



## Kinetic Evaluation of Simultaneous CNP Removal in an up-Flow Aerobic/Anoxic Sludge Fixed Film (UAASFF) Bioreactor

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**Abstract:** The kinetics of simultaneous removal of carbon, nitrogen and phosphorus from a synthetic wastewater in an innovative up-flow aerobic/anoxic sludge fixed film (UAASFF) bioreactor was investigated. The kinetic analysis was performed using the experimental data obtained in an earlier study where the UAASFF bioreactor was examined under different operating conditions by changing three independent variables, HRT, COD:N:P ratio and aeration time. In the analysis, different kinetic models (Monod, first-order, second-order and Stover-Kincannon models) were evaluated. The maximum removal efficiency of COD, total nitrogen (TN) and phosphorus (TP) were obtained to be 95.42, 79 and 79.1 %, respectively. All the models examined, gave high correlation coefficients for carbon, nitrogen and phosphorus removal. Biokinetic coefficients were determined as  $Y=0.417-0.496$  g VSS/g COD,  $k_d=0.027-0.053$  d<sup>-1</sup>,  $\mu_{max}=1.36$  g VSS /g VSS.d,  $K_B=37.96$  g/l.d,  $U_{max}=38.46$  g/l.d,  $K_B(N)=0.271-7.2$  g/l.d,  $U_{max}(N)=0.33-5.4$  g/l.d,  $K_B(P)=0.09-0.89$  g/l.d,  $U_{max}(P)=0.07-0.42$  g/l.d.

**Key words:** Simultaneous nutrients removal kinetics • UAASFF bioreactor • Monod • Grau second-order model • Stover-Kincannon model

### INTRODUCTION

Discharge of untreated wastewater containing nitrogen and phosphorus into receiving rivers result in environmental and human health problems such as fish poisoning by ammonia and eutrophication in water bodies. It is, therefore, necessary to remove these substances from wastewaters for reducing their harms to environments. Biological treatment has been accepted as one of the most feasible, eco-friendly and cost-effective options for the treatment of pollutants [1-3]. The performance of biological wastewater treatment systems can be improved by maintaining a high biomass concentration; because of the wastewater treatment capacity is proportional to the total biomass of the bioreactor.

In the recent years, substantial attention has been paid towards the compact high-rate bioreactors for wastewater treatment to meet the strict constraints with

respect to space, odor, view and biosolids production. The integrated bioreactors which combine the aerobic and anaerobic processes in a single bioreactor are seen as a viable alternative and enhancing the overall removal efficiency [4]. A number of integrated bioreactors (such as; a anaerobic-aerobic granular biofilm bioreactor, anoxic/oxic-membrane bioreactor (A/O-MBR), nano-filtration membrane bioreactor (NF-MBR), staged anaerobic-aerobic membrane bioreactor (MBR), rotating biological contactor and activated sludge (RBC-AS) and integrated anaerobic-aerobic fixed-film reactor (FFR)) have been developed which allow the coexistence of anaerobic and aerobic populations inside the same reactor [4-10].

Biomass concentration in biological reactors can be increased by variety techniques. Passive immobilization and intermittent aeration and effluent discharge in are two approaches for obtained this purpose [11-12]. The intermittent aeration strategy can also reduce the cost of treatment operation and demand for rbCOD contained in

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the influent wastewater, so that PAOs will obtain sufficient rbCOD for anaerobic P release, which is beneficial to biological P removal. In addition, in an intermittently aerated bioreactor, the organic C stored by PAOs could be used by denitrifiers for denitrification in subsequent anoxic periods [13], resulting in less dependence of denitrification on the rbCOD content in the influent wastewater. Therefore, stable and efficient N and P removal can be achieved in intermittently aerated bioreactors. On the basis of the above consideration, an innovative up-flow aerobic/anoxic sludge fixed film (UAASFF) bioreactor with intermittent aeration as a hybrid reactor which is a combination of an activated sludge (AS) and an immobilized cell or fixed film (FF) reactor. The UAASFF was established and applied as a single treatment unit for carbon, nitrogen and phosphorus removal. The possibility to achieve high biomass concentration, no requirement for additional equipment to circulate the mixed liquor between aerobic and anaerobic compartments and, consequently, the application of short hydraulic retention time and wide variety COD:N:P ratio are the advantages of this type reactor.

Process modeling is an accepted route for describing the performance of biological treatment systems and predicting their performance. In order to achieve the correct design of a bioreactor as well as the reactor's maximum performance, the kinetic coefficients should be taken into consideration at the process of engineering design instead of using only empirical methods. The types of substrates and microorganisms and environment surrounding in a bioreactor are associated with value of the kinetic coefficients. Many models for the biomass growth processes have appeared in the wastewater treatment literature [14, 15]. Global parameters such as COD, BOD and  $\text{NH}_4\text{-N}$  were used as substrate for evaluation under the assumption that the removal was exclusively due to aerobic biodegradation [16]. First-order substrate removal model [16-18] and second-order model often known as Optaken-Grau model are some of those which are used to test the kinetics of organic and nitrogen removal in bioreactors [19-20].

As the UAASFF system is involving a complex process (including anoxic and aerobic reactions in a single system with attached and suspended microbial growth), determination of the kinetic constants is of great importance in practical point of view. Therefore, this study is aimed to determine the biokinetic parameters of the process removing carbon, nitrogen and phosphorus using experimental data obtained under different operational conditions (varying HRT, aeration time and

COD/N/P ratio). Different mathematical models including Monod, first-order, second-order and Stover-Kincannon model were employed to predict the kinetic coefficients.

### Theoretical Development

**Mass Balance Model:** For an UAASFF reactor without biomass recycle, the rate of change of biomass in the system can be expressed as Eq. (1):

$$\frac{dX}{dt}V = QX_0 - [(Q - Q_w)X_e + Q_wX_w] - VYr_{su} - V k_d X \quad (1)$$

If it is assumed that the influent biomass concentration can be neglected and the condition is steady-state ( $dX/dt = 0$ ), Eq. (2) which was derived by rearranging and simplifying the Eq. (1),

$$\frac{(Q - Q_w)X_e + Q_wX_w}{VX} = -Y \frac{r_{su}}{X} - k_d \quad (2)$$

The inverse of the term on the left-hand side of Eq. (2) is defined as the average solid retention time (SRT) and then Eq. (2) is rewritten as:

$$\frac{1}{SRT} = -Y \frac{r_{su}}{X} - K_d \quad (3)$$

In the Eq. (3), the term  $(-r_{su}/X)$  is known as the specific substrate utilization rate,  $U$ , which is calculated as follows:

$$U = \frac{r_{su}}{X} = \frac{Q(S_0 - S)}{VX} = \frac{S_0 - S}{(HRT).(X)} \quad (4)$$

By Substituting ( $\mu=1/SRT$ ) and  $U$  into Eq. (3) will have:

$$\mu = YU - k_d \quad (5)$$

The kinetic parameters ( $Y, K_d$ ) can be obtained by plotting Eq. (5).

