Combined effect of plate pulsation parameters and phenol concentrations on the phenol removal efficiency of a pulsed plate bioreactor with immobilized cells

K. V. Shetty, M. R. Kedargol and G. Srinikethan

ABSTRACT

Continuous aerobic biodegradation of phenol in synthetic wastewater with phenol at different concentrations (200, 300, 500, 800 and 900 ppm) was carried out in a pulsed plate column, which is used as a bioreactor with immobilised cells of *Nocardia hydrocarbonoxydans* (NCIM 2386) at a dilution rate of 0.4094 h^{-1} and amplitude of 4.7 cm at various frequencies of pulsation (0, 0.25, 0.5, 0.75 and 1 s^{-1}). The effect of frequency of pulsation on the steady state performance of the bioreactor for phenol biodegradation at different influent concentrations was studied. Percentage degradations were observed to be a combined effect of volumetric phenol loading, reactor residence time, mass transfer limitations and phenol inhibition effect. At 500 ppm influent phenol concentration the effect of frequencies of pulsation on the steady state percentage degradation at different amplitudes was studied. The percentage degradation increased with increase in frequency and almost 100% degradation was achieved at 0.75 s^{-1}, 0.5 s^{-1} or 0.25 s^{-1}, with 3.3, 4.7 or 6.0 cm amplitudes respectively and hence the vibrational velocity (amplitude * frequency) was found to influence the steady state performance of the reactor. It was found that optimum vibrational velocities need to be fixed for maximum removal efficiency of the bioreactor depending on the influent phenol concentration.

Key words | amplitude, frequency, *Nocardia hydrocarbonoxydans*, phenol, pulsed plate bioreactor, vibrational velocity

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INTRODUCTION

Industrialization and technological advancement have led to the introduction of hazardous chemicals into the environment. During the last few decades, an array of foreign compounds to biological systems called "xenobiotics" has been introduced into the environment. In the last decade, the production of synthetic chemicals has continuously grown all over the world. Such a massive production and use of chemicals is the main cause of environmental pollution. These industrial plants generate increasing amounts of wastewater, contaminated with different toxic and hazardous organic compounds, which serve problems to the environment.

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Phenol is one of the most widely used compounds in existence and it ranks in the top 50 in production volumes for chemicals produced in the United States. Table 1 shows the theoretical distribution in the environment resulted for phenol using the distribution model according to Mackay: level 1 (ECB 2006)

The hydrosphere is therefore the target compartment for phenol in the environment. The pollution of the surface water and ground water with phenol is a highly important environmental problem, first of all because of the propagation of the pollution, and second because of its unfavorable consequences on the aquatic and human life, on the

Table 1 | Distribution of phenol

Compartment	Percentage
Air	0.8
Water	98.8
Soil	0.2
Sediment	0.2

organoleptic properties and uses of water. Phenol is considered to be a toxic compound by the Agency for Toxic Substances and Disease Registry (ATSDR 2006). Phenol is classified as a priority pollutant by the US Environmental Protection Agency owing to its high toxicity and wide spread environmental occurrence (USEPA 2006c).

It is a common constituent of wastewater originating from numerous industries including oil refining, chemical, petrochemical, textiles, dye stuffs, resins and plastics, agricultural chemicals, tanning, coal coking, coal gasification,steel industries, fiberboard production and paint stripping operations, pulp and paper, pesticides, medications, pharmaceuticals, food processing industries and from surface runoff from coal mines (Entezari & Petrier 2004; Lathasreea *et al.* 2004; Beltran *et al.* 2005; Entezari & Petrier 2005; Mahamuni & Pandit 2005; Vinod & Reddy 2006). These wastewaters frequently contain high concentrations of phenolic compounds (Chang *et al.* 1998), which represent a serious ecological problem due to their widespread use, toxicity and occurrence throughout the environment (Fava *et al.* 1995). A water-soluble compound, phenol ultimately find ways into rivers, lakes and oceans and generally found to contaminate the water bodies that are situated near, or are, the receiving waters of industrial factories. This contamination can affect the water beneath the surface of the earth, ground water. Comprising over 70% of the Earth's surface, water is undoubtedly the most precious natural resource that exists on our planet. As a result, governments have passed laws limiting the amounts and kinds of wastes that can be dumped into water.

