Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 589–594
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Kinetic analysis of waste activated sludge hydrolysis and short-chain fatty acids production at pH 10

FENG Leiyu, YAN Yuanyuan, CHEN Yinguang*

State key Laboratory of Pollution Control and Resources Reuse, School of Environmental Science and Engineering, Tongji University, Shanghai 200092, China. E-mail: fengleiyu2001@yahoo.com.cn

Received 20 June 2008; revised 13 August 2008; accepted 28 August 2008

Abstract

The accumulation of short-chain fatty acids (SCFAs), a preferred carbon source for enhanced biological phosphorus removal microbes, was significantly improved when waste activated sludge (WAS) was fermented at pH 10. The kinetics of WAS hydrolysis and SCFAs production at pH 10 was investigated. It was observed that during WAS anaerobic fermentation the accumulation of SCFAs was limited by the hydrolysis process, and both the hydrolysis of WAS particulate COD and the accumulation of SCFAs followed first-order kinetics. The hydrolysis and SCFAs accumulation rate constants increased with increasing temperature from 10 to 35°C, which could be described by the Arrhenius equation. The kinetic data further indicated that SCFAs production at pH 10 was a biological process. Compared with the experiment of pH uncontrolled (blank test), both the rate constants of WAS hydrolysis and SCFAs accumulation at 20°C were improved significantly when WAS was fermented at pH 10.

Key words: waste activated sludge; hydrolysis; short-chain fatty acids; kinetics; alkaline pH

DOI: 10.1016/S1001-0742(08)62312-8

Introduction

Biological wastewater denitrification and phosphorus removal require sufficient carbon in wastewater. In some areas of China, especially in the southern China, however, the influent of wastewater treatment plants (WWTPs) generally contains insufficient soluble COD so that limits the application of biological nutrient removal (BNR) processes. Short-chain fatty acids (SCFAs) are the preferred carbon source for biological nutrient removal microbes (Thomas *et al*., 2003; Chen *et al*., 2004) and sufficient concentration of SCFAs in wastewater allows high rate of denitrification and low effluent phosphorus concentration (GonCaves *et al*., 1994).

On the other hand, large amounts of waste activated sludge (WAS) are produced in WWTPs. As there is great deal of protein and carbohydrate in WAS, the SCFAs can be accumulated during the anaerobic fermentation of WAS organics (Chen *et al*., 2007). It is, therefore, meaningful to produce SCFAs from WAS through the WAS reduction and BNR carbon source production, which are accomplished in WWTPs.

It has been shown in our previous publication that the accumulation of SCFAs during WAS anaerobic fermentation was significantly improved when pH was controlled at 10 (Yuan *et al*., 2006). It is attributed to that the alkaline pH causes more WAS hydrolysis and decreases the activity of methanogens. More soluble substrates are provided for SCFAs production and less SCFAs are consumed by methanogens, which result in high SCFAs accumulation at pH 10.

Most of the previous studies on sludge hydrolysis and SCFAs accumulation were conducted at acidic or neutral pH with primary sludge (PS) or its mixture with WAS as the substrate (Moser-Engeler *et al*., 1999; Miron *et al*., 2000; Ferreiro and Soto, 2003; Mahmoud *et al*., 2004). The kinetic analysis of WAS fermentation at alkaline pH has not been explored. It is well known that three stages including hydrolysis, acidification and methanogenesis are involved during WAS anaerobic treatment. Nevertheless, there is no obvious methane generation at pH 10. In order to have a deep understanding of the process of WAS fermentation at pH 10, the kinetics of both WAS hydrolysis and SCFAs accumulation were investigated in this study.

1 Material and methods

1.1 Sludge

The WAS used in this study was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant in Shanghai, China. The sludge was concentrated by settling it at 4°C for 24 h and the main composition of concentrated WAS is presented in Table 1. The protein and carbohydrate are the first and second dominant organic compounds in WAS, respectively.

* Corresponding author. E-mail: yg2chen@yahoo.com

Table 1 Characteristics of the concentrated waste activated sludge (WAS) used in this study

Parameter	Value	SD
pH	6.8	0.1
TSS (total suspended solids) (mg/L)	13808	743
VSS (volatile suspended solids) (mg/L)	10815	159
$SCOD_0$ (soluble chemical oxygen demand in raw inlet WAS) (mg/L)	41	21
$TCOD_0$ (total chemical oxygen demand in raw inlet WAS) (mg/L)	13407	573
Total carbohydrate (as COD) (mg/L)	1522	332
Total protein (as COD) (mg/L)	8180	103
Soluble carbohydrate (as COD) (mg/L)	25	4
Soluble protein (as COD) (mg/L)	66	8
Lipid and oil (as COD) (mg/L)	131	8

SD: standard deviation.

