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Enhanced biodegradation of pyrene by immobilized bacteria on modified biomass materials

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ABSTRACT

The degradation of pyrene (PYR) by *Mycobacterium gilvum* immobilized on peanut shell powder (PSP) was improved by chemically modifying the PSP. The physicochemical properties of the modified biomass (M-PSP) were characterized with surface area analysis, X-ray diffraction, Fourier transform infrared and solid-state CP/MAS ^{13}C NMR spectra. The results showed that the chemical modification decreased the crystallinity of the biomass and destroyed the benzene rings in the cellulose, which improved the porosity of PSP. Fluorescein diacetate activity (FDA) of cells immobilized on the M-PSP decreased more slowly than cells immobilized on PSP. The PYR degradation efficiencies achieved by bacteria immobilized on M-PSP was higher than that achieved by immobilized bacteria on PSP after 7 d. It revealed that chemical modification of biomass could further improve bacterial activity and enhance the degradation ability of the cells compared with the raw one, as we expected.

In summary, this study shows that M-PSP is a good immobilizing bio-mass for supporting pollutant-degrading bacteria and could be employed as an effective material for PYR biodegradation.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), a group of persistent organic pollutants (POPs), are of worldwide concern because of their long-term harmful effects associated with their persistence, bioaccumulation, cytotoxicity and carcinogenicity (Cui et al., 2014). Microbial bioremediation, the utilization of microbes to remove pollutants, has been proposed as a promising method for treating PAHs in the environment (Yang et al., 2011) due to its higher efficiency, simplicity and cost-effectiveness when compared to other methods (Ojo, 2006). However, the use of free bacteria in remediation was usually adversely affected by valued biotic and abiotic elements in the environment (Prabua and Thatheyus, 2007). To overcome these limitations on the use of exogenous bacteria for pollutant bioremediation, the microbial cells are often immobilized, a modification that can increase the biodegradation rate by protecting the cells from sudden exposure to higher concentrations of PAHs, thereby avoiding the direct toxicity of the pollutant on the effector cells (Sarma and Pakshirajan, 2011) and improving their tolerance against the toxic compound (Wang et al., 1997; Baskaran and Nemati, 2006).

Agricultural waste for use as a low-cost bio-carrier to produce immobilized cells has attracted considerable attention as it can serve as an abundant carbon source for bacteria; e.g., corncob powder (Labana et al., 2005), sugarcane bagasse (Plangklang and Reungsang, 2009) and peanut hull powder (Xu and Lu, 2010). However, most of these previous researches have only used the raw material as a carrier, whose adsorption ability is limited by the material's low surface area (Zheng et al., 2010b). In some studies, the agricultural waste has been modified in order to increase adsorption capacity. For example, sodium hydroxide can break ether linkages between lignin and polysaccharides in treated corn stalks, enabling the extraction of cellulose from corn (Xiao et al., 2001). Sodium hydroxide treatment can also improve the adsorption capacity of corn stalk (Šćiban et al., 2008).

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Peanut shell is a suitable carrier material for immobilizing bacteria due to its high granularity, absorbent properties, biodegradability, and low cost (Xu and Lu, 2010). In this study, we treated peanut shell powder (PSP) with sodium hydroxide and hydrogen peroxide solution to improve the surface area of the biomass and enhance the adsorption capacity of cells. This modified peanut hull powder (M-PSP) was used as the carrier for immobilized cells (IC) of bacteria to further improve PAH biodegradation efficiency. These results were compared with both the results from IC on PSP and the results using support-free cells (SFC). Pyrene (PYR) was used as the representative compound for PAHs since its structure is found in most of the PAHs (Sarma and Pakshirajan, 2011). The PAH biodegradation efficiency achieved by IC on M-PSP was determined and compared with that of previous works. In addition, the structure of the support material was investigated by X-ray diffraction, Fourier transform infrared and solid-state CP/MAS ^{13}C NMR spectra. To our knowledge, this is the first time that the structure of PSP treated with sodium hydroxide and hydrogen peroxide solution has been examined and that the material has been used as a support for immobilized bacteria to degrade PAHs.

2. Materials and methods

2.1. Materials and microorganisms

PYR (Aldrich, purity above 98%) was used as the model PAH compound (Sarma and Pakshirajan, 2011). PYR was dissolved in acetone and stored in brown bottles at 4 °C. First of all, 400 mg of solid PYR was weighted by an electronic scale, and then dissolved in 1 L acetone as. Before use, 0.5 ml of the PYR solution will be taken to volatilize in a flask and the final weight of PYR in each flask is 0.2 mg. After that, the PYR we want to use was quantified.

The microorganism used for PYR degradation in mineral salts medium (MSM) was *Mycobacterium gilvum* CP13. CP13 was isolated from activated sludge in a coking plant in Shaoguan, Guangdong, China (Wu et al., 2014). The bacterium was cultured in fresh nutrient broth for 2–3 days to an optical density (OD) of 2. The nutrient broth containing cultured bacterium was centrifuged and the supernate was discarded. The cells were then resuspended with 0.9% NaCl. Repeated 2–3 times, the cells were finally resuspended in 0.9% NaCl for immobilization.

