Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 149 (2007) 452–459
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

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

K. Vidya Shetty *, R. Ramanjaneyulu, G. Srinikethan

Department of Chemical Engineering, National Institute of Technology Karnataka, Surathkal, P.O. Srinivasanagar, 575025 Karnataka, India

Received 17 October 2006; received in revised form 24 March 2007; accepted 3 April 2007
Available online 12 April 2007

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.

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].

* Corresponding author. Tel.: +91 824 2222358; fax: +91 824 2474033.
E-mail addresses: vidyaks68@yahoo.com, vidyaks95@nitk.ac.in (K. Vidya Shetty), srini@nitk.ac.in (G. Srinikethan).

0304-3894/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2007.04.024

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

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 LPM to ensure proper supply of oxygen to the microorganisms and dissolved oxygen concentration was maintained at around 5–6 mg/l. The frequency of pulsation was set at 0.5 s^{-1} using the variable voltage speed regulator and the amplitude was set at 4.7 cm, by changing the position of the crankshaft. 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. When the phenol concentration attained a steady state value, the biofilm thickness and attached biomass dry weight also attained a constant value [27].

2.5. Experimentation

The performance of the bioreactor for biodegradation of phenol in a synthetic wastewater containing different phenol concentrations (S_i = 300, 500, 800 and 900 ppm) was studied for varying dilution rates (D = 0.4094, 0.6141, 0.8188 and 1.0235 h^{-1}) at a frequency of pulsation (f) of 0.5 s^{-1} and amplitude of pulsation (A) set at 4.7 cm. Continuous biodegradation studies were conducted in the reactor at these operating conditions, to study the effect of dilution rate and influent phenol concentration on the percentage degradation of phenol, biofilm thickness, attached biomass dry weight and biofilm density at steady state. After the steady state is attained, average biofilm thickness on the glass beads, attached biomass dry weights in the reactor and biofilm density were estimated [23].

3. Results and discussion

3.1. Start-up studies

The effect of dilution rate on the reactor effluent phenol concentration during start up of the bioreactor, at different influent phenol concentrations of 300, 500, 800 and 900 ppm are shown in Figs. 1–4. It is evident from these time course variations that, the rate of degradation during start up decreases with increase in dilution rate. From these plots, it can be seen that, during the start up of the bioreactor, there is a sharp drop in phenol concentration at the initial period, followed by a period of slowly decreasing concentration and then again a sharp decrease to reach steady state concentration. Initial sharp drop may be due to adsorption of phenol onto the biofilm surface. In biofilms, possible sorption sites are extra cellular polymeric substances, cell walls, cell membranes and cytoplasm. These sites contribute to the sorption properties of biofilms for organic and inorganic substances [28,29]. A faster sorption step due to physicochemical interactions between the organic chemicals and microbial cell walls has been reported [30–37]. During this phase the removal by biodegradation may be less significant. Organisms are initially acclimatized to and then grown, in respective inlet phenol concentrations under batch conditions, before immobilization. Immobilization and hence initial biofilm formation is also done

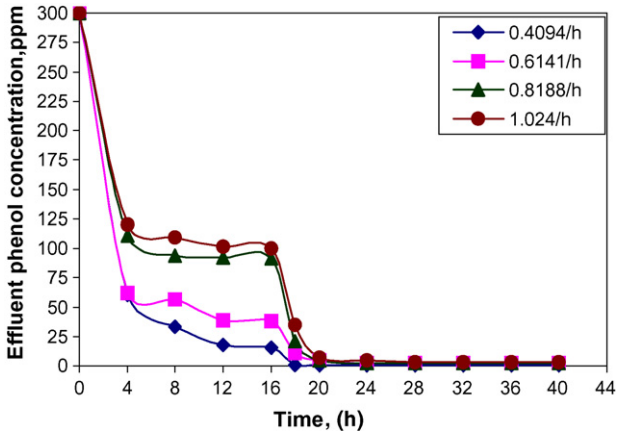

Fig. 1. Effect of dilution rate during start up of the bioreactor. Conditions: Si = 300 ppm; f = 0.5 s^{-1}; A = 4.7 cm.

