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ScienceDirect
Journal of Hazardous Materials 140 (2007) 346–352
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Performance of pulsed plate bioreactor for biodegradation of phenol

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Received 4 February 2006; received in revised form 12 September 2006; accepted 14 September 2006
Available online 24 September 2006

Abstract

Biodegradation of phenol was carried out using *Nocardia hydrocarbonoxydans* immobilised on glass beads, in a pulsed plate bioreactor. The effect of operating parameters like frequency of pulsation and amplitude of pulsation on the performance of pulsed plate bioreactor for biodegradation of phenol in a synthetic wastewater containing 500 ppm phenol was studied. Axial concentration profile measurements revealed that the pulsed plate bioreactor shows continuous stirred tank behaviour. As the amplitude was increased, percentage degradation increased, reaching 100% at amplitude of 4.7 cm and higher. Introduction of pulsation is found to increase the percentage degradation. Percentage degradation has increased with increase in frequency and 100% degradation was achieved at 0.5 s^{-1} and above. Biofilms developed in a non-pulsed bioreactor were thicker than those in the pulsed plate bioreactor. But biofilm thickness remained almost constant with increasing frequency. Biofilm density was found to be influenced by pulsation. The time required to reach steady state was more for pulsed reactor than the non-pulsed reactor and this start-up time had increased with increase in frequency of pulsation. The performance studies reveal that the pulsed plate bioreactor with immobilized cells has the potential to be an efficient bioreactor for wastewater treatment.

Keywords: Biodegradation; Biofilm; Pulsed plate bioreactor; Immobilised cells; Amplitude; Frequency

1. Introduction

Phenol is a persistent pollutant found in waste waters from many industries like petrochemical and petroleum refineries, coal coking, polymer production, dye synthesis, wood processing plants, pharmaceuticals, pesticides, etc. [1–11]. Phenol is a serious water pollutant due to its toxicity even at very low concentrations [2,12]. It is responsible for serious damage to the flora and fauna, aquatic life, animals as well as human beings in many ways [1,9]. Physical and chemical methods for phenol removal [3,13–17] are costly and cannot remove phenol completely or convert it into some other form, which may be harmful. Biodegradation has been studied as an alternative technology, due to the low cost associated with this option, as well as the possibility of complete mineralization of the compound [3,5,8,18,19]. The concentration of phenols in wastewater varies from 10 to 3000 ppm [6]. Wastewaters containing phenol in the range of 5–500 ppm are considered suitable for treatment by biological processes [5,7]. Microbial strains such as *A. eutrophus*, *B. stearothermophilus*, *S. setonii*, *T. cutaneum*, *Pseudomonas putida*, *Pseudomonas pictorum*, *Phormidium valderianum*, *Pseudomonas fluorescens*, *Pseudomonas cepacia*, arthrobacter, *Candida tropicalis*, *Pseudomonas aerogenosa*, *Bacillus brevis*, *Nocardia hydrocarbonoxydans* [8,12,20–38] have been reported to be suitable for degrading phenol. *N. hydrocarbonoxydans* is an actinomycetes, which was found to effectively degrade phenol [27].

Biological treatment of phenols has been studied in various fixed film as well as suspended growth bioreactor systems. Suspended growth bioreactor requires a sludge separation unit and the dilution rate is limited by maximum growth rate, to avoid cell washout. 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 [39]. Immobilization binds the microorganism to a solid support and the sludge separation unit is not required. The immobilised cell reactors with various reactor configurations namely packed bed or trickle bed reactors [4,40–46], fluidised bed reactors [3,39,47–55] have

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doi:10.1016/j.jhazmat.2006.09.058

been reported for the biodegradation of phenol. The use of packed bed reactors or trickle bed reactors with immobilized cells in biotechnological processes presents a series of problems. Among them major drawbacks are (a) mass transfer limitations, (b) the accumulation of evolved gas, which reduces the useful volume, (c) the formation of preferential paths or channelling as well as short circuiting and (d) clogging. [56–58]. The fluidised bed bioreactors involve scaling up problems due to the difficulties in controlling the bed expansion and biofilm thickness, influent distribution devices and/or oxygen saturation systems [56] and may encounter hydrodynamic problems [58].

The application of a perturbation in the form of pulsation into the fixed bed reactor can minimise these problems, due to the generation of the slow movements of the bed, which cause the renewal of the interfacial area and favours the distribution of the substrate as well as the elimination of gaseous metabolites that cause the dead zones [57].

