Determination the Biochemical Kinetics of Natural and Synthetic Estrogens in Moving Bed Bioreactor

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Abstract

Aim: Estrogenic compounds as a group of endocrine disruptive compounds can interfere with endocrine system beings. In the present work, an attempt has been made, to characterize the kinetic coefficients of natural and synthetic estrogens, in a pilot-scale moving bed biofilm bioreactor (MBBR). **Materials and Methods:** The substrate removal rates were investigated at different organic loading rates, and hydraulic retention times. By applying some biokinetic models including first order, second order, Stover–Kincannon, and the Monod equation, the kinetic constants (m, Ks, k, Y, and Kd) were determined. **Results:** Estrogen-specific removal rate was between 0.22 and 1.45 µg. g VSS-1.d-1 for natural and synthetic hormones. The experimental data showed that the Stover–Kincannon model and second-order model were the fit models and have high correlation coefficients more than 99%. **Conclusion:** These findings indicated that theses mathematical models could be promising models for effectively predicting kinetic parameters for performance of MBBR reactors.

Keywords: Estrogens, kinetic constants, mathematical models

INTRODUCTION

Endocrine disruptive compounds (EDCs) are a wide range of natural and synthetic substances, which dispersed in the environment.[1] Steroid estrogens (SE) as a main class of EDCs have the most potent adverse health effects on wildlife especially in aquatics.[2] Some of the undesirable effects that are attributed to these pollutants include reduced fertility, bioaccumulative and intensely toxic on organisms, teratogenic, feminization, and carcinogenic, even in low concentrations.[3] Therefore, the Economic Partnership Agreement and European Union have listed SE as emerging contaminants. These priority pollutants included natural estrogens such as estrone (E1) and 17β-estradiol (E2) and synthetic steroid 17α-ethinyl estradiol (EE2).[4] Most of conventional wastewater treatment processes are designed to remove the organic matter and other pollutants with concentration in range of mg/L.[5] As regards the concentration of these emergency pollutants is very low ranging from a few ng/L to several µg/L, the removal efficiency of many of these micropollutants during wastewater treatment process is insufficient and imperfect.[6,7] The presence of these contaminants in to receiving water is the result of the effluent discharge flow from sewage treatment plants and due to estrogenic activity considered as a risk for aquatic ecosystem.[8]

During conventional WWTPs, the removal efficiency of estrogenic compounds is not sufficient and perfect. Nevertheless, numerous study illustrate a wide range from 76% to > 90% for EE2, 19% to 98% for E1, and 62% to 98% for E2.[9] Optimizing the performance and stable operation are design criterion for the biological wastewater treatment. Recently, the proper design of bioreactors affected by empirical and logic parameters based on biological kinetic equations. Biokinetic parameters make useful information about the rate of microbial growth and consumption of substrate.[10] These coefficients are calculated to understanding well the process

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control and predict the implementation of a biological process. For getting the high efficiency of bioreactor, it necessary to considered the kinetic coefficients instead of empirical methods. However, uncomplicated models with few variables are more suitable for monitoring and field applications of biological reactors.[11] Specific growth rate (μ), maximum rate of substrate utilization per unit mass of microorganisms (k), half-velocity constant, or substrate concentration at one-half the maximum specific growth rate (K_s), maximum cell yield (Y), and endogenous decay coefficient (k_d), are major biological kinetic coefficients that used for design the activated sludge processes.[10] Numerous studies have been carried out to evaluate the kinetic constants in the different wastewater treatment processes. These values are compared in Table 4. Borghei determined biokinetic coefficients for a biomass reactor for treating a synthetic wastewater including sugar manufacturing. He reported the Stover–Kincannon model and Grau model showed the most coordination.[11] Fikret Kargi evaluated the kinetic constants of synthetic wastewater containing 2, 4-dichlorophenol by rotating perforated tubes biofilm reactor.[12] The biological kinetics for activated sludge process in municipal wastewater was determined by Mardani *et al*.,[10] Wong *et al*., evaluated the biokinetic coefficients for palm oil mill effluent on anaerobic stabilization pond treatment. These findings indicated that Y, k_d, Ks and μ_{max} coefficients were of 0.990 g VSS/g chemical oxygen demand (COD), 0.024 day^{-1}, 0.524 day^{-1}, 203.433 g COD l^{-1}, respectively.[13] Among the biological process for wastewater treatment, the most effective and benefits are attached growths. The moving bed biofilm reactor (MBBR) is an attached growth process that was constructed based on activated sludge process. Advantages of MBBRs include the reduction in space as compared to conventional activated sludge, facilitate, and enhance the growth of slow-growing microorganisms due to high SRT, redox conditions within biofilm that enhance the removal of micropollutants.[14]

