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Biological phenol removal using immobilized cells in a pulsed plate bioreactor: Effect of dilution rate and influent phenol concentration

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Abstract

The continuous aerobic biodegradation of phenol in synthetic wastewater was carried out using *Nocardia hydrocarbonoxydans* immobilized over glass beads packed between the plates in a pulsed plate bioreactor at a frequency of pulsation of 0.5 s^{-1} and amplitude of 4.7 cm. The influence of dilution rate and influent phenol concentration on start up and steady state performance of the bioreactor was studied. The time taken to reach steady state has increased with increase in dilution rate and influent phenol concentration. It was found that, as the dilution rate is increased, the percentage degradation has decreased. Steady state percentage degradation was also reduced with increased influent phenol concentration. Almost 100% degradation of 300 and 500 ppm influent phenol could be achieved at a dilution rate of 0.4094 h^{-1} and more than 99% degradation could be achieved with higher dilution rates. At a higher dilution rate of 1.0235 h^{-1} and at concentrations of 800 and 900 ppm the percentage degradation has reduced to around 94% and 93%, respectively. The attached biomass dry weight, biofilm thickness and biofilm density at steady state were influenced by influent phenol concentration and dilution rate.
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Keywords: Biodegradation; Dilution rate; Biofilm; Pulsed plate bioreactor; Immobilization

1. Introduction

Phenol is an aromatic pollutant, which is present in the waste waters of numerous industries including oil refining, chemical, petrochemical, textiles, dye stuffs, coal coking, coal gasification and steel industries [1–3]. These wastewaters frequently contain high concentrations of phenolic compounds [4], which represent a serious ecological problem due to their widespread use, toxicity and occurrence throughout the environment [5]. Aerobic processes of biological treatment are generally preferred to degrade these substances [6], due to the low costs associated with this option, as well as the possibility of complete mineralization of the xenobiotic [7].

Decades of operation involving the leaking of gaskets, improper handling and technical troubles can contaminate soil and ground waters with phenolic compounds. During cleaning and washing periods, the concentrations in the wastewaters may exceed the limiting value that can be treated by conventional biological methods using activated sludge [8] without disturbing its normal function [9]. It has been demonstrated that various toxic organic compounds are not eliminated by the conventional biological effluent treatment systems, due to the presence of relatively high concentrations of easily biodegradable substances [10]. Furthermore, the treatment of small volumes of concentrated toxic compounds at the site of emission, using specific microbial strains and better reactors, is preferable as this procedure allows a higher control over the process and higher removal efficiencies than those obtained in conventional treatment plants [11]. Aerobic biodegradation of many classes of aromatic compounds is common and proceeds through the key intermediate, catechol. Many microbial strains capable of degrading phenol [12–20] have been cited in Table 1. Most of the cultures tested are capable of degrading phenol at low concentrations. However phenol is toxic to most types of microorganisms at sufficiently high concentration and can be a growth rate inhibitory to even those species, which have the metabolic capability of using it as a substrate for growth. So, for achieving satisfactory performance, phenol concentration needs to be maintained below toxic limits and acclimatization of organism to the wastewater environment is required [21].

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Table 1
Microbial strains capable of degrading phenol

Culture	T (°C)	pH	S_{max} (ppm)	S_{tol} (ppm)	Reference
Pseudomonas putida CCRC14365	30	6.8	96.43	610	[12]
P. putida DSM 548	30	6.8	18.3	100	[13]
P. putida	–	–	44.39	800	[14]
P. putida ATCC17514	–	–	49.86	–	[15]
P. putida ATCC 700007	30	7	87.98	200	[16]
P. fluoroescens	–	–	131.18	–	[17]
Acinetobacter	30	–	19.36	350	[18]
Trichosporon cutaneum R57	–	–	204.45	–	[19]
Candida tropicalis	–	–	49.32	–	[20]

Since phenol is an inhibitory substrate for most species, the Haldane equation [22] has been frequently used to model phenol degradation. According to this model, maximum growth rate occurs at phenol concentration, $S_{max} = \sqrt{K_s K_I}$ [22] where K_s is the half saturation coefficient (ppm) and K_I is the substrate inhibition coefficient (ppm). At phenol concentrations greater than S_{max}, specific growth rate decreases with increasing concentration. The phenol concentration above which, substrate inhibition occurs (S_{max}) has been calculated using the values of K_s and K_I reported in literature for different species that degrade phenol. Table 1 shows S_{max} values and the maximum concentration being able to be degraded (S_{tol}) for different species.

