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Biodegradation of phenol by *Pseudomonas putida* immobilized on activated pumice particles

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Abstract

Pseudomonas putida was acclimized to phenol by increase in concentration and the degradation rate was calculated as approximately 0.042 g l^{-1} h^{-1} in batch shake flask cultures. Phenol degradation by *P. putida* immobilized on several silica based support materials was investigated. Pumice was chosen as a support material and then partially characterized physically and chemically. The cell adsorption ratio was 91% with Zr-activated pumice. The biocatalyst completely degraded 1.0 g l^{-1} phenol in the batch shaking system in 22 h and it was also used in recycled and continuous mode packed bed bioreactors for phenol degradation. The performance of the bioreactor was tested by running five times and it was observed that the degradation rates did not change for the first four runs. The biodegradation rate becomes high and concentration-independent in recycled packed bed bioreactors for phenol degradation. The reactor performance at high phenol concentration for the reactor tolerance was 1.25 g phenol l^{-1}. Continuous degradation of phenol in a packed bed bioreactor was also studied. The phenol concentrations in the feed tank was increased with a constant dilution rate (2.27 h^{-1}) from 0.065 to 0.820 g l^{-1}. The maximum phenol degradation level of 99% was reached at a phenol loading rate of 0.001–0.002 g l^{-1}. The biocatalyst could be stored at 4 °C for 6 months without significant decrease in activity.
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Keywords: Phenol biodegradation; *Pseudomonas putida*; Immobilization; Pumice

1. Introduction

Phenols are common components of industrial streams from polymeric resin producing companies, coal gasification, oil refining, and coking plants [1]. Wastewater with high concentrations of phenol can be treated by physicochemical [2] or conventional biological methods using activated sludges [3] or anaerobic cultures [4,5]. However, these detoxifying methods do not appear to be entirely satisfactory because they produce secondary effluent problems. Moreover, the use of pure cultures of microorganisms, especially adapted to metabolize the contaminant, can be envisaged as an attractive alternative [6,7].

Phenol uptake by *Pseudomonas putida* cultures has been reported by several authors [8–13]. Some publications treated phenol degradation and purification of phenol containing waste waters using immobilized microorganisms [14–19].

Natural porous silica based material, pumice, offers several advantages including mechanical strength, resistance to organic solvents and microbial attack, easy handling and regenerability.

The objective of this study was to investigate the usage of the pumice for the immobilization of *P. putida* (DSMZ 50026) for biological phenol treatment systems. Pumice, as a carrier, was compared to other silica based support materials and its activation methods were searched.

2. Material and methods

2.1. Microorganism

A pure strain of *P. putida* 50026 was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) and used after accli-

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mization studies for the aerobic degradation of phenol. The culture was maintained on nutrient agar (NA) slants at 4 °C.

2.2. Medium and culture conditions

Bacteria were grown on nutrient agar (OXOID CM3). For adaptation experiments of the cells to phenol, which was also used as a sole carbon source, a simple minimal salts medium (MSM) was used [20]. The activated culture on NA was inoculated into 50 ml of MSM containing 1.0 g glucose l^{-1} and incubated at 28 °C, 150 rpm. After 24 h, cells were transferred to (0.8 g glucose + 0.2 g phenol) l^{-1} MSM medium as 2% inocula and this culture was inoculated in to flasks including gradually increasing phenol concentrations and also gradually decreasing glucose concentrations in MSM and finally tertiary cultures of *P. putida* in 1.0 g phenol l^{-1} MSM were used for next phenol biodegradation studies.

MSM and apparatus were autoclaved at 121 °C for 15 min, and glucose and phenol were separately sterilized by membrane filter.

2.3. Dry cell weight determinations

Biomass was determined turbidometrically at 560 nm and converted to dry cell weight with a standard conversion curve.

2.4. Support material

Pumice, obtained from the region of Nevsehir from Turkiye, was ground and sieved. Prior to immobilization, carriers (12–16 US mesh; average diameter, 774 μm; and 10–100 g) were treated 3 M HCl and then 0.5 M and finally washed with distilled water and dried at room temperature [21,22]. Morphological, physical and chemical properties of pumice particles are given in Table 1 [23].

2.5. Activation of support materials

2.5.1. Silanization

Acid washed pumice particles were silanized in toluene. Pumice (10 g) was added to 40 ml of 3-aminopropyltriethoxysilane (γ-APTS) solution (10% (v/v)) in toluene and refluxed for 18 h. The support material was then washed with toluene and acetone, respectively, and dried in vacuum oven [21].

2.5.2. Zr-activation

Acid washed pumice particles were treated with 0.65 M $ZrOCl_2$ in 1.0 M HCl. The mixture was dried in an oven for 48 h at 55 °C and then washed three times with distilled water [24].

Table 1
Chemical and physical properties of pumice

Chemical properties of pumice	
Elemental composition of pumice (%)	
Oxygen	62.75
Sodium	3.22
Aluminium	5.88
Silica	24.57
Chlorine	1.16
Potassium	2.44
Chemical composition of pumice (%)	
SiO_2	74.0
Al_2O_3	15.6
Na_2O	6.1
K_2O	2.4
Others	1.9
Physical properties of pumice particles	
Average particle diameter (μm)	774
Specific surface area ($m^2\ g^{-1}$)	1.210
Density ($g\ cm^{-3}$)	1.9573

2.6. Cell immobilization

Immobilization was carried out for about 3 h in flasks, which contained the activated and sterilized (120 °C, 20 min) pumice (1 g) on a shaker with a controlled speed (60 rpm). The number of adsorbed cells of *P. putida* was estimated by determining the difference in cell numbers before and after immobilization [25].

2.7. Phenol determination

Phenol determinations were performed using a colorimetric method employing 4-aminoantipyrine (4-AAP) as colour reagent [23]. The method is based on the reaction between phenol and 4-AAP in the presence of ferricyanide at pH 10 to form a coloured antipyrine dye. The absorbance of the dye was measured at 500 nm [26].

2.8. Bioreactor

2.8.1. Batch-recirculation system

P. putida cells were suspended in 50 ml MSM and percolated through a bed comprised of 5.0 g of support material in a glass column (Fig. 1). The cell suspension was recirculated at 28 °C with a peristaltic pump.

2.8.2. Continuous system

Cultivation conditions were similar in continuous and batch-recirculation mode experiments. The entire closed system consisting of the packed column, feed vessel, waste vessel, and connecting tubing and filter vents were sterilized by autoclaving. Phenol was then added to the reactor feed reservoir. Fig. 2 represents a scheme of the immobilized cell packed bed column biorector.

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