1.2 Kinetic experiments of waste activated sludge hydrolysis and acidification

In this study, all reactors were made of plexiglass with working volume of 4.0 L each, and were equipped with the stainless steel stirrer.

Within the same reaction time, when the environmental conditions (such as temperature and pH) maintain steady, the relation between the initial concentration of reactant and its conversion efficiency was as follows: with positive correlation during the zero-order reaction, be independent during the first-order reaction and with negative correlation during the second-order reaction (Hill, 1977). In order to get the reaction progression of WAS hydrolysis, the effect of initial COD concentration of WAS particulate on its conversion efficiency was conducted. Three identical reactors with initial COD concentration of WAS particulate 6683, 13366, or 20049 mg/L (by diluting or concentrating the WAS described in Table 1) were maintained at 20 ± 1°C. The pH value in all reactors was controlled at 10 by adding 2 mol/L NaOH or 2 mol/L HCl. After WAS was added to the reactors, the operational time was recorded.

To study whether the SCFAs accumulation was limited by the hydrolysis of particulate COD, especially protein (the most dominate compound in WAS), the production and degradation rates of amino acids were examined by the batch fermentation tests with synthetic wastewater of bovine serum albumin (BSA, model protein used in this study) and L-alanine (model amino acid), respectively. BSA, the molecular weight is 69000 g/mol, consists of an elliptic protein molecule containing of a chain of 607 amino acids. It has been reported that only 0.061 g alanine /(g BSA) can be obtained when amino acids were produced from BSA (Rogalinski *et al*., 2005). Thus, in this study the BSA of 1000 mg and L-alanine of 61 mg were dissolved respectively into 900 mL of tap water, and the pH values in two reactors were adjusted to 10. Then, 100 mL of WAS was added to each reactor as an inoculum with a final sludge concentration of 1200 mg/L. The two batch reactors were maintained at 20 ± 1°C. If the degradation rate of amino acid in L-alanine reactor exceeded the production rate of amino acid in BSA reactor, it is believed that the amino acid generated from protein hydrolysis is rapidly converted into SCFAs and the hydrolysis of particulate COD (mainly protein) to soluble substances is the rate-limiting step of SCFAs production. The process of SCFAs production from soluble COD can thus be assumed as a first-order reaction, otherwise a zero-order one.

The effect of temperature on the kinetics of WAS fermentation was carried out in four identical reactors. The temperature in reactors 1–4 was set at 10, 20, 30, and 35°C, respectively, and the solution pH was set at 10.

In order to study the effect of pH 10 on the hydrolysis rate constant and the SCFAs accumulation rate constant, two identical reactors were operated. For one reactor the pH was controlled at 10, the other one without any adjustment as control. Both reactors were maintained at 20°C and were mixed for 8 d.

To verify the kinetic models of WAS hydrolysis and acidification, three identical reactors were operated at temperature of 15, 25, and 32°C. The pH in three reactors was maintained at 10.

1.3 Analytical methods

The determination of carbohydrate, protein, lipid, methane, COD, soluble orthophosphate (PO_4^{3-}-P), and ammonia nitrogen (NH_4^+-N) was the same as described in our previous publications (Yuan *et al*., 2006; Jiang *et al*., 2007). The alanine content were analyzed and quantitatively determined using HPLC (Waters System Interface module 501, Hewlett Packard, USA) as described by Pico-tag method (Rogalinski *et al*., 2005).

For the quantification of SCFAs, the filtrate was collected in a 1.5-mL gas chromatography (GC) vial, and acidified with 3% H_3PO_4 to pH 4 before assayed on an Agilent 6890N GC with flame ionization detector and CPWAX52CB column (30 m × 0.32 μm × 0.53 mm). Nitrogen was used as carrier gas and the flux was 25 mL/min. The injection port and the detector were maintained at 220 and 250°C, respectively. The oven of GC was programmed to begin at 110°C, held for 2 min, then increase to 220°C with a rate of 10°C/min, and held for an additional 2 min. The sample injection volume was 1.0 μL.

