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Modified ADM1 for modeling free ammonia inhibition in anaerobic acidogenic fermentation with high-solid sludge

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ABSTRACT

Anaerobic acidogenic fermentation with high-solid sludge is a promising method for volatile fatty acid (VFA) production to realize resource recovery. In this study, to model inhibition by free ammonia in high-solid sludge fermentation, the anaerobic digestion model No. 1 (ADM1) was modified to simulate the VFA generation in batch, semi-continuous and full scale sludge. The ADM1 was operated on the platform AQUASIM 2.0. Three kinds of inhibition forms, *e.g.*, simple inhibition, Monod and non-inhibition forms, were integrated into the ADM1 and tested with the real experimental data for batch and semi-continuous fermentation, respectively. The improved particle swarm optimization technique was used for kinetic parameter estimation using the software MATLAB 7.0. In the modified ADM1, the K_s of acetate is 0.025, the $k_{m,ac}$ is 12.51, and the K_{I_NH3} is 0.02, respectively. The results showed that the simple inhibition model could simulate the VFA generation accurately while the Monod model was the better inhibition kinetics form in semi-continuous fermentation at pH 10.0. Finally, the modified ADM1 could successfully describe the VFA generation and ammonia accumulation in a 30 m^3 full-scale sludge fermentation reactor, indicating that the developed model can be applicable in high-solid sludge anaerobic fermentation.

Introduction

The amount of waste activated sludge (WAS) has increased significantly due to the rapid growth of wastewater treatment during the past few decades, which imposes a big burden on the environment. It is well known that anaerobic fermentation is an effective method to resolve WAS by reduction of its volume and generation of valuable products (Appels et al., 2011). Among the valuable products, volatile fatty acids (VFAs) attract intensive interest because of their wide range of applications such as in the fields of bioenergy, bioplastics and biological nutrient removal from wastewater (Lee et al., 2014). In traditional anaerobic digestion, the WAS is fermented with the total solid (TS) concentration between 4% and 6% (400–600 g TS/L), which requires a large volume for the digester and low efficiency (Hansen et al., 1998). Recently, high-solid anaerobic fermentation, with the TS content over 8% or even as high as 15% of the sludge feedstock, is becoming more prevalent due to its merits such as higher loading, smaller reactor volume, lower energy consumption and so on (Dai et al., 2014).

Ammonia is produced by the biological degradation of nitrogenous matter, mostly in the form of proteins and urea (Kayhanian, 1999). In high-solid fermentation, there will be a high concentration of ammonia in the fermentation liquid, which will inhibit the anaerobic fermentative microorganisms

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in the digester and consequently limit the VFA production. Among the four types of anaerobic microorganisms, the methanogens are the least tolerant to ammonia inhibition. There is conflicting information in the literature about the sensitivity of acetoclastic and hydrogenotrophic methanogens. The fermentative and acidogenic populations in granular sludge were hardly affected by the free ammonia (FA) (Chen et al., 2008).

Because alkaline fermentation has been widely demonstrated as the optimal condition for the production of VFAs from sludge (Yuan et al., 2006), a high pH value further promotes the generation of FA, and this may lead to a stronger inhibition of VFA fermentation. However, there is less information about the mathematic modeling regarding the inhibition impacts of high NH_3 on VFA generation from sludge by anaerobic fermentation, which limits the scale-up of anaerobic digesters and industrial application.

The anaerobic digestion model No. 1 (ADM1) proposed in 2002 is a structural model used to establish a common platform for modeling anaerobic digestion processes and for further model development and validation studies (Batstone et al., 2002). The ADM1 has become a widely used application tool for modeling anaerobic digestion. By using the model, anaerobic processes can be simulated and predicted in a variety of operating conditions. Many studies based on the original ADM1 have been applied to describe the whole process and results (Batstone, 2006; Rodriguez et al., 2006; Girault et al., 2012). However, in the conventional ADM1 structure, inhibition by FA only considered the inhibitor concentration and the inhibitor parameter (Batstone et al., 2002). Since the hydrophobic ammonia molecule may diffuse passively into the cell, causing proton imbalance and/or potassium deficiency in acidogenic microorganisms, the inhibition by ammonia with high concentration should be re-considered and integrated into the ADM1 to elaborate the model structure. Therefore, it is necessary to compare and integrate proper kinetics forms to simulate the inhibition due to FA in WAS fermentation systems for VFA production. Several algorithms have been used to estimate the parameters for simulating the biochemical reactions (Ni et al., 2011). Particle swarm optimization (PSO) is a novel evolutionary algorithm which has many advantages over the other algorithms in some aspects (Kennedy, 2010). However, few papers have applied PSO in ADM1 for parameter estimation.

