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Biodegradation Kinetics of Bi-substrate Solution of Phenol and Resorcinol in an Aerobic Batch Reactor

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Abstract

Phenol and resorcinol compounds are found to co-exist in real-life wastewater, especially in petrochemical, coking and coke-oven wastewater. An indigenous mixed microbial culture isolated from effluent treatment plant of a coke oven industry has been employed to investigate for its biodegradation capacity of bi-solute mixture of phenol and resorcinol under aerobic batch reactor operation. A 2^2 full factorial design with the two substrates at two different levels of initial concentration ranges (high and low) was explored to design the biodegradation experiments. The effect of individual substrate concentrations and their interaction on rate of phenolics biodegradation were also determined. The phenol and resorcinol as substrates were completely utilized after 22 hrs when the solutes are present at low concentrations of 100 mg/L each. But the culture has taken total 58 hrs to biodegrade completely higher initial concentrations i.e., 400 mg/L of each substrate. This study also observed that both specific growth rate of the culture and the specific substrate degradation rate have descended to lower value in presence of phenol and resorcinol as dual substrate in the solution compared to their presence as single substrate, showing the interaction and inhibition by each substrate. Sum kinetic model was used to describe the variation in the specific substrate degradation rates by the mixed culture. From the interaction parameters obtained from this model, it has been observed that resorcinol inhibits specific substrate degradation rate to a higher extent than inhibition caused by phenol ($I_{\text{Resorcinol, Phenol}} = 0.5$, $I_{\text{Phenol, Resorcinol}} = 0.1$, RMSE = 0.04361)

Keywords: *biodegradation, inhibition kinetics, phenol, resorcinol, dual substrate, 2^2 factorial design of experiments*

1. Introduction

Phenol and its derivatives are common water pollutants contained in various industrial waste streams such as polymeric resin producing companies, coal gasification plants, oil refining, coke oven industries, fiber glass units, pharmaceuticals, explosive manufacturer and varnish industries (Yan *et al*., 2006; Juang *et al*., 2006). It is also listed as the priority organic pollutants given by the Environmental Protection Agency, USA (Yan *et al*., 2006). Phenol and resorcinol are two major toxic organic pollutants that occupied the top of the list for their inherent nature of posing inhibition during their degradation. These compounds have high stability, high toxicity and carcinogenic in nature. They cause considerable damage and threats to the ecosystem and even at very low concentration it will produce unacceptable odor and taste of water. Wastewater containing phenol and resorcinol needs appropriate treatment before they are discharged to the receiving water bodies.

The removal of phenolic compounds from wastewater is widely used, although ongoing economical cost studies have shown, physico-chemical studies are costly and many times produce secondary effluent even more toxic (Klein and Lee, 1978; Talley and Sleeper, 1997). Elimination or bio-degradation of such xenobiotic compounds from wastewater became a challenging job to the researchers worldwide. These xenobiotic compounds rarely undergo self-degradation naturally and most of the cases remain in the environment as persistent organic pollutants (Juang *et al.*, 2006). Thus biological phenol and resorcinol removal would be a useful alternative of wastewater treatment.

Industrial wastewater may contain different range of phenolic compounds as varying concentration and so may be considered as multi-substrate mixture. Hence mixed substrates containing structurally related compounds affect the growth of organisms and each other's metabolism either synergistically or antagonistically (Swaminathan *et al*., 1999). Aerobic degradation of phenol by pure microbial culture has been studied extensively (Hill and Robinson, 1975; Kotturi *et al.*,1991; Nikakhtari and Hill, 2006; Agarry *et al*., 2008). Few scientists have studied resorcinol biodegradation under anaerobic condition (Latkar *et al*., 2003; Godbole and Chakraborty, 1991). However a very limited works have been done on simultaneous phenol and resorcinol biodegradation by mixed bacterial culture under aerobic condition.

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Kumaran and Paruchuri (1997) have estimated biokinetic constants of an activated sludge removing phenolics (phenol, catechol, resorcinol, cresol, hydroquinone) in wastewater and compared those kinetic constants for pure culture in synthetic wastewater containing phenolic compounds. Later, biokinetics parameter estimation of phenolic compounds on activated sludge was done by respirometric method by Orupold *et al.* (2001). The bio-oxidation of phenol, catechol, resorcinol, *m*-cresol and 5-methylresorcinol on activated sludge was investigated by them. Latkar *et al.* (2003) evaluated the biokinetic constants for resorcinol, catechol and hydroquinone biodegradation in an anaerobic upflow fixed-film fixed bed reactor. Their study highlighted the biochemical sensitivity acquired by microbial consortia when exposed to different inhibitory substrates for acclimatization. Their study revealed that maximum substrate removal of 98.13% was observed for resorcinol corresponding to an influent concentration of 750 mg/L. In catechol and hydroquinone acclimated reactors maximum substrate removals of 93.11% (influent catechol concentration 720 mg/L) and 83.02% (influent hydroquinone concentration 600 mg/L) were obtained respectively, beyond which the removal efficiencies decreased. Godbole and Chakrabarti (1991) have studied the degradation of resorcinol, catechol and phenol as mono and binary substrate mixtures in upflow anoxic Fixed Film–Fixed Bed (FFFB) reactors acclimated to these compounds. They reported that, in the resorcinol acclimated reactor phenol and catechol in binary mixture feed negatively interacted with each other, the interaction being more pronounced at higher substrate loading rates. However, interacting role on inhibition of biodegradation of these phenolic compounds due to presence of two or more such compounds has been reported. In a study by Guedes *et al.* (2011), observed that *Penicillium chrysogenum* strain previously isolated from a salt mine could able to grow at 1,000 mg/L of resorcinol on solid medium. This strain was able to remove phenol faster than resorcinol. They also found that when phenol and resorcinol were in binary substrate matrices, phenol enhanced resorcinol degradation, and organic load decreased with respect to the mono substrate matrices. Cross feeding studies by Swaminathan *et al.* (1999) have revealed that phenol was poorly degraded in resorcinol acclimated reactor whereas it was readily degraded in catechol acclimated reactor. But addition of resorcinol along with phenol in a COD ratio of 1:3 in resorcinol reactor increased phenol removal efficiency to 95% indicating that resorcinol speeds up phenol degradation.

