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Studies on Phenol degradation using Pseudomonas putida in Fluidized bed Bioreactor: A Waste water treatment

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Abstract: The present investigation deals with the biological degradation of Phenolic bulk drug waste with *Pseudomonas putida*, a Phenol degrading microorganism in a draft tube fluidized bed bioreactor and estimation of phenol transfer rates from bulk liquid to bioparticle. The concentration of highly harmful Phenol is found very much in the wastewaters of most of the Chemical and Pharmaceutical industries. The *Pseudomonas putida* was immobilized on plastic beads in a draft tube Fluidized bed bioreactor. Experiments have been conducted at different feed flow rates, feed concentrations and air flow rates. The temperature and P^H were maintained at 7.0 at 30^oC respectively in all the runs. The concentration of phenol in the outlet of rector was analyzed after every one hour until steady state is reached. The exponential growth phases of the microorganism and the Biokinetic parameters have been determined. The transfer rates of Phenol through liquid phase to bioparticle for different feed flow rates, feed concentrations and air flow rates have also determined.

Key words: Phenolic Bulk drug effluent, *pseudomonas putida*, Mass transfer studies, mathematical modeling and Fluidized bed bioreactor.

Introduction¹⁻¹⁰:

The contamination of water heavily by phenol has been investigated as an issue of growing importance in recent years. Organic pollutants consist of chemicals hazardous to human health. Many of these are resistant to degradation. They are also capable of long range transportation, bioaccumulation in human and animal tissue and biomagnifications in food chain.Phenol is resistant to natural degradation and hence persists in environment. Phenol attributes carbolic odor to river water and can be toxic to living bodies and hence waste water treatment has become essential nowadays to meet the acceptable water quality standards. Phenols are hydroxy compounds of aromatic hydrocarbons. Phenol derivatives are widely used as raw materials in many industries such as petrochemical, petroleum refineries, chemical and pharmaceutical. The effects of gas and liquid velocities, solid and liquid properties on the heat and mass transfer coefficients in three-phase fluidized beds have been determined. The various correlations and models to predict the heat and mass transfer coefficients in the literature have been examined and the unified correlations based on the concepts of surface renewal theory and energy dissipation rate in the beds have been proposed. The analogy between the heat and the mass transfer in three-phase fluidized beds has been discussed. The areas wherein future research should be undertaken to improve the state of the present knowledge are defined with recommendations.It is also widely used in pulp and paper mills, coking operations, coal refining, tannery and foundries. Phenolic compounds are among the most frequently found pollutants in rivers, industrial effluents, and landfill runoff waters. These compounds are toxic either by ingestion or by contact or inhalation even at low concentrations. Acute exposure of phenol causes central nervous system disorders, which leads to collapse and coma. Muscular convulsions with significant reduction in body temperature are also noted due to phenol toxicity, and this is known hypothermia. Renal damage and salivation may be induced by continuous exposure to phenol.

Materials and Methods¹⁻¹⁰:

The liquid broth prepared was poured into 75 conical flasks, 30ml in each. All these conical flasks were inoculated with *pseudomonas putida* culture. At two hours intervals of time, the biomass of any two flasks is measured with the turbidity meter. And the average value of this two is noted. The draft tube fluidized bed bioreactor shown in fig.1 has been

used in the present investigation. The reactor up of glass was provided with a glass sparger at the bottom of the reactor through which air can be sparged. The total volume of the reactor was about 2.67 l. The top of the glass reactor was closed with a plate through which all the probes and sensors were inserted into the reactor. An over flow line has been provided near the top for the reaction medium to flow out of the reactor in continuous operation. Two peristaltic pumps have been provided each for media and feed into the reactor. The reactor was provided with a glass jacket to maintain the temperature of the reactor system. A pH meter and a controller have been provided for maintaining the pH of the system by the addition of acid or base from the tanks provided at the top. Oxygen required for the microorganism has been supplied by a compressor. Fixed flow rate of broth containing the culture and growth medium of known inlet phenol concentration was introduced continuously into the reactor. The temperature was maintained at 30°C with a heating or cooling circuit. P^{H} was maintained at 7.0 using 0.1N HCl and 0.1N NaOH. The concentration of phenol in the overflow was analyzed Idometrically after every one hour until steady state was reached. The experiment was repeated for feed flow rates of 300, 400, 500 and 600 ml/h, feed concentrations of 20, 30, 40, 50 and 60 ppm and air flow rates of 2, 3 and 4 lpm.