The objective of this study is to develop a modified ADM1 model to better characterize the FA inhibition and simulate the VFA accumulation in anaerobic fermentation with both experimental and modeling approaches. Furthermore, the modified ADM1 was also used to simulate the VFA production in a semi-continuous digester and 30 m^3 full-scale sludge fermentation reactor.

1. Materials and methods

1.1. Sludge characteristics and pre-treatment method

The WAS used in the study was dewatered sludge obtained from the Taihu Xincheng Wastewater Treatment Plant, Wuxi, China. The sludge was diluted to TS of 75 g/L with distilled water. Pre-treatment of sludge by thermal alkaline treatment was conducted as follows: the pH of the sludge was adjusted to 11.0 using 20 mol/L NaOH solution from an initial pH of 7.0, and then stirred for 2 hr at 90°C (Liu et al., 2012). The process of the acclimation and re-reactivation of seeding sludge was followed as per the previous literature (Wang et al., 2013).

The characteristics of the fermentation sludge and seeding sludge are shown in Table 1. In order to fit the application in the model, the organic matter is converted to COD with the equivalent coefficient.

1.2. Fermentation experiments

The batch and semi-continuous fermentation of sludge were carried out in a 30 L anaerobic reactor (GUJS-30C, Zhengjiang East Co., China). 18.9 L pre-treated sludge and 2.1 L seeding sludge were pumped into the reactor for anaerobic fermentation. During the fermentation, the pH of the sludge will deviate from the target because of the VFA generation, so the pH should be maintained at 11.0 by periodic adjustment. The pH of the sludge was adjusted to pH 11.0 every 48 hr by using 20 mol/L NaOH when it was lower than 11.0 or 12 mol/L HCl solution when it exceeded 11.0, respectively. Nitrogen gas was sparged into the reactor for 10 min to remove the oxygen from the headspace after sampling and pH adjustment. The temperature was kept at 37 ± 1°C.

In the semi-continuous experiment, a 21.0 L sludge mixture was pumped into the anaerobic reactor, and the other operation conditions were the same as those in batch fermentation. After reaching the stable stage, the fermentation was converted to a semi-continuous experiment. 4.2 L fermented sludge was discharged and 4.2 L fresh pre-treated sludge was pumped into the reactor every 2 days, so the sludge retention time was 10 days. Samples were taken every 2 days for analysis.

The full scale sludge fermentation was conducted in Shuofang Wastewater Treatment Plant, Wuxi, China. During the fermentation, 21 m^3 pre-treated sludge (TS = 8%) and 0.2 m^3 seeding sludge were pumped into the fermentation reactor. The methods of sludge pretreatment and seeding sludge acclimation were the same as the methods used in the laboratory experiment. The whole fermentation was divided into a start-up period (days 0–18) and stable operation period (days19–48). The start-up period was batch fermentation and

Table 1 – The characteristics of the pretreated sludge liquid.

Parameters	Value (mg/L)	Input values of model (kg COD/m^3)
Total COD	36,982.58	36.98
Soluble COD	30,884.62	30.86
Total COD (seeding sludge)	31,381.54	31.38
Proteins	9341.67	21.52
Carbohydrates	2240.01	3.45
Lipids	952.07	2.77
Total nitrogen	2089.75	——
Ammonia nitrogen	1217.88	——

"——"No unit conversion.

the stable operation period was semi-continuous fermentation. In the start-up period, the fermentation was batch mode, so the VFAs will eventually accumulate to a plateau. After the VFAs stopped increasing, the fermentation was switched into semi-continuous fermentation mode. During the semi-continuous fermentation, 2.1 m^3 fresh sludge was pumped into the reactor each day after the same volume of sludge was pumped out from the reactor. The VFAs will maintain stability if the fermentation is operated well. Based on this operation mode, when the fluctuation of VFA yield was less than 10%–15% of the average yield, the fermentation was considered to be in stable status. During the fermentation, the VFAs, total nitrogen and ammonia nitrogen were measured every day.

