

## Investigation of biodegradation and growth kinetics of dairy wastewater in a batch reactor

T. T. Bayram<sup>1</sup>, A. Nuhoğlu<sup>2</sup>, E. Aladağ<sup>1\*</sup>

<sup>1</sup>Department of Environmental Engineering, Faculty of Engineering, Van Yuzuncu Yil University, Van, Turkey

<sup>2</sup>Department of Environmental Engineering, Faculty of Engineering, Ataturk University, Erzurum, Turkey

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In this study, biodegradation of dairy wastewater was investigated under aerobic conditions in a batch reactor. A minimum concentration of 100 mg L<sup>-1</sup> and a maximum concentration of 1000 mg L<sup>-1</sup> of COD in the wastewater were used. The culture substrate removal kinetics was followed and the specific growth rate was fitted to the Monod model. The kinetic coefficients  $K_S$  and  $\mu_{max}$  were found to be 46.55 mg L<sup>-1</sup> and 0.0344 h<sup>-1</sup>, respectively. The regression coefficient was 0.99. The compatibility of actual and predicted results of microbial growth and substrate removal was compared with this model. Results indicated that predicted and actual values fitted each other with 89% compatibility.

**Keywords:** Dairy wastewater; Biological treatment; Aerobic processes; Batch reactor; Bio-kinetic parameters.

### INTRODUCTION

Wastewater pollution is increasing as a result of rapid industrialization and increasing human population. This inhibits the metabolic function of aquatic life and ecological balance. Usable water reserves are consistently being contaminated, as a result of which the water turns unusable. When such a situation is encountered, wastewater needs to be treated and serious precautions must be taken.

Wastewater produced during the dilution of milk and dairy products in the dairy industry is among the substantial pollution sources for natural aquatic environments. Considerably different treatment systems have been developed in order to treat wastewater released from the dairy and dairy products industry but generally physicochemical and biological methods are reported in the literature [1-6].

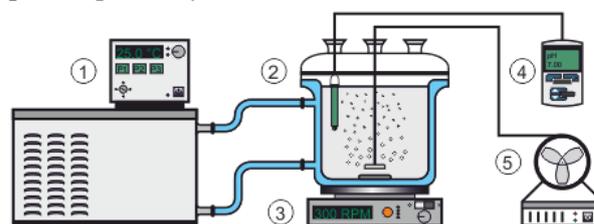
This study proposes investigating of the biological COD removal performance from dairy wastewater under aerobic conditions in a batch system and comparing the compatibility of predicted (proposed by the model) and actual (experimental) values for the removal of organic material and the growth of microorganisms with the Monod model.

### EXPERIMENTAL

#### Materials

All chemicals used in the study were purchased from Merck and Sigma. Wastewater was collected from the pilot dairy production and processing plant operated by Ataturk University Agriculture Faculty

Food Engineering Department. The plant produces several products including feta cheese, cheddar (kashar), thread cheese (civil), yoghurt, pasteurized milk, daily milk and ice cream. The plant capacity is approximately 900 kg day<sup>-1</sup> of milk and dairy products. The wastewater capacity of the plant ranges between 1.5–2 m<sup>3</sup> wastewater ton<sup>-1</sup> processed milk. Activated sludge samples were taken from the secondary settling tank of the wastewater treatment facility in Erzincan city and used for the adaptation of microorganisms to wastewater by feeding with dairy industry wastewater. The experiments were carried out in a completely mixed batch reactor with a working volume of 2.0 L. Experimental setup can be seen in Fig. 1. Aeration was provided by means of air bubble diffusers at a volumetric flow rate of 1 vvm. During the experiments, pH, temperature and stirring rate were adjusted to 7±1, 25±1 °C and 300 rpm, respectively.



**Fig. 1.** Schematic diagram of the batch reactor system (1) Water circulator, (2) Batch reactor, (3) Magnetic stirrer, (4) pH meter, (5) Air pump.

#### Method

The COD analysis was carried out using the methods stated in the Standard Methods [7]. The microorganisms concentrations were measured spectrophotometrically using Spekol 1100 (Analytik Jena AG). The calibration curve was prepared at the

\* To whom all correspondence should be sent:  
E-mail: erdincaladag@gmail.com

wavelength of 525 nm and the concentration of the microorganisms was determined using that curve. The pH and temperature of the medium were continuously controlled using a WTW multiline P4 multi-parameter measurement device.

## RESULTS AND DISCUSSION

### Effect of initial concentration on biodegradation

Batch experiments were conducted by adding wastewater with COD concentrations of 100, 250, 500, 750 and 1000 mg L<sup>-1</sup> to the medium. Biodegradation of different initial wastewater concentrations *versus* time is shown in Fig. 2. It can be seen that when COD concentration increased from 100 to 1000 mg L<sup>-1</sup>, the time of biodegradation raised from 10 to 48 h.

