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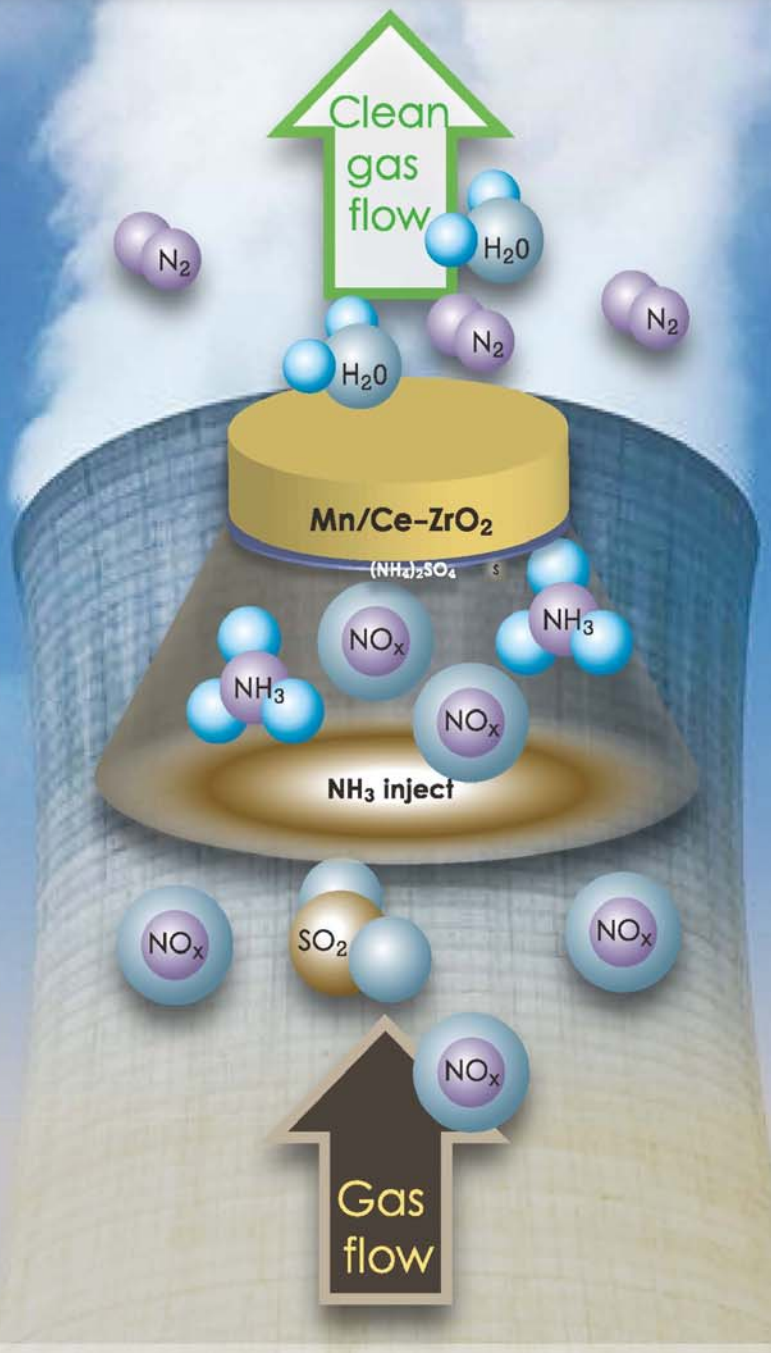

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Performance study and kinetic modeling of hybrid bioreactor for treatment of bi-substrate mixture of phenol-*m*-cresol in wastewater: Process optimization with response surface methodology

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Abstract

Performance of a hybrid reactor comprising of trickling filter (TF) and aeration tank (AT) unit was studied for biological treatment of wastewater containing mixture of phenol and *m*-cresol, using mixed microbial culture. The reactor was operated with hydraulic loading rates (HLR) and phenolics loading rates (PLR) between 0.222–1.078 $m^3/(m^2 \cdot day)$ and 0.900–3.456 $kg/(m^3 \cdot day)$, respectively. The efficiency of substrate removal varied between 71%–100% for the range of HLR and PLR studied. The fixed film unit showed better substrate removal efficiency than the aeration tank and was more resistant to substrate inhibition. The kinetic parameters related to both units of the reactor were evaluated and their variation with HLR and PLR were monitored. It revealed the presence of substrate inhibition at high PLR both in TF and AT unit. The biofilm model established the substrate concentration profile within the film by solving differential equation of substrate mass transfer using boundary problem solver tool 'bvp4c' of MATLAB 7.1©software. Response surface methodology was used to design and optimize the biodegradation process using Design Expert 8 software, where phenol and *m*-cresol concentrations, residence time were chosen as input variables and percentage of removal was the response. The design of experiment showed that a quadratic model could be fitted best for the present experimental study. Significant interaction of the residence time with the substrate concentrations was observed. The optimized condition for operating the reactor as predicted by the model was 230 mg/L of phenol, 190 mg/L of *m*-cresol with residence time of 24.82 hr to achieve 99.92% substrate removal.

Key words: hybrid bioreactor; bi-substrate; phenol; *m*-cresol; response surface methodology; biofilm model

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Introduction

Aerobic biological treatments of wastewater are based on either suspended growth or attached growth system. However combination of the two types of growth in one system has created interest of the researchers. Incorporation of biofilm growth along with suspended growth system in a single hybrid reactor has been proven to be more efficient for wastewater treatment especially for removal of toxic substrate by microorganisms even at low temperature (Tsuno et al., 1992; Lessel, 1994; Hamoda and Al-Sharekh, 2000). As biofilm system is capable of handling shock load, so system stability and sludge properties are improved in reactor having biofilm (González-Martínez and Duque-Luciano, 1992; Wang et al., 2000). Combination of attached growth and suspended growth also offers simplicity of operation and economic advantage compared to other processes (Harison et al., 1984).

Some researches have been done on hybrid reactor combining an attached growth and activated sludge or anaerobic sludge blanket system for removal of toxic substrates from wastewater (Misra and Gupta, 2001; Majumder and Gupta, 2003; Zinatizadeh et al., 2006; Yeom, 2007). Limited researches have been carried out on removal of phenolic compounds by aerobic hybrid reactors using mixed culture bacteria. Co-degradation of phenol and cresols was studied by Ramakrishnan and Gupta (2006). They examined granulation and performance of four similar anaerobic hybrid reactors combining upflow anaerobic sludge blanket unit and anaerobic filter for treatment of synthetic coal wastewater containing phenol (490 mg/L); *m*-, *o*-, *p*-cresols (123.0, 58.6, 42.0 mg/L); 2,4-, 2,5-, 3,4- and 3,5-dimethyl phenols (6.3, 6.3, 4.4

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and 21.3 mg/L) as major phenolic compounds. They found that, efficiency of phenolics and COD removal was 93% and 88%, respectively at volumetric loading rate of 2.24 g COD/(L·day) and hydraulic retention time (HRT) of 24 hr. Eker and Kargi (2007) investigated the performance of hybrid loop reactor consisting of a packed column biofilm and an aerated tank bioreactor with effluent recycle for treatment of 2,4,6-tri-chlorophenol (TCP). They found that volumetric and specific removal rates of TCP and COD decreased with increasing HRT due to increase in biomass concentration and decrease in flow rates at high HRT levels. Further study by Eker and Kargi (2009) showed that specific rates of TCP and COD removals increased with the feed TCP due to low biomass concentrations at high TCP contents.

Performance of a wastewater treatment reactor is judged by its pollutant removal efficiency, which in turn depends on pollutant loading and residence time provided. Thus optimization of the process variables is very crucial. The shortcomings of the classical optimization technique can be overcome by collecting all the affecting parameters of a process collectively using statistical experimental design methods like response surface methodology (RSM). As far the knowledge of present research group, no comprehensive study on optimization of the hybrid reactor treating phenolic wastewater (phenol and *m*-cresol) has been made by RSM.