Most of the countries specify the maximum allowable concentration of phenol in the industrial effluent streams to be less than 1 mg/l (Mahamuni & Pandit 2005). Central Pollution Control Board, India, has set the limit for phenol in effluent to be discharged to water bodies as less than 1 mg/l. There is a proposal for making the norms more stringent. Proposed minimal effluent standard for petroleum oil refinery is less than 0.35 mg/l phenol. With the increasingly stringent national and international regulations governing wastewater treatment in today's environment, there is a great need to develop innovative and more efficient wastewater treatment methods to reduce phenol concentration in wastewater to acceptable levels. The problem of cleaning/recovery of the phenolic wastewater was and still is a matter of concern for the researchers and the designers, and they are either searching for new methods, or trying to improve the existing ones.

Different methods of treatment are available for reduction of phenol content in wastewater (Throop 1975/1976; Vinod & Reddy 2003). The treatment processes must guarantee the elimination or recuperation of the pollutant in order to reach the strict authorized levels for the discharge of these effluents. The probable technologies are, adsorption, solvent extraction, incineration, wet oxidation, electrochemical oxidation, conventional chemical oxidation processes and advanced oxidation processes as well as biological oxidation process. Physical and chemical methods for phenol removal are costly, of limited applicability at high concentration or they transfer it from one phase to another, cannot remove phenol completely or they convert it into some other form, which may be harmful causing secondary pollution. Biological oxidation or biodegradation is especially attractive because it has the potential to almost completely degrade phenol while producing innocuous end products. In addition, it has the advantage of reduced capital and operating cost because of operation at ambient conditions. In the absence of high concentration of toxic substances, or in instances of their successful prior removal, biological treatment is widely employed for treatment of wastewater containing intermediate phenol levels (5–500 mg/L) (Patterson 1985). It has been demonstrated that various toxic organic compounds are not eliminated by the conventional biological effluent treatment systems like activated sludge process, due to the presence of relatively high concentrations of easily biodegradable substances (Ordaz *et al.* 2001). 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 (Schröder *et al.* 1997).

Many microbial strains were reported to be capable of degrading phenol (Shetty *et al.* 2007*b*). *Nocardia hydrocarbonoxydans*, an actinomycete, is a prominent soil microorganisnm and was found to effectively degrade phenol (Vidyavathi 1998; Shetty *et al.* 2007*a*) and is resistant to contamination (Vidyavathi 1998). It has higher inhibitory concentration level, as compared to many microbial species degrading phenol (Shetty *et al.* 2007*b*). Further, very little work was reported on the application of actinomycetes in biodegradation in general and the biodegradation of phenol, in particular. Especially, studies on continuous biodegradation of phenol using actinomycetes are very scarce. Hence it has been chosen in the present study for the biodegradation of phenol.

The phenomena of mixing and mass transfer of substrates and oxygen to microorganisms greatly affect any aerobic biochemical reactions. A suitable bioreactor is to be developed considering the efficiency of aeration and agitation of a mixing device for a particular application. The parameters considered for the evaluation of the suitability and efficiency are the homogeneity in the reactor, oxygen mass transfer rate, the shear stress produced by the mixing element and the ease of scale-up. Recently considerable attention has been focused towards using immobilized cells for the degradation of stable organic compounds present in the industrial effluents in continuous reactors, due to high cell density in the reactor, even beyond washout conditions and an increased resistance to the detrimental effects of toxic shock loadings (Worden & Donaldson 1987). Immobilization binds the microorganism to a solid support and the sludge separation unit is not required. Many researchers compared phenol removal using free cells and attached growth systems in laboratory experiments (Chen *et al.* 2002; Prieto *et al.* 2002; Tziotzios *et al.* 2005). In all these cases the efficiency of the immobilized cells was higher than that of the free suspended cells. In order to eliminate the sludge separation unit and cell washout problems of suspended cell systems and to overcome the difficulties of fixed bed systems with immobilized cells like mass transfer limitations, an innovative bioreactor was developed recently for the biodegradation of phenol (Shetty *et al.* 2007*a*,*b*). It is a pulsed plate column, with the space between the plates packed with glass beads as carrier particles on which the microorganisms are immobilized.

The present work deals with the combined effect of influent phenol concentrations and the plate pulsation parameters such as amplitude and frequency on the phenol removal efficiency of this bioreactor with immobilized cells of *Nocardia hydrocarbonoxydans* at steady state.

METHODS

Microorganism and subculture

Nocardia hydrocarbonoxydans (NCIM 2386) chosen for the present study was obtained from NCIM, a division of National Chemical Laboratories, Pune, India. The strains were periodically sub cultured once in fifteen days on agar slants and were stored at 4°C.

