REPEATED BATCH OPERATION OF INTERNAL LOOP AIRLIFT BIOREACTOR IN DEGRADING PHENOLICS AS SINGLE AND MIXED SUBSTRATE BY USING PREDOMINANTLY PSEUDOMonas SP.

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ABSTRACT

An internal loop airlift bioreactor (ILALR) was developed and studied for biodegradation of phenol and m-cresol as single and mixed substrate system using Pseudomonas sp. under repeated batch operation. The results showed that the culture was able to degrade phenol and m-cresol with a cumulative concentration of 600 mg L⁻¹ each in 13 and 17 h respectively. A maximum of four steps was adopted in the study, with a substrate concentration of 125 mg L⁻¹ for each step. The degradation rates of both the substrates were increased without lag in the degradation profile. The specific growth rates of the culture at various phenol and m-cresol concentrations were fit to Monod model. The biokinetic constants estimated using this model showed good potential of the Pseudomonas sp. in treating phenol and m-cresol in an ILALR under repeated batch mode.

INTRODUCTION

The increased concern about the environment and health leads to development of more rigorous control methodologies, ultimately leads to a new treatment technologies capable of dealing with the toxic pollutants. A major fraction of wastewater is polluted with the highly toxic organic compounds even at low concentrations like phenolics. Such wastewater was disposed of from a variety of industries, involving in production of steel, resins, ceramic, fiberglass, metallurgical extraction, textile processing, fungicides and herbicides [1-3]. Owing to their toxic properties, removal of these phenolics to significantly low levels is of great importance. As a result, it has become mandatory to treat this wastewater before safe final disposal into the water [2,3]. For more than a decade various pure cultures of microbial strains have been tested for degrading phenolic compounds. The study pertaining to these processes using indigenous mixed microbial in a reactor is limited, although such mixed microbial community seems promising in terms of mineralization of these phenolics to CO₂ and H₂O without producing any toxic residues.

For many decades numerous technologies have been used in the environment profession to bioremediate recalcitrant pollutants in aqueous system [4,5]. Nonetheless, microbial degradation is slow and efficient mass transfer of nutrients and oxygen to the cell culture remains as the main stumbling block in the industrial biotreatment plants. Such problem has been successfully overcome by implementing a new bioreactor system called airlift bioreactors [6]. For decades, such kinds of reactors were employed mainly for fermentation process; but in recent years, they have become popular in treating wastewater containing organics by employing suitable microbes. This kind of study was carried out in order to explore the adoptability of such bioreactor for wastewater treatment. Since this kind of reactors never requires mechanical
agitation, only aeration is required that enhances degradation of the pollutants [2].

In recent years, many researchers have focused their attention to evaluate airlift bioreactor performance in degrading organics with pure and mixed species of microbes. Quan et al. [7] studied the biodegradation of 2,4-dichlorophenol and phenol in an internal loop airlift bioreactor (ILALR) immobilized with Achromobacter sp. In their study, they performed the biodegradation of mixed substrate in fed batch and in continuous mode. In the process of fed batch operation, removal rate of 2,4-dichlorophenol decreased with increase in the run number, while the phenol was just to the contrary [7]. They operated the reactor with the pollutants concentration less than 100 mg L\(^{-1}\). Moreover, a study pertaining to such repeated batch biodegradation in ILALR, with a treatment objective is again limited.

Hence, the present study focused on biodegradation of phenol and m-cresol using predominantly Pseudomonas species, isolated from sewage treatment plant, in an ILALR operated under repeated batch mode.

**MATERIALS AND METHODS**

1. **Chemicals and Reagents**

    Phenol and m-cresol used in the study were of analytical grade; glucose and inorganic salts used in preparing microbial growth media were of reagent grade. All the chemicals and reagents were purchased from Merck, India.

