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Efficient treatment of phenolic wastewater with high salinity using a novel integrated system of magnetically immobilized cells coupling with electrodes

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HIGHLIGHTS

- A novel integrated system was built to treat phenolic wastewater at high salinity.
- Integrated system showed higher removal efficiency than the sum of single systems.
- Integrated system kept high removal efficiency during six recycles.

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ABSTRACT

A novel integrated system in which magnetically immobilized cells coupled with a pair of stainless iron meshes-graphite plate electrodes has been designed and operated to enhance the treatment performance of phenolic wastewater under high salinity. With NaCl concentration increased, phenol, *o*-cresol, *m*-cresol, *p*-cresol and COD removal rates by integrated system increased significantly, which were obviously higher than the sum of removal rates by single magnetically immobilized cells and electrode reaction. This integrated system exhibited higher removal rates for all the compounds than that by single magnetically immobilized cells during six cycles for reuse, and it still performed better, even when the voltage was cut off. These results indicated that there was a coupling effect between biodegradation and electrode reaction. The investigation of phenol hydroxylase activity and cells concentration confirmed that electrode reaction played an important role in this coupling effect.

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1. Introduction

Recently, a large volume of phenolic wastewater is generated as a result of petrochemical refineries, pesticide, coal coking and textile industries etc., leading to significant ecological impact (Babich and Stotzky, 1985; Arutchelvan et al., 2006). Phenolic wastewater is one of the most refractory wastewaters and contains large quantities of phenol and its derivatives such as *o*-cresol, *m*-cresol, *p*-cresol, etc. Besides, phenolic wastewater also contains significant amounts of inorganic dissolved salts (Ramos et al., 2015). Phenolic wastewater is poisonous for human beings, animals and aquatic organism due to their toxic, carcinogenic and mutagenic effects, and cannot be discharged to the environment directly before it have been effectively processed (Ersu and Ong, 2008). In contrast to physical and chemical processes, biological method is widely employed as a cost-effective and environmental friendly method to remove phenolic wastewater (González et al., 2001; Ramos et al., 2015, 2016). However, high salinity may decrease the activity and metabolism ratio of microorganism, which is detrimental to biological method (Rinzema et al., 1988; Rene et al., 2008). Thus, there is a crucial need to isolate salt-tolerant phenol-degraders. The recent studies are mostly focused on the isolation of salt-tolerant phenol-degraders, as well as its basic physiological and biochemical characteristics including phenols tolerance and degree of salt tolerance (Hinteregger and Streichsbier, 1997; Peyton et al., 2002; Wang et al., 2009), and rarely on the reutilization of salt-tolerant phenol-degraders to degrade phenols with the presence of salinity.

According to the previous studies, the use of immobilized microorganisms rather than free cells in biodegradation is to enhance the stability of biocatalyst and to facilitate its recovery and reuse (Wang et al., 2007). These advantages have encouraged researchers to investigate the application of immobilized cells in

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the biodegradation of phenols (Dwyer et al., 1986; Ahmad et al., 2012). However, mass transfer limitation involved in substrate diffusion to the reaction system is still the major drawback in the application of an entrapment technique. To reduce this mass transfer limitation, magnetic nanoparticles as a new generation of environmental remediation technologies have been introduced into the immobilized process to prepare the magnetically immobilized cells, which can not only reduce the mass transfer resistance of traditional immobilization processes, but also improve the stability of biocatalyst and promote its recovery in reuse processes (Wang et al., 2007; Shi et al., 2014, 2015; Jiang et al., 2015). It has been proved as a novel aspect of microbial cells immobilization and has been increasingly attracting attention due to their reutilization in the bioremediation of industrial wastewaters (Shi et al., 2014, 2015; Jiang et al., 2015). However, the application of magnetically immobilized cells in industrial wastewaters under a high salt content has not been investigated so far.

Although the presence of salts in industrial wastewater can inhibit the bioremediation, salt is of high conductivity, which makes it possible to develop electrochemical methods for enhanced treatment of industrial wastewater with high salinity (Zhang et al., 2012a). As it is well known, the integration of two or more conventional technologies has been proved as a promising strategy and caused increased interest in the bioremediation of industrial wastewaters. Recently, many studies focused on the integration of biological treatment with electrochemical methods to improve the treatment of wastewater containing nitrogen, azo dye, volatile fatty acids, and chlorinated organic compounds at the anaerobic or anoxic conditions (Thrash et al., 2007; Zhang et al., 2012a,b,c; Shen et al., 2012; Wen et al., 2013; Lu et al., 2014). However, few studies were tried to enhance the treatment of wastewater by the integration of biological treatment with electrochemical methods at aerobic conditions and little investigation was conducted about the effect of salinity on the integrated system.

