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The highest inhibition coefficient of phenol biodegradation using an acclimated mixed culture

Mojtaba Mohseni, Payman Sharifi Abdar and S. Mehdi Borghei

ABSTRACT

In this study a membrane biological reactor (MBR) was operated at 25 ± 1 °C and pH = 7.5 ± 0.5 to treat synthetic wastewater containing high phenol concentrations. Removal efficiencies of phenol and chemical oxygen demand (COD) were evaluated at four various hydraulic retention times (HRTs) of 24, 12, 8, and 4 hours. The removal rate of phenol (5.51 kg-Phenol kg-VSS^{-1} d^{-1}), observed at HRT of 4 h, was the highest phenol degradation rate in the literature. According to COD tests, there were no significant organic matter in the effluent, and phenol was degraded completely by mixed culture. Substrate inhibition was calculated from experimental growth parameters using the Haldane, Yano, and Edward equations. The results show that the Haldane equation is fitted to the experimental data in an excellent manner. Kinetic parameters were derived by nonlinear regression with a correlation coefficient (R^2) of 0.974. The values for Haldane constants μ_{max}, K_s, and K_i were 0.3085 h^{-1}, 416 mg L^{-1} and 1,886 mg L^{-1}, respectively. The K_i value is the highest value obtained for mixed cultures degrading phenol under batch conditions.

Key words | aerobic process, biodegradation, growth kinetics, membrane bioreactor, phenol, wastewater treatment

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INTRODUCTION

Although phenolic compounds are rarely present in municipal wastewaters, they are found in various concentrations in the effluents of many chemical industries. Industries such as oil refineries, petrochemical industries, ceramic plants, steel plants, coal conversion processes, phenolic resin producers, and pharmaceutical manufacturers consume or produce high concentrations of phenolic substances and thus phenolic compounds are present in their effluents (Kumar *et al.* 2013). The inevitability of removal of phenol from industrial wastewaters before their discharge into the environment has been advised by many scholars because of its toxicity to flora and fauna and it is a prerequisite to sustain a healthy ecology (Basak *et al.* 2014a).

Different methods of treatment, such as adsorption on activated carbon, ion exchange, solvent extraction, and chemical oxidation, have been suggested for removing phenols, but most of these methods are expensive and only suitable for low level concentrations. Some of these processes may even cause secondary problems due to formation of complex molecules. Biological treatment is often preferred because of its economic and environmental advantages as well as degradation into harmless products (Basak *et al.* 2013; Mandal *et al.* 2013). However, microbial biodegradation suffers from the inhibitory effects of phenols at higher concentrations and represents a bottleneck for successful bioremediation. Several approaches have been suggested to overcome the substrate inhibition at higher phenol concentration, including acclimation of cells to higher phenol concentration, application of genetically engineered microorganisms and immobilisation of microbial cells (Basak *et al.* 2014a). Among these approaches, acclimation of cells is a more practical method for large amounts of wastewater, and the limits of the biological process depend on the acclimation of the biomass to the degradation of phenol.

The typical metabolic pathway for the phenol degradation occurs via catechol derivates, before ring cleavage through an *ortho*- or *meta*-oxidation. *Ortho*-pathway involves production of intermediates such as catechol, cis, cismuconic acid, while *meta*-pathway involves ring cleavage of catechol to form 2-hydroxymuconic semialdehyde (2-HMSA). Depending upon the metabolic pathway employed by the organism to degrade the substrate and consequently the intermediates generated, the situation and composition of wastewater also vary (Basak *et al.* 2014b).

Since microorganisms responsible for phenol degradation have a relatively low growth rate, biofilm processes

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where microorganisms are attached to porous materials to improve biomass retention in the digesters (Sá & Boaventura 2001) are a reasonable choice. However, membrane biological reactors (MBRs) can hold a large biomass and hence assure the retention of phenol degrading microorganisms without risk of an eventual wash-out of biomass. In fact, MBR technology has led to a very good biomass retention capacity in reactors working with microbial consortiums characterised by slow growth rates, and its industrial application has gained attention because of these features and because of the robustness of the process that allows for an operation with shock loading rates and hydraulic fluctuations (Trigo *et al.* 2006).

