J. Biochem. **136**, 177–182 (2004)
DOI: 10.1093/jb/mvh110

Anaerobic Treatment of Antibiotic Production Wastewater and Kinetic Evaluations

İlda Değirmentaş and Nuran Deveci[*]

Chemical Engineering Department, Chemical and Metallurgical Faculty, Istanbul Technical University, 34469, Maslak, Istanbul, Turkey

Received April 14, 2004; accepted May 11, 2004

In this study, the anaerobic treatment of high-strength antibiotic production wastewater and the development of a mathematical model for the treatment were attempted. Anaerobic treatability was investigated using synthetic solutions and original wastewater of which the initial chemical oxygen demand (COD) was determined. Initial COD of solutions was increased from 3,000 to 43,000 mg O_2/liter in an anaerobic bioreactor. The bioreactor pH was maintained at 6.5–7.5. The temperature was kept constant at 37 ± 1°C. Raw materials and original wastewater containing penicillin antibiotics were obtained from Fako Pharmaceutical Factory (Fako) in Istanbul, Turkey. Anaerobic sludge used for treatment was obtained from Pakmaya Baker's Yeast Producing Factory (Pakmaya) in Izmit, Turkey and the Fako. A mathematical model based on substrate (total COD) concentration was developed assuming that only three consecutive reactions, namely, hydrolysis, acidogenesis and methanogenesis, are significant. From the experimental data, a model that can be used for COD calculation as a function of time was developed using the first- and the second-order kinetic approaches. Making use of the developed model equation, it was proved that the anaerobic treatment of high strength (COD > 25,000 mg O_2/liter) antibiotic production wastewater fits the second-order kinetics.

Key words: anaerobic, antibiotic, kinetics, modeling, wastewater treatment.

Anaerobic digestion is a multistage biochemical process that can stabilize many different types of organic material. Digestion occurs in three basic stages. In the first stage, the hydrolysis phase, extracellular enzymes break down solid complex organics, cellulose, proteins, lignins, and lipids into soluble organic fatty acids, alcohols, carbon dioxide, and ammonia. In the second stage, the acidogenesis phase, microorganisms convert the products of the first stage into acetic acid, propionic acid, hydrogen, carbon dioxide, and other low molecular weight organic acids. In the third stage, the methanogenesis phase, two groups of methane-forming bacteria work. One group converts hydrogen and carbon dioxide to methane; the other converts acetate to methane and bicarbonate (*1*, *2*).

Since wastewaters from pharmaceutical companies have high biochemical oxygen demand (BOD) and suspended solid (SS) concentrations, and high flow rate, they need to be treated with the most suitable method before discharge. However, research into the treatment of antibiotic production wastewaters or the treatment of extremely contaminated wastewater has not been reported in the literature. So our research will clarify these points.

This paper presents a mathematical model developed for anaerobic treatment of high-strength antibiotic production wastewater by using data from laboratory experiments.

MATERIALS AND METHODS

Raw materials and original antibiotic wastewater were obtained from the Fako Medicine Producing Factory at Istanbul, Turkey. The characteristics of high-strength process effluents from Fako antibiotic production factory (Fako) are presented in Table 1.

Wastewater was stored at 4°C to avoid biodegradation and treated as soon as possible. Anaerobic sludge was obtained from Pakmaya Baker's Yeast Producing Factory (Pakmaya) at Izmit, Turkey and Fako.

Anaerobic treatability was investigated using synthetic solutions and original wastewater, of which the initial chemical oxygen demand (COD) was determined at the beginning of experiments. The anaerobic biodegradation was investigated in a laboratory-scale bioreactor (Fig. 1) of 2 liters in volume. The stirred reactor was suitable for nutrient addition, pH and temperature control. The bioreactor contents (penicillin wastewater) were maintained at pH 6.5–7.5. The temperature was kept constant at 37 ± 1°C, and mixing speed 150 rpm.

