



## Substrate Removal Kinetics of an Anaerobic Hybrid Reactor Treating Pharmaceutical Wastewater

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### ABSTRACT

A laboratory-scale anaerobic hybrid reactor was operated at mesophilic temperature (30 – 35°C) for treating fermentation based pharmaceutical wastewater at six hydraulic retention times (HRTs) of 30, 18, 12, 8, 6, 3 h. The COD removal efficiencies decreased from 91.25 to 68.00 % with decreasing the HRT from 30 to 3 h. In this study, the mathematical model such as Monod, Grau second order, modified Stover-Kincannon and first order kinetic models were applied to determine the substrate removal kinetic of anaerobic hybrid reactor. Kinetic parameters were determined through linear regression using the experimental data. The predicted COD concentrations were calculated using the kinetic constants. It was found that these simulated data were in good agreement with the observed ones in Grau second order and modified Stover-Kincannon models. Furthermore, the correlation coefficient value ( $R^2$ ) obtained for the experimental and predicted effluent COD concentration also confirmed the suitability of the kinetic models.

*Keywords:* Anaerobic hybrid reactor; Pharmaceutical wastewater; Kinetic model; Hydraulic retention time

### 1. INTRODUCTION

Pharmaceutical manufacturing industry produces a wide range of products to be used as human and animal medications. Manufacturing can be characterised by five main processes; fermentation, extraction, chemical synthesis, formulation and packaging. Although wastewater streams from all five processes have the potential to contain high organic load, fermentation and synthesis operations usually generate larger volumes of wastewater, and the wastewaters generated usually contain higher organic load (Oktem et al., 2007). It is estimated that approximately

half of the pharmaceutical wastewaters produced worldwide are discarded without specific treatment (Enick and Moore, 2007). Disposing of pharmaceutical wastewater has attracted much public and research attention, as around 80–100 pharmaceuticals and their metabolites have been measured in both effluent and surface waters in numerous countries (Ashton et al., 2004; Chen et al., 2008). The biological wastewater treatment is one of the viable effective methods in eliminating organic matter present in pharmaceutical wastewaters. The crucial issues of energy consideration and environmental protection have escalated interests on anaerobic treatment of industrial wastewater in particular to

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those from pharmaceutical units (Rao et al., 2004; Mullai et al., 2011).

The anaerobic degradation of complex organic material involves sequence of subsequent steps which are carried out by different kinds of bacteria in a mixed population (Tramsek et al., 2007). The performance of the bacteria in degrading the organic matter can be described by kinetics. Further, kinetics is an important tool to understand the anaerobic degradation, reactor design and operation (Chen et al., 2001). The most successful anaerobic systems include the anaerobic suspended film contact reactor (Mohan et al., 2001), expanded granular sludge bed (EGSB)-based anaerobic reactors (Enright et al., 2005), fluidized bed reactor (FBR) (Saravanane et al., 2001), anaerobic continuous stirred tank reactor (CSTR) (Oz et al., 2002), anaerobic baffled reactor (ABR) (Zhou et al., 2006), anaerobic filter (AF) (Chen et al., 1994; Nandy and Kaul, 2001; Ince et al., 2002) and upflow anaerobic sludge blanket (UASB) reactor (Ince et al., 2001). In the present study, an anaerobic hybrid reactor (AHR) was used to evaluate the feasibility of treatment of pharmaceutical wastewater.

AHR is one of high rate anaerobic reactors (HRAR) originally proposed by Maxham and Wakamiya in 1981 and is a conglomeration of the positive features of both the anaerobic filter (AF) and upflow anaerobic sludge blanket (UASB) reactor. The attached growth on the media in the upper portion of the reactor together with the formation of a granular or flocculent sludge bed in the bottom section add up significant biomass inventories leading to increased process stability, improved gas/solid/liquid separation and higher removal efficiency. Another advantage of this kind of design is its ability; even without granular sludge it retains high amounts of biomass inside the reactor. Although hybrid reactor design is expected to work efficiently without granular sludge, it is

desirable to cultivate granular biomass (Tilche and Vieira, 1991).