Based on the above discussion, the main objective of this study was to provide a biological solution to treat high strength phenolic wastewater. This type of wastewater exists in the Persian Gulf region where many petrochemical plants discharge large amounts of effluents high in phenolic compounds, presenting a real hazard to the local environment. To this goal, biomass adaptation with high concentrations of phenol was seen as a priority in a MBR system. Optimisation of a suitable biological reactor holding a well-acclimated biomass capable of achieving a high efficiency of chemical oxygen demand (COD) degradation, was the ultimate goal. Besides, this paper addresses the evaluation of biokinetic constants μ_{max}, K_s and K_i of the acclimated mixed consortia. In this study batch assays also were carried out with different phenol concentrations in the medium to determine the growth and kinetic parameters, using the three models of Haldane, Edward, and Yano.

MATERIALS AND METHODS

Experimental setup

A membrane bioreactor with an immersed hollow fiber membrane was used in this study. The reactor used was a 12 L cubic flask, made from plexiglass. Air was introduced into the bottom of the reactor through a coarse bubble spargers to supply oxygen and proper agitation to reduce membrane fouling. The dissolved oxygen concentration was kept less than 5 mg L^{-1} by an adjusting aeration rate. A peristaltic pump was used to withdraw the effluent from the reactor. The reactor was run at a temperature of 25 ± 1 °C throughout the experiment. Wastewater pH was held at 7.5 ± 0.5 by using 0.1 N sodium hydroxide and sulfuric acid.

Chemical washing of the membrane was carried out according to known protocols. The membrane element was parted from the reactor and washed with distilled water in a flask. Then, it was placed in a bottle of sodium hypochlorite (0.2–0.4%) for 2 hours, and finally before it was returned to the reactor, the membrane was washed with water.

This study was performed in two phases. For better and faster acclimation, in the first phase the biomass was adapted to phenol in a batch MBR system with F/M < 0.2 (Melin *et al.* 2006) for 7 weeks; for this purpose, an inoculum from an activated sludge tank of an oil refinery (Behran Oil Refinery) was provided. After adaptation, the second phase was carried out in a continuous mode by use of the obtained acclimated sludge. The biomass concentration of continuous mode was 8,500 ± 300 mg L^{-1}, and the sludge retention time (SRT) was adjusted to maintain this value over the operation (Table 1).

Analytical methods

Phenol concentration was determined by the direct photometric method based on rapid condensation with 4-aminoantipyrine, followed by oxidation with potassium ferricyanide under alkaline conditions to give a red colour product. Colour measurement was carried out with a UV/VIS spectrometer 'Lambda/PERKIN ELMER'. The quantitative determination of phenol, COD, total suspended

Table 1 | Operational conditions and feed specifications at different phases

Phase	Day	Inflow phenol concentration (mg L^{-1})	COD inflow (mg L^{-1})	Hydraulic retention time (h)	pH	Temperature (°C)
1	1–42	0–3,300	7,200	Batch MBR – Adaptation	7.5 ± 0.5	29 ± 2
2	43–74	140–2,400	305–5,230	24	7.5 ± 0.5	25 ± 2
3	75–115	900–3,300	1,960–7,200	12	7.5 ± 0.5	25 ± 2
4	116–161	2,000–4,100	4,360–8,940	8	7.5 ± 0.5	25 ± 2
5	162–233	3,400–5,900	7,400–12,860	4	7.5 ± 0.5	25 ± 2
6	233–240	150–1,200	330–2,600	Batch condition – Kinetic constant	7.5 ± 0.5	25 ± 2

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solids (TSS), and volatile suspended solids (VSS) were all carried out according to *Standard Methods* (APHA 2005).

Synthetic wastewater

The reactor feed was prepared synthetically. Commercial phenol was used as the main ingredient of the synthetic wastewater. Phenol was considered as the only carbon source of the wastewater in continuous mode, while other nutrients were supplied by the addition of urea (CH_4N_2O) and potassium diphosphate (K_2HPO_4) as sources of nitrogen and phosphorus, respectively. The addition of nutrients was necessary in order to keep a balance of COD:N:P proportion of 100:5:1 as often used in similar studies.