Table 1: Definition of the parameters used in this study

Nomenclature	**Description**
k_1	First order kinetic constant (/day)
E	The substrate removal efficiency (%)
K_{max}	Maximum specific substrate utilization rate (mg COD (mg VSS/day))
Ks (G)	Grau second-order substrate removal rate constant (d^{-1})
A	Total specific surface area of packed media in reactor (m^2)
X (A)	Attached biomass per area (g VSS/m^2)
M	Constant for Grau second-order model (d^{-1})
n	Constant for Grau second-order model (dimensionless)
K_B	Saturation value constant (g (l/day))
U_{max}	Maximum substrate removal rate (mg COD (l/day))
r_{su}	Change in the substrate utilization rate (g m^3 d1)
Rg	Specific rate of growth (g VSS m^2 d1)
VSS	Volatile suspended solid (mg/L)
V	Reactor volume (l)
Q	Inflow rate (l/day)
X	Concentrations of biomass in the reactor (g VSS l^{-1})
HRT	Hydraulic retention time (day)
Xatt	Attached biomass (g VS)
VS	Volatile solid (mg/L)
S0	Influent substrate concentration (mg/L)
S	Effluent substrate concentration (mg/L)
k	Overall reaction rate (d1)
Kd	Biomass decay rate (d1)
Ks	Half saturation constant (mg/L)
μ_{max}	Maximum specific growth rate (d^{-1})
Y	Biomass yield coefficient (g VSS produced/g substrate utilized)

VSS: Volatile suspended solids, COD: Chemical oxygen demand

There is also not enough information in the literature to analytically determine the biokinetic coefficient of natural and synthetic hormones in MBBR. Four common mathematical models such as first-order, second-order, Monod, Stover-Kincannon are used for evaluate the biodegradability of SE in MBBR. There has also been little effort dedicated toward the development of a better fundamental and conceptual illustrating of kinetic parameters of natural and synthetic hormones in biological wastewater treatment. The main objective of this article is to assessment the elimination efficiency of E1, E2, and EE2 in MBBR and development a kinetic model to represent the performance of this process. The target analytes were extracted by dispersive liquid liquid microextraction, and identified by gas chromatography followed with mass spectrometry (GC-MS).

MATERIALS AND METHODS

Experimental set-up

It can be seen a schematic of the moving bed bioreactor (MBBR) Figure 1. The Polypropylene carriers had specific surface 400 m^2/m^3 and density 0.97 g/cm^3.

Synthetic wastewater composition is illustrated in Table 3. Wastewater spiked with target analytes at different organic loading rate was introduced to the reactor through pump (Etatron-Italy). COD spiked with hormones was considered as influent substrates for biokinetic study [Table 3]. By modifying the flow rate of the influent, HRT was controlled.

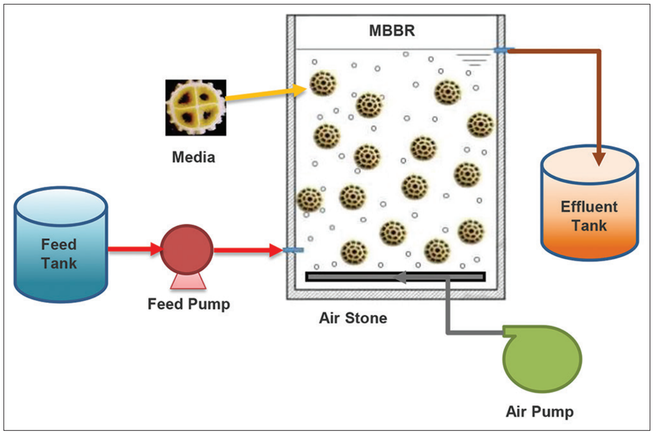

Figure 1: Schematic diagram of the lab scale moving bed biofilm reactor system

The parameters required for the biokinetic values calculation summarized in Nomenclature.

Analytical methods

Common operating parameters including COD, sCOD, rbCOD, MLSS, TSS, and VSS were measured according to the standard methods.[15] The attached-growth biofilm was determined by procedure was described by Amin *et al*.[16]

For extraction, the target analytes from wastewater samples, 5 ml of effluent spiked with 10 µL of n-Octyl Phenol as internal standard, 100 µL of chloroform (extractive solvent) and 500 µL of methanol (dispersive solvent) injected rapidly into tube. Then, cloudy solution centrifuged for 5 min at 5000 rpm. The lower phase extracted and transferred into a 2 mL vial to dryness under a gentle flow of nitrogen.[17] The dry residue was derivatized with 10 µL of BSTFA containing 1% of TMCS (as derivative agent) and 20 µL pyridine and heated at 70°C for 30 min in a water bath.[18]

GC–MS analysis was carried out using a gas chromatograph (7890A Agilent Technologies, USA) interfaced with a mass spectrometry (5975C series). For qualitative and quantitative analysis, The MS was operated in SIM scan mode from m/z, 50–600.[19] The ratio of m to z (m/z) was 342, 416 and 425 correspond to E1, E2 and EE2, respectively.[20] Figure 2 shows the chromatogram of 17-α ethynil estradiol, estrone and 17-β estradiol. All experiments were performed in triplicates.