The pulsed plate (reciprocating plate) column or plate pulsed column, since its development by Karr [59], have been used increasingly as liquid–liquid extractor and gas–liquid contactor. Pulsed plate column is a column of pulsating plates, in which the pulsation is generated by the rising-descending motion of the pile of perforated plates by means of an electric motor connected via an accentrical crank rod which links the pile of plates. It has the potential to be used as a commercial bioreactor because of the numerous advantages over the more conventional stirred tank or bubble column type of gas–liquid contactor such as enhanced mass transfer, uniform turbulence intensity, uniform mixing of the phases, more overall productivity and easy scale up [60]. Brauer, has developed a pulsed plate column, named reciprocating jet bioreactor with suspended cells and has applied it for the biological wastewater treatment [61] and for growth of fungi and bacteria [62].

In the present work, a pulsed plate column with the space between the plates packed with glass particles immobilized with the cells, is used as a bioreactor for the biodegradation of phenol. This study will focus on the steady state and start-up performance of the pulsed plate bioreactor for continuous biodegradation of phenol in water, under different operating conditions like frequency and amplitude of pulsation.

2. Materials and methods

2.1. Materials

N. hydrocarbonoxydans (NCIM 2386), an actinomycetes, chosen for the present study by virtue of its effectiveness to degrade phenolic waste [27] was obtained from NCIM, a division of National Chemical Laboratories, Pune, India. The strains were periodically subcultured once in 15 days on agar slants and were stored at 4 °C. All chemicals (except 4-amino antipyrene) were obtained from Nice Chemicals, Cochin, India. 4-Aminoantipyrene was obtained from Central Drug House, New Delhi, India. Glass beads of 3 mm size were procured from Rolex Chemicals, Bangalore, India.

2.2. Nutrient media

Organisms were grown on phenol as the sole carbon and energy source and the nutrient 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 (0.50 g/l) in distilled water. The solution was adjusted to pH 7.0 by 0.1N NaOH.

2.3. Acclimatized inoculum preparation

Organisms were acclimatized for 500-ppm phenol. A loop full of test organism from a freshly subcultured slant was inoculated into a 100 ml of sterile media, containing 100 ppm phenol and all the required nutrients. The culture was incubated in a shaker at 30 °C for 3 days. This formed the primary culture. The secondary acclimatized inoculum was prepared in the same way, wherein 1 ml of primary culture was used instead of the subculture, to inoculate the medium and the culture was incubated for 48 h. This was continued for the third and fourth acclimatization. The 100 ppm acclimatized culture thus obtained, was further acclimatized gradually to 200, 300, 400 and then 500 ppm phenol using the above procedure. The 500 ppm acclimatized culture was then stored at 4 °C.

2.4. Cell immobilization

Cell immobilization on glass beads of 3 mm size was done by adsorption. Cell suspension cultured for 48 h was prepared from the 500 ppm acclimatized culture. To every gram of glass beads, 5 ml of cell suspension was added and it was refrigerated at 4 °C for 2 days with occasional stirring. The immobilized glass beads were then filtered, washed and used for the experiment. The immobilization of biomass on glass beads were carried out at different times following the same procedure and the biomass dry weight was measured using the procedure presented in Section 2.6. Initial immobilized biomass dry weight was found to be the same with a difference of around ±5%. The average initial biomass dry weight for 6400 glass beads was approximately 2.4 g.

2.5. Phenol analysis

Phenol was analysed by the photometric method using 4-aminoantipyrene as the colouring agent and measuring the absorbance at 510 nm with a Hitachi UV spectrophotometer [63].

2.6. Determination of biomass dry weight

Samples of 100 glass beads with the attached biomass were taken from different stages in the reactor. These bio particles were washed with distilled water and dried at 105 °C for 24 h and weighed. The bio films were then completely removed from glass beads by heating the dried particles in 0.25 M NaOH solu-

tion. Then the beads were washed with distilled water, dried at 105 °C for 24 h and weighed. The process was repeated till constant weight was obtained. Difference in weights of dried bio particles and dried bare particles gave the weight of biomass on 100 particles [54]. Attached biomass dry weight in the entire reactor was then calculated.

