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Kinetics of styrene biodegradation in synthetic wastewaters using an industrial activated sludge

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ABSTRACT

Kinetics of styrene biodegradation in synthetic wastewaters, containing either styrene or styrene together with ethanol, by an industrial activated sludge obtained from the wastewater treatment unit of a petrochemical complex was studied. The kinetic data could be fitted using the Haldane kinetic model. This model was previously used to predict kinetic data for biodegradation of styrene by pure or mixed microbial cultures isolated from biofilters, but the values of the model parameters reported in these studies was substantially different from that obtained for the industrial activated sludge. The presence of ethanol did not affect the kinetics of styrene biodegradation by the industrial activated sludge; however, it increased the rates of styrene biodegradation due to the resulting higher microbial growth rates. Styrene concentration was found to affect the specific growth rate in a manner similar to its effect on the styrene degradation rate. No lag phase was observed in styrene biodegradation by industrial activated sludge for styrene concentrations up to 100 mg/L. Lag phase was observed for municipal activated sludge even at 50 mg/L styrene concentration but the rate of styrene biodegradation after the lag phase was similar to that achieved by the industrial activated sludge.

1. Introduction

Styrene is a volatile organic compound which is used widely in many petrochemical and polymer-processing industries [1]; therefore, the liquid and gaseous effluents from many units in a petrochemical complex contain styrene. However, styrene and its metabolites are known to have serious negative effects on human health [2,3]; therefore, before discharge to the environment, both the liquid and gaseous effluents of petrochemical complexes should undergo an appropriate treatment to decrease the concentration of styrene to below toxic levels [4].

The gaseous effluents of petrochemical complexes contain VOCs such as styrene and, before discharge, the level of the VOCs in gas phase can be reduced through the use of treatments such as biofiltration. VOCs such as styrene also exist in petrochemical wastewaters at above emission standards. Many different methods have been used for the treatment of petrochemical wastewaters including physical processes such as adsorption with activated carbon, chemical processes such as Advanced Oxidation Processes like ozonation, photocatalytic degradation and the Fenton process [5], and aerobic biological processes such as the conventional activated sludge processes [6], rotating biological contactors [7], immobilized biofilm processes [8], biological activated carbon processes [5], and membrane bioreactors [9]. The use of physical and chemical processes incurs high capital and operating costs and usually results in the production of secondary effluents and does not always reduce the pollutant concentration in the wastewater to acceptable levels.

The most widely used method for the treatment of petrochemical wastewaters at industrial scale is the conventional activated sludge process [10]. This is a cost-effective process and, because of the presence of a mixed bacterial population in the activated sludge, can potentially result in complete mineralization of the organic pollutants to CO_2 and other substances [11].

Microbial growth and pollutant biodegradation kinetics aids in proper prediction and optimization of activated sludge processes at an industrial scale. The most commonly employed kinetic model for biodegradation of substances, which above a certain concentration inhibit microbial growth, is the Haldane kinetic model. This model has been successfully used to fit the data for biodegradation of some aromatic pollutants such as phenol, 4-chlorophenol, benzene, toluene and xylene by pure, as well as mixed, bacterial populations in activated sludge [11–16]. However, the validity of the Haldane kinetic model has been found to depend on the concentration range of the toxic compound employed; for example, Nuhoglu and Yalcin [17] have managed to fit the Haldane model to phenol biodegrada-

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tion data by a mixed bacterial population derived from an activated sludge only for phenol concentrations <100 mg/l. Furthermore, in the case of phenol biodegradation [12] the value of the biokinetic parameters of the Haldane model has also been found to depend on the type of microbial culture employed (e.g. species type and whether pure or mixed bacterial population is employed). The type of toxic compound also strongly influences the value of the biokinetic parameters of the Haldane model [18].

Kinetic modeling studies are usually carried out in batch systems [11–18,25–27]. The models usually relate rate of biodegradation of a toxic compound (per unit of biomass) to its concentration. Such models can be used to estimate both the cycle time of batch biological wastewater treatment processes, such as Sequencing Batch Reactors, as well as the HRT of continuous processes such as activated sludge reactors [19].

