African Journal of Biotechnology Vol. 8 (4), pp. 644-649, 18 February, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Continuous phenol removal using *Nocardia hydrocarbonoxydans* in spouted bed contactor: Shock load study

M.A. Dabhade[1]*, M.B. Saidutta[2] and D.V.R. Murthy[2]

[1]Institute of Petrochemical Engineering, Lonere-402 103, Raigad, Tal-Mangaon, Dist-Raigad, Maharashtra, India.
[2]Chemical Engineering Department, National Institute of Technology Karnataka, Surathkal, Srinivasnagar-575025, Karnataka, India.

Accepted 5 November, 2008

Shock load studies are essential to investigate the suitability of biocontactors in degradation of pollutants. In the present work, the degradation of phenol by immobilized *Nocardia hydrocarbonoxydans* in a spouted bed contactor was conducted. Granular activated carbon (GAC) and polymer beads were tested for the immobilization of cells of *N. hydrocarbonoxydans*-NCIM 2386. Initially, batch immobilization study was conducted to know the quantity of immobilized microorganisms per gram of solids and then the immobilized solids were used in the spouted bed contactor for phenol degradation. Also, the shock loading of phenol and hydraulic shock load test was performed to check the stability of operation. The immobilized *Nocardia* cells sustained the shock load and hydraulic load of phenol. Increase of influent phenol concentration and dilution rates increased the steady state effluent phenol concentration. Almost 95% degradation at maximum phenol loading of 0.73 $gL^{-1}h^{-1}$ was achieved. GAC has more attached biomass weight compared to polymer beads.

Key words: Immobilization, *Nocardia hydrocarbonoxydans,* biodegradation, spouted bed contactor, phenol biodegradation**.**

INTRODUCTION

Phenol is a toxic compound found in many industrial waste water. Industrial effluent can contain as little as 10 mg/L to greater than 5000 mg/L of phenol. Phenol is considered to have toxic effects on human health even when present in small concentration in water. The ingestion of such contaminated water in the human body causes protein degeneration, tissue erosion and paralysis of the central nervous system, vomiting, and lung failure and also damages the kidney, liver and pancreas. Adsorption through the skin can occur. Exposure of 400 cm^2 of skin to phenol for 30 min may cause death. Phenol is a local anesthetic, therefore there is no initial pain upon contact and by the time burning is sensed, the phenol would have penetrated the skin. Loss of pain does not indicate removal of phenol from the body. Immediate washing of the skin with ethyl alcohol solution and warm water usually removes the phenol without serious consequences (Kirk and Othmer, 1982). Discharge limit of phenol is 1 mg/L in inland surface water and 5 mg/L in marine coastal area (Gupta et al., 2006). Hence it must be removed from waste stream before discharge. Biodegradation of phenol is a widely used method as it is economical and easy to operate as compared to chemical, physical, electrochemical or advanced oxidation process.

Attached growth processes have advantage of retaining more biomass in the contactor. With immobilization high throughput of liquid can pass through the reactor while the cells are retained within, and no cell washout occurs. Different solids like sand, glass beads, Ca-alginate, granular activated carbon (GAC), polymer beads etc. are used for immobilization of cells. Different contactors have been used for the treatment of phenolic wastewater such as fluidized bed (Livingston and Chase,

*Corresponding author. E-mail: dabhadema@rediffmail.com. Tel: +91-9420947765; +91-9422494173. Fax: +91 2140 275103.

Table 1. Properties of GAC and polymer.

Property of solid	GAC	Polymer bead
Average size	2.4 mm	2.16 mm
Bulk density	639 kg/m^3	833 kg/m^3
True density	1378.7 kg/m^3	1279.6 kg/m^3
Specific surface area	270.74 m^2/g (BET)	11.433 cm^2/g

1989), packed column (Sheeja and Murugesan, 2002), rotating biological contactor (Israni et al., 2002), trickling filters, air lift reactors and membrane bioreactors. Large numbers of microorganisms are able to utilize phenol as a source of carbon and degrade it (Shetty et al., 2007b).