2.7. Estimation of biofilm thickness and biofilm density

The biofilm thickness of 10–15 glass beads with the attached biomass taken from the reactor was estimated by Labomed optical microscope equipped with a micrometer. The average thickness was determined. The biofilm density was calculated based on the following equation [55]:

$$\rho_f = \frac{W}{N(\pi/6)[(d_p + 2\delta)^3 - d_p^3]}$$

where ρ_f is the biofilm density, W the biomass dry weight in the reactor, d_p the diameter of glass beads, N the number of glass beads in the reactor and δ is the average biofilm thickness.

2.8. Experimental bioreactor

The experimental pulsed plate bioreactor is as shown in Fig. 1. It consisted of a vertical Perspex tube of 5.8 cm i.d., 6.3 cm o.d. and 77 cm height. The plate stack consisted of five perforated brass plates of 5.4 cm diameter, 1.5 mm thick with holes of 2 mm diameter in square pitch, mounted over a central rod. The pulsation of the plate stack is generated by a variable speed motor with frequency controller, through a slider/crank arrangement. The entire stack of plates can be pulsated at the required frequency and amplitude through this arrangement. Spacing between the plates was 3 cm. The entire circumference of the plate stack was covered with a 1 mm × 1 mm nylon mesh of thickness 0.5 mm. 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). The

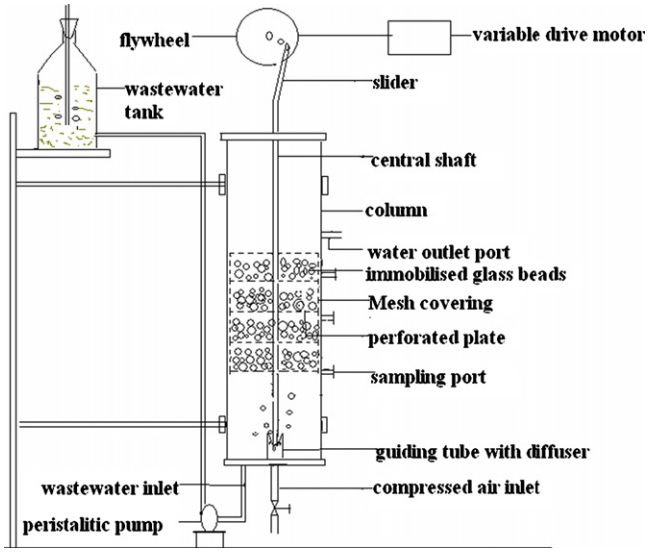

Fig. 1. Schematic diagram of experimental set-up.

central rod with the packed stages is inserted in the outer column with the bottom portion of the central rod positioned in the guiding tube. Four sampling ports are provided along the length of the column at 37, 31, 25 and 19 cm from the bottom of the column. Water and compressed air inlets are provided at the bottom of the column. The reactor outlet is through a port at 37 cm. The working volume of the reactor is 0.977 l. Synthetic phenol solution in tap water with phenol at 100 or 500 ppm concentration and other nutrients in concentrations as indicated in Section 2.2, was pumped from the bottom using a peristaltic pump. Compressed air was continuously passed from the bottom at 1.7 lpm to ensure proper supply of oxygen to the microorganism and dissolved oxygen concentration was maintained at around 5–6 mg/l. The required frequency of pulsation was set using the variable voltage speed regulator and the amplitude was set, by changing the position of the crankshaft. The concentration of phenol in the effluent from the column (the reactor outlet at 37 cm), were analysed at regular intervals of time during start-up till steady state was attained.

2.9. Experimentation

To study the mixing behaviour of the reactor, axial concentration profile measurement study was conducted with synthetic wastewater containing 100 ppm phenol, at a dilution rate (D) of 0.4094 h^{-1}, frequency (f) of 1.0 s^{-1} and amplitude (A) of 4.7 cm. Water samples were collected at the four sampling ports, during start-up and at steady state, and then analysed for phenol.

Different experiments were conducted sequentially at different frequency of pulsation (0.25, 0.5, 1.0 s^{-1}) and amplitude of pulsation (3.3, 4.7, 6.0 cm) to study the performance of the pulsed plate bioreactor for continuous biodegradation of phenol in a synthetic wastewater containing 500 ppm phenol and at a dilution rate of 0.4094 h^{-1}. The concentration of phenol in the effluent from the column (the reactor outlet at 37 cm), were analysed at regular intervals of time during start-up till steady state was attained. Water samples were collected at the four sampling ports at steady state, and then analysed for phenol. Steady state biofilm thickness and biomass dry weight were measured.