There are a large number of microorganisms capable of metabolizing styrene including *Pseudomonas* spp. [20,21], *Corynebacterium* [22], *Mycobacterium* [23], *Rhodococcus rhodochrous* [24] and *Exophiala jeanselmei* [25]. The styrene biodegradation kinetics has been studied for the fungus *E. jeanselmei* [25] and a novel strain of the bacteria *Rhodococcus pyridinovorans* PYJ-1 [26], both isolated from biofilters used for treating synthetic waste gases containing styrene. Both these authors managed to fit the Haldane kinetics to their data. Styrene biodegradation data for a mixed bacterial culture obtained from a biofilter used for treating synthetic waste gases containing low concentrations of styrene has also been successfully modeled using Haldane kinetics [27]. However, to the knowledge of the authors, kinetic models for the biodegradation of styrene by industrial activated sludge have not been reported previously.

It can therefore be concluded that kinetic models, or the value of the biokinetic parameters of the model, obtained based on data with pure or mixed microbial cultures that have been exposed to low styrene concentrations, such as those isolated from biofilter beds, are not necessarily appropriate for describing the kinetics of styrene biodegradation by the mixed bacterial population, such as those existing in the activated sludge of biological treatment units of petrochemical plants. This means that there is a need for studies aimed at elucidating kinetic model for styrene biodegradation in the liquid phase by such activated sludge.

Petrochemical wastewaters contain a number of organic pollutants including normal-alkanes, phenol, toluene, xylene, styrene and indene [10]. Previous studies have shown that the kinetics of biodegradation of an aromatic pollutant can be affected by the presence of other organic chemicals in the media [21,28,29]. However, to the knowledge of the authors, there is no previous study on the kinetics of biodegradation of styrene in presence of other potential petrochemical wastewater pollutants.

The purpose of the present study was the elucidation of the styrene biodegradation kinetics in synthetic wastewaters containing styrene as the sole carbon source, or styrene in the presence of ethanol, using activated sludge cultures obtained from an industrial biological wastewater treatment unit of a petrochemical plant. Ethanol was chosen as an organic chemical which is commonly found in petrochemical wastewaters and has a structure dissimilar to styrene. Furthermore, through the addition of ethanol the COD of the synthetic wastewater was set in a range similar to that reported for petrochemical wastewaters [10]. Finally, the performance of the industrial activated sludge in terms of styrene biodegradation was also compared with that of a municipal activated sludge.

Table 1
Composition of the mineral medium.

Constituent	Concentration (mg/L)
K_2HPO_4	25.61
KH_2PO_4	20.02
NH_4Cl	174.41
$MgSO_4{\cdot}7H_2O$	13
$CaCl_2{\cdot}2H_2O$	7
$NiCl_2$	0.0094
$MnSO_4$	0.014
Na_2MoO_4	0.423
$CoCl_2$	0.094
$FeCl_3$	5
$ZnSO_4$	2
EDTA	7
$NaHCO_3$	500

2. Materials and methods

2.1. Culture and media

Two kinds of activated sludge (industrial and municipal) were used in the present study. The industrial sludge was obtained from the return sludge line of the activated sludge treatment unit of a petrochemical complex situated in Tabriz in north western Iran. After transporting to the laboratory, this sludge was frozen using 15% (w/w) glycerol and then stored at −20 °C. Before inoculating into the serum bottles, the frozen activated sludge was thawed and grown under aeration in the mineral media presented in Table 1 plus ethanol as carbon source, for several days. In some of the runs a municipal activated sludge obtained from a municipal wastewater treatment unit in Tehran was employed. This sludge was also aerated for several days immediately after transfer to the laboratory and inoculated into serum bottles.

The synthetic wastewater employed during the kinetic experiments had mineral composition as presented in Table 1. The mineral media was supplemented with either styrene on its own in the concentration range of 0–200 mg/L, or styrene and ethanol (at a concentration of 245.7 mg/L equivalent to a COD of 512.8 mg/L).

2.2. Analytical techniques

The styrene concentrations in the gas phase of serum bottles were analyzed by using a gas chromatograph (GC) (Young Lin, ACME-6100) equipped with a Helium Ionization Detector (HID) and a capillary column TRACSIL TRB-5 (30 m × 0.53 mm × 3.0 μm). The oven temperature was maintained at 70 °C for 1 min and raised to 140 °C. The temperatures of the injector and the detector were fixed at 200 and 240 °C, respectively. After various test time intervals, 100 μL of the gas sample was extracted from each of the serum bottles by means of a 250 μL gas-tight syringe. A calibration curve was used to determine the styrene concentration.

The concentration of styrene in the liquid phase was estimated using the partition coefficient of styrene over styrene/mineral medium at 32 °C. To evaluate the partition coefficient 0.2, 0.5, 1, 2, 3, and 5 μL of liquid styrene were added to 118 mL serum bottles containing 16 mL mineral medium. The bottles were sealed by Teflon-coated silicone septa and aluminum crimp caps. After reaching vapor–liquid equilibrium, the styrene concentration in the gas phase was determined by gas chromatography, and the partition coefficient was calculated from the total amount of styrene added [26].