Table 1. **Characteristics of high-strength process effluents from Fako Medicine Producing Factory, Istanbul-Turkey.**

Parameter	Range
COD (mg O_2/l)	10,000–43,000
pH	7.75–10.62
TS (mg/l)	16,800–23,800
VTS (mg/l)	6,210–8,240
SS (mg/l)	120–580
VSS (mg/l)	100–560

---

[*] To whom correspondence should be addressed.Tel: +90212-285-34-28, +90212-466-54-56, Fax: +90212-285-29-25, E-mail: nuran@itu.edu.tr

EXPERIMENTAL STUDY

In the experimental studies, the wastewater samples were prepared by diluting the original wastewater and preparing synthetic solutions to obtain various COD values between 3,000 and 43,000 mg O_2/liter. During the experiments, COD, volatile fatty acids (VFA), total solids (TS), suspended solids (SS), dissolved solids (DS), volatile total solids (VTS), and volatile suspended solids (VSS) were determined according to standard methods (*3*). pH control was carried out in the samples taken at start-up, shut-down and periodically during the treatment. A mathematical model of anaerobic treatment was developed. Reaction rate constants k_1, k_2 and k_3 (given in italics) were calculated according to the first-order kinetic equation, given by Ciftci (*4*). The constants of the developed model were calculated according to the second-order kinetic approach by using the Levenberg-Marquardt algorithm (Sigma Plot Scientific Graph System Program, Jandel Scientific Co.).

Model Development—In the literature, kinetic analyses of anaerobic treatment of industrial wastewaters have been reported. Kinetic models that have been successfully tested include the Monod model, the Haldane model, the Stover-Kincannon model, and the Contois model. Of these, the Monod model seems to be employed most frequently to describe the anaerobic treatment kinetics. However, in some situations, the Monod model is insufficient to explain the treatment kinetics (*5–10*).

In a recent study, a mathematical model for the anaerobic treatment of Baker's yeast effluents was developed (*4*). The purpose was to develop a practical mathematical model for the anaerobic digestion of the wastewater taken from the baker's yeast industry and to describe the reduction of COD by using the data obtained from laboratory experiments. According to this study, the biodegradation of the baker's yeast effluents fitted first-order kinetics, and could be defined with Eq. 1 given below (COD of low and medium strength wastewater of 3,000–22,500 mg O_2/liter):

$$S_T = S_{A0} \cdot e^{-k_1 t} + k_1 S_{A0}\left(\frac{e^{-k_2 t} - e^{-k_1 t}}{k_1 - k_2}\right) + k_1 k_2 S_{A0}\left(\frac{(k_2 - k_3) \cdot e^{-k_1 t} - (k_1 - k_3) \cdot e^{-k_2 t} + (k_1 - k_2) \cdot e^{-k_3 t}}{(k_1 - k_2)(k_2 - k_3)(k_1 - k_2)}\right) \tag{1}$$

where k_1 is the hydrolysis stage reaction rate constant, k_2 is the acidogenesis stage reaction rate constant, and k_3 is the methanogenesis stage reaction rate constant according to the first-order kinetic model.

This study also made use of this equation (*i.e.*, Eq. 1) for describing the biodegradation reaction of antibiotic wastewater with respect to first-order kinetics.

The purpose of this study was to develop a mathematical model for anaerobic treatment of the high-strength antibiotic production wastewater by using laboratory data. Therefore, the following assumptions were made to simplify the development of the model.

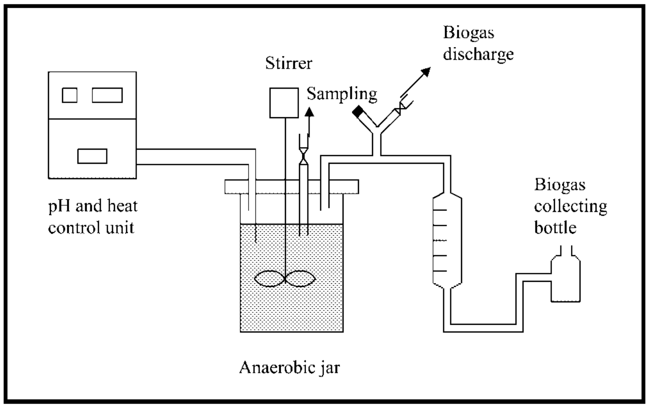

Fig. 1. **Experimental apparatus.**

(1) Anaerobic degradation of organic matter is a complicated biological process, and the conversion of organic matter through microbial activities to methane and carbon dioxide consists of several independent, consecutive and parallel reactions. From a practical point of view, it may be accepted that only the three major steps, hydrolysis, acidogenesis and methanogenesis, are significant. The proposed mechanism is given below:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_3} D \tag{2}$$

where A is the original complex organic material, B represents hydrolysis products, C volatile fatty acids, D biogas, and k_1, k_2, k_3 are reaction rate constants.