Continuous biodegradation of phenol present in the synthetic wastewater containing 500 ppm phenol was also carried out in the same bioreactor under nonpulsed conditions, at a dilution rate of 0.4094 h^{-1}. The reactor effluent concentrations during start-up and at steady state were measured. Steady state biofilm thickness and biomass dry weight were also measured.

The number of glass particles with immobilised cells in the reactor was 6400 for all the runs. The average initial biomass dry weight in the reactor for all the experimental runs was approximately 2.4 g with a difference of ±5%.

3. Results and discussion

3.1. Axial concentration studies

The results of axial concentration measurement studies conducted with 100 ppm influent phenol concentration are presented in Fig. 2. The phenol concentrations measured at various sam-

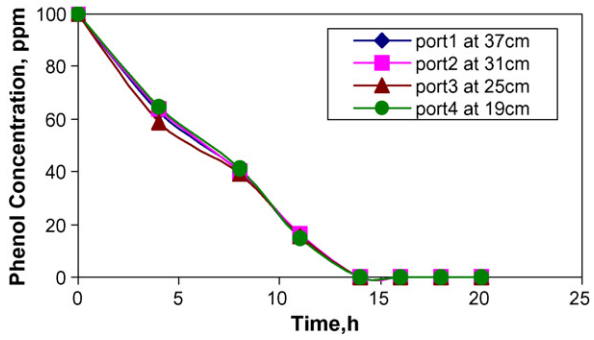

Fig. 2. Axial concentration profile in a pulsed plate bioreactor with Si = 100 ppm; $D = 0.4094\ h^{-1}$; $f = 1.0\ s^{-1}$; $A = 4.7$ cm.

pling ports situated along the length of the pulsed plate bioreactor, during start-up and at steady state, showed that the concentrations are uniform along the entire bioreactor. This may be because of the turbulence and hence back mixing caused by the pulsation of plates and the air flow rate, leading to uniform concentration throughout the reactor. Steady state phenol concentrations measured at various sampling ports situated along the length of the pulsed plate bioreactor, with 500 ppm influent phenol concentration and at different operating conditions tested in this work, also showed that the concentrations are same in samples from all the ports under pulsed conditions. This shows that the pulsed plate bioreactor behaves like a CSTR with perfect mixing at all the operating conditions considered in this work. These results are supported by the RTD studies conducted on the column using tracer experiments at the similar operating conditions [64].

3.2. Effect of frequency of pulsation

The effect of frequency of pulsation on the performance of the reactor during start-up is presented in Fig. 3. Phenol concentrations plotted are the concentrations measured at the reactor outlet (Port1 provided at 37 cm from the bottom of the reactor). Rate of phenol degradation during start-up, is lesser for pulsed plate bioreactor than for non-pulsed bioreactor. It is also found that this rate decreases with increasing frequency. The time required to reach steady state is more for pulsed reactor than the non-pulsed reactor. And the time increases with increase in

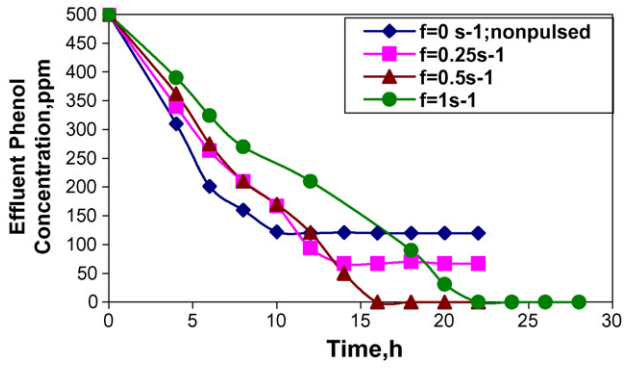

Fig. 3. Effect of frequency on phenol biodegradation during start-up for Si = 500 ppm; $D = 0.4094\ h^{-1}$; $A = 4.7$ cm.

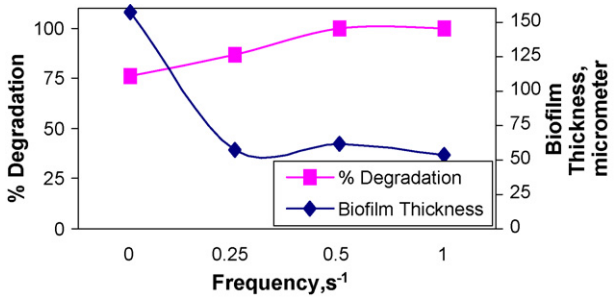

Fig. 4. Effect of frequency on steady state performance for Si = 500 ppm; $D = 0.4094\ h^{-1}$; $A = 4.7$ cm.

frequency of pulsation. This may be because of continuous formation and erosion of the biofilm due to shear during start-up and hence it may take more time for the formation of a stable biofilm.