Kinetic models are normally divided into two classes: structured and unstructured one. Structured models take metabolic pathways into consideration and are generally complicated. On the other hand, the unstructured kinetic models are much simpler than the structured ones (Bailey and Ollis, 1986). In the unstructured kinetic models microorganisms are usually considered to be a component or reactant in the system. The unstructured kinetic models are the most frequently employed for modelling microbial systems because they are simple, but are good enough for technical purposes (Hu et al., 2002). Kinetic analysis is an accepted route for describing the performance of biological treatment systems and for predicting their performance (Yetilmezsoy and Sakar, 2008; Debik and Coskun, 2009). Of the several kinetic models available in literature four different models such as the Monod, Grau second order, modified Stover-Kincannon, and first order kinetic models were applied to determine the substrate removal kinetics of AHR using pharmaceutical wastewater and verified the validity of the models by comparing the experimental and predicted data at decreasing HRTs.

## **2. MATERIALS AND METHODS**

### **2.1 Substrate**

Pharmaceutical wastewater collected from penicillin-G unit, Cuddalore, Tamil Nadu, India was used as a substrate.

### **2.2 Inoculum**

Sewage sludge and cow dung slurry were mixed and sieved with a mesh of 1mm to remove large debris and inert impurities of

larger size. The obtained filtrate was used as inoculum.

### 2.3 Anaerobic Hybrid Reactor

A laboratory scale anaerobic hybrid reactor (AHR) used in this study was made of perspex tube with an internal diameter of 10.4 cm and overall height of 60 cm with a total capacity of 5 litres. The top third of the reactor (10 cm) was filled with polypropylene spherical beads. This packed section performed dual functions of retaining the suspended sludge within the reactor and exerting a polishing effect on the wastewater through the activity of bio-film developed on the packing material. At the lower part of the reactor an inlet was fixed. At the upper part of the reactor, above the packing column, an outlet for the effluent was made. The third outlet fixed on the topmost part of the reactor was meant for the flow of gas, and a gas flow meter was connected to it to measure the biogas collected.

### 2.4 Reactor Operation

The reactor was operated continuously at six hydraulic retention times (HRTs) of 30, 18, 12, 8, 6 and 3 h and the corresponding organic loading rates were 3.20, 5.32, 8.04, 11.988, 16.048, and 32.256 kg COD m<sup>-3</sup> d<sup>-1</sup> by keeping the initial substrate concentrations constant, closely around 4.0 g COD/L.

### 2.5 Analytical Techniques

Analyses of chemical oxygen demand (COD), alkalinity, volatile fatty acid (VFA), volatile suspended solids (VSS) and pH of influent and effluent samples were carried out by following the Standard Methods (APHA, 1995).

## 3. RESULTS AND DISCUSSION

### 3.1 Reactor Performance

#### 3.1.1 COD removal efficiency

At 30 h HRT, on the first day, the lowest COD removal efficiency was 50% (Figure not shown). This might be due to limited time available for acclimatisation. The highest COD removal efficiency of 91.25% achieved on the 12<sup>th</sup> day might be attributed to the formation of more biomass (Table 1). Sreekanth et al. (2009) reported similar pattern on bulk drug pharmaceutical wastewater treatment. When the HRT was brought down to 18, 12, 8, 6 and 3 h, the steady state values of COD removal efficiency were 89.9, 88.5, 86.54, 84.5 and 68.0% respectively (Table 1). The COD removal efficiencies decreased as HRT was decreased. This was because the lowering of HRT led to shorter residence time. Moreover, pharmaceutical wastewaters containing a high proportion of spent fermentation broths have been shown to require long HRT for efficient treatment (LaPara et al., 2002) presumably on account of their complex organic carbon content and this probably limited the AHR performance at 3h HRT. Similar trend of fall in the COD removal efficiency concomitant to decrease in HRT was documented by Vijayaraghavan and Ramanujam (2000) and Yu et al. (1998). The lowest ever COD removal efficiency of 68% achieved at 3 h HRT with an OLR of 32.256 kg COD/m<sup>3</sup>·day could be attributed to the disintegration and washing away of granular biomass along with the effluent due to high mixing intensities (Lettinga et al., 1980). This is an indicative of total failure of the system as noticed by Fang and his coworkers (Fang et al., 1990). When the HRT was restored to 6 h, at 16.016 kg COD/m<sup>3</sup>·day, a revival of the system was noticed.