Mathematical model development

Monod equation

The Monod equation is a mathematical model, which has been widely used for the microbial growth and the kinetics, to explain the biodegradation of pollutants. This model is used for obtaining the empirical coefficients k_s and K.

$$V\frac{dS}{dt}=QS_0-QS-A(r_{su}) \tag{1}$$

$$r_{su}=-\frac{dS}{dt}=\frac{KSX}{K_S+S} \tag{2}$$

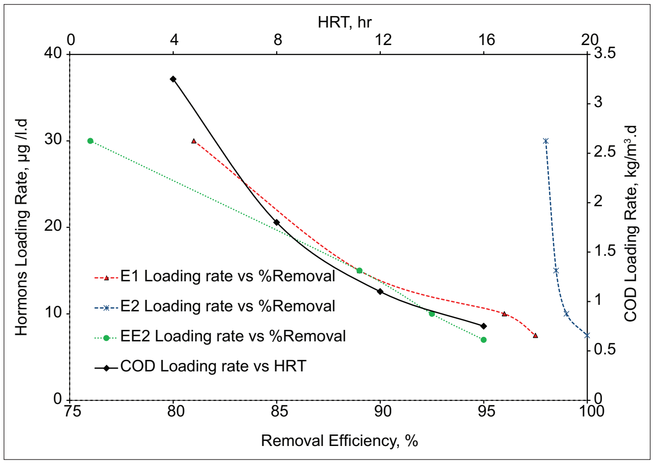

Figure 2: Removal efficiency of chemical oxygen demand, E1, E2, and EE2 at different HRTs

By considering the steady state conditions, the changing rate of substrate concentration can be neglected (ds/dt = 0) and Equations (1) and (2) can be rewritten as Equation (3):

$$\frac{1}{S}=\frac{K}{K_S}\left(\frac{A.X(\mathrm{A})}{Q(S_0-S)}\right)-\frac{1}{K_S} \tag{3}$$

K_s and k half which are saturation constant and the maximum rate of substrate consumption, respectively, obtained by plotting the 1/S versus X_{att}/(Q (S_0-S)). In equation 3, (A. X_A) is known as X_{att}, the slope of this graph is K/k_s, and the intercept is 1/ks. The Y and K_d coefficients were derived by the mass balance equation and the monod growth kinetic for biomass, as rewritten in Equations (4) and 5):

$$V\frac{dX}{dt}=QX_0-QX+A\left(r_g\right) \tag{4}$$

$$r_g=Y\left(r_{su}\right)-K_d AX\left(A\right) \tag{5}$$

As above-mentioned, under steady state conditions, the term of dx/dt is negligible (dx/dt = 0), and by integrating of Equations (4) and (5) rearranged Equation (6) as follows:

$$\frac{(S_0-S)}{X}=\frac{K_d}{Y}\left(\frac{X_{att}}{QX}\right)+\frac{1}{Y} \tag{6}$$

By the linear regression of (S_0-S)/X versus X_{att}/QX, (Y) and (K_d) can be determined subsequently. The maximum specific growth rate coefficient (µm) is attained by Equation (7) as follow:[21]

$$\mu_m=KY \tag{7}$$

First order kinetic

In complete mix reactor, the rate of changes in substrate concentration complies with first-order kinetic, which expresses as follow:

$$-\frac{dS}{dt}=\frac{Q}{V}S_0-\frac{Q}{V}S-k_1S \tag{8}$$

If the steady state conditions predominated in the complete mixed reactor, the left section of equation 8 removed and the Equation (8) is simplified to Equation (9):

$$\frac{S_0-S}{HRT}=k_1S \tag{9}$$

The k_1 value can be achieved from the slope of line which plotted the ((S_0-S)/HRT) versus S.

Stover–Kincannon model

In this model, the substrate utilization rate for biofilm reactors is a function of organic loading rate Equation (10), and Equation (11) can be obtained from the linearization of Equation 10 as follows:

$$\frac{dS}{dt}=\frac{U_{max}(Q\frac{S_0}{V})}{K_B+(Q\frac{S_0}{V})} \tag{10}$$

$$\frac{V}{Q(S_0-S)}=\frac{K_B}{U_{max}}.\frac{V}{QS_0}+\frac{1}{U_{max}} \tag{11}$$

Second-order kinetics (Grau model)

The general equation of second-order kinetic model which presented by Optaken (Optaken, 1982) and Grau *et al*.(Grau *et al*., 1975) is demonstrated in Equation (12).