In the present work, phenol degradation was carried out in a spouted bed contactor using *Nocardia hydrocarbonoxydans* immobilized on GAC. Objective of the work was to use GAC and polymer beads for immobilization, compare the immobilization capacity of GAC with polymer beads, carry out continuous degradation study using both the solids in gas-liquid-solid three phase spouted bed contactor and compare their performance. Also, the stability of microorganisms in the spouted bed contactor during shock load and hydraulic load of phenol would be tested.

Spouted bed contactor

Earlier, the use of spouted bed technology was common for contacting gas with solids (Mathur and Epstein, 1974). Fan et al. (1984) used spouted bed as a gas-liquid-solid contactor. Silva and Yang (1998) used gas-solid spouted bed bioreactor for production of amylases from rice by solid state fermentation. The first report on hydrodynamic study of three phase spouted bed system was presented by Vukovic et al. (1973).

Spouted bed contactor has the following well defined regions.

i) Inlet zone where influent stream mixes with gas and enters into the lower conical section of reactor.

ii) Riser: Riser is centrally located up flow region from where air, solids and liquid moves upward.

iii) The Disengaging Zone: when solid, liquid and gas is moving up-ward from riser reaches the solid surface, a spout is formed, due to which solids move upward above the solid surface and forms spout, which reaches certain height, then solids falls down in annular region and gas moves up, disengaging from soids and liquid.

iv) Down comer: Here some gas bubble, part of liquid and solids from spout are recycled back to the inlet zone. Because of the pressure difference between the down comer outlet and the riser (at the bottom), the solids moves downward. The internal recycling of liquid and solids creates turbulence and dispersion in the liquid. This avoids segregation of biomass taking place in spouted bed contactor (Toumi et al., 2008).

Fluidized bed has a gas distributor plate at the bottom which keeps all the solids floating, whereas in spouted bed, a single orifice is used at the bottom in the center of the bed and only fraction of solids is fluidized (Karamanev et al., 1992). Increasing liquid flow rate in three phase spouted bed lowers the maximum bed pressure drop and minimum spouting velocity. This reduces the cost of operation (Vukovic et al., 1974).

MATERIALS AND METHODS

The properties of GAC and polymer beads used in the study are as shown in Table 1. A strain of microorganism *N. hydrocarbonoxydans*, (NCIM-2386), an actinomycetes was procured from National Collection of Industrial Microorganisms, a division of National Chemical Laboratory, Pune. All the required chemicals were obtained from NICE, Cochin, India.

Initially, a fresh slant was prepared using agar-agar and bacteriological agar. From a parent slant a loop was struck on a freshly prepared slant and then the cells were allowed to grow by keeping a slant in an incubator at 30^{o}C. Once the cells grew on the slant without any contamination, a liquid suspension culture was then prepared from this slant by striking a loop and adding it in nutrient medium and phenol. This was further sub cultured in the low phenol concentration of 50 mg/L. This suspension culture was then further acclimatized to subsequently increase in the phenol concentration slowly from 50 mg/L to higher phenol concentration. While preparing a second acclimatization, 1 mL of cell suspension from primary culture was used instead of a loop from a slant. A third acclimatized culture was used in immobilization study.

Nutrient medium composition consisted of 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. This nutrient medium composition is suitable for the microorganism N. hydrocarbonoxydans used in biodegradation studies. All standard procedure was followed for subsequent acclimatization and immobilization (Shetty et al., 2007). Phenol concentration was measured according to standard method, (APHA, 1975) using a UV-Visible Spectrophotometer (HITACHI-2000). Absorbance of sample was measured at 510 nm. Initially standard calibration plot of absorbance against known phenol concentration was prepared. It showed linear relationship up to 5 mg/L of phenol concentration. Suspended biomass concentration was measured optically at 610 nm using spectrophotometer.