**Table 1** Operational parameters and steady state results during the study period

Time (d)	HRT (h)	OLR (kg COD m <sup>-3</sup> d <sup>-1</sup> )	COD removal efficiency (%)	VFA (mg acetic acid/l)	Alkalinity (mg CaCO <sub>3</sub> /L)	pH	Biogas production rate (ml/d)	CH <sub>4</sub> (%)
0-12	30	3.200	91.25	319	2625	7.7	1200	70
13-21	18	5.320	89.90	307	2768	7.8	2581	71
22-42	12	8.040	88.50	303	2892	7.8	4450	69
43-64	8	11.988	86.54	281	3139	7.9	5775	68
65-84	6	16.048	84.50	290	3425	7.9	8780	68
85-95	3	32.256	68.00	598	3265	7.4	7890	63
96-113	6	16.016	82.50	436	3583	7.5	8695	67

The increased COD removal efficiency of 82.5% was resumed on the final day of the experiment. Augoustinos et al. (1989) in their research on petroleum waste using the HUASB reactor made similar observations.

### 3.1.2 VFA, pH and alkalinity

As VFAs are potential inhibitors to the anaerobic process, their determination is important in the control of anaerobic wastewater treatment processes. Alkalinity and pH measurements were used in evaluating the buffering capacity of the wastewater. In this study, the values of VFA, pH and alkalinity were in the ranges of 237-1372 mg acetic acid/l, 7.0-7.9 and 2428-3538 mg CaCO<sub>3</sub>/L, respectively. The VFA concentrations which were in the ranges of 237-698 mg acetic acid/L, abruptly shot up to 1372 mg acetic acid/L at 3 h HRT at an OLR of 32.256 kg COD/m<sup>3</sup>·day. Subsequently, the values of pH dropped from 7.9 to 7.2 and the alkalinity levels also reduced from 3425 to 2832 mg CaCO<sub>3</sub>/L at 3 h HRT. Owing to such changes in these parameters, the process instability occurred and as a result the COD removal efficiency declined to 51.7% (Figure not shown). This might be due to short residence time for the effluent in the reactor, which could have prevented the activity of the methanogens on the effluent. For the rest of

the days (86-95) at the same OLR, the values of VFA gradually decreased and reached a steady state value of 598 mg acetic acid/l (Table 1). Augoustinos et al. (1989) also explained the impact of high accumulation of VFA on the reactor performance. When the HRT was restored to 6 h, the steady state values of VFA, pH and alkalinity reached to 436 mg acetic acid/L, 7.5 and 3583 mg CaCO<sub>3</sub>/L, respectively.

## 3.2 Substrate Removal Kinetics

Kinetic studies are very important for industrial anaerobic reactor design. Generally, the results of kinetic studies obtained from experimental studies can be used for estimating treatment efficiencies of full-scale reactors with the same operational conditions. For this purpose, various kinetic models such as, Monod, Grau second order, modified Stover-Kincannon, and first order were applied to determine the substrate removal kinetics.

### 3.2.1 Monod model

For an AHR without biomass recycle, the rate of change of biomass and substrate in the system can be expressed as Eq. (1)

$$\frac{dX}{dt} = \frac{Q}{V} \times X_0 - \frac{Q}{V} \times X_E + \mu \times X - K_d \times X \quad (1)$$

$$-\frac{dS}{dt} = \frac{Q}{V} \times S_0 - \frac{Q}{V} \times S - \frac{\mu \times X}{Y} \quad (2)$$

where  $X_0$ ,  $X$  and  $X_E$  are the concentrations of biomass in the feed, reactor and reactor effluent respectively (g VSS/L);  $Q$  is the inflow rate (l per day);  $V$  is the reactor volume (l);  $\mu$  is the specific growth rate (per day);  $K_d$  is the death rate constant (per day);  $S_0$  and  $S$  are the substrate concentrations in the feed and effluent (g COD/L);  $Y$  is the yield coefficient (g VSS gCOD<sup>-1</sup>). The ratio of the total biomass in the reactor to biomass wasted per given time represent the average time called as mean cell-residence time ( $\theta_C$ ) and calculated from the Eq. (3) for AHR.