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^{2+} and Mg^{2+}, 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 0.1 N NaOH. Organisms were acclimatized gradually for different phenol concentrations according to the procedure explained elsewhere (Shetty *et al.* 2007*a*). 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 (Shetty *et al.* 2007*a*).

Phenol analysis

Phenol analysis was done by measurement of absorbance at a wavelength of 510 nm using Hitachi UV-VIS

Spectrophotometer, after colour development by the 4-aminoantipyrene method (Standard Methods 1975).

Experimental bioreactor

The bioreactor used is a pulsed plate column containing five perforated plates fixed onto a central shaft at a plate spacing of 3 cm. The entire plate stack is covered with a nylon wire mesh. The space between the plates, forming each stage in the bioreactor, was filled with 1,600 (approximately 40 g) glass beads, immobilized with *Nocardia hydrocarbonoxydans* (NCIM 2386) acclimatised previously to the corresponding phenol concentrations in synthetic waste water. The entire stack of plates can be pulsated at the required frequency(f) and amplitude(A). The schematic diagram of the experimental pulsed plate bioreactor and the detailed description of the bioreactor is given elsewhere (Shetty *et al.* 2007*a*). The frequency of pulsation was set at the required value using the variable voltage speed regulator and the amplitude was set, by changing the position of the crankshaft. The working volume of the reactor was 0.977 L. Synthetic phenol solution in tap water with different concentrations of phenol and all the other nutrients in concentrations as indicated in an earlier section, were pumped from the bottom using a peristaltic pump. Compressed air was continuously passed from the bottom through a constant air pressure regulator, at a flow rate of 1.7–1.8 LPM to ensure proper supply of oxygen to the microorganisms and dissolved oxygen concentration was maintained at around 5–6 mg/l. The concentrations of phenol in the effluent from the column (from port at 37 cm from bottom) were analysed at regular intervals of time during start-up till steady state was attained. Steady state conditions were considered when the phenol concentration in the effluent remained constant for a period of 12 h (Shetty *et al.* 2007*b*)

RESULTS AND DISCUSSION

Effect of frequency of pulsation on phenol degradation at different influent phenol concentrations

Continuous aerobic biodegradation of phenol in synthetic waste water containing phenol at different concentrations (200, 300, 500, 800 and 900 ppm) were carried out in the pulsed plate bioreactor at a dilution rate(D) of 0.4094 h^{-1} and amplitude of 4.7 cm at various frequencies of pulsation (0, 0.25, 0.5, 0.75 and 1 s^{-1}). Figure 1 presents the effect of frequency of pulsation on steady state percentage degradation at different influent concentrations (Si) and at amplitude of 4.7 cm. Almost 100% degradation of 200 and 300 ppm influent phenol were achieved both under non-pulsed condition and pulsed condition of all frequencies. Introduction of pulsation or increased pulsation decreases the mass transfer resistance across the liquid film, around the biofilms formed by immobilized cells. But mass transfer limitations do not play a role at low influent phenol concentrations of 200 and 300 ppm since the volumetric phenol loading is very low and the residence time provided in the reactor are more than sufficient for the complete degradation of phenol. At 500, 800 and 900 ppm influent phenol concentrations, percentage degradations achieved under pulsed conditions were higher than the non-pulsed condition. At 500 ppm influent phenol concentration as the frequency of pulsation was increased the percentage degradation increased and almost 100% degradation was achieved at frequencies greater than or equal to 0.5 s^{-1}.

As shown in Figure 1, with 800 and 900 ppm influent phenol concentrations, as the frequency of pulsation was increased up to 0.5 s^{-1}, the percentage degradation increased. But with further increase in frequency to 0.75 s^{-1}, the percentage degradation decreased and then remained almost constant. The maximum percentage degradation of almost 97% occurred at $f = 0.5\ s^{-1}$ for both 800 and 900 ppm influent phenol. With 800 or 900 ppm influent phenol concentrations, the mass transfer resistance

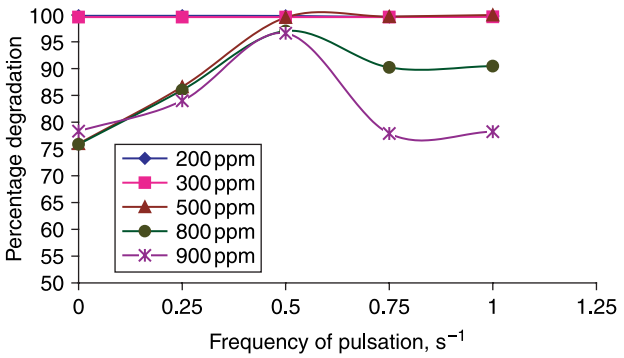

Figure 1 | Effect of frequency of pulsation on steady state percentage degradation of phenol at different influent phenol concentrations. Conditions: D = 0.4094 h^{-1}; A = 4.7 cm.