The effect of frequency of pulsation on the performance of the reactor at steady state is presented in Fig. 4. Percentage degradation calculated from the phenol concentration measurements in the reactor effluent (Port1). Steady state percentage degradation of phenol is only 76% for nonpulsed bioreactor. But as the pulsation is given, the percentage degradation is increased, reaching 100% degradation at $0.5\ s^{-1}$. Further increase in frequency to $1\ s^{-1}$, did not alter the steady state percentage degradation. Complete continuous degradation of 500 ppm influent phenol was achieved at $0.5\ s^{-1}$. 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 maximum degradation is achieved at $0.5\ s^{-1}$. The biofilm developed in the non-pulsed reactor was very thick, with around 157 μm. Figs. 4 and 5 show that as the pulsation of $0.25\ s^{-1}$ was provided, the biofilm thickness was reduced in spite of the biomass dry weight remaining almost constant. This may be because of shear stress due to pulsation, which brings about the combined effect of film attrition and increase in biofilm density caused by the tight packing of the cells in the biofilm to resist the shear. But biofilm thickness remained

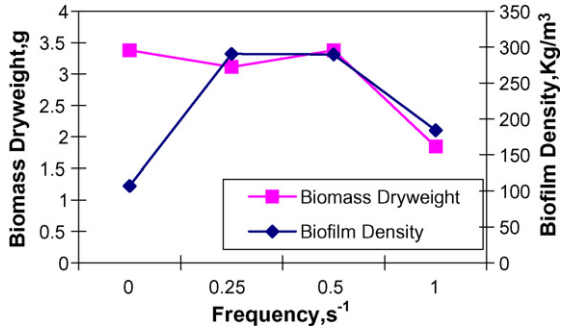

Fig. 5. Effect of frequency on biomass dryweight and biofilm density at Si = 500 ppm; $D = 0.4094\ h^{-1}$; $A = 4.7$ cm.

almost constant with increasing frequency. Fig. 5 shows that the biomass dry weight has remained constant for frequencies from 0 to 0.5 s^{-1} and has decreased with further increase in frequency to 1 s^{-1}. Biofilm density has reduced as the frequency was increased to 1 s^{-1}. The microorganisms keep themselves in tightly packed condition up to a frequency of 0.5 s^{-1} in an effort to resist shear. *N. hydrocarbonoxydans*, being an actinomycetes has mycelia similar to fungi. The organisms might have failed to resist shear of higher magnitude corresponding to 1 s^{-1} resulting in leakage of intracellular material to the environment without the mycelia being damaged [65]. This might have caused reduction of biomass dry weight. Reduction in mass transfer resistance with increased reciprocation would have increased the growth rate of organisms. This increased growth rate would have been balanced by the increased detachment rate caused by higher reciprocation, resulting in no variation of biofilm thickness with increased frequency. Decreased biomass dry weight but constant thickness has resulted in decrease in biofilm density at 1 s^{-1}. The increase in the density of biofilm with increased pulsation would have decreased the diffusivity through the biofilm, hence increasing the concentration gradient per unit length of the film. The maximum rate of degradation will be at the surface of the biofilm as the concentration is highest at the surface compared to the interior of the film. So the rate of degradation will decrease with increase in thickness from the surface of the biofilm. The denser films should have steeper concentration profiles than the less dense ones. And hence rate of degradation in the interior will be lesser in denser films than in loose films. But as we see from Figs. 4 and 5, the percentage degradation has increased when the pulsation is given, though the biofilm density has increased. This may be because the rate at the surface will be more in pulsed system than in non-pulsed system, due to higher substrate concentration at the biofilm surface in the pulsed system resulting from decreased external mass transfer resistance through the liquid film. This shows that internal diffusional resistance of the biofilm has less pronounced effect on the rate than the external mass transfer limitation. Hence the changes in biofilm density with frequency would not have changed the diffusivity through the biofilm to a considerable extent.

3.3. Effect of amplitude of pulsation

The effect of amplitude of pulsation on the performance of the reactor during start-up is presented in Fig. 6. Phenol concentrations plotted are the concentrations measured at the reactor outlet (Port1 provided at 37 cm from the bottom of the reactor). The rate of degradation during start-up was slower and time taken to reach steady state was lower for the non-pulsed system than the pulsed system of any amplitude. But no considerable change in the rate of degradation during start-up and the time taken to reach steady state (14–16 h) was observed with increasing amplitude.