Although other micro-nutrients may be required to enhance bacterial growth, it was assumed that normal tap water would provide the necessary trace elements.

Batch degradation tests

For the batch experiment, microbial consortium adjusted to phenol having the ability to remove 250 mg L^{-1} h^{-1} of phenol in continuous MBR reactor was used as inoculum. The biomass concentration of inoculum was almost 12,500 mg L^{-1}. All batch biodegradation tests were carried out in 500 mL Erlenmeyer flasks containing 120 mL of liquid medium with different concentrations of phenol solutions (150–1,200 mg L^{-1}). 30 mL of bacterial culture was inoculated into flasks. The samples were then incubated on a rotary shaker at 180 rpm, 25 °C and pH 7.5. Each experiment was performed twice to ensure data quality. Aqueous samples were periodically collected for measurement of the residual phenol concentration, biomass content, and pH.

Phenol degradation kinetic

Among several available kinetic models, the most used and suitable models namely, the Haldane model (Haldane 1965), the Yano model (Yano *et al.* 1966), and the Edward model (Edwards 1970) were fitted to the experimental data in order to select the best model(s) to represent the degradation kinetics of phenol by acclimated mixed consortia in the present study. The specific growth rate, μ (h^{-1}), for those models are represented in. The S_0 value is the initial substrate concentration (mg L^{-1}), μ_{max} is the maximum specific growth rate (h^{-1}), K_s is the substrate-affinity constant (mg L^{-1}), K_i is the substrate-inhibition constant (mg L^{-1}), and K is the Yano constant.

The microbial flora adapted to the inhibitory substance tends to follow Monod's growth kinetics as μ increases initially with an increasing substrate but at a certain inhibitory threshold concentration it tended to decrease due to the increasing toxic effect of phenol. A larger K_i value indicates that the culture is less sensitive to substrate inhibition (Edwards 1970). The specific growth rate, μ, for each concentration of phenol was calculated from the slope of linear logarithmic plots of biomass growth against time. From the values of μ versus S_0, the values of the kinetic parameters for various models were obtained using nonlinear regression analysis in MATLAB R2009b.

RESULTS AND DISCUSSION

After the adaptation phase the reactor was run in a continuous mode over a period of over 200 days to evaluate the performance of the acclimated biomass in removal of phenol and organic matter in terms of COD. During this period various phenol loading rates of 0.02 to 6.17 kg-Phenol kg-VSS^{-1} d^{-1} were applied to investigate the inhibitory effect of phenol on biomass growth. The reactor was kept at each organic loading rate (OLR) for several days, until a stable condition was reached. For evaluating the performance of the MBR system at high phenol concentration and low HRT, phenol loading was progressively increased to find the breaking point of the efficiency curve. After that, batch assays also were carried out with different phenol concentrations in the medium to determine growth and kinetic parameters.

Phenol and COD removal

At HRTs of 24 and 12 hours, the inlet phenol loading rate varied between 0.02 and 0.9 kg-Phenol kg-$VSS^{-1}d^{-1}$. As shown in Figure 1, the rates of phenol removal are high, showing that the biomass has adapted well to the high concentrations of phenol. In the HRT at 24 h the inlet concentration was increased to 2,400 mg L^{-1} (corresponding to a phenol loading rate of 0.32 kg-Phenol kg-VSS^{-1} d^{-1}) without any inhibitory effects on phenol degradation. Due to the high efficiency of phenol removal at the HRT of 24 h, the HRT was decreased to 12 h on day 75.

