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Removal of Acid blue 113 dye in a moving bed biofilm reactor using isolated bacterial species

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Acid blue 113 (AB113), an azo dye, is found in the wastewater released from leather, textile, tannery, and paper printing industries. It is toxic, carcinogenic, and mutagenic, causes adverse effects on the eco-system. In this study, polypropylene-polyurethane foam (PP-PUF) was used as carriers in a lab-scale moving bed biofilm reactor (MBBR) for the removal of AB113. The process variables i.e. pH, dye concentration, and hydraulic retention time (HRT) were optimized by central composite design (CCD) of response surface methodology (RSM). The optimum value of pH and HRT were obtained as 7.0 and 20 h, respectively, for the maximum removal of dye at the highest concentration of 300 mg/L of AB113. At optimized conditions, the maximum removal of AB113 and chemical oxygen demand (COD) were found to be 72.76 and 58.84%, respectively.

Keywords: Carrier, optimization, moving bed biofilm reactor, Acid blue 113, microbial consortium.

Introduction

The potable water demand is increasing day by day due to the rapid growth of industrialization. A large amount of wastewater containing toxic and harmful pollutants is released in water bodies. The synthetic dyes are the most common raw material used in textiles, chemicals, leather, cosmetics, tanneries, and paper printing industries[1,2]. The untreated wastewater released from these industries contains pigments and dyes, which lead to harmful effects on human beings, and aquatic animals[3]. This wastewater can deteriorate the surface water quality by reducing the dissolved oxygen and photosynthesis process[4,5]. In specific, Acid blue 113 (AB113) is an azo dye used in the wool, textile, paper, and leather industries, causes adverse effects on the aquatic plants and microorganisms. Moreover, acute exposure causes cancer, neurological disorder, and dermatitis in human beings[3]. Therefore, effective and cost benign techniques should be developed for the removal of AB113 from wastewater.

In this direction, various physico-chemical processes such as coagulation, adsorption, ozonation, UV-Fenton, and photocatalysis have been employed[3,5]. However, the major limitations of these methods include excess sludge generation, production of intermediate pollutants, and high cost. Various researchers have widely studied the biodegradation process due to its merits, such as low cost, eco-friendly, complete degradation, and less sludge generation as compared to physico-chemical methods[2]. Generally, free and immobilized cells are employed in the biodegradation of pollutants. The immobilized (attached) cells offer high removal efficiency and protect microorganisms from the adverse conditions than free cells[6]. The materials such as calcium alginate, polyvinyl alcohol gel, biochar, polyurethane foam, etc., have been used as the support for the attached cells process[1,5]. In this direction, the bioreactors such as packed bed bioreactor (PBR), moving bed biofilm reactor (MBBR), and trickling biofilter have been extensively used[6,7]. MBBR is preferred as efficient bioreactors due to high biomass growth, no need for fluid recirculation, effective substrate diffusion[7]. However, limited research has been reported on AB113 removal in a MBBR using polypropylene-polyurethane (PP-PUF) as carrier.

In the present study, microbial consortium immobilized PP-PUF carriers were used in a MBBR to remove AB113 dye from wastewater. The process parameters such as pH, dye concentration, and HRT were optimized using CCD RSM.

Material and methods

Chemicals and bacterial culture:

AB113 (CAS number 3351-05-1) dye was brought from

Sigma-Aldrich, India, and other chemicals used for the mineral salt media (MSM) were purchased from Himedia, India. The composition of MSM was prepared as follows (g/L): K_2HPO_4 (2.0); KH_2PO_4 (0.4); $CaCl_2.2H_2O$ (0.01); $FeSO_4.7H_2O$ (0.05); NaCl (0.5); $MgSO_4.7H_2O$ (0.2); $C_6H_{12}O_6$ (3). The synthetic wastewater (SW) was prepared by adding different amounts of dye in MSM. The pH of the SW was adjusted by using 0.1 *N* HCl or NaOH.

