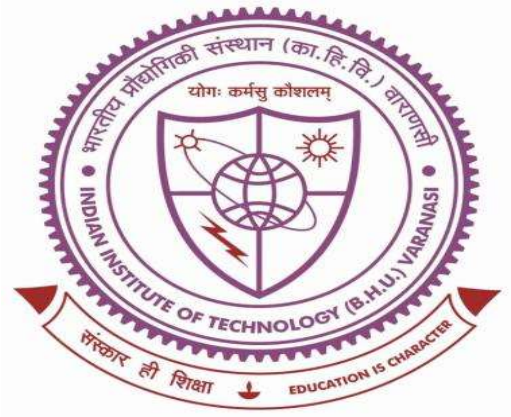
Process Design and Optimization of Bioreactors for the Treatment of Aromatic Hydrocarbon and Dye using Isolated Bacterial Species



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DOCTOR OF PHILOSOPHY

By

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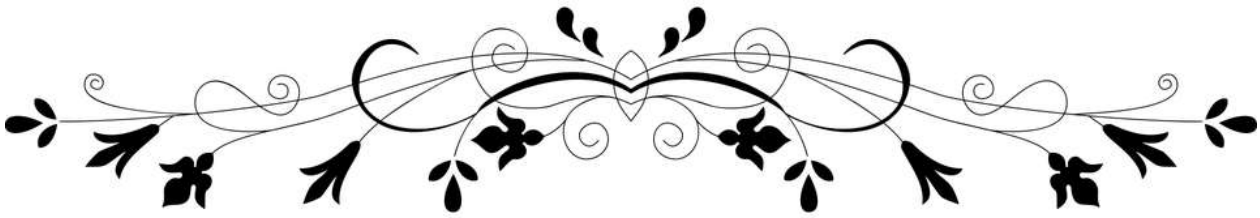
6.4. Conclusions

The biodegradation of Congo red dye was performed using polyurethane foam-polypropylene immobilized *Bacillus* sp. MH587030.1 in MBBR. The response surface methodology was used to optimize the process parameters, namely pH, Congo red concentration, and media filling ratio, and the optimum conditions were observed to be 7.0, 50 mg/L, and 45%, respectively in batch MBBR. The performance of a continuous MBBR was studied at various inlet loading rates, and more than 90% of RE and 57.6 mg/L.day of EC were found under optimum conditions. A modified Stover-Kincannon model examined the biodegradation kinetics of Congo red dye and proposed kinetic correlations can be used for the prediction of effluent (dye) concentration and reactor volume to scale-up of the process. Finally, the *Vigna radiata* seeds germinated in the dye treated wastewater showed better growth than untreated wastewater.



CHAPTER 7

Summary of the thesis and scope for future work

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7.1. Summary of the work

In the present work, various potential bacterial species were isolated from petroleum-contaminated and dye-contaminated soil for the biodegradation of aromatic hydrocarbon (naphthalene) and dye (Congo red) in different types of bioreactors. At the outset, ten bacterial species (RKS1, RKS2, RKS3, RKS4, RKS5, RKS6, RKS7, RKS8, RKS9, and RKS10) were isolated from petroleum-contaminated soil for naphthalene biodegradation. Amongst them, RKS3, RKS4, and RKS8 were found to be more effective for naphthalene biodegradation than other bacterial species, and these were identified as *Exiguobacterium* sp. *Bacillus* cereus, and *Stenotrophomonas* sp., respectively, using 16S rRNA. The most potential bacterial species, i.e. *Bacillus* cereus RKS4 (MH681588.1) was used for naphthalene biodegradation in a lab-scale integrated aerobic treatment plant (IATP). The experiments were designed using a central composite design (CCD) of response surface methodology (RSM) to optimize the process variables such as pH, temperature, and naphthalene concentration. The maximum naphthalene removal efficiency of 96.1% was found at optimum conditions (pH of 7.0, naphthalene concentration of 10 mg/L, and temperature of 32.0 ◦C). The performance of an IATP was evaluated at different inlet loading rates under optimized conditions. It was found that the removal efficiency of naphthalene was highest at the lowest inlet loading rate or vice versa. The intermediates were analyzed by GC-MS and catechol ($C_6H_6O_2$) and 2-naphthol ($C_{10}H_8O$) were the major intermediates obtained. The growth-inhibition kinetic study by Monod and Teissier-Edwards models have clearly indicated that the specific growth rate (μ) was initially increased with increasing naphthalene concentration up to 30 mg/L and beyond this concentration, the value of μ

was decreased. The decreased in the value of μ may be due to the substrate inhibition beyond 30 mg/L. The *Cicer arietinum* seeds germinated in treated wastewater showed better growth than untreated wastewater.