Biomass concentration in the serum bottles was determined by the measurement of the OD (Optical Density) of the liquid inside the serum bottles at 550 nm using a spectrophotometer (Jasco V-550). A calibration curve was used to relate the absorbance of the culture to the biomass concentration. The extension coefficient was determined as 0.002. MLSS, MLVSS, and COD analyses were carried out according to standard methods [30]. The pH was measured by a pH-meter (Hach Instrument). All measurements were carried out in duplicate.

2.3. Experiments

Batch experiments were conducted using 16 mL of the mineral medium in 118 mL sterile serum bottles sealed with Teflon-coated silicone septa and aluminum crimp caps in the rotary shaker at 150 rpm. The temperature and pH of the medium were fixed at 32 °C and 7, respectively and all experiments were carried out in duplicate [26]. Sampling of the overlying gas phase and the liquid phase was carried out by insertion of syringes into the Teflon membrane. To determine the possibility of chemical degradation and volatilization of styrene during the kinetic runs, parallel control tests were conducted in serum bottles without the introduction of biomass. Results showed negligible change in styrene concentration under the studied conditions for all initial styrene concentrations.

In the kinetic experiments in which styrene was used as the sole carbon source, in order to accelerate biodegradation rate of styrene the initial biomass concentration was set at an initial value of 650 ± 10 mg/L. This use of a high value for biomass concentration reduced the time necessary for complete styrene biodegradation and eliminated any change in the characteristics of the biomass as a result of long term exposure to styrene. In the experiments in which styrene and ethanol were biodegraded simultaneously, initial biomass concentration was in the range 18–20 mg/L and COD/biomass ratio ranged from 26.1 to 44.5 on COD basis. This range allows determination of the intrinsic growth-associated kinetic parameters, which represents the capabilities of the members of the microbial community in the activated sludge with the fastest growth kinetics [11,31]. In the experiments for comparing the styrene biodegradation rate for two kinds of sludge, initial biomass concentration was set around 20 mg/L [11,32].

Styrene and ethanol were added to the serum bottles by using a 10 μL syringe inserted into the Teflon membrane and the gas phase concentrations were measured during incubation.

3. Results and discussion

3.1. Styrene biodegradation kinetics in a media containing styrene as the sole carbon source

Kinetics tests on seven initial concentrations of styrene in the liquid phase, namely 8.5, 20, 40, 60, 82.4, 106 and 123.4 mg/L, were conducted in order to obtain the specific degradation rate (SDR) by the industrial activated sludge (initial biomass concentration = 650 ± 10 mg/L) and incorporate this parameter into a model. The concentration of gaseous phase was monitored during incubation until complete degradation of styrene was attained.

Fig. 1 shows time-course variation in styrene concentration in the liquid phase during the batch incubations. As can be seen from Fig. 1, complete removal of styrene was achieved at all concentrations studied. The microbial community present in the industrial activated sludge was able to degrade 8.5–123.4 mg/L of styrene in 0.325–9.58 h at pH 7 and 32 °C.

Gas chromatogram at different time intervals during the batch incubations at all initial styrene concentrations tested showed diminution in the area of the peak relating to styrene, without the appearance of any peak at any other retention time. This suggests complete mineralization of styrene during the experiments. Complete mineralization of styrene during microbial biodegradation has also been claimed in previous reports [27]. Kuhlmeier [33] has reported that styrene biodegradation under aerobic environment results in harmless end products.

In Fig. 2, specific degradation rates (SDRs) at various initial styrene concentrations are shown. As can be seen from Fig. 2, the SDR curve can be divided into two parts: at low styrene concentrations SDR increases with increase in the concentration of styrene,

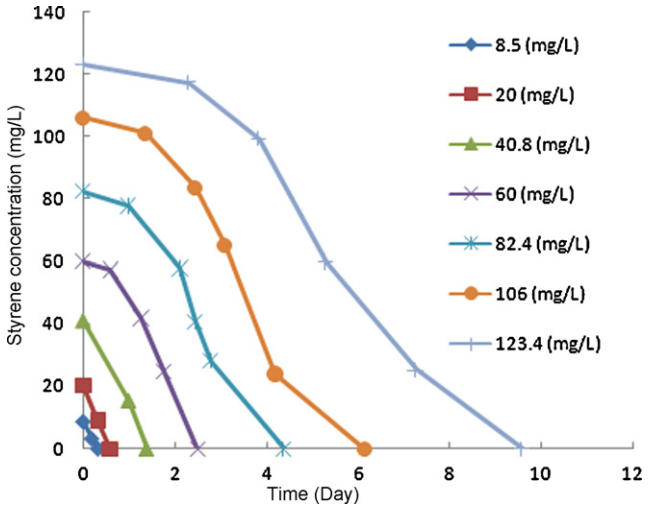

Fig. 1. Concentrations of styrene in the liquid phase as a function of time for different initial styrene concentrations.