would have reduced with increase in frequency up to 0.5 s^{-1}, so percentage degradation has increased. When the frequency was increased further to 0.75 s^{-1}, the mass transfer resistance would have reduced further, so biofilm surface concentrations of phenol may be very close to bulk concentrations. These concentrations to which the organism gets exposed are inhibitory concentrations. The steady state bulk concentrations under these cases are higher than 74.26 ppm. Growth of *Nocardia hydrocarbonoxydans* is inhibited at phenol concentrations >74.26 ppm i.e. the growth kinetics for *Nocardia* fits Haldane substrate inhibition model (Shetty *et al.* 2007*b*). So at influent concentrations of 800 and 900 ppm, growth of organisms might have got inhibited at frequencies of 0.75 s^{-1} and above, leading to reduction in percentage degradation.

Effect of frequency of pulsation on phenol degradation at different amplitudes

Continuous biodegradation of phenol were carried out with 500 ppm influent phenol concentration and the dilution rate of 0.4094 h^{-1} and the effect of frequencies of pulsation on the steady state percentage degradations at different amplitudes (3.3, 4.7 and 6.0 cm) were studied. The steady state percentage degradations obtained at different frequencies and amplitudes are presented in Figure 2.

At a fixed amplitude of 3.3 cm or 4.7 cm, as the frequency of pulsation was increased the percentage degradation increased and almost 100% degradation was achieved at frequencies greater than or equal to 0.75 s^{-1} or 0.5 s^{-1} respectively. Similarly at an amplitude of 6.0 cm, almost

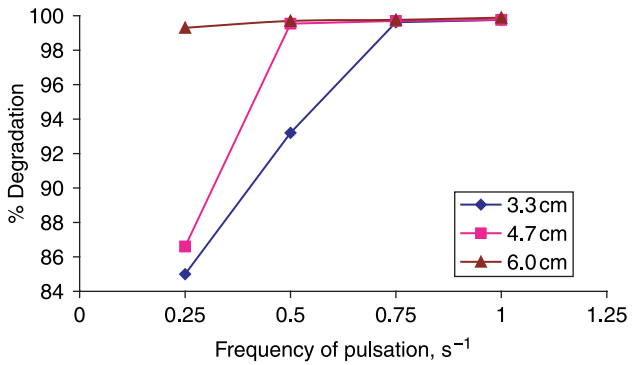

Figure 2 | Effect of frequency of pulsation on steady state percentage degradation at different amplitudes. Conditions: D = 0.4094 h^{-1}, Si = 500 ppm.

100% degradation was achieved at all frequencies greater than or equal to 0.25 s^{-1}. When the influent phenol concentration was 500 ppm, the residence time in the reactor may be sufficient for the complete removal of phenol loading to the reactor, provided, mass transfer limitations are completely overcome. At a frequency $\geq$0.25 s^{-1} with 6.0 cm amplitude, frequency $\geq$0.5 s^{-1} with 4.7 cm amplitude and frequency $\geq$0.75 s^{-1} with 3.3 cm amplitude, the turbulence created was sufficient to overcome the mass transfer limitations for the almost complete removal of 500 ppm phenol. As the frequency was increased, the mass transfer resistance for phenol and oxygen transfer, through the external liquid film around the biofilm was reduced, by thinning down of the liquid film, resulting in higher mass flux across the film.

So the concentration at the biofilm surface comes closer to the bulk concentration, making higher substrate and oxygen concentrations available in the vicinity of the microorganism for their growth. This enhances the percentage degradation with increased frequency and almost 100% degradation was achieved at 0.75 s^{-1}, 0.5 s^{-1} or 0.25 s^{-1}, with 3.3, 4.7 or 6.0 cm amplitudes respectively. When the frequency is increased, the mixing time may be reduced. But when the amplitude is increased, the mixing zone or zone of influence of pulsation increases. Frequency of pulsation contributes to turbulence whereas amplitude contributes to region of influence of turbulence. In other words, increase in amplitude increases the pulsed volume of liquid or volumetric rate of movement of liquid. This will increase the rate of mass transfer of oxygen to the liquid phase and the transfer of phenol and oxygen to the solid phase. More of the reactor liquid will be in repeated contact with bio particles, hence increasing the active volume of the reactor. At higher amplitudes, volumetric rate of movement of liquid, brought about even at lower frequencies, may be sufficient to overcome the mass transfer limitations in the column. So the frequency, at or above which, almost 100% degradation of 500 ppm phenol could be achieved, has decreased with increase in amplitude.