$$-\frac{dS}{dt} = K_{(2)S} X \left(\frac{S}{S_0}\right)^2 \qquad (12)$$

By integrating and linearizing Equations (12) and (13) is demonstrated as:

$$\frac{S_0 HRT}{S_0 - S} = \frac{S_0}{K_{(2)S} X} + HRT \qquad (13)$$

If the first term of the right part of Equation 13, is considered constant, and $(S_0\text{-}S)/S_0$ accepts as the substrate removal efficiency and represented with E, the final equation can be summarized as follows:

$$\frac{HRT}{E} = a + bHRT \qquad (14)$$

RESULTS

Moving bed biofilm reactor operation

The biodegradability of steroid hormones and the biokinetic coefficient evaluation carried out in MBBR. Table 2 summarizes the steady state operation of MBBR at various HRT of 4, 8, 12, 16 h. The removal efficiency of COD and sCOD corresponding to HRT is illustrated in Figure 2. By decreasing the COD loading rate (from 3 to 0.75 kg/m^3.d), COD removal was increased. In addition, COD removal was increased from 86% to 97% by decreasing the loading of target analytes. According to these results, COD and sCOD removal efficiency was increased by increasing the HRT. In addition, increasing the loading of E1, E2 and EE2 cause to reduce the removal of COD. These results indicated the high removal of COD was acceded in all of experiment (88%–97%) and the sCOD concentration in effluent was lower than 20 mg/L. Minimum removal rates of E1, E2, and EE2 (81, 98.5 and 76%, respectively) was achieved at HRT 4 h. By gradual increasing the HRT, removal efficiency of E1, E2, and EE2 augmented and obtained 98, 99.9, and 95%, respectively, at high HRT (16 h). In general, during the operation of MBBR, the elimination rate of natural and synthetic estrogens was more than 90%. As can be seen, the removal efficiency of steroid hormones was not much significance difference at the higher HRT of 12 and 16 h. SRT and HRT are two critical parameters for operating of MBBR. At high SRT, the microbial consortium for degradation of steroid hormones enhanced the biodiversity of microbial for degradation of rebellious pollutants such as EE2.[22]

First order kinetic

As shown in Figure 3, the coefficient of first-order kinetic for substrate removal was obtained by plotting between $(S_0\text{-}S)/HRT$ versus S. According to Eq 9, from the slope of this line k_1 coefficient was achieved. This value for different concentration (5, 10, and 50 µg/L) was 16.76, 17.87, and 19.67 per day, respectively. The performance of MBBR can't be predicted by this model, because the correlation coefficient was very low for all concentrations (<0.8).

Second-order kinetic (Grau model)

Figure 4 pinpoints the second order model (Grau model) for elimination of substrate. The kinetic coefficients of a, b and $k_{(2)\ s}$ at Equation (13) was achieved by plotting the $(S_0.HRT)/(S_0\text{-}S)$ versus HRT. At concentration of E1, E2, and EE2 equal to 5 µg/l, the values of a, b, and $k_{(2)\ s}$ were found to be 0.052, 1.057, and 0.472, respectively. The correlation coefficient was 0.996. For concentration 10 µg/l of target analytes, these coefficients were obtained 0.0472, 1.0311, and 0.572, respectively, with high correlation coefficient (0.997). Finally, at concentration 50 µg/l of steroid hormones, the value of kinetic coefficient were 0.053, 0.9721 and 0.546 d^{-1}, respectively. In addition, (R^2) was 0.999. It seems the Grau model have a good suitability for predicting the MBBR performance.

Stover– kincannon

Figure 5 indicates the linear regression of Stover-Kincannon modified model which achieved by plotting the $\frac{V}{Q(S_0 - S)}$

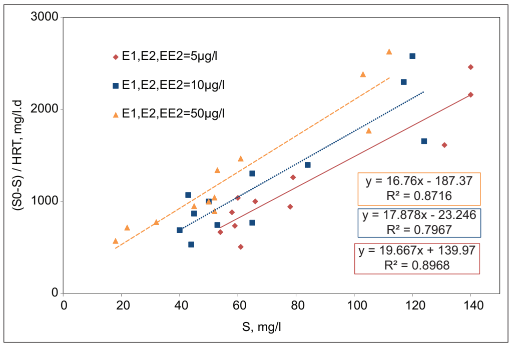

Figure 3: First order kinetic model for wastewater containing E1, E2, and EE2

Table 2: Characteristics of the synthetic wastewater used in this study

Compounds	**Glucose**	**$(NH_4)_2SO_4$**	**KH_2PO_4**	**K_2HPO_4**	**$CaCl_2{\cdot}2H_2O$**	**$MgSO_4{\cdot}7H_2O$**	**$MnCl_2{\cdot}4H_2O$**
Concentration (mg/l)	600	90	9	8.4	4.4	12.2	0.05
Compounds	$ZnSO_4{\cdot}7H_2O$	$FeCl_3$	$CuSO_4{\cdot}5H_2O$	$CoCl_2{\cdot}6H_2O$	$Na_2MoO4{\cdot}2H_2O$	KI	H_3BO_3
Concentration (mg/l)	0.132	18.2	0.01	0.04	0.15	0.054	0.045

against $\frac{V}{QS_0}$. The value of U_{max}, which was computed from the equation line in graph 4 at influent concentrations (5, 10, and 50 µg/l) of hormones, was 5.66, 10.17, and 11.6 g/l.d, respectively. This finding illustrated, by increasing the concentration of these micropullatants, the maximum substrate elimination was obtained. The K_B constant values were 5.9,105 and 11.5 g/l.d. Moreover, the high value of correlation coefficient of 0.97, 0.991, and 0.997 declared the Conformity of this model with high precision for the MBBR performance.