Peanut shells, obtained from local farmers in Guangzhou, China, were ground in a milling machine, air-dried, and sieved through a 2-mm mesh to obtain PSP. M-PSP was prepared by soaking PSP in a sodium hydroxide and hydrogen peroxide solution (1% respectively, m/m) with stirring at room temperature for 14 h. PSP and M-PSP were used as the immobilization carriers of CP13. The samples of the carrier were then put into polyethylene bags resistant to high voltage and high temperature and sterilized by autoclaving at 121 °C, 1×10^5 pa for 20 min before use. The immobilization of the microbes was carried out by mixing the carrier with the free inoculum at room temperature (Labana et al., 2005).

The stability of cells immobilized on peanut hull powder was measured by the procedure of Xu and Lu (2010). Briefly, the dry powder containing immobilized cells was kept at room temperature for 4, 8, 12, and 16 weeks, respectively. After that, the powder was used to determine total hydrolytic activity in the powder by fluorescein diacetate (FDA) hydrolysis, using the method described previously (Lu et al., 2009). The FDA hydrolytic activity was measured from the absorbance of supernatant at 490 nm (A_{490}). The value of A_{490} per gram soil was used as a measure of the microbial activity in the carrier. Results were expressed as A_{490} mg^{-1} carrier.

2.2. Modification and characterization of materials

To confirm bacterial immobilization, both PSP and M-PSP were collected and treated using the method described by Wu et al. (2003); i.e., 2.5% glutaraldehyde treatment for 48–72 h, with repeated dehydrations using 20% acetone for 0.5 h, 50% acetone for 1 h and 100% acetone for 7 h. These samples were observed with a scanning electron microscope (SEM QUANTA 400). The acceleration voltage (Acc. V) was 10.0 KV. The surface area (BET method) of PSP before and after modification was determined using surface area analyzer (ASAP 2020M).

The chemical structures of PSP and M-PSP were characterized by a Fourier transform infrared (FT-IR) spectrophotometer and solid-state CP/MAS ^{13}C NMR spectroscopies (Bruker AVANCE 400 spectrometer) at the frequency of 100 MHz with 4 mm MAS BBO prode. The crystallinity of PSP and M-PSP was characterized using X-ray diffraction (XRD) (D/max-IIIA, Rigaku). The sample was exposed to X-rays with the 2θ angle varying between 5 and 80°.

2.3. Biodegradation of PYR from mineral salts medium (MSM)

Degradation of PYR in aqueous solution by CP13 as SFCs and as ICs in PSP and M-PSP were conducted in batch experiments in 100-ml shake flasks containing 20 ml of mineral salts medium (MSM) supplemented with 10 mg L^{-1} of PYR as the sole carbon source. ICs in PSP and M-PSP (0.5 g) were added into the flasks to a cell concentration of 10^7 CFU ml^{-1}. Flasks were then incubated at 30 °C while shaken at 150 rpm. The MSM consisted of the following: 2.5 g L^{-1} K_2HPO_3, 0.77 g L^{-1} KH_2PO_4, 100 mg L^{-1} $(NH_4)_2SO_4$, 20 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 10 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 1.2 mg L^{-1} $FeSO_4 \cdot 7H_2O$, 0.3 mg L^{-1} $MnSO_4 \cdot H_2O$, 0.3 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, and 0.1 mg L^{-1} $CoSO_4 \cdot 7H_2O$, 0.1 mg L^{-1} $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. Flasks were sampled on days 0, 1, 2, 3, 5 and 7. PYR in the SFC and IC treatments was extracted with methanol and *n*-hexane, respectively.

2.4. Statistical analysis

The statistical analyses were performed with one-way analysis of variance (ANOVA) using Minitab 16 Software. A multiple comparison Tukey test was applied to assay the differences among the treatments.

3. Results and discussion

3.1. Scanning electronic microscopy of support materials and immobilized cells

Scanning electronic microscope (SEM) micrographs of the support materials and ICs are shown in Fig. 1. The photograph showed that the M-PSP had greater porosity than PSP, which allowed for better adsorption of cells onto the carrier.

The immobilization of cells in M-PSP was confirmed by SEM, and the images are shown in Fig. 1c. The photograph evidently showed that the bacteria were firmly immobilized on the M-PSP, as seen by their rod shape of approximately 1 μm in length and 0.3 μm in diameter.

3.2. Characterization of PSP and M-PSP

The surface area results of PSP and M-PSP determined by Brunauer, Emmett and Teller (BET) method are shown in Table 1. M-PSP had greater BET surface area than unmodified PSP, reflecting the greater porosity of the M-PSP. These data illustrate favorable conditions for adsorption and survival of cells on the M-PSP,