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

$$\mu = \frac{\mu_{max}.S}{K_s + S} \quad (6)$$

$$\mu = \frac{1}{SRT} + k_d \quad (7)$$

$$\frac{\mu_{max}.S}{K_s + S} = \frac{1}{SRT} + k_d \quad (8)$$

The value of  $\mu_{\max}$  and  $K_s$  are determined by plotting Eq. (9), which is derived by rearranging Eq. (8).

$$\frac{SRT}{1 + k_d SRT} = \frac{K_s}{\mu_{\max}} \cdot \frac{1}{S} + \frac{1}{\mu_{\max}} \quad (9)$$

**First-Order Substrate Removal Model:** The rate of change in substrate concentration in the system with assuming the first order model for substrate removal could be expressed as follows:

$$-\frac{dS}{dt} = \frac{QS_0}{V} - \frac{QS}{V} - k_1 S \quad (10)$$

Under pseudo-steady-state conditions, the rate of change in substrate concentration due to accumulation ( $-dS/dt$ ) is negligible and the equation given above can be modified as:

$$\frac{S_0 - S}{HRT} = k_1 S \quad (11)$$

The value of  $k_1$  can be obtained by plotting  $((S_0 - S)/HRT)$  versus  $S$  in Eq. (11).

**Second-Order Substrate Removal Model:** The general equation of a second-order model is given below [20] :

$$-\frac{dS}{dt} = k_{2(s)} \cdot \left(\frac{S}{S_0}\right)^2 \quad (12)$$

If Eq. (12) is integrated and then linearized, Eq. (13) is resulted:

$$\frac{S_0 HRT}{S_0 - S} = HRT + \frac{S_0}{k_{2(s)} X} \quad (13)$$

If the second term of the right part of this equation is accepted as a constant, equation will be modified as below:

$$\frac{S_0 HRT}{S_0 - S} = a + bHRT \quad (14)$$

$(S_0 - S)/S_0$  expresses the substrate removal efficiency and is symbolized as  $E$ . Therefore, Eq. (15) can be written as follows:

$$\frac{HRT}{E} = a + bHRT \quad (15)$$

**Stover-Kincannon Model:** Stover-Kincannon is one of the most widely used mathematical models for determining the kinetic constants in immobilized systems. The model has been applied to continuously operated mesophilic and thermophilic upflow anaerobic filters for the treatment of paper-pulp liquors [21] and simulated starch wastewater [22], anaerobic filter for soybean wastewater treatment [22], nitrogen removal in an anammox non-woven membrane reactor [24] upflow aerobic immobilized biomass (UAIB) reactor treating simulated sugar-manufacturing wastewater [25] and anaerobic migrating blanket reactor treating a synthetic wastewater [26].

In this model, the substrate utilization rate is expressed as function of the organic loading rate by monomolecular kinetic for biofilm reactors such as rotating biological contactors and biological filters. Equations of the Stover-Kincannon model are as follows:

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

$$\frac{dS}{dt} = \frac{U_{\max} (QS_0/V)}{k_B + (QS_0/V)} \quad (17)$$

If  $(dS/dt)^{-1}$  is taken as  $V/[Q(S_0 - S)]$ , which is the inverse of the removed substrate loading rate and this is plotted against the inverse of the total loading rate  $V/Q \cdot S_0$ , a straight line portion of intercept  $1/U_{\max}$  and a slope of  $K_B/U_{\max}$  resulted.

$$\left(\frac{dS}{dt}\right)^{-1} = \frac{V}{Q(S_0 - S)} = \frac{k_B}{U_{\max}} \cdot \frac{V}{QS_0} + \frac{1}{U_{\max}} \quad (18)$$

The substrate balance for the reactor at steady-state can be written as follows:

$$QS_0 = QS + V \frac{dS}{dt} \quad (19)$$

Substituting of Equation (17) into (19) gives

$$QS_0 = QS + \frac{U_{\max} (QS_0/V)}{K_B + (QS_0/V)} * V \quad (20)$$

This expression can then be solved for either the effluent substrate concentration (Eq. (21)) or the removal efficiency of the reactor (Eq. (22)) by substituting kinetic constants  $U_{\max}$  and  $K_B$ .

$$S = S_0 - \frac{U_{\max} S_0}{K_B + (QS_0/V)} \quad (21)$$

$$E = \frac{S_O - S}{S_O} = \frac{U_{max}}{K_B + (QS_0/V)} \quad (22)$$

**MATERIALS AND METHODS**

**Synthetic Wastewater (SWW):** SWW was prepared based on the three different COD:N:P ratios (1000:250:50, 1000:83.3:35 and 1000:50:20). The synthetic wastewater was composed of glucose as simple carbon source, NH<sub>4</sub>Cl as nitrogen source, KH<sub>2</sub>PO<sub>4</sub> as phosphorus source and mineral nutrients such as MgSO<sub>4</sub> (0.2 g/l), FeSO<sub>4</sub> (0.01 g/l), CaCl<sub>2</sub> (0.2 g/l) and NaHCO<sub>3</sub> (0.073-1.45 g/l).

**Bioreactor Configuration and Start up:** A lab-scale UAASFF bioreactor was used in this study (Fig. 1). The glass bioreactor column was fabricated with an internal diameter of 5.2 cm and a liquid height of 122 cm. The working volume (total liquid volume excluding volume of the pall rings in fixed bed section) was 2500 ml. The column consisted of three sections; bottom, middle and top. The bottom part of the column, with a height of 80 cm was operated as an upflow activated sludge reactor, the middle part of the column with a height of 25 cm was operated as a fixed film (FF) reactor and the third part is for providing sufficient volume at the top of the reactor in order to continuous feeding and intermediate discharge. The middle section of the column was packed with a

plastic media (supplied by JiangXi Transung Chemical Packing Co. China). The voidage of the packed-bed reactor was 85.45% and the specific surface area of the packing material was 500 m<sup>2</sup>/m<sup>3</sup>. The UAASFF reactor was operated under room temperature (20±2°C). In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. Air was introduced into the reactor with two bubble air diffusers at the bottom of the reactor. The air flow rate and the aeration time were controlled with an air flow-meter and timer that connected to the blower.

The reactor was inoculated with activated sludge taken from an aeration tank (municipal wastewater treatment plant, Kermanshah, Iran). The inoculum sludge had a sludge age of about 15 d, a mixed liquor volatile suspended solids (MLVSS) concentration of 5.8 g/l. After an initial dilution, 2.5 L activated sludge was seeded to the reactor, resulting in an initial MLVSS concentration of 3.8-4.0 g/l in the reactor.