The soil sample was collected from a dye-contaminated site located in Bhadohi, India, to isolate potential bacterial culture. The soil was collected in a sterile container and stored at 4°C for further application. Initially, 15 g soil was added in an Erlenmeyer flask (250 mL) containing 100 mL MSM and 50 mg/L of dye. The flask was incubated at 30°C and 100 rpm for five days to enrich the microorganisms. After five days, 15 mL of inoculum was transferred to a new flask containing MSM and dye (100 mg/L). The flask was again incubated for five days. This procedure was repeated three times more with gradually increasing the concentration of dye by 50 mg/L in each stage. Finally, bacterial species were isolated by serial dilution method[7].

MBBR setup and immobilization procedure:

A lab-scale MBBR was developed for the biodegradation of wastewater containing AB113. The 2 L bioreactor was made of borosilicate glass. The MBBR was associated with the feed tank, air compressor, rotameter, and effluent tank (Fig. 1). The wastewater was fed to the MBBR by a peristaltic pump (Miclins PP 30EX). The MBBR was filled with a 45% volume of PP-PUF carriers. The detailed descriptions of polypropylene-polyurethane foam (PP-PUF) carriers were reported in our previous work[7]. The PP-PUF carriers were developed by inserting polyurethane foam cubes (2.0 cm length) into the alternate holes of polypropylene. The average dried mass of the PP-PUF carrier was 1.18 ± 0.03 g. For the immobilization of dye degrading species on PP-PUF carriers, the bioreactor was run at a batch mode for 15 days with glucose (1.0 g/L) as a carbon source and 20 mL (10^7 CFU/mL) of the microbial consortium. After 15 days, scanning electron microscopy (SEM) technique was performed to study the morphological characteristics of the carriers.

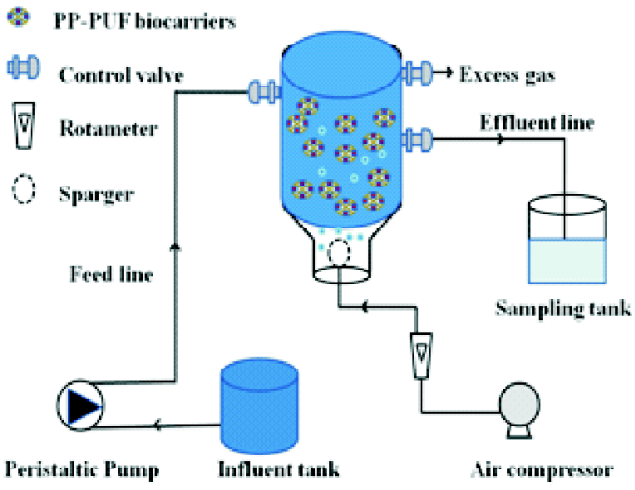

Fig. 1. Schematic representation of MBBR for the removal of Acid blue 113.

Process optimization:

The independent process variables were optimized by central composite design (CCD) of response surface methodology (RSM). RSM is a cost-effective and time-saving tool as it reduces the number of experiments. The mathematical and statistical tool is designed to predict the optimum responses from a wide range of independent variables. Moreover, the statistical method provides empirical correlations between the process variables with responses[8]. Design-Expert software (Version 11) (Stat-Ease Inc., Minneapolis, USA) was used for process optimization. A wide range of process variables such as pH (5.0–9.0), dye concentration (100–300 mg/L), and hydraulic retention time (HRT) (10–30 h) were used for the optimization process. According to the expression 2^n+2n+n_0 (where, n is the number of process variables, and n_0 is the repetitions of experiments at central points), a total of 20 runs were performed, and the corresponding responses were summarized in Table 1. Finally, the obtained responses were analyzed by ANOVA statistics, model summary statistics, and 3-D model graphs to evaluate the optimum condition.

Analytical methods:

The residual dye concentration of AB113 was determined using a UV-Vis spectrophotometer (SL-210, ELICO). Before analysis, the effluent samples were centrifuged at 8000 rpm for 5 min, and the supernatant was analyzed for the determination of dye and COD. The COD was determined using the standard protocol[7]. The pH and dissolved oxygen (DO) were measured using pH meter and DO meter, respectively. The biofilm growth onto the carriers was analyzed by a scanning electron microscope (SEM, QUANTA 200F, Netherland).