Fig.1. Fluidized Bed Bioreactor



Determination of exponential growth phase time

Fig. 2. Growth of the microorganism (measured in terms of turbidity) versus time

Results and Discussion:

When the microorganism is in the exponential growth phase, each cell of *p.putida* divides into two of new cells and as a result, the concentration of the living biomass will be high. Consequently, the substrate degradation rate is also high. So it is necessary to determine the time required to reach this exponential growth phase (lag phase) after the inoculation of cells into the sterilized growth medium. The average turbidity meter reading, proportional to the biomass concentration Vs time in hours is plotted in fig.2. From the graph the time

required for exponential growth for microorganism is noted as 8 hrs.

The Bio-kinetic parameters have been calculated. The concentration of the phenol was monitored at regular intervals to measure the rate of biodegradation of phenol. Simultaneously the biomass concentration in the flasks was also monitored. The time versus biomass concentration data for different initial phenol concentrations have been plotted. These plots gave specific growth rates (μ) for different phenol concentrations. From this the bio-kinetic parameters were determined using the following correlation by rearranging it into a linear form. The variation of biomass with time in figs 3-5.

Table.	l. Exper	imental	results	of	Batch	operations
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S. No	Initial substrate	Final substrate	Initial Bio-	Final Bio	Specific
	Conc. (S_0) ,Ppm	Conc. (S),Ppm	mass(X ₀),ppm	mass(X ₀),Ppm	Growth rate μ ,(hr)
1	20.3	14.6	440	486	0.0332
2	39.8	15.06	480	589	0.0267
3	60.5	15.9	490	652	0.0393
4	76.5	24.3	620	745	0.236
5	100.9	38.4	204	319	0.0376
6	123.1	29.0	314	488	0.0316
7	142	43.4	428	616	0.0327
8	161	63.8	510	773	0.030

ln X=0.0267t+6.1691





ln X = 0.0376t+5.3586

Fig.4. Degradation of phenol for Culture concentration 100-ppm with time.



ln X= 0.0327t+ 6.0768

Fig. 5. Phenol degradation for a Culture concentration of 160-ppm with time



Under Steady state conditions, the rate of phenol degradation in the bioreactor is equated to the total rate of mass transfer to the bio-particle in fluidized bed bio-reactor and the diffusion rates of phenol were determined. The diffusion rate balance gives, $X_f V_b K S_s = Q (S_f - S_b)$, for feed the given Q, S_s is calculated. Mass Balance over bio-particle gives, $N_p A_p K_L (S_b - S_s) = Q (S_f - S_b)$, from which K_L is determined. It is observed from the figs. 6 & 7 that the mass transfer coefficient was increasing with increase in feed flow rate, feed concentration and air flow rate.

Conclusions:

The time required to reach exponential growth phase after the inoculation of cells into the sterilized



Fig 6. Bulk phase concentrations Vs Inlet phenol concentration



Fig. 7. Mass transfer coefficient VS inlet phenol concentration

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growth medium was determined. The concentration of the living biomass was found to be high and as a result, the substrate degradation rate is also high. The Biokinetic parameters were also determined. The determination of mass transfer coefficient for phenol from the bulk of the liquid to the solid particle in the fluidized bed is based on the assumption that the phenol is diffusing from the bulk phase to the solid particle through a liquid film surrounding the solid particle. The mass transfer coefficient for phenol was found to increase with increase in feed flow rate, concentration and air flow rate. bubble contamination kinetics. Chem. Engg. Proc. 43 (2004) 823-830.

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