**Bioreactor Operation:** In the first stage (reactor start-up), after adding the prepared inoculums, the bioreactor was operated under continuous flow regime. At intermittent aeration conditions at temperature, HRT, COD:N:P ratio and aeration time were 20±2°C, 6.5 h, 1000:83.3:35 and 40 min/h, respectively. It should be explained that each operation cycle was included three steps

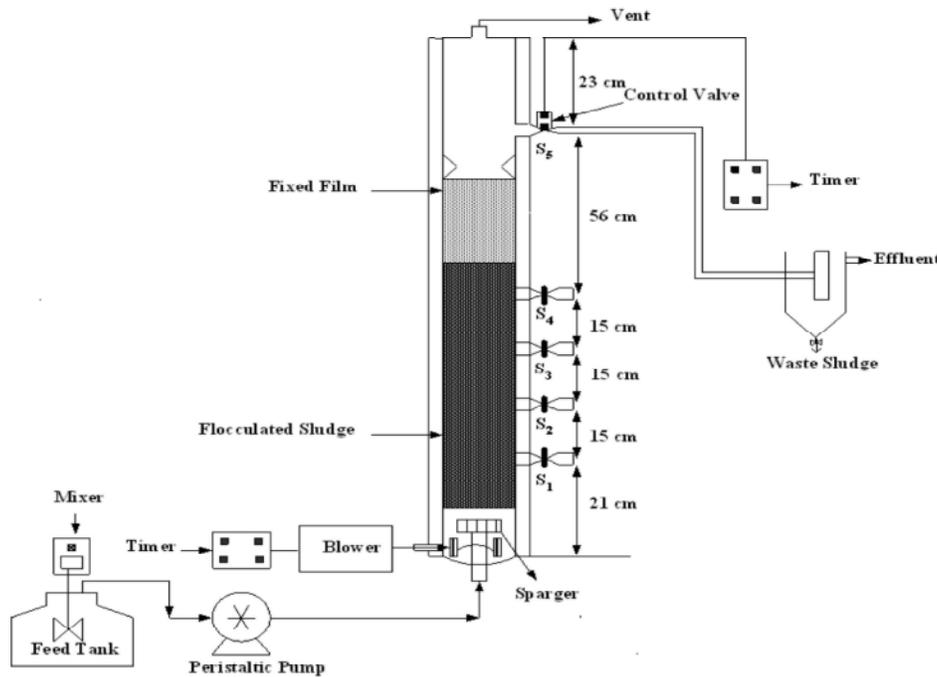


Fig. 1: Experimental set up

Table 1: Experimental range and levels of the independent variables

Variable(s)	Range and levels					
	-1	0		1		
HRT(h)	2	4.25		6.50		
COD:N:P ratio	4	12		20		
Aeration time(min/h)	30	40		50		
	Aeration	Settling	Aeration	Settling	Aeration	Settling
	12 min	12 min	10 min	7 min	16 min	4 min

Table 2: Experimental data obtained under steady state conditions.

Run	Variables			Influent											Effluent				Responses			
	HRT h	COD:TKN:TP g:g:g	Aeration time min/h	Q <sub>in</sub> l/d	X <sub>i</sub> gVSS/l	Q <sub>w</sub> l/d	X <sub>cs</sub> g/l	X <sub>w</sub> g/l	COD <sub>c</sub> g/l	TKN <sub>cs</sub> g/l	NO <sub>3in</sub> g/l	NO <sub>3c</sub> g/l	TN <sub>c</sub> g/l	TP <sub>c</sub> g/l	μ (1/SRT) d <sup>-1</sup>	COD Rem. %	TN Rem. %	TP Rem. %				
1	2	1:0.25:0.05	30	30	4	0.74	0.011	8	0.406	0.138	0.017	0.003	0.139	0.03	0.62	59.4	45.3	39.2				
	4.25	1:0.25:0.05	30	14.11	4	0.48	0.006	8	0.134	0.111	0.017	0.004	0.112	0.017	0.39	86.6	55.9	65.6				
	6.5	1:0.25:0.05	30	9.23	4	0.31	0.008	8	0.083	0.73	0.017	0.002	0.073	0.01	0.25	91.7	71	79.1				
2	2	1:0.05:0.02	30	30	4	0.84	0.009	8	0.35	0.031	0.017	0.001	0.032	0.012	0.70	65	41.2	41.3				
	4.25	1:0.05:0.02	30	14.11	4	0.49	0.006	8	0.134	0.026	0.017	0.003	0.028	0.007	0.40	86.6	50.3	65.6				
	6.5	1:0.05:0.02	30	9.23	4	0.33	0.006	8	0.064	0.011	0.017	0.006	0.012	0.006	0.27	93.6	77.7	71.7				
3	2	1:0.083:0.035	40	30	4	0.77	0.11	6.5	0.316	0.47	0.017	0.001	0.048	0.023	0.82	68.4	45.2	34				
	4.25	1:0.083:0.035	40	14.11	4	0.71	0.011	6.5	0.117	0.027	0.017	0.007	0.029	0.014	0.47	88.3	66.81	59				
	6.5	1:0.083:0.035	40	9.23	4	0.44	0.008	6.5	0.009	0.017	0.017	0.005	0.018	0.013	0.29	91	79	63.7				
4	2	1:0.25:0.05	50	30	4	0.28	0.35	5.5	0.27	0.117	0.017	0.003	0.012	0.037	0.98	73	53.6	25.4				
	4.25	1:0.25:0.05	50	14.11	4	0.63	0.12	5.5	0.146	0.093	0.017	0.016	0.097	0.029	0.51	85.4	61.7	41.7				
	6.5	1:0.25:0.05	50	9.23	4	0.57	0.03	5.5	0.088	0.073	0.017	0.048	0.083	0.031	0.34	91.2	67.1	37.3				
5	2	1:0.05:0.02	50	30	4	0.29	0.35	5.5	0.248	0.018	0.017	0.019	0.023	0.015	0.99	75.2	57.3	23.3				
	4.25	1:0.05:0.02	50	14.11	4	0.65	0.12	5.5	0.146	0.016	0.017	0.016	0.019	0.012	0.52	85.4	63.9	37.3				
	6.5	1:0.05:0.02	50	9.23	4	0.59	0.03	5.5	0.071	0.013	0.017	0.024	0.018	0.011	0.35	92.9	65.2	46.2				

(aeration, settling and effluent discharge) which are intermittently carried out while the influent was continuously fed. This was continued until providing steady state condition. Intermittent effluent discharge was provided by using a programmable control valve at the bioreactor output. The time of discharge was adjusted by giving the time program with regard to the operating condition. The range studied for the HRT, COD:N:P ratio and intermittent cycling program for the aeration and settling time is presented in Table 1.

In the second stage, the UAASFF bioreactor was operated with synthetic wastewater under continuous flow regime and various experimental conditions by changing three independent variables viz. HRT, COD:N:P ratio, aeration time designed using Design Expert software (ver. 6.0) as shown in Table 2. The region of exploration for the process was taken as the area enclosed by hydraulic retention times (2, 4.25 and 6.5 h), COD: N: P ratios (1000:50:20, 1000:83.3:35 and 1000:250:50) and aeration times (30, 40 and 50 min/h) boundaries.