2. **Microorganism and Culture Conditions**

    The microorganism used in this study was a mixed culture capable of degrading phenol and m-cresol. It was isolated and enriched from a sewage treatment plant located in Guwahati, India. The isolated species was later identified as a predominantly Pseudomonas species based on routine biochemical and staining tests; scanning electron microscopy also confirmed the results [8]. The culture was cultivated in a 250 mL flask containing 100 mL of mineral salt medium (MSM) in an orbital shaker at 150 rpm and 27 °C. The MSM composed of (in mg L\(^{-1}\)) \((\text{NH}_4\text{H}_2\text{SO}_4 \text{ (230), CaCl}_2 \text{ (8.0), FeCl}_3 \text{ (1.0), MnSO}_4\text{H}_2\text{O (100), MgSO}_4\text{H}_2\text{O (100), K}_2\text{HPO}_4 \text{ (500), KH}_2\text{PO}_4 \text{ (250) and glucose 2 g L}^{-1}\text{ and pH 7.0 under agitation condition (150 rpm). The culture was then acclimatized, over a period of one month, to grow in MSM containing phenol and m-cresol as the sole carbon source up to a concentration of 800 and 1000 mg L\(^{-1}\) respectively. Thus, the acclimatized culture was utilized in the present study. The acclimatization phase is shown in Fig. 1. From the profile, a lag phase was evident at a phenol concentration greater than 500 mg L\(^{-1}\) and more lag was observed for higher concentration of 800 mg L\(^{-1}\) with a longer utilization time by the culture.**

3. **Biodegradation Experiments in an Internal Loop Airlift Bioreactor**

    An ILALR made of perspex acrylic material with a working volume of 2.5 L was used throughout the study. The reactor consisted of two concentric tubes, where the inner tube was removable draft tube (40 × 5 cm); the external tube had dimensions of 60 × 8 cm. The top and bottom of the reactor was sealed with a flange made of stainless steel. Compressed air was fed from the bottom of the reactor via a nozzle of diameter 0.8 cm. Sterile air was fed in the reactor by filtering it through a sterile air filter (0.45 µm). The nozzle was placed inside the inner tube and the superficial gas flow was measured with a rotameter with a needle valve to maintain the flow. The superficial gas flow was fixed at 2 L min\(^{-1}\) throughout all the experiments. This was an optimal gas flow rate as determined from the hydrodynamic study. The experiments were performed in the reactor by maintaining the temperature at 26 °C. However, no attempts were made to control the temperature inside the reactor but it was monitored to be 26 ± 1 °C.

4. **Repeated Batch Biodegradation Operation of ILALR**

    The reactor was operated in repeated batch mode in order to evaluate the reactor performance in degrading phenolics. The reactor study was initially started in batch mode with a phenol and m-cresol concentration of 100 mg L\(^{-1}\) each respectively. An inoculum size of 250 mL of freshly-grown culture was added to start up the degradation experiment. The reactor was then switched to repeated batch mode up to a cumulative concentration of phenol and m-cresol to 600 mg L\(^{-1}\) each with increment in both medium and substrate concentration (125 mg L\(^{-1}\) each) in four repeated steps. The dilution factor was taken care and substrate concentration was calculated and then fed in to the reactor.

![Fig. 1. Phenol and m-cresol degradation profile followed during the enrichment period.](image-url)
as step input. The details of operation adopted in the study are shown in Table 1.

5. Analytical Methods

Cell density in the samples was estimated with Diode Array Spectrophotometer (Spekol 1200, Analytik Jena, Germany) by measuring its absorbance (OD) at 600 nm wavelength. OD<sub>600</sub> reading was then converted to dry cell weight by a calibration curve, which was obtained by plotting dry weight of biomass per milliliter against OD<sub>600</sub>. For determination of biomass concentration as dry weight, samples were centrifuged for 10 min at 10,000 g at room temperature (Biofuge Stratos, Heraeus instruments, Kendro, Hanau, Germany) in Ependroff tubes. The pellets were dried at 50 °C for 24 h, cooled in a desiccator at room temperature and weighed. The tubes were washed, dried and re-weighed to determine its empty weight. The difference between the first and second weight was used to determine the dry weight of biomass in mg L<sup>-1</sup>.

High performance liquid chromatography (HPLC 200 series UV/Vis, Perkin Elmer, Waltham, MA) was employed to quantify phenol and m-cresol concentrations in the biomass free samples. The analysis was performed with C18 column (150 mm × 4.6 mm × 5 μm; chromatopak) with acetonitrile/water (60/40) as the mobile phase at a flow rate of 1 mL min<sup>-1</sup>, and the detection was done with a UV detector set at 275 nm. The retention period for phenol and m-cresol were 2.75 and 3.25 min, respectively.