$$\theta_C = \frac{V \times X}{Q \times X_E} \quad (3)$$

The relationship between the specific growth rate and the rate limiting substrate concentration can be expressed by the Monod Eq. (4) as follows:

$$\mu = \frac{\mu_{max} \times S}{K_s + S} \quad (4)$$

If it is assumed that the concentration of biomass in the influent can be neglected at steady-state conditions ( $dX/dt = 0$  and  $-dS/dt = 0$ ) and HRT ( $\theta_H$ ) is defined as the volume of the reactor divided by the flow rate of the influent, following equations can be obtained by substituting and rearranging Eqs. (3) and (4) into Eqs. (1) and (2);

$$\mu = \frac{1}{\theta_C} + K_d \quad (5)$$

$$\frac{\mu_{max} \times S}{K_s + S} = \frac{1}{\theta_C} + K_d \quad (6)$$

The kinetic parameters  $Y$  and  $K_d$  for Monod model can be obtained by rearranging equations as shown below:

$$\frac{(S_0 - S)}{\theta_H \times X} = \frac{1}{Y} \times \left( \frac{1}{\theta_C} \right) + \frac{1}{Y} \times K_d \quad (7)$$

The experimental data under steady-state conditions were used and kinetic parameters were evaluated using the linear expressions. Figure 1 was plotted from Eq. (7) for determining the values of  $Y$  and  $K_d$  for Monod model and the corresponding values were 0.0175 g VSS g COD/L and 0.5618 per day, respectively with high regression coefficient ( $R^2 = 0.9938$ ). The decay coefficient value,  $K_d$  which was higher than the reported value (Hwang et al., 1992; Isik et al., 2005; Singh and Ohja, 2002) might be due to substantial decay of cells that occur as a result of endogenous respiration (Table 2). It also heavily depends on the substrate used. Furthermore, the higher yield coefficient obtained in the present investigation could be attributed to relatively larger proportion of biodegradable organic waste that got synthesized into new cells. In general, the model parameters are specific to the configuration and operational mode of the reactor.

The values of  $\mu_{max}$  and  $K_s$  were determined from Figure 2 by plotting Eq. (8), which was derived by rearranging Eq. (6).