**Chemical Analysis:** The concentrations of chemical oxygen demand (COD), Total Kjeldahl nitrogen (TKN), nitrate, total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS) were determined by using standard methods [27]. For COD, a colorimetric method

with closed reflux method was developed. Spectrophotometer (DR 5000, Hach, Jenway, USA) at 600 nm was used to measure the absorbance of COD samples. Total Kjeldahl nitrogen (TKN) was determined by TKN meter Gerhardt model (Vapodest 10, Germany). The pH meter model HANNA-pH 211 was used to measure the pH.

## RESULTS AND DISCUSSION

### SCOD Removal

**Process Description:** The experimental values for SCOD removal obtained from a process performance studies were used to determine the kinetic coefficients. Table 2 summarizes the experimental conditions, effluent parameters and the process responses. Influent COD concentration was kept constant about 1000 mg/l. The range of HRT studied corresponds to food to microorganism (F/M) and feed flow rate 2.31-7.5 g COD/g VSS.d and 9.23-30 l/d, respectively. The maximum value of the COD removal was obtained to be 93.6% at COD: N: P ratio and aeration time 1000:50:20 and 30 min/h, respectively. The most significant factor effective on the COD removal was determined to be HRT. The effect of HRT at lower values of the aeration time was greater than those with higher aeration times. The interaction showed

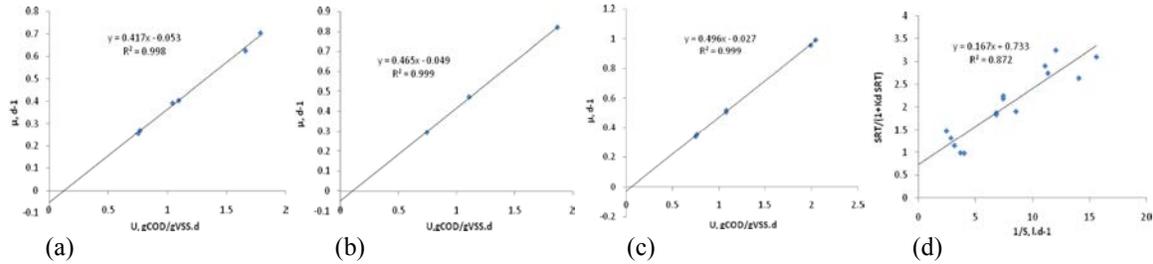


Fig. 2: Estimation of the yield coefficient of biomass ( $Y$ ) and bacteria decay rate ( $k_d$ ) for SCOD removal in, (a) aeration time =30 min/h, (b) aeration time =40 min/h, (c), aeration time =50 min/h, (d) Estimation of the half velocity coefficient ( $K_s$ ) and maximum rate of substrate degradation ( $U_{max}$ ) for SCOD removal.

that HRT and aeration time played an important role in COD removal in the process. From the results presented in the Table 2, the response increased upon increasing the aeration time at lower HRT while at higher HRT, aeration time did not show significant effect on COD removal. It was attributed to sufficient aeration time at higher HRT which makes the response independent to aeration time in the design space studied. As a result, as the HRT increases, less aeration time is needed. The COD /N ratio (in the range of 4-20) did not show a strong effect on the process, as only a small difference is observed in the results obtained with different COD/N ratio. However, the response showed a little increase as the COD/N ratio was increased. Many methods have been used to describe the overall kinetics of organic removal in biological treatment systems. Here, the Monod model, first-order model, Stover-Kincannon model and second-order model (based on Substrate) were selected for describing COD removal rate in the UAASFF reactor.

### Kinetics Evaluation for SCOD Removal

**Mass Balance-based (Monod) Model:** In order to estimate the cell yield coefficient ( $Y$ ) and biomass decay coefficient ( $k_d$ ), the relationship between the inverse SRT ( $\mu = 1/SRT$ ) and the specific substrate utilization rate ( $U$ ) (Eq. (5)) in the different conditions of aeration times for COD removal was plotted in the Fig. 2a-c.  $Y$  and  $k_d$  values were determined to be in the range of 0.417-0.496 g VSS g/COD and 0.027-0.053  $d^{-1}$ , respectively, as shown in Table 3. As noted in the Table 3, an increase in aeration time (from 30 to 50 min/h) caused an increase in  $Y$  due to higher sludge production resulted from high COD consumption rate in the higher aeration time. Dissolved oxygen serves only as an electron acceptor for heterotrophic aerobes in wastewater.

The  $k_d$  is of little significance when the retention time is short, being an order of magnitude less than  $\mu$ . However, when the system is operated in the endogenous

growth phase,  $k_d$  is important in the calculation of the net amount of microorganisms produced and the oxygen utilization rate [28]. The lower values of  $k_d$  in this study resulted in short hydraulic retention time. The value of  $Y$  relates to concentration of biomass in the bioreactor and amount of excess sludge wasted. The higher values of  $Y$  obtained from the present work compared to the other study was because of short HRT (corresponding to higher OLR) and more biodegradability of substrate in this research.

The maximum growth rates ( $\mu_{max}$ ) and substrate saturation constant ( $K_s$ ), were computed by plotting the experimental data as  $SRT/((1+k_d*SRT))$  vs.  $1/S$  (Fig. 2d). The values of kinetics coefficients,  $\mu_{max}$  and  $K_s$ , were 1.36 g VSS produced /g VSS and 0.22 gCOD/l, respectively (Table 3). The  $K_s$ , as an apparent kinetic constant, represent the half velocity coefficient of bacteria which has reverse relationship with yield coefficient ( $Y$ ) and bacteria decay rate ( $k_d$ ) [29]. Also  $K_s$  determines how rapidly  $\mu$  approaches  $\mu_{max}$  and it is defined as the substrate concentration at which  $\mu$  is equal to half of  $\mu_{max}$  ( $\mu = \mu_{max}/2$ ) [30]. This is the basis for all continuous flow treatment processes in biological wastewater treatment in which microorganisms are continuously cultivated but the overall rate of metabolism is controlled by the substrate concentration.

Table 4 shows the kinetic coefficients reported from different studies. As presented in Table 4, the value of the  $\mu_{max}$  (1.36  $d^{-1}$ ) obtained in this study was higher than the  $\mu_{max}$  value found by Carta-Escobar *et al.* [16] but smaller than the values reported by Kaewsuk *et al.* [28] (Table 4). The most likely reason for the differences in the kinetic coefficients compared with the values is the significant discrepancy in reactor configurations and wastewater composition [31]. According to the Monod equation, high  $\mu_{max}$  value relates to the high substrate removal rate, indicating relatively high removal rate in the UAASFF bioreactor.

