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Biokinetic coefficients determination of a MBR for Styrene and Ethylbenzene as substrate based on the Andrews model

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ABSTRACT

In this study, a lab-scale membrane bioreactor (MBR) was operated for a period of more than 10 months to determine the biokinetic coefficients of the system under the hydraulic retention times (HRT) of 20, 15 and 10 hrs and sludge retention times (SRT) of 5-20 days. The results revealed that the biological removal efficiency of styrene and ethylbenzene at a solid retention time of 20 day and a hydraulic retention time of 15 hr was higher compared to a SRT of 10 day and at the same HRT. The results also showed that the yield (Y), the endogenous decay coefficient (k_d), the maximum specific growth rate (μ_{max}), and the saturation constant (K_s) for styrene and ethylbenzene as substrate were 0.60 and 0.60 mg/mg, 0.25 and 0.25 day⁻¹, 0.188 and 0.363 h⁻¹, and 0.146 and 2.82 mg /l, respectively. Furthermore, ethylbenzene was more appropriate as a source of carbon to activated sludge in the membrane bioreactor than the styrene which had a lower μ_{max} than ethylbenzene.

1. Introduction

Many different chemical industries in Iran produce wastewater that contains volatile organic compounds (VOCs). These materials are man-made and/or naturally occurring highly reactive hydrocarbons [1]. These contaminants not only have destructive effects on the environment but also raise health concerns for workers. Wastewater treatment processes have been established to respond appropriately and relieve various anxieties concerning public health. These methods include physical techniques such as activated carbon adsorption [2], chemical procedures such as ozonation [3], and biological methods such as conventional activated sludge process [4], rotation biological contactor (RBC) processes [5], and stabilized biofilm [6]. Among the biological methods, conventional activated sludge has been employed in many industries as well as in the petrochemical industry. It was reported that biodegradation, stripping, and absorption are three main mechanisms that facilitate volatile organic

compound removal in the conventional activated sludge systems [1]. Due to low mixed liquor suspended solid (MLSS) concentration in conventional activated sludge plants (CASPs), they are one of the most significant VOC emission sources. A membrane bioreactor involves an activated sludge process in which the sedimentation unit is replaced with a membrane. The MBR process can achieve a higher MLSS concentration compared to CASP; therefore, these systems reduce VOC emissions. The main disadvantage of a MBR is the reduction of output flux due to the clogging of the membrane. Previous works show that membrane fouling is related to operating parameters such as hydraulic retention time, sludge retention time, and sludge specifications [6,7,8] HRT and SRT are the main parameters of biological processes such as CASP and MBR for wastewater treatment [9,10]. Further, defining the degradation kinetics of VOCs by bacterial populations is one of the principle steps to forecast and optimize the activated sludge processes on an industrial scale. Mathematical models have been developed to evaluate the



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biodegradation rates of organic contaminants that include substrate utilization, bacterial growth and decay, and the utilization of electron acceptors. Hence, the employment of an appropriate kinetic model is necessary. For instance, the models derived from Monod simulation are employed for population growth studies in the case of the microbial growth kinetics [9]. Generally, the Monod kinetic model is used in reports with a pure culture, limited substrate, and non-inhibitory biomass growth [10,11]. However, modifications of the Monod model, which includes an inhibition term, are used when substances are above a certain concentration. In addition, these models have been used to investigate the effects of substrate inhibition on biomass growth at high substrate concentrations [12, 13,14]. Up to now, there have been only a few investigations concerning styrene and ethylbenzene removal via MBRs. The literature survey showed that there is a lack of information related to the biokinetic coefficients of membrane bioreactors. Furthermore, no attempt has been made to determine the biokinetic coefficients of styrene and ethylbenzene in the MBR. The main goal of this study was to determine the biokinetic coefficients of membrane bioreactors for styrene, ethylbenzene and optimum HRT, which would enable process engineers to determine the minimum reactor volume and recognize the process control through reactor simulation. In order to achieve this aim, a lab scale membrane bioreactor was operated at various HRTs and SRTs.