Experimental

Figure 1 shows the schematic of experimental set up. It consisted of lower conical section made up of brass and upper cylindrical section of acrylic. Expanded end of the conical section was connected to cylindrical section. The lower end of the conical section was connected to the nozzle of diameter 4 mm. Cylindrical section has 25 mm inner diameter and height of 50 cm. At a height of 26 cm outlet for effluent was provided. The active volume of contactor is 136.4 mL. Using peristaltic pump, phenolic water along with nutrients was pumped

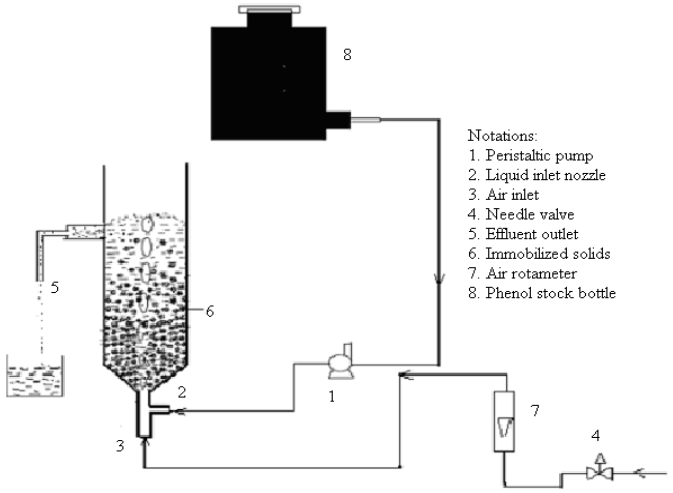

Figure 1. Schematic diagram of experimental set up.

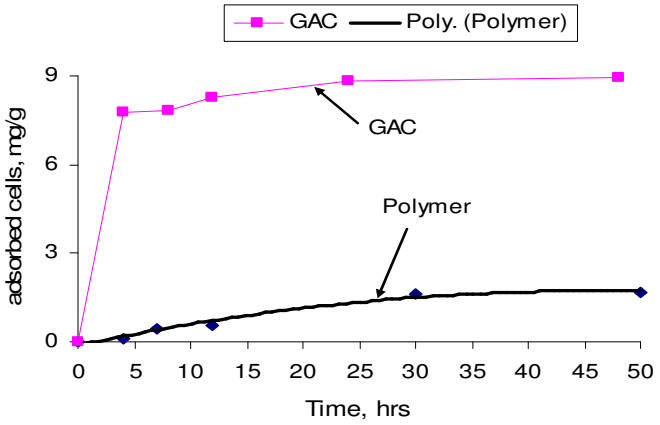

Figure 2. Plot of time against Immobilization of cells/ gm of solid.

through the side entry of nozzle and compressed and filtered air was allowed to enter the contactor through the air pipe provided at the bottom part of the conical section.

Air flow rate was maintained at 1.6 LPM. This air flow was sufficient for the solids to spout and have its recirculation inside the contactor. Initially the immobilized solids were carefully transferred into the contactor. The phenolic solution of required concentrations was synthetically prepared. The stock bottle was blackened to avoid any photo-degradation. The phenolic liquid and air flow rates were adjusted and the experiment continued till steady state was reached. At regular interval of time, effluent samples were collected and filtered using Whattman filter paper No1. The samples were stored in a refrigerator at 4°C and then analyzed for phenol within 24 h.

RESULTS AND DISCUSSION

Microorganism immobilization-batch study

To measure the immobilization capacity of a support material the batch studies were carried out in a flask. Initially the precultures were incubated at atmospheric temperature of 28°C on a shaker rotated at 150 RPM using a 500 mg/L phenol containing medium. After complete consumption of phenol, i.e. after 2 days, they were harvested for immobilization. *Nocardia* species was used for immobilization. The working volume was 100 mL in a 250 mL flask. A known weight of activated carbon and polymer beads were used for the immobilization study. During immobilization the flasks were kept in refrigerator. The growth of suspended microorganisms was determined by measuring the optical density of suspension at 610 nm. The quantity of immobilized microorganisms was calculated by the difference of cells in suspension before and after immobilization on the solids i.e. after two days of immobilization (Ehrhardt and Rehm, 1985). A control experiment without solids was also conducted. It showed no substantial change in the cells concentration.