whereas when styrene concentration exceeds a threshold value, SDR decreases with increase in liquid phase styrene concentration. This implies that above a certain concentration styrene acts as an inhibitor of bacterial activity. The fact that styrene, above a certain concentration, can be toxic to yeasts and bacteria has been previously reported [34,35].

Regarding the inhibitory effect of styrene on its own transformation the kinetic model of Haldane was used. The Haldane model is represented by Eq. (1) [19]:

$$\mathrm{SDR} = \frac{q_m S}{K_S + S + (S^2/K_i)} \quad (1)$$

where S is the concentration of substrate mg/L, q_m is the maximum specific degradation rate for Haldane model (mg/(g MLVSS h)), K_S is the half velocity constant mg/L and K_i is the substrate inhibition constant mg/L. The effect of a toxic compound on its biodegradation is quantified by the term K_i. It should be noted that when K_i is very large the Haldane equation simplifies to the Monod equation [19]. So, low values of K_i show that the inhibition effect can be observed at low substrate concentrations.

Application of the experimental data to Haldane equation gave excellent fit to the experimental data as R^2 was observed to be 0.989. The least-square error method with the help of Matlab 7.6 was used to obtain kinetic parameters. The parameters of Haldane

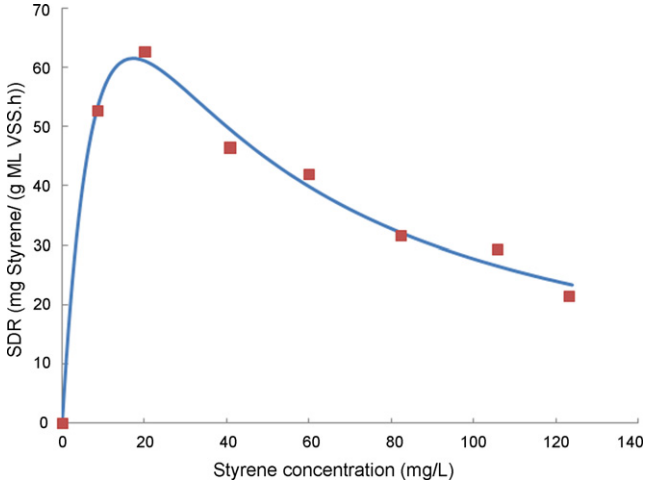

Fig. 2. Specific degradation rates as a function of initial styrene concentration.

Table 2
Kinetic constants for biodegradation of styrene in different studies.

Strain	Conc. range studied (mg/L)	q_m (mg/(g MLVSS h))	K_S (mg/L)	K_i (mg/L)	$S_{threshold}$ (mg/L)	T (°C)	pH	References
Industrial activated sludge	8.5–123.4	160.13	13.8	21.57	17.25	32	7	This study
Biofilter mixed culture	4.5–36	23.22[a]	3.49	26.55	9.62	25	–	[27]
Exophiala jeanselmei	0.78–104.15	630[b]	0.78	21.8	4.12	25	5.7	[25]
Rhodococcus pyiridinovorans PYJ-1	0.006[c]–0.5[c]	210[b]	0.154[c]	0.186[c]	0.17[c]	32	7	[26]

[a] This value was calculated from the data presented in the paper using the following equation: $q_m = \mu_m/Y_{X/S}$.
[b] Protein amount was assumed to be one half of the biomass.
[c] These values were converted from ppm to (mg/L) and represent the gas concentration.

equation for activated sludge were obtained as follows:

$q_m = 160.13\ \text{mg/g MLVSS h}$

$K_S = 13.8\ \text{mg/L}$

$K_i = 21.57\ \text{mg/L}$

Therefore, the Haldane kinetic equation for the biodegradation of styrene by the industrial activated sludge can be represented as follows (Eq. (2)):

$$\frac{dS}{dt} = -\text{SDR} \cdot X = -\frac{q_m SX}{K_S + S + (S^2/K_i)} = -\frac{16.13\,SX}{13.8 + S + (S^2/21.57)} \quad (2)$$

In Eq. (2) X represents the biomass concentration (g MLVSS/L).