The fermentation temperature was controlled at 35 ± 2°C, with stirring rate of 48 r/min and pH at 10.0–11.0. During the operation, sludge samples were taken every day or two days for analysis.

1.3. Analytical methods

Before the VFA assay, the sludge samples were centrifuged 10 min at 10,000 r/min at room temperature and the supernatants were used for VFA measurement. The VFAs were measured by a gas chromatograph (GC-2010, Shimadzu Co., Japan) equipped with an auto injector (AOC-20i, Shimadzu Co., Japan). The liquid sample was pretreated by filtration through a 0.45 μm filter membrane (Liu et al., 2011). A flame ionization detector was used and the column was a fused silica capillary (PEG-20 M, 30 m × 0.32 mm × 0.51 m, China); 4-methyl-valeric acid was added as an internal standard and the samples were acidified by 3 mol/L phosphoric acid. The initial temperature of the GC column was 80°C and was held for 3 min, then increased by 15°C/min to a final temperature of 210°C, and then held for 2 min.

1.4. Model modification and inhibition functions

The ADM1 model contains the disintegration, hydrolysis, acidogenesis and methanogenesis process in the WAS anaerobic fermentation. Due to the strong inhibition caused by the FA released from the high-solid sludge, the inhibition due to FA on VFA generation was considered in the modified ADM1 model. Modifications of the main inhibition form in the model are listed in Appendix A Table S2. The ADM1 was simulated by AQUASIM 2.0 in this study. The stoichiometric coefficients were calculated from the concentrations of the substrate components in Table 2. The values of other coefficients used in modeling are from Batstone et al. (2002). Additional parameters and the initial state in the model calculation are listed in Appendix A Tables S3–S4. Typically, inhibition such as that due to long-chain fatty acids was classified as toxic, while ammonia inhibition was considered as non-competitive inhibition. A simple inhibition form was proposed by Lehninger and given in Eq. (1) (Granger and Lehninger, 1982).

$$I = \frac{1}{1 + {}^{S_I}/_{K_I}} \tag{1}$$

In the above form, non-competitive inhibition was only influenced by the concentration of inhibitors and the influence of substrate concentration was ignored. Also, studies have reported that the Monod kinetics form can also be used to describe the inhibition process. This is shown in Eq. (2) (Sprott and Patel, 1986).

$$r_j = \frac{k_{m,i} X S}{K_{S,i} + S\left(1 + {}^{K_I}/_{S_i}\right)} \tag{2}$$

The two forms of inhibition equations and definition of parameters are listed in Appendix A Table S1.

The determination coefficient (R^2) values were used to evaluate the accuracy of the model simulation and experimental data of different forms of FA inhibition. If the model is more accurate, the R^2 is closer to 1, indicating the simulated data are in agreement with the actual data.

1.5. Parameter estimation

During the parameter optimization, ADM1 takes the specific parameters $k_{m,i}$, and $K_{s,j}$ as variables. The difference between the true and simulated values was taken as the objective function. Then, we minimized the objective function to improve the accuracy of simulation.

Among the available algorithms, PSO is a widely used algorithm for global space search in several algorithms that are used in estimating. For parameter estimation, an improved PSO method was used for ADM1 optimization (Bai et al., 2015). The estimation was conducted using the platform of MATLAB 7.0. The Monod maximum uptake rate parameters $k_{m,i}$ and Monod half saturation constant $K_{s,j}$ in the modified ADM1 model were estimated with the experimental data using the PSO algorithm. With the estimation based on the improved κ-PSO, the optimal values of the parameters are shown in Appendix A Fig. S1. As shown in Fig. S1A, B and C, after 500 iterations by κ-PSO, the K_s of acetate is 0.025, the $k_{m,ac}$ is 12.51, and the $K_{I\text{-}NH3}$ is 0.02, respectively.

Table 2 – Stoichiometric coefficients for ADM1.