Table 3: Kinetic parameters for COD removal in UAASFF reactor

Variables		Kinetic parameters															
		Mass balance (Monod) model							First order model	Stover-Kin cannon model	Second order model						
Run	HRT h	COD: TKN:TP g:g:g	Aeration time min/h	Regression equation	R <sup>2</sup>	Y COD	k <sub>d</sub> VSS.d	Regression equation	R <sup>2</sup>	μ <sub>max</sub> g new cell/g cell.d	K <sub>s</sub> gCOD/l	k <sub>1</sub> d <sup>-1</sup>	K <sub>0</sub> g.l.d <sup>-1</sup>	U <sub>max</sub> g.l.d <sup>-1</sup>	k <sub>20</sub> d <sup>-1</sup>	a	b
1	2	1:0.25:0.05	30	Y=0.417x-0.053	0.99	0.417	0.053					12.09					
	4.25	1:0.25:0.05	30														
	6.5	1:0.25:0.05	30														
2	2	1:0.05:0.02	30														
	4.25	1:0.05:0.02	30														
	6.5	1:0.05:0.02	30														
3	2	1:0.083:0.035	40	Y=0.465x-0.049	0.99	0.465	0.049	Y=0.167x+0.733	0.91	1.36	0.22		37.65	38.46	5.95	0.042	0.928
	4.25	1:0.083:0.035	40									19.48					
	6.5	1:0.083:0.035	40														
	2	1:0.25:0.05	50														
4	4.25	1:0.25:0.05	50	Y=0.496 - 0.027	0.99	0.496	0.027					30.71					
	6.5	1:0.25:0.05	50														
	2	1:0.05:0.02	50														
5	4.25	1:0.05:0.02	50														
	6.5	1:0.05:0.02	50														

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

Models	Substrate	Type of reactor	CODin g/l	HRT d	Kinetic parameters				Reference
					Y	k <sub>d</sub>	μ <sub>max</sub>	K <sub>s</sub>	
Monod	Domestic wastewater	MBR	-	-	0.25 - 0.40	0.04-0.075	-	-	Huang <i>et al.</i> , 2001
	Dairy wastewater	AS	-	2.3-2.4	0.26	0.032	0.440	0.141	Escobar <i>et al.</i> , 2004
	Dairy wastewater	MSBR	2.5	10	0.23	0.14	1.69	0.174	Kaewsuk <i>et al.</i> , 2010
	Synthetic wastewater	UAASFF	1	0.083-0.271	0.417-0.496	0.027-0.053	1.36	0.22	This study
First-order	sugar-manufacturing wastewater	UAIB	0.75-4.5	0.5-1.0		14.549			Borghei <i>et al.</i> , 2007
	Synthetic wastewater	UAASFF	1	0.083-0.271		12.09 -30.71			This study
Stover-Kincannon	sugar-manufacturing wastewater	UAIB	0.75-4.5	0.5-1.0		U <sub>max</sub> 101		K <sub>B</sub> 106.8	Borghei <i>et al.</i> , 2007
	Synthetic wastewater	MBBR	0.75-4.5	1		8.3		9.45	Borghei and Hosseiny, 2002
	Soybean wastewater	AF	7.5-11.45	1-1.45		83.3		85.5	Yu <i>et al.</i> , 1998
	Synthetic wastewater	UAASFF	1	0.083-0.271		38.46		37.88	This study
Second order (Grau)	Synthetic wastewater	UAIB	0.75-4.5	0.5-1.0		k <sub>2</sub> (s) 3.582		a 0.047 b 1.007	Borghei <i>et al.</i> , 2008
	Molasses	RBC	2-15	0.5-2.0		10.81		0.033 1.192	Optaken ,1982
	Synthetic wastewater	UAASFF	1	0.083-0.271		5.95		0.042 0.928	This study

**First Order Model:** The majority of biological wastewater treatment processes are described by first-order kinetics. Reaction orders can differ when there is variation in the microorganisms, the substrate or environmental conditions and they must be measured experimentally. The BOD and COD removal has been traditionally modeled as a continuous first order reaction [32]. In this type of reaction, the rate of breakdown is at first rapid when the organic content is high, but gets progressively slower as the organic material is utilized.

Fig. 3a-c shows the correlation between the (S<sub>0</sub>-S)/HRT and the substrate concentration (S) in the bioreactor drawn based on the first-order equation (Eq. (11)). The data fitted well with an R<sup>2</sup> > 0.93. The high values of the determination coefficients (R<sup>2</sup>) clearly indicate that first-order kinetics can be applied with good

degree of precision. The values of first-order kinetic constant (k<sub>1</sub>) were calculated to be in the range of 12.09 - 30.71 d<sup>-1</sup> ( Table 3). It was found that with an increase in the aeration time, the first-order kinetic constant (k<sub>1</sub>) was increased favoring the biodegradation reaction of the substrate. As data presented in the Table 4, in a similar work, the first-order model was applied for the process kinetics of in a lab-scale upflow aerobic immobilized biomass (UAIB) reactor treating simulated sugar-manufacturing wastewater [25]. In this study, k<sub>1</sub> obtained 14.549 d<sup>-1</sup> with correlation coefficient of 0.742. The difference between k<sub>1</sub> values obtained from the two studies might be attributed to the difference in type of the wastewater used as feed and the OLR applied. The results indicated that the UAASFF reactor described in this study was capable to biodegrade the organic matter up to 93 % at a low HRT (6.5 h).

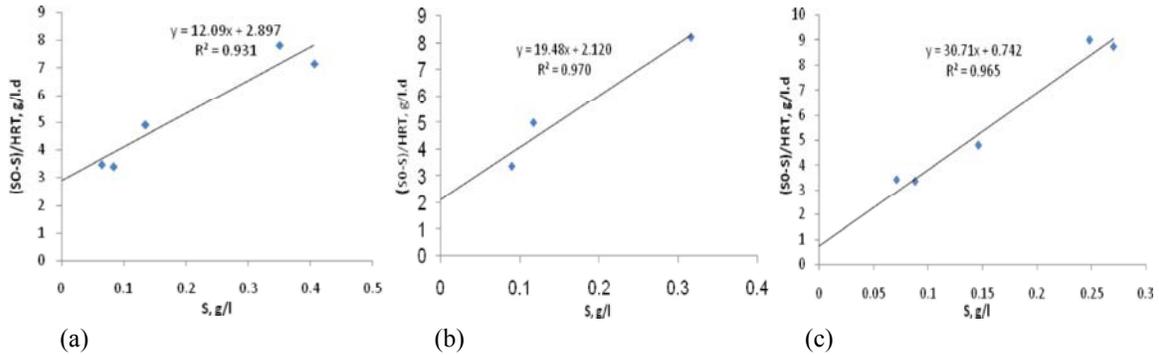


Fig. 3: First-order kinetics model plot for SCOD removal in, (a) aeration time =30 min/h , (c) aeration time= 40 min/h, (d) aeration time =50 min/h.

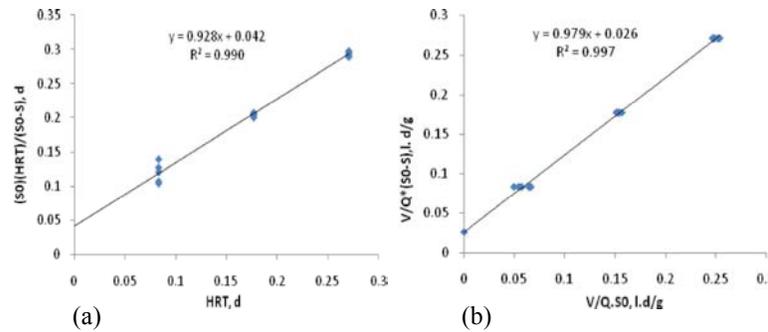


Fig. 4: (a) Second-order kinetics model plot for SCOD removal (b) Stover-Kincannon model plot for SCOD removal.