At the HRT of 12 h, the inlet phenol concentration varied between 900 and 3,300 mg L^{-1}. This reduction of HRT increased the phenol loading of the system (up to 0.9 kg-Phenol kg-VSS^{-1} d^{-1}), but no effect on phenol removal efficiency was observed and it remained above

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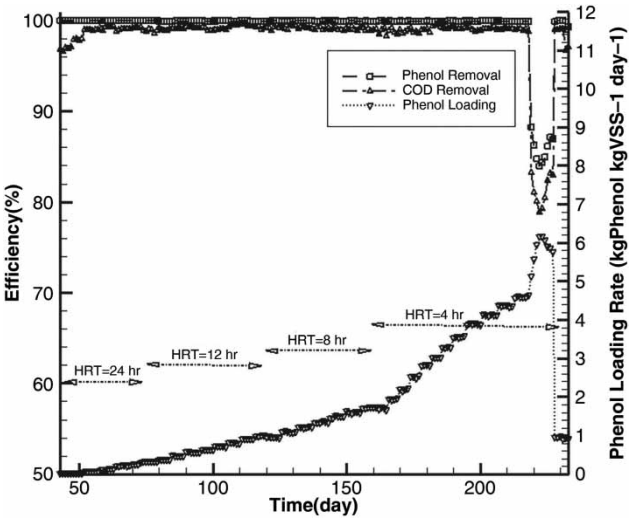

Figure 1 | Phenol and COD removal efficiency in various inlet phenol loading rates.

99.7%. It appeared that the reactor performance was remarkably high in phenol degradation at soaring loads and showed no sign of being subdued.

To investigate the reactor's tolerance, the reactor's HRT was reduced to 8 h, under which conditions the inlet phenol concentration ranged between 2,100 mg L^{-1} and 4,100 mg L^{-1}. This meant that the phenol loading rate was progressively increased from the previous HRT. At this HRT, the maximum phenol loading rate was 1.73 kg-Phenol kg-VSS^{-1} d^{-1}. This value for phenol loading rate is quite high, but as is apparent from Figure 1, the phenol removal efficiency is still above 99.7%. These results proved that the capability of the reactor is remarkably high with an acclimated biomass that could degrade phenol completely at a rate of 1.73 kg-Phenol kg-VSS^{-1} d^{-1}.

No breaking point at the phenol removal efficiency curve was observed so higher loading rates were applied by reducing HRT to 4 h on day 162.

The reactor was operated under the new conditions for several weeks where phenol loading was up to 5.51 kg-Phenol kg-VSS^{-1} d^{-1} (Figure 1). On day 219, when increasing the phenol concentration from 5,500 mg L^{-1} to 5,900 mg L^{-1} the efficiency of phenol removal decreased from 99.8 to 88.2%, meaning that the acclimated biomass was unable to degrade the inlet phenol completely. At the breaking point, the biomass concentration decreased suddenly to 7.8 g L^{-1}, showing the severe inhibitory effects on biomass performance. The inlet concentration of phenol remained at 5,900 mg L^{-1} for another week.

According to Figure 1, due to the inhibitory effect of phenol, initially the decay rate of the biomass was very high and the biomass concentration decreased rapidly (the phenol loading increased in the same concentration due to the decrease in the biomass) and the efficiency was reduced to little more than 84%. However after the first impact, the biomass started to grow under this critical concentration and the removal efficiency increased slightly to 86%.

Therefore it was concluded that the inhibitory concentration of phenol was reached at HRT of 4 h. The performance of the microbial community showed remarkable potential in degradation of phenol, indicating that microorganism consortium were perfectly adapted to phenol. The maximum removal rate of phenol in this study was 4.64 kg-Phenol kg-VSS^{-1} d^{-1} which has not been reported by any publication so far. However it should be emphasised that this performance was only possible in a MBR bioreactor where the biomass concentration can be held at high values; hence the MBR is good choice for phenol acclimation of biomass.