Effect of vibrational velocity (A * f)

Results discussed in previous section reveal that the performance of pulsed plate bioreactor depends on the combined

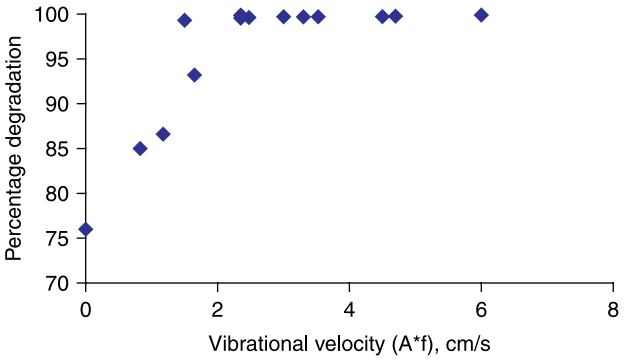

Figure 3 | Effect of vibrational velocity on percentage degradation. Conditions: D = 0.4094 h^{-1}1; Si = 500 ppm.

effect of the frequency and amplitudes of pulsation. So a combination parameter called vibrational velocity (A ∗ f) which was found to have profound effect on the mass transfer characteristics of the bioreactor, is considered and its effect on the performance of the bioreactor was studied. Effect of vibrational velocities obtained by varying the frequency and amplitude, on the steady state performance of the reactor at a dilution rate of 0.4094 h^{-1} for 500 ppm influent phenol concentration is presented in Figure 3.

Different frequency and amplitude combinations and the corresponding vibrational velocities (A ∗ f) used for different runs are shown in Table 2. Figure 3 shows that the percentage degradation has increased with the increase in vibrational velocity,reaching almost 100% at a vibrational velocity of 2.35 cm/s and remained constant at almost 100% degradation with further increase in vibrational velocity. The experiments were also carried out at different frequency and amplitude combinations that gave same vibrational velocity of 2.35 cm/s and the results are shown in Table 3.

Table 2 | Vibrational velocities at different frequency and amplitude combinations used for different runs at Si = 500 ppm; D = 0.4094 h^{-1}

Amplitude, cm	Frequency, s^{-1}	Vibrational velocity (A ∗ f)
0	0	0
3.3	0.25	0.825
4.7	0.25	1.175
6	0.25	1.5
3.3	0.5	1.65
4.7	0.5	2.35
3.3	0.75	2.475
6	0.5	3
3.3	1	3.3
4.7	0.75	3.525
6	0.75	4.5
4.7	1	4.7
6	1	6
3.3	0.712	2.35
6	0.392	2.35

Table 3 | Effect of different frequency and amplitude combinations at same vibrational velocity on percentage degradation at Si = 500 ppm; D = 0.4094 h^{-1}

Amplitude, cm	Frequency, s^{-1}	Vibrational velocity (A ∗ f)	Percentage degradation
4.7	0.5	2.35	99.54
3.3	0.712	2.35	99.86
6	0.392	2.35	99.86

Table 3 shows that the percentage degradation obtained were almost same at the same vibrational velocities of 2.35 cm/s, though the amplitude and frequencies were different for different runs. So, optimum vibrational velocities need to be fixed for maximum removal efficiency of the bioreactor depending on the influent phenol concentration.

CONCLUSIONS

The combined effects of pulsation parameters like frequency and amplitude of pulsation as well as influent phenol concentrations on the steady state performance of pulsed plate bioreactor for the biodegradation of phenol in synthetic wastewater with immobilized cells of *Nocardia hydrocarbonoxydans* were studied. Percentage degradation of phenol was observed to be a combined effect of volumetric phenol loading, reactor residence time, mass transfer limitations and phenol inhibition effect. The performance of pulsed plate bioreactor depends on the combined effect of the frequency and amplitudes of pulsation. So a combination parameter called vibrational velocity (A ∗ f) which was found to have profound effect on the mass transfer characteristics of the bioreactor, is considered and its effect on the performance of the bioreactor was studied. The vibrational velocity (amplitude ∗ frequency) was found to influence the steady state performance of the reactor. It was found that the percentage degradation obtained were almost same with

different frequency and amplitude combinations which provide the same vibrational velocities. It was found that optimum vibrational velocities need to be fixed for maximum removal efficiency of the bioreactor depending on the influent phenol concentration.

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