Journal of Hazardous Materials 164 (2009) 720–725

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel

Muftah H. El-Naas*, Shaheen A. Al-Muhtaseb[1], Souzan Makhlouf

Chemical & Petroleum Engineering Department, U.A.E. University, P.O. Box 17555, Al-Ain, United Arab Emirates

ARTICLE INFO

Article history:
Received 20 March 2008
Received in revised form 19 August 2008
Accepted 20 August 2008
Available online 26 August 2008

Keywords:
Biodegradation
Immobilization
Phenol
Wastewater
Refinery

ABSTRACT

Batch experiments were carried out to evaluate the biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel pellets in a bubble column bioreactor at different conditions. The bacteria were activated and gradually acclimatized to high concentrations of phenol of up to 300 mg/l. The experimental results indicated that the biodegradation capabilities of *P. putida* are highly affected by temperature, pH, initial phenol concentration and the abundance of the biomass. The biodegradation rate is optimized at 30 °C, a pH of 7 and phenol concentration of 75 mg/l. Higher phenol concentrations inhibited the biomass and reduced the biodegradation rate. At high phenol concentration, the PVA particle size was found to have negligible effect on the biodegradation rate. However, for low concentrations, the biodegradation rate increased slightly with decreasing particle size. Other contaminants such heavy metals and sulfates showed no effect on the biodegradation process. Modeling of the biodegradation of phenol indicated that the Haldane inhibitory model gave better fit of the experimental data than the Monod model, which ignores the inhibitory effects of phenol.

1. Introduction

Petroleum refineries generate huge amounts of wastewater that usually go through a combination of treatment steps to reduce the concentrations of the different contaminants to acceptable discharge levels. The main contaminants of refinery wastewater include phenols, polycyclic aromatic hydrocarbons (PAHs) as well as heavy metals. Of these toxic pollutants, phenols are considered to be among the most hazardous, and they are certainly the most difficult to remove. Phenol may be fatal by ingestion, inhalation, or skin absorption, since it quickly penetrates the skin and may cause severe irritation to the eyes and the respiratory tract. It is listed among the priority organic pollutants by the US Environmental Protection Agency [1]. It is considered to be potentially carcinogenic to humans and may be lethal to fish at concentrations of 5–25 mg/l [2]. The UAE has one of the most stringent environmental regulations, especially those related to discharge levels. Abu Dhabi National Oil Company (ADNOC) has set a desirable limit of phenol discharge concentration of 0.01 mg/l compared to that set by the EPA (USA) of 0.168 mg/l.

It is essential, therefore, that phenol concentrations in refinery effluents be reduced to environmentally acceptable and harmless levels through utilizing effective and practical treatment methods. Many treatment techniques have been employed in the past few years to reduce the concentrations of phenols, including biodegradation, adsorption, ion exchange and the use of bioactive activated carbon. Biological treatment has proved to be the most promising and economical method for the removal of phenol from wastewater. It is believed to lead to complete mineralization of phenol [3] and can handle a wide range of concentrations. The biodegradation of phenols by different types of microbial cultures has attracted the attention of many researchers during the past two decades. Many types of aerobic bacteria, including *Pseudomonas putida*, are believed to be capable of consuming aromatic compounds as the only source of carbon and energy. *P. putida* is a rod-shaped, Gram-negative bacterium that has been known for its ability to degrade organic solvents, especially its high removal efficiency of phenol [4]. Numerous other types of bacteria and biosorbents were reported to be utilized for the biodegradation or the removal of phenol. These include: *Rhodococcus erythropolis* [5]; *Bacillius* sp. [6]; *Alcaligenes faecalis* [7]; *rhizobium Ralstonia taiwanensis* [8]; *Nocardia hydrocarbonoxydans* [9]; *Candida tropicalis* [10] and activated sludge [11].

In recent years, the strain of *P. putida* has been the most widely used type of bacteria for phenol biodegradation. Under aerobic conditions, phenol may be converted by the bacterial biomass to carbon

* Corresponding author. Tel.: +971 3 713 3637; fax: +971 3 762 4262.
E-mail address: muftah@uaeu.ac.ae (M.H. El-Naas).
[1] Present address: Department of Chemical Engineering, Qatar University, Doha, Qatar.

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.08.059

dioxide; other intermediates such as benzoate, catechol, *cis–cis*-muconate, β-ketoadipate, succinate and acetate are known to be formed during the biodegradation process [12]. *P. putida* has been studied by many researchers in free and immobilized forms, using different types of bioreactors [2,13–23].

Immobilization of bacterial biomass for the degradation of phenol is an important and effective technique that is usually employed to serve several purposes, including protection of the bacteria from high phenol concentrations as well as ease of separation and reutilization of the biomass. The overall efficiency of the bacterial biomass in the biodegradation of phenol may be affected by many factors such as phenol concentration [24], temperature [25], the presence of other nutrients [26], the presence of other pollutants [27] and bacterial abundance [28].

The main objective of this study is to assess the biodegradation of phenol by *P. putida* immobilized in polyvinyl alcohol (PVA) gel matrix at different conditions in a bubble column bioreactor. The article examines many important factors that have not been addressed in the literature including the immobilization of *P. putida* in a PVA gel matrix, the effect of other contaminants on the biodegradation rate of phenol as well as the effect of particle size and hence mass transfer limitations on the biodegradation rate.