Therefore, it is found that although some researches have been carried out for studying the performance of hybrid reactor for phenolics removal, but evaluation of performance based on kinetic principles has not yet been elucidated. In the present study, performance of a hybrid reactor composed of trickling filter at the top and activated sludge unit at the bottom section in series has been studied extensively for removal of phenol-*m*-cresol mixture from synthetic wastewater. Kinetic evaluation have been performed to predict the reaction rate equations and rate constants in attached growth system, substrate flux in biofilm, effectiveness factor of biofilm, kinetics constants applicable to suspended growth system. A novel approach has been made to model and optimize the process variables affecting the hybrid reactor performance for treatment of phenol and *m*-cresol containing wastewater by RSM. This RSM technique was also used to predict at what extent the removal of phenolics was affected by the change of the input variables and which of the factors influenced the output the most. A mathematical relation has been formulated to relate the removal of phenolics with the affecting factors.

1 Materials and methods

1.1 Collection and acclimatization of sludge

A mixed microbial sludge was collected from an effluent treatment unit of a coke oven plant situated in Durgapur, India. The sludge was maintained in suspended condition with sufficient aeration in that unit. After collection, the sludge was grown in mineral salt media (MS) with glucose (0.5 g/L) and beef extract (0.5 g/L) in the laboratory. The composition of MS media is given as (mg/L): $(NH_4)_2SO_4$ 230.0, $CaCl_2$ 7.5, $FeCl_3$ 1.0, $MnSO_4{\cdot}H_2O$ 100.0, $MgSO_4{\cdot}7H_2O$ 100.0, K_2HPO_4 500.0, KH_2PO_4 250.0 (pH 7.0). Then the concentration of glucose and beef extract were decreased by 100 mg/L each and supplemented by phenol of 10 mg/L in every batch. After a few batch of operation, the sludge was able to grow in MS media with phenol as sole carbon source upto 700 mg/L, without glucose and beef extract. Thus the culture was acclimatized to phenol. For the acclimatization of sludge with *m*-cresol, the concentration of phenol in MS media was gradually decreased along with gradual increase in *m*-cresol by 10 mg/L in each batch. After 3 months of operation the sludge could grow in MS media with *m*-cresol as sole carbon source upto 700 mg/L of concentration. After acclimatization of sludge with single phenolic substrate, the culture was fed with binary mixture of phenol-*m*-cresol. The batch experiment resulted that the sludge could degrade upto 400 mg/L of each substrate in the dual substrate matrices (data not shown).

1.2 Analytical method

Phenol and *m*-cresol concentrations were analytically estimated by high performance liquid chromatography (LC-20AT, Shimadzu, Japan) equipped with Ultraviolet-Visible (UV-Vis) detector (SPD-20A, Shimadzu, Japan) and C18 column. The mobile phase used was acetonitrile and water mixture (volume ratio 60:40). The flow rate of the eluent was set to 1 mL/min and the detection wavelength was 275 nm. The retention period of phenol and *m*-cresol were 3.967 and 4.827 min, respectively.

1.3 Experimental set-up

The hybrid reactor was consisted of a trickling filter (TF) (top section) and an aeration tank (AT) unit (bottom section) leading a combination of attached growth and suspended growth system (**Fig. 1**). The reactor was made of a perplex column with diameter of 101.5 mm and height of 609.6 mm for trickling filter unit. The aeration tank was also of cylindrical shape with same diameter and 508 mm height. The reactor was operated at down-flow mode with wastewater inlet point situated at 50.8 mm below the top of trickling filter. This TF unit was packed with packing material that acted as biomass support to facilitate attached growth of biomass. The packing material was number of similar looking pieces of burnt clay rings having hollow cylindrical shape. Each of the clay rings had inside diameter 12.5 mm, outside diameter 16.5 mm and length of 25.4 mm. The clay rings were chemically inert in nature. The height of packing bed was 457.2 mm. The TF section had three sampling ports located at 152.4, 304.8 and 457.2

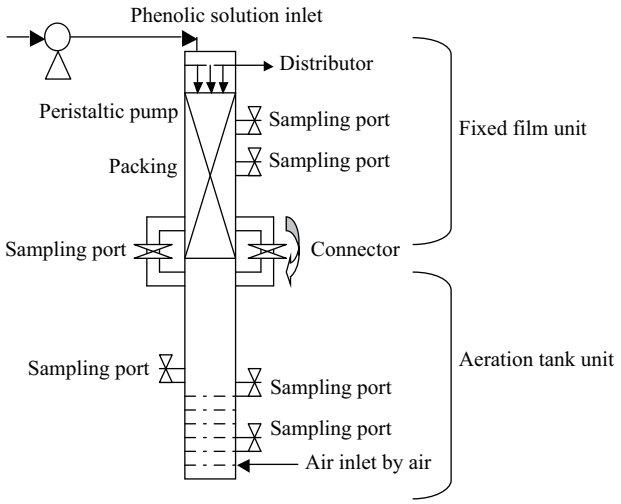

Fig. 1 Schematic diagram of reactor set up.

mm from the top of packing. Oxygen was supplied by natural ventilation along with a small aqua-compressor at a rate of 0.25 L/min. The synthetic wastewater i.e. MS media with phenol-*m*-cresol mixture was fed continuously with a peristaltic pump through the inlet port and sprayed on the packing by a distributor. The void fraction of packing after the biomass growth was 0.70. The effective surface area and specific surface area of TF were 1.15 m^2 and 575 m^2/m^3, respectively. The volume of the aeration tank was 0.0042 m^3. Its effective volumes were adjusted to 0.0010 m^3 and 0.0020 m^3 and 0.0026 m^3 by placing three sampling ports at a distance of 13.0 mm and 26.0 mm and 32.0 mm from the bottom of tank, respectively. Air compressor was used for aeration at a rate of 5.00 L/min.

The partially treated wastewater coming out from the botto*m*-most sampling port of TF was directly fed to the inlet port of aeration tank. At steady state, the rates of flow in and out from both TF and AT were the same. The effluent was collected from any one of the sampling port of AT as mentioned above.

1.4 Experimental procedure

1.4.1 Seed culture growth

For the start-up of the reactor, the TF was operated in batch mode and fed with 200 mL of acclimatized sludge with MS media containing phenol and *m*-cresol of 200 mg/L each. The solution was taken out from the TF after 24 hr. The biomass coming out from the TF was recycled totally to the inlet with fresh phenolics feed. After 4 weeks of operation, thin biofilm growth was observed on the packing material. For the growth of biomass in AT, an actively grown seed was fed to the AT in presence of MS media with phenolics of above-mentioned composition, under continuous air supply. Everyday, aeration was stopped for half an hour, supernatant was discarded from the reactor after settlement of biomass and fresh feed media (2 L) was added. After sufficient growth of biomass in both TF and AT, the whole operation was changed to continuous mode.

1.4.2 Hydraulic loading rate and residence time calculation in trickling filter unit

The reactor was operated at five different flow rates of wastewater (1.25, 2.50, 3.50, 4.50, 6.0 mL/min) at hydraulic loading rates (HLRs) of 0.222, 0.444, 0.622, 0.800 and 1.067 $m^3/(m^2 \cdot day)$. The mean residence time of the wastewater in TF under each flow rate was determined by tracer injection technique. A 2 g/L of potassium-dichromate solution was fed to the reactor as step input through the sample inlet port at the flow rates same as those selected for reactor operations. Optical density of the solution coming out the botto*m*-most sampling port of the TF was measured with time at 520 nm by UV-Vis spectrophotometer. From the step input data, "*F*" curve and "*E*" curve ($E = \frac{dF}{dt}$) was plotted. From the *E* curve, mean residence time (θ_{TF}) and dispersion number (D/uL) were calculated for every different flow rates by using the following equations (Levenspiel, 1991):

$$\theta_{TF} = \int_0^\infty tE\,dt = \sum t_1 E_1 \Delta t \quad (1)$$

$$\sigma^2 = \int_0^\infty (t - \theta_{TF})^2 E\,dt \quad (2)$$

$$\sigma_\theta^2 = \frac{\sigma^2}{\theta^2_{TF}} = 2\left(\frac{D}{uL}\right) \quad (3)$$

where, θ_{TF} (hr) is the mean residence time, t (hr) is time, σ^2 is the variance, D is longitudinal or axial dispersion coefficient, u (m/hr) is fluid velocity, L (m) is path length and the fraction of the fluid that spends a given duration, t_1 (hr) inside the reactor is given by the value of $E_1 \Delta t$.