Symbol	Definition	Values
f_{si_XC}	Fraction of soluble inert from components	0.085
f_{xi_XC}	Fraction of insoluble inert from components	0.165
f_{ch_XC}	Fraction of particulate carbohydrates from components	0.091
f_{pr_XC}	Fraction of particulate proteins from components	0.581
f_{li_XC}	Fraction of particulate lipids from components	0.075

2. Results and discussion

2.1. Modeling simulation in batch fermentation

A high concentration of FA is one of inhibiting factors in the fermentation of high-solid WAS for VFA generation. To evaluate the simulation by the modified ADM1 that integrated simple inhibition, Monod inhibition or non-inhibition forms of high FA inhibition, the model was first tested with the experimental data in the batch fermentation. The experimental data for acetate, propionate, butyrate and valerate accumulation and the simulation results are shown in Fig. 1.

As shown in Fig. 1, the acetate, propionate, butyrate and valerate concentrations increased from the initial value of 0 to 13.40 kg COD/m^3, 0.98 kg COD/m^3, 7.80 kg COD/m^3 and 1.02 kg COD/m^3 on day 22, respectively. They all increased rapidly during days 4–6 and then maintained stability during the rest of the fermentation. The generation of acetate, propionate, butyrate and valerate was simulated by AQUASIM 2.0 with the simple inhibition, Monod and non-inhibition forms respectively. For each modified model structure, the parameters were estimated by the improved PSO algorithm. Through structure modification and proper parameter estimation, the ADM1 model has been demonstrated to successfully simulate the VFA accumulation under FA inhibition conditions in sludge fermentation for VFA production (Bai et al., 2015).

It was shown that the simple inhibition model could simulate the production of VFAs better than the other two models in the batch reactor at pH 6.5 over 22 days. It can be seen that the data predicted by ADM1 with the non-inhibition form are always much lower than the experimental data (Fig. 1). This is because a high concentration of FA not only inhibits the acidogenic microorganisms, but also inhibits the methanogens very seriously (Chen et al., 2008). In the absence of FA inhibition in the ADM1 model, the methanogens will consume the acetate, and this causes lower predicted values for VFA data than the real determined data.

VFAs are intermediates during the anaerobic fermentation of sludge, and the generation process is a series of biochemical reactions driven by different functions of facultative and anaerobic microbes, such as fermentative bacteria, syntrophic acetogens and homoacetogens. (Jabłoński and Łukaszewicz, 2014). Many factors will influence the activity of these microorganisms, which then influences the VFA generation. In this study, we focused on the effect of high concentrations of FA on the VFA generation and simulated the process by the modified ADM1. The simulated results in Fig. 1 show that a proper FA inhibition form in the ADM 1 structure is important for VFA simulation.

2.2. Modeling simulation in semi-continuous fermentation

After the evaluation of the modified ADM1 in batch fermentation, it was further applied in the simulation of the VFA profiles in semi-continuous fermentation. The experimental data and the simulation results from the modified ADM1 are plotted in Fig. 2.

As shown in Fig. 2a, the concentration of acetate increased from 6 kg COD/m^3 to 9 kg COD/m^3 and remained at that level during the whole fermentation. In Fig. 2b and d, the trends of propionate and valerate concentrations during the whole semi-continuous fermentation process are similar to those for acetate, but their concentrations (0.75 kg COD/m^3 and 0.55 kg COD/m^3, respectively) are much lower than acetate. The butyrate concentration also followed the change trend of acetate, and its final concentration was about 5.5 kg COD/m^3 (Fig. 2c). The production of acetate, propionate, butyrate and valerate were simulated with the simple inhibition form, Monod and non-inhibition form, respectively. It was shown that the Monod model can simulate the