The present study deals with biodegradation performance by indigenous mixed microbial culture that can degrade phenol and resorcinol after acclimatization with each of the substrates, under binary mixed substrate condition. Statistically valid 2^2 full factorial design of experiments was employed to carry out experimental investigation for the kinetics of simultaneous biodegradation of phenol and resorcinol. The interaction and inhibition by the substrates on the growth and their biodegradation have been investigated. This statistical based full factorial design of experiments that give meaningful indication towards the main effect and the interaction effect of the factors involved in the study (Montegomery, 2004) has been carried out.

2. Materials and Method

2.1 Microorganisms and Culture Condition

A mixed microbial sludge was collected from an effluent treatment plant of a coke oven industry in Durgapur, India. The culture was initially grown in 3 L of batch reactor containing 1 L of Mineral Salt (MS) medium with glucose as substrate. The composition of MS media is given as (mg/L): $(NH_4)_2SO_4$ 230, $CaCl_2$ 7.5, $FeCl_3$ 1.0, $MnSO_4$. H_2O 100, $MgSO_4.7H_2O$ 100, K_2HPO_4 500, KH_2PO_4 250 (pH 7.0) under stirring condition by a mechanical stirrer (Remi, India). For acclimatization of sludge with phenol, first the culture was grown at very low concentration of phenol (5 mg/L) in presence of high concentration of glucose (2 g/L) in MS media. Then glucose concentration was gradually decreased and supplemented by increased concentration of phenol. After six month of acclimatization period, the sludge was changed to mineral salt medium (MS media) with phenol as sole carbon source. After acclimatization with phenol, the sludge was acclimatized to resorcinol as sole carbon source in MS media. The culture was considered to be predominated by the organisms that can metabolize both phenol and resorcinol in its feed solution.

2.2 Analytical Procedure

Phenol and resorcinol concentration were analytically estimated by High Performance Liquid Chromatography (HPLC) (Shimadzu) equipped with Ultraviolet-Visible (UV-VIS) detector and C18 column. The mobile phase used was acetonitrile and water mixture (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 was 3.967 min and for resorcinol, it was 2.923 min. The biomass growth in the sample was monitored by measuring its absorbance at 600 nm wavelength using UV-VIS Spectrophotometer (Shimadzu). Then biomass concentration was calculated from a standard graph plotted as dry cell mass of microbial culture vs. optical density measured at 600 nm.

2.3 Batch Biodegradation Study

The biodegradation study was carried out for examining the ability of microbial mass developed and acclimatized in laboratory condition to biodegrade phenol and resorcinol in dual substrate mode. All biodegradation experiments using the culture were performed in batch bioreactor containing MS media with both phenol and resorcinol as sole carbon and energy sources at different initial concentration ranges. 60ml inoculum was added to batch bioreactor for each set of experiment. Fig. 1 represents the flowchart of the whole method used in this study. This was accomplished by transferring directly (under aseptic conditions), freshly acclimatized culture to MS media containing phenol and resorcinol. In the previous study it was found that phenol present as the sole carbon source inhibited the growth of the culture

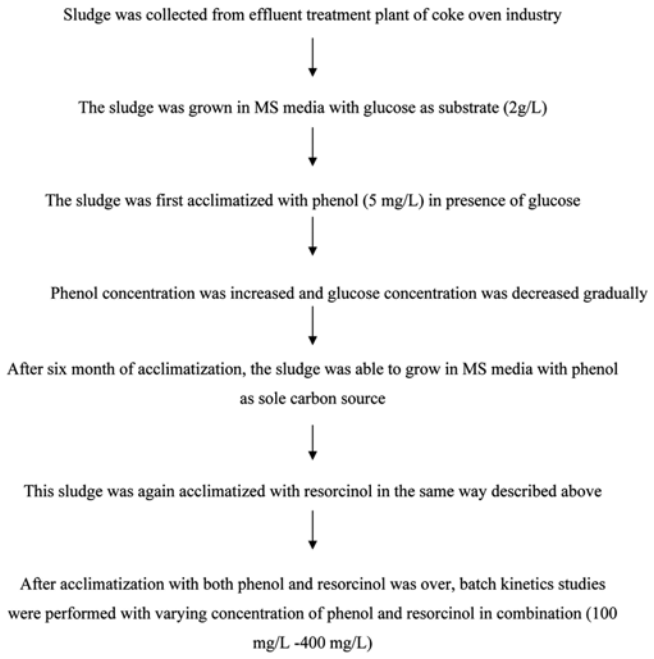

Fig. 1. Flowchart of the Work Done in the Present Study

Table 1. 2^2 Full Factorial Design Matrix for Low and High Initial Concentration Ranges