The effect of amplitude of pulsation on the performance of the reactor at steady state is presented in Fig. 7. Percentage degradation calculated from the phenol concentration measurements in the reactor effluent (Port1). As the amplitude was increased, the percentage degradation increased and 100% degradation

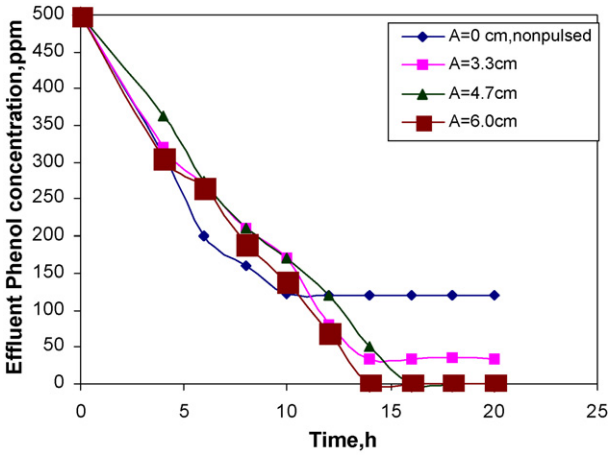

Fig. 6. Effect of amplitude on phenol biodegradation during start-up for Si = 500 ppm; $D = 0.4094\ h^{-1}$; $f = 0.5\ s^{-1}$.

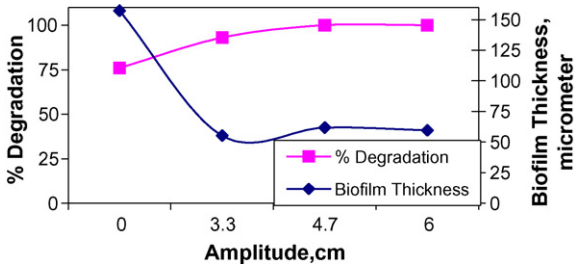

Fig. 7. Effect of amplitude on steady state performance for Si = 500 ppm; $D = 0.4094\ h^{-1}$; $f = 0.5\ s^{-1}$.

was achieved at amplitudes of 4.7 cm and higher. 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. So the percentage degradation increases with amplitude. Biofilm thickness at steady state remained almost constant with increasing amplitude. Fig. 8 shows the effect of amplitude of pulsation on biomass dry weight and biofilm density. Increase in amplitude has not altered the biomass dry weight and biofilm density.

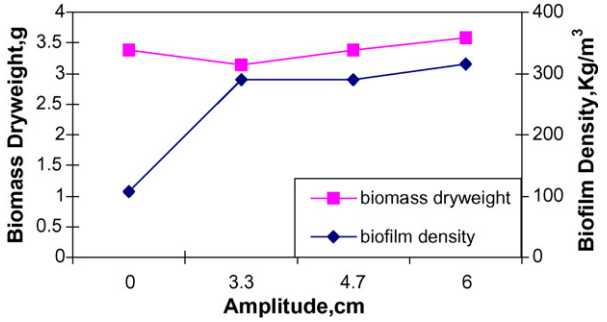

Fig. 8. Effect of amplitude on biomass dryweight and biofilm density at Si = 500 ppm; $D = 0.4094\ h^{-1}$; $f = 1\ s^{-1}$.

4. Conclusion

Axial concentration profile measurements revealed that the pulsed plate bioreactor shows continuous stirred tank behaviour. About 100% degradation of 500 ppm phenol in wastewater was achieved with the pulsed plate bioreactor consisting of immobilized cells, operating with frequencies at and above 0.5 s^{-1} and amplitudes at and above 4.7 cm. Therefore frequency of 0.5 s^{-1} and amplitude of 4.7 cm are considered the optimum conditions for the biodegradation of wastewater containing 500 ppm phenol. It is also found that internal diffusional resistance of the biofilm has less pronounced effect on the rate of degradation than the external mass transfer limitation. Biofilms developed in a non-pulsed bioreactor were thicker than those in the pulsed plate bioreactor. Percentage degradation was found to increase with the increase in frequency and amplitude of pulsation. Hence the performance studies reveal that the pulsed plate bioreactor with immobilized cells has the potential to be an efficient bioreactor for wastewater treatment.

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