1.1. Biokinetic coefficients determination

Basic equations that describe the interaction between the growth of microorganisms and the utilization of the growth limiting substrate in activated sludge processes are based on the Monod model; Monod was the first to initially suggest the idea of microbial growth kinetics controlled by an empirical model (Eq. 1).

$$\mu_{i} = \frac{\mu_{\max_{i}} S_{i}}{K_{s_{i}} + S_{i}}$$
(1)

where the specific growth rate of biomass is μ_i (h⁻¹), μ_{max_i} is the maximum specific growth rate of biomass (h^{-1}), S_i is the substrate concentration (mg/L), and K_{s_i} is the substrate half-saturation constant (i.e. substrate concentration at half μ_{max_i}). The Monod model presented the concept of a growth limiting substrate through the parameters μ_{max} and Ks [14]. However, this model becomes unsatisfactory when a substrate prevents its own biodegradation. Therefore, a modified version of the Monod model, which is named the Andrews model, was employed to deliver an improved fitting for the data achieved from the sole substrate tests. In this case, the Andrews model, shown as Eq. (2), was used for substrate inhibition [15]. This model was justified and employed in this study based on the satisfactory/reliable results from previous reports [15,16], the toxic nature of VOCs (in this

case styrene and ethylbenzene), and the possibility of substrate inhibition.

$$\mu_{i} = \frac{\mu_{\max_{i}} S_{i}}{K_{s_{i}} + S_{i} + \frac{S_{i}^{2}}{K_{I}}}$$
(2)

Again, in Eq. (2), μ_{max_i} is the maximum specific growth rate (h⁻¹), K_{s_i} is the half velocity constant and K_I is the substrate inhibition constant, which quantified the influence of a toxic compound on its biodegradation. The three kinetic parameters, μ_{max_i} , K_{s_i} and K_I could be estimated by fitting Eq. (2) to experimentally obtained specific growth rates as a function of substrate concentration. It is important to note that the Andrews model is nonlinear. The existence of mixtures of chemicals in industrial and municipal wastewaters is a significant issue in biodegradation or bioremediation developments. The complexity of the degradation models sharply increased based on the growing number of substrates caused by the interaction between them. Furthermore, the kinetic parameters for a single substrate were not able to illustrate the phenomena observed during the mixture biodegradation. Uncompetitive inhibition, non-competitive inhibition and competitive inhibition are some interactions that can occur when multiple substrates are present [17]. Hence, several models have been established in order to define the specific growth rate through the degradation of multiple interacting substrates. One of the most common types of these models is obtained through the summation of specific growth rates. For instance, during competitive inhibition, substrates compete for binding sites to be metabolized by the mix culture; in this environment, a sum kinetics model incorporating purely competitive substrate kinetics is useful and is shown in Eq. 3 [18,22].

$$\mu_{tot} = \mu_1 + \mu_2 = \frac{\mu_{max_1} S_1}{K_{s_1} + S_1 + (\frac{K_{s_1}}{K_{s_2}})S_2} + \frac{\mu_{max_2} S_2}{K_{s_2} + S_2 + (\frac{K_{s_2}}{K_{s_1}})S_1}$$
(3)

Nevertheless, there is a model that accounts for substrate interactions without directly specifying the type of interaction [21]. This model is formulated by incorporating an interaction parameter $I_{i,j}$ as an unknown and is shown in Eq. 4.

$$\mu_{\text{tot}} = \mu_1 + \mu_2 = \frac{\mu_{\text{max}_1} S_1}{K_{s_1} + S_1 + I_{2.1} S_2} + \frac{\mu_{\text{max}_2} S_2}{K_{s_2} + S_2 + I_{1.2} S_1}$$
(4)

This model is known as sum kinetics with interaction parameters or SKIP. $I_{i,j}$ Specifies the degree to which substrate i affects the biodegradation of substrate j. According to the model, the stronger inhibition has a direct relationship with the large value of j [19]. The value of the interaction parameter is calculated by fitting the SKIP model

to a binary of mixture data sets. Therefore, the specific growth rate of the biomass from the utilization of substrate i can be expressed by:

$$\mu_{i} = \frac{\mu_{max_{i}}S_{i}}{K_{s_{i}} + S_{i} + I_{j,i}S_{j} + I_{k,i}S_{k} + \cdots}$$
(5)

This work employed three substrates as the carbon source: 1) styrene 2) ethylbenzene and 3) ethanol. The biological removal of ethylbenzene and styrene are important due to their low biodegradability compared to ethanol; the value of the interaction parameters for styrene and ethylbenzene are presented in Table 1.