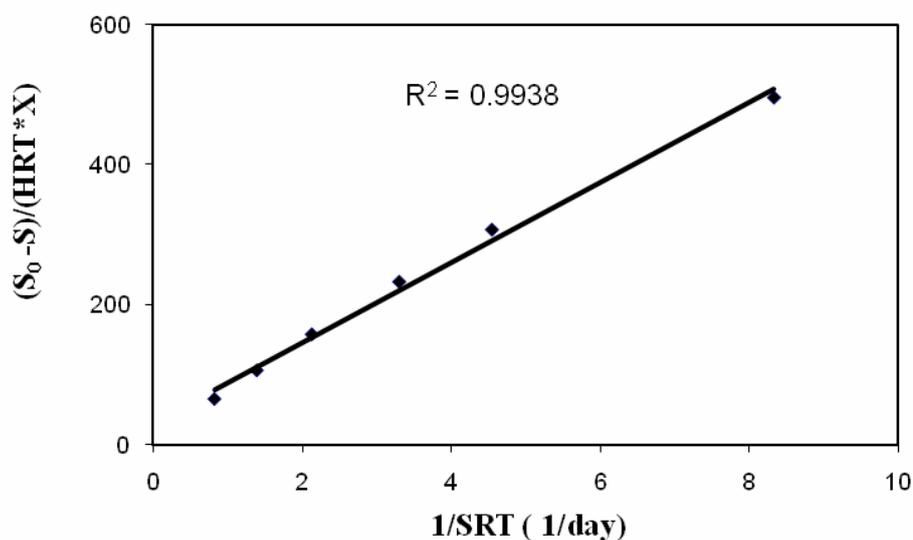
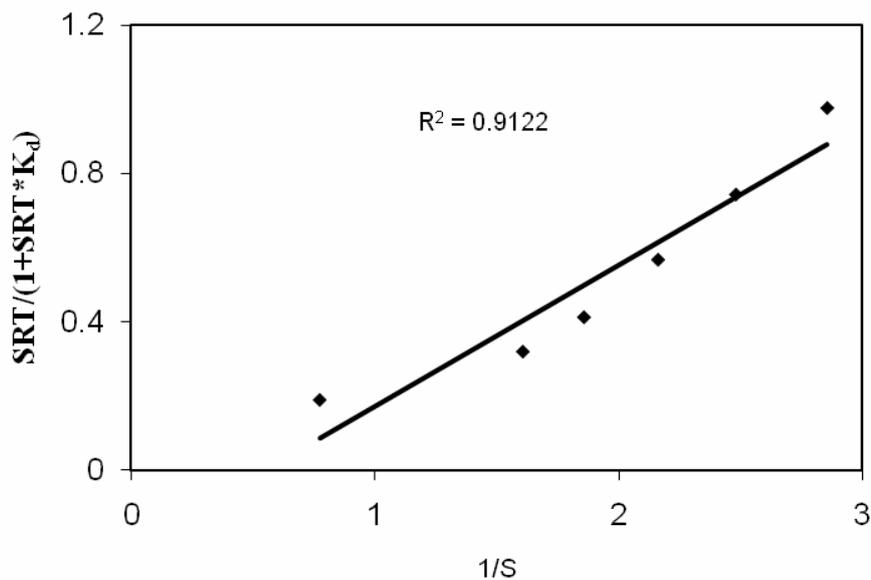
$$\frac{\theta_C}{1 + \theta_C \times K_d} = \frac{K_s}{\mu_{max}} \times \frac{1}{S} + \frac{1}{\mu_{max}} \quad (8)$$

$K_{max} = \frac{\mu_{max}}{Y}$ . The substrate removal rate,  $K_{max}$ ,

indicated the hydraulic retention time actually required for complete waste stabilization. Figure 2 indicates negative  $K_s$  value with regression coefficient of 0.9122. As stated by Converti et al. (1999), the negative values of these constants might be ascribed to maximum degree of degradation.

**Table 2** Comparison of kinetic constants in the Monod model

Feed and reactor used	$K_s$ (g COD/L)	$K_{max}$ (g COD/g VSS/ d)	Y (g VSS/ g COD)	$k_d$ (per day)
Pharmaceutical wastewater hybrid UASB	>1.83	2.63	0.0175	0.5618
Whey permeate UASB	0.773	0.941	0.153	0.022
Simulated wastewater UASB	>4.0	0.84	0.125	0.0065
Whey permeate UASB	0.845	31.6456	0.0885	0.0952

**Figure 1** Determination of yield coefficient (Y) and decay rate constant for Monod model**Figure 2** Determination of maximum specific growth rate ( $\mu_{max}$ ) and half saturation constant ( $K_s$ ) for Monod model

### 3.2.2 Grau second-order multicomponent substrate removal model

The general equation of a second-order kinetic model is illustrated in Eq. (9).

$$-\frac{dS}{dt} = k_s \times X \times \left(\frac{S}{S_0}\right)^2 \quad (9)$$

If Eq. (9) is integrated and then linearized, Eq. (10) will be obtained

$$\frac{S_0 \times \theta_H}{S_0 - S} = \theta_H - \frac{S_0}{k_s \times X} \quad (10)$$

of *a* and *b* (dimensionless Grau second-order constant) were calculated from the intercept and slope of the straight line on the graph (Figure 3) as 0.03 L/d and 1.067 respectively, with high correlation coefficient (*R*<sup>2</sup>) of 0.999. The multicomponent Grau second-order substrate removal rate constant (*k<sub>s</sub>* (d<sup>-1</sup>)) was calculated from the equation *a* = *S<sub>0</sub>* / (*k<sub>s</sub>* × *X*). Table 3 summarizes the constants determined from the applicable Grau second-order models in previous studies. The possible reasons for the differences might be due to variation in reactor configuration, wastewater characteristics and microorganisms used in the study.