In the next study, the performance of a packed bed bioreactor (PBBR) was evaluated at different inlet flow rates for naphthalene biodegradation. The low-density polyethylene (LDPE) immobilized with *Exiguobacterium sp*. RKS3 (MG696729.1) was used as the packing media in the PBBR. The SEM analysis demonstrated the biofilm was successfully developed onto the surface and pores of the LDPE. The most affecting process variable, namely temperature, salinity, and pH were optimized under the batch mode and found to be 30 °C, 4 g/L, and 7.0, respectively. Also, an attached-growth bioreactor (PBBR) has shown better performance than the suspended-growth bioreactor. An effort has also been made to examine the correlation between the external mass transfer (EMT) coefficient and mass flux at various inlet flow rates (IFRs). A new mass transfer correlation was developed ($J_D = 5.71{N_{Re}}^{-0.1}$) to predict the external mass transport aspects, and it could be helpful to estimate the external biodegradation rate of naphthalene. The substrate inhibition study by Andrews-Haldane model revealed that the substrate inhibition was perceived at high concentration of substrate. The bio-kinetic parameters, μ_{max}, K_s, and K_i were estimated and found to be 0.386 per day, 13.6 mg/L, and 20.54 mg/L, respectively.

Three plastic carriers, namely polypropylene (PP), low-density polyethylene-polypropylene (LDPE-PP), and polyurethane foam-polypropylene (PUF-PP), were developed and used separately in three different moving bed bioreactors (MBBRs) for naphthalene biodegradation. The enriched bacterial consortium isolated from petroleum-contaminated soil was used as an inoculant in MBBR. The SEM analysis demonstrated the successful growth of biofilm onto carriers. At optimum pH (7.0) and

HRT (5 days), the maximum COD removal efficiencies were obtained as 72.4%, 84.4%, and 90.2% for MBBR filled with PP, LDPE-PP, and PUF-PP carriers, respectively. MBBR filled with PUF-PP carriers has shown the highest performance at the optimum conditions than PP and LDPE-PP. The values of maximum substrate removal rate (U_{max}) were found to be 0.476, 0.666, and 0.769 g/L.day for PP, LDPE-PP, and PUF-PP carriers, respectively. Similarly, the values of saturation constant K_B were found to be 0.565, 0.755, and 0.874 g/L.day for PP, LDPE-PP, and PUF-PP carriers, respectively. The kinetic constants evaluated by the modified Stover–Kincannon model demonstrated that the MBBR filled with PUF-PP carrier had shown the highest substrate removal rate (U_{max}) than MBBR filled with PP and LDPE-PP.

Further, PUF-PP immobilized *Bacillus* sp. (MH587030.1) was used for the biodegradation of Congo red dye in MBBR at different inlet loading rates. *Bacillus* sp. (MH587030.1) was isolated from dye-contaminated soil. The CCD of RSM designed the experiments to optimize the process variables such as pH, Congo red concentration, and media filling ratio. The optimum pH, Congo red concentration, and carrier filling ratio were obtained as 7.0, 50 mg/L, and 45%, respectively in batch MBBR. More than 90% of dye removal efficiency and 57.6 mg/L.day of elimination capacity were found under optimum conditions. The value of kinetic parameters K_B and U_{max} were obtained to be 0.253 and 0.263 g/L.day, respectively. A modified Stover-Kincannon model examined the biodegradation kinetics of Congo red dye and proposed kinetic correlations can be used for the prediction of effluent (dye) concentration and reactor volume to scale-up of the process. The *Vigna radiata* seeds germinated treated wastewater showed better growth in germination, shoot, and root length than untreated wastewater and suggested that the treated wastewater could be used for irrigation purposes.

7.2. Scope for further work

Biodegradation is a promising option to treat dyes, aromatic hydrocarbons, and other pollutants in an eco-friendly and cost-effective manner. Enzymes or genetically modified microbe could be developed to enhance the biodegradation rate. The bioreactor is the heart of any biochemical process (e.g., biodegradation of pollutants) to obtain optimal microbial growth and resulting the high biodegradation rate of pollutants. Further, the development of cutting-edge combined bioreactor designs is necessary to provide the desired solution to existing complex pollutants. To understand the operation of bioreactors, a knowledge of bio-reaction kinetics, mass transfer, design and modeling of the bioreactors, and selection of carriers is crucial. Hence, there is a need to focus on continuous innovation in bioreactor configurations to enhance the performance of bioreactors for pollutants removal. Further, the study is required to scale-up the process from laboratory to pilot scale and finally at the industrial level.