Results and discussion

Morphological characteristics of PP-PUF carriers:

SEM analysed the morphology of PP-PUF before and after the immobilization. Before immobilization (control), several micropores were observed on the surface of PP-PUF (Fig. 2a). However, after 15 days of operation, a layer of biofilm was developed on the surface of PP-PUF (Fig. 2b), which confirmed the successful immobilization of biofilm. Moreover, PUF provides a large surface area for the entrapment of the microorganisms, which enhances the high biomass growth in the PP-PUF carrier.

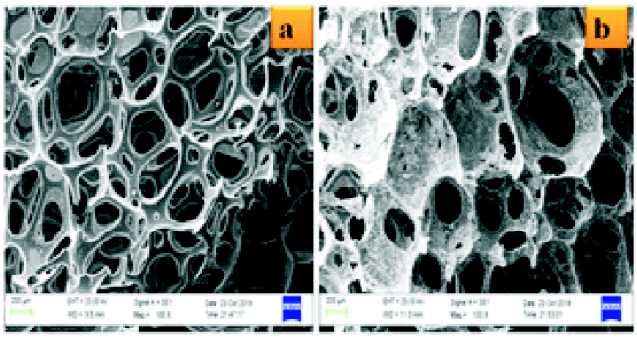

Fig. 2. SEM images of PP-PUF carrier: (a) before, (b) after immobilization.

Optimization study:

The process parameters such as pH, dye concentration, and HRT were optimized using CCD of RSM. The obtained responses are summarized in Table 1. The correlations developed between the independent process and the responses are expressed in the form of a second-order polynomial equation as:

$$\text{Dye removal (\%)} = 78.68 + 3.82A + 15.74B - 7.37C + 0.8125AB + 0.7950AC + 1.51BC - 23.05A^2 - 12.55B^2 + 3.94C^2 \quad (1)$$

$$\text{COD removal (\%)} = 65.16 + 4.34A + 14.94B - 8.76C + 1.42AB + 0.0037AC + 0.5612BC - 21.18A^2 - 9.70B^2 + 3.56C^2 \quad (2)$$

The value of R^2, adjusted R^2, and predicted R^2 were obtained to be 0.987, 0.977, and 0.901, respectively for dye removal. Similarly, R^2 of 0.982, adjusted R^2 of 0.966, and predicted R^2 of 0.897 were obtained for COD removal. The obtained values of R^2 are greater than 0.8, which represent that the quadratic model was in reasonable agreement with the experimental data[1].

Table 1. Experimentally obtained responses at various conditions used in RSM optimization

Run	*A*: pH	*B*: HRT (h)	*C*: Conc. (mg/L)	Dye removal (%)	COD removal (%)
1	5	10	100	39.4	29.5
2	9	30	100	70.6	63.9
3	5	20	200	50.5	39.2
4	7	30	200	87.1	76.0
5	5	30	300	51.8	38.5
6	9	10	100	41.8	34.5
7	7	20	200	79.1	67.0
8	7	10	200	45.6	38.3
9	7	20	200	78.9	65.1
10	7	20	200	77.6	64.3
11	9	30	300	60.7	49.2
12	9	10	300	28.2	17.0
13	7	20	200	78.6	61.2
14	5	30	100	62.5	53.8
15	7	20	200	78.2	63.9
16	7	20	300	72.7	58.8
17	9	20	200	61.3	52.2
18	7	20	200	78.1	62.3
19	5	10	300	20.2	12.5
20	7	20	100	93.0	82.0

Moreover, ANOVA analysis was carried out to analyze the significance and consistency of the model. The higher value of *f* and the lower value of *p* (< 0.05) show that the parameters are more significant[9]. The obtained value of *p* for pH, HRT, and dye concentration was less than 0.05, representing the significant role of the process parameters in the model (Table 2).

Simultaneous effect of pH, dye concentration, and HRT on dye and COD removal.

The effect of pH and initial dye concentration on the dye and COD removal were represented in Fig. 3a and Fig. 4a, respectively. The maximum dye removal was observed at a pH of 7.0. The dye and COD removal were obtained of 79.18 and 67.02% at a pH of 7.0 (HRT = 20 h, dye concentration = 200 mg/L).