In a previous study, the continuous aerobic degradation of phenol, mixed with readily degradable synthetic wastewater was studied over a period of 400 days at a temperature of 25 ± 5 °C in a fixed bed biofilm reactor by Bajaj *et al.* (2008). The phenol concentration added to the reactor ranged from 190 to 5,170 mg L^{-1} and was achieved by a gradual increase of phenol in the wastewater. A maximal removal rate of 2.92 g Phenol L^{-1} d^{-1} at a HRT of 22.8 h was observed (Bajaj *et al.* 2008). In this condition, the total OLR and the phenol concentration was 15.3 g-COD L^{-1} d^{-1} and 4,900 mg L^{-1}, respectively. While in our study, the maximum removal efficiency was 33 g-phenol L^{-1} d^{-1} at a HRT of 4 h. Adav *et al.* (2007) cultivated aerobic granules that could degrade phenol at a constant rate of 49 mg-phenol g-VSS^{-1} h^{-1} up to 1,000 mg L^{-1} of phenol (Adav *et al.* 2007). In comparison, the maximal phenol degradation rate was achieved in this investigation is 193 mg-phenol g-VSS^{-1} h^{-1} (four times higher than what was reported by Adav *et al.* (2007)). This value for the phenol removal rate is higher than values that were obtained from granular studies.

As it is apparent from Figure 1, the COD removal manner is similar to phenol, for the only carbon source in the bioreactor was phenol. It should be noted that analysis of the solution showed that a solution of 1 g commercial phenol has (on average) a soluble COD of 2.18 g.

Batch profile of phenol degradation

Getting better understanding about the kinetic behaviour of a microorganism or mixed culture growing on high concentration of phenol as sole source of carbon and energy is a prerequisite as each microorganism or culture has its

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unique growth dynamics which can predict degradative capability of that particular microorganism or consortia (Basak *et al.* 2014b).

The results of batch experiments show that the degradation rate for the continuous-phenol-acclimated biomass enhanced as the initial phenol concentration increased to 800 mg L^{-1} phenol (Figure 2). The degradation rate of phenol closely follows a zero-order kinetic behaviour without lag phase, yielding a maximum degradation rate constant of 114 ± 2 mg L^{-1} h^{-1} at phenol concentration of 800 mg L^{-1}. Moreover, phenol was depleted quickly as microbial growth increased, and was completely degraded at about 7 h.

At 930–1,200 mg L^{-1} phenol, a lag phase occurred and the phenol degradation rate decreased, indicating substrate inhibition on the cells. The biomass had a short lag phase of 2 and 4 h at 900 and 1,200 mg L^{-1}, respectively. After lag phase, by increasing the biomass concentration, the degradation rate was increased from about 59± 2 mg L^{-1} h^{-1} (at lag phase) to 127.5 ± 2 mg L^{-1} h^{-1}.

The increase in lag phase depended on the size of the inoculum and on the inhibition constant (Bajaj *et al.* 2009). In comparison to a similar study (Saravanan *et al.* 2008) with an indigenous mixed microbial consortium selected from a sewage treatment plant and previously adapted to phenol up to 800 mg L^{-1}, the lag times ranged from 5 h (at substrate concentration 100 mg L^{-1}) to 35 h (at substrate concentration 800 mg L^{-1}). Also in another (Bajaj *et al.* 2009), the microbial flora was adapted to phenol in a laboratory-scale fixed bed aerobic reactor (Bajaj *et al.* 2008), the lag phase was 3, 4 and 6 h for initial concentration of about 280, 380 and 660 mg L^{-1}, respectively, while in our study maximum lag phase was 4 h at initial concentration of 1,200 mg L^{-1}.

The results of the plot of cell biomass concentration versus time for acclimated mixed consortia (Figure 3) indicate that at lower initial phenol concentrations (150 to 800 mg/L), the mixed consortia that degrades phenol immediately have no lag phase, indeed the consortia has a lag phase of 4 h (Figure 3) at 1,200 mg/l of initial phenol concentration.

At each of the initial phenol concentrations a period of exponential growth period was observed which is further confirmed by the substrate being consumed at faster rate. The same observation was made in our experimental studies during the exponential growth phase of the mixed consortia.

Effect of inoculum concentration

A sufficient quantity of inoculum ensures rapid proliferation and biomass synthesis in cultivation (Sabu *et al.* 2006). Phenol degradation at an initial concentration of 600 mg L^{-1} was achieved at an inoculum concentration of 625(5), 1,000(8), 1,500(12), 1,875(15), and 2,500(20) mg L^{-1} (%v/v) at pH 7.5 and 25 °C.