Table 1. The value of interaction parameters for styrene and ethylbenzene

Interaction parameter	I _{1,2}	I _{1,3}	I _{2,1}	I _{2,3}
Value	0.4	0.08	1.64	0.12

Not: Styrene: 1, Ethylbenzene: 2 and Ethanol: 3

The equations describing the performance of the reactor are the mass balance equations of both the biomass and substrate. The biomass balance can be expressed by:

$$V \frac{dX_i}{dt} = \mu_i X_i V - k_{di} X_i V - Q_w X_i$$
(6)

where V= reactor volume (m³); X_i = biomass concentration that is produced from the utilization of substrate i in the reactor (mg/l); k_{d_i} = biomass decay coefficient for fraction i (1/d); and Qw =wastage flow rate (m³/d); and t = time (d). At steady-state conditions, $\frac{dX_i}{dt}$ = 0; hence, Eq. 6 becomes:

$$\mu_{i} - k_{d_{i}} = \frac{Q_{w}}{V}$$
⁽⁷⁾

Since the SRT is defined as:

$$SRT = \theta_c = \frac{VX}{Q_w X} = \frac{V}{Q_w}$$
(8)

Therefore, Eq. 7 becomes:

$$\mu_{i} = k_{d_{i}} + \frac{1}{\theta_{c}} \tag{9}$$

Substituting the value of μ_i from Eq. 9 into Eq. 5 yields the following equation that describes the steady-state condition of the substrate concentration in the reactor:

$$S_{i} = \frac{\left(k_{d_{i}} + \frac{1}{\theta_{c}}\right)\left(K_{s_{i}} + I_{j,i}S_{j} + I_{k,i}S_{k} + \cdots\right)}{\mu_{\max_{i}} - \left(k_{d_{i}} + \frac{1}{\theta_{c}}\right)}$$
(10)

On the other hand, the substrate balance can be expressed as:

$$V\frac{dS_{i}}{dt} = QS_{0i} - \mu_{i}\frac{XV}{Y_{i}} - S_{i}(Q - Q_{w}) - Q_{w}S_{i}$$
(11)

where Y_i is the maximum cell yield for substrate i; S_{0i} = initial concentration of substrate i (mg/L); and S_i is concentration of substrate i in the reactor. At the steady state, $\frac{dS_i}{dt} = 0$, therefore :

$$\frac{Q}{V} = (S_{0i} - S_i) = \frac{\mu_i X}{Y_i}$$
(12)

Substituting Eq. 9 into Eq. 12 results in:

$$\frac{Q(S_{0_i} - S_i)}{VX} = \frac{1}{Y_i} \left(\frac{1}{\theta_c}\right) + \frac{k_{d_i}}{Y_i}$$
(13)

Eq. 12 is plotted as $\frac{Q(S_{0i}-S_i)}{VX}$ versus $\frac{1}{\theta_c}$; the biokinetic coefficients k_{d_i} and Y_i can be determined from the slope and the Y-intercept of the equation. To determine the biokinetic coefficients, μ_{\max_i} and K_{s_i} , Eq. 10 can be rearranged to become:

$$\frac{\theta_{c}}{1 + k_{d_{i}}\theta_{c}} = \frac{\left(K_{s_{i}} + I_{j,i}S_{j} + I_{k,i}S_{k} + \cdots\right)}{\mu_{max_{i}}} \left(\frac{1}{S_{i}}\right) + \frac{1}{\mu_{max_{i}}}$$
(14)

Substituting the value of k_{d_i} in Eq. (14) and plotting $\frac{\theta_c}{1+k_{d_i}\theta_c}$ versus $\frac{1}{S_i}$, the biokinetic coefficients, μ_{\max_i} and K_{s_i} can also be examined from the slope and the Y-intercept of the equation. Similarly, it could be applied to other substrates; videlicet μ_{\max_j} , μ_{\max_k} , K_{s_j} and K_{s_k} can also be determined by applying the above method.