Experimental run	Factor concentrations (mg/L)		Factor levels	
	Phenol	Resorcinol	Phenol	Resorcinol
1	100	100	-1	-1
2	400	400	+1	+1
3	100	400	-1	+1
4	400	100	+1	-1

when concentration exceeded 300 mg/L (Dey and Mukherjee, 2010); similarly resorcinol inhibited the culture growth also beyond 300 mg/L of concentration also (Dey and Mukherjee, 2011). Based on the result of single substrate degradation study, concentration range of phenol and resorcinol were chosen accordingly as 100 mg/L to 400 mg/L for both of them. Table 1 shows the concentration combination of each substrate and the design matrix employed in the study for factorial design. Total 4 combinations (experimental runs) of initial concentrations of phenol and resorcinol were investigated for studying their biodegradation and culture growth in low and high concentration ranges adopted in this study.

All biodegradation experiments using the mixed microbial culture were carried out in a 3 L bioreactor with provisions for air supply for necessary aeration. In every batch the total volume of wastewater was 1L. Compressed air was fed in the reactor at a rate of 2.5 L/min. For each concentration, culture was incubated at 30°C. Samples were withdrawn at regular time interval, analyzed for biomass concentration and subsequently centrifuged (10,000 × *g* for 3 min) and the resulting supernatant was analyzed for residual phenol and resorcinol concentration, until complete degradation of the substrates.

2.4 Microbial Growth Kinetics Study

The experimental data on the microbial growth at various combinations of initial phenol and resorcinol concentrations were utilized for calculating specific growth rate of culture and specific substrate degradation rates. The specific growth rate and the microbial yield were determined from the following formula:

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{1}$$

$$Y_{X/S} = \frac{(X_M - X_0)}{(S_0 - S_M)} \tag{2}$$

where μ is the specific cell growth rate (hr^{-1}), X is the biomass concentration (mg/L), t is time (hr), X_M and X_0 are the maximum and initial dry cell mass concentration (mg/L), S_M and S_0 are the substrate concentration (mg/L) at the maximum cell concentration and initial time.

2.5 Sum-Kinetic Model

Sum kinetics model, proposed by Yoon *et al.* (1977) was slightly modified to fit the experimental data on specific degradation rates of the substrates, wherein the specific growth rate μ in the original equation was replaced by specific substrate degradation rate q, as represented in Eq. (3). This model is utilized to evaluate the relative interaction effects on the individual degradation rates:

$$q = \frac{q_{\max,1} S_1}{K_{s,1} + S_1 + \frac{S_1^2}{K_{i,1}} + I_{2,1} S_2} + \frac{q_{\max,2} S_2}{K_{s,2} + S_2 + \frac{S_2^2}{K_{i,2}} + I_{1,2} S_1} \tag{3}$$

and q is defined as:

$$q = \frac{1}{X}\frac{dS}{dt} \tag{4}$$

$$q_1 = \frac{q_{\max,1} S_1}{K_{s,1} + S_1 + \frac{S_1^2}{K_{i,1}} + I_{2,1} S_2} \tag{5}$$

$$q_2 = \frac{q_{\max,2} S_2}{K_{s,2} + S_2 + \frac{S_2^2}{K_{i,2}} + I_{1,2} S_1} \tag{6}$$

where X is the biomass concentration (mg/L), t is time (hr), S is the substrate concentration (mg/L of either phenol or resorcinol and in some cases total phenolic compound as phenol + resorcinol) the interaction parameter Ii,j indicates the degree by which substrate i affects the biodegradation of substrate j, large value of the parameter indicate stronger inhibition on the substrate (Yoon *et al.*, 1977).

3. Results and Discussion

3.1 Simultaneous Degradation of Phenol and Resorcinol in Dual Substrate System

A series of biodegradation experiments containing different initial concentration of phenol and resorcinol was conducted. The biodegradation patterns are shown in Fig. 2 to Fig. 4. Fig. 3 and Fig. 4 also provide the comparison of simultaneous biodegradation of phenol and resorcinol of unequal concentration with their single substrate degradation profile at respective concentration. The cell growth rates and biodegradation rates in bi-substrate system were different as it appeared under single substrate condition. It could be observed that for the equal initial concentration (mg/L) of both the substrates (100P + 100R, 200P + 200R, 300P + 300R, 400P + 400R), the culture took more time to utilize resorcinol than phenol (Fig. 2), but both of them were degraded simultaneously. Preferential uptake of phenol over resorcinol for given concentration of substrates, indicates that phenol compared to resorcinol is a much simpler carbon source for the culture.