2. Materials and methods

2.1. Preparation of microbial culture

A special strain of the bacterium *P. putida* was obtained in an Amnite (P300) cereal form from Cleveland Biotech Ltd., UK. A 100 g of the cereal was mixed in a 1000 ml of 0.22% sodium hexametaphosphate buffered with Na_2CO_3 to a pH of 8.5. The mixture was homogenized in a blender for about 1 h, decanted and kept in the refrigerator at 4 °C for 24 h. Bacteria slurry was prepared by four consecutive steps of low speed centrifugation at 6000 rpm for 15 min. The supernatants were collected and centrifuged again at 10,000 rpm for 20 min. The precipitated amount from the three centrifugations (which contains the harvested bacteria cells) were collected, suspended as slurry in distilled water and kept in the refrigerator for subsequent immobilization. A similar extraction procedure was found to be effective for the extraction of microorganisms from soil [29].

2.2. Immobilization

Polyvinyl alcohol gel was used for immobilizing the bacteria cells. As a synthetic polymer, PVA has better mechanical properties, and it is more durable than Ca-alginate which is biodegradable and can be subject to abrasion [30]. A homogenous 10 wt% PVA viscous solution was prepared by mixing 100 g of PVA powder with 900 ml of distilled water at about 70–80 °C. The 10% mixture is known to result in a good quality polymer matrix with high porosity [31]. The formed mixture was allowed to cool to room temperature before adding 10 ml of the previously prepared bacterial suspension. It was then well stirred for 10–15 min to insure the homogeneity of the whole solution. The mixture was then poured into special molds and kept in a freezer at −20 °C for 24 h before it was transferred to the refrigerator and allowed to thaw at about 4 °C. This gives the gel lower thawing rate and enhances the crystalline area formation, which increases the mechanical strength of the formed polymer. The freezing–thawing process was repeated 3–4 times for 5 h for each cycle. This improves the cross-linking in the polymerized PVA gel structure. The frozen molds were then cut into the specified sizes, washed with distilled water to remove any uncross-linked chains, and then sent for acclimatization.

Table 1
Composition of nutrient mineral salt medium

Component	Concentration (mg/l)
$MgSO_4 \cdot 7H_2O$	300
K_2HPO_4	250
$CaCl_2 \cdot 2H_2O$	150
$(NH_4)_2CO_3$	120
$FeSO_4 \cdot 7H_2O$	3.5
$ZnSO_4 \cdot 7H_2O$	1.3
$MnCl_2 \cdot 4H_2O$	0.13
$CuSO_4 \cdot 5H_2O$	0.018
$CoCl_2 \cdot 6H_2O$	0.015
$Na_2MoO_4 \cdot 2H_2O$	0.013

2.3. Acclimatization of bacteria

A portion of the cut PVA gel (containing immobilized bacteria) was placed in a 1 l solution containing 100 mg/l of glucose (as an easily biodegradable source of organic compounds) in addition to other essential mineral nutrients (with concentrations shown in Table 1) with continuous aeration. The bacteria cells were activated by gradually increasing the concentration of glucose to 1000 mg/l over a period of 5 days. The activation of the bacteria was confirmed through microscopic analysis as well as observed reduction in the glucose concentration. Once activated, the bacteria cells were then slowly acclimatized to phenol concentrations of up to 300 mg/l. This process was achieved by gradual increase in the phenol concentration (10 mg/(l day)) in combination with a gradual reduction in the glucose concentration. The increase of phenol (accompanied with the decrease of glucose) is made after confirming that the bacteria has consumed all the organics in the previous batch (both glucose and phenol) and washing the PVA gel pieces with distilled water before being placed in a solution containing the new concentrations of phenol and glucose. This process allows the biomass to gradually adapt to the biodegradation of phenol as a substitute organic compound.

Once the maximum concentration (300 mg/l) of phenol is reached, the concentration of glucose would be dropped to zero. At this stage, the bacteria are considered fully acclimatized to phenol with concentrations up to 300 mg/l as the only source of organic compounds. The PVA pellets with the phenol acclimated bacteria were then used for the experimental study to evaluate the effect of different parameters on the biodegradation rate. A 300 ml of these pellets were placed in a 1 liter glass bubble column with 700 ml of synthetic phenol solution. The concentration of the mineral nutrients, which were added at the beginning of each run, was always kept constant at 82.6 mg/l.

2.4. Reagents

Analytical grade phenol was purchased from BDH Chemicals, UK. Synthetic phenol solutions were prepared for the desired concentration in distilled water before each experimental run. The solutions were always kept in brown flasks inside a dark cabinet to avoid light oxidation of the phenol. All other chemicals and PVA powder were of analytical grade and were also obtained from BDH, UK.

2.5. Analytical methods

Phenol concentration in the biomass free samples was determined quantitatively using Chrompack Gas Chromatograph, Model CP9001. The accuracy of the analyzer was checked to be within ±0.5 mg/l and confirmed for low concentrations (less than 50 mg/l) using a Shimadzu UV Spectrophotometer, Model UV-2450. Mea-