2. Materials and methods

2.1. Experimental setup

The dimensions of the membrane bioreactor for this setup were 60×22×6.5 cm (Figure 1). The effective volume in the reactor was 7 L. The membrane used in this study was a Micro-Filtration (MF) type with an effective area of 0.1 m^2 , a pore nominal diameter of 0.4 μ m, and an A4 sheet size. The membrane was produced by the SINAP Company and was made of Poly-Ethylene (PE). The aeration process in the MBR was done for two purposes: first, to supply the oxygen needed for biological processes; and secondly, to clean the membrane surface and reduce the fouling rate. To achieve the second goal, a poly (methyl methacrylate) (PMMA) plate was used as a baffle to keep the air bubbles near the membrane surface so that they can make proper tensions with it and wash the sediments out of the surface. The aerobic sludge used in the MBR basin was supplied from the activated sludge of the Tabriz Petrochemical Company and then adapted with synthetic feed for one month.



Fig. 1. Schematic of lab-scale experimental setup

2.2. Influent wastewater

The synthetic wastewater used in this research was formulated to simulate petrochemical industrial wastewater in terms of chemical oxygen demand (COD), styrene, and ethylbenzene concentrations which were 1200, 50-100 and 50-100 mg/L respectively. Ethanol was used as a carbon source which created a COD concentration of about 1200 mg/L. The synthetic wastewater compositions used in the present study are described in Table 2.

Table 2.	The synthetic	wastewater	compositions
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Components	Concentration (mg/L)
Ethanol	370-400
Styrene (STR)	50-100
Ethylbenzene (EB)	5-100
NH ₄ Cl	560
K ₂ HPO ₄	35
KH ₂ PO ₄	45
MgSO ₄ .7H ₂ O	13
CaCl ₂ .2H ₂ O	7
FeCl₃	5
ZnSO ₄	2
NaHCO ₃	500
EDTA (C ₁₀ H ₁₆ N ₂ O ₈)	7

2.3. Analytical methods and operation parameters

The styrene and ethylbenzene concentrations were analyzed using a gas chromatograph (GC). The GC (Young Lin, ACME-6100) was set with a Flame Ionizing Detector (FID) and an attached silica capillary column (DB-5, 0.53 mm I.D., 30 m length, 1 mm film thickness) that was designed to be well suited for the analysis of volatile components. The carrier gas was helium flowing at 15 mL/min. The oven temperature was maintained at 70 °C for a 1 min duration and then raised to 140 °C. The temperatures of the injector and the detector were fixed at 200 and 240 °C, respectively. The styrene and ethylbenzene concentrations in the liquid phase were estimated using the head-space method [20]. The gas flow rate from the bioreactors headspace was measured using a flow meter. The MLSS, MLVSS, and COD were estimated according to standard methods [21].

3. Results and discussion

3.1. Experimental results for MBR

3.1.1. Styrene, ethylbenzene and COD removal efficiency in HRT=20 hr and SRT= 20 d

The styrene, ethylbenzene, and COD removal efficiency is presented in Figure 2. As it can be seen, in the steady state condition for a HRT of 20 hrs, the COD removal efficiency in the reactor was around 98 percent; the styrene and ethylbenzene removal was more than 99 percent. In addition, the concentration of ethylbenzene and styrene in the exit air from the reactor was measured daily. Figure 3 shows that in an HRT of 20 hrs, the concentrations of ethylbenzene and styrene in the reactor exit air in a steadystate condition was 0.7 ppm (equal 1.16% stripping removal) and 1ppm (equal 1.65% stripping removal), respectively. This fact indicated that the mechanism of removal in the reactor was not a consequence of the volatility of styrene and ethylbenzene. Also, the absorption of a pollutant by a microbial culture can only be considered as an important mechanism whenever the partition coefficient of octanol - water (log K_{ow}) was more than 4 [20],

while this coefficient for styrene and ethylbenzene was about 3.15 and 2.85, respectively [7,21]. Moreover, previous studies revealed that the styrene absorption by sludge as a removal mechanism was insignificant [23]. Therefore, the removal mechanism in the reactor was mainly through a biodegradation mechanism.