(2) All three reactions are assumed to be irreversible and follow second-order kinetics because of the big differences between the model and experimental data for the anaerobic treatment of high strength (COD > 25,000 mg O_2/liter) antibiotic production wastewater. For this reason, it is thought that the overall mechanism cannot follow first-order kinetics.

(3) Microbial growth is negligible in the stoichiometry.

(4) The model is based on the unit of COD due to the complexity and variability of substrate concentrations. Because of the compatibility between the consecutive anaerobic digestion stages (*i.e.*, the product of each stage is the reagent of the following stage) during the treatment reaction; A (the total COD) is converted into a measurable quantity C (*i.e.*, VFA) without accumulation of B in the system.

(5) Methane is insoluble in the liquid.

Using the assumptions above, the material balances for the four components may be shown as follows:

$$\frac{dS_A}{dt} = -k_1 S_A^2 \tag{3}$$

$$\frac{dS_B}{dt} = k_1 S_A^2 - k_2 S_B^2 \tag{4}$$

$$\frac{dS_C}{dt} = k_2 S_B^2 - k_3 S_C^2 \tag{5}$$

$$\frac{dS_D}{dt} = k_3 S_C^2 \tag{6}$$

Table 2. **Second-order reaction rate constants k_1, k_2 and k_3.***

COD (mg O_2/l)	$k_1 \times 10^7$ (l/mg·h)	$k_2 \times 10^4$ (l/mg·h)	$k_3 \times 10^{-2}$ (l/mg·h)
3,000–10,000	2.0460–9.3360	0.3071–9.8530	2.7480–75.1600
11,000–22,500	0.8957–3.5980	0.0825–2.6510	6.6020–287.700
31,500–43,000	0.4459–0.9207	0.0342–0.0397	605.800–719.800

*The rate constants k_1, k_2, and k_3 were calculated for the given COD interval.

where S_A is hydrolysable substrate concentration, S_B is hydrolysis products concentration, S_C is volatile fatty acids concentration, and S_D is biogas concentration.

Solving the above equations for these consecutive reactions, the following equations can be obtained:

$$S_A = \frac{S_{A0}}{1 + k_1 S_{A0} t} \tag{7}$$

$$\begin{aligned} S_B = {} & 1 / 2k_2(1 + tk_1S_{A0}) \times [-\sqrt{k_1}\sqrt{k_1 + 4k_2}\,S_{A0} \\ & + ((2\sqrt{k_1}\sqrt{k_1 + 4k_2}S_{A0}) / (1 + (1 + tk_1S_{A0})^{-\sqrt{k_1+4k_2}/\sqrt{k_1}} \\ & (k_1S_{A0} + \sqrt{k_1}\sqrt{k_1 + 4k_2}\,S_{A0} - 2k_2S_{B0}) / \\ & (-k_1S_{A0} + \sqrt{k_1}\sqrt{k_1 + 4k_2}\,S_{A0} + 2k_2S_{B0})))] \\ & + k_1S_{A0} / 2k_2 + 2tk_1k_2S_{A0} \end{aligned} \tag{8}$$

$$S_C = \frac{k_1 S_{A0}}{2k_3 + 2tk_1k_3S_{A0}} + \frac{\sqrt{k_1(k_1^2 + 4k_1k_2 + 4k_2k_3)S_{A0}^2}}{2\sqrt{k_1 + 4k_2}\,k_3(1 + tk_1S_{A0})} \tag{9}$$

$$\begin{aligned} S_D = {} & ((((k_1^2 + 4k_1k_2 + 2k_2k_3)S_{A0} \\ & + \sqrt{k_1 + 4k_2}\sqrt{k_1(k_1^2 + 4k_1k_2 + 4k_2k_3)S_{A0}^2}) \\ & (-1 + (k_1 + 4k_2)^2k_3^2(1 + tk_1S_{A0}))) / \\ & 2(k_1 + 4k_2)k_3(1 + tk_1S_{A0})) \end{aligned} \tag{10}$$

where S_{A0} is the initial substrate (total COD) concentration (mg O_2/liter). Because of the mathematical complexity of the above equations, it is extremely difficult to determine the concentration of each component for each step. However, the effluent concentration (S_T), which is measured in the units of COD, represents the total concentration of all components in the system.