Monod equation

Monod's equation explain the dependence of microbial degradation rate on the of biomass concentration. A mass balance for microbial mass and Monod equation can be used for calculating the kinetic coefficients of K, k_s, Y, K_d and μ_{max} in biofilm systems. The K_s and K value for synthetic wastewater (COD = 500 mg/l) containing E1, E2, and EE2 = 5 µg/l was calculated as 49.07 and 0.326 mg/L, respectively. These coefficients for concentrations of 10 and 50 µg/l were 12.32, 0.218, and 7.25, 0.2 as mg/L, respectively. High correlation coefficient (more than 95%) for these concentrations as depicted in Figure 6, illustrates a good model for calculating kinetic coefficients in biological process. Figure 7 shows the graph plotted between reciprocal of X_{att}/Q. X versus the $(S_0\text{-}S)/X$ for computing the Y, K_d and μ_{max}. The Y, K_d coefficients for 5 µg/L were 0.515 and 0.018 d^{-1}. These values for 10 and 50 µg/L were 0.7, 0.17 and 0.64, 0.01 d^{-1}, respectively. The (K_s) value for SEs in 5, 10, and 50 µg/l was 39.07, 12.3, and 7.2 mg/L, respectively. In addition, (k) value was 0.27, 0.22, and 0.21 d^{-1} for estrogen compounds as substrate, respectively [Figure 6].

DISCUSSION

Evaluation of kinetic models

Table 4 summarizes the constants coefficient evaluated on COD basis determined from the kinetic models in this study and compared with other studies. This result has highlighted, for prediction the performance of MBBR, the Stover–Kincannon and Grau second-order kinetics were more conformity. The Monod and Stover–Kincannon ($R^2 > 0.9$), illustrates that the modified Stover–Kincannon model were more appropriate model for describing the kinetics of the MBBR treating estrogens wastewater. The constant coefficients of Stover–Kincannon model (K_B and U_{max}) were lower than those reported by others [Table 4].[23] Hosseini and Borghei were reported similar observations for synthetic wastewater containing beet sugar molasses.[11] Nonetheless, Ahmadi *et al.* reported the higher values of U_{max} and K_B for DEP and DAP.[24]

According to the second-order model (Grau model) results, the $k_{(2)\ s}$ coefficient measured in this research was in the range of $k_{(2)\ s}$ values that acquired in other researches. According to concentration of influent substrate and the biomass in the reactor, the $k_{(2)\ s}$ value will be increased by the removal rate of substrate. In conclusion, the $k_{(2)\ s}$ coefficient gradually decreased by increasing the target analytes concentrations, show conformity to recorded results from the Stover–Kincannon model. Kinetic constants K_s, k, Y, k_d, μ_{max} were

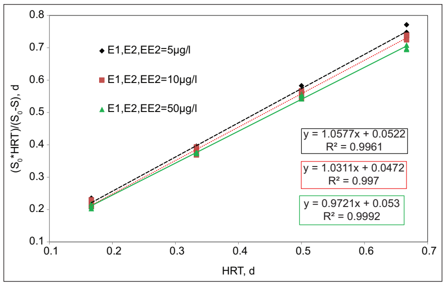

Figure 4: Second-order model (Grau model) for wastewater containing E1, E2, and EE2

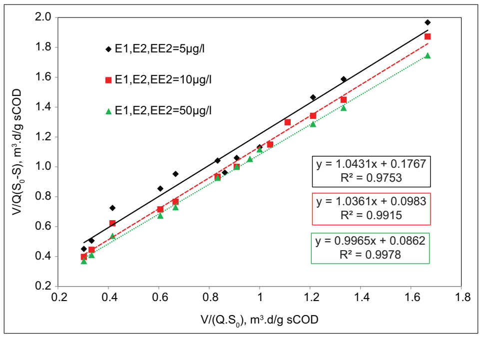

Figure 5: Stover–Kincannon model for wastewater containing E1, E2, and EE2

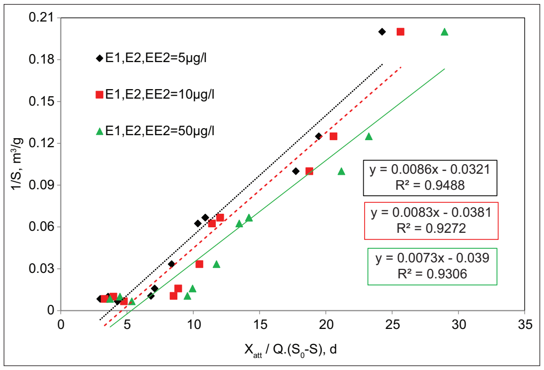

Figure 6: Linear regression for determination of (K_s) and (k) for wastewater containing E1, E2, and EE2