### 3.2.3 Modified Stover-Kicannon model

Equation of the modified Stover-Kicannon model is as follows:

$$\frac{dS}{dt} = \frac{R_{max} \times (Q \times \frac{S_0}{V})}{K_B + (Q \times \frac{S_0}{V})} \quad (13)$$

where dS/dt is defined in Eq. (14):

If the second term of the right part of Eq. (10) is accepted as a constant, the Eq. (11) will be obtained,

$$\frac{S_0 \times \theta_H}{S_0 - S} = b \times \theta_H + a \quad (11)$$

*S<sub>0</sub>* - *S* / *S<sub>0</sub>* expresses the substrate removal efficiency and is symbolized as *E*. Therefore, the last equation can be written as follows:

$$\frac{\theta_H}{E} = a + b \times \theta_H \quad (12)$$

In order to determine the kinetic coefficients, Eq. (12) was plotted in Figure 3. The values

$$\frac{dS}{dt} = \frac{Q}{V} \times (S_0 - S) \quad (14)$$

Eq. (15) obtained from linearization of Eq. (14) as follows:

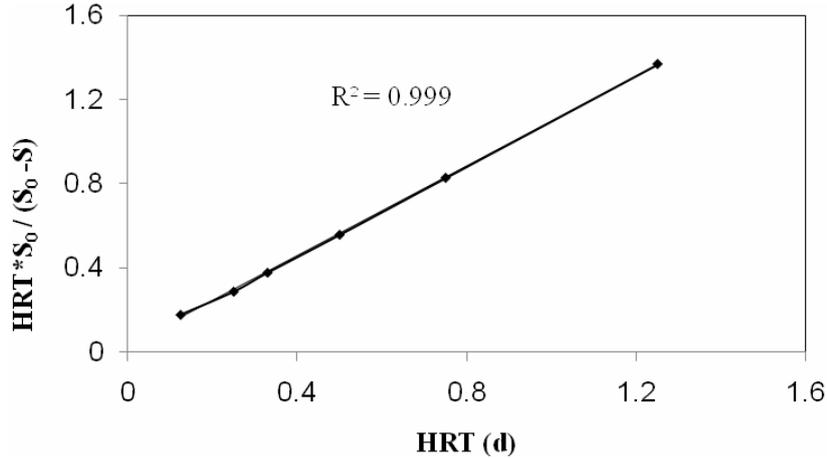
$$\frac{V}{Q \times (S_0 - S)} = \frac{K_B}{R_{max}} \frac{V}{Q \times S_0} + \frac{1}{R_{max}} \quad (15)$$

Saturation value constant (*K<sub>B</sub>*) and maximum utilization rate (*R<sub>max</sub>*) were calculated from Figure 4 as 115.66 g/L·d and 108.69 g/L·d with high regression coefficient (*R*<sup>2</sup> = 0.997). From *R*<sup>2</sup> value (Figure 4), the experimental data are found to align with this model.

The *R<sub>max</sub>* and *K<sub>B</sub>* values obtained in this study were higher than values found by Isik and Sponza, (2005), Yu et al. (2000) and Priya et al. (2009) (Table 4). The maximum utilization rates increase the reactor efficiency. Stover- Kicannon model suggested that the substrate removal rates (COD) were affected by the organic loading rate entering the reactor.

