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Burkholderia cepacia immobilized on eucalyptus leaves used to simultaneously remove malachite green (MG) and Cr(VI)

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ABSTRACT

A multifunctional biomaterial capable of simultaneously removing malachite green (MG) and Cr(VI) was prepared by immobilizing *Burkholderia cepacia* (*B. cepacia*) on eucalyptus leaves (EL). The maximum uptake of MG (60 mg/L) and Cr(VI) (20 mg/L) were 94.8% and 71.9% respectively, which was more efficient than when using EL or free cells alone. SEM-EDS demonstrated that *B. cepacia* was attached to EL and that Cr(VI) was biosorbed into the immobilized cells. FTIR showed that the degradation by functional groups of immobilized cells was in keeping with the products, detected by GC–MS, which suggested that MG could be degraded to 4-dimethylamino benzophenone and 4-dimethylamino phenol. The removal of both MG and Cr(VI) by EL immobilized cells fit the pseudo-second order adsorption kinetic model well (with both $R^2 > 0.983$). The equilibrium adsorption capacity of MG was 9.59, 18.67 and 28.64 mg/g for initial MG concentrations of from 30, 60, 90 mg/L, respectively when the concentration of Cr(VI) was held constant at 20 mg/L. The adsorption capacity of Cr(VI) increased from 3.49, 7.68 to 9.79 mg/g as the initial Cr(VI) concentrations increased (10, 20, 30 mg/L) while the MG concentration was kept constant at 60 mg/L. The results showed that eucalyptus leaves as a low cost and eco-friendly material have some potential to be an effective immobilization for environmental applications.

1. Introduction

More than 10,000 different synthetic dyes are routinely used in a range of industries, including food, leather, paper, printing and textile industries due mainly to their ease and cost effectiveness in synthesis, high stability towards light, temperature, detergent and microbial attack [1]. Heavy metals, which are common additives in the textile industry are also often found in dye effluents which leads to highly colored and toxic, wastewater/dye effluent that being resistant to degradation poses a significant threat to the environment [2,3]. In particular, malachite green (MG), a triphenylmethane dye, which is often used for dying cotton and treating microbial infections in fish, interferes with the penetration of visible light in waters, resulting in decreased photosynthesis and thus inhibition of biological activity. MG not only causes serious water pollution but also affects living organisms via carcinogenic, teratogenic, mutagenic, and allergenic properties [4]. Similarily, Cr(VI) is toxic to living organisms even at low concentrations and has carcinogenic and mutagenic effects on humans [5]. Thus due to the common co-existence of a complex recalcitrant dye together with a low biodegradable heavy metal in many wastewater streams, the simultaneous removal of MG and Cr(VI) before wastewater discharge has been of topic of some interest.

In recent years, many traditional physical, chemical, biological and combined methods, including physical adsorption, ion exchange, membrane filtration techniques, chemical precipitation, flocculation, oxidation and aerobic/anaerobic treatment, have been used to remediate dye effluent streams containing a complex mix of dyes and heavy metals [6]. Among of these methods, adsorption is one of the most successful techniques in wastewater treatment because of its significant advantages such as design simplicity, easy operation technique, and high separation efficiencies [7]. Now, the green synthesis biocompatible nanosorbent, which is based on hydrophilic supermagnetic imprinted biopolymer, can simultaneously extract and preconcentrate the target compound from real complicated samples [8–10]. However, chemical adsorption can be limited by the operational and economic considerations [1]. Consequently, biosorption which uses dead or inactive microorganisms as absorbents, has recently been considered as a more economic and effective technology for the simultaneous removal of dyes and/or heavy metals from wastewater. Thus, in recent years, numerous studies have focused on investigating the biosorption

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characteristics of natural materials such as lignocellulosic biomass, agriculture byproducts, and plant wastes for pollutant removal [7,8]. For example, *Solanum melongena* leaf was used as an absorbent remove Pb(II) from aqueous solution [11] and coffee grounds were used to remove Rhodamine dyes [12]. However, limited research has been conducted on the simultaneous removal of multiple pollutants. One study reported the adsorption of Cu(II) and Methylene Blue on wheat straw using both single and binary systems [13]. However, although the contaminants were rapidly adsorbed to wheat straw, they could not be degraded. Consequently, to increase degradation rates, studies combining adsorbents with microorganisms have been conducted.

Thus, this study aims to develop a low cost and eco-friendly multifunctional biomaterial, which composed of a living microorganism and a natural biomaterial support for the simultaneous removal and degradation of MG and Cr(VI) from wastewater. This was achieved by initially immobilizing a living microorganism (*Burkholderia cepacia*) on a natural support (eucalyptus leaves). Subsequently, the ability of the produced multifunctional biosorbent to remove MG and Cr(VI) was assessed in single and mixed contaminant systems; and compared to the removal efficiencies of either free cells or eucalyptus leaves (EL) alone.

2. Materials and methods

2.1. Microorganism and medium

Burkholderia cepacia (*B. cepacia*) was isolated from the Fuzhou Textile Printing Plant (Fujian, China). The bacteria were cultured in a phosphate-buffered basal medium (BM), which was composed of K_2HPO_4 (1.8 g), $NaH_2PO_4{\cdot}12H_2O$ (3.5 g), $MgSO_4$ (0.1 g), KNO_3 (1.0 g), glucose (6.0 g) and $FeCl_3{\cdot}7H_2O$ (0.01 g) per litre of distilled water, and the pH was adjusted to 6.0.