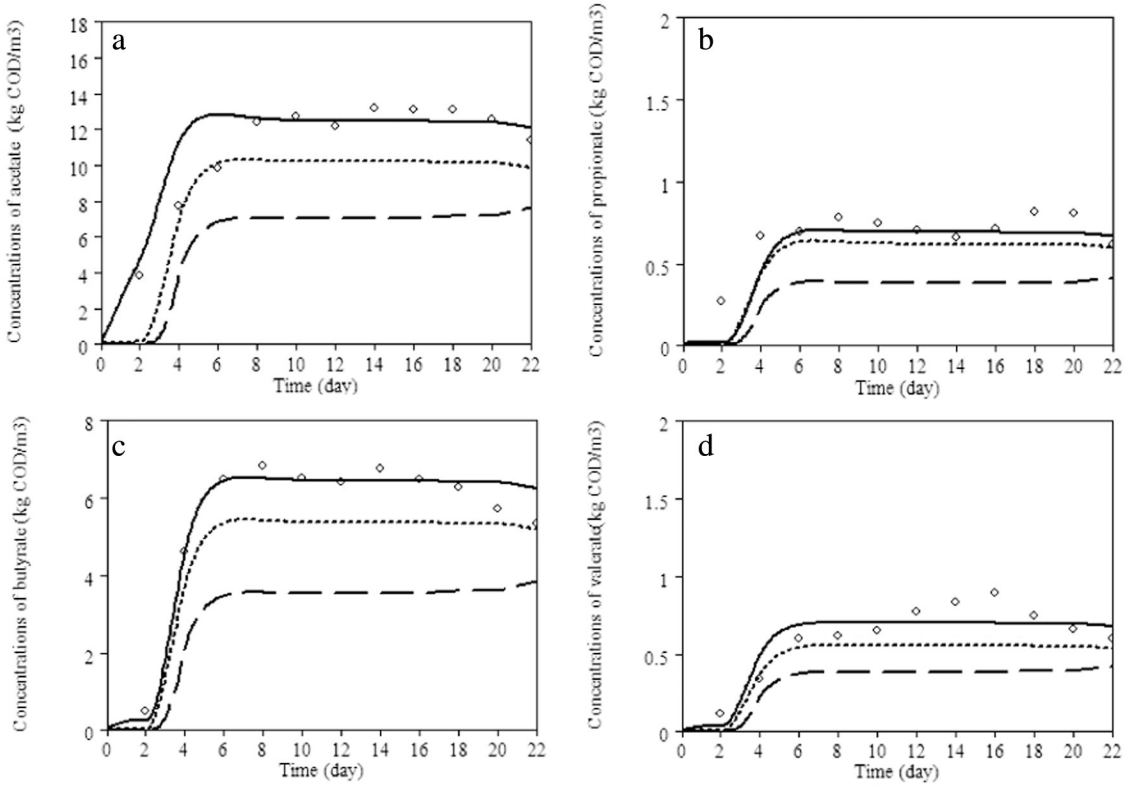

Fig. 1 – Modeling results (line) and the experiment results (open cycle) of volatile fatty acid (VFA) production in the anaerobic batch fermentation.