**Table 3** Comparison of kinetic constants in the Grau second-order model

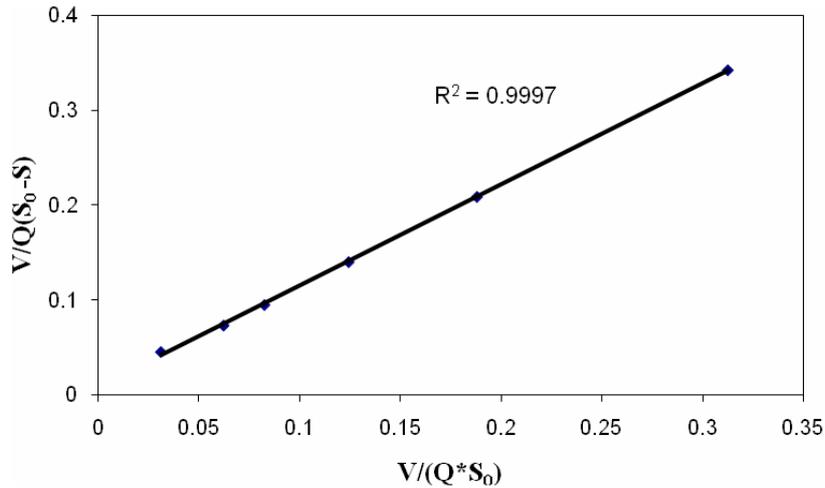
Feed and reactor used	S (g COD/L)	HRT	a (per day)	b	R <sup>2</sup>
Pharmaceutical wastewater AHR	4.0-4.5	0.125-1.25	0.031	1.067	0.999
Simulated wastewater UASB	4.214	0.25-4.16	0.562	1.095	0.995
formaldehyde containing wastewater UAFB	0.100-2.0	0.42-1.0	0.64	9.36	0.96



**Figure 3** Determination of kinetic constants for Grau second-order multicomponent substrate removal model

**Table 4** Comparison of kinetic constants in the modified-Stover Kincannon model

Feed and reactor used	S (g COD/L)	HRT	R <sub>max</sub> (g/L·d)	K <sub>B</sub> (g/L·d)	R <sup>2</sup>
Pharmaceutical wastewater AHR	4.0-4.5	0.125-1.25	108.69	115.66	0.9997
Simulated wastewater UASB	4.214	0.25-4.16	7.5	8.2	0.995
Soybean wastewater AF	7520-11450	1-1.45	83.3	85.5	-
formaldehyde containing wastewater UAFB	0.100-2.0	0.42-1.0	3.4	4.6	0.9617



**Figure 4** Determination of kinetic constants for modified Stover-Kicannon model

**3.2.4 First order substrate removal model**

The rate of change in substrate concentration in the system assuming the first order model for substrate removal could be expressed as follows:

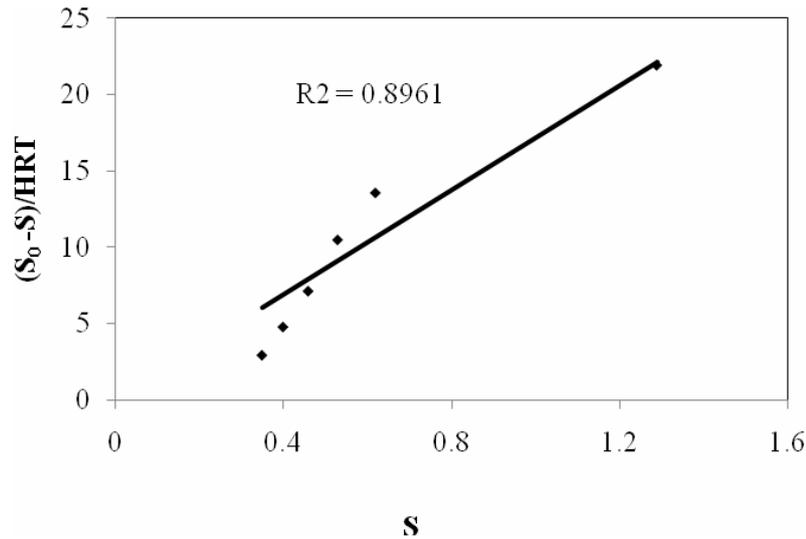
$$-\frac{dS}{dt} = \frac{Q}{V} \times S_0 - \frac{Q}{V} S - k_1 S \quad (16)$$