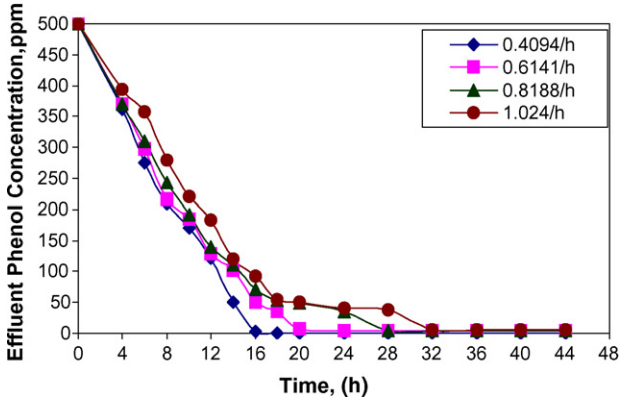

Fig. 2. Effect of dilution rate during start up of the bioreactor. Conditions: Si = 500 ppm; f = 0.5 s^{-1}; A = 4.7 cm.

under batch status. When these cells, which are acclimatized, grown and immobilized under batch conditions, are placed in the continuous reactor, they are exposed to increase in shear stress caused by frequently pulsing and flowing liquid as well as continuously flowing air. This may result in sloughing of the biomass. Sloughing is the detachment of large particles of biomass [38]. Sudden increase of shear stress on a biofilm causes

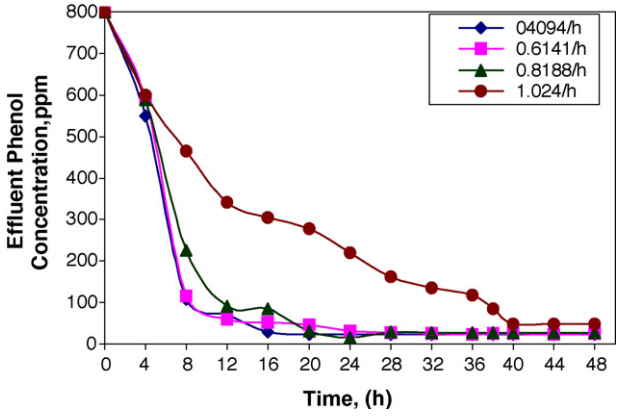

Fig. 3. Effect of dilution rate during start up of the bioreactor. Conditions: Si = 800 ppm; f = 0.5 s^{-1}; A = 4.7 cm.

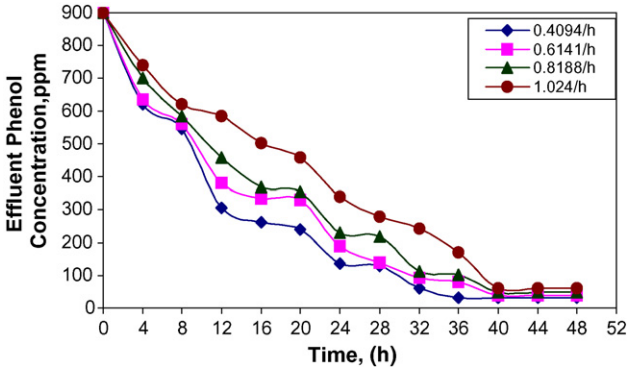

Fig. 4. Effect of dilution rate during start up of the bioreactor. Conditions: Si = 900 ppm; $f = 0.5\ s^{-1}$; $A = 4.7$ cm.

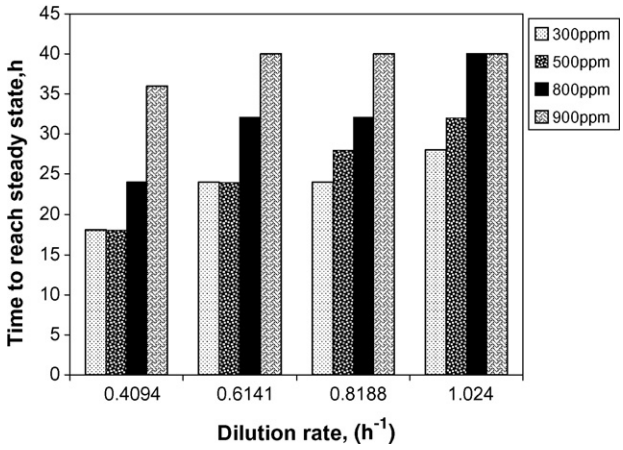

Fig. 5. Effect of dilution rate and influent concentrations on time taken to reach steady state.

sloughing and stimulates the secretion of EPS–polysaccharides [39]. The attached biomass dry weight may decrease during the adsorption phase due to sloughing. When the entire exposed surface of the biofilm gets saturated with phenol or reaches closer to saturation, then removal by adsorption becomes less significant as seen in the second phase. During the second phase of slowly decreasing concentration, biodegradation becomes significant. But the attached biomass dry-weight may be low and hence rate of biodegradation is lower. During this phase there may be drastic increase in the EPS–polysaccharides, as a direct response of the microbial communities to help biofilm reestablish after most of the biofilm was sloughed off [39]. EPS contribute to mechanical stability of the biofilms. Not only the shear stress, i.e. external forces, is decisive for the detachment but also the internal forces, i.e. the biofilm strength, contribute to the detachment [40]. When the biofilm reestablishes, detachment may be by erosion, but not by sloughing. Erosion refers to the continuous removal of individual cells or small groups of cells from the surface of the biofilm and is presumed to be the results of shear forces exerted by the moving fluid in contact with the biofilm surface [38]. Once the biofilm reestablishes, attached biomass dry weight will be higher and hence rate of biodegradation increases. So a third phase of sharp drop in phenol concentration is seen. Steady state biofilm thickness will be achieved when the net rate of growth of cells becomes equal to the rate of detachment of cells.