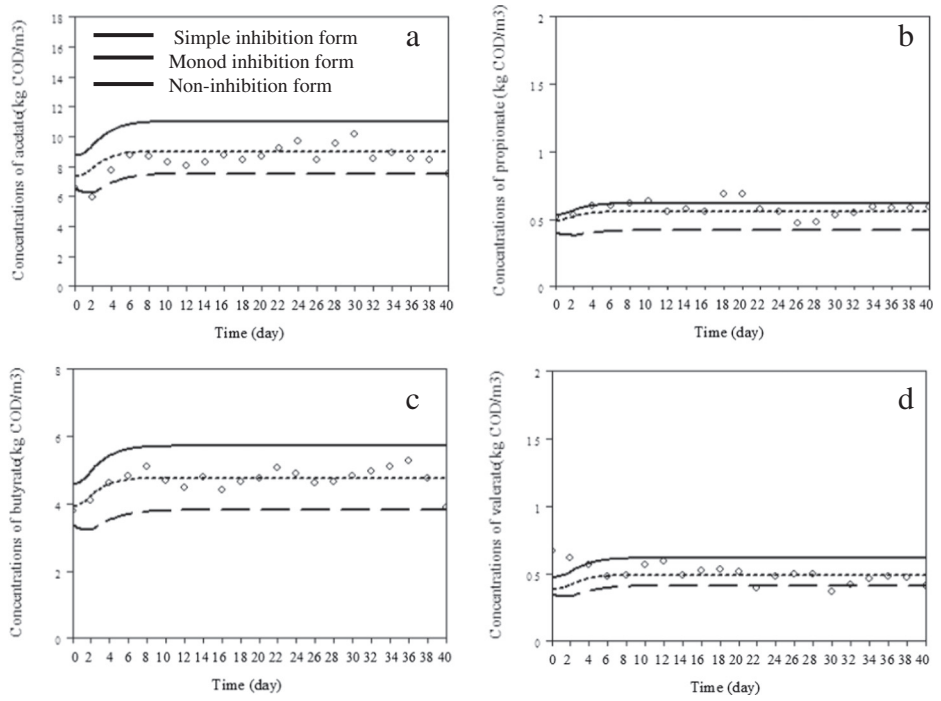

Fig. 2 – Modeling results (line) and the experiment results (open cycle) of VFAs production in semi-continuous fermentation. a: acetate; b: propionate; c: Butyrate; d: valerate.

production of VFAs better than the other two models in semi-continuous fermentation.

Traditionally, ammonia inhibition in anaerobic fermentation has been modeled based on the simple form of ammonia inhibition in ADM1, which was proposed by Lehniner, assuming that the inhibition rates depend on inhibition parameters and concentrations (Granger and Lehninger, 1982). However, for complex substrates with high concentrations of FA, more complex kinetic forms should be more applicable.

2.3. The determination coefficient analysis

The R^2 values for model simulation and experimental data of different FA inhibition forms are shown in Table 3. If a model accurately predicts the experimental result, R^2 should be close to 1.0. The R^2 values for the simple inhibition, Monod and non-inhibition forms for acetate in the batch reactor at pH 10.0 were 0.9842, 0.9558 and 0.7988, respectively.

The R^2 results showed that the simple inhibition model was the proper form for simulation of FA inhibition in the batch fermentation experiment. The other determination coefficient values for propionate, butyrate and valerate showed that the simple inhibition model can also simulate the production of VFAs very well. However, in the semi-continuous fermentation experiment, the R^2 values for the simple inhibition, Monod and non-inhibition forms for acetate are 0.9750, 0.9923 and 0.9310, respectively. The R^2 results showed that the Monod inhibition model was the proper form for simulation of FA inhibition.

2.4. Modeling simulation in full scale fermentation

According to the modified ADM1 model, the fit between the experimental data for acetate, propionate and butyrate accumulation as well as the ammonia accumulation in a 30 m^3 full-scale anaerobic digester and the simulation results are plotted in Fig. 3. The full-scale fermentation was divided into a start-up stage (days 0–16) and semi-continuous stage (days 17–48).

As shown in Fig. 3a, the acetate increased rapidly in the start-up stage and maintained at about 4.5 kg COD/m^3 in the

Table 3 – The determination coefficient values (R^2) of the different models.

$R^2$of VFAs	Batch fermentation				Semi-continuous fermentation			
	Acetate	Propionate	Butyrate	Valerate	Acetate	Propionate	Butyrate	Valerate
Simple form	0.9842	0.9345	0.9953	0.9792	0.9750	0.9750	0.9625	0.9336
Monod form	0.9558	0.9184	0.9664	0.9287	0.9923	0.9923	0.9948	0.9694
Non-inhibition form	0.7988	0.7079	0.7840	0.7695	0.9310	0.9310	0.9445	0.9231