In dual substrate mode, both phenol and resorcinol took longer time for their complete biodegradation (Table 2) compared to their respective single substrate degradation (Dey and Mukherjee, 2010; Dey and Mukherjee, 2011) under identical operating condition and similar initial concentration. As 100 mg/L of phenol was mixed with 100 mg/L of resorcinol, culture took 12.5

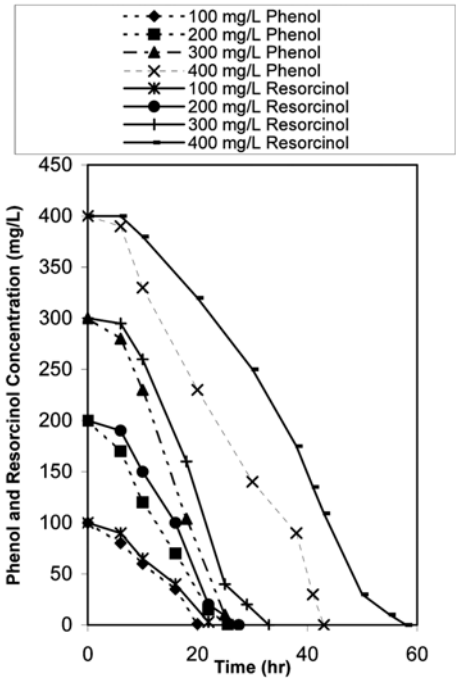

Fig. 2. Biodegradation Profile of Phenol and Resorcinol at Their Equal Initial Concentration Combination with Respect to Time

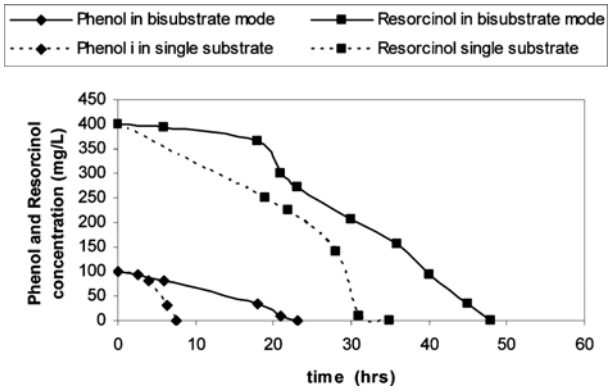

Fig. 3. Comparison of Time Profile for Simultaneous Biodegradation of 100 mg/L of Phenol in Mixture with 400 mg/L of Resorcinol, with Single Substrate Condition

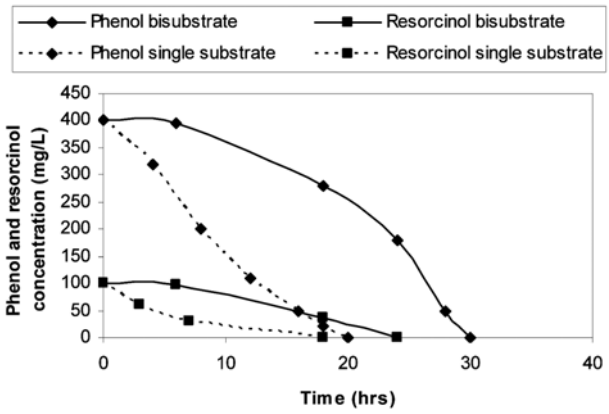

Fig. 4. Comparison of Time Profile for Simultaneous Biodegradation of 100 mg/L of Resorcinol in Mixture with 400 mg/L of Phenol, with Single Substrate Condition

Table 2. Comparison of the Time Needed for Biodegradation of Substrates under Single and Mixed Substrate Conditions

Initial substrate concentrations (mg/L)	Time for complete biodegradation of the substrate (hr)	Initial substrate concentration (mg/L)	Time for complete biodegradation of the substrate (hr)	Initial substrate concentrations (mg/L)	Time for total biodegradation (hr)	
Single substrate mode		Single substrate mode		Dual Substrate mode	Phenol	Resorcinol
100 (P)	7.5	100 (R)	18	100 (P) + 100 (R)	20	22
200 (P)	11	200(R)	26	200 (P) + 200 (R)	25.5	27.5
300 (P)	12.08	300(R)	28	300 (P) + 300 (R)	29	33
400 (P)	20	400(R)	35	400 (P) + 400 (R)	43	58
				100 (P) + 400 (R)	23	48
				400 (P) + 100 (R)	30	24

(P)→ Phenol, (R)→ Resorcinol

hrs more to degrade phenol than 100 mg/L of phenol as single substrate, but resorcinol (100 mg/L) degradation was delayed by only 4 hrs than without phenol. The similar observation was found when 200 mg/L of phenol is mixed with 200 mg/L of resorcinol. In such condition, the culture took 14.5 hrs more time to degrade 200 mg/L of phenol in dual substrate mixture compared to 200 mg/L of phenol as single substrate; where as, delay of resorcinol degradation (200 mg/L) in dual substrate was by only 1.5 hrs than that of 200 mg/L of resorcinol without phenol. It proves that at low concentration of phenol, the inhibition by resorcinol on phenol biodegradation was very acute. But inhibition of resorcinol biodegradation in presence of low concentration of phenol was negligible. As the phenol concentration was gradually elevated (upto 400 mg/L) in the mixture along with resorcinol, then phenol also started inhibiting the resorcinol degradation to a large extent. On the other hand, the high concentration of phenol could not overcome the ill effect of resorcinol. The fact was corroborated from the following observation. As 400 mg/L of phenol was mixed with 400 mg/L of resorcinol, phenol took 43 hrs for its 100% degradation which was 23 hrs more than its single substrate (400 mg/L) degradation time; while resorcinol took 58 hrs for its complete biodegradation (dual substrate mode) which was also 23 hrs more than the case when 400 mg/L of resorcinol was the single carbon source in the growth media.