At low inoculum concentrations of 625 and 1,000 mg L^{-1}, microbial growth had a prolonged lag phase of 4 h and 6 h, respectively. The phenol was completely removed after more than 12 h of incubation. When the inoculum concentration was increased to 1,500 and 1,875 mg L^{-1}, the lag phase was largely eliminated. The phenol added was completely

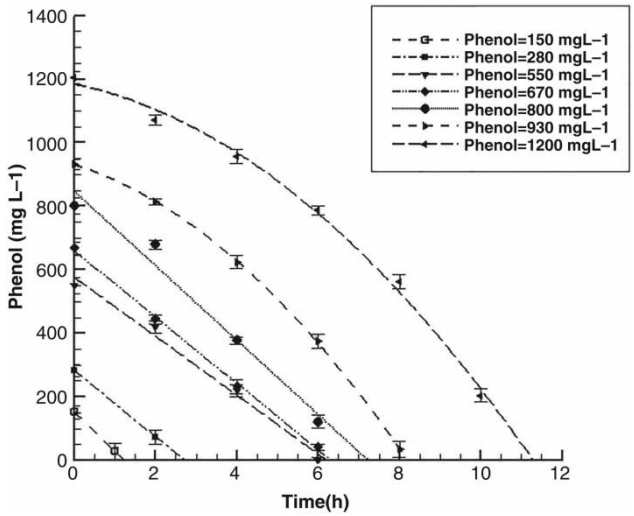

Figure 2 | Effect of initial phenol concentration on phenol biodegradation.

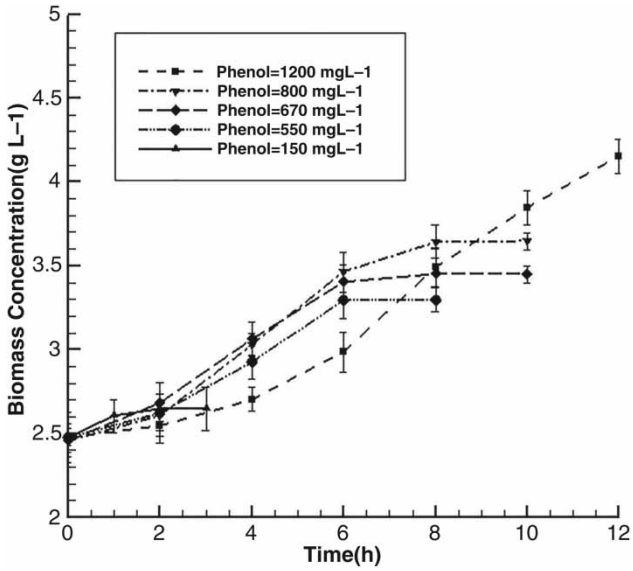

Figure 3 | Cell growth in presence of different initial phenol concentrations.

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removed at 6–8 h. With an inoculum concentration of 2,500 mg L^{-1} roughly 93% of the added phenol was depleted at 4.5 h. A sufficient quantity of inoculum was required to minimise the duration of the lag phase, increase the degradation rate, and induce the exponential growth phase after seeding. These experimental findings are similar to those reported by Artuchelvan (Arutchelvan *et al.* 2006) using Bacillus brevis to degrade high-strength phenol and Ho (Ho *et al.* 2009) using *Corynebacterium* sp. DJ1 aerobic granules.

Kinetic analysis of phenol degradation

The specific growth rates (μ) were calculated for various S_0 values used in the biodegradation of phenol by mixed consortia and are shown in Figure 4.

The relation between the specific growth rate and the initial substrate concentration is described by a set of empirically derived rate equations referred to as theoretical models. Three different models, namely those of Haldane, Yano, and Edward were used in the present study to relate the specific growth rate with the initial phenol concentration. For better comparison between these three models which are suitable for inhibitory substrate and models which do not cover the inhibitory effect, the Monod equation was also used.