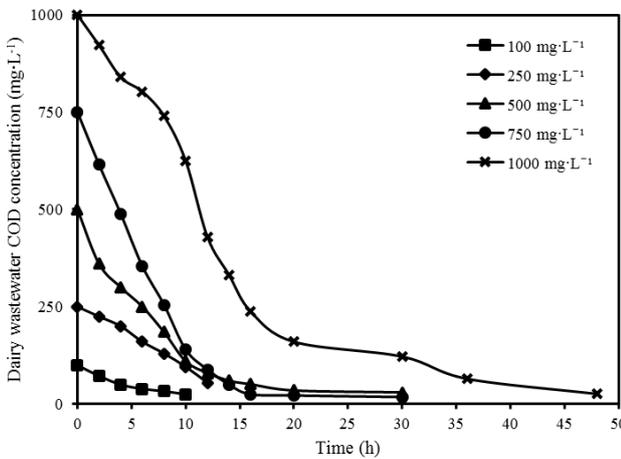


Fig. 2. Biodegradation of different initial COD concentrations in wastewater *versus* time.

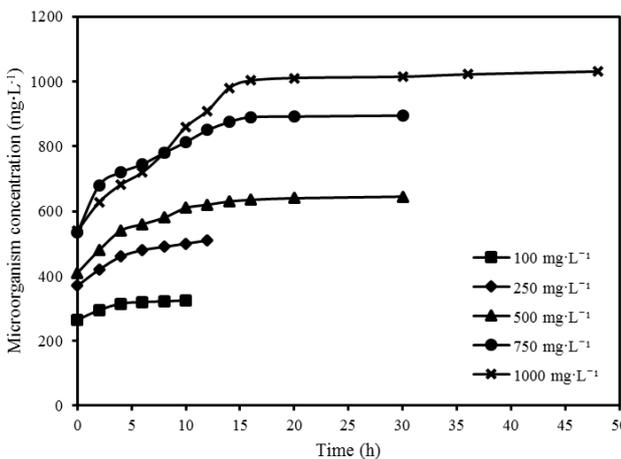


Fig. 3. Microorganisms concentrations as a function of time at different initial dairy COD concentrations in wastewater

### Effect of initial concentration on the growth of the culture

Fig. 3 shows microorganisms concentrations as a function of time at different initial dairy wastewater concentrations. According to the experimental results, the maximum specific growth rate was registered at a COD concentration of 1000 mg L<sup>-1</sup>.

### Modeling the growth kinetics of the microorganisms

One of the most important points to be taken into consideration when determining growth kinetics of microorganisms is to adopt an equation showing the relationship between the specific growth rate and the substrate concentration.

$$\frac{dX}{dt} = \mu X \quad (1)$$

Equation (1), where  $X$  is the biomass concentration (mg L<sup>-1</sup>), was used to calculate the specific growth rate of microorganisms. The constant  $\mu$  which represents the specific growth rate (h<sup>-1</sup>) can be estimated through linearization of the equation. The plotted data of the linearized results are shown in Fig. 4. The constant  $\mu$  can be obtained from the linear interception of  $\ln(X)$  *versus*  $t$ .

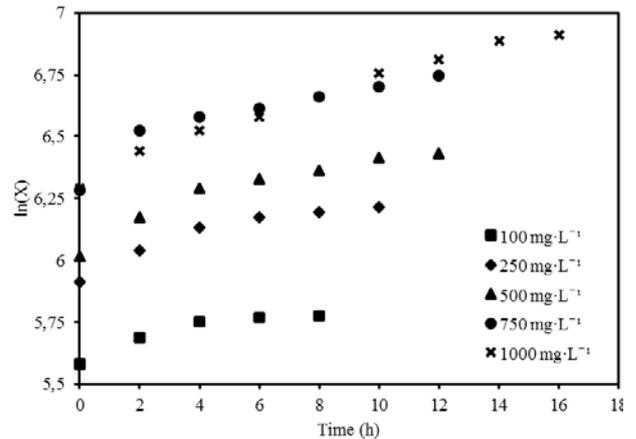


Fig. 4. Variation of microorganisms concentration *versus* time.