Figure 2 shows the immobilization of cells of *Nocardia* (mg/g) on to GAC and polymer beads with time. It indicates that on to GAC, immobilization of cells takes place very fast compared to the polymer. Also the quantity of immoblized cells attached is dependant on time. In case of activated carbon, the immobilization took around 10 h during which the adsorption balance was reached. The immobilization of cells to polymer beads shows slow adsorption and also the quantity of cells immobilized onto the polymer surface is less compared to the GAC. It was found that the immobilization of *N. hydrocarbonoxydans* cells on to GAC was 8.92 mg/g of GAC, whereas the immobilization of same cells on to polymer beads was 1.68 mg/g of polymer. This can be explained based on the surface property of the materials.

The activated carbon has higher surface area than the polymer beads. Activated carbon is porous material. It has micro pores, meso pores and also macro pores. The microorganism *N. hydrocarbonoxydans* is actinomycetes. It has structure of fungi. It has long tail like shape. Hence the adsorption onto porous material is faster and higher as compared with polymer material which is soft and nonporous one. This shows that the porous material like GAC can attach more cells than nonporous materials like polymer beads.

Degradation study in spouted bed contactor

Figures 3 and 4 show the effluent phenol concentration during start up period when GAC and polymer beads were used in the contactor. During the use of GAC in the first stage, effluent concentration dropped suddenly. In the second stage, the concentration increased and in the last stage it dropped reaching to steady state concentration. This can be explained as follow.

Activated carbon contains a highly developed pore structure and is a good adsorbent for phenol. Initial decrease in effluent concentration was due to adsorption of phenol. Second stage indicates the growth period of microorganisms during which they get adjusted with new

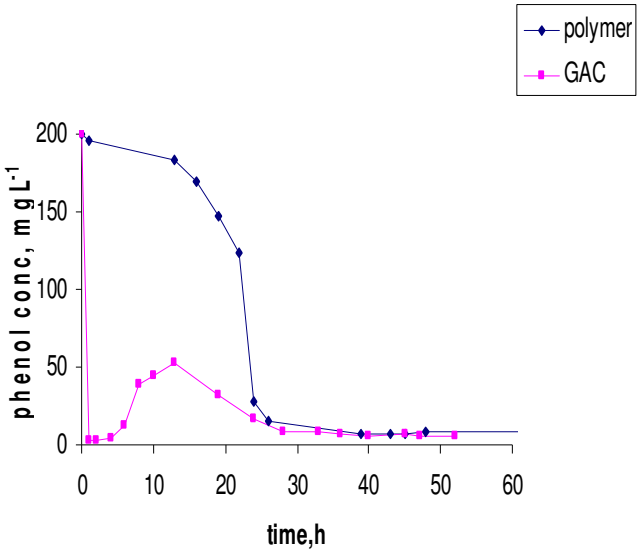

Figure 3. Effluent phenol concentration during startup period (Influent phenol conc. = 200 mg/L; dilution rate = 0.733 h^{-}).

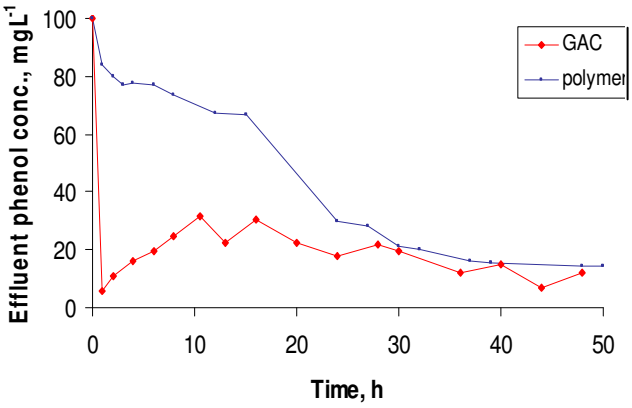

Figure 4. Effluent phenol concentration during startup period (influent phenol conc. = 100 mg/L; dilution rate = 2.2 h^{-1}).