The threshold styrene concentration, at which the maximum value of SDR is achieved, can be calculated by taking the derivative of Eq. (1) with respect to S and setting the result equal to zero (Eq. (3)):

$$S_{\text{threshold}} = \sqrt{K_S K_i} \quad (3)$$

In Table 2 the values of Haldane kinetic parameters of styrene biodegradation by activated sludge were compared with those previously reported for pure and mixed cultures isolated from biofilters used for treating styrene-containing waste air streams. The K_S values obtained in previous studies is lower than that obtained in the present study for the industrial activated sludge; this is to be expected since the environment of a biofilter used for treating styrene-containing waste gas streams favours microbial species which have a relatively higher growth rate at lower styrene concentrations (i.e., those with low K_S). However, the comparison of the $S_{\text{threshold}}$ values shows that the mixed bacterial population present in the industrial activated sludge has a higher tolerance of styrene compared to both pure and mixed microbial cultures isolated from biofilters.

The q_m of styrene by the industrial activated sludge was lower than those reported for the pure yeast and bacterial cultures; this is to be expected since a unit mass of activated sludge includes both microbial species that are capable and those that are incapable or poorly capable of metabolizing styrene. However, q_m obtained for the industrial activated sludge is much higher than that reported for the mixed microbial population present in a biofilter, which shows that microbial species with a higher styrene degrading capability are selected in the environment of an activated sludge plant used for treating petrochemical wastewaters.

3.2. Modeling of styrene biodegradation in media containing both styrene and ethanol as carbon sources

Time-course variations in biomass concentration, presented as OD, and styrene concentration were followed at various initial concentrations of styrene in the presence of ethanol (at a concentration of 512.8 mg/L on COD basis). An example of such a trend, for initial styrene concentration of 69.25 mg/L, is presented in Fig. 3(A) and (B) respectively. It can be seen that styrene is fully consumed by around 25 h (Fig. 3(B)) whereas the growth of sludge proceeds up to 50 h (Fig. 3(A)). This trend was also observed in the data of the runs at other initial styrene concentrations employed in the present study. This suggests that in the latter phase of the experiments the mixed bacterial population in the industrial activated sludge were only using ethanol as carbon source. Since ethanol was not monitored during the experiments no definite comments can be made as to whether ethanol was used as carbon source together with styrene during the initial phase of the experiments or not. However, theoretically, styrene would have been consumed independently of ethanol only if it either inhibited or repressed enzymes involved in the catabolism of ethanol. But if this was the case the growth curve should resemble a diauxie growth curve, characterized by a lag phase after the exhaustion of styrene, in which the enzymes

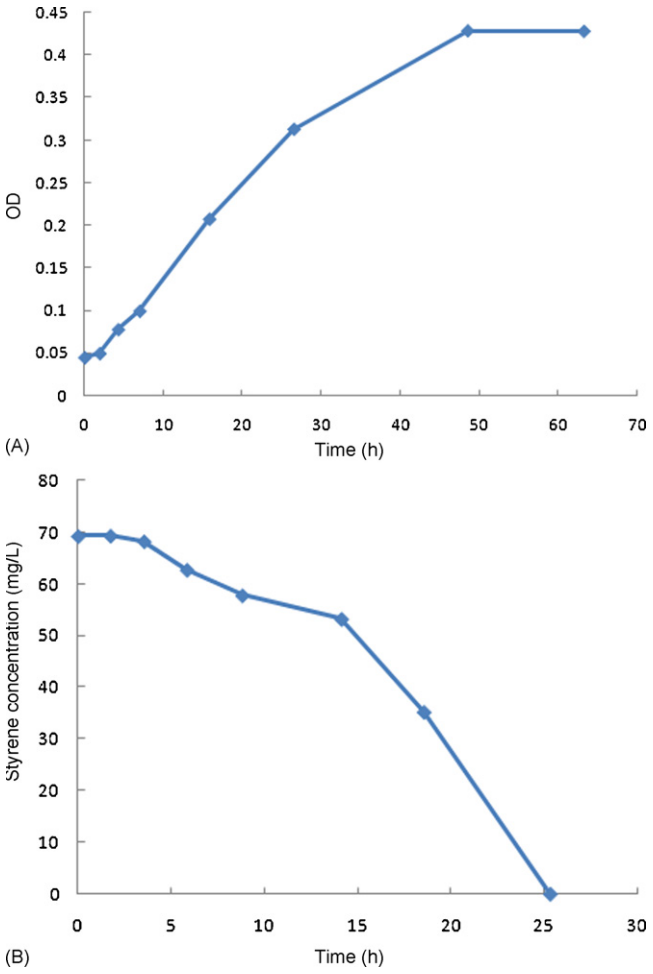

Fig. 3. Time-course variation in OD (A) and styrene concentration (B) at 69.25 mg/L initial styrene concentration.