1.4.3 Phenolics loading rates and hydraulic retention time study

For each HRT, phenolics of five different influent concentrations were fed to the reactor. The reactor performance was studied in terms of percentage of total phenolics removal measured for the effluent collected from the sampling port of AT when fed with phenol-*m*-cresol mixture. The phenolics loading rates were varied between 0.090–3.456 $kg/(m^3 \cdot day)$. The details of HLR and phenolics loading rates (PLR) other than those selected for design of experiments are also investigated (**Table 3**).

HRT studies were conducted at 8.74 hr (3.19 hr TF + 5.55 hr AT), 12.15 hr (4.75 hr TF + 7.4 hr AT), 15.76 hr (6.24 hr TF + 9.54 hr AT), 21.01 hr (7.68 hr TF + 13.33 hr AT) and 28.03 hr (14.7 hr TF + 13.33 hr AT) to optimize the reactor performance. As per design of experiment by RSM, the HRT was also set to 18.39 hr (6.24 hr TF + 12.15 hr AT), by adjusting the volume of the aeration tank to 2.6 L. The void volume of TF after the biofilm formation was assumed to be steady at 2 L. The volume of aeration tank was adjusted to 1 L for HLR of 0.222 $m^3/(m^2 \cdot day)$. For all other HLRs and PLRs, this volume was adjusted to 2.0 L.

The effluent samples were collected from all the three sampling ports of the TF and the outlet port of AT. The sample collected from the bottommost port of TF was analyzed for residual phenolics concentration to check the efficiency of TF. The samples collected from the other sampling ports of TF were used to check the phenolics concentration profile along the depth of the filter. The sample from the outlet of the AT was used to measure the efficiency of the whole reactor. The efficiency (r, %) of the reactor was calculated by Eq. (4):

$$r = \frac{m_{\text{in}} - m_{\text{out}}}{m_{\text{in}}} \times 100\% \tag{4}$$

where, m_{in} (mg/L), m_{out} (mg/L) are concentrations of phenol-*m*-cresol mixture entering the reactor and coming out of the reactor, respectively.

The mixed liquor suspended solid (MLSS) concentration in AT was maintained at three different values: 750, 1500 and 2500 mg/L to check the effect of MLSS concentration on substrate removal efficiency.

1.4.4 Kinetic analysis in both trickling filter and aeration tank

As proposed by Eckenfelder (1989) the mean residence time of the fluid in the trickling filter (θ_{TF}) is related to the filter depth, HLR and nature of support as follows:

$$\theta_{\text{TF}} = \frac{C \times D}{\text{HLR}^n} \tag{5}$$

where, D (m) is the filter depth, C and n are constants that depend upon the biomass support nature and specific surface area. Calculating θ_{TF} from the residence time distribution of the TF at five different HLR, C and n can be determined by fitting the data at Eq. (5).

A simple kinetic model to represent the phenolics degradation kinetics was outlined. Assuming, substrate removal kinetics followed the Haldane form; the substrate removal rate was given by Eq. (6) (Andrews, 1968):

$$-\frac{\text{d}S}{\text{d}t} = \frac{k \times S \times X}{K + S + S^2/K_i} \tag{6}$$

where, S (mg/L) is the total phenolic substrates concentration, k is substrate degradation rate constant, K (mg/L) is the substrate concentration at which specific substrate degradation rate is half of maximum, t (hr) is time and X (mg/L) is biomass concentration.

Assuming X to be constant and equal to average biomass concentration of the TF and integrating Eq. (5) from $t = 0$ to $t = \theta_{\text{TF}}$ and substituting θ_{TF} from Eq. (5), leads to

$$K\ln S_{\text{out}} + S_{\text{out}} + S_{\text{out}}^2/2K_i = K\ln S_{\text{in}} + S_{\text{in}} + S_{\text{in}}^2/2K_i - k'D/L^n \tag{7}$$

where, $k' = k \times X \times C$.

In this form (Eq. (7)), the data can be linearized by assuming K and K_i and plotting each $K\ln S_{\text{out}} + S_{\text{out}} + S_{\text{out}}{}^2/2K_i$ vs. $K\ln S_{\text{in}} + S_{\text{in}} + S_{\text{in}}{}^2/2K_i$ for every HLR and total filter depth and choosing those values of K and K_i that yield a straight line with unity slope and maximum coefficient of determination (Gerrard et al., 2006; Dey and Mukherjee, 2010). Equation (7) was solved and correspondingly K and K_i values were found out by MATLAB 7.1©.

Plotting $K \ln \frac{S_{\text{in}}}{S_{\text{out}}} + (S_{\text{in}} - S_{\text{out}}) + \frac{1}{2K_i}(S_{\text{in}}^2 - S_{\text{out}}^2)$ vs. filter depth D for a particular HLR, the slope of the straight line indicated the value of k'/HLR^n for that HLR. By knowing the value of n from reactor hydrodynamics (Eq. (5)), k' was calculated as a function of L.

The removal of phenolics in aeration tank followed the equation (Metcalf and Eddy, 1995):

$$\frac{X\theta}{S_0 - S} = \frac{K_{\text{s}}}{k_1 S} + \frac{1}{k_1} \tag{8}$$

where, S_0 (mg/L) is the influent concentration, S (mg/L) is the effluent concentration, θ (day) is HRT, k_1 (day^{-1}) is the maximum specific rate of substrate utilization , K_{s} (mg/L) is half velocity constant.

1.4.5 Biofilm modeling

The general equation of mass transfer of substrate in biofilm is represented by the following expression (Beyenal and Lewandowski, 2005):

$$D_{\text{f}z}\frac{\text{d}^2S}{\text{d}Z^2} + \zeta\frac{\text{d}S}{\text{d}z} = \frac{\mu_{\max} S X_{\text{f}z}}{Y_{x/s}(K + S)} \tag{9}$$

where, $\zeta = \frac{\text{d}D_{\text{f}z}}{\text{d}Z}$ and $D_{\text{f}z}$ (m^2/sec) is the diffusivity of the substrate in biofilm in the Z direction.

For homogeneous biofilm (where effective diffusivity gradient $\zeta = 0$, average diffusivity is D_{fav} and average biofilm concentration is X_{av}), the nutrient continuity equation is simplified to the form:

$$D_{\text{fav}}\frac{\text{d}^2S}{\text{d}Z^2} = \frac{\mu_{\max} S X_{\text{av}}}{Y_{x/s}(K + S)} \tag{10}$$

Defining dimensionless parameters-distance Z^*, substrate concentration S^* and Monod half rate constant β as follows: $Z^* = \frac{z}{L_{\text{f}}}$, $S^* = \frac{S_{\text{f}}}{S_{\text{s}}}$, $\beta = \frac{K}{S_{\text{s}}}$, where L_{f} (m) is biofilm thickness, S_{s} (mg/L) and S_{f} (mg/L) are phenolics concentration at the surface of biofilm and within the biofilm.