RESULTS AND DISCUSSION

1. Effect of Initial Concentration on Phenol or m-Cresol Biodegradation

Figure 2 shows the removal of phenol and m-cresol as a single substrate under repeated batch operated mode in an ILALR. It was observed that both the substrates were completely degraded by the culture within 13 and 17 h, respectively. Initially the reactor was operated with phenol and m-cresol concentration of 100 mg L<sup>-1</sup> each, which was degraded in 5 and 8 h, respectively. Then the substrates was increased in 4 repeated steps with a concentration of 125 mg L<sup>-1</sup> in each step. This resulted in a cumulative concentration of 600 mg L<sup>-1</sup> of both phenol and m-cresol. In comparing the degradation profile of phenol and m-cresol, the m-cresol profile showed much greater lag and took more time than the pervious one. This could be reasoned that the phenol in comparison with m-cresol is easily assimilated by the culture and has less toxicity, while the later has more toxic and difficult to be assimilated. Operating reactor in repeated batch mode took only 13 and 17 h for complete degradation of the phenolics. Moreover it could be observed when compared with the simple batch mode, the degradation time was much less for the repeated batch mode even at the higher concentration. The lag obtained in the batch degradation was completely eliminated in the present mode of operation The culture growth profile is shown in Fig. 3. From the figure it is clear that an enriched biomass was produced in the repeated batch, than that of the simple batch operation. The maximum biomass output shows the effective and quick uptake of the phenolics by the isolated culture.

2. Kinetics of the Culture Growth as Single Substrate

In order to establish the effect of phenol concentration on growth of this culture, specific growth rates of the culture at different phenol concentrations were calculated with well known Monods model. It was observed from the estimated biokinetic parameters that the μ<sub>max</sub> is 0.55 h<sup>-1</sup> for phenol with K<sub>s</sub> 152 mg L<sup>-1</sup>. Correspondingly 0.49 h<sup>-1</sup> and 145 mg L<sup>-1</sup> for m-cresol. In general higher growth rate value denotes the potential of the culture in degrading the or-
organic waste. The obtained culture showed a higher $\mu_{\text{max}}$: Monterio et al. [9] in their observation on phenol biodegradation by *Pseudomonas putida* 548 in a batch reactor obtained $\mu_{\text{max}}$ of 0.44 h$^{-1}$. Similarly Banerjee et al. [10] in their study on biodegradation of phenol with *P. putida* in batch shake flasks adopted Haldane model reported the value of $\mu_{\text{max}}$ to be 0.06 h$^{-1}$, which was very low. Kumar et al. [11] obtained $\mu_{\text{max}}$ of 0.31 h$^{-1}$ for the biodegradation of phenol with *P. putida* MTCC 1194. In comparison with the previous literature report, the obtained $\mu_{\text{max}}$ value in the present study showed a better potential of *Pseudomonas* species in degrading phenol and *m*-cresol as single substrate. Figure 4 shows the experimental $\mu_{\text{max}}$ for both substrates.

Hopper and Taylor [12] studied the pathways for the degradation of *m*-cresol and *p*-cresol by *P. putida* and observed that catechol, 3-methylcatechol, and 4 methylcatechol were formed in the *m*-cresol biodegradation. They also proposed the pathway for metabolism of 3,5-xylene, *m*-cresol and *p*-cresol by *P. putida*. Similarly, Bayly and Wigmore [13] studied and proposed the biodegradation pathway for phenol.

3. Co-biodegradation of Phenol and M-cresol

The co-biodegradation of phenol and *m*-cresol as mixed substrate in the bioreactor was also studied in repeated batch mode (Fig. 5). It can be seen that phenol and *m*-cresol of initial concentration 50 mg L$^{-1}$ at the first run was degraded within 7 h. In the second run, the 50 mg L$^{-1}$ of both substrates phenol was degraded faster within 1.2 h while *m*-cresol was degraded in 1.3 h.