In the present study, it was observed that phenol and resorcinol inhibited the substrate degradation rate of each other when present in mixed substrate condition both in low and in high concentration. The inhibition effect of each of the substrates always simultaneously existed, and competed with each other. The study done by Guedes *et al.* (2011) showed that phenol enhanced the degradation rate of resorcinol when present in binary substrate matrices in presence of *Penicillium chrysogenum* strain. This strain removed phenol faster than resorcinol similar to our study. But in the present study, none of the substrate had accelerated the degradation of the other at any concentration combination level. This result was quite similar to Godbole and Charaborty (1991) where they found that the binary mixture feed of phenol and catechol interacted negatively with each other in a resorcinol acclimatized FFFB reactor. They found that the interaction was more pronounced at higher phenolics loading rate which supported the result of the present study.

3.2 Microbial Growth in Presence of Phenol and Resorcinol Mixture

The microbial growth profile for equal and unequal combinations of initial concentration of phenol and resorcinol are shown in Fig. 5 and Fig. 6. In the previous study for single phenolic substrate biodegradation (Dey and Mukherjee, 2010), fitting the batch kinetics data in Haldane model (Andrews, 1968), it was found that μ_{max} for phenol was 0.3059 hr^{-1} (Dey and Mukherjee, 2010) where as μ_{max} for resorcinol was 0.5 hr^{-1} (Dey and Mukherjee, 2011). It indicated that though phenol was much simpler carbon source than resorcinol, yet the cell mass production was much more facilitated in presence of resorcinol. The reason might be

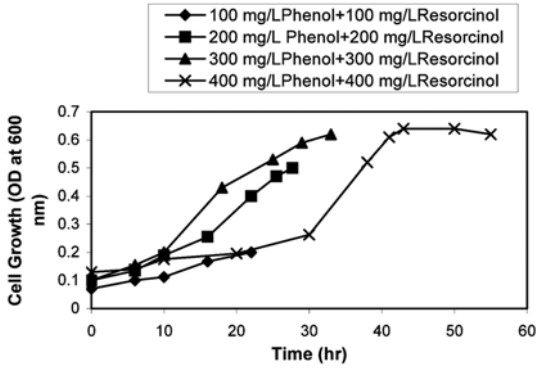

Fig. 5. Microbial Growth Profile in Presence of Phenol and Resorcinol as Dual Substrate at Their Equal Initial Concentration Combination, with Time

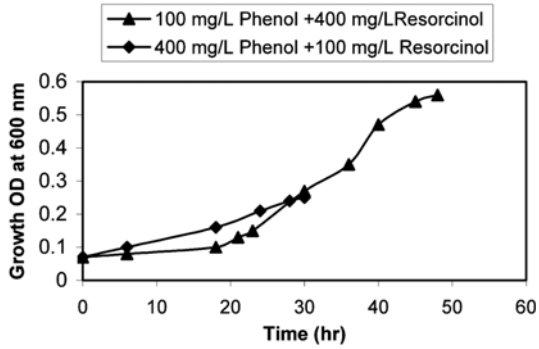

Fig. 6. Microbial Growth Profile in Presence of Phenol and Resorcinol as Dual Substrate at Their Unequal Initial Concentration Combination, with Time

that, as the biodegradation pathway of phenol and resorcinol are not identical, probably higher amount of carbon molecules in supplied phenol were utilized for forming the intermediate products and thus lesser amount of carbon molecules were left for formation of cell mass. Where as, in case of resorcinol biodegradation, it might happen that, intermediate metabolites contained less carbon atoms compared to phenol biodegradation intermediates and thus higher amount of carbon were available for cell growth in this case. Evaluation of yield coefficient ($Y_{X/S}$) for single substrates also proved that yield of microbes in phenol was 0.718 mg/mg of substrate utilized where as yield in presence of resorcinol was having higher value of 0.835 mg/mg. Under the same condition, the culture could biodegrade more phenol than resorcinol and the value of $(S_0 - S_M)$ for phenol was much greater than that of resorcinol. At the same time, the value of $(S_0 - S_M)$ increased much quickly than the value of $(X_M - X_0)$ for phenol. So biomass yield for resorcinol was much more than phenol. The similar observation was found by Yao *et al.* (2011) for degradation of m-cresol and pyridine by *L. cresolivorans*, where the organism could degrade m-cresol much easily than pyridine, but yield of organism under m-cresol was lesser than that of pyridine under single substrate condition.

In dual substrate condition, irrespective of concentration combination of phenol and resorcinol, the specific cell growth

Table 3. Comparison of Microbial Growth and Substrate Degradation Rate at Different Combination of Initial Phenol and Resorcinol in Dual Substrate Mixture

Initial Substrate concentration (mg/L)	Microbial Yield coefficient (Yx/s mg/mg)	Microbial specific growth rate μ (hr^{-1})	Specific phenol degradation rate q_{phenol} (hr^{-1})	Specific resorcinol degradation rate $q_{resorcinol}$ (hr^{-1})	Specific total phenolic degradation rate $q_{phenol+resorcinol}$ (hr^{-1})
100(P)+100(R)	0.393179	0.047719	0.118092	0.107356	0.2147
200(P)+200(R)	0.60489	0.0585	0.12961	0.120232	0.240464
300(P)+300(R)	0.524238	0.055	0.17094	0.15029	0.300581
400(P)+400(R)	0.378056	0.0272	0.118267	0.087703	0.175405
100(P)+400(R)	0.592792	0.0433	0.102683	0.196809	0.246011
400(P)+100(R)	0.21776	0.0424	0.314894	0.098408	0.393617