Plugging these dimensionless parameters into Eq. (10) and defining Thiele modulus φ as:

$$\varphi = \sqrt{\frac{\mu_{\max} L_{\text{f}}^2 X_{\text{av}}}{Y_{x/s} S_{\text{s}} D_{\text{fav}}}} \tag{11}$$

Eq. (10) yields

$$\frac{d^2S^*}{dZ^{*2}} = \varphi^2 \frac{S^*}{\beta + S^*} \quad (12)$$

The boundary conditions to solve Eq. (12) are:

$$Z^* = 1, S^* = 1 \text{ and } Z^* = 0, \frac{dS^*}{dZ^*} = 0$$

Equation (12) was solved by boundary value solver (bvp4c) of MATLAB 7.1©. To calculate the values of φ and η, D_{fav} for phenol-*m*-cresol mixture was calculated by equation proposed by Wilke and Chang (1955). Putting the values of necessary parameters, D_{fav} for phenolic mixture is 85 cm^2/day. $Y_{x/s}$ and μ_{max} values were obtained from batch kinetic experiments.

The effectiveness factor, η is given by the Eq. (13) (Beyenal and Lewandowski, 2005), η = Diffusion limited substrate uptake rate/Diffusion free substrate uptake rate.

$$\eta = \frac{(\beta + 1)}{\varphi^2} \frac{dS^*}{dZ^*_{\text{at } Z^*=1}} \quad (13)$$

The substrate concentration at the biofilm surface is defined as S_s (mg/L). It can be calculated by considering mass transfer equation in the bulk liquid and within the biofilm by the following Eqs. (14) and (15).

$$Q(S_0 - S_b) = J_f \times A_f \quad (14)$$

where, S_0 (mg/L) and S_b (mg/L) are inlet and bulk phenolics concentration, J_f is biofilm flux. If no external mass transfer resistance exists, then $S_s = S_b$.

$$D_{fav} \frac{d^2S_f}{dz^2} + r = 0 \quad (15)$$

where, S_f (mg/L) is substrate concentration within film. The differential equation (Eq. (15)) can be solved analytically for zero order or first order reaction rate (r). But for typical saturation kinetics for the biological system, the substrate volumetric reaction rate (r) is given by:

$$r = \frac{q_{max} S_f}{S_f + K} X_{av} \quad (16)$$

where, q_{max} (day^{-1}) is the maximum specific substrate conversion rate.

Composite flux of substrate through the biofilm is given by Eq. (17) (Eberl et al., 2006):

$$J_f = \frac{S_s}{S_s + K} J(0) + (1 - \frac{S_s}{S_s + K}) J(1) \quad (17)$$

where, $J(0)$ is flux for zero order kinetics and $J(1)$ is the flux for first order kinetics. $J(0)$ and $J(1)$ were calculated by formula proposed by Eberl et al. (2006). Replacing J_f in Eq. (14) with Eq. (17) and putting $S_b = S_s$, the value of S_s was determined.

In the present experiment, the biofilm thickness was calculated as 0.78 mm. Biofilm mass was calculated by the difference of dry weight (dried at 105°C) of clay rings before and after biofilm formation. The biofilm density was computed by dividing the dry mass of biofilm by biofilm volume. The biofilm density thus obtained was 3 kg/m^3.

1.5 Optimization of the process by response surface methodology

RSM is a statistical method that used quantitative data from appropriate experiments to determine and simultaneously solve multivarient equations. It is used to determine the optimum combination of the factors that yield a desired response and describes the response near then optimum. It also determines how a specific response is affected by the changes in the level of the other factors over the specified levels of interest. The most popular RSM design is the central composite design (CCD). A CCD has three groups of design points: (a) two-level factorial (–1 and +1) or fractional factorial design points, (b) axial points (sometimes called "star" points) and (c) center points. It's well suited for fitting a quadratic surface, which usually works well for process optimization.

In the present experiment, the removal percentage of the phenolics is represented as a response which depends on three prime process factors: phenol concentration, *m*-cresol concentration and residence time. The process has been optimized by Face Centered Central Composite Design (CCF) by Design Expert Software (Version 8) using 8 factorial points, 6 center points and 6 axial points. The lower level (–1) and upper level (+1) of the concentrations (phenol and *m*-cresol both) and residence time are selected as 100 mg/L and 400 mg/L, 8.75 hr and 28.03 hr, respectively.

Few models like: Linear, Two factorial, Quadratic and Cubic models have been tested for the present experiment. ANOVA response of the models have been compared and the best model was selected for predicting the model equation of the system, main effect, interaction effect of the process factors and the optimized selection of the parameters.

2 Results and discussion

2.1 Reactor hydrodynamics

The concentration profiles of the tracer with time in the biofilter section of the reactor at different flow rates are shown in **Fig. 2**. The mean residence time (θ_{TF}), variance (σ) and dispersion numbers (D/uL) at different flow rates of tracer are listed in **Table 1**. It is observed that as the flow rate was increased, the mean residence time decreased and dispersion number increased gradually. It indicated that

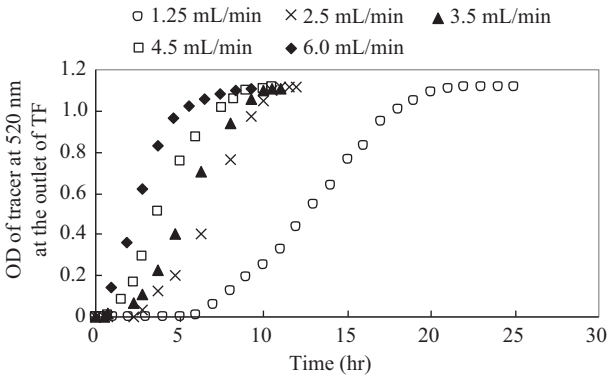

Fig. 2 '*F* Curve' for tracer profile for mean residence time study in trickling filter.

Table 1 Result of residence time distribution study of trickling filter

Q (mL/min)	HLR ($m^3/(m^2 \cdot day)$)	θ_{TF} (hr)	σ	*D/uL*
1.25	0.222	14.7	4.312772	0.043038
2.5	0.444	7.68	2.695292	0.052295
3.5	0.622	6.24	2.347318	0.071645
4.5	0.8	4.75	2.252856	0.112663
6	1.076	3.19	1.866553	0.171585

Q: flow rate; HLR: hydraulic loading rates; θ_{TF}: mean residence time; σ: variance; *D/uL*: dispersion number.

higher was the flow rate, more was the deviation from ideal plug flow behavior.

Taking the natural logarithm at both sides of Eq. (5) and plotting $\ln\theta_{TF}$ vs. lnHLR (**Fig. 3**), the slope provided the value of *n* as 0.936. Using the value of total filter depth (*D*) as 0.4572 m, the value of *C* was determined as 8.05 $m^{-0.06}$ $day^{0.06}$.

2.2 Effect of HRT, HLR and PLR on TF performance

TF performance was measured at five different HRTs as mentioned in **Table 1**. The extent of substrate removal at different HLR and PLR as designed by RSM are listed in **Table 2**. Performance of the reactor with few more combinations of HLR and PLR were studied to obtain kinetic coefficients and listed in **Table 3**. It shows that for a particular influent phenolics concentration, the

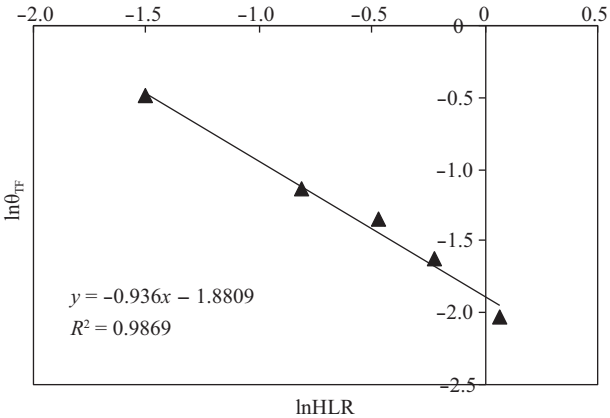

Fig. 3 Plot to determine *C* and *n* for the trickling filter.