Fig. 5 shows the influence of dilution rate and influent phenol concentrations on the time taken to reach steady state. The time taken to reach steady state has increased with increase in dilution rate at any influent phenol concentration. As the dilution rate increases, flow velocity increases and may lead to increase in rate of biofilm shearing, as compared with the rate of cell growth. Due to higher cell washout during start-up, at higher dilution rates, longer time was taken to achieve steady and stable biofilm.

At any dilution rate, the time to reach steady state has increased with the increase in influent phenol concentration. As the influent phenol concentration increases, the initial monolayer of immobilized cells on the glass beads present in the reactor are exposed to higher and inhibitory phenol concentrations. Growth of *N. hydrocarbonoxydans* is inhibited at phenol concentrations >74.26 ppm i.e. the growth kinetics for Nocardia fits Haldane substrate inhibition model [25]. In Haldane model kinetics, at substrate concentrations greater than S_{max} (74.26 ppm in this case) the growth rate decreases with increasing phenol concentration. Though the bulk substrate concentrations at steady state were lesser than the inhibitory concentrations in all these experimental runs, during start up, the biofilm is exposed to inhibitory phenol concentrations. When the influent phenol concentration is higher, the biofilm is exposed to higher inhibitory phenol concentrations during start up. So higher growth inhibition is resulted at higher influent phenol concentrations and the growth rates of these cells will be lower. As the organisms grow during start up, the attached biomass dry weight increases and hence biofilm thickness increases as a function of time. But at higher influent concentrations, effect of inhibitory concentrations reaches even greater depth of biofilms. So the net growth rates in the biofilm are lowered as influent concentration is increased. So the time taken for the formation of a steady biofilm increases and the reactor takes longer time for the attainment of steady state, as the influent concentration is increased.

3.2. *Effect on steady state percentage degradation*

The effect of dilution rate and influent phenol concentration on the percentage degradation of phenol at steady state are shown in Fig. 6. The results in Fig. 6 indicate that as the dilution rate is increased, the percentage degradation is

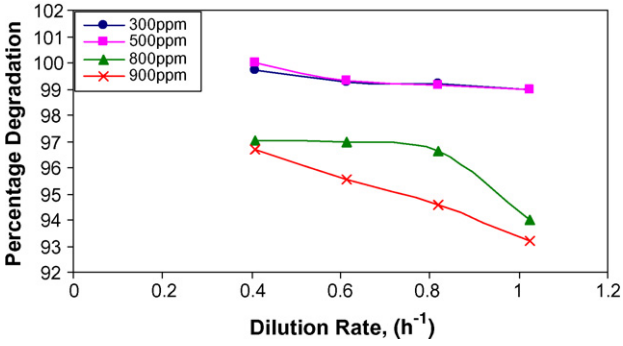

Fig. 6. Effect of dilution rate and influent phenol concentration on steady state percentage degradation of phenol. Conditions: $f = 0.5\ s^{-1}$; $A = 4.7$ cm.

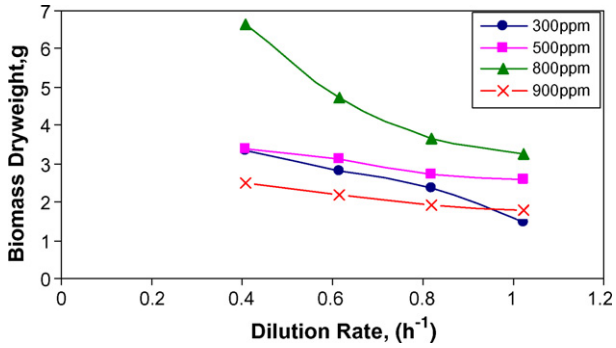

Fig. 7. Effect of dilution rate and influent phenol concentration on steady state biomass dry weight in the reactor. Conditions: $f = 0.5\ s^{-1}$; $A = 4.7$ cm.