Table 3
Biokinetic coefficients at various concentrations of styrene.

Initial styrene concentration (mg/L)	$Y_{X/S}$[a] (mg MLVSS/mg COD)	μ[b] (h^{-1})
0	0.33 ± 0.002	0.107 ± 0.004
14.94	0.304 ± 0.012	0.124 ± 0.0031
31.32	0.279 ± 0.018	0.115 ± 0.064
54.81	0.251 ± 0.021	0.106 ± 0.010
69.25	0.236 ± 0.023	0.102 ± 0.007
90.1	0.221 ± 0.034	0.093 ± 0.21

[a] $Y_{X/S} = (x_{max} - x_0)/(COD_0 - COD_{min})$ [11].
[b] $\mu = (1/t)\ln(x/x_0)$ [19].

involved in ethanol catabolism would be reactivated or expressed [19]. However, no such lag phase is observed in either Fig. 3(B) (data of run at 69.25 mg/L initial styrene concentration) or Fig. 5 (data of run at 90.1 mg/L initial styrene concentration) suggesting that most probably styrene and ethanol were consumed together. Same trend was observed in runs at all other initial styrene concentrations. The fact that the growth data over the entire range of experiments could be reasonably fitted with a logistic growth model (see below) is a further confirmation of the simultaneous uptake of ethanol and styrene by the mixed bacterial population in the industrial activated sludge.

For each initial concentrations of styrene, specific growth rate (μ) was calculated using the exponential growth phase curve predicted by the logistic equation using the OD data [19]. The μ values, together with calculated yield coefficients of the biomass with respect to the COD ($Y_{X/S}$), are presented in Table 3. The results show that $Y_{X/S}$ decreases with increase in styrene concentration (in the range 0–90.1 mg/L). This could be due to the lower yield coefficient for styrene compared to ethanol. Another explanation is that increase in the concentration of styrene results in increased utilization of the substrates for maintenance, instead of growth, which in turn results in the reduction in the yield coefficient for ethanol. Jorio et al. [27], based on a combination of theoretical considerations and experimental results, have calculated a yield coefficient for styrene ($Y_{X/styrene}$) equal to 0.696 g biomass/g styrene for a mixed bacterial culture isolated from a biofilter. In this study, due to the low growth of the activated sludge in the presence of styrene alone, $Y_{X/styrene}$ values for growth on styrene could not be determined. However, the $Y_{X/S}$ values presented in Table 3 suggests that $Y_{X/styrene}$ values for the industrial activated sludge are much lower than the value reported by Jorio et al. [27]; this could be due to the difference between the species of microbial degraders that are selected in the environment of a biofilter compared to that selected in an activated sludge plant used for the treatment of petrochemical wastewaters. It should be pointed out that a high value for $Y_{X/S}$ in activated sludge treatment plants results in increased excess sludge production; if in industrial treatment plants sludge is to be disposed of low $Y_{X/S}$ is desirable whereas if it is anaerobically converted to biogas high $Y_{X/S}$ is preferable.

Results presented in Table 3 also show that the trend of change in μ with increase in styrene concentration (in the range 0–90.1 mg/L) is similar to the trend of change in SDR with styrene concentration and that the threshold styrene concentration for inhibition of growth is somewhere in the range 14.94–31.32 mg/L which includes the corresponding value for the inhibition of styrene degradation ($S_{threshold}$).

In order to study the effect of the presence of ethanol on the biodegradation kinetic of styrene by the mixed bacterial population present in activated sludge, the validity of Eq. (2) was checked by applying the observed model to the results of all the experiments in which ethanol and styrene were biodegraded simultaneously. Variations in biomass concentration were modeled using a logistic