P→ Phenol, R→ Resorcinol

rate was less compared to similar concentration ranges of either phenol or resorcinol as single substrate. This phenomenon proves that inhibition of cell growth took place due to interaction of substrate mixture. In the previous study (Dey and Mukherjee, 2010; Dey and Mukherjee, 2011), single substrate kinetics showed that initial substrate concentration at which highest specific cell growth rate achieved was 300 mg/L for both phenol and resorcinol. But in presence of both of the substrates in the media, 200 mg/L of each substrate (200 mg/L phenol+200 mg/L resorcinol) yielded maximum experimental specific growth rate (0.0585 hr^{-1}). The μ value increased with increase of the total phenolic loading starting from 100 mg/L of phenol with 100 mg/L of resorcinol upto 300 mg/L of phenol with 300 mg/L of resorcinol combinations. But μ decreased drastically (Table 3) when initial substrate concentration was 400 mg/L of phenol in mixture with 400 mg/L of resorcinol, indicating substrate inhibition at high substrate loading (total 800 mg/L of phenolics). Presence of low concentration of phenol (100 mg/L) with high concentration of resorcinol (400 mg/L) slowed down the rate of degradation of phenol considerably due to inhibition of phenol biodegradation by resorcinol. Therefore, though at the end of biodegradation period, total biomass production was high (OD_{600nm} = 0.56), but time needed for that amount of biomass growth was high enough and as a result, μ came down to 0.0433 hr^{-1}. It was also observed that combination of high phenol (400 mg/L) with low resorcinol (100 mg/L), the total time needed for complete biodegradation was lesser than the above mentioned case, because high phenol could overcome the inhibition caused by resorcinol on substrate biodegradation rate. But as the presence of high phenol inhibited the growth of the culture a lot, so the μ value in this case also came down to 0.0424 hr^{-1}. The yields of microbial growth for each of the initial substrate mixture combinations were calculated. It was found that maximum value of $Y_{X/S}$ was obtained (0.605 mg/mg) at medium level of the substrates (200 mg/L phenol + 200 mg/L resorcinol). Yield value was similarly high for combination of 100 mg/L of phenol with 400 mg/L of resorcinol (0.593 mg/mg), but lowest (0.218 mg/mg) at the combination of 100 mg/L of resorcinol and 400 mg/L of phenol. This proves that phenol concentration at low level does not inhibit cell mass production, but at its high concentration level, inhibits the cell growth to a large extent (Table 3).

3.3 Sum Kinetic Model Fitting of Experimental Specific Substrate Degradation Rate

Based on the experimental data, the model equation Eq. (3) was solved by MATLAB 7.1© using non-linear least square programme. First the individual phenol and resorcinol degradation rates obtained in the experiment (bisubstrate condition) were fitted in the Eq. (5) and Eq. (6). From that the values of q_{max1}, Ks_1, $K_{i,1}$, q_{max2}, Ks_2, $K_{i,2}$ were obtained as 0.5841 hr^{-1}, 348.5 mg/L, 158.6 mg/L, 0.4496 hr^{-1}, 253 mg/L, 160.1 mg/L, respectively. Using these values in Eq. (3) the values of $I_{2,1}$ and $I_{1,2}$ were obtained as 0.5 and 0.1 with RMSE = 0.04361, where q defined the total phenolic substrate degradation rates (Fig. 7). A larger value of $I_{2,1}$ indicates that resorcinol inhibited phenol degradation a larger extent than phenol inhibited resorcinol degradation, which was in accordance with the experimental result.

Many substrate-inhibitory effects in mixed substrate systems have been reported previously. For example, Wang and Loh (2001) have studied the degradation of mixed phenol and sodium glutamate by *P. putida* ATCC 49451 at 30C. They found that the specific rate of phenol degradation was lower in the presence of glutamate, but more cells were generated with simultaneous utilization of glutamate and phenol. Klecka and Maier (1988) observed that phenol or 2,4,5-trichlorophenol reduced the rate of pentachlorophenol degradation by an enrichment culture. Reardon *et al.* (2000) had examined the degradation of benzene, toluene

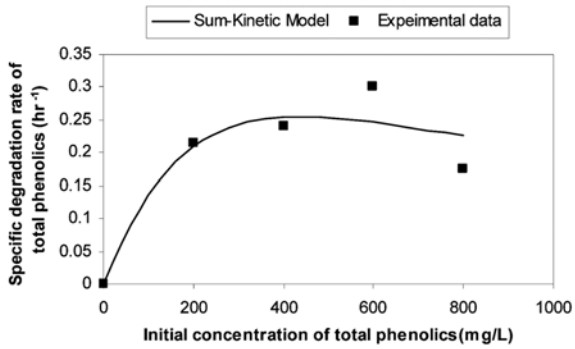

Fig. 7. Sum Kinetic Model Prediction and Experimentally Determined Specific Total Phenolics Degradation Rate at Initial Phenolics Concentration of 200-800 mg/L (100P + 100R to 400P + 400R)

and phenol as single and mixed substrates by *P. putida* F1. They found that toluene and benzene enhanced the degradation of phenol, while phenol did not affect the degradation of toluene and benzene significantly and, there was no significant interaction between benzene and toluene. They used Monod kinetics to describe the specific growth rate in this low substrate level range. Juang *et al.* (2006) studied the degradation of phenol in presence of sodium salicylate. They found that sodium salicylate (SA) inhibited the specific rate of phenol removal, in a competitive and uncompetitive manner, much more than phenol inhibited the specific rate of SA removal.