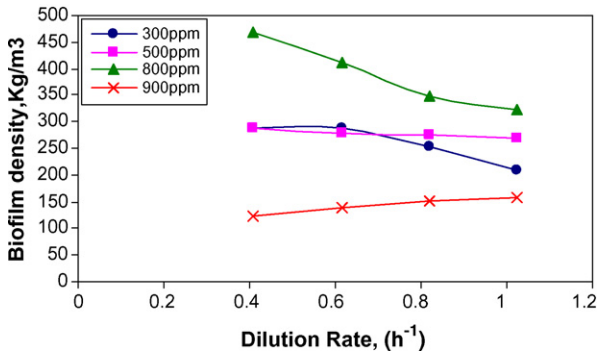

Fig. 9. Effect of dilution rate and influent phenol concentration on biofilm density at steady state. Conditions: $f = 0.5\ s^{-1}$; $A = 4.7$cm.

decreased. As the dilution rate increases, the residence time in the reactor decreases and this will decrease the time of contact between the cells and the substrate. This has resulted in decrease in percentage degradation of phenol. Fig. 6 also shows that at a dilution rate of 0.4094 h^{-1} and at influent phenol concentrations of 300 and 500 ppm, almost 100% degradation of phenol could be achieved. Even at higher dilution rates, more than 99% degradation of 300 and 500 ppm influent phenol was obtained. At a given dilution rate, the steady state percentage degradation has decreased with increase in influent phenol concentration. At a high 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. Reduction of percentage degradation with increased influent phenol concentration may be due to increase in the volumetric phenol-loading to the reactor. When the phenol degradation rate is less than the volumetric phenol-loading rate to the reactor, then complete removal of phenol does not take place. The percentage removal will depend on the ratio between the phenol degradation rate and the volumetric phenol-loading rate.

3.3. Effect on attached biomass dry weight, biofilm thickness and biofilm density at steady state

The effect of dilution rate and influent phenol concentration on the attached biomass dry weight, biofilm thickness and biofilm density at steady state are shown in Figs. 7–9. As the dilution rate is increased, the attached biomass dry weight and the biofilm thickness have decreased. With increase in dilution rate, the time of contact between the cells and substrate (residence time in the reactor) has reduced and this would have resulted in reduced cell growth. As the dilution rate increases, the flow velocity past the particle increases. This increased flow velocity might have resulted in higher erosion of the biomass from the biofilm surface. Reduced cell growth and increased cell detachment by erosion, would have resulted in reduction in attached biomass dry weight and biofilm thickness, with increased dilution rates.

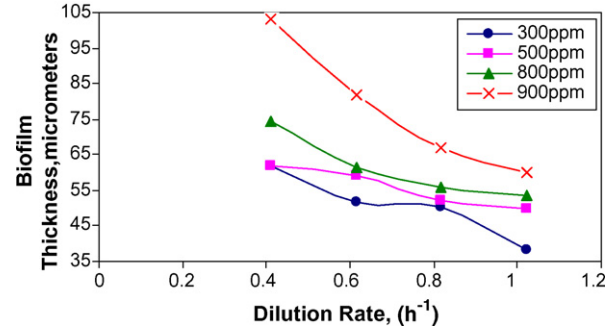

Fig. 8. Effect of dilution rate and influent phenol concentration on biofilm thickness at steady state. Conditions: $f = 0.5\ s^{-1}$; $A = 4.7$ cm.

Figs. 7 and 8show that, as the influent phenol concentration is increased from 300 to 800 ppm at the same dilution rate, the attached biomass dry weight and biofilm thickness have increased. At higher influent phenol concentration, the bulk phenol concentration at steady state is also higher. Though the concentrations of 300–800 ppm are inhibitory to the organisms, the bulk concentrations prevailing in the reactor at steady state are less than the inhibitory concentrations ($S_{max} = 74.26$ ppm). The growth rate of organisms is higher at higher bulk phenol concentrations, since all the steady state bulk concentrations are lower than S_{max}. So the biomass dry weight and biofilm thickness have increased with increase in influent phenol concentration. But with 900 ppm influent concentration, even though the bulk concentrations were lesser than the inhibitory concentrations, and greater than the bulk concentrations at 300–800 ppm influent concentrations, the biomass dry weight has reduced and the biofilm thickness has increased. It may probably be due to the structure or morphology of the biofilm formed, owing to the exposure of microorganisms to high substrate concentrations during start up. During start up, higher and inhibitory substrate concentrations would have reached deeper into the biofilm, making more of the biofilm influenced by substrate inhibition. This reduces the net growth rate of organisms and lead to reduction in biomass dry weight when influent phenol concentration is 900 ppm. The structure of biofilm formed at 900 ppm influent concentration may be such that, even under steady state conditions, they exhibit lower activity and hence reduced growth. Up to 800 ppm influent phenol concentrations, the start up bulk concentrations involved would not have changed the structure of the biofilm to that extent as to lower the activity.