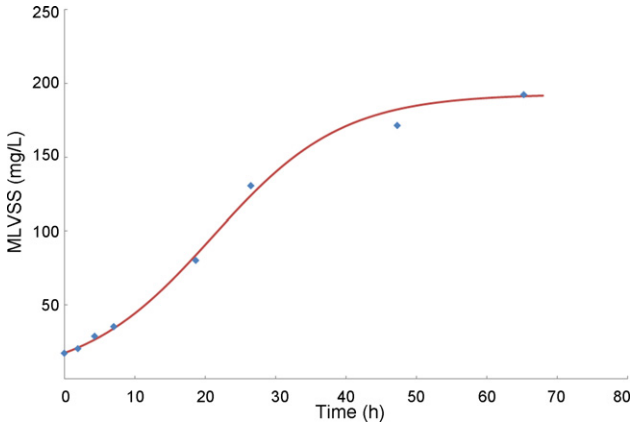

Fig. 4. Biomass growth in the presence of ethanol at 90.1 mg/L initial styrene concentration.

growth equation (Eq. (4)) [19]:

$$X = \frac{X_0 \exp(kt)}{1 - (X_0/X_\infty)(1 - \exp(kt))} \quad (4)$$

where k, X_0 and X_∞ are growth-associated constant (h^{-1}), initial biomass concentration (mg MLVSS/L) and carrying capacity of the system (mg MLVSS/L), respectively. The values of X_∞ were determined from the experimental growth curve data, whereas the k values were determined by fitting the experimental results to Eq. (4). For example, for an initial styrene concentration of 90.1 mg/L, X_∞ and k values were found to be 192.6 mg/L and 0.1088 (R^2 = 0.994), respectively. In Fig. 4 experimental data and logistic equation for biomass growth are shown at an initial styrene concentration of 90.1 mg/L.

Combination of Haldane model and logistic growth equation gives Eq. (5):

$$\frac{dS}{dt} = -\frac{q_m S}{K_S + S + (S^2/K_i)} \times \frac{X_0 \exp(kt)}{1 - (X_0/X_\infty)(1 - \exp(kt))} \quad (5)$$

The solution of Eq. (5) using the obtained kinetic parameters gives the time-course variations in styrene concentration. Dependence of the time on styrene concentration is represented by Eq. (6) and the amount of $f(S)$ is calculated using Eq. (7):

$$t = \frac{1}{k}\left(1 - \frac{X_\infty}{X_0}(1 - \exp(f(S))\right) \quad (6)$$

$$f(S) = \frac{k}{X_\infty q_m} \times \left(K_S \ln\left(\frac{S_0}{S}\right) + (S_0 - S) + \frac{1}{2K_i}(S_0^2 - S^2)\right) \quad (7)$$

In Fig. 5, time-course variations in styrene concentration predicted by the model are compared with experimental data and show a reasonable fit between the model and experimental results.

Therefore, it can be concluded that the presence of ethanol does not increase the specific biodegradation rate of styrene and the higher biodegradation rates obtained in presence of ethanol can be solely accounted for by the resulting increase in the microbial growth rates. Such an observation was also reported by Sahinkaya and Dilek [11] for the effect of presence of proteose-peptone on biodegradation of 4-chlorophenol by activated sludge.

3.3. Comparison of the styrene biodegradation performance of industrial and municipal activated sludge

For assessing the effect of previous growth conditions of activated sludge on its biodegradation capacities, the performance of two kinds of sludge, in terms of styrene biodegradation, were compared. The initial styrene concentrations used in these experiments were in the range 50–200 mg/L. In Fig. 6, results for industrial

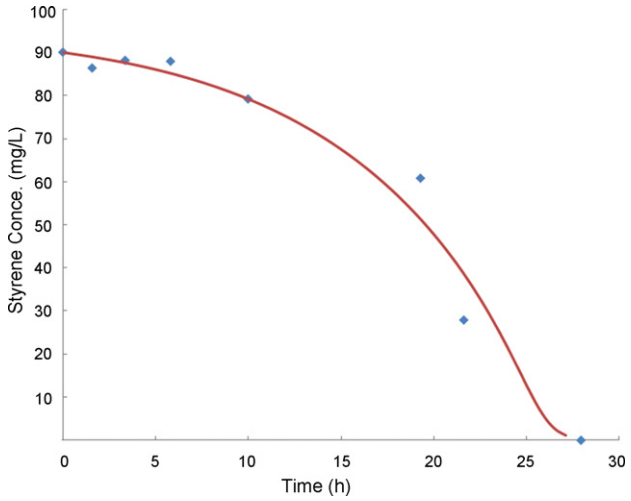

Fig. 5. Styrene biodegradation in the presence of ethanol at 90.1 mg/L initial styrene concentration.