2.2. Preparation and immobilization method

Eucalyptus leaves (EL) were collected locally (Fujian, China) and repeatedly washed with distilled water prior to oven drying at 60 °C until a constant weight was obtained. Dried leaves were ground into a fine powder and passed through 20–30 mesh sieves to obtain uniform sized particles. The surface area of EL particles was about 0.3745 m^2/g by BET measurement. The treated leaves were used as the support material for immobilized *B. cepacia*.

EL powder (0.05 g) and a suspension of 5% (v/v) *B. cepacia* were added into capped Erlenmeyer flasks containing sterilized culture medium (15 mL) and incubated on a shaker at 30 °C and 150 rpm for 18–22 h. Cells were concentrated through centrifugation by removing the supernatant and all cells attached to the surface and interior of EL, were considered to be immobilized cells.

2.3. Experiments

Batch experiments were conducted to evaluate the adsorption efficiency of MG and Cr(VI) by EL in single and mixed systems and the simultaneous removal of MG and Cr(VI) using EL immobilized cells.

For the adsorption experiments, EL (0.05 g) was weighed into a 50 mL flasks and pH 6.0 sterilized medium (15 mL) containing either 1) MG (60 mg/L) and Cr(VI) (20 mg/L) 2) MG (60 mg/L) alone or 3) Cr (VI) (20 mg/L) alone was added. The suspensions were then shaken at 150 rpm and 30 °C for 360 min to achieve equilibrium.

Similarly, for adsorption experiments involving simultaneous removal of both MG and Cr(VI) by EL immobilized cells, culture medium (15 mL) containing MG (60 mg/L) and Cr(VI) (20 mg/L) were added to a 50 mL flask containing immobilized cells (0.05 g) and after capping were incubated under the same conditions as other adsorption experiments (i.e. 150 rpm and 30 °C for 360 min). In this study, the removal of MG and Cr(VI) by free cells and EL alone were used as controls.

The temporal variation of both MG and Cr(VI) was monitored periodically in kinetic studies for different initial MG and Cr(VI) concentrations. For the removal of MG, kinetics studies used a series of flasks filled with a constant concentration of Cr(VI) (20 mg/L) and three different initial concentrations of MG (30, 60 and 90 mg/L). For Cr (VI) removal kinetic studies a constant MG concentration (60 mg/L) was used with three different concentrations of Cr(VI) (10, 20 and 30 mg/L) under the same condition. All the experiments were performed in triplicate.

Residual MG concentrations were determined using a UV-spectrophotometer (722 N, Shanghai, China), at a maximum absorbance of 618 nm. Residual Cr(VI) concentrations were determined using an atomic absorption spectrometer (AA-250, China). During kinetic studies the residual concentrations of MG and Cr (VI) were determined every 6 h and percentage removal and adsorption capacity of either MG or Cr (VI) was calculated using the following equations :

$$\text{Removal}(\%) = \frac{(C_0 - C_t)}{C_0} \times 100 \quad (1)$$

$$\text{Adsorption capacity (mg/g)} = \frac{(C_0 - C_t)}{W} V \quad (2)$$

Where C_0 is the initial concentration of MG or Cr(VI) (mg/L), C is the residual solution concentration of MG or Cr(VI) after adsorption (mg/L), V is the volume of solution (L) and W is the weight of EL (g).

2.4. Characterization

The morphological characteristics and elemental composition of EL and immobilized cells were analyzed by scanning electron microscopy (SEM) combined with an X-ray energy-dispersive spectrometer (EDS). The samples were collected and fixed in the phosphate buffers (PBS, pH 7.2) containing 2.5% (w/v) glutaraldehyde at 4 °C for 12 h. Then they were washed with PBS several times, 15 min per time prior to dehydration in a series of gradually increasing ethanol concentrations (30%, 50%, 70%, 80%, 90% and 100%). Each step was allowed to equilibrate for 10 min. The samples were finally dried in an oven at 30 °C for 24 h. Images of EL and immobilized cells were taken at an operating voltage of 3 kV by a Philips-FEI XL30 ESEM-TMP (Philips Electronics Co., Eindhoven, Netherlands). The elements detected in the sample were confirmed using EDS (Oxford Instruments, UK).

A fourier transform infrared spectrometer (FTIR Nicolet 5700, Thermo Corp, USA) was used to characterize the presence of various functional groups in both EL and immobilized cells. Samples were centrifuged and dried, and finely ground with KBr at a proportion of 1:100 and pressed into a translucent sample slice. FTIR spectra were recorded in the wavenumber range of 500–4000 cm^{-1}.

MG degradation products were detected by gas chromatography-mass spectrometry (GC–MS, Thermo, USA). The analytical column was a TR-35MS capillary column with 30 m × 0.25 μm ID and a film thickness of 0.25 μm. The injector temperature was 250 °C and the oven temperature was kept isothermal at 50 °C for 1 min, then raised to 290 °C at a rate of 15 °C min^{-1} and held at 290 °C for 15 min. The GC–MS system was operated in full scan (m/z 50–650).

3. Results and discussion

3.1. Adsorption of MG and Cr (VI) by EL

The amount of MG and Cr(VI) adsorbed by EL in both single and mixed system increased with time (Fig. 1). While the percentage removal of MG was 98.1% after 36 h in the single system, only 20.2% of Cr(VI) was removed in the single system for the same period (Fig. 1). The mixed system resulted in slight variations in the adsorption of both MG and Cr(VI), so that a slight decrease in MG removal (92.1%) and an increase in Cr(VI) removal (26.8%) were observed after 36 h. However regardless of whether a single of mixed system was used, MG was