Under steady-state conditions, the rate of

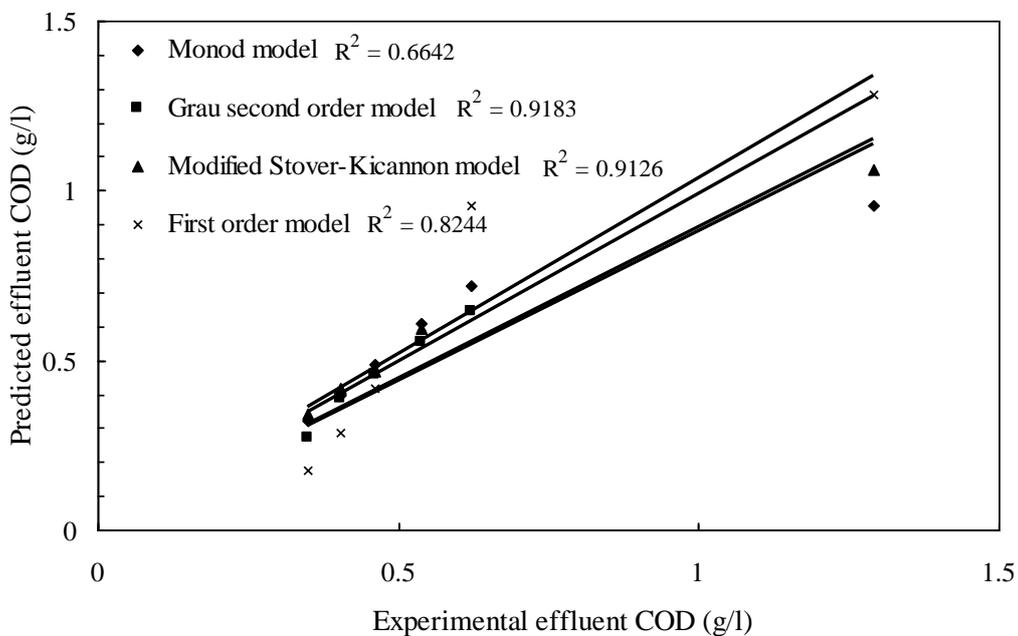
change in substrate concentration (-dS/dt) is negligible and the equation given above can be reduced to the Eq. (17):

$$\frac{S_0 - S}{\theta_H} = k_1 S \quad (17)$$

The value of  $k_1$  was obtained from the slope of the line by plotting  $(S_0 - S / \theta_H)$  versus  $S$  (Figure 5) in Eq. (17) with the lower regression coefficient of 0.8961.



**Figure 5** Determination of kinetic constant for first order model



**Figure 6** Comparison of predicted and experimental COD values

**3.2.5 Prediction and validation**

In order to test the validity of the model the results obtained from the experimental effluent COD values were compared with the predicted values obtained from the models. From the obtained high statistical quality of the modelling ( $R^2 = 0.9183$  and  $0.9126$  for Grau second-order and Stover-Kincannon model respectively, between experimental and predicted values), it could be inferred that predicted results are in good agreement with

the experimental data in case of Grau second-order and Stover- Kincannon models (Figure 6). The Monod and first order substrate removal model were not suitable for predicting the COD values compared to the other investigated models with lower regression coefficient of  $0.6642$  and  $0.8244$  respectively.

**CONCLUSIONS**

Pharmaceutical wastewater could be effectively treated using the AHR. By conducting

experiments at the HRTs of 30, 18, 12, 8, 6 and 3 h, it was found that the maximum COD removal efficiency of 91.25% was recorded at 30 h HRT. Kinetic parameters were determined through linear regression using the experimental data. It was found that these simulated data were in good agreement with the observed ones in the models, such as, Grau second-order and Stover- Kincannon models. The obtained high statistical quality of the kinetic modeling (regression coefficient ( $R^2$ ) values between experimental and simulated values of substrate concentration), confirmed the suitability of the models. The results indicated that the kinetic models are capable of describing the bio-kinetic behaviour of the reactor.

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