Table 2 Design of experiments suggested by RSM using Design Expert 8 software

Phenol (mg/L)	*m*-Cresol concentration (mg/L)	Residence time (hr)	Removal (%)
250.00 (0)	250.00 (0)	18.39 (0)	94.5
250.00 (0)	250.00 (0)	18.39 (0)	94
250.00 (0)	250.00 (0)	18.39 (0)	95
250.00 (0)	250.00 (0)	18.39 (0)	95.55
250.00 (0)	250.00 (0)	18.39 (0)	95.1
250.00 (0)	250.00 (0)	18.39 (0)	96.8
250.00 (0)	250.00 (0)	28.03 (+1)	99.7
250.00 (0)	250.00 (0)	8.75 (–1)	85.75
250.00 (0)	400.00 (+1)	18.39 (0)	88.25
100.00 (–1)	250.00 (0)	18.39 (0)	96.1
250.00 (0)	100.00 (–1)	18.39 (0)	97.2
400.00 (+1)	250.00 (0)	18.39 (0)	90
400.00 (+1)	400.00 (+1)	28.03 (+1)	99
400.00 (+1)	100.00 (–1)	8.75 (–1)	83
100.00 (–1)	400.00 (+1)	8.75 (–1)	80.5
100.00 (–1)	100.00 (–1)	8.75 (–1)	99
400.00 (+1)	100.00 (–1)	28.03 (+1)	99.8
100.00 (–1)	100.00 (–1)	28.03 (+1)	99.9
100.00 (–1)	400.00 (+1)	28.03 (+1)	99.7
400.00 (+1)	400.00 (+1)	8.75 (–1)	71

Removal was calculated with MLSS = 2500 mg/L.
(+1): upper level concentration; (–1): lower level concentration; (0) middle level or center point of factor.

concentration of effluent of TF decreased with increase in mean HRT. Because, as higher HRT was provided, more time for biodegradation was available for the microorganism, which resulted more phenolics degradation. Similar observation was found by Majumder and Gupta (2003) for removal of nitrobenzene by hybrid reactor. At HLR of 0.222 $m^3/(m^2 \cdot day)$ (HRT = 14.7 hr), almost 99% removal of total phenolics (phenol-*m*-cresol) was achieved for PLR of 0.090, 0.180, 0.360, 0.540 $kg/(m^3 \cdot day)$. At the same HRT, as the PLR increased to 0.720 $kg/(m^3 \cdot day)$, the removal percentage sharply came down to 88.25%. It was observed that under that PLR, the removal percentage was higher for HLR of 0.444 $m^3/(m^3 \cdot day)$ (98%) than that of 0.222 $m^3/(m^2 \cdot day)$ (88.25%). The reason may be that when PLR and HLR were of 0.720 $kg/(m^3 \cdot day)$ and 0.444 $m^3/(m^2 \cdot day)$ respectively, then influent phenolics concentration was set at 200P + 200C (mg/L), therefore no inhibition took place. But at lower HLR (0.222 $m^3/(m^2 \cdot day)$), the same PLR was achieved at substrate concentration of 400P + 400C (mg/L), which was high enough to cause substrate inhibition leading to lower substrate removal. With the increase of PLR beyond 0.720 $kg/(m^3 \cdot day)$ upto 3.456 $kg/(m^3 \cdot day)$, the removal lied between 89.17%–55.25%. For HLR of 0.444 $m^3/(m^2 \cdot day)$ and above, a sudden drop of removal percentage was observed at influent phenolics concentrations of 300P + 300C (mg/L) or higher, indicating intense substrate inhibition in the system. The result was in accordance with the batch reactor data, where it was found that specific growth rate of the culture decreased significantly when influent phenolics concentration was set at 300P + 300C (mg/L) or more. TF

was observed to remove major amount of the phenolics irrespective of HRT and PLR, thus proved to be more efficient than AT.

2.3 Effect of HRT, HLR and PLR on AT performance

The performance of the AT was tested for HRT of 13.33, 9.50, 7.40 and 5.56 hr. The HRT was set to 13.33 hr for HLR of 0.222 and 0.444 $m^3/(m^2 \cdot day)$, by adjusting the effective volume of the reactor to 1 and 2 L, respectively. It was observed that rate of substrate removal at AT depended not only on HRT, but also on its influent substrate concentration. Thus, with 750 mg/L of MLSS and 13.33 hr of HRT, the influent streams of 65 and 94 mg/L phenolics showed 84.61% and 92.55% removal, respectively. It indicated that more was the influent phenolics concentration, higher was the rate of substrate removal. The substrate removal efficiency was better for MLSS of 2500 than 750 or 1500 mg/L (**Table 3**).

2.4 Result of kinetic analysis in trickling filter

It was observed that for HLR below 0.800 $m^3/(m^2 \cdot day)$, the values of K gradually increased from 11.48 to 150.00 mg/L which was the result of decrease in affinity of the culture for the substrate with elevation of loading. But for HLR at 1.076 $m^3/(m^2 \cdot day)$, the K drastically shouted up to 225.00 mg/L (**Table 4**). The reason may be that at high HLR, the value of PLR was also high which caused inhibition to substrate degradation.

Table 5 represents the variation of phenolics concentration along the filter depth at different HLR, both experimentally and modeled, which are observed to be in well agreement with each other. **Figure 4** shows the plot of $K \ln \frac{S_{in}}{S_{out}} + (S_{in} - S_{out}) + \frac{1}{2K_i}(S_{in}^2 - S_{out}^2)$ vs. filter depth for HLR at 0.800 $m^3/(m^2 \cdot day)$, the slope of which provided the value of k/L^n at that HLR (Eq. (7)). The similar plots for the other HLR showed that the value of k/L^n decreased with increase in HLR. Similar observation was found by Sá and Boaventura (2001) for degradation of phenol by *Pseudomonas putida* DSM 548 in a trickling bed reactor. Putting $n = 0.936$, the values of k values were calculated for different HLR. Replacing C and X with 8.05 $m^{-0.06}$ $day^{0.06}$ and 3 kg/m^3, respectively in Eq. (6), values of k were also determined (**Table 4**). It was observed that value of k increased with the increase in HLR. As k represents the maximum specific substrate degradation rate, therefore it can be concluded that the culture degraded the substrates at a faster rate when high HLR was provided.

2.5 Result of biofilm modeling

The Thiele modulus (φ) is defined as the ratio of the rate of reaction and the rate of diffusion. In biofilm, reaction rate is controlled by biofilm density. To check the nutrient concentration profile within the biofilm, φ values were calculated according to Eq. (11) at the entrance of the TF. The surface concentration at the entrance of the reactor

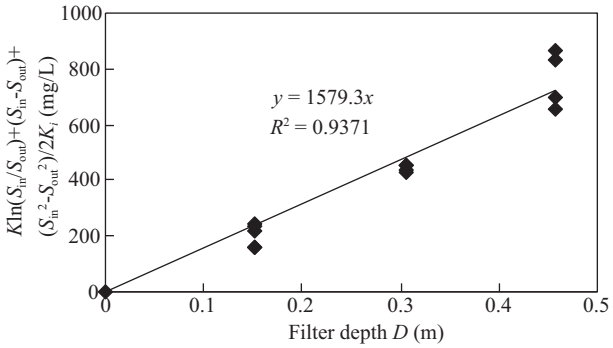

Fig. 4 Plot to find value of k for HLR = 0.800 $m^3/(m^2 \cdot day)$.

was chosen to ensure the presence of sufficient substrate for diffusion and reaction. The values of φ (**Table 4**) were calculated for different phenolics influent concentrations where μ_{max} and $Y_{x/s}$ were substituted with 0.0842 (per day) and 0.59 (mg/mg), respectively as obtained from batch kinetic analyses, with D_{fav} value as 0.85 cm^2/day. It was observed that the values of φ did not vary with HLR, but its value decreased with increase in influent concentration upto total phenolics concentration of 600 mg/L, and then remained almost constant. This happened because of the fact that increase in influent phenolics concentration upto 600 mg/L increased the rate of diffusion much rapidly than the increase in reaction rate, but then became steady at higher influent value. The concentration profiles within the biofilm as plotted in **Fig. 5** (HLR = 0.222 and 1.076 $m^3/(m^2 \cdot day)$) showed similar trend. The concentration profiles for the other HLRs were almost similar to each other and not shown here. The phenolic substrates penetrated to the whole depth, did not reduce to zero concentration in the biofilm which indicated that increase in diffusion rate was higher than the increase in rate of reaction. It was also observed that for a particular HLR, the substrate concentration at the innermost layer of biofilm ($Z^* = 0$) was lowest for total influent phenolics concentration of 200 mg/L (100P + 100C), but the other profiles overlapped with each other irrespective of the HLR. Therefore it can be concluded that at low concentration of influent substrate (200 mg/L), the culture consumed the substrate quickly showing low S^* value within the biofilm. But the rate of substrate consumption was almost same for all higher influent concentration resulting very close S^* values at a particular depth of biofilm. The effectiveness factor (η) calculated according to Eq. (12) are also listed in **Table 4**.