Table 3: Experimental data in the different loading rates

Parameters	**HRT (h)**			
	4	**8**	**12**	**16**
Organic loading rate (kg COD/m³ day)	3.3	1.65	1.1	0.75
Influent COD (mg/l)	600	600	600	600
Effluent sCOD (mg/l)	70	58	35	18
COD removal efficiency (%)	88.3	90.3	94.16	97

HRT: Hydraulic retention times, COD: Chemical oxygen demand

Table 4: Comparison of kinetic constants in the different models cited in the literature with results of the present study

Models	**Substrate**	**Kinetic parameters**					**Reference**
Monod		**Y (mg VSS/ mg COD)**	**kd (d^{-1})**	**μmax (d^{-1})**	**Ks (mg/l)**	**k (d^{-1})**	
	Sewage wastewater	0.64	0.24	1.5	5		Sollfrank and Gujer (28)
	Activated sludge	-	-	1-8	2.5-4	1.69	Kappeler and Gujer (29)
	Tannery Wastewater	0.68	0.1	2	12	7.34	Karahan and Dogruel (30) by means of sequential filtration/ ultrafiltration, respirometric analysis and model evaluation. PSD profiles were determined in physical segregation experiments, using eight membrane discs, each with different pore sizes between 2 and 1600nm. Biodegradability-related COD fractionation was determined at each size interval by model simulation and calibration of the corresponding oxygen uptake rate (OUR
	Synthetic Wastewater containing Phthalic acid	0.6112	0.0047	0.0371	8	0.65-0.7	Meghdad Pirsaheb (31)
	Synthetic Wastewater containing Dimethyl phthalate	0.7875	0.0025	0.0249	1.1	1.21	Meghdad Pirsaheb (31)
	Refinery wastewater	0.222-0.276	0.07-.09	0.653-1.2	396.62-659.42		Al-Malack, M. H (25)
	Pharmaceutical Wastewater	0.481-1.029	0.045-0.06	0.77-0.83	1596-2680		Y. Anjaneyulu (33)
	Domestic Wastewater	0.3-0.6	0.06-0.15	2-10	10-60		Metcalf&Eddy (32)
	Municipal wastewater	0.46-0.6	0.05-0.16	5.6-8.1	250-3720		Al-Malack (25)
	Municipal wastewater	0.49-1.25	0.017-0.039	0.23-3.17	13.8-508	0.366-3.17	Mardani (13)
	Synthetic Wastewater containing E1, E2 & EE2	0.515-0.647	0.01-0.018	0.121-0.153	25.62-31.2	0.187-0.268	This study
First-order				**K1 (d-1)**			
	sugar- manufacturing wastewater			14.549			Borghei *et al*.,(15)
	Synthetic Wastewater			12.09-30.71			Mansouri *et al*.,(23)
	Synthetic Wastewater containing phthalate			36.1-37.5			Ahmadi (24)
	Synthetic Wastewater containing E1, E2 & EE2			16.76-19.67			This study
Stover-Kincannon		**U max (mg/l.d)**			**KB (mg/l.d)**		
	sugar- manufacturing wastewater	101			106.8		Borghei *et al*., (15)
	Soybean Wastewater	83.3			85.5		Yu *et al*., 1998 (31)
	Synthetic Wastewater	38.46			37.88		Mansouri *et al*., (23)
	Synthetic Wastewater containing phthalate	35.6-41.1			37.1-47.8		Ahmadi, (24)

Contd...

Table 4: Comparison of kinetic constants in the different models cited in the literature with results of the present study

	Synthetic Wastewater containing E1, E2 & EE2	5.66-11.6		5.9-11.5	This study
Second order (Grau)		**K (2) S (d-1)**	**a (d-1)**	**b**	
	sugar- manufacturing wastewater	3.582	0.047	1.007	Borghei *et al*., (15)
	Synthetic Wastewater	5.95	0.042	0.928	Mansouri *et al*., (23)
	Synthetic Wastewater containing phthalate	2.28-3.03	0.037-0.042	1.032-1.034	Ahmadi, (24)
	Synthetic Wastewater containing E1, E2 & EE2	0.472-0.572	0.0472-0.053	0.972-1.058	This study

