ABSTRACT

Phenolic waste water is discharged by many industries like petroleum refineries , gas and coke industries , fiberglass units , plastic industries , pharmaceuticals and chemical industries, paint and varnish industries , textile industries, etc causing severe environmental pollution . Though various types of treatment methods are available, biological processes are found to be most suitable for the treatment of those wastes which are in dilute conditions . The thesis presents an experimental investigation on the acclimatized culture of ***Pseudomonas putida*** MTCC 1194 used for phenol degradation with free cell and immobilised cell systems under different process variables to identify the optimum conditions. It was also desired to develop some kinetic models to interpret the experimental data obtained from batch and continuous flow bioreactors . Since the immobilised solid matrix is important for phenol degradation , it is imperative to study the characteristic of the solid matrix in terms of structural strength and reactivity, so that the same can be used in commercial reactors like packed bed , fluidised bed, etc.

Acclimatization of ***Pseudomonas putida*** (MTCC 1194) was done by repeated subculturing with gradually increasing phenol concentration (range 100-1000 ppm) in the medium over a prolonged period. The inhibition effects of phenol as substrate have become predominant above the concentration of 500 ppm. The optimum temperature and initial pH required for maximum phenol biodegradation were 30^0C and 7.0 respectively. The activation energy (Ea) was found to be equal to 13.8 kcals/ g mole substrate reacted. The most suitable inoculum age and volume for highest phenol degradation were 12 hr. and 7 % v/v respectively. Surfactants had negligible effect on phenol biodegradation process by this microorganism . Monod model has been used to interpret the free cell data on phenol degradation . The kinetic parameters have been estimated upto initial concentration of 500 ppm. μ_{max} and K_S gradually increased with higher concentration of phenol . However, beyond

phenol concentration of 500 ppm , the inhibition became predominant . Thus μ_{max} has been found to be a strong function of initial phenol concentration. The simulated and the experimental phenol degradation profiles have good correspondence with each other.

Calcium alginate has been used as solid matrix for the immobilization of whole cells. In case of immobilized cells, the inhibition effect become prominent above the phenol concentration of 750 ppm. The optimum temperature and pH were same as those of free cell systems. The activation energy is less compared to the free cell system. Phenol degradation increased with the higher solid loading but the stability of the immobilised solid matrix was seriously affected. 2-3 % solid loading was recommended for the commercial reactors like the fluidised bed or air lift fermenter. The phenol removal was enhanced with higher cell loading but it is apprehended that excessive cell loading in the solid matrix may lead to cell leakage. The highest rate of phenol biodegradation was observed with the smallest particle size of 2.5 mm, compared to bigger size particle. The kinetic studies based on modified Michaelis - Menten Equation indicate that v'_{max} has been found to decrease with higher phenol concentration whereas the reverse trend has been observed with respect to K_m . v'_{max} increased with pH but K_m is least affected by the variation of pH . The diffusional effects of immobilised beads of bigger size have been demonstrated in terms of Thiele parameter ϕ for different size of particles (3 mm , 5 mm, 7.5mm) . The simulation studies indicate that the system is sufficiently influenced by diffusion. The correspondence between experimental and predicted data are very much satisfactory upto the initial phenol concentration of 500 ppm. However , at 750 ppm initial phenol concentration sufficient deviations of the experimental from the calculated ones have been observed . Hardening of the immobilised beads has been achieved by changing the concentration of the sodium alginate and calcium chloride and by treatment with glutaraldehyde. . The estimated D_{es} values for the same size of particle (2.5 mm) but having different structural strength have different values . With solid matrix of various strengths , it was

observed that the reactivity of the immobilised system decreased with the higher strength of the beads . So, compromising between reactivity and structural strength an optimum concentration of 3% solution of each of the components (Sodium alginate and Calcium chloride) has been used to prepare the beads . Higher concentration of glutaraldehyde produces higher strength of bead. However lowest glutaraldehyde concentration of 0.01 % yielded highest degradation rate though the highest rate has been achieved without treatment . Lowest time of exposure in glutaraldehyde increased the rate of phenol degradation . In the reusability studies , it was observed that the extent of phenol degradation decreased with the next consecutive batches . In the shelf life study (at 4 ^{0}C in mineral medium) 50% loss of phenol biodegradation activity was observed after five months .

Furthermore, studies on phenol biodegradation were carried out in a packed bed reactor (PBR) . The phenol conversion were 90 % , 65% , 20% and 2% . corresponding to the initial phenol concentration 50 , 100, 250 and 500 ppm respectively with an average residence time of 1 hour . Direct aeration into the system has distinctly beneficial effect on phenol degradation , indicating the important role of dissolved oxygen . When the pH was changed from 7 to 5.5 and again to 8.0 the phenol conversions dropped from 95% to 65% and 45% respectively. These changes are not identical with those carried out in the batch immobilised and free cell systems . Using a particular flow rate and residence time, phenol degradation were 90 % , 75%, and 72 % for particle sizes of 2.5mm , 5 mm and 7 mm diameter respectively . In case of using glutaraldehyde treated beads the phenol conversion efficiency was decreased by 10 % as compared to untreated ones . The experimental data have been correlated by the two models which have been considered in the present studies . The first one is a simple plug flow model with modified Michaelis Menten kinetics for the immobilised cell reactions and the other is a plug flow model with axial dispersion with Michaelis -Menten kinetics modified to first order kinetics with $K_M >> S$. There is fairly good agreement between the experimental and predicted conversion particularly in the first part of the reactor .