Experimentally, the biofilm flux (J_f) was determined from Eq. (17) by replacing S_b with S_s (experimentally measured), as it was assumed that no external mass transfer resistance existed. The J_f value calculated from Eq. (17) and the experimental ones are plotted against PLR (R^2 = 0.90) (**Fig. 6**). It shows that as PLR was increased, the substrate flux within biofilm increased upto certain limit, and then slightly decreased due to substrate inhibition caused by high substrate loading. **Figure 6** also shows the comparative value of surface concentration of the substrate

Table 3 Performance of reactor with variation of phenolics loading rate (HLR) and hydraulic loading rate (PLR)

HLR ($m^3/(m^2 \cdot day)$)	PLR ($kg/(m^3 \cdot day)$)	Phenol + *m*-cresol concentration (mg/L)					
		Inlet of TF (mg/L)	Outlet of TF	Inlet of AT	Outlet of AT		
					MLSS 750 mg/L	MLSS 1500 mg/L	MLSS 2500 mg/L
0.222	0.090	100 (50P + 50C)	0	–	–	–	–
(HRT = 28.03 hr)	0.180	200 (100P + 100C)	0	–	–	–	–
	0.360	400 (200P + 200C)	0.1	0.1	0	0	0
	0.540	600 (300P + 300C)	8	8	0	0	0
	0.720	800 (400P + 400C)	94	94	7	0	0
0.444	0.180	100 (50P + 50C)	0	–	–	–	–
(HRT = 21.01 hr)	0.360	200 (100P + 100C)	0	–	–	–	–
	0.720	400 (200P + 200C)	2	2	0	0	0
	1.080	600 (300P + 300C)	65	65	10	0	0
	1.440	800 (400P + 400C)	225	225	120	98	65
0.622	0.352	100 (50P + 50C)	0	0	–	–	–
(HRT = 15.76 hr)	0.704	200 (100P + 100C)	0	0	–	–	–
	1.408	400 (200P + 200C)	14	14	0	0	0
	2.112	600 (300P + 300C)	146	146	75	58	30
	2.816	800 (400P + 400C)	262	262	182	160	148
0.800	0.324	100 (50P + 50C)	0	0	–	–	–
(HRT = 12.15 hr)	0.648	200 (100P + 100C)	10	10	0	0	0
	1.260	400 (200P + 200C)	22	22	4	0	0
	1.944	600 (300P + 300C)	185	185	115	95	0
	2.592	800 (400P + 400C)	295	295	234	201	185
1.076	0.216	100 (50P + 50R)	0	–	–	–	–
(HRT = 8.75 hr)	0.864	200 (100P + 100R)	15	15	0	0	0
	1.728	400 (200P + 200R)	184	184	146	125	105
	2.592	600 (300P + 300R)	256	256	196	170	148
	3.456	800 (400P + 400R)	320	320	270	250	232

P: phenol, C: *m*-cresol.

Table 3 does not contain the combination of HLR and PLR combinations proposed by Design of Experiments by RSM except all the factors (phenol concentration, *m*-cresol concentration, residence time) are at their lowest (–1, –1, –1) and highest (+1, +1, +1) level.

Table 4 Kinetic constants related to trickling filter

HLR ($m^3/(m^2 \cdot day)$)	$\frac{k'}{L^n}$	k'	k (day^{-1})	K (mg/L)	K_i (mg/L)	Influent concentration of phenol + *m*-cresol (mg/L)							
						100P + 100C		200P + 200C		300P + 300C		400P + 400C	
						φ	η	φ	η	φ	η	φ	η
0.222	1.727	0.422	0.017	11.48	860.0	8.2	0.03514	4.53	0.09649	4.1	0.1102	4.15	0.10611
0.444	1.718	0.803	0.033	64.18	1097.1	8.2	0.03462	4.53	0.09650	4.1	0.10936	4.15	0.10485
0.622	1.588	1.018	0.042	95.00	1335.0	8.2	0.03764	4.53	0.09970	4.1	0.11340	4.15	0.10733
0.800	1.579	1.281	0.053	150.00	1500.0	8.2	0.03764	4.53	0.09970	4.1	0.11340	4.15	0.10733
1.076	1.458	1.561	0.064	225.00	1550.0	8.2	0.05577	4.53	0.01256	4.1	0.13276	4.15	0.12255

Table 5 Experimental (Exp.) and modeled (Mod.) phenolics concentrations along the filter depth

HLR ($m^3/(m^2 \cdot day)$)	Filter depth (m)	Phenolics concentration (mg/L)									
		50P + 50C		100P + 100C		200P + 200C		300P + 300C		400P + 400C	
		Exp.	Mod.	Exp.	Mod.	Exp.	Mod.	Exp.	Mod.	Exp.	Mod.
0.222	0.1524	7	0.05	80	72.00	230	225.00	415	418.00	620	632.00
	0.3048	1	0.00	6	0.10	70	58.00	220	222.00	350	398.00
	0.4572	0	0.00	0	0.00	0.1	0.05	8	16.00	90	110.00
0.444	0.1524	25	12.00	90	77.00	240	232.00	420	400.00	645	642.00
	0.3048	2	0.00	8	3.00	90	78.50	190	190.00	435	440.00
	0.4572	0	0.00	0.1	0.00	2	2.50	65	69.00	225	202.00
0.622	0.1524	25	23.00	90	80.00	250	245.00	445	443.00	655	652.00
	0.3048	6	2.00	25	13.00	120	112.00	315	304.00	450	449.00
	0.4572	0	0.00	0	0.00	14	14.50	146	145.00	265	270.00
0.800	0.1524	50	46.00	95	98.00	275	277.00	500	480.00	655	652.00
	0.3048	10	8.00	35	28.00	145	138.00	235	315.00	525	515.00
	0.4572	0	0.00	10	7.00	22	25.00	185	178.00	295	305.00
1.076	0.1524	55	42.00	145	118.00	325	302.00	530	502.00	725	693.00
	0.3048	28	10.00	92	58.00	270	244.00	436	403.00	646	615.00
	0.4572	0	0.00	15	11.00	184	128.00	256	178.00	320	318.00

measured experimentally and modeled (R^2 = 0.93). The modeled S_s value was that value for which J_f obtained from Eq. (14) and Eq. (17) was same. The trend of S_s vs. PLR showed that initially S_s value increased with increase

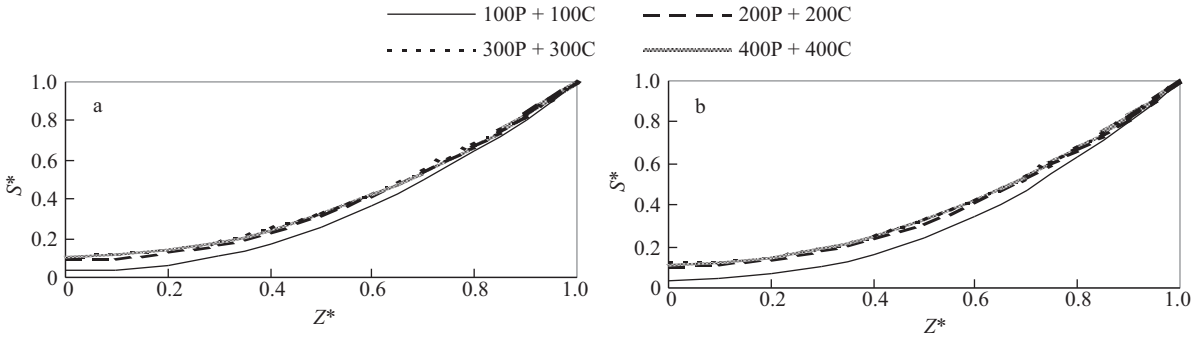

Fig. 5 Phenolics concentration profile within biofilm plotted as dimensionless substrate concentration vs. dimensionless distance for (a) HLR = 0.222 $m^3/(m^2 \cdot day)$ and (b) 1.076 $m^3/(m^2 \cdot day)$.