Structure and morphology of the biofilm depends on the conditions at which they are formed. Slowly growing organisms produce more of exopolymers than the rapidly growing ones [41]. The EPS molecules are the exopolymers, which provide the forces responsible for cohesion of the biofilm and adhesion to the substratum. It seems that the composition of EPS is not constant but influenced by growth conditions and environmental stress [42]. Biomass is believed to detach when the tensile forces caused by the external shear exceed the tensile strength of the EPS matrix that holds together the biofilm [43,44] where the tensile strength of biomass matrix depends on physical conditions inside the biofilm [45]. At 900 ppm influent phenol concentration, due to substrate inhibition, organisms may be growing slowly during start up and hence produce more of exopolymers, which hold together the biofilm and imparts high tensile strength needed to resist shear at steady state. This would have resulted in increase in steady state biofilm thickness in spite of decreased biomass dry weight.

Fig. 9shows that as the influent phenol concentration is increased from 300 to 800 ppm, the biofilm density has increased. When the influent phenol concentration is 300, 500 and 800 ppm, the biofilm density has decreased with the increase in dilution rate. But at 900 ppm influent concentration, the biofilm density has increased with increase in dilution rate. At any dilution rate and at 900 ppm influent concentration, biofilm density is lesser than the densities of the biofilms obtained at lower concentrations of 300–800 ppm. Biofilm density will depend on the biofilm structure or morphology. The structure of the biofilms will be influenced by the substrate loading on the biofilm surface, the hydrodynamic conditions including shear stress on the biofilm and growth rate. It may be that biological mechanisms create an optimum structure that maximizes mass transport to deep layers within the biofilm and mechanical strength needed to resist detachment [45–48]. At steady state the biofilm structure and density adopts to the shear forces in such a manner that the detachment is balanced by the growth [46]. Earlier investigations of biofilm have shown that many biofilms possess a heterogeneous structure [49]. At the substratum, the biofilm cells can be arranged in a thin, dense layer of cells, to which are bound some dense, roundly shaped, micro colonies, filled with extra cellular polymers packed with microorganisms, and separated by interstitial voids. The space within the voids is filled with water or low concentrated extra cellular polymers. The interstitial voids are interconnected; forming a network of channels that create a characteristic porous structure [50–52]. Growth will not directly lead to uniform outgrowth of cells. In general protuberances or filamentous structures are formed. Convective transport inside the biofilm may be significant sometimes due to the presence of holes and channels connected to the bulk of liquid [53–56]. Many researchers used terms like “channels”, “patches”, “fluffy”, “rough”, “smooth” to describe biofilm morphology [57–66]. Therefore changes in biofilm density may be because of changes in their structure and morphology caused due to changes in substrate loading at the surface, hydrodynamic shear and growth rates as a combined effect of influent concentration and dilution rates.

4. Conclusions

It is evident from the start up studies that, the rate of degradation during start up decreases with increase in dilution rate. The time taken to reach steady state increases with increase in dilution rate and influent phenol concentration. Around 100% degradation could be achieved with 300 and 500 ppm influent phenol concentrations and at very low dilution rate of 0.4094 h^{-1} But at a high 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%. So the percentage degradation of phenol decreases with increase in both influent phenol concentration and dilution rate. Reduced cell growth and increased cell detachment by erosion, has resulted in reduction in attached biomass dry weight and biofilm thickness with increased dilution rates. As the influent phenol concentration is increased from 300 to 800 ppm at the same dilution rates, the biomass dry weight and biofilm thickness has increased owing to increase in growth rate of organisms at higher phenol concentrations. At a concentration of 900 ppm, the biomass dry weight has reduced but biofilm thickness has increased. Due to substrate inhibitory effect during start up, the structure of biofilm formed at 900 ppm influent concentration may be such that, even under steady state conditions, they exhibit lower activity but high tensile strength. Changes in biofilm density may be due to changes in the structure and morphology of biofilms caused due to changes in substrate loading at the surface, hydrodynamic shear and growth rates as a combined effect of influent concentration and dilution rates.