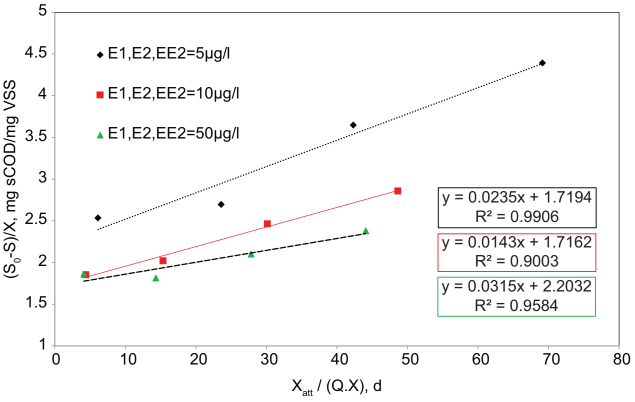

Figure 7: Linear regression for determination of (*y*) and (K_d) for wastewater containing E1, E2, and EE2

obtained by using the modified Monod's equation at different concentrations. It can be seen; the value of (k_d) Had a declining rate by increasing the concentrations of steroids and based on the COD were 0.06 and 0.045, respectively. As illustrated in Table 2 the effluent substrate concentration showed the direct effect on k_d and K_s values while had inverse effect on μ_{max} value. In the study of Hamoda and Al-Attar, it was concluded that the values of k_d for activated sludge and fresh waters were 0.3 and 0.16, respectively. Related findings were described the K_s values affected by the nature of the substrate.[10] The maximum specific growth rate is agree with the studies, which investigated by Mardani *et al*.,[10] Al-Malack,[25] YU,[26] Samuel Suman Raj.[27]In general, it is clear from Table 3 that the change of SE concentrations coefficients did not affect the coefficients. It can be concluded that the presence of estrogenic compounds did not have inhibitory effect on biological treatment. The potential degradation of natural and synthetic estrogens by various isolated bacterial strains from activated sludge confirmed by many publications. In addition, numerous study characterized the estrogens can be used as only source of energy and carbon which metabolized by bacterial strains in wastewater treatment plant. On the other hand, the strain could be cultivated on estrogens. However, this variability might be originated from the nature of the system itself to select a process and obtained kinetic coefficient from different species.[25] The same occurrence happened at other concentrations also.

CONCLUSION

The result of this study demonstrated that the natural and synthetic SEs could be treated effectively through MBBR. With respect to the bio-kinetic coefficients of the MBBR process, the findings indicated the coefficients, except that of k_s, were accommodated with the conventional activated sludge processes recorded in the literature. The biokinetic coefficients that achieved from the experiments will be useful for prediction the overall efficiency in treatment plants. It was also postulated that overall biodegradation of estrogenic compounds was influenced by increasing of HRT. It is also concluded that MBBR could be an excellent alternative as attached growth process for treating estrogen wastewaters. Results from the whole experiments, indicated that the biodegradability of hormones in order E2, E1 and EE2. Accordingly, EE2 and E2 are recalcitrant and easily estrogenic hormones for biodegradation, respectively.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Cai D, Chen J, Fu J, Zheng Y, Song Y, Yan J, *et al*. Study on contamimation of endocrine disrupting chemicals in aquatic environment of Qiantang River. Wei Sheng Yan Jiu 2011;40:481-4.
2. Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Stanford BD, Snyder SA. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. Environ Sci Technol 2009;43:597-603.
3. Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, *et al*. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ

