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Decolorization of high concentration crystal violet by periphyton bioreactors and potential of effluent reuse for agricultural purposes

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ABSTRACT

Current wastewater treatment biomeasures are dominated by single microbial species, which can only decolorize low concentrations (≤150 mg L^{-1}) of crystal violet (CV). Considering the advantages of microbial aggregates, with micro-porous structure and complete hierarchical trophic communities, several innovative bioreactors based on periphyton (i.e. epiphyton, metaphyton and epilithon) were examined. The results showed that periphyton could tolerate high concentration of CV (1000 mg L^{-1}) and the immobilized periphyton bioreactors could completely decolorize up to 1000 mg L^{-1} of CV within 168 h showing 50–100% removal by bioreactors. The removal of CV was a synergistic process accomplished by adsorption (with insignificant desorption) followed by the dominant mechanism of biodegradation. The CV was converted into non-toxic aliphatic compounds in the presence of periphyton. Phytotoxicity and microbial toxicity tests showed that biodegradation of CV did not produce any toxic secondary metabolite, leading to environmentally benign effluents meeting the standards of agricultural irrigation. These results provide an insight into the processes involved in removing CV by periphyton or similar microbial aggregates, and indicate the potential of periphyton as a green material to purify water contaminated by high concentrations of CV.

1. Introduction

On the basis of production rate of industrial dyes, triphenylmethane dyes are ranked third, after azo and anthraquinone dyes (Liu et al., 2015). Hexamethyl pararosaniline chloride (molecular formula $C_{25}H_{30}N_3Cl$ and molecular weight 407.98), better known as crystal violet (CV), is one of the most extensively used cationic triphenylmethane dyes and is widely employed in biological (He et al., 2010; Kumar and Ahmad, 2011) and industrial processes (Ayed et al., 2009). CV is classified as a "rebellious" molecule which has been demonstrated to be mitotically, genetically and physiologically toxic for a range of exposed organisms (Senthilkumaar et al., 2006).

In the past few decades, many approaches, including precipitation (Pavan et al., 2014), photodegradation (Gupta et al., 2011), adsorption (Mittal et al., 2010), advanced oxidation processes (Parshetti et al., 2011), ozonation (Hu et al., 2016), flocculation and ion-pair extraction (Zhang et al., 2016) have been used for the remediation of CV contaminated wastewater. These systems vary in removal efficacy, operational competency and capacity of treatment. In addition, these methods are also costly, only applicable to smaller volume of effluents, and results in the production of by-products with potential to trigger carcinogenic side effects (Parshetti et al., 2011; Yang et al., 2011). Although, adsorption is the most commonly used method amongst all, however, it is also not very efficient for the removal of higher concentrations of dyes (Table 1).

Biological treatment methods have gained attention due to their economic feasibility and environmental sustainability (Hu et al., 2016; Zhang et al., 2016). Microbial remediation systems have high capacity for adaptation and are capable of decolorizing and biotransforming dyes by reduction reactions (Ayed et al., 2010; Shabbir et al., 2017b). Due to recalcitrant nature of CV, however, the microbial systems have some drawbacks including variable substrate transfer (removal rates), operational instability, and lack of microbial survival and adaptability (Ha et al., 2009). Furthermore, microorganisms are unable to remove CV completely due to the presence of dimethyl amino groups (Jang et al., 2005),

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prolonged degradation time (Chen et al., 2007) and intolerance to high CV concentrations (El-Naggar et al., 2004; Gan et al., 2014).

To date, most biological systems have been designed to deal with lower concentrations of CV ($\leq$150 mg L^{-1}) using single microbial species (bacteria, yeast and microalgae) or combinations of aerobic and anaerobic conditions (Angelova et al., 2016; Casas et al., 2009) which are incapable of complete CV removal over short time spans. Furthermore, the growth of most microbial species is inhibited by higher concentrations of dye (Gan et al., 2014) and the dye is ultimately converted to toxic metabolites including anilines (Liu et al., 2014). The practical implementation of a single microbial species or a community requires initial laboratory-scale screening (Shahid et al., 2013). The inoculum must have the capability to exist in a stable form and to compete with the indigenous flora in contaminated wastewater environments (Khalid et al., 2010). Therefore, there is a need to develop a new eco-friendly approach that can be employed on a large scale, be highly efficient, and can degrade CV to harmless products.