$$S_T = S_A + S_B + S_C + S_D \tag{11}$$

So the following mathematical model can be developed:

$$S_T = \left(\frac{S_{A0}}{1 + k_1S_{A0}t}\right) + \left(\frac{k_1S_{A0}}{2k_2 + 2tk_1k_2S_{A0}} + \frac{1}{2k_2(1 + tk_1S_{A0})}\right.$$

$$\{-\sqrt{k_1}\sqrt{k_1 + 4k_2}S_{A0} + \frac{2\sqrt{k_1 + 4k_2}S_{A0}}{1 + \left(\frac{(1 + tk_1S_{A0})^{-\sqrt{k_1+4k_2}/\sqrt{k_1}}(k_1S_{A0} + \sqrt{k_1}\sqrt{k_1 + 4k_2}S_{A0})}{-k_1S_{A0} + \sqrt{k_1}\sqrt{k_1 + 4k_2}S_{A0}}\right)})\}$$

$$+ \left(\frac{k_1S_{A0}}{2k_3 + 2tk_1k_3S_{A0}} + \frac{\sqrt{k_1(k_1^2 + 4k_1k_2 + 4k_2k_3)S_{A0}^2}}{2\sqrt{k_1 + 4k_2}\,k_3(1 + tk_1S_{A0})}\right)$$

$$\begin{aligned} & + ((((k_1^2 + 4k_1k_2 + 2k_2k_3)S_{A0} \\ & + \sqrt{k_1 + 4k_2}\sqrt{k_1(k_1^2 + 4k_1k_2 + 4k_2k_3)S_{A0}^2}) \\ & (-1 + (k_1 + 4k_2)^2k_3^2(1 + tk_1S_{A0}))) / \\ & 2(k_1 + 4k_2)k_3(1 + tk_1S_{A0})) \end{aligned} \tag{12}$$

Reaction rate constants k_1, k_2 and k_3 were calculated according to the second-order kinetic equation (12) by using the Levenberg-Marquardt algorithm. These rate constants are given in Table 2.

Furthermore, the maximum specific growth rate (μ_{max}), half-velocity constant (K_S), growth yield coefficient (Y) and microbial decay coefficient (k_d) (*11*) were calculated according to equations 13–21 proposed by Monod, and the calculated values of these kinetic constants are given in Table 3. Here μ is specific growth rate (h^{-1}) and q is specific substrate utilization rate (h^{-1}).

$$\mu = \mu_{max}\frac{S}{K_S + S} \tag{13}$$

$$\mu = \frac{dX}{dt} \cdot \frac{1}{X} \tag{14}$$

where, dX/dt = rate of microbial growth (mg/liter·h); X= concentration of microorganism (mg/liter).

Specific substrate utilization in microbial cultures is expressed by

$$\frac{dS}{dt} \cdot \frac{1}{X} = q \tag{15}$$

where dS/dt is substrate utilization rate (mg/liter·h).

Growth yield Y is defined mathematically as

$$Y = \frac{(dX/dt)}{(dX/dt)} \tag{16}$$

It is possible to develop a relationship between specific substrate utilization rate, specific growth rate, and growth yield which has the form

$$\frac{(dS/dt)}{X} = q = \frac{\left(\frac{(dX/dt)}{X}\right)}{\left(\frac{dX}{dS}\right)} = \frac{\mu}{Y} \tag{17}$$

Substrate utilization can be described by an equation similar to Monod, where q_{max} is maximum specific substrate utilization rate (h^{-1}),

$$q = q_{max}\frac{S}{K_S + S} \tag{18}$$

In microbial systems used for wastewater treatment, the distribution of cell ages is such that not all the cells in the system are in the log-growth phase. When the specific growth rate decreases, observed biomass production decreases. Therefore the expression for the rate of growth must be corrected to account for the energy required for

Table 3. **Values of kinetic constants.**[*]

COD (mg O_2/l)	μ_{max} (h^{-1})	$K_s \times 10^{-3}$ (mg/l)	$Y \times 10^2$ (mg/mg)	$k_d \times 10^2$
10,000–22,500	36–100	16–50	8.20–11.80	4.09–5.88
31,500–43,000	125–132	75–296	7.25– 4.23	21.80–21.10