environment and grow. Hence, the effluent concentration starts increasing with time. When the microorganisms are in log growth phase, the biodegradation becomes predominant, and the effluent phenol concentration gets reduced reaching steady state value. At this stage microorganisms might form a stable film on GAC. In this case around 97.5% degradation was observed. To differentiate the adsorption and the biodegradation of phenol using GAC, an experiment was performed in earlier work (Dabhade et al., 2008) and it was shown that adsorption dominates at the initial stage and biodegradation dominates after reaching the adsorption equilibrium.

When polymer beads were immobilized with the microorganisms and used in the contactor under similar conditions, it showed that initially for 3 to 4 h the effluent concentration got reduced very slowly. In the log growth phase of the microorganism the effluent concentration started decreasing at faster rate before reaching the steady state value. In this case up to 96% degradation was achieved. While using GAC, it took less time for the system to reach steady state compared to the use of polmer.

The difference in the percentage degradation in case of GAC and polymer was due to the surface area of material. As surface area of GAC is higher than the polymer, more microorganisms can be immobilized on to it as indicated earlier. GAC is a good adsorbent for phenol. Adsorbed phenol can easily be taken by the attached microorganism. Hence the biomass concentration in the contactor is higher in case of GAC. Also the GAC is lighter than the polymer; hence the spouting velocity required is less i.e., the cost of spouting is less. Hence, GAC is a better material that could be used for immobilization of microorganisms.

Continuous biodegradation of phenol–shock load study

In continuous mode of contactor operation, granular activated carbon was used for immobilization. Initially the cell immobilization was performed in shake flask using third acclimatized culture. The cell immobilized activated carbon was then carefully charged into the contactor. Synthetic influent having phenol concentration of 50 mg/L along with the nutrient medium was introduced into the contactor.

The dilution rate of 1.465 h^{-1} and air flow rate of 1.6 LPM was adjusted so as to have proper spouting. The experiment was continued till the contactor attained steady state. The contactor reached steady state after six hours of continuous operation. This was indicated by the constant phenol effluent concentration. At this time, no phenol was detected in the effluent stream. Then, the influent phenol concentration was increased to 100 mg/L, and operation was continued till the new steady was reached. It took around 44 h for the contactor to attain steady state. Again the influent phenol concentration was increased to 200 mg/L, and operation continued till the steady state was reached, after which the influent concentration was increased to 300 mg/L. The influent phenol concentration was increased progressively up to 500 mg/L (Figure 5). At this concentration the effluent phenol concentration was higher; hence, the influent phenol concentration was then suddenly reduced to 100 mg/L. It was found that when the influent concentration was lowered to 100 mg/L, the contactor reached steady state in 42 h. But, the steady state concentration was slightly higher than the initial steady state condition at 100 mg/L. This indicates that the contactor can be operated continuously for phenol biodegradation.

The time taken to reach steady state was higher when influent concentration was increased. Also the microorganisms could sustain and grow in the environment when step change in concentration took place which is

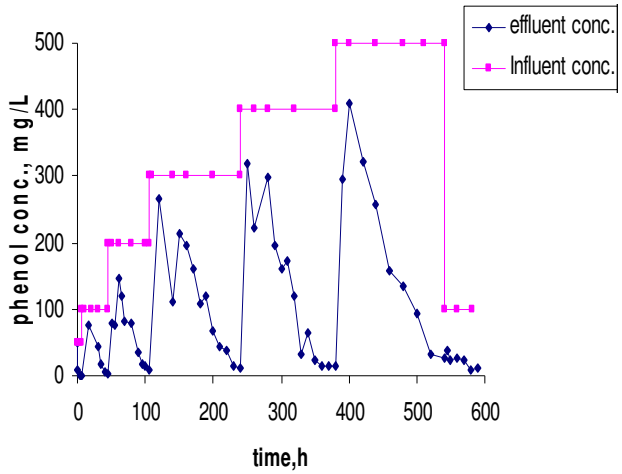

Figure 5. Influent phenol concentration and steady state effluent concentration with time for dilution rate of 1.465 h^{-1}.