**Second-Order Model (Grau Model):** The second-order model was also used to evaluate the rate of the biodegradation process occurred in the UAASFF bioreactor. In order to determine the kinetic coefficients (a, b and  $k_{2(s)}$ ), Eq. (14) was plotted as shown in Fig. 4a. It must be noted that the substrate concentration is of high importance in second order reactions. Table 4 presents a comparable data for second-order kinetics from different studies [19, 25]. The values of  $K_{2(s)}$  reported in the previous studies were in the range of 3.58-10.81  $d^{-1}$ , where the value of  $k_{2(s)}$  was 5.95( $d^{-1}$ ). The obtained results in this study, conforming the reported date in literature. The correlation coefficient ( $R^2$ ) was 0.99, indicating the excellent agreement between the experimental and the modeled data.

Based on the Eq. (14), the relationship between effluent COD concentration and HRT is described as follows:

$$S = S_0 \left( 1 - \frac{HRT}{0.042 + 0.928HRT} \right) \quad (23)$$

and the substrate removal efficiency is represented by

$$E = \frac{HRT}{0.042 + 0.928HRT} \quad (24)$$

**Stover-Kincannon Model:** Stover-kincannon model investigates the effect of the influent OLR on the removed OLR. Fig. 4b shows the graph plotted between inverse the removed OLR,  $V/(Q(S_0 - S))$ , vs. inverse OLR<sub>m</sub>,  $V/(QS_0)$ . According to Eq. (18), saturation constant ( $K_B$ ) and maximum total substrate utilization rate ( $U_{max}$ ) were calculated (Fig. 4b). The constant values for  $K_B$  and  $U_{max}$  were obtained 37.65 and 38.46 g/l.d, respectively (Table 4). It is resulted from the experimental data that the maximum removed OLR (OLR<sub>rem</sub>) in the reactor was 22.56 g/l.d, implying that the reactor possessed an excellent COD removal capacity. The Stover- Kincannon model can also be used to determine the volume required to decrease the influent nutrient concentration from  $S_0$  to  $S$  or to determine the effluent nutrient concentration for a given volume of the UAASFF bioreactor and influent nutrient concentration. From substrate mass balance equation (Eq. (21)), the following equation can be obtained:

$$S = S_0 - \frac{38.46S_0}{37.65 + (QS_0/V)} \quad (25)$$

The model developed for prediction of the total substrate removal efficiency in the present work is as follows:

$$E = \frac{38.46}{37.65 + (QS_0/V)} \quad (26)$$

Consequently, the results of the kinetic studies obtained from the lab-scale experiments can be used for estimating treatment efficiency of a full-scale process under similar operational conditions. Therefore, the Stover-Kincannon model could be used in the design of the UAASFF bioreactor. As seen in Table 4, a wide range of kinetic constants ( $U_{max}$  and  $K_B$ ) were determined depending on the characteristics of the studied wastewaters and experimental conditions. Higher values of the constants have been reported for treating readily biodegradable substrates, such as molasses and glucose, while the lower values result from the presence of several recalcitrant inorganic compounds, complex components and other undesirable impurities in the wastewaters [22, 33-34]. The  $U_{max}$  value (38.46 g/l.d) obtained in this study was smaller than the value found by Borghei and his coworkers [25] (101 g/l.d) and larger than that obtained by Borghei and Hosseiny [35] (8.3 g/l.d). The wide range reported for  $U_{max}$  is attributed to different factors like type of reactor, OLR applied, wastewater characteristics and microorganisms used in the studies.

### TN Removal

**Process Description:** During the course of the system's operation, the removal of the nitrogen contents was also monitored. The influent and effluent concentrations of TN, TKN and nitrate for steady state conditions are presented in Table 2. The concentration of ammonium nitrogen in this study was in the range of 50-250 mg/l. The performance of the UAASFF reactor under different HRT, COD:N:P ratio and aeration time is shown in Table 3. The results indicate that high efficiencies of TN removal were attained at high HRT and low aeration time. A reverse impact of the aeration time on TN removal was observed as the variable increased (Table 2). An increase in the aeration time (from 30 to 40 min/h) caused an increase in the response due to higher  $NO_3^-$  production as well as the favored condition for denitrification resulted from high DO consumption rate. Further increment in the variable (from 40 to 50 min/h) decreased the response. This was due to domination of nitrification over denitrification process, which was originated from much shortened time of settling. The maximum TN removal efficiency was found to be 79% at HRT, COD/N ratio and aeration time of 6.5 h, 12 and 40 min/h, respectively.

It has been argued that the TN removal process is so complex that it cannot be adequately described solely by the first-order reaction equation, with good degree of

precision [24,36]. As the fraction of nitrifiers and denitrifiers in the biomass contents of the bioreactor was not investigated, so the Monod model could not be reliably employed to describe the TN removal process. Therefore, in this study the kinetic of the TN removal was studied using second-order and Stover-Kincannon models.

### Kinetic Evaluation for TN Removal

**Second-Order Substrate Removal Model:** Grau second-order model coefficients were determined by plotting Eq. (14) (Fig. 5a-e). The values of  $k_{2(s)}$ , a and b are presented in Table 5. The  $R^2$  of the second-order kinetic model was in the range of 0.79-1.0. Coefficients (a and b) for different aeration time and COD/N ratio studied are shown in Table 5. It is clear from the results that the both variables were effective on the  $k_{2(s)}$ . It was found that with an increase in aeration time, the average  $k_{2(s)}$  was increased while with an increase in COD/N ratio the average  $k_{2(s)}$  was decreased.

**Stover-Kincannon Model:** This model is capable of predicting substrate removal at any loading conditions, no matter which order kinetics [36]. Fig. 6a-e depicts the graphs plotted as inverse removed nitrogen loading rate,  $[V/(Q(TN_{in} - TN_{out}))]$ , versus the inverse total nitrogen loading rate,  $[V/(Q TN_{in})]$ , at different conditions of aeration times (30, 40 and 50 min/h) and COD/N ratios (4, 12 and 20). As biokinetic data are presented in Table 5, the saturation constant ( $K_B$ ) and the maximum utilization rate ( $U_{max}$ ) for different conditions were computed to be in the ranges between 0.271-7.25 and 0.333-5.43 g/l.d, respectively.