2014;473-474:619-41.
4. Hamid H, Eskicioglu C. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. Water Res 2012;46:5813-33.
5. Sim WJ, Lee JW, Shin SK, Song KB, Oh JE. Assessment of fates of estrogens in wastewater and sludge from various types of wastewater treatment plants. Chemosphere 2011;82:1448-53.
6. Mcavoy K, Lane B. Occurrence of Estrogen in Wastewater Treatment Plant and Waste Disposal Site Water Samples. marrine pollution bulletin. Vol 36.NO 10. 833-839. 1988.
7. Auriol M, Filali-Meknassi Y, Tyagi RD, Adams C. Endocrine Disrupting Compounds Removal from Wastewater , Process Biochemistry 41 (2006) 525–539.
8. Ternes TA, Kreckel P, Mueller J. Behaviour and occurrence of estrogens in municipal sewage treatment plants--II. Aerobic batch experiments with activated sludge. Sci Total Environ 1999;225:91-9.
9. Mes T De, Zeeman G, Lettinga G. Occurrence and fate of estrone , 17 b -estradiol and 17 a -ethynylestradiol in STPs for domestic wastewater. Reviews in Environmental Science and Bio/Technology volume 4, Article number: 275. 2005;275–311.
10. Mardani S, Mirbagheri A, Amin MM, Ghasemian M. Determination of biokinetic coefficients for activated sludge processes on municipal wastewater. Iran J Environ Heal Sci Eng 2011;8:25-34.
11. Borghei SM, Sharbatmaleki M, Pourrezaie P, Borghei G. Kinetics of organic removal in fixed-bed aerobic biological reactor. Bioresour Technol 2008;99:1118-24.
12. Eker S, Kargi F. Kinetic modeling and parameter estimation in biological treatment of 2,4-dichlorophenol containing wastewater using rotating perforated tubes biofilm reactor. Enzyme Microb Technol 2006;38:860-6.
13. Wong YS, Kadir MO, Teng TT. Biological kinetics evaluation of anaerobic stabilization pond treatment of palm oil mill effluent. Bioresour Technol 2009;100:4969-75.
14. Kermani M, Bina B, Movahedian H, Amin MM, Nikaein M. Application of moving bed biofilm process for biological organics and nutrients removal from municipal wastewater. Am J Environ Sci 2008;4:675-82.
15. APHA, WEF and AWWA, Standard Methods for the Examination of Water and Wastewater.23rd edition. 2017, American Public Health Association, Washington, DC.
16. Azzouz A, Ballesteros E. Trace analysis of endocrine disrupting compounds in environmental water samples by use of solid-phase extraction and gas chromatography with mass spectrometry detection. J Chromatogr A 2014;1360:248-57.
17. Chang CC, Huang SD. Determination of the steroid hormone levels in water samples by dispersive liquid-liquid microextraction with solidification of a floating organic drop followed by high-performance liquid chromatography. Anal Chim Acta 2010;662:39-43.
18. Liu R, Zhou JL, Wilding A. Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction-gas chromatography-mass spectrometry. J Chromatogr A 2004;1022:179-89.
19. Liu R, Zhou JL, Wilding A. Microwave-assisted extraction followed by gas chromatography-mass spectrometry for the determination of endocrine disrupting chemicals in river sediments. J Chromatogr A 2004;1038:19-26.
20. Estrada-Arriaga EB, Mijaylova PN. Influence of operational parameters (sludge retention time and hydraulic residence time) on the removal of estrogens by membrane bioreactor. Environ Sci Pollut Res Int 2011;18:1121-8.
21. Nabizadeh R, Mesdaghinia A. Simulation of microbial mass and its variation in biofilm systems using STELLA. J Chem Technol Biotechnol 2006;81:1209-17.
22. Amin MM, Bina B, Ebrahim K, Yavari Z, Mohammadi F. Biodegradation of natural and synthetic estrogens in moving bed bioreactor. Chinese Journal of Chemical Engineering. Vol 26, Issue 2, 2018, 393-399;
23. Mansouri AM, Zinatizadeh AAL, Akhbari A. Kinetic Evaluation of Simultaneous CNP Removal in an up-Flow Aerobic/Anoxic Sludge Fixed Film (UAASFF) Bioreactor. Iranica Journal of Energy & Environment 5 (3): 323-336, 2014
24. Ahmadi E, Yousefzadeh S, Ansari M, Ghaffari HR, Azari A, Miri M, *et al*. Performance, kinetic, and biodegradation pathway evaluation of anaerobic fixed film fixed bed reactor in removing phthalic acid esters from wastewater. Sci Rep 2017;7:41020.
25. Al-Malack MH. Determination of biokinetic coefficients of an immersed membrane bioreactor. J Member Sci 2006;271:47-58.
26. Yu H, Tay J. Kinetic analysis of an anaerobic filter treating soybean wastewater. Water Research. 1998;32(11):3341–52.
27. Raj DS, Anjaneyulu Y. Evaluation of biokinetic parameters for pharmaceutical wastewaters using aerobic oxidation integrated with chemical treatment. Process Biochem 2005;40:165-75
28. Sollfrank U, Gujer W. Characterisation of domestic wastewater for mathematical modelling of the activated sludge process. Water Sci Technol 1991;23:1057-66.
29. Kappeler J, Gujer W. Estimation of kinetic parameters of heterotrophic biomass under aerobic conditions and characterization of wastewater for activated sludge modelling. Water Sci Technol 1992;25:125-39.
30. Karahan O, Dogruel S, Dulekgurgen E, Orhon D. COD fractionation of tannery wastewaters--particle size distribution, biodegradability and modeling. Water Res 2008;42:1083-92.
31. Pirsaheb M, Mesdaghinia AR, Shahtaheri SJ, Zinatizadeh AA. Kinetic evaluation and process performance of a fixed film bioreactor removing phthalic acid and dimethyl phthalate. J Hazard Mater 2009;167:500-6.
32. Tchobanoglous G, Burton FL, Stensel HD. Wastewater Engineering: Treatment and Reuse. 3rd ed., Vol. 4. New York: Metcalf and Eddy, Inc., McGraw-Hill; 2003. p. 1819. Available from: http://www.amazon.com/dp/007124140X. [Last accessed on 2017 Jun 03].
33. Ahmadi E, Gholami M, Farzadkia M, Nabizadeh R, Azari A. Study of moving bed biofilm reactor in diethyl phthalate and diallyl phthalate removal from synthetic wastewater. Bioresour Technol 2015;183:129-35.