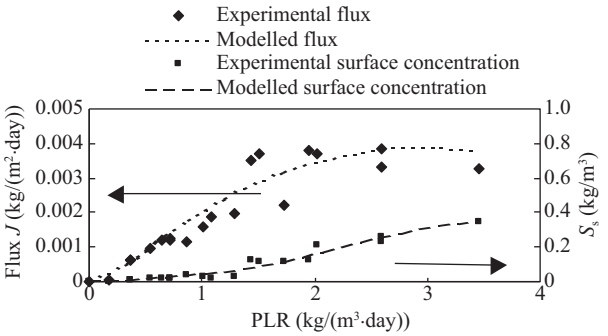

Fig. 6 Comparison of experimental and modeled flux and substrate concentration at biofilm surface.

Table 6 Kinetic constants related to aeration tank

MLSS (mg/L)	k_1 (day^{-1})	K_s (mg/L)
750	0.2856	11.152
1500	0.1893	4.751
2500	0.1428	3.456

in PLR, and then became constant. The reason may be that when PLR was low, the reaction was of first order. So increase in PLR increased the S_s value. But after crossing certain limit of PLR, two opposite effect regulated the reaction rate: (1) reaction rate accelerated with increase in phenolics concentration, because of first order reaction, (2) substrate inhibition decelerated the reaction rate at high phenolics loading. As a result, the reaction rate remained constant and S_s value became steady.

2.6 Result of kinetic analysis of aeration tanks

From the intercept and slope of plot $\frac{X\theta}{S_0-S}$ vs. $\frac{1}{S}$ (Eq. (8)) the values of k_1 and K_s were obtained for different MLSS (**Fig. 7**). The variation of these values with MLSS concentration (**Table 6**) showed that with the increase in MLSS, K_s value decreased, leading to better rate of substrate removal and thus lower effluent concentration. It was also obtained that k_1 values decreased with increase in MLSS. As k_1 represents the maximum specific substrate degradation rate $(1/X)(dS/d\theta)$, thus it can be stated that, at higher MLSS, the rate of substrate degradation increased to some extent but that increase in rate $(dS/d\theta)$ was lower than the proportionate increase in MLSS, and therefore k_1 value decreased.

Table 7 ANOVA result for modeling of process

Parameter	Sum of squares	*F* value	*P* value
Model	1118.21	82.96	< 0.0001
Phenol	104.98	70.09	< 0.0001
m-Cresol	163.62	109.25	< 0.0001
Residence time	621.73	415.11	< 0.0001
Phenol × *m*-Cresol	4.35	2.91	0.1191
Phenol × Residence time	76.26	50.92	< 0.0001
m-Cresol × Residence time	108.78	72.63	< 0.0001
$Phenol^2$	2.04	1.36	0.2702
$m\text{-}Cresol^2$	3.87	2.58	0.1390
$Residence\ time^2$	3.87	2.58	0.1390

Standard deviasion: 1.22, R^2 = 0.9868, Adj R^2 = 0.0.9749.

2.7 Result for statistical analysis of the process by CCF

Among the models tested, the software suggested the quadratic model to be best fit for the present study. The result for statistical analysis are listed in **Table 7**.

The final equation predicted by the software is as Eq. (18):

$$\begin{aligned}\text{Phenolics removal} = [&105.24475 + (-0.049888\ C_p) \\ &+ (-0.055657\ C_c) + (0.115720) + (3.2778 \times 10^{-5} \times C_p \times C_c) \\ &+ (2.13409 \times 10^{-3} \times C_p \times \theta) + (2.54882 \times 10^{-3} \times C_c \times \theta) \\ &- (3.82828 \times 10^{-5} \times C_p^2) - (5.27273 \times 10^{-5} \times C_c^2) - \\ &(0.012753 \times \theta^2)] \times 100\% \end{aligned} \quad (18)$$

where, C_p and C_c are phenol and *m*-cresol concentration respectively, θ is residence time.

The effects with *p*-values higher than 0.05 are not significant at the 95% confidence level. In this case C_p, C_c, θ, $C_p\theta$, $C_c\theta$, are significant model terms. The sign of the effect marks the performance of the response. In this way, when a factor has a positive effect, the response is higher at the high level and when a factor has a negative effect the response is lower at high level.

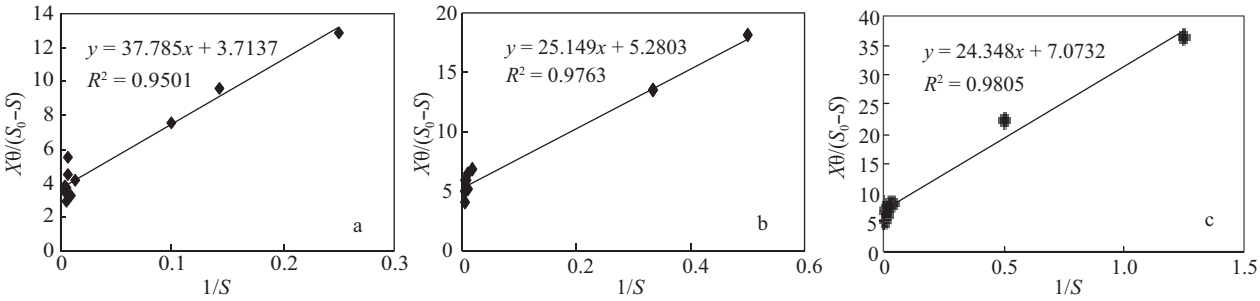

Fig. 7 Plot to determine the kinetic constants (k_1 and K_s) in aeration tanks for MLSS of 750 (a), 1500 (b), and 2500 mg/L (c).

2.7.1 Effect of phenolics concentration on removal percentage

Total 20 experimental runs had been studied for CCF statistical analysis. The main effects of the parameters studied here shows that phenol and *m*-cresol concentration have insignificant effect on the removal if the residence time of watsewater in the reactor is kept at its highest level. In that case, removal percentage reaches to near 100% irrespective of the substrate concentration levels (**Fig. 8c**). But as the residence time decreases to 8.75 hr with *m*-cresol concentration kept at its low level (100), removal decreases sharply from 98.43% to 84.85% at phenol concentration 100 and 400 mg/L respectively. At this minimum residence time of 8.75 hr and phenol concentration of 100, increase in *m*-cresol concentration from 100 to 400 mg/L shows sharp decrease in removal from 98.43% to 81.76% (**Fig. 8a**). The removal percentage drops to a value of 70.77% when phenolics concentration are 400 mg/L each at residence time of 8.75 hr. Keeping residence time at its centre point (18.39 hr), the change of phenol concentration from 100 to 400 mg/L shows a decrease of removal percentage from 100% to 92.34% and 91.1% to 85.78% for *m*-cresol concentration of 100 and 400 mg/L, respectively (**Fig. 8b**). The removal percentage changes from 90.97% to 78.23% for phenol concentration varying between 100 to 400 mg/L with *m*-cresol concentration and residence time are at 250 mg/L and 8.75 hr respectively (**Fig. 8d**). But for the same residence time, if the *m*-cresol concentration varies from 100 to 400 mg/L, then removal changes from 91.93% to 76.74%, at phenol concentration of 250 mg/L (**Fig. 8e**). It can be concluded that both of the substrates negatively affect the removal of phenolics. The effect of *m*-cresol is a little more than that of phenol as evident from the estimated coefficients of the model parameters .