References

[1] P.C. Singer, F.K. Pfaender, J. Chincilli, J.C. Lamb, Composition and biodegradability of organics in coal conversion wastewaters, Symp. Proc. Environ. Aspects Fuel Convers. Technol. 3 (1977) 461–468.
[2] A. Maleki, A.H. Mahvi, F. Valzi, R. Nabizadeh, Ultrasonic degradation of phenol and determination of the oxidation byproducts toxicity, Iran J. Environ. Health. Sci. Eng. 2 (3) (2005) 201–206.
[3] A. Venu Vinod, G. Venkat Reddy, Mass transfer correlation for phenol biodegradation in a fluidised bed bioreactor, J. Hazard. Mater. 136 (3) (2006) 727–734.
[4] Y.H. Chang, C.T. Li, M.C. Chang, W.K. Shieh, Batch phenol degradation by *Candida tropicalis* and its fusant, Biotechnol. Bioeng. 60 (1998) 391–395.
[5] F. Fava, P.M. Armenante, D. Kafkewitz, Aerobic degradation and dechlorination of 2-chorophenol, 3-chlorophenol and 4-chlorophenol by a *Pseudomonas pickettii* strain, Lett. Appl. Microbiol. 21 (1995) 307–312.
[6] P.M. Fedorak, S.E. Hrudey, The effects of phenol and some alkyl phenolics on batch anaerobic methanogenesis, Water Res. 18 (1984) 361–367.
[7] L.D. Collins, A.J. Daugulis, Biodegradation of phenol at high initial concentrations in two-phase partitioning batch and fed-batch bioreactors, Biotechnol. Bioeng. 55 (1) (1997) 155–162.
[8] K. Watanabe, S. Hino, N. Takahashi, Responses of activated sludge to an increase in phenol loading, J. Ferment. Bioeng. 82 (1996) 522–524.
[9] A.M. Gerrard, J. Páca Júnior, A. Kostečková, J. Páca, M. Stiborová, C.R. Soccol, Simple models for the continuous aerobic biodegradation of phenol in a packed bed reactor, Br. Arch. Biol. Technol. 49 (4) (2006) 669–676.
[10] N.R. Ordaz, J.C.R. Lagunez, J.H.C. González, E.H. Manzano, E.C. Urbina, J.G. Ayer, Phenol Biodegradation using a repeated batch culture of *Candida tropicalis* in a multistage bubble column, Revista Latinoamericana de Microbiología 43 (2001) 19–25.
[11] M. Schröder, C. Müller, C. Posten, W.D. Deckwer, V. Hecht, Inhibition kinetics of phenol degradation from unstable steady-state data, Biotechnol. Bioeng. 54 (1997) 567–576.