Recently, ubiquitous periphyton biofilms have been utilized to remove a number of organic and inorganic pollutants from environmental and/or waste waters (Wu et al., 2010a, 2014, 2010b). Periphyton is a type of naturally growing biofilm, attached to submerged substrates in water with sections moving freely between substrates (Azim et al., 2002). These biofilms are entangled biomasses of heterotrophic (bacteria and protozoa) and autotrophic microorganisms, primarily dominated by phototrophic microorganisms, including algae and cyanobacteria, along with detritus (Larned, 2010). Their multilayered nature is supported by extracellular polymeric substances (EPS), produced by the intact microbial community (Larned, 2010). Periphyton biofilms have been widely used for the bioremediation of various contaminants, including heavy metals (Duong et al., 2010), nutrients (Wu et al., 2010a) and organic compounds (Wu et al., 2014). Furthermore, the unique characteristic of periphyton biofilms to remove the contaminant by the dual process of adsorption and biodegradation makes them an ideal biomaterial for the removal of otherwise persistent toxic organic chemicals including dyes (Wu et al., 2010b). To the best of our knowledge, the role of periphyton biofilms in the adsorption and/or biodegradation of triphenylmethane dyes has not been evaluated.

In this study, simple innovative laboratory-scale suspended bioreactors were designed by individually immobilizing three different types of periphyton biofilms to detoxify CV in contaminated water. The technique of using microbial cells, immobilized on different substrates, is potentially more effective due to their higher levels of activity and resistance to the environmental perturbations (Cheng et al., 2012). Compared to other microbial systems, immobilized periphyton biofilms are advantageous due to their innate capacity to autoregulate under varied conditions, their ability to degrade numerous toxins, and the potential for up-scaling to industrial (commercial) scales.

2. Materials and methods

2.1. Dyestuff and chemicals

Analytical grade crystal violet (98%) and other chemicals were purchased from Sinopharm Chemical Reagents, Shanghai. A stock solution (1000 mg L^{-1}) of CV was prepared by dissolving the requisite quantity of CV in deionized water. The stock solution was filter sterilized and stored at 4 °C. This stock solution was then diluted to make a range of concentrations as required.

2.2. Immobilized periphyton bioreactors

2.2.1. Periphyton collection and periphyton bioreactor design

Three types of periphyton biofilms were collected from a hypereutrophic lake (Xuan Wu Lake, China), to obtain the natural microbial community needed to form the required complex structures and to permit up-scaling of the system for practical implication for CV removal. Among three types of periphyton, epiphyton were obtained from macrophyte surfaces, epilithon from stone surfaces, and metaphyton from sediments. The average lake conditions at sampling times were total nitrogen (TN) 2.10 mg L^{-1}; total phosphorous (TP) 0.25 mg L^{-1}; pH 7.75; ammonia 0.61 mg L^{-1}; and nitrate 0.75 mg L^{-1}.

To facilitate the formation of a sufficiently large biomass for complete removal (or at least decolorization) of CV, several immobilized periphyton bioreactors were designed. Three types of periphyton were individually “inoculated” on two types of biofilm substrates - Industrial Soft Carriers (ISC, diameter 12 cm and length 55 cm, Jineng Environmental Protection Company of Yixing, China) and artificial aquatic mats (AAM, diameter 1 cm and length 9 cm). These carriers were designated as ISC-periphyton and AAM-periphyton, respectively. Consequently, six types of periphyton bioreactors were designed, namely three AAM bioreactors (epiphyton, epilithon and metaphyton) and three ISC bioreactors (epiphyton, epilithon and metaphyton). Initially, the biofilms were submerged in 250 mL water obtained from the collection site. Thereafter, the collected biofilms (~10 g wet weight) were added into simulated water containing substrates. The experiments were performed in triplicate under natural light and temperature (25–30 °C) conditions in a green house. After 80 days of incubation, the biofilms were stabilized on the surface of substrates and no further increase in the thickness of periphyton biomass was observed thereafter.

After reaching stability, the periphyton biofilms along with their substrates were used for subsequent testing. The structure and composition of biofilms were determined by peeling the biofilms off the surface of the substrates and then observing under scanning

Table 1
Comparison of different adsorbents implemented for dye decolorization to the present.

Type of adsorbent	Concentration of dye	Percentage decolorization	Reference
Modified-Alumina	200 ppm	20-80%	Adak et al. (2005)
Kaolin	10–100 mg L^{-1}	65-95%	Nandi et al. (2008)
Ananas comosus (pineapple)	20–100 mg L^{-1}	62-95%	Chakraborty et al. (2012)
Nostoc linckia	100–200 mg L^{-1}	72%	Mona et al. (2011)
Coniferous pinus bark powder	10–50 mg L^{-1}	17.5–75%	Ahmad (2009)
Cyperus rotundus	10–30 mg L^{-1}	60-95%	Suyamboo and Srikrishnaperumal (2014)
Surfactant modified magnetic nanoparticles	10–50 ppm	64-74%	Muthukumaran et al. (2016)
Sagaun sawdust	6–12 mg L^{-1}	74–89.45%	Khattri and Singh (2012)
Epiphyton biofilm	100–1000 mg L^{-1}	91.17-complete (followed by degradation)	Present Study
Epilithon biofilm	100–1000 mg L^{-1}	91.17-complete (followed by degradation)	Present Study
Metaphyton biofilm	100–1000 mg L^{-1}	91.17-complete (followed by degradation)	Present Study