The correlation coefficients ( $R^2$ ) were over 0.87. The maximum values of  $K_B$  and  $U_{max}$  were determined to be 7.25 and 5.43 g/l.d, respectively. At aeration time and COD/N ratio of 50 min/h and 4 while the minimum values were obtained 0.271 and 0.333 at aeration time and COD/N ratio of 30 min/h and 12, respectively (Table 5). The results showed that  $K_B$  and  $U_{max}$  were very sensitive to aeration time and COD/N ratio such that with an increase in aeration time, the values of  $K_B$  and  $U_{max}$  were increased while an increase in COD/N ratios caused a decrease in the constants. It was attributed to that the nitrogen serves as an essential nutrient for all living organisms, including the heterotrophic bacteria that remove organic pollutants from wastewater. Therefore, as aeration time increases, a greater percentage of nitrogen is removed via bacterial growth and reproduction (i.e. assimilation into new cell mass).

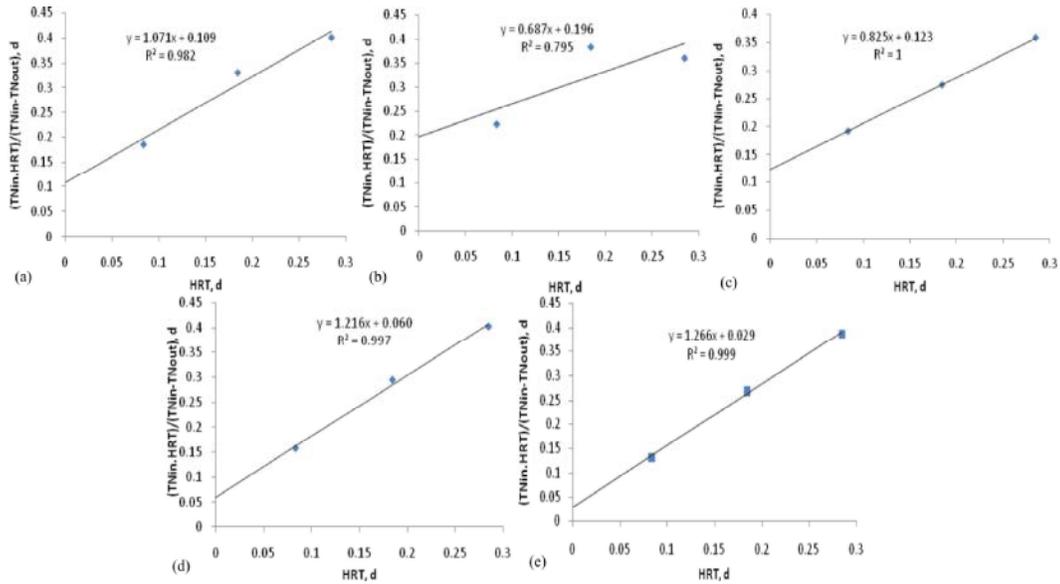


Fig. 5: Second-order kinetics model plots for TN removal in, (a) aeration time =30 min/h and COD/N ratio= 4, (b) aeration time= 30 min/h and COD/N ratio= 20, (c) aeration time = 40 min/h and COD/N = 12, (d) aeration time =50 min/h and COD/N ratio= 4, (e) aeration time =50 min/h and COD/N ratio= 4.

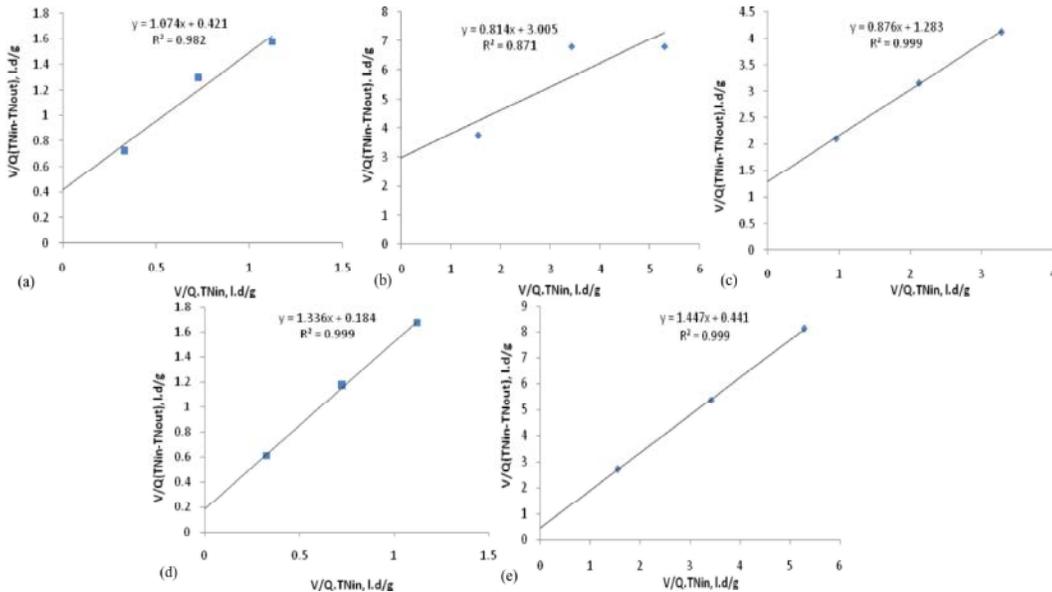


Fig. 6: Stover-Kincannon model plots for TN removal in, (a) aeration time =30 min/h and COD/N ratio= 4, (b) aeration time= 30 min/h and COD/N ratio= 20, (c) aeration time = 40 min/h and COD/N = 12, (d) aeration time =50 min/h and COD/N ratio= 4, (e) aeration time =50 min/h and COD/N ratio= 4.

The Kinetic coefficients  $K_B$  and  $U_{max}$  for TN removal (0.271-7.25 and 0.333-5.43 g/l.d respectively) were lower than the values found by Jin and Zheng [36] (12 and 12.4 g/l.d, respectively). This could be attributed to the relatively high nitrogen loading rate applied to the bioreactor in this study.

### TP Removal

**Process Description:** Removal of phosphorus in wastewater is closely dependent upon the phosphorus release in anaerobic conditions and on the subsequent uptake process of the excess phosphorus including that contained in wastewater in aerobic conditions.

Table 6: Kinetic parameters for biological phosphorus removal in UAASFF reactor

Run	Variables			Models								
				Second order model					Stover-Kin cannon model			
	HRT h	COD:TKN:TP g:g:g	Aeration time min/h	Regression equation	R <sup>2</sup>	k <sub>2(s)</sub> d <sup>-1</sup>	a	b	Regression equation	R <sup>2</sup>	U <sub>max</sub> g.l.d <sup>-1</sup>	K <sub>B</sub> g.l.d <sup>-1</sup>
1	2 4.25 6.5	1:0.25:0.05	30	Y=0.69x+0.152	0.99	0.08	0.152	0.69	Y=0.69x+3.05	0.99	0.33	0.31
2	2 4.25 6.5	1:0.05:0.02	30	Y=0.94x+0.117	0.99	0.04	0.117	0.94	Y=0.94x+5.86	0.99	0.16	0.17
3	2 4.25 6.5	1:0.083:0.035	40	Y=0.96x+0.153	0.98	0.057	0.153	0.96	Y=0.96x+4.38	0.98	0.23	0.22
4	2 4.25 6.5	1:0.25:0.05	50	Y=2.12x+0.117	0.96	0.11	0.117	2.12	Y=2.12x+2.36	0.96	0.42	0.89
5	2 4.25 6.5	1:0.05:0.02	50	Y=1.22x-0.256	0.99	0.019	0.256	1.2	Y=1.22x-12.8	0.99	0.07	0.09

