Biodegradation of phenol and its derivatives in packed and moving bed bioreactors using *Bacillus* sp. isolated from petroleum site

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By

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CHAPTER 5

CONCLUSIONS

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5. 1. Conclusions

In this study, the potential phenol degrading microbial species were isolated from the petroleum-contaminated site (IOCL refinery Mathura, India, 27º30’12” N, 77º40’19” E and 181 m elevation above sea level). Among the isolated species, GS1 and GS2 had high phenol degradation ability, and these species were identified as *Bacillus flexus* GS1 IIT (BHU) and *Bacillus cereus* GS2 IIT (BHU). First, a free cell study was performed to optimize the process parameters such as pH (5.0 – 9.0), temperature (25 – 40 ℃), and phenol concentration (50 – 500 mg/L) for the removal of phenol using *Bacillus flexus* GS1 IIT (BHU). The optimum pH, temperature, and phenol concentration were obtained to be as 7.0, 30 ℃, and 100 mg/L, respectively. The substrate inhibition was observed at high concentration of phenol which was studied by the Andrew-Haldane kinetic model. The inhibition model implied that the specific growth rate of the bacterial species decreased after 200 mg/L due to the toxic effect of phenol. After the free cell study, a lab-scale packed bed bioreactor (PBBR) was fabricated and operated at the optimized process conditions by varying the inlet feed flow rate from 15 – 60 mL/h. Low-density polyethylene (LDPE) immobilized with the *Bacillus flexus* GS1 IIT (BHU) was used as packing material in the PBBR. The maximum removal efficiency (RE) of 99.13 % and elimination capacity (EC) of 35.69 mg/L/d were obtained at 100 mg/L of the initial concentration of phenol. The impact of external mass transfer resistance on the biodegradation rate of phenol in a packed bed bioreactor (PBBR) was studied. A new non-dimensional empirical correlation, $J_D = 3.114\mathrm{N}_{\mathrm{Re}}^{-0.64}$, based on Colburn factor was developed to determine the external mass transfer coefficient of phenol in the PBBR. The above mass transfer correlation predicted the experimental data accurately and its use is recommended to quantify external film diffusion effect for the continuous bioconversion in a PBBR.

In the next study, the performance of a moving bed biofilm reactor (MBBR) with bio-carriers made of polypropylene-polyurethane foam (PP-PUF) was evaluated for the collective removal of phenol and ammonia. Three independent variables, including pH (5.0-8.0), retention time (2.0-12.0 h), and airflow rate (0.8-3.5 L/min) were optimized using central composite design (CCD) of the Response Surface Methodology (RSM). The maximum removal of phenol and ammonia was obtained to be 92.6 and 91.8%, respectively, in addition to the removal of 72.3% in the chemical oxygen demand (COD) level at optimum conditions. A second-order model was found to be appropriate for predicting reaction kinetics in the MBBR. The values of second-order rate constants were obtained to be 2.35, 0.25, and 1.85 L^2/g.VSS.gCOD.h for phenol, COD, and ammonia removal, respectively. The intermediate such as 2-hydroxy muconic semialdehyde was detected by GC-MS, which confirmed that the *Bacillus* sp. could utilize phenol via the meta-cleavage pathway.

The effect of peptone on biodegradation of 4-chlorophenol (4-CP) was studied in a moving bed biofilm reactor (MBBR) packed with *Bacillus* sp. immobilized polyurethane foam-polypropylene biocarriers. It was found that the growth of biomass increased with the increase of peptone concentration, resulting in the higher removal of 4-CP. At the optimized process conditions, the maximum removals of 4-CP and chemical oxygen demand (COD) were obtained to be as 91.07 and 75.29 %, respectively. The two kinetic models, i.e., Monod and modified Stover-Kincannon models, were employed to investigate the behaviour of MBBR during 4-CP biodegradation. The high regression coefficients obtained by the modified Stover-Kincannon models showed better substrate utilization rates predictably. In addition, the phytotoxicity test revealed that the treated wastewater offered less toxicity and higher growth (plumule and radicle) compared to the untreated wastewater.

Finally, a comparative analysis was carried out to evaluate the performance of the MBBR and PBBR under identical conditions. In this direction, a sponge-modified carrier, i.e., polypropylene-polyurethane foam (PP-PUF) was used in the bioreactors, MBBR and PBBR, for the biodegradation of 4-CP. The maximum removal efficiencies (REs) of 4-CP were obtained as 94.34 and 90.62 % in MBBR and PBBR, respectively, at an initial 4-CP concentration of 50 mg/L. Similarly, the chemical oxygen demand (COD) REs were found to be 83.26 and 79.37 % in MBBR and PBBR, respectively. The higher REs of 4-CP and COD were observed in MBBR due to the high growth of biomass. The total biomass found in the MBBR and PBBR was in the range of 1595 – 1752 mg/L and 1468 – 1640 mg/L, respectively. The present study confirms a higher efficacy of the MBBR, in comparison to the PBBR, for the biodegradation of phenol derivative under similar conditions. A relatively low efficiency of the PBBR was attributed to high substrate diffusion resistance and channeling of fluid causing poor biomass-substrate contact in the bioreactor.

5. 2. Scope for future work

The following future works are recommended based on this study:

1. Genetic modification of *Bacillus* species to further improve phenol and COD removal efficiency
2. Investigation of the effect of internal pore diffusion on phenol biodegradation in immobilized cell bioreactors
3. Chemical modification of the plastic biocarriers to enhance bio-adhesion
4. Development of hybrid bioreactors to accelerate the rate of biodegradation