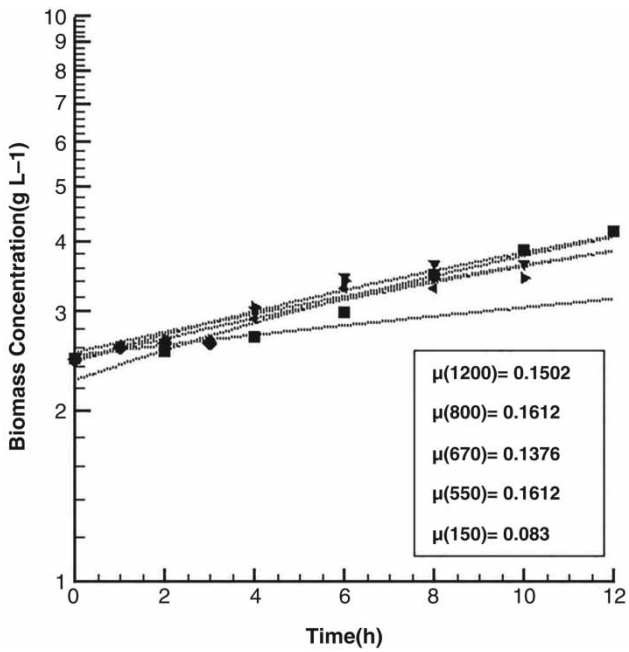

Figure 4 | Time profile of growth with different phenol concentrations: semi-logarithmic presentation.

The experimentally obtained values of the substrate specific growth rate at various S_0 values were used to fit the above models using the nonlinear regression analysis in Matlab 2009b for estimating the kinetic parameters. The values of the biokinetic parameters obtained from the fitting of the above models are summarised in Table 2.

Table 2 shows that the Monod equation has the lowest correlation coefficient (R^2) among the models. It shows the inhibitory effect of phenol and inability of Monod equation in predicting of experimental data. Among three others, the inhibitory constant (K_i), that was achieved by the Yano and Edward equations, is so high and illogical. Thus, the Haldane equation is the best model; and it perfectly describes the phenomena of the combined effect of S as a growth substrate and at higher concentrations as an inhibitor (Arutchelvan *et al.* 2006; Marrot *et al.* 2006).

The curve obtained for the Haldane model had a high correlation coefficient (R^2) of 0.974. It indicated that the maximal growth rate of the mixed phenol-degrading enrichment was obtained at 850 mg L^{-1} phenol. Below this concentration growth seemed to be suboptimal due to substrate limitation and above this concentration growth was declined increasingly due to substrate inhibition. At high phenol concentrations some cells were damaged, leading to a decreased metabolic activity. In addition, death of a part of the cells seemed to contribute further to apparent inhibition of growth at high concentrations. Thus 'negative' values for the growth rate have to be assumed at very high substrate concentrations (Straube *et al.* 1990).

The value of K_i for the phenol-degrading mixed culture in the present study is the highest among the reported studies for mixed cultures (Table 3) and the second highest value if compared with *K*i values for pure cultures. The only higher value of *K*i was reported by Arutchelvan *et al.* (2006) for a culture of *Bacillus brevis*. The *Ki* of up to 2,434.7 mg L^{-1} was determined by them, which was unusually high for a pure culture. No other report indicating such high *K*i values with pure cultures seems to exist.

The μ_{max} of our mixed consortia was comparable to that of other mixed cultures (Table 3). The value for K_s (416 mg L^{-1}) was also the highest among the all reports, indicating that the microorganisms were able to grow at higher phenol concentrations (Table 3). The variation of kinetic parameters may be explained by differences in inoculum selection and its cultivation (Bajaj *et al.* 2009). For example, Bajaj *et al.* (2009) used inocula were taken from the phenol treating continuous fixed bed reactor and then maintained as 10% culture. Saravanan *et al.* (2008) for instance, took inocula directly from a wastewater treatment plant and exposed these to phenol for 1 month before conducting the study. In the present study

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Table 2 | Operational kinetic parameters of phenol degradation obtained by various models