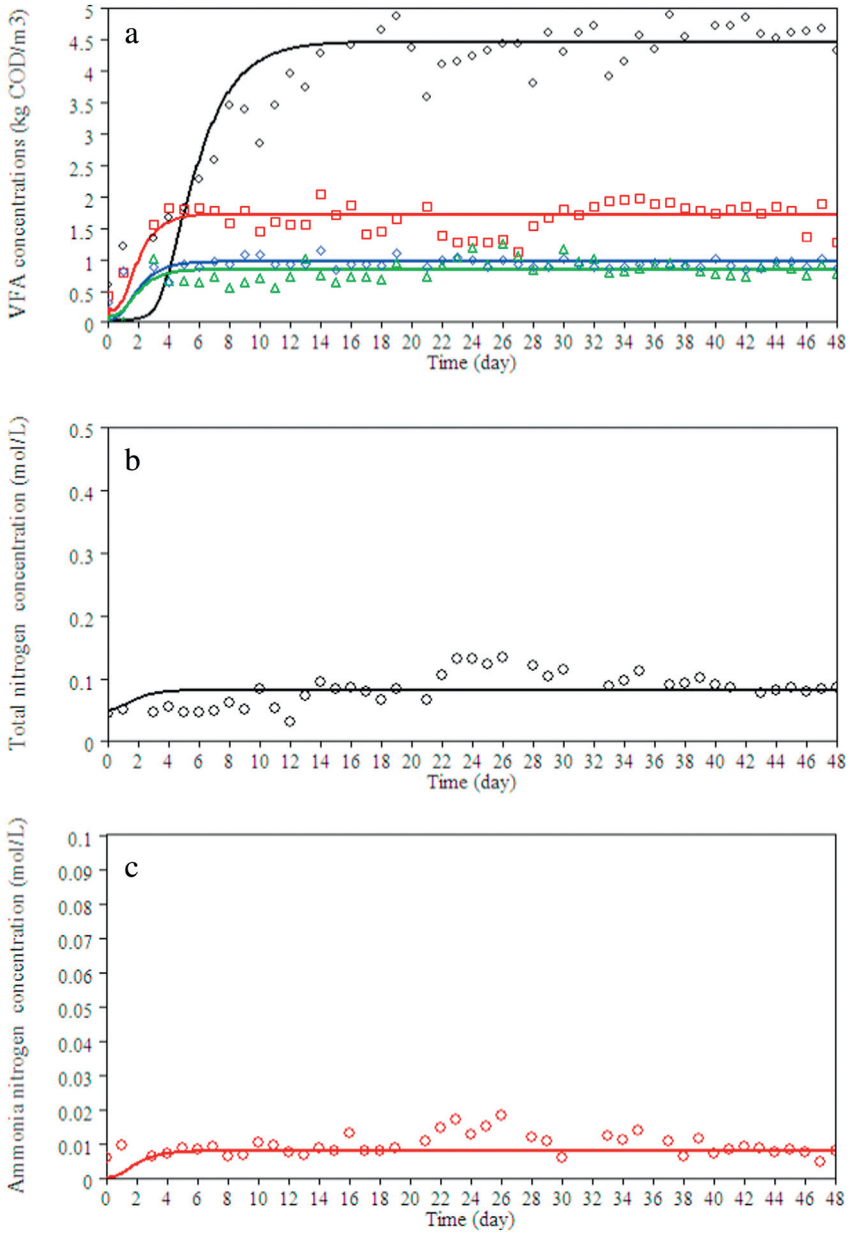

Fig. 3 – Modeling results (line) and the experiment results (open cycle) of the VFAs (a) and total nitrogen (b) and ammonia nitrogen (c) in full scale sludge fermentation.

semi-continuous stage. Meanwhile, the change trends of propionate, butyrate and valerate were similar to that of acetate, and their average concentrations were 0.85 kg/m^3, 1.77 kg/m^3 and 0.68 kg/m^3 (calculated as COD), respectively. The experimental data were simulated with the simple inhibition form (in the start-up stage) and Monod inhibition form (semi-continuous stage) of the modified ADM1 model. According to the simulation results, it can be seen that the two forms of models can fit the acetate, propionate, butyrate and valerate data well, indicating that the modified ADM1 model with the two FA inhibition forms can simulate the practical experimental data even in full-scale sludge fermentation for VFA production.

The total nitrogen and ammonia nitrogen concentrations and their simulation results are plotted in Fig. 3b and c. As shown in Fig. 3b, the total nitrogen increased from 0.04 mol/L in the start-up stage and then maintained at 0.09 mol/L in the semi-continuous fermentation stage. The total nitrogen starts off with a high concentration during the fermentation because of the thermo/alkalinity pretreatment of the sewage sludge, which caused the release and accumulation of total nitrogen. It should be noted that due to the changes in influent sludge concentrations and operation conditions, the ammonia nitrogen concentrations fluctuated greatly in the semi-continuous fermentation stage. The ammonia nitrogen concentrations were close to 0.01 