Fig. 2. Variations of COD, styrene and ethylbenzene removal during the operation of the MBR (HRT of 20h (days 0-43th), 15h (days 44- 85th) and 10h (days 86-125th)

3.1.2. Styrene, Ethylbenzene and COD Removal Efficiency in HRT=15 & 10 h and SRT =20 d

In an HRT of 15 hr, except during the first few days (days 44 to 47th) in which the removal efficiency in the reactor was extremely reduced, the removal efficiency of COD, styrene and ethylbenzene increased and after reaching a steady state was 98, 99.9 and 99.9%, respectively. Because of an unexpected boost in the amount of organic load that entered the system on the 44th day, the microorganisms were under shock and for this reason, a declining trend was observed. Following this stage, the microorganisms adapted themselves to the new conditions which gradually increased the efficiency of the system and eventually reached a steady state condition. After a change in the retention time from 20 to 15 hours, the stripping removal efficiency of styrene and ethylbenzene in the reactor slightly decreased. This could be attributed to styrene and ethylbenzene concentrations in the exit air which decreased from 1 and 0.7 ppm to 0.8 and 0.5 ppm, respectively. In a previous study, it was also reported that HRT reduction decreased the removal efficiency through volatility [10]. After the change in the retention time from 20 to 15 hours, the biological removal efficiency of styrene and ethylbenzene in the reactor increased. Thus, when the retention time was

reduced, two parameters affected the removal efficiency. Firstly, organic loading increased slightly and as a consequence, the amount of MLSS grew in the reactor. The other parameter was the drop in contact time between the contaminants and the sludge. It was obvious that an increase in MLSS had a positive effect and a reduction in the contact time had a negative influence on the biological removal efficiency. Nevertheless, since the MLSS concentration in the reactor increased, the negative effect of the contact time was neutralized and the removal efficiency increased. When the HRT declined to 10 hrs, the organic load rate in the system increased; on the other hand, the contact time between the activated sludge and wastewater decreased significantly compared to the previous states (e.g. in HRTs of 20 and 15 hrs). Therefore, the removal efficiency in the reactor was reduced significantly. Under this circumstance, the removal efficiency of COD, styrene and ethylbenzene was 90, 99.9, and 99.9 percent, respectively, but the biological removal for styrene and ethylbenzene was 93 and 94%. Further, the styrene and ethylbenzene concentration in the exit air of the reactor was 4 and 3 ppm, respectively. It should be noted that the concentration of exit gases also increased because of the fall of the MLSS value. Therefore, in an HRT of 10 hr, the biological removal efficiency in the reactor was reduced significantly compared to an HRT of 15 and 20 hr.

3.1.3. Styrene, ethylbenzene biological removal efficiency in HRT=15 h and SRT = 10 day

The biological removal efficiency of styrene and ethylbenzene at a SRT of 10 and a HRT of 15 hrs was measured in a steady state. The biological removal of styrene and ethylbenzene was about 94.6% and 98.7% while the SRT of 20d was about 99% and 99%, respectively. Moreover, the concentration of ethylbenzene and styrene in the reactor exit air was measured daily under this condition. The results showed that in the SRT of 10 day, the concentrations of ethylbenzene and styrene in the reactor exit air was 3.2 and 4.9 ppm, respectively. The compression of biological removal efficiency and VOCs concentration for styrene and ethylbenzene in two SRTs (10 and 20) are presented in Table 3.