This basic information indicates that control of anaerobic (or anoxic) and aerobic conditions are of great importance to biological phosphorus removal. In the present study, as the system is intermittently aerated, a micro anaerobic environment seems to be provided in the biofloc formed in the process. The maximum observed phosphorus removal efficiency was about 79.1 % at COD:P ratio and aeration time 20 and 30 min/h, respectively. While, the minimum value of the response (23.3 %) was obtained at COD:P ratio and aeration time 50 and 50 min/h, respectively. As shown in Table 3, the response decreased upon increasing the aeration time. An increase in aeration time causes a decrease in the anaerobic time when the PAOs accumulate polyhydroxy butyrate (PHB) from volatile fatty acids (VFAs) produced. In this process, glucose as the individual source of VFAs requires sufficient time for acidification [37]. The reason for the decrease in phosphorous removal at high aeration time was due to the presence of nitrate, which inhibits the fermentation processes producing VFAs in the anaerobic zone.

#### Kinetic Evaluation for TP Removal

**Grau Second-order Substrate Removal Model:** The second-order kinetic coefficients ( $k_{2(s)}$ ,  $a$  and  $b$ ) are summarized as shown in Table 6. Very high agreement was found between the experimental and the model data ( $R^2=0.96$ ). The second-order substrate removal rate constant values  $k_{2(s)}$  ( $a = P_{in}/(k_{2(s)}X)$ ), were in the range of 0.04-0.11d<sup>-1</sup> (Table 6). Almost the same trend in  $k_{2(s)}$  as what obtained for TN removal was found for P removal with smaller amount, indicating slower reaction rate compared to N removal rate.

**Stover-Kincannon Model:** Stover-Kincannon model was also used to assess the kinetic P removal process. The

different coefficient ( $U_{max}$  and  $K_B$ ) were computed as presented in Table 6. Determination coefficient showed very good regression ( $R^2=0.96$ ). The values of  $U_{max}$  and  $K_B$  were in the range of 0.07-0.42 g/l.d and 0.09-0.89 g/l.d, respectively. From the results, the maximum values of  $U_{max}$  and  $K_B$  were obtained to be 0.42 and 0.89 g P/l.d for aeration time and COD/P ratio 50 min/h and 20, respectively; while the minimum values of these parameters were 0.07 g/l.d and 0.09 g/l.d for aeration time and COD/P ratio, 50 min/h and 50, respectively.

#### CONCLUSION

Kinetic analysis of the UAASFF bioreactor using the experimental data obtained under different HRT, COD: N: P ratio and aeration time was successfully preformed. The maximum removal efficiencies of COD, TN and TP were obtained 93.6, 79 and 79.1 %, respectively. All the models examined, gave high correlation coefficients, for carbon, nitrogen and phosphorus removal. The maximum  $U_{max}$  for COD, TN and TP were found to be 22.56, 5.43 and 0.42 g/l.d, respectively. From the obtained biokinetic data suggesting that the reactor possessed an excellent COD, N and P removal capacity.

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**Nomenclature**

TKN	Total Kjeldahl nitrogen	g/l
MLVSS	Mixed liquor volatile suspended solids	g/l
rbCOD	Readily biodegradable chemical oxygen demand	g/l
TN	Total nitrogen	g/l
VSS	Volatile suspended solid	g/l
SRT	Solid retention time	d <sup>-1</sup>
OLR	Organic loading rate	g/l.d
Y	Growth yield coefficient	g VSS/g COD
k <sub>d</sub>	Microbial decay rate constant	d <sup>-1</sup>
μ <sub>max</sub>	Maximum specific biomass growth rate	g VSS produced /g VSS present. d
K <sub>s</sub>	Half-velocity constant	g/l
U <sub>max</sub>	Maximum substrate utilization rate constant	g/l.d
K <sub>B</sub>	Saturation constant	g/l.d
r <sub>su</sub> = ds/dt	Rate of change in the substrate concentration due to utilization	g/l. d
X	Biomass concentration	g/l
S <sub>0</sub>	Influent substrate concentration	g/l
S	Substrate concentration	g/l
μ	Specific biomass growth rate	g VSS produced /g VSS present. d
k <sub>1</sub>	First-order substrate removal rate constant	d <sup>-1</sup>
k <sub>2(S)</sub>	Second -order substrate removal rate constant	d <sup>-1</sup>
Q <sub>0</sub>	Influent flow rate	l/d
Q	Effluent flow rate	l/d
Q <sub>w</sub>	Waste sludge flow rate	l/d
X <sub>e</sub>	Effluent biomass concentration	g/l
X <sub>w</sub>	Effluent biomass concentration	g/l
V	Reactor volume	L
U = r <sub>su</sub> /X	Specific substrate utilization rate	g COD/g VSS. d

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### Persian Abstract

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#### چکیده

در این مطالعه، حذف همزمان کربن، نیتروژن و فسفر از فاضلاب سنتزی در یک بیوراکتور با جریان رو به بالای هوازی/انوکسیک با فیلم ثابت لجن (UAASFF) مورد بررسی قرار گرفت. تحلیل سینتیکی با استفاده از داده های آزمایشگاهی به دست آمده از مطالعه قبلی که در آن بیوراکتور UAASFF تحت شرایط راهبری مختلف با تغییر سه متغیر مستقل HRT، نسبت COD:N:P و زمان هوادهی مورد آزمایش قرار گرفته بود، انجام شد. در این تحلیل مدل های مختلف سینتیکی (مونود، درجه اول، درجه دوم؛ و استوور کین کین) مورد ارزیابی قرار گرفتند. حداکثر راندمان حذف برای COD، نیتروژن کل (TN) و فسفر کل (TP) به ترتیب ۷۹، ۹۵/۴۲ و ۷۹٪ به دست آمد. تمام مدل های آزمایش شده، ضرایب همبستگی بالایی را برای حذف کربن، نیتروژن و فسفر نشان دادند. ضرائب سینتیکی به دست آمده در این مطالعه به صورت زیر می باشند:

$$Y = 0.417-0.496 \text{ g VSS/g COD}, k_d = 0.027-0.053 \text{ d}^{-1}, \mu_{\max} = 1.36 \text{ g VSS/g VSS.d}, K_B = 37.96 \text{ g/l.d}, U_{\max} = 38.46 \text{ g/l.d}, \\ K_B(N) = 0.271-7.2 \text{ g/l.d}, U_{\max}(N) = 0.33-5.4 \text{ g/l.d}, K_B(P) = 0.09-0.89 \text{ g/l.d}, U_{\max}(P) = 0.07-0.42 \text{ g/l.d}$$

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