[*] μ_{max}, K_s, Y and k_d were calculated for the given COD interval.

cell maintenance. This decrease is often identified in literature as the endogenous decay (in the log-growth phase k_d is equal to zero). The endogenous decay term can be formulated as follows:

$$\frac{dX}{dt} = -k_d \cdot X \tag{19}$$

$$\mu = Y \cdot q - k_d \tag{20}$$

In Eq. 20, μ corresponds to the net specific growth rate,

$$q = \mu \frac{1}{Y} + \frac{k_d}{Y} \tag{21}$$

RESULTS AND DISCUSSION

In this study, the main objective was to develop a mathematical model for anaerobic treatment of antibiotic wastewater. By use of Eq. 12, calculation of total COD as a function of time was possible, especially for wastewaters having COD exceeding 25,000 mg O_2/liter.

For antibiotic production wastewaters (of COD values ranging between 3,000–43,000 mg O_2/liter), the model-calculated values were in satisfactory accord with experimental data as shown in Figs. 2–4.

For this reason, it can be stated that the anaerobic treatment kinetics of high strength (COD > 25,000 mg O_2/liter) antibiotic production wastewater fits the second-order biodegradation model.

Also, according to this model equation, k_1, k_2 and k_3, (*i.e.*, the rate constants of the anaerobic biodegradation reactions) were determined. Because of the conformity of the anaerobic degradation of medium strength (COD = 10,000–20,000 mg O_2/liter) antibiotic production wastewater to the first-order kinetic equation given by Ciftci (*4*), reaction rate constants k_1, k_2 and k_3 (given in italics) were calculated according to the latter equation presented in the literature. Thus, the advantage of the developed second-order model equation is mainly its applicability to fairly concentrated solutions of high COD content.

The maximum biodegradation efficiency of the anaerobic treatment obtained in approximately 60 d was 97 %. In all the anaerobic degradations, except the blank experiment with distilled water, there was an inert COD. This inert COD had values of 725–3,250 mg O_2/liter. One of the reasons of low efficiency in the treatment of low-strength antibiotic production wastewater (COD =

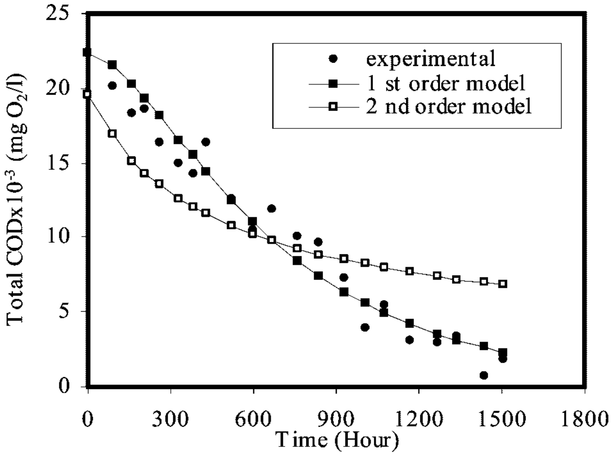

Fig. 3. **Decline of total COD with time, as experimentally measured, and theoretically predicted according to first- and second-order kinetic models (inital COD = 22,326 mg O_2/liter).**

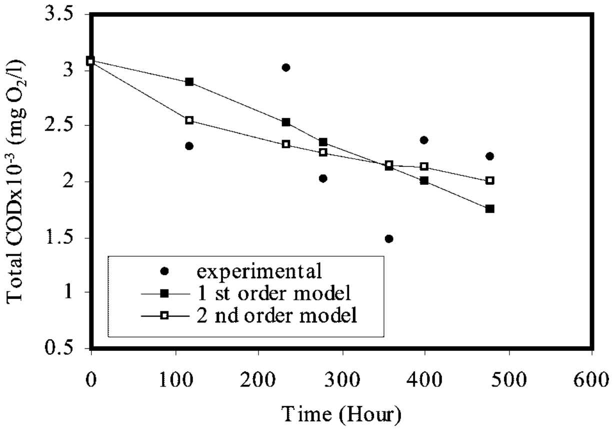

Fig. 2. **Decline of total COD with time, as experimentally measured, and theoretically predicted according to first- and second-order kinetic models (inital COD = 3,077 mg O_2/liter).**