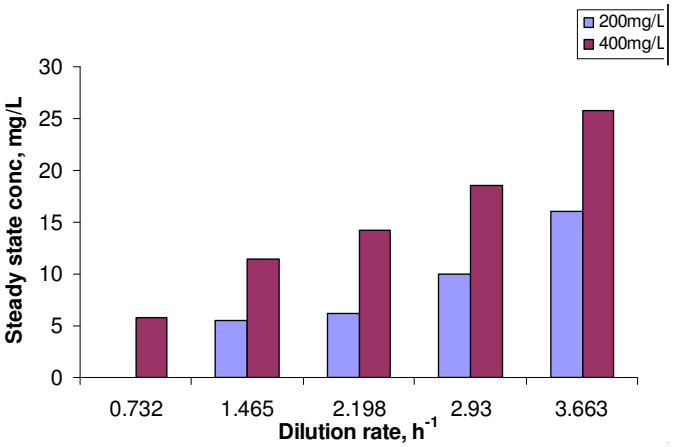

Figure 7. Comparison of steady state effluent phenol concentration for different dilution rate at 200 and 400 mg/L influent phenol concentration.

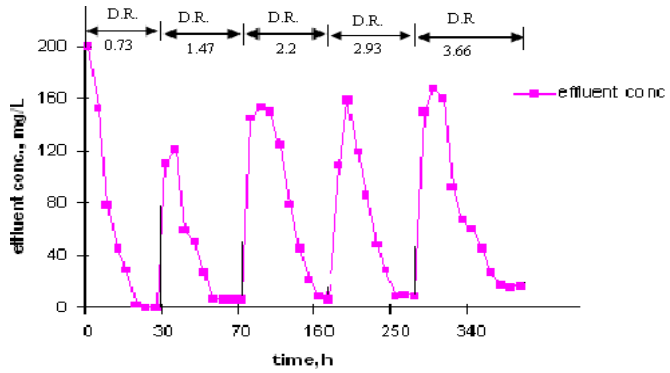

Figure 6. Effect of dilution rate on steady state effluent concentration.

commonly found in industrial waste water streams. The removal efficiency of 95% was observed for the maximum phenol loading of 0.73 g/L/h. The contactor performance was also tested at constant dilution rate of 2.96 h^{-1}. It was found that the microorganisms could take the shock load even at higher dilution rate; however it took more time for the system to attain steady state with poor removal efficiency. The removal efficiency of 93.6% was observed for the maximum phenol loading of 0.73 g/L/h.

Effect of dilution rate (hydraulic shock)

The effect of hydraulic shock was studied by varying the dilution rate from 0.73 to 3.66 h^{-1} at constant phenol concentration of 200 and 400 mg/L. Figure 6 shows the effect of dilution rate for 200 mg/L influent phenol concentration and Figure 7 shows the comparison of steady state effluent phenol concentration for various dilution rate when influent concentration was 200 and 400 mg/L. It was observed that with increase of the dilution rate the steady state effluent concentration increased. At higher influent concentration, the steady state concentration was higher and it took more time to attain the steady state.

This could be due to the fact that the increase of the dilution rate decreases the residence time of the phenol in the contactor. This reduces the contact time of substrate with microorganisms and results in poor degradation and also takes longer time to reach the steady state. At higher dilution rate there are chances of biomass washing out due to sloughing of biofilm. In such a contactor, continuous sloughing and reestablishment of biofilm occurs and hence it takes more time for a contactor to reach steady state

Conclusions

Based on this work the following conclusions are drawn.