Model	Equation	μ_{max} (h^{-1})	K_s (mg L^{-1})	K_i (mg L^{-1})	K (mg L^{-1})	R^2	RSME
Haldane	$\mu = \frac{\mu_{max} S_0}{K_s + S_0 + \frac{S_0^2}{K_i}}$	0.3085	416	1,886	–	0.974	0.011
Yano	$\mu = \frac{\mu_{max} S_0}{K_s + S_0 + \frac{S_0^2}{K_i}\left[1 + \frac{S_0}{K}\right]}$	0.286	398	9,600	333	0.972	0.0126
Edward	$\mu = \mu_{max}\left[\exp\left(\frac{-S_0}{K_i}\right) - \exp\left(\frac{-S_0}{K_s}\right)\right]$	0.1895	258	6,395	–	0.973	0.0111
Monod	$\mu = \frac{\mu_{max} S_0}{K_s + S_0}$	0.1905	179	–	–	0.963	0.0119

Table 3 | Comparison of phenol degradation kinetics predicted by the Haldane model in batch experiments with pure and mixed culture

Culture	Maximum phenol concentration (mg L^{-1})	μ_{max} (h^{-1})	K_s (mg L^{-1})	K_i (mg L^{-1})	Reference
Bacillus brevis	750–1,750	0.026–0.078	2.2–29.3	868–2434.7	Arutchelvan *et al.* (2006)
Sulfolobus solfataricus 98/2	745	0.094	77.7	319.4	Christen *et al.* (2012)
Paecilomyces variotii JH6	1,800	0.312	130.4	200	Wang *et al.* (2010)
Corynebacterium sp. DJ1	2,500	0.656	33.1	1,470	Ho *et al.* (2009)
Candida tropicalis PHB5	2,400	0.3407	15.81	169	Basak *et al.* (2014b)
Mixed culture	800	0.308	44.92	525	Saravanan *et al.* (2008)
Mixed culture	2,500	0.438	29.5	72.4	Marrot *et al.* (2006)
Mixed culture	350	0.66	76.1	205.4	Hamitouche *et al.* (2012)
Mixed culture	659	0.3095	74.65	648.13	Bajaj *et al.* (2009)
Mixed culture	1,200	0.3085	416	1,886	This study

inocula were taken from the phenol treating continuous MBR system, while the biomass could degrade phenol at the rate of 35 mg-phenol g-VSS^{-1} h^{-1}, and then was maintained as 20% culture transfer in phenol containing mineral medium in order to select a phenol resistant microbial consortium.

CONCLUSION

The active sludge, taken from the oil refinery, was adapted to phenol over a period of 42 days and then was used in a MBR. The MBR was able to sustain a high concentration of biomass without inhibitory effects from the phenol. Throughout the study clogging of the membrane element was not observed and the reactor operated without any significant problem. Various loading rates, with different concentrations of phenol, were applied and a maximum removal rate of up to 33 g L^{-1} d^{-1}, at influent phenol concentration of 5,500 mg L^{-1} and HRT of 4 h, was achieved. It is therefore concluded that wastewaters with as high as 5,500 mg L^{-1} of phenol can be treated efficiently by a MBR system. Selection of a suitable culture, well adapted to phenol, appeared to be the key to successful operation of the MBR. High COD degradation indicated that most probably phenol intermediates, possibly as products of phenol molecule breakup, are absent and phenol degradation reaches completion in the reactor. At 150–1,200 mg L^{-1} phenol, the kinetic parameters for the acclimated mixed consortia using the Haldane, Yano, and Edward models were calculated. The results show that the Haldane equation is the best model for predicting experimental data. The values for Haldane constants were $\mu_{max} = 0.3085\ h^{-1}$, $K_s = 416$ mg L^{-1}, and $K_i = 1{,}886$ mg L^{-1}. High

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K_i and large μ_{max} values indicate that the acclimated mixed consortia effectively degraded large amounts of phenol.

Finally, it appeared that a MBR is an excellent choice for treatment of difficult industrial wastewaters, such as petrochemical effluents, where hazardous chemicals such as phenols are present; therefore, the MBR has led to a very good biomass retention capacity in reactors working with microbial consortiums characterised by slow growth rates.

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