1.4 Calculation

According to Miron *et al*. (2000), 1 g protein (assumed as $(C_4H_{6.1}O_{1.2}N)_x$) equals to 1.5 g COD, 1 g carbohydrates (assumed as $C_6H_{12}O_6$) equals to 1.07 g COD, and 1 g lipid equals to 2.91 g COD. The calculation follows the Eqs. (1)–(3).

$$X_0 = TCOD_0 - SCOD_0 \quad (1)$$

$$X = TCOD_t - SCOD_t \quad (2)$$

$$x_C = \frac{X_0 - X}{X_0} \times 100\% \quad (3)$$

where, X_0 (mg/L) is the initial concentration of WAS particulate COD, X (mg/L) is the concentration of WAS particulate COD at fermentation time t, $TCOD_t$ (mg/L) is the total COD at fermentation time t (particulate plus dissolved COD), $SCOD_t$ (mg/L) is the soluble COD at fermentation time t, and x_C (%) is the conversion efficiency of WAS particulate COD.

1.5 Kinetics equations

According to the principle of reaction kinetics, temperature, concentration, and pressure are the main factors which influence the reaction rates. However, in most of reactions, temperature and concentrations of reactants are the dominant factors (Hill, 1977). Thus, the kinetics equation is usually expressed as:

$$r_i = f(C, T) \tag{4}$$

where, r_i is the reaction rate, C is the concentration of reactant, and T is the reaction temperature. Furthermore, in most cases the variables of temperature and concentrations of reactants can be separated from each other. Then Eq. (4) can be changed into the following form:

$$r_i = f_T(T) f_C(C) \tag{5}$$

where, f_T (T) is the temperature effect of reaction rate, and f_C (C) is the concentration effect.

Based on Eq. (5), in the case of temperature keeping constant, the batch hydrolysis process of particulate matters in WAS can be expressed as:

$$r_{\mathrm{h}} = k f_C(C) = -\frac{\mathrm{d}X}{\mathrm{d}t} = k_{\mathrm{h}} X^n \tag{6}$$

where, r_{h} (mg COD/(L·d)) is the hydrolysis rate of particulate COD in WAS, k_{h} (d^{-1}) is the hydrolysis rate constant of particulate COD and n is the reaction progression. Similarly, the relationship between SCFAs production rate and SCFA concentration can be written as follows:

$$r_{\mathrm{a}} = \frac{\mathrm{d}S_{\mathrm{a}}}{\mathrm{d}t} = k_{\mathrm{a}} S_{\mathrm{a}}^n \tag{7}$$

where, r_a (mg COD/(L·d)) is the SCFAs production rate, k_{a} (d^{-1}) is the rate constant of SCFAs production, and S_{a} (mg COD/L) is the SCFAs concentration at fermentation time t.

2 Results and discussion

2.1 Preliminary analysis of WAS hydrolysis and SCFAs production

During WAS fermentation at pH 10 and 20°C, the changes of x_{C} with fermentation time at different X_0 are illustrated in Fig. 1. Clearly, almost the same x_{C} was obtained with the same fermentation time at different X_0. It seems that x_{C} was independent of X_0 at the same fermentation time. With the increase of fermentation time from 1 to 8 d the x_{C} increased at any initial COD concentration of WAS particulate. Nevertheless, with a further increase of fermentation time from 8 to 12 d, no significant increase of x_{C} was observed. Thus, when WAS was fermented at pH 10 within fermentation time of 8 d, the hydrolysis of particulate COD can be assumed to obey the first-order kinetics.

When WAS particulate COD (protein as the most dominant compound) was hydrolyzed at pH 10, the amino acid was produced, which was further fermented to SCFAs during acidification. Figure 2 describes the production rate of amino acid from the hydrolysis of BSA (model protein used in this study) and the degradation rate of L-alanine (model amino acid compound) with fermentation time at pH 10. It can be seen that during the entire fermentation time the degradation rate of amino acid exceeded the production rate, which indicated that the hydrolyzed products of particulate COD was not enough for SCFAs production by acidogenic bacteria. Apparently, the SCFAs production was limited by the hydrolysis of WAS particulate COD. At pH 10, the SCFAs accumulation was therefore assumed to be a first-order kinetics reaction.