sludge were compared with the corresponding trends for municipal sludge for initial styrene concentrations of 50–200 mg/L. The low degradation rates in this set of experiments are due to the low biomass concentrations employed. At initial concentrations of 50 and 100 mg/L styrene was completely degraded by the industrial activated sludge, although, due to toxic effect of styrene, the time needed for this significantly increased when initial styrene concentration was raised to 100 mg/L. In the case of municipal activated sludge, at 50 mg/L styrene concentration, an initial lag phase was observed after which styrene biodegraded at roughly the same rate as that achieved by the industrial activated sludge. This suggests that microbial species capable of metabolizing styrene are present in municipal activated sludge but have to be enriched and/or adapted to toxic effects of styrene. There has been indirect evidence for the induction of a stress response mechanism in the mixed bacterial population present in industrial activated sludge as a result of increase in styrene organic loading rates in a membrane bioreactor [36]. The induction of a stress response mechanism, called the glutathione-gated potassium efflux (GGKE) system, in bacterial populations existing in activated sludge in response to shock loading of thiol reactive toxic chemicals has been previously demonstrated [37]. The absence of a substantial lag phase at both 50 and 100 mg/L initial styrene concentrations for the experiments with industrial activated sludge indicates that previous exposure of the mixed bacterial community in this sludge to environment containing styrene and other aromatic compounds has to some extent adapted it to the inhibitory effect of styrene.

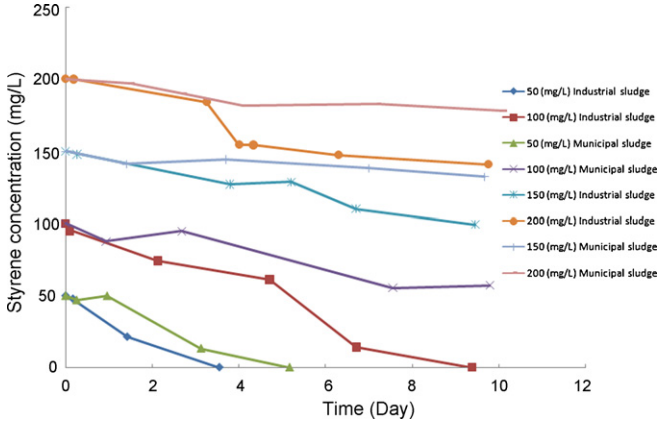

Fig. 6. Time-course variation of styrene concentration during batch experiments employing industrial sludge and municipal sludge (for initial styrene concentration of 50–200 mg/L).

Previous studies have also shown that the ability of the mixed bacterial community in activated sludge to biodegrade inhibitory substrates can be enhanced by exposure of this community to an environment containing this substrate. For example, Sahinkaya and Dilek [11] reported that the acclimation of activated sludge to high concentrations of 4-chlorophenol improved the biodegradation capacities compared to unacclimated sludge. Previous studies have also indicated difference in biodegradation capability of industrial activated sludge obtained from wastewater treatment units of two different plants [32].

At higher styrene concentrations in the range 150–200 mg/L, a substantial lag phase appeared in the results of experiments with industrial activated sludge, suggesting that at high styrene concentrations previous exposure to a styrene-containing environment is not enough to enable the microbial community in activated sludge to counteract the toxic effects of styrene. However, it is possible that if the bacterial community in the industrial activated sludge had been exposed to higher styrene concentration or loading rates they would have exhibited a better styrene degrading performance; but, the validation of this statement requires further study. In the case of municipal activated sludge, for initial styrene concentrations in the range 150–200 mg/L, the lag phase extended to at least 10 days, as insignificant styrene removal was obtained even after 10 days of incubation.

4. Conclusions

Haldane model successfully predicted kinetic data obtained from batch experiments with synthetic wastewaters containing styrene (in the concentration range 8.5–123.4 mg/L) or styrene and ethanol with an industrial activated sludge obtained from the wastewater treatment unit of a petrochemical complex. The presence of ethanol in the media did not affect the inherent kinetic parameters of the Haldane model but only increased the rate of biodegradation through increasing the biomass concentration. Increase in styrene concentration (in the range 0–90.1 mg/L) in media containing ethanol resulted in decrease in the overall $Y_{X/S}$, whereas the trend of change of μ with styrene concentration was very similar to that of SDR vs styrene concentration. Industrial activated sludge showed no lag phase in styrene biodegradation for styrene concentrations up to 100 mg/L which was attributed to previous exposure of the bacterial community in industrial activated sludge to a styrene-containing environment; however, at higher styrene concentrations, a very long lag phase was observed. In case of municipal activated sludge lag phase was observed even at styrene concentrations of 50 mg/L, although complete biodegradation of styrene was achieved in around 5 days.

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