3.4 Interaction Effects Indicated by 2^2 Full Factorial Design

To justify the interaction effects of the substrates, 2^2 full factorial design was employed according to the design matrix shown in Table 1. Table 3 shows the calculated specific degradation rates of phenol and resorcinol and specific growth rates of the culture in each initial concentration combination of the two substrates. In general, the main effect plot describes the individual effect of any factor (plotted as abscissa) at its low and high level on the dependent variable (plotted as ordinate). The interaction effect plot, on the other hand, illustrates the significance of interaction of any two factors involved in the study where dependent variable is plotted on the ordinate and one of the factors on the abscissa. The remaining factor is plotted for high and low values of both factors (Saravaran, 2008). The result of the main effect of the substrates on the total phenolics degradation rate is shown in Table 4. The main effect plot of phenol on specific total substrate degradation rate ($q_{phenol+resorcinol}$) shows that as initial phenol concentration is increased from low level to high level, the $q_{phenol+resorcinol}$ value increases (Fig. 8). The main effect plot of resorcinol on $q_{phenol+resorcinol}$ shows the negative effect, i.e. increase of resorcinol concentration decreases the specific total phenolic substrate degradation rate (Fig. 8). Fig. 9 shows strong interaction effect of the two substrates on the specific degradation rate of phenolics, where the lines are completely unparallel and cross each other. The interaction plot shows that when initial resorcinol concentration is kept low, increasing the phenol concentration can increase the $q_{phenol+resorcinol}$ value. This proves that higher level of initial phenol concentration helps to overcome the inhibitory effect of resorcinol at low concentration level. But the presence of high initial resorcinol concentration with phenol in mixture inhibits the phenolics degradation at such a high extent that phenol at any level (high or low) cannot overcome the inhibitory effect, and thus the specific substrate degradation rate falls. This result is in accordance with the experimental findings and the interaction parameters ($I_{i,j}$) values estimated from the sum kinetic model. From the interaction plot of Fig. 10, it is observed that at low phenol concentration, resorcinol either high or low level suppresses the specific degradation rate of phenol (q_{phenol}). But as initial phenol concentration is kept at its high level, it can accelerate its individual specific degradation rate highly at low resorcinol concentration. This acceleration effect is almost negligible in presence of inhibitory resorcinol at high level. The study regarding the specific degradation rate of resorcinol (Fig.

Table 4. Data to Study the Main Effect of the Substrates on Total Phenolics Degradation

Initial substrate concentration (mg/L)	Specific substrate degradation rate (hr^{-1})
Main effect data	
100 (phenol)	0.230
400 (phenol)	0.285
100 (resorcinol)	0.275
400 (resorcinol)	0.210

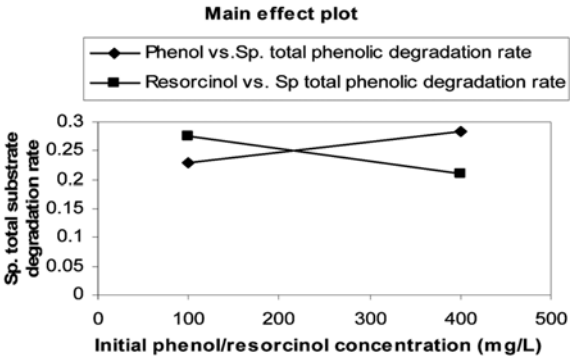

Fig. 8. Effect of Initial Substrate Concentration on Specific Total Substrate Degradation Rate

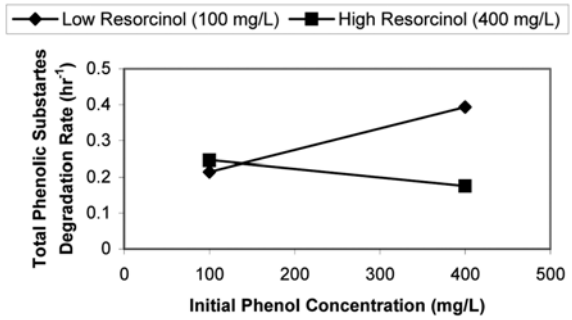

Fig. 9. Interaction Effect between Phenol and Resorcinol on Specific Total Substrate Degradation Rate

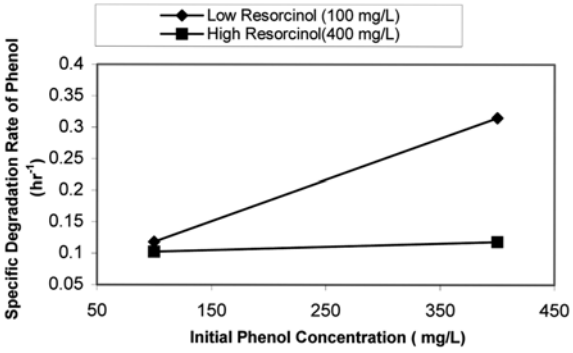

Fig. 10. Interaction Effect between Phenol and Resorcinol on Specific Phenol Degradation Rate in Dual Substrate Mode