At similar conditions of dye concentration and HRT, dye removals were obtained of 61.31 and 50.57% at a pH of 9.0

Table 2. ANOVA analysis for the quadratic model responses

Response 1: Dye removal					
Source	Sum of squares	D_f	Mean square	*F*-value	*p*-value
Model	7610	9	845.56	90.51	<0.0001
A: pH	145	1	145.62	15.59	0.0027
B: HRT	2477	1	2477.79	265.23	<0.0001
C: Dye conc.	542	1	542.73	58.1	<0.0001
Residual	93	10	9.34		
Lack of fit	91	5	18.37	57.72	0.0002
Pure error	1.5	5	0.3182		
Cor. Total	7703	19			
Response 2: COD removal					
Model	6555	9	728.44	61.61	<0.0001
A: pH	188	1	188.44	15.94	0.0025
B: HRT	223	1	2232.93	188.86	<0.0001
C: Dye conc.	768	1	768.08	64.96	<0.0001
Residual	118	10	11.82		
Lack of fit	97.4	5	19.49	4.68	0.0577
Pure error	20.8	5	4.16		
Cor. Total	6674	19			

and 5.0, respectively.

The dye and COD removal were sharply declined when the pH of the SW varied either acidic or alkaline. The maximum removal of pollutants were obtained at a neutral pH[1,7]. At acidic or alkaline pH, the dye and COD removal of the wastewater were decreased due to the inhibition of the metabolic activity of the microorganisms. Optimum dye removals of 93.09, 79.18, and 72.76% were obtained for the initial dye concentration of 100, 200, and 300 mg/L, respectively, at a pH of 7.0 and HRT of 20 h.

Fig. 3b and Fig. 4b represent the interactive effect of HRT and dye concentration on the dye and COD removal, respectively. The plots show that dye removal was increased with an increase of HRT.

Keeping the HRT at 30 h, the maximum dye removal was obtained to be 87.18% (pH = 7.0, dye concentration = 200 mg/L). However, at similar conditions, dye removal was decreased with decreasing HRT. Similarly, COD removal was declined from 82.07 to 58.84% with an increase of initial dye concentration from 100 to 300 mg/L (pH = 7.0, HRT = 20 h) (Table 1). Sekar *et al.*[10] have investigated the biodegradation of AB113 dye and reported that the dye removal rate

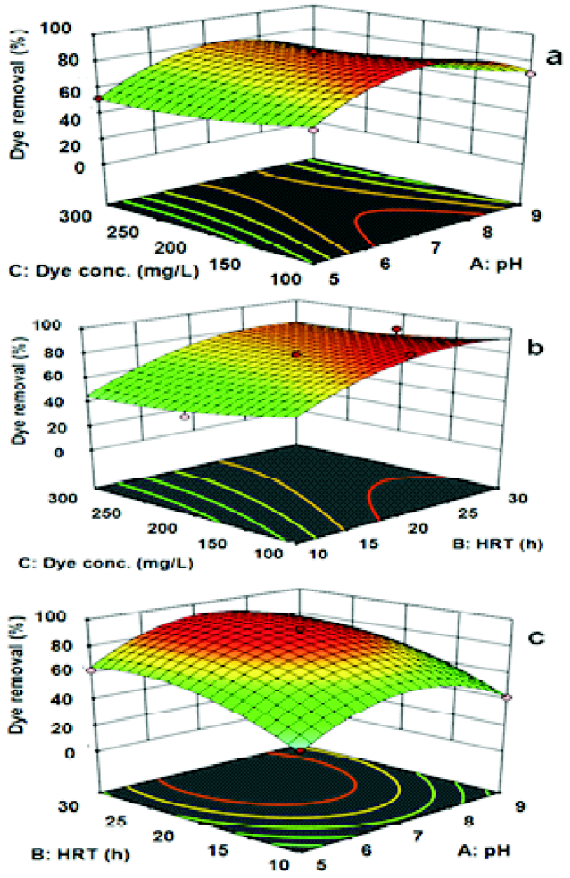

Fig. 3. 3-D surface plots for the removal (%) of Acid blue 113 dye: (a) effect of pH and dye concentration; (b) effect of HRT and dye concentration; (c) effect of pH and HRT.

was continuously decreased with the increase of initial dye concentration.