In this study, a novel integrated system in which magnetically immobilized cells coupled with a pair of stainless iron meshes-graphite plate electrodes has been designed and operated to enhance the treatment performance of phenolic wastewater containing high concentration of phenol, *o*-cresol, *m*-cresol and *p*-cresol under high salinity.

2. Materials and methods

2.1. Chemicals

Phenol, *o*-cresol, *m*-cresol and *p*-cresol were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). *r*-Fe_2O_3 nanoparticle (diameter < 20 nm, 99.9%) was purchased from DKnano Scientific Ltd. (China).

2.2. Bacterial strain and cultivation conditions

Comamonas sp. JB was grown in mineral salt medium (MSM) at 150 rpm and 30 °C, which included (g L^{-1}) NH_4Cl 2.0, KH_2PO_4 3.7, Na_2SO_4 1.0, K_2HPO_4 $3H_2O$ 5.2, $MgSO_4$ 0.1, phenol 0.4, 1 mL of trace metal solution and NaCl 20. For preparing cell suspensions, cells were harvested by centrifugation (10,000*g* for 5 min) in exponential phase, washed twice with 0.1 M Tris-HCl buffer (pH 7.0) and then resuspended in the same buffer to form cell suspension (the optical density was about 2.5 at 660 nm).

2.3. Preparation of immobilized cells

The gellan gum (1% wt vol^{-1}) and cell suspension were mixed at ratio of dry polymers powder to cell wet weight of 1:3 (wt:wt) as previously described (Wang et al., 2007). Nonmagnetically immobilized cells were prepared by extruding the mixture through a syringe into 0.2 M $CaCl_2$ and letting it solidify for 2 h. Magnetically immobilized cells was prepared by adding 120 mg L^{-1} of *r*-Fe_2O_3 nanoparticle suspension to the above-mentioned mixture.

2.4. The effects of salinity on the bioremediation of phenolic wastewater

In this study, phenolic wastewater was the synthetic wastewater, which contained (g L^{-1}) phenol 1, *o*-cresol 0.3, *m*-cresol 0.3, *p*-cresol 0.3, COD 5.285, NaCl 0–50, and NH_4Cl 2.0. The effects of NaCl concentration (0–50 g L^{-1}) on the performance of free cells, nonmagnetically immobilized cells and magnetically immobilized cells of strain JB were carried out in polymethyl methacrylate reactor with 650 mL phenolic wastewater in 12 h as previous described (Jiang et al., 2016). The aeration was 0.2 L min^{-1}. A pair of stainless iron meshes electrode (anode, 7 cm length × 4 cm width) and graphite plate electrode (cathode, 7 cm length × 4 cm width) inserted into the above reactor with magnetically immobilized cells of strain JB was to build an integrated system. The electrodes were supplied by DC power source of 0.75 V. The integrated system without magnetically immobilized cells was used as the single electrode reaction. The effects of voltage (0.5–1.25 V) on performance of the integrated system were conducted. The recycling experiments by the integrated system and single magnetically immobilized cells were conducted as previously described (Shi et al., 2014). After six cycles, the voltage supplied to the electrodes was cut off.

2.5. Analytical methods

All experiments were performed in triplicate. Samples were taken at intervals to monitor the concentration of phenol, *o*-cresol, *m*-cresol and *p*-cresol. To determine the concentration of phenol, *o*-cresol, *m*-cresol, and *p*-cresol, the sample was extracted with ethyl acetate and then tested by high-performance liquid chromatography (HPLC). HPLC was equipped with a Hypersil ODS2 column (5 μm, 250 mm × 4.6 mm) with CH_3OH/H_2O as the mobile phase. A linear gradient of 30–45% CH_3OH (v/v, in H_2O) over 20 min was used with a flow rate of 1 mL min^{-1} and UV detection was set at 254 nm. COD was tested according to the standard tool. After the reaction, cells were collected, one portion of cells was disrupted by sonication (225 W at 4 °C for 30 min, Ultrasonic processor CPX 750) and then the cell debris was removed by centrifugation at 22,000 r min^{-1} for 20 min at 4 °C to determine the activity of phenol hydroxylase as previously described (Qu et al., 2012). A second portion of cells was used to investigate the concentration of strain JB by qPCR assays as previously described (Jiang et al., 2015). The acute toxicity of effluent and influent samples was assessed by Microtox bioassays using the luminescent bacteria *Vibrio fischeri* (NRRL B-11177) as the previously described (Shi et al., 2014).

3. Results and discussion

3.1. The performance of magnetically immobilized cells at different salinity

The previous experiment suggested that strain JB could use phenol, *o*-cresol, *m*-cresol or *p*-cresol as the sole carbon source and energy source under NaCl concentration of 20 g L^{-1}, respectively (data not shown). In this study, the impact of NaCl concentration on the performance of magnetically immobilized cells of JB with gellan gum as the support was initially carried out. The

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