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Bioremediation of coking wastewater containing carbazole, dibenzofuran and dibenzothiphene by immobilized naphthalene-cultivated *Arthrobacter* sp. W1 in magnetic gellan gum

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HIGHLIGHTS

- Cometabolic degradation CA, DBF and DBT simultaneous by magnetically immobilized cells.
- Coking wastewater was rapidly treated by magnetically immobilized cells with zeolite.
- Magnetically immobilized cells kept high bioremediation activity during seven recycles.

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ABSTRACT

In this study, the cometabolic degradation of carbazole (CA), dibenzofuran (DBF), and dibenzothiphene (DBT) by immobilized *Arthrobacter* sp. W1 cells pregrown with naphthalene was investigated. Four kinds of polymers were evaluated as immobilization supports for strain W1. After comparison with agar, alginate, and κ-carrageenan, gellan gum was selected as the optimal immobilization support. Furthermore, magnetic Fe_3O_4 nanoparticle was selected as most suitable nanoparticle for immobilization and the optimal concentration was 80 mg/L. The relationship between specific degradation rate and the initial concentration of CA, DBF and DBT was described well by Michaelis–Menten kinetics. The recycling experiments demonstrated that the magnetically immobilized cells coupling with activation zeolite showed highly bioremediation activity on the coking wastewater containing high concentration of phenol, naphthalene, CA, DBF and DBT during seven recycles. Toxicity assessment indicated the treatment of the coking wastewater by magnetically immobilized cells with activation zeolite led to less toxicity than untreated wastewater.

1. Introduction

Carbazole (CA), dibenzofuran (DBF), and dibenzothiophene (DBT) as the predominant nitrogen-, oxygen-, and sulfur-heterocyclic compound have been detected in environments contaminated by coal tar, crude oil, and creosote (Lim et al., 2003; Zhang et al., 1998; Zhu et al., 2009). They have been found to be toxic and mutagenic, and have become a great threat to the environment (Zhu et al., 2009). It is necessary to establish effective methods to clean up these heterocyclic compounds to protect the environment. Many researchers have focused their studies on the isolation of CA-, DBF- and DBT-degrading bacteria belonging to the genera *Sphingomonas*, *Terrabacter*, *Pseudomonas*, *Ralstonia*, etc. (Gai et al., 2007; Das et al., 2012; Iida et al., 2002; Schneider et al., 2000) . However, because the polycyclic aromatic compounds (PACs) including CA, DBF and DBT are widespread and usually coexist in the polluted environments, cometabolic degradation of some PACs by bacteria growing with some others should be common, and currently there have been many studies on the cometabolic degradation of these heterocyclic compounds by naphthalene-, fluorene-, and biphenyl-utilizing strains (Shi et al., 2013, 2014; Grifoll et al., 1995; Li et al., 2009a,b). Nevertheless, these studies are mostly focused on the cometabolic degradation pathways, as well as the genes and enzymes involved, and rarely on the development of an immobilization method for the cometabolic degradation.

Immobilized microbial cells are frequently used in the biodegradation of a series of aromatic compounds, due to their better

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operational stability, easier separation from products for possible reuse, and satisfactory efficiency in catalysis compared to free cells (Tan et al., 2014; Xu et al., 2013; Li et al., 2013; Rodríguez Couto et al., 2004; Lin et al., 2010). However, mass transfer limitation involved in substrate diffusion to the reaction system is still the major drawback in the application of immobilization by entrapment technique (Wang et al., 2007). Recently, nanoparticles that represent a new generation of environmental remediation technologies have been used in the studies of immobilized microbial cells, which not only reduced the mass transfer resistance of traditional immobilization processes, but also facilitated the recovery of immobilized cells in the reuse processes (Li et al., 2009a,b; Fernando Bautista et al., 2010; Qiu et al., 2010; Liu et al., 2012). For example, Shan et al., reported that *Pseudomonas delafieldii* R-8 coated by magnetic Fe_3O_4 nanoparticles not only had the same desulfurizing activity on DBT as free cells but could also be reused more than five times (Shan et al., 2005a,b). A study by Wang et al. also showed *Sphingomonas* sp. strain XLDN2-5 immobilized in magnetic gellan gum gel beads could keep high degradation activity on CA during the eight recycles (Wang et al., 2007).

As is well known, coking wastewater is generated from coal coking, coal gas purification, and by-product recovery processes of coking, which commonly contains considerable amounts of phenol, naphthalene, and low levels of CA, DBF, and DBT (Wang et al., 2012; Fang et al., 2013; Zhu et al., 2009). In this study, the cometabolic degradation of CA, DBF and DBT by *Arthrobacter* sp. W1 cells immobilized in nanoparticles was studied. The selection of immobilization supports and nanoparticles were also investigated. The reuse of magnetically immobilized cells and nonmagnetically immobilized cells with activation zeolite for the bioremediation of real coking wastewater containing CA, DBF, DBT and phenol and naphthalene were tested.