Nocardia hydrocarbonoxydans, an actinomycetes, was found to effectively degrade phenol [23,24] and is resistant to contamination [24]. For acclimatized *N. hydrocarbonoxydans* S_{max} was found to be 74.26 ppm [25]. It has higher inhibitory concentration level, as compared to many microbial species degrading phenol (Table 1). So it has been chosen for the present study.

A pulsed plate column with the space between the plates packed with glass particles immobilized with the cells has been used as a bioreactor for the biodegradation of phenol. The potential of this column to be used as a bioreactor, the advantages of using immobilised cells and the advantages of this bioreactor over other kinds of bioreactors have been reported earlier [23]. The present work deals with the effect of dilution rate and influent phenol concentrations on start up and steady state performance of the pulsed plate bioreactor, with the immobilised cells of *N. hydrocarbonoxydans*, used for biodegradation of phenol.

2. Materials and methods

2.1. Microorganism and subculture

N. hydrocarbonoxydans (NCIM 2386) chosen for the present study by virtue of its effectiveness to degrade phenolic waste was obtained from NCIM, a division of National Chemical Laboratories, Pune, India. The strains were periodically sub cultured once in 15 days on agar slants and were stored at 4 °C.

2.2. Nutrient media and culture preparation

Organisms were grown on phenol as the sole carbon and energy source and the mineral medium of following composition was used: ammonium nitrate (1 g/l), ammonium sulphate (0.50 g/l), sodium chloride (0.50 g/l), di-potassium hydrogen orthophosphate (1.5 g/l), potassium di-hydrogen orthophosphate (0.5 g/l), ferrous sulphate (0.002 g/l), calcium chloride (0.01 g/l), magnesium sulphate (0.5 g/l) in distilled water. To prevent the precipitation of Ca++ and Mg++, a solution of calcium chloride and magnesium sulphate were prepared as concentrated solution B and the solution of other chemicals as solution A, both being steam sterilized separately and then mixed in sufficient volumes aseptically, when cooled. pH of the solution was adjusted to 7.0 by using 0.1N NaOH. Organisms were acclimatized gradually for different phenol concentrations according to the procedure explained elsewhere [23]. Phenol concentrations used for acclimatisation were 100, 200, 300, 400, 500, 600, 800 and 900 ppm. The acclimatized cultures were then immobilized on glass beads following the method described elsewhere [23]. Satisfactory growth of *N. hydrocarbonoxydans* was found up to an initial phenol concentration of 900 ppm under batch conditions. The organisms were found to grow in 1000 ppm phenol sometimes but not always. So 900 ppm can be considered as the tolerance limit for the organism. So in the present work a maximum concentration of 900 ppm was used.

2.3. Analytical procedures

Procedures for estimation of Biofilm thickness, attached biomass dry weight and biofilm density are described elsewhere [23].

Phenol analysis was done by measurement of absorbance at a wavelength of 510 nm using Hitachi UV–vis spectrophotometer, after colour development by 4-aminoantipyrene method [26].

2.4. Experimental bioreactor

The schematic diagram and detailed description of the experimental pulsed plate bioreactor are given elsewhere [23]. The space between the plates, forming each stage in the bioreactor, was filled with 1600 (approximately 40 g) glass beads, immobilized with *N. hydrocarbonoxydans* (NCIM 2386) acclimatised previously to the corresponding phenol concentrations of synthetic wastewater. The reactor outlet is through the port at 37 cm from the bottom of the column. The working volume of the reactor is 0.977 l. Synthetic phenol solution in tap water with different concentrations of phenol and all the other nutrients in concentrations as indicated in Section 2.2, were pumped from

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