2.7.2 Effect of residence time on removal percentage

As per the model prediction, 100% removal is possible achieve for residence time more than 17 hr for phenol and *m*-cresol concentration of 100 mg/L each, but removal percentage decreases to 98.43% when residence time provided is 8.75 hr. The model also predicts that, for residence time kept at its highest level, near about 99% removal can be always achieved irrespective of the substrate concentration, but removal percentage will decrease sharply with the decrease in residence time and increase in phenolics concentration.

2.8 Optimisation of process parameters

Process optimisation by the statistical analysis of CCF design with the software was performed. For treatment of wastewater with higher substrate concentration, the following criteria has been set: the initial substrate concentration: 'maximise', residence time: 'in range' and response: 'maximise'. Thus the optimised parameters predicted by the software are 400 mg/L of each substrate with residence time of 28.03 hr and this combination will give 99.44% substrate removal. As predicted by the software, if the system has to run at low substrate concentration but with low residence time, then the optimized parameters are 100 mg/L of each substrate with 8.75 hr residence time (98.24% removal). If the process is allowed to run at any flow rate between 100–400 mg/L, then the best combination which will give 99.92% substrate removal rate is 230 mg/L phenol, 190 mg/L *m*-cresol with residence time of 24.82 hr.

RSM method also predicts that the input parameter residence time has the greatest positive effect on the removal of phenolics. Thus the surface of the plot between removal percentage vs. either phenol or *m*-cresol concentration and residence time, dropped drastically towards the lower side of the residence time axis (**Fig. 8d** and **e**). This change in the surface nature is more than the changes observed in **Fig. 8a–c**. This can be also confirmed from the equation relating the input parameters and the removal percentage, predicted by the RSM. The coefficient of residence time has the highest value (+0.11572) among all the coefficients associated with the other input variables.

3 Conclusions

The present study examined the performance of a hybrid reactor comprising of trickling filter and aeration tank units for treatment of bi-substrate mixture of phenol and *m*-cresol in wastewater by mixed microbial culture. The culture was capable of forming biofilm on specially designed inert clay rings serving as packing material in the trickling filter. The performance of the each unit of the

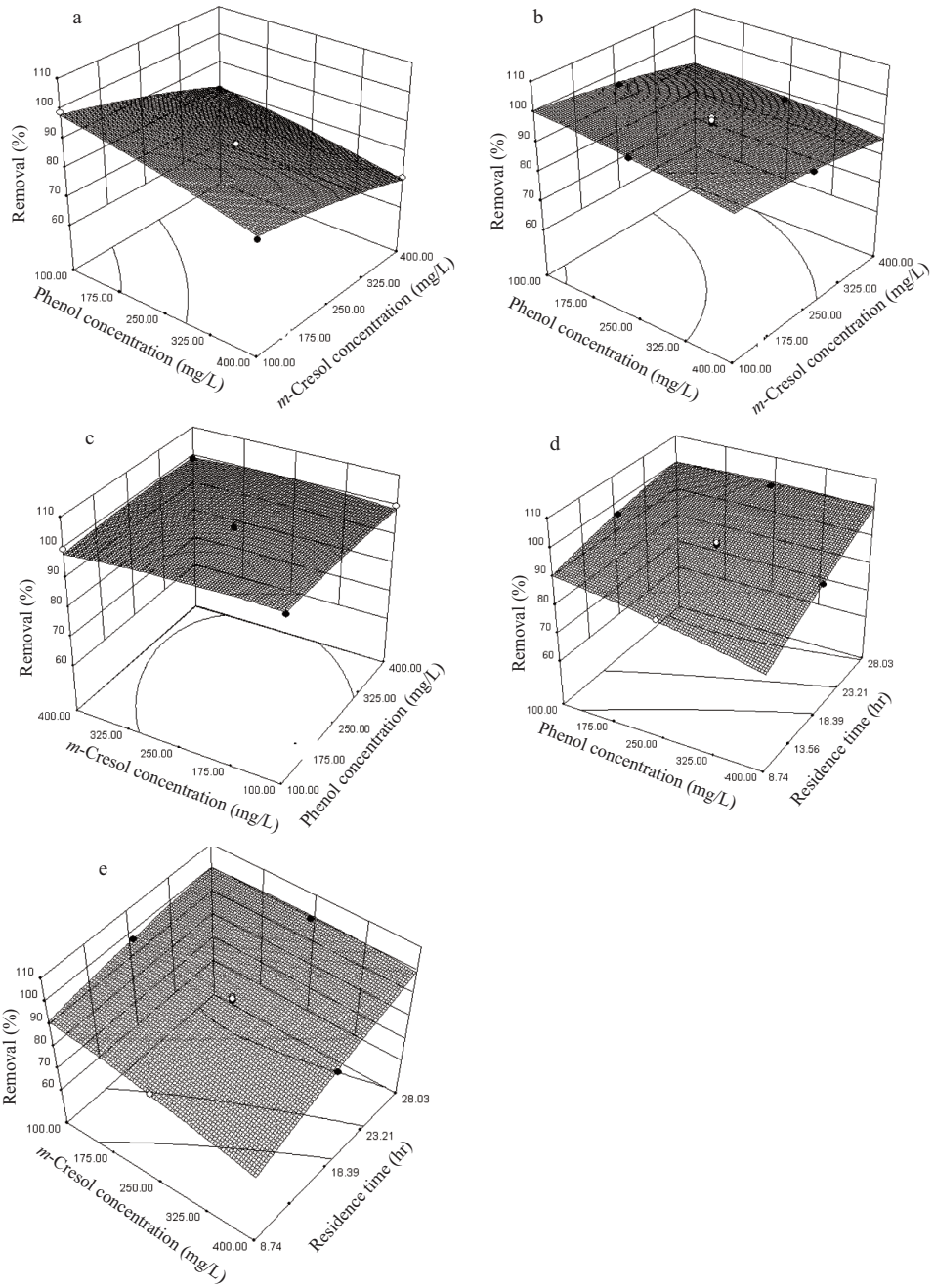

Fig. 8 Effects of substrate concentrations on the removal of phenolics as shown by surface response plot. Effect of phenol and *m*-cresol concentration on removal of phenolics when residence time were kept at 8.75 (a), 18.39 (b), and 28.03 (c) hr; effect of phenol concentration and residence time on phenolics removal when *m*-cresol concentration is kept at 250 mg/L (d); effect of *m*-cresol concentration and residence time on phenolics removal when phenol concentration is kept at 250 mg/L (e).

reactor and the total reactor in terms of total phenolics removal percentage reveals that trickling filter performs better than the aeration tank. Hydraulic study of the reactor shows that as the flow rate of wastewater increases, axial dispersion also increases, indicating deviation from ideal plug flow in the trickling filter unit. Almost 100% substrate removal is possible to achieve upto flow rate of 3.5 mL/min and substrate concentration upto 200P + 200C (mg/L). The efficiency of substrate removal varies between 71%–100% for the range of HLR and PLR studied here. A steady-state biofilm model has been also established to find the kinetic parameters related to biodegradation in biofilm. The model shows reasonable agreement with the experimental data in terms of substrate flux within biofilm and surface concentration of the film. Variation of the kinetic parameters with the HLR and PLR proves the existence of substrate inhibition at high phenolics input. The performance of the reactor has been optimized by Face-centered Central Composite Design of Response Surface Methodology using Design Expert 8 software. The design of experiment

shows that a quadratic model can be fitted best for the present experimental study with significant interaction of the residence time with the substrates concentrations. The actual substrate removal and the predicted values are in well agreement with each other. The optimized condition for operating the reactor as predicted by the model is 230 mg/L phenol, 190 mg/L *m*-cresol with residence time of 24.82 hr to achieve 99.92% substrate removal .

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