In both these cases, no lag was observed in the degradation profile. In the third step, the reactor was operated with a concentration of 50 mg L$^{-1}$ each substrate and it was observed that phenol was preferentially degraded first within 1.2 h while it took 1.3 h for *m*-cresol. At the final step, the substrate concentration was increased to 125 mg L$^{-1}$ each and a slight lag was observed for both phenol and *m*-cresol. However, the substrates were completely degraded in 15 h. Overall, in these steps, no lag phase was observed in the degradation profile of phenol and phenol was preferentially degraded faster than *m*-cresol.

It was observed that operating ILALR in a repeated batch mode resulted in a complete degradation of phenolics in short duration without any lag. Quan et al. [7] in their biodegradation study of 2,4-dichlorophenol and phenol in an airlift reactor immobilized with *Achromobacter* sp. observed that the degradation rate of 2,4-dichlorophenol decreased with increase in run while that of phenol was increased. But in their study the concentration variation in each step was less than 80 mg L$^{-1}$ for phenol and 20 mg L$^{-1}$ for 2,4-dichlorophenol.

In the present study such lag or reduction in rate was not observed even at slightly higher concentrations of substrate. The biomass output for the respective operation is presented in Fig. 6. The results obtained in the repeated batch mode showed better performance in degrading phenol and *m*-cresol as single and mixed substrate in an ILALR.
4. Co-biodegradation Model Fitting of Experimental Specific Growth Rate

A sum kinetics model proposed by Yoon et al. [14] was adopted in the present study to predict its variations due to various combinations of concentrations of substrates: phenol and m-cresol. The each step in the dual degradation experiments was considered as batch and the obtained data were fitted to the sum kinetic model. This model used for evaluation of the interaction between phenol and m-cresol on the growth of the culture, and to estimate the relative effects of the two substrates on their individual uptakes (degradation of phenol and m-cresol). The form of this model is shown in Eq. 1.

$$\mu = \frac{\mu_{\text{max1},i} S_{1i}}{K_{S,i} + S_{1i} + \frac{S_{2i}^2}{K_{I,i}} + I_{i,j} S_{2i} + \frac{S_{2i}^2}{K_{I,i}} + I_{i,j} S_{1i}}$$

The model equation is almost similar to that of Haldane’s with an additional interaction parameter. The interaction parameter $I_{i,j}$ indicates the degree to which substrate $i$ affects the biodegradation of substrate $j$; a large value of the parameter indicates a strong inhibition on the substrate uptake by the microorganism [14]. The other kinetic parameters $\mu_{\text{max}1}$, $K_{S}$, and $K_{I}$ in the equation are the same as those for any single substrate system. A non-linear regression technique involving constraints for positive integer values of the parameters was employed for solving the model equation using MATLAB version 7. Very high determination coefficient ($R^2$) value (0.96) was obtained by fitting the model equation to the experimental growth rate values of the culture. The $I_{i,j}$ values were found to be 12.5 for $I_{\text{phenol-m-cresol}}$ and 8.9 for $I_{\text{m-cresol-phenol}}$. Again, the $I_{\text{phenol-m-cresol}}$ represents the effect of phenol on m-cresol degradation with respect $\mu_{\text{max}}$ and the $I_{\text{m-cresol-phenol}}$ represents the effect of m-cresol on phenol degradation. From the values of these parameters, it could be concluded that both phenol and m-cresol at 100 mg L$^{-1}$ exhibit stronger inhibition on m-cresol degradation, than that of m-cresol on phenol degradation. In literature, a maximum $I_{ij}$ value of 5.16 was reported for the effect of toluene on benzene degradation by a P. putida strain [15]. The $I_{ij}$ values obtained from the model render a fair knowledge on the inhibitory effects of dual substrate degradation. From both the single and dual substrate kinetics the culture showed its good potential in degradation the phenolics either as a single or dual substrate.

CONCLUSIONS

This study revealed the applicability of an ILALR for treating phenolics in repeated batch mode operation. The employed Pseudomonas species degraded both phenol and m-cresol to a cumulative concentration of 600 mg L$^{-1}$ each. A complete degradation was achieved in the co-biodegradation of phenol and m-cresol. In both the cases, the substrates were degraded well in short time duration. The results showed the potential of the ILALR in completely degrading phenolics using predominantly Pseudomonas species.

REFERENCES

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Discussions of this paper may appear in the discussion section of a future issue. All discussions should be submitted to the Editor-in-Chief within six months of publication.

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