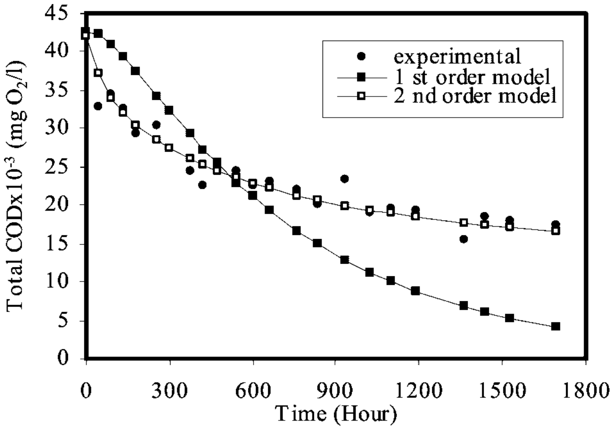

Fig. 4. **Decline of total COD with time, as experimentally measured, and theoretically predicted according to first- and second-order kinetic models (inital COD = 42,623 mg O_2/liter).**

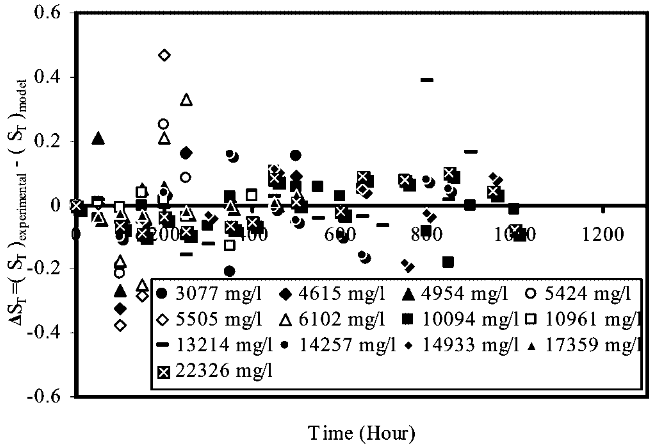

Fig. 5. **Statistical evaluation of ΔS_T inverted time data (according to first-order kinetics; COD of 3,000–11,000 mg O_2/liter).**

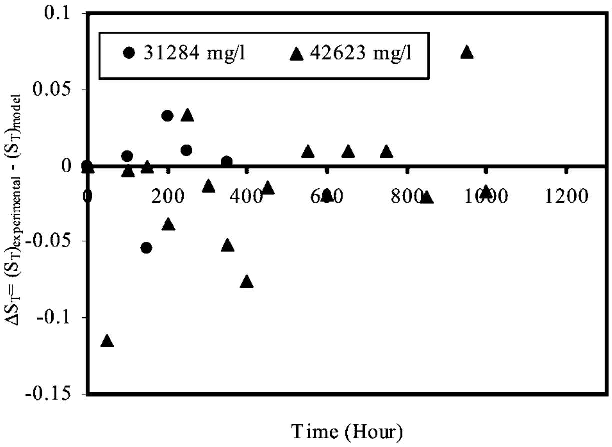

Fig. 6. **The statistical evaluation of ΔS_T inverted time data (according to the second-order kinetics; COD > 25,000 mg O_2/liter).**

3,000–10,000 mg O_2/liter) was this inert COD. However, this inert COD has not affected the efficiency of the anaerobic treatment of the high-strength antibiotic production wastewater because of its relatively small magnitude.

Regarding the results of this study, using the second-order kinetic and first-order kinetic equations, statistical evaluations for comparing theoretical and experimental values were made (*12*). As a result of these studies, which are shown in Figs. 5 and 6, the difference between experimental and calculated S_T values along the time axis shows a random deviation with no systematic correlation, and it was concluded that there is no missing term in the model equations (Eqs. 2 and 12, see Figs. 5 and 6). Added to this, the linear correlation coefficient (R) of ΔS_T with respect to time was 0.99–1.0, given in Table 4, which proves that the suggested model is appropriate and the biodegradation of anaerobic treatment of high-strength antibiotic production wastewater fits the second-order kinetics. The magnitude of R shows that the proposed model Eq. 12 is valid for high-strength antibiotic wastewater, and does not apply or dilute solutions.