The microbial growth can be represented by a simple Monod equation [8].

$$\mu = \frac{\mu_{max} S}{K_S + S} \quad (2)$$

where  $\mu$  is the specific growth rate (h<sup>-1</sup>),  $S$  is the substrate concentration (mg L<sup>-1</sup>),  $\mu_{max}$  is the maximum specific growth rate (h<sup>-1</sup>),  $K_S$  is the half saturation coefficient (mg L<sup>-1</sup>). As can be seen, the Monod equation has two kinetic parameters  $\mu_{max}$  and  $K_S$ . Numeric values of these two parameters should be calculated and used in the mathematical modeling

studies. A non-linear regression method was used instead of a linear method to calculate the numeric values. The most important point to be considered in the calculation of parameters using a nonlinear regression method is to draw the best fitting curve using the experimental data. Non-linear estimation module of the Statistical Package for the Social Sciences (SPSS) software was used for nonlinear regression calculation.  $K_S$  and  $\mu_{max}$  were determined to be 46.55 mg L<sup>-1</sup> and 0.0344 h<sup>-1</sup>, respectively. The regression coefficient was found to be 0.99 for both estimated and experimental  $\mu$  values. The curve drawn based on these values and experimental data is presented in Fig. 5.

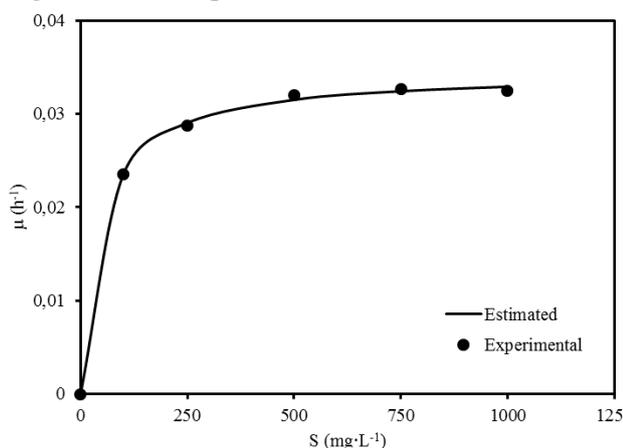


Fig. 5. Predicted and actual specific growth rate.

It is known that the removal rate of an organic compound is a function of its molecular structure, the species of microorganisms that use it as a carbon and energy source and the time required by these microorganisms to develop the necessary enzymes for its utilization. In Table 1 a comparison is given between growth kinetic parameters discussed in the literature including this study.

Table 1. Comparison of the growth kinetic parameters discussed in literature

System	$K_S$ (mg L <sup>-1</sup> )	$\mu_{max}$ (h <sup>-1</sup> )	Reference
Batch reactor	46.55	0.0344	This study
MSBR	174	0.070	[9]
AS	141	0.018	[10]
Two-phase anaerobic	134	0.412	[11]
Anaerobic digestion	420	0.032	[12]

Kinetic coefficients found in previous studies show great variations when compared to the values obtained from the pilot facility wastewater in this study and depend on its characteristics. All compounds involved in dairy industry wastewater are biodegradable. About the lower  $K_S$  values

calculated is, it can be said that the wastewater has higher organic material content and lower biodegradation rate in comparison with other studies.

In the study [11],  $\mu_{max}$  was reported to be 0.412 h<sup>-1</sup> and it was stated that the mixed culture used for the treatment of dairy wastewater adapted more rapidly to wastewater and more quickly consumed the carbon sources.

#### Evaluation of the compatibility of biodegradation with the model

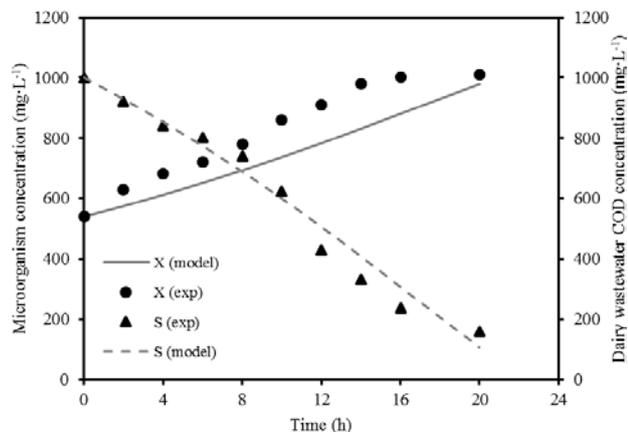
The mathematical model of the system was numerically solved determined by means of the coefficients in the Monod model obtained using the 8.3.18 version of Berkeley Madonna software program and simultaneously solving Equations (3) and (4) considering the Runge-Kutta method.