1. GAC is a better solid matrix for immobilization of microorganisms as compared to polymer beads, as it has higher surface area and gives higher percentage degradation.
2. *N. hydrocarbonoxydans* immobilized on GAC degrades phenol effectively.
3. Spouted bed contactor can be used as bioreactor for the degradation of phenol.
4. Microorganisms immobilized on to GAC and used in spouted bed contactor can take the shock load of phenol from 50 to 500 mg/L. The removal efficiency of 95% was observed for the maximum phenol loading of 0.73 g/L/h. Increase of substrate concentration increased the effluent phenol concentration. Also increase of dilution rate resulted in increase of effluent phenol concentration. At dilution rate of 1.465 h^{-1}, phenol removal efficiency was above 95%. At higher dilution rate biomass washout can occur resulting in poor degradation.

REFERENCES

APHA, Standard Methods for the Examination of Water and Waste Water, 14th ed. (1975). American Public Health Association /American Water Works Association/Water Environment Federation, Washington, D.C., USA.

Dabhade MA, Saidutta MB, Murthy DVR (2008). Modeling of phenol degradation in spouted bed contactor using artificial neural network (ANN). Chem. Prod. Proc. Modeling, 3" 1-13.

Ehrhardt HM, Rehm HJ (1985). Phenol degradation by microorganisms adsorbed on activated carbon. J. Appl. Microbiol. Biotechnol. 21: 32-36.

Fan LS, Hwang IJ, Matsuura A (1984). Hydrodynamic behavior of a draft tube gas-liquid-solid spouted bed. Chem. Eng. Sci. 39: 1677-1688.

Gupta SK, Gupta SK, Hung YT (2006). Treatment of Pharmaceutical Waste, In: Wang, LK., Hung YT, Howard HL, Yapijakis C (eds.) Waste Treatment in the process Industries, CRC Taylor and Francis pp. 167-233.

Israni SH, Koli SS, Patwardhan AW, Melo JS, D'souza SF (2002) Phenol degradation in rotating biological contactors, J. Chem. Technol. Biotechnol. 77: 1050-1057.

Karamanev D, Nagamune G, Endo I (1992). Hydrodynamic and mass transfer study of a gas-liquid-solid draft tube spouted bed bioreactor. J..Chem Eng Sci, 47 13/14: 3581-3588.

Kirk-Othmer (1982). Kirk Othmer Encyclopedia of Chemical Technology, 3rd ed., p. 17.

Livingston AG, Chase HA (1989). Modeling phenol degradation in a fluidized bed. Bioreactor AIChE J 35(12): 1980-1991.

Mathur KB, Epstein N (1974) Spouted Bed, Academic Press, Inc., N.Y.

Shetty KV, Kalifathulla I, Srinikethan G (2007). Performance of pulsed plate bioreactor for biodegradation of phenol, J. Hazard. Mater. 140(2): 346-352.

Shetty KV, Ramanjaneyulu R, Srinikethan G (2007b). Biological phenol removal using immobilized cells in a pulsed plate bioreactor: Effect of dilution rate and influent phenol concentration J Hazard Mater. 149(10): 452-459.

Sheeja RY, Murugesan T (2002). Mass transfer studies on the biodegradation of phenols in up-flow packed bed reactors, J. Hazard. Mater. B89: 287-301.

Silva EM, Yang ST (1998) .Production of amylases from rice by solid-state fermentation in a gas-solid spouted bed bioreactor, Biotechnol Progr. 14(4): 580-587.

Toumi LB, Fedailaine M, Khedidja A (2008). Modeling three-phase fluidized bed bioreactor for wastewater treatment, Int. J. Chem. React Eng, p. 6.

Vukovic DV, Zdanski FK, Vunjak GV (1973). The three phase spouted bed-A new system in chemical engineering processing. A.I.C.H.E. meeting, Detroit, paper no11d, AIChE, New York.

Vukovic DV, Zdanski FK, Vunjak GV, Grbavcic ZB (1974). Pressure drop, bed expansion and liquid hold up in a three phase spouted bed contactor, Can. J. Chem. Eng. 52: 180-184.