The three-dimensional plots were shown in Fig. 3c and Fig. 4c represent the simultaneous effect of pH and HRT on the removal of AB113 dye and COD in MBBR. The maximum dye and COD removal were observed ata pH of 7.0. However, at adverse pH (pH other than 7.0), the dye removal was decreased (Table 1). Pillai *et al.*[11] have reported that at highly acidic or alkaline conditions, the enzymatic activity of the microorganisms could be inhibited, which results in a decrease in the removal of the textile azo blue dye. Further, HRT plays a vital role in the biodegradation process as it controls the biomass growth in the bioreactor[11]. At a lower HRT, the removal rate of the pollutant was decreased due to the insufficient time for the completion of the metabolic activity of the microorganisms[12].

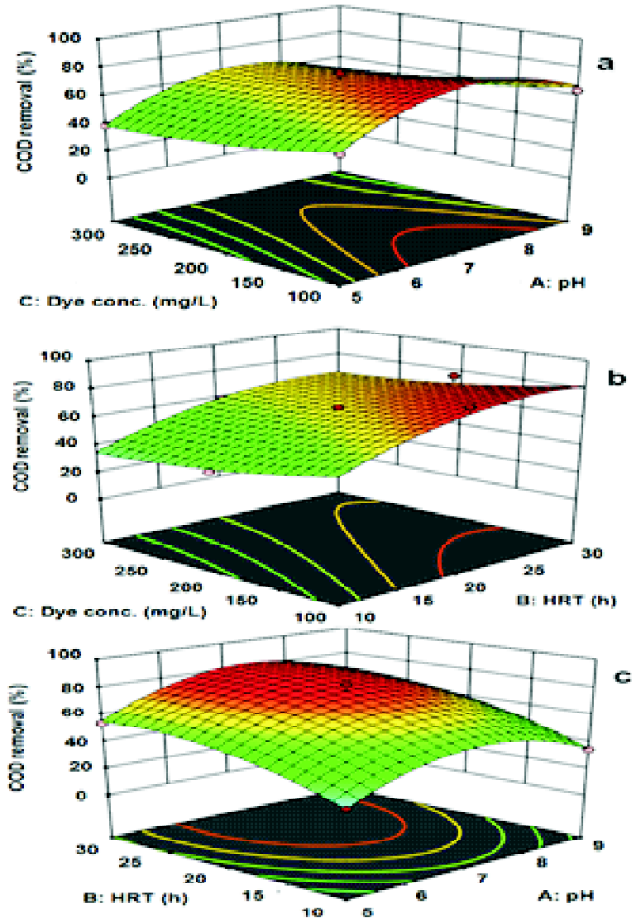

Fig. 4. 3-D surface plots for the removal (%) of COD: (a) effect of pH and dye concentration; (b) effect of HRT and dye concentration; (c) effect of pH and HRT.

Verification of the model:

The process variables were optimized using CCD of RSM at a favorable pH, a maximum concentration of the AB113 dye, and a minimum HRT. The optimum process conditions were predicted as a pH of 7.0, dye concentration of 300 mg/L, and HRT of 20 h. At optimized conditions, the removal efficiencies of AB113 and COD were obtained as 72.7% and 58.8%, respectively. Further, a set of experiments were performed to validate the model. The experimental values of dye and COD removal were found to be 70.45±0.5 and 56.08±0.3, respectively. The error between the model and experimental values was found to be in the range of 3%.

Conclusion

The potential bacterial species were isolated from a dye contaminated site and used as inoculum in MBBR for the removal of AB113 from wastewater. The MBBR with immobilized PP-PUF carriers shows effective removal of AB113 and COD. The important process parameters, such as pH, dye concentration, and HRT, were optimized by the RSM technique. The low concentration of dye, neutral pH, and long HRT was favorable for the highest performance of MBBR. The present study could be effectively used for the scale-up of the process to remove the dye wastewater with minimum effort.

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