deionized water. The periphyton was added into each tank with substrate containing modified WC media (composition in Supplementary material). The periphyton were grown in a greenhouse to minimize temperature fluctuations (25–30 °C) until the substrate surface appeared to be evenly covered with biofilm (60 days).