$$\frac{dS}{dt} = -\frac{\mu_{max}SX}{[K_S + S]Y} \quad (3)$$

$$\frac{dX}{dt} = \frac{\mu_{max}SX}{K_S + S} - bX \quad (4)$$

By simultaneously solving Equations (3) and (4), the variables of substrate removal and microbial growth in the reactor with time were also determined using the model. The curve fit module of Berkeley Madonna software was used to reduce deviations between the coefficients calculated in the model and the experimentally measured values. The software was operated by considering the ranges for microorganisms decay rate ( $b$ ) from 0.001 to 0.003 h<sup>-1</sup> and the yield coefficient ( $Y$ ) from 0.25 to 0.75 (mg microorganism.mg<sup>-1</sup> substrate). Best compatibility was observed when the values of  $b$  and  $Y$  were entered into the program as 0.001 h<sup>-1</sup> and 0.52 (mg microorganism.mg<sup>-1</sup> substrate), respectively. The model was operated at initial COD concentration in the wastewater of 1000 mg.L<sup>-1</sup> and the graph obtained is given in Fig. 6. It can be seen from the figure that estimated and actual values are compatible with each other. Table 2 shows the specific growth rate and the regression coefficients for different initial wastewater COD concentrations. The profiles found using the same model kinetic coefficients for removal of substrate and growth of microorganism gave better results for the substrate, while the values of some microorganism profiles showed deviations. A lower increase in the concentration of microorganisms was calculated than that predicted by the model. This situation can be explained by the occurring biofilm on the wall of the continuously stirred reactor. Even though great

care was taken to prevent such a situation, it can be stated that the measured microorganisms concentration does not accurately reflect the increase in the amount of microorganisms in the reactor.



**Fig. 6.** Predicted versus actual values of dairy wastewater COD removal at initial concentration (S) of 1000 mg L<sup>-1</sup> and microbial growth (X).

**Table 2.** Specific growth rate and regression coefficients for different initial wastewater COD concentrations.

S (mg L <sup>-1</sup> )	100	250	500	750	1000
μ (h <sup>-1</sup> )	0.024	0.029	0.032	0.033	0.033
R <sup>2</sup>	0.89	0.90	0.92	0.88	0.94

Mathematical models are extensively used in the optimum design and control of any given process since effects of operational parameters on system performance can be estimated through such models without any experimental work. Mathematical models offer significant advantages for the estimation of the performance in biological wastewater treatment systems where days or even months are needed to find balance conditions even if only one parameter is changed. For microbial growth, even simple models dependent on Monod criteria can give accurate results for scaling a reactor [13, 14].

### CONCLUSIONS

In this study, the biological COD removal performance from dairy wastewater under aerobic conditions was investigated in a batch system and the compatibility of predicted (proposed by model) and actual (experimental) values for the removal of organic material and the growth of microorganisms using the Monod model were compared.

Batch experiments were carried out with 100, 250, 500, 750 and 1000 mg L<sup>-1</sup> initial COD concentrations. The equilibrium time at an initial concentration of 100 mg L<sup>-1</sup> is 10 h and at an initial concentration of 1000 mg L<sup>-1</sup> - 48 h. The Monod

kinetic parameters  $K_s$  and  $\mu_{max}$  were calculated to be 0.0344 h<sup>-1</sup> and 46.55 mg L<sup>-1</sup>, respectively. The regression coefficient was 0.99 for the curve drawn by using the Monod equation and the values of the kinetic parameters. Taking into consideration the results above, it can be concluded that the kinetic parameters relationships derived from the batch experiments and proposed by the model were compatible with each other. Additionally, it can be stated that the dairy wastewater is easily biodegradable by the microorganisms.

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## ИЗСЛЕДВАНЕ НА БИОРАЗГРАЖДАНЕТО И КИНЕТИКАТА НА РАСТЕЖА НА МЛЕЧНИТЕ ОТПАДНИ ВОДИ В СТАНДАРТЕН РЕАКТОР

Т. Т. Байрам<sup>1</sup>, А. Нухоглу<sup>2</sup>, Е. Аладаг<sup>1\*</sup>

<sup>1</sup>*Катедра по Инженерство на околната среда, Факултет по инженерство, Юзунку Гил Университет, Ван, Турция*  
<sup>2</sup>*Департамент по Инженерство на околната среда, Факултет по инженерство, Ататюрк Университет,  
Ерзурум, Турция*

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(Резюме)

В това проучване биоразграждането на млечните отпадъчни води е изследвано при аеробни условия в стандартен реактор. Използвани са концентрации от минимум 100 и максимум 1000 mg L<sup>-1</sup> COD в отпадните води. Кинетиката на отстраняване на субстратната култура и специфичната скорост на растеж са пригодени към Monod модел. Кинетичните коефициенти  $K_S$  и  $\mu_{max}$ , са съответно 46.55 mg L<sup>-1</sup> и 0.0344 h<sup>-1</sup>. Регресионният коефициент е 0.99. Съвместимостта на действителните и прогнозираните резултати от микробния растеж и отстраняването на субстрата са сравнени с помощта на този модел. Резултатите показват, че съответствието между прогнозираните и действителните стойности е 89%.