2. Methods

2.1. Chemicals

CA, DBF, and DBT were purchased from J&K Scientific Ltd. (China). Magnetic Fe_3O_4 nanoparticle (diameter <20 nm, 98%) was purchased from Tianyuxiangrui science and technology Co., Ltd. (China). Single-walled carbon nanotubes (SWNTs) (diameter <2 nm, 95%) and multi-walled carbon nanotubes (MWNTs) (diameter <10 nm, 97%) were purchased from Xfnan Co., Ltd. (China). Activation zeolite as the ammonium exchangers was obtained from Zhengjiang Shengshi Mining Industry Co., Ltd. (China). All other commercially available chemicals were of analytical grade.

2.2. Bacterial strain and cultivation conditions

Arthrobacter sp. W1 was isolated from the sludge samples of a sewage plant in China and deposited as a bacterium in China General Microorganism Culture Center with the accession number CGMCC 4376. Strain W1 was routinely grown in mineral salt medium (MSM) as previously described with a minor modification (Shi et al. 2014). CA, DBF and DBT were dissolved in dimethyl sulfoxide (100 mM) and added to the medium at a suitable concentration. All cultures or cell suspensions were incubated at 30 °C on a reciprocal shaker at 150 rpm.

2.3. The effect of nanoparticles on the growth of strain W1 and degradation

Cell suspensions of naphthalene-grown W1 were prepared separately by centrifugating the cultures in late exponential phase at 10,000×*g* for 5 min, washing cell pallets twice with MSM, and resuspending cells in MSM to get different turbidities.

The effect of magnetic Fe_3O_4 nanoparticle, SWNT and MWNT (0.5–10 mg/L) on the growth of strain W1 were tested with naphthalene (1 mM) using the naphthalene-grown W1 cell suspension with a turbidity at 660 nm of 0.1, respectively. The effect of magnetic Fe_3O_4 nanoparticle, SWNT and MWNT (2 mg/L) on the growth of strain W1 also were tested with naphthalene (1 mM) plus CA (0.2 mM), DBF (0.2 mM) and DBT (0.2 mM). The cell growth and degradation in the culture without nanoparticles were detected as controls.

2.4. Preparation of gel beads and immobilized cells

Gellan gum, agar, κ-carrageenan and calcium alginate were used as adsorbents in adsorption experiments. The ionotropic method described by Woodward was used to form gel beads from gellan gum and alginate (Woodward, 1988). The interphase technique described by López et al. was used to form gel beads from agar and κ-carrageenan (López et al., 1997) The polymers (1% wt/vol) and cell suspension of strain W1 were mixed at ratio of cell wet weight to dry polymers powder of 3 (wt/wt). Nonmagnetically immobilized cells were formed by extruding the mixture through a syringe into 0.2 M $CaCl_2$ and letting it solidify for 2 h as previously described (Wang et al., 2007). For preparing the immobilized cells with nanoparticles, an appropriate Fe_3O_4, SWNT or MWNT particle suspension was added to the above mentioned mixture of polymers and cell suspension, and the procedure was the same as that for nonmagnetically immobilized cells. The immobilized cells with Fe_3O_4 nanoparticle was designed as magnetically immobilized cells. Immobilized inactive cells with or without nanoparticles was prepared as described above.

2.5. Absorption of CA, DBF and DBT by polymers

All the adsorption experiments were carried out in 100 mL flasks containing 20 mL MSM. In each experiment, CA (0.5 mM), DBF (0.5 mM), or DBT (0.5 mM) was added to MSM and gel beads (1 g, wet weight) served as the adsorbent. Samples were taken at intervals to monitor the concentrations of CA, DBF and DBT as described below.

2.6. Biodegradation of CA, DBF and DBT by immobilized cells

Studies on the simultaneous degradation of CA, DBF and DBT (0.75 mM) by immobilized cells in gellan gum, and gellan gum with nanoparticles (40 mg/L) were carried out at a function time. The effect of the concentration of magnetic Fe_3O_4 nanoparticle (40–120 mg/L) on the degradation of CA, DBF and DBT (0.75 mM) was also tested. Free cells of strain W1, gellan gum gel beads without cells, magnetically immobilized inactive cells and nonmagnetically immobilized inactive cells were added to MSM with substrates as controls. The degradation of CA, DBF and DBT (from 0.25 to 1.5 mM) by magnetically immobilized cells was also tested, respectively. The relationship between specific degradation rate and initial concentration of CA, DBF and DBT was described with Michaelis–Menten kinetics by GraphPad Prism 5 software.

2.7. Reuse of magnetically immobilized cells on the bioremediation of coking wastewater

Raw coking wastewater was obtained from the adjusting tank of the coking wastewater treatment plant in Shanxi province of China. The wastewater quality was as follows: COD 1700 mg/L, phenol 200 mg/L, naphthalene 58.9 mg/L, CA 12.5 mg/L, DBF 11.7 mg/L, DBT 10.8 mg/L and NH_3-N 86 mg/L. The raw wastewater