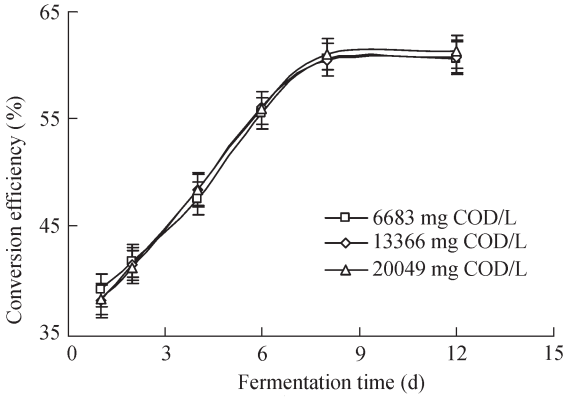

Fig. 1 Changes of conversion efficiency (x_{C}) of particulate COD with fermentation time at different initial concentrations of WAS particulate COD.

Shimizu *et al*. (1993) studied the bioconversion mechanism and kinetics model during the anaerobic digestion of WAS, and their results showed that the hydrolysis of WAS biopolymer, the acid accumulation and the conversion of acetic acid to methane followed the first-order kinetics. Moser-Engeler *et al*. (1999) reported that the accumulation of SCFAs during primary sludge anaerobic fermentation was limited by the hydrolysis process, and the production of SCFAs could be described by the first-order kinetics. It should be noted that all these kinetic studies of sludge fermentation were conducted without any pH control.

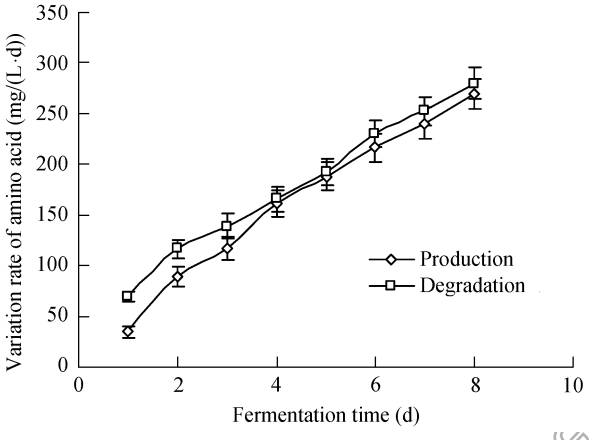

Fig. 2 Production rate of amino acid from BSA hydrolysis and the degradation rate of L-alanine with fermentation time at pH 10.

2.2 Kinetic study of WAS hydrolysis

According to the above discussion, the hydrolysis of WAS particulate COD can be described by the following first-order kinetic equation:

$$r_h = -\frac{dX}{dt} = k_h X \quad (8)$$

$$\ln X = -k_h t + b \quad (9)$$

where, b is the constant of integration. By plotting $\ln X$ versus t, the slope and the intercept can be obtained, which corresponded respectively to the value of k_h and b in Eq. (9). At different temperatures, the values of k_h and b are shown in Table 2. It is clear that the goodness of fit values at different temperatures were generally good in the range of 0.97–0.99, which further indicated that the hydrolysis of particulate COD in WAS obeyed the first-order kinetics. Moreover, the hydrolysis rate constant increased with temperature showing that for the same x_C more fermentation time was needed at low temperature.

Table 2 Kinetic constants at different temperatures (pH 10)

Temperature (°C)	k_h (d^{-1})	b	R^2
10	0.017	9.5	0.98
20	0.072	9.6	0.97
30	0.12	9.3	0.99
35	0.16	9.4	0.99

As the hydrolysis process is affected by several factors, such as pH, temperature, particle size and its distribution, and sludge source, different k_h values were reported. For example, for primary sludge, the k_h value at 20°C was 0.095 d^{-1} in the study of Ferreiro and Soto (2003), and was 0.152 d^{-1} in the study of Moser-Engeler *et al*. (1998). Thus, it is difficult to compare the k_h values obtained in this study with those in the previous publications.

In order to understand the effect of pH on the k_h of WAS hydrolysis, the hydrolysis of WAS at pH 10 was compared with that at pH un-adjusted (blank test). It was observed that the hydrolysis of WAS particulate COD in the blank test also followed the first-order kinetics at 20°C (k_h was 0.011 d^{-1}). As shown in Table 2, at pH 10 and 20°C the k_h value was 0.072 d^{-1} which was 6 times that in the blank test. It is clear that the hydrolysis rate was significantly accelerated at pH 10. One possible reason for pH 10 improving WAS hydrolysis was that the alkaline pH resulted in the dissociation of acidic groups of sludge extracellular polymeric substances (EPS) and the repulsions between the negatively charged EPS.