[12] T.P. Chung, H.Y. Tseng, R.S. Juang, Mass transfer effect and intermediate detection for phenol degradation in immobilized *Pseudomonas putida* systems, Process Biochem. 38 (10) (2003) 1497–1507.
[13] A.M.G. Monteiro, R.A.R. Boaventura, A.E. Rodrigues, Phenol biodegradation by *Pseudomonas putida* DSM 548 in a batch reactor, Biochem. Eng. J. 6 (1) (2000) 45–49.
[14] S.J. Wang, K.C. Loh, Modeling the role of metabolic intermediates in kinetics of phenol biodegradation, Enzyme Microb. Technol. 25 (1999) 177–184.
[15] S. Dikshitalu, B.C. Baltzis, G.A. Lewandowaski, Competition between two microbial populations in a sequencing fed-batch reactor: theory, experimental verification, and implications for waste treatment applications, Biotechnol. Bioeng. 42 (1993) 643–656.
[16] T. Abuhamed, E. Bayraktar, T. Mehmetoglu, U. Mehemetoglu, Kinetics model for growth of *Pseudomonas putida* F1 during benzene, toluene and phenol biodegradation, Process Biochem. 39 (2004) 983–988.
[17] P. Kumaran, Y.L. Paruchuri, Kinetics of phenol biotransformation, Water Res. 31 (1) (1997) 11–22.
[18] O.J. Hao, M.H. Kim, E.A. Seagren, Kim, Kinetics of phenol and chlorophenol utilization by *Acinetobacter* species, Chemosphere 46 (6) (2002) 797–807.
[19] Z. Alexievaa, M. Gerginova, P. Zlateva, N. Peneva, Comparison of growth kinetics and phenol metabolizing enzymes of *Trichosporon cutaneum* R57 and mutants with modified degradation abilities, Enzyme Microb. Technol. 34 (3–4) (2004) 242–247.
[20] J. Yan, W. Jianping, L. Hongmei, Y. Suliang, H. Zongding, The biodegradation of phenol at high initial concentration by the yeast *Candida tropicalis*, Biochem. Eng. J. 24 (3) (2005) 243–247.
[21] A. Venu Vinod, G. Venkat Reddy, Dynamic behaviour of a fluidized bed bioreactor treating wastewater, Indian Chem. Eng. 45 (1) (2003) 20–27.
[22] G.A. Hill, C. Robinson, Substrate inhibition kinetics: phenol degradation by *Pseudomonas putida*, Biotechnol. Bioeng. 17 (1975) 1599–1615.
[23] K.V. Shetty, I. Kalifathulla, G. Srinikethan, Performance of pulsed plate bioreactor for biodegradation of phenol, J. Hazard. Mater. 140 (1–2) (2007) 346–352.
[24] N. Vidyavathi, Bioremediation of industrial and domestic effluents by microorganisms, PhD Thesis, Department of Chemical Engineering, KREC Surathkal, Mangalore University, India, 1998.
[25] D.K. Verma, Modeling of phenol degradation in a pulsed plate bioreactor, MTech Thesis, National Institute of Technology Karnataka Surathkal, India, 2004.
[26] Standard Methods for the Examination of Water and Wastewater, 14th ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA, 1975.
[27] B.Y. Santoshkumar, Steady state and dynamic performance of pulsed plate bioreactor for the biodegradation of phenol, MTech Thesis, National Institute of Technology Karnataka Surathkal, India, 2006.
[28] Z. Aksu, Application of biosorption for the removal of organic pollutants: a review, Process Biochem. 40 (2005) 997–1026.
[29] H.C. Fleming, Sorption sites in biofilms, Water Sci. Technol. 32 (8) (1995) 27–33.
[30] J. Wu, H.Q. Yu, Biosorption of 2,4-dichlorophenol by immobilized white-rot fungus *Phanerochaete chrysosporium* from aqueous solutions, Bioresour. Technol. 98 (2007) 253–259.
[31] J. Wu, H.Q. Yu, Biosorption of 2,4-dichlorophenol from aqueous solution by *Phanerochaete chrysosporium* biomass: isotherms, kinetics and thermodynamics, J. Hazard. Mater. B 137 (2006) 498–508.
[32] C. Quintelas, E. Sousa, F. Silva, S. Neto, T. Tavares, Competitive biosorption of *ortho*-cresol, phenol, chlorophenol and chromium(VI) from aqueous solution by a bacterial biofilm supported on granular activated carbon, Process Biochem. 41 (2006) 2087–2091.
[33] J.R. Rao, T. Viraraghavan, Biosorption of phenol from an aqueous solution by *Aspergillus niger* biomass, Bioresour. Technol. 85 (2) (2002) 165–171.
[34] W. Jianlong, Q. Yi, N. Horan, Ed. Stentiford, Bioadsorption of pentachlorophenol (PCP) from aqueous solution by activated sludge biomass, Bioresour. Technol. 75 (2000) 157–161.
[35] C.J. Daughney, J.B. Fein, Sorption of 2,4,6-trichlorophenol by *Bacillus subtilis*, Environ. Sci. Technol. 32 (6) (1998) 749–752.
[36] P. Benoit, E. Barriuso, R. Calvet, Biosorption characterization of herbicides, 2,4-D and atrazine, and two chlorophenols on fungal mycelium, Chemosphere 37 (7) (1998) 1271–1282.
[37] M. Tsezos, J.P. Bell, Comparison of the biosorption and desorption of hazardous organic pollutants by live and dead biomass, Water Res. 23 (5) (1989) 561–568.
[38] C. Nicolella, S. Chiarle, R.D. Felice, M. Rovatti, Mechanism of biofilm detachment in fluidised bed reactors, Water Sci. Technol. 36 (1) (1997) 229–235.
[39] P. Ramasamy, X. Zhang, Effects of shear stress on the secretion of extracellular polymeric substances in biofilms, Water Sci. Technol. 52 (7) (2005) 217–223.
[40] B. Manz, F. Volke, D. Goll, H. Horn, Investigation of biofilm structure,flow patterns and detachment with magnetic resonance imaging, Water Sci. Technol. 52 (7) (2005) 1–6.
[41] J.A. Robinson, M.G. Trulear, W.G. Characklis, Cellular reproduction and extracellular polymer formation by *Pseudomonas aerogenosa* in continuous culture, Biotechnol. Bioeng. 26 (1984) 1409–1417.
[42] J. Schmitt, D. Nivens, D.C. White, H.C. Flemming, Changes of biofilm properties in response to sorbed substances—an FITR-ATR study, Water Sci. Technol. 32 (8) (1995) 149–155.
[43] A. Ohashi, H. Harada, Characterisation of detachment mode of biofilm developed in an attached growth reactor, Water Sci. Technol. l30 (11) (1994) 35–45.
[44] W.K. Kwok, C. Picioreanu, S.L. Ong, M.C.M. van Loosdrecht, W.J. Ng, J.J. Heijnen, Influence of biomass production and detachment forces on biofilm structures in airlift suspension reactor, Biotechnol. Bioeng. 58 (4) (1998) 400–407.
[45] R. Sudarshan, K. Milferstedt, E. Morgenroth, H.J. Eberi, Quantification of detachment forces on rigid biofilm colonies in a roto-torque reactor using computational fluid dynamic tools, Water Sci. Technol. 52 (7) (2005) 149–154.
[46] M.C.M. van Loosdrecht, D. Eikelboom, A. Gjaltema, A. Mulder, L. Tijhuis, J.J. Heijnen, Biofilm structures, Water Sci. Technol. 32 (8) (1995) 35–43.
[47] H. Beyenal, Z. Lewandowski, Internal and external mass transfer in biofilms grown at various flow velocities, Biotechnol. Progr. 18 (1) (2002) 55–61.
[48] B.M. Peyton, Effects of shear stress and substrate loading rate on *Pseudomonas aerogenosa* biofilm thickness and density, Water Res. 30 (1) (1996) 29–36.
[49] J.W. Costerton, Z. Lewandowski, D. DeBeer, D. Caldwell, D. Korber, G. James, Minireview: biofilms, the customised micronich, J. Bacteriol. 176 (8) (1994) 2137–2142.
[50] Z. Lewandowski, D. Webb, M. Hamilton, G. Harkin, Quantifying biofilm structure, Water Sci. Technol. 39 (7) (1999) 71–76.
[51] G.M. Wolfaardt, J.R. Lawrence, R.D. Robarts, D.E. Caldwell, Multicellular organisation in a degradative biofilm community, Appl. Environ. Microbiol. 60 (1994) 434–446.
[52] A.A. Massol-Deya, J. Whallon, R.F. Hickey, J.M. Tiedje, Channel structures in aerobic biofilms of fixed film reactors treating contaminated ground water, Appl. Environ. Microbiol. 61 (2) (1994) 769–777.
[53] D. DeBeer, P. Stoodley, Z. Lewandowski, Liquid flow in heterogeneous biofilms, Biotechnol. Bioeng. 44 (1994) 636–641.
[54] P. Stoodley, D. Debeer, Z. Lewandowski, Liquid flow in biofilm systems, App. Environ. Microbiol. 60 (1994) 2711–2716.
[55] S.W. Hermanowicz, U. Schindler, P. Wilderer, Fractal structure of biofilms: new tools for investigation of morphology, Water Sci. Technol. 32 (8) (1995) 99–105.
[56] D. DeBeer, A. Sachramm, Microenvironments and mass transfer phenomena in biofilms studied with microsensors, Water Sci. Technol. 39 (7) (1999) 173–178.
[57] F.R. Christensen, G.H. Kristensen, J.L.C. Jansen, Biofilm structure—an important and neglected parameter in waste water treatment, Water Sci. Technol. 21 (8/9) (1989) 805–814.
[58] P.J. Bremer, G.G. Geesey, B. Drake, Atomic force microscopy examination of the topography of a hydrated bacterial biofilm on a copper surface, Curr. Microbiol. 24 (1992) 223–230.