CONCLUSIONS

The proposed mechanism, which was developed for the anaerobic treatment of high-strength antibiotic production wastewater, gave a good fit to experimental results. Consequently, the developed model can be used in such systems, provided that they do not show substrate inhibition.

Table 4. **Data of linear regression analysis.**

COD (mg O_2/l)	The linear correlation coefficient (R^*)	
	First-order kinetics	Second-order kinetics
3,000–6,000	0.13–0.97	0.31–0.77
10,000–22,500	0.96–1.00	0.52–0.86
31,500–43,000	—**	0.99–1.00

*The linear correlation coefficients were calculated for the given COD range. **Since the reaction does not follow first-order kinetics; the coefficients have big deviations, and therefore are not acceptable.

According to the model equation that follows second-order kinetics, the rate constants, k_1, k_2 and k_3, of the anaerobic biodegradation reactions were determined. As a result of this study, k_1, the rate constant for the hydrolysis step, was found to be smaller than the k_2 and k_3 values. For this reason, it can be concluded that hydrolysis is the rate-limiting step in the anaerobic treatment reactions of antibiotic production wastewater.

The developed model can be used for the calculation of total COD at certain times of treatment (for wastewaters having COD exceeding 25,000 mg O_2/liter) and also this model equation follows second-order kinetics.

According to the results of this study, the anaerobic biodegradation of medium strength (10,000 ≤ COD ≤ 20,000 mg O_2/liter) antibiotic production wastewater fits the first-order kinetics, and the anaerobic biodegradation of the high strength (COD > 25,000 mg O_2/liter) wastewater fits the second-order kinetics. However, neither rate-equation precisely describes the biodegradation of low-strength (COD < 10,000 mg O_2/liter) wastewater.

It is suggested that making use of half order (such as 1/2, 3/2, *etc.*) reaction approximations, similar studies can be done to precisely determine the order of the overall reaction.

Generally, the hydrolysis step fits the first-order kinetics in the anaerobic treatment systems.

If the following steps (acidogenesis and methanogenesis) assume second-order kinetics, the overall reaction order may be fractional, and this argument may form the basis of developing further models.

REFERENCES

1. Gray, N.F. (1989) *Biology of Wastewater Treatment*, Oxford Science Publications, London
2. Pavlostathis, S.G. and Giraldo, G.E. (1991) Kinetics of anaerobic treatment. *Water Sci. Technol.* **24**, 35–59
3. APHA, AWWA, WPCF (1989) *Standard Methods for the Examination of Water and Wastewater*, American Public Health Assoc., Washington, DC
4. Ciftci, G. (1997) A mathematical model for the anaerobic treatment of Baker's yeast effluents, Ph.D. Thesis, Istanbul Technical University, Istanbul

5. Christ, O., Wilderer, P.A., Angerhöfer, R., and Faulstich, M. (2000) Mathematical modeling of the hydrolysis of anaerobic processes. *Water Sci. Technol.* **41**, 61–65
6. Elnekave, M. (1999) Formaldehyde and Iso-Propanol treatment in an anaerobic fluidized bed reactor and kinetic evaluations, Ph.D. Thesis, Istanbul Technical University, Istanbul
7. Yu, H., Wilson, F., and Tay, J. (1998) Kinetic analysis of an anaerobic filter treating soybean wastewater. *Water Res.* **32**, 3341–3352
8. Jeyaseelan, S. (1997) A simple mathematical model for anaerobic digestion process. *Water Sci. Technol.* **35**, 185–191
9. Timur, H. (1997) Anaerobic treatment kinetics of municipal landfill leachate, Ph.D. Thesis, Istanbul Technical University, Istanbul
10. Kınacı, C. (1987) Investigation the effect of flow conditions on the efficiency of bioreactor, Ph.D. Thesis, Istanbul Technical University, Istanbul
11. Degirmentas, I. (2001) Anaerobic treatment of high strength antibiotic production wastewater and kinetic evaluations, unpublished Ph.D. Thesis, Istanbul Technical University, Istanbul
12. Wonnacott, R.J. and Wonnacott, T.H. (1985) *Introductory Statistics*, John Wiley & Sons, Canada