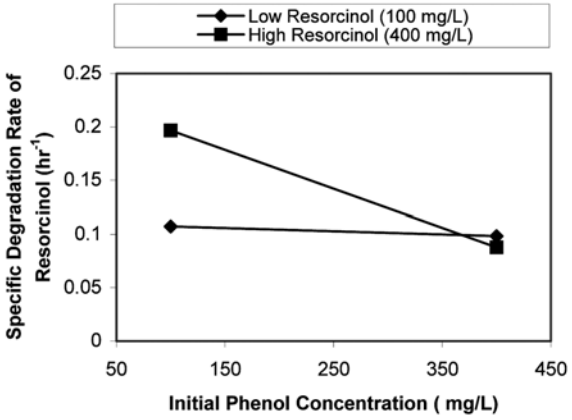

Fig. 11.Interaction Effect between Phenol and Resorcinol on Specific Resorcinol Degradation Rate in Dual Substrate Mode

11) shows that increase in phenol concentration decreases the specific resorcinol degradation rate ($q_{resorcinol}$) in lesser extent when resorcinol is present at its low concentration. It proves that the inhibition of resorcinol by phenol is very low. But increase of phenol concentration in presence of high concentration of resorcinol decreases the specific degradation of resorcinol significantly, which is the outcome of inhibition by phenol as well as self substrate inhibition of resorcinol at its high level (Fig. 11). The Figs. 9-11 show that as the lines in every plot are unparallel to each other, the phenol and resorcinol are interacting a lot among themselves to regulate the specific degradation rate of either the total phenolic compounds or any of the substrates in dual substrate degradation experiments.

By 2^2 full factorial design of experiments and calculating the main effect and interaction effect coefficients, it is obtained that the equation relating the specific degradation rate of total phenolic compounds ($q_{phenol+resorcinol}$) in the substrate with the phenol (S_{phenol}) and resorcinol concentration ($S_{resorcinol}$) is as follows:

$$q_{phenol+resorcinol} = 0.2574 + 0.027S_{phenol} - 0.0336S_{resorcinol} - 0.075S_{phenol}.\ S_{resorcinol} \quad (7)$$

where, $q_{phenol+resorcinol}$ is the specific total phenolic substrate degradation rate (hr^{-1}), S_{phenol} is the initial concentration of phenol (mg/L) and $S_{resorcinol}$ is the initial concentration of resorcinol (mg/L) in the mineral salt media.

4. Conclusions

In the present study, an indigenous mixed microbial culture was explored for simultaneous phenol and resorcinol biodegradation study in a batch reactor under aerobic condition in dual substrate liquor. The kinetics of microbial growth and specific degradation rate of substrate showed that phenol was preferentially uptaken by the culture than resorcinol. The specific growth rate of microbes was high (μ_{max} experimental = 0.0585 hr^{-1}) at lower concentration combination of phenol and resorcinol. But at high concentration of the substrates mixture (400 mg/L phenol + 400 mg/L resorcinol), inhibition played predominant role and specific growth rate of the culture fell sharply (μ = 0.027 hr^{-1}). By fitting the experimental data of the specific substrate degradation rate at different combination of phenol and resorcinol in sum kinetic model, it was observed that inhibition of phenol biodegradation by resorcinol was more than inhibition of resorcinol degradation by phenol ($I_{Resorcinol,\ Phenol}$ = 0.5, $I_{Phenol,\ Resorcinol}$ = 0.1, RMSE = 0.04361). Using 2^2 full factorial design of experiments, it was obtained that the substrates interacted with each other very strongly to regulate the substrate degradation rate. A linear equation to represent experimental work was also constructed which showed that the phenol had a positive effect whereas resorcinol had a negative effect on specific substrate degradation rate. So it can be concluded that the culture is efficient enough to biodegrade completely upto total 800 mg/L of phenolic compounds in mixture. In future this culture can be used in bioreactor to study its performance for the large scale wastewater treatment under continuous mode of operation.

Notations

$I_{2,1}$ = Inhibitory coefficient of resorcinol on phenol
$I_{1,2}$ = Inhibitory coefficient of phenol on resoecinol
$Ki_{,1}$ = Substrate (phenol) inhibition coefficient (mg/L)
$Ki_{,2}$ = Substrate (resorcinol) inhibition coefficient (mg/L)
$K_{S,1}$ = Half saturation concentration of substrate phenol (mg/L)
$K_{S,2}$ = Half saturation concentration of substrate resorcinol (mg/L)
q = Specific substrate degradation rate (hr^{-1})
$q_{max,1}$ = Maximum specific phenol degradation rate (hr^{-1})
$q_{max,2}$ = Maximum specific resorcinol degradation rate (hr^{-1})
q_{phenol} = Specific phenol degradation rate (hr^{-1})
$q_{phenol+resorcinol}$ = Specific phenol and resorcinol degradation rate (hr^{-1})
$q_{resorcinol}$ = Specific resorcinol degradation rate (hr^{-1})
RMSE = Root Mean Square Error
S = Substrate concentration for the biomass (mg/L)
S_0 = Substrate concentration (mg/L) at the initial time
S_M = Substrate concentration (mg/L) at the maximum cell concentration
X = Biomass concentration (mg/L)
X_0 = Initial dry cell mass concentration (mg/L)
X_M = Maximum dry cell mass concentration (mg/L)
$Y_{X/S}$ = Yield coefficient of biomass (mg/mg)
μ = Specific cell growth rate (hr^{-1})
μ_{max} = Maximum specific cell growth rate (hr^{-1})

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