[59] Y.S. Cao, G.J. Alaerts, Influence of reactor type and shear stress on aerobic biofilm morphology, population and kinetics, Water Res. 29 (1995) 107–118.
[60] T.T. Eighmy, D. Maratea, P.L. Bishop, Electron microscopic examination of wastewater biofilm formation and structural components, Appl. Environ. Microbiol. 45 (1983) 1921–1931.
[61] S. Kugaprasatham, H. Nagaoka, S. Ohgaki, Effects of turbulence on nitrifying biofilms at non-limiting substrate conditions, Water Res. 26 (1992) 1629–1638.
[62] Z. Lewandowski, G. Welser, Influence of hydrodynamics on biofilm accumulation, Proc. Environ. Eng. Conf. ASCE EE Div., Reno, NV, July 8–10, 1991.
[63] A.A. Massoldeya, J. Whallon, R.F. Hickey, J.M. Tiedje, Channel structures in aerobic biofilms of fixed film reactors treating contaminated groundwater, Appl. Environ. Microbiol. 61 (1995) 769–777.
[64] H. Siegrist, W. Gujer, Mass transfer mechanism in a heterotrophic biofilm, Water Res. 19 (11) (1985) 1369–1378.
[65] W. Zahid, J. Ganczarczyk, A technique for a characterisation of RBC biofilm surface, Water Res. 28 (1994) 2229–2231.
[66] T.C. Zang, P.L. Bishop, Density, porosity and pore structure of biofilms, Water Res. 28 (11) (1994) 2267–2277.