It is well known that the relationship between reaction rate constant and temperature can be expressed by the Arrhenius equation (Eq. (10)).

$$k = A\exp(-\frac{-E_a}{RT}) \quad (10)$$

where, A is the preexponential factor, E_a (kJ/mol) is the activation energy of reaction and T (K) is the absolute temperature. When WAS was hydrolyzed at pH 10 and temperature in the range of 10–35°C, the relationship between $\ln k_h$ and $1/T$ is shown in Fig. 3a. Obviously, the first-order hydrolysis rate constants obtained in this study followed an Arrhenius type of behavior ($\ln k_h$ = –7458.3 + 22.5, R^2 = 0.94), and the activation energy was 62.0 kJ/mol, which was a typical value of enzyme kinetics (Roels, 1983; Veeken and Hamelers, 1999). In our previous study, it has been reported that all four types of hydrolytic enzymes (protease, α-glucosidase, alkaline and acid phosphatases) were active when WAS was fermented at pH 10 (Yuan *et al*., 2006).

Thus, the value of the first-order hydrolysis constant, k_h, can be expressed by Eq. (11).

$$k_h = 5.88\times10^9\exp(-\frac{6.20\times10^4}{RT}) \quad (11)$$

From Table 2 it can be seen that the value of b remained almost constant. The average value of b in the temperature range 10–35°C is 9.43. Equation (9) has then be expressed as the following form which obtained within fermentation time less than 8 d and temperature range 10–35°C:

$$X(t) = 1.25\times10^4\exp(-5.88\times10^9\exp(-\frac{6.20\times10^4}{RT})t) \quad (12)$$

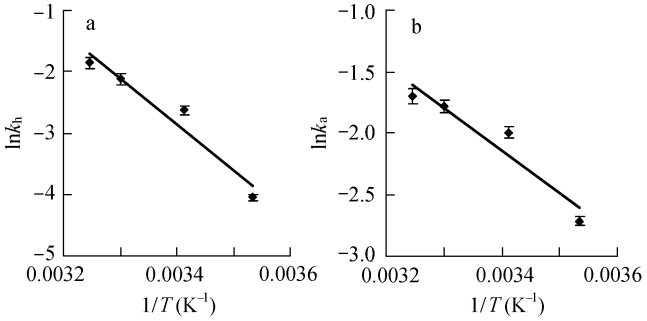

Fig. 3 Relationship between $\ln k$ and $1/T$ during WAS hydrolysis (a) and SCFAs accumulation (b) at pH 10.

2.3 Kinetic study of SCFAs production

As discussed above, the accumulation of SCFAs during WAS fermentation at pH 10 followed first-order kinetics, which can be described by Eq. (13) or (14):

$$r_a = \frac{dS_a}{dt} = k_a S_a \quad (13)$$

$$\ln S_a = k_a t + c \quad (14)$$

At different temperatures, the kinetic data involved in SCFAs accumulation are summarized in Table 3. With a increasing of temperature from 10 to 35°C, the k_a value increased from 0.066 to 0.18 d^{-1}, but the value

Table 3 Kinetic data relevant to SCFAs accumulation at pH 10 and different temperatures

Temperature (°C)	k_a (d^{-1})	c	R^2
10	0.066	6.7	0.97
20	0.14	6.9	0.98
30	0.17	6.8	0.99
35	0.18	7.1	0.98