3.2. Determination of biokinetic coefficients

The determination of the biokinetic coefficients at an MLSS concentration of 4000 mg/L was initiated using an SRT of 20 days. During the investigation, the SRT was varied between 20 and 5 days. Table 4 shows the steady-state data obtained at an MLSS concentration of 4000 mg/L, while Figures 3 and 4 show the determination of the coefficients using Eqs. 13 and 14. The values of the biokinetic coefficients were found to be as follows: 1. for styrene substrate: Y = 0.599 mg/mg, $k_d = 0.25 \text{ day}^{-1}$, $\mu_{max} = 0.188 \text{ h}^{-1}$, and $K_s = 1.457 \text{ mg/L}$; and 2. for ethylbenzene: Y = 0.599 mg/mg, $k_d = 0.25 \text{ day}^{-1}$, $\mu_{max} = 0.364 \text{ h}^{-1}$, and $K_s = 2.821 \text{ mg/L}$.

Table 3. Comparison of biological removal efficiency and VOCs concentration in the exit air for two SRTs

SRT (d)	HRT (hr)	Biological removal of STR	Biological removal of EB	STR concentration in air	EB concentration in air
10	15	93.6	95.7	4.9	3.2
20	15	98.5	99.1	0.8	0.5

Note: Styrene (STR), Ethylbenzene (EB)

 Table 4. The steady-state data obtained at an MLSS concentration of about 4000 mg/L

SRT (d)	HRT (h)	X (mg/L)	Q (L/d)	S_0 (mg/L) for STR & EB	S (mg/L) for STR	S (mg/L) for EB
20	20	4000	0.20	100	0.12	0.10
15	20	5000	0.20	100	0.13	0.11
10	15	5100	0.27	80	0.14	0.12
5	18	3500	0.22	80	0.19	0.15
3	15	3400	0.27	80	0.25	0.21



Fig. 3. Determination of (a) Y and kd (b) μ_{max} and Ks for styrene substrate



Fig.4. Determination of (a) Y and kd (b) μ_{max} and Ks for ethylbenzene substrate

The results showed that the yield (Y) and the endogenous decay coefficient (k_d) was the same for styrene and ethylbenzene as the substrate, but the maximum specific growth rate (μ_{max}) for ethylbenzene was more than styrene. As can be seen in Table 5, in comparison to prior

research that considered the special pure culture to evaluate the kinetics of biodegradation, the biokinetic coefficients obtained for both styrene and ethylbenzene in this study were different from the previously gained values [21,22]. This clearly showed that the type of substrate and bacterial consortium can have a significant effect on the determination of the biokinetic coefficients. The μ_{max} value showed the capability of the microbial culture in MBR to use the special pollutant as a source of carbon and energy. Although some microorganisms showed an excessive ability to biodegradation, the other culture cannot appropriately use these components as a source of energy. Therefore, the abundance of the microorganisms led to a competition between the bacterium cultures for the common substrate [23,24]. Furthermore, it can be seen in this case that the ethylbenzene was more appropriate as a source of carbon to activated sludge in the MBR than the styrene, which had a lower μ_{max} than the ethylbenzene. In addition, the different values of μ_{max} demonstrated different pathways in order to completely catabolize the selected components using the microbial species picked to attack and catabolized the carbon sources [22].

4. Conclusions

The operation of a MBR system for the biological removal of volatile organic compounds such as styrene and ethylbenzene demonstrated the 15-hour time as an optimum HRT value at a SRT of 20 day. The operation of a lab scale MBR confirmed that it can be a feasible procedure to reduce VOC emissions from petrochemical wastewater. The results showed that the yield (Y) and the

endogenous decay coefficient (k_d) was the same for styrene and ethylbenzene as for the substrate, but the maximum specific growth rate (μ_{max}) for ethylbenzene was more than the styrene. Further, the values of the biokinetic coefficients, except that of k_d, were within the normal range reported for these components.

Strain	Substrate	µ _{max} (1/h)	Y(mg/mg)	K _s (mg/L)	k _d (d ⁻¹)	рН	References
Activated sludge in MBR	Styrene	0.188	0.6	0.146	0.25	7	This study
Exophiala jeanselmei	Styrene	0.630	-	0.1	3.3	5.7	[27]
Activated sludge in MBR	Ethylbenzene	0.363	0.6	2.82	0.25	7	This study
Pseudomonas putida F1	Ethylbenzene	0.260	-	1.5	20	-	[28]

Table 5. Comparison between kinetic parameters estimated for the biodegradation of styrene and ethylbenzene in different studies

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