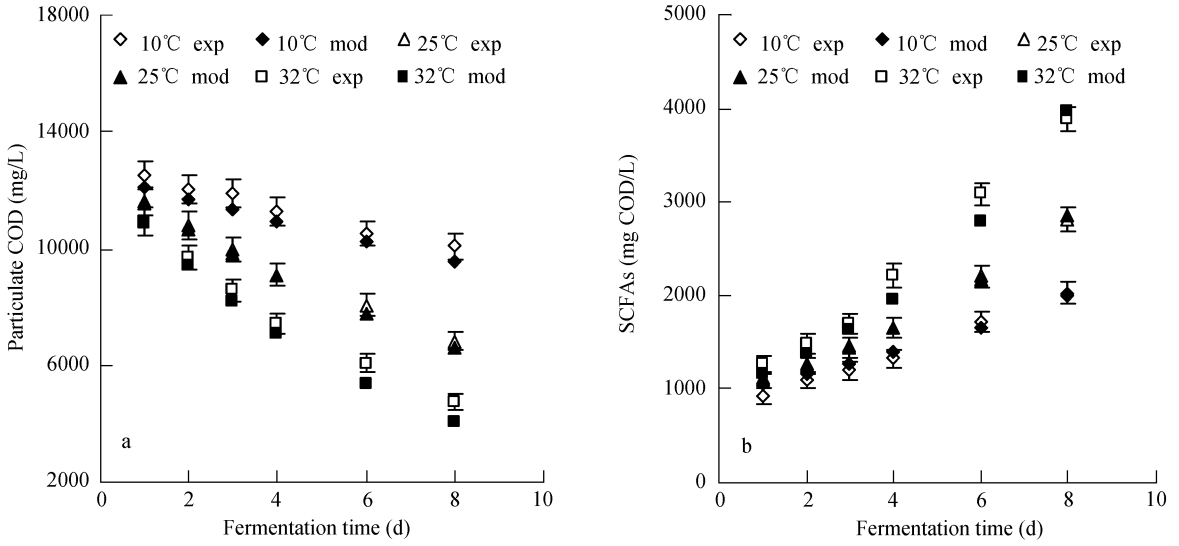

Fig. 4 Comparison between the experimental and model value of particulate COD hydrolysis (a) and SCFAs production (b) at pH 10 and given temperatures (15, 25 and 32°C).

of c remained relatively stable. Clearly, the accumulation of SCFAs can be described by the first-order kinetics. Some researchers also observed that during primary sludge anaerobic fermentation, the hydrolysis of particulate fermentable components was the rate-limiting step and the accumulation of SCFAs obeyed first-order kinetics (Lilley *et al*., 1990; Moser-Engeler *et al*., 1999; Ferreiro and Soto, 2003).

The relationship between $\ln k_a$ and $1/T$ at pH 10 is shown in Fig. 3b, from which the Arrhenius activation energy can be obtained. In this study the Arrhenius activation energy of SCFAs accumulation was 28.8 kJ/mol. According to Table 3, the average value of c can be calculated as 6.87. Thus, within fermentation time 8 d and temperature range 10–35°C, the first-order SCFAs accumulation constant (k_a) and the accumulation of SCFAs can be expressed by Eqs. (15) and (16), respectively.

$$k_a = 1.51 \times 10^4 \exp(-\frac{2.88 \times 10^4}{RT}) \quad (15)$$

$$S_a(t) = 966 \exp(1.51 \times 10^4 \exp(-\frac{2.88 \times 10^4}{RT})t) \quad (16)$$

It was also observed that the accumulation of SCFAs followed first-order kinetics when the fermentation pH was not adjusted (blank test). Nevertheless, the k_a value in the blank test was much lower than that at pH 10. At 20°C, for example, the k_a value was 0.14 d^{-1} at pH 10, while it was 0.11 d^{-1} when the fermentation pH was not controlled.

2.4 Model verification

To verify the kinetic models of WAS hydrolysis and acidification, three identical reactors with initial particulate WAS COD of 13366 mg/L were operated at 15, 25, and 32°C, respectively (pH 10). It can be seen from Fig. 4 that the both experimental values of particulate COD hydrolysis and SCFAs accumulation gave good conformity with the model ones. Thus, Eqs. (12) and (16) will be helpful for designing and operating bioreactors for anaerobic WAS hydrolysis and SCFAs production at pH 10.

3 Conclusions

The hydrolysis of WAS particulate COD at pH 10 was a first-order kinetic reaction, and the hydrolysis rate constant increased from 0.017 to 0.16 d^{-1} with the increasing of temperature from 10 to 35°C. It was also observed that the accumulation of SCFAs at pH 10 obeyed the first-order kinetics, and the SCFAs accumulation rate constant was 0.066, 0.14, 0.17and 0.18 d^{-1} at 10, 20, 30 and 35°C, respectively. The influences of temperature on the hydrolysis and SCFAs accumulation rate constants could be described by the Arrhenius equation. The accumulation of SCFAs during WAS fermentation at pH 10 was a biological effect. The model of both WAS hydrolysis and SCFAs production at given temperatures (15, 25 and 32°C) showed a good conformity with the experimental data.

Acknowledgments

This work was financially supported by the Hi-Tech Research and Development Program (863) of China (No. 2007AA06Z326), the Key Projects of National Water Pollution Control and Management of China (No. 2008ZX07315-003, 2008ZX07316-002) and the Key Laboratory of Environmental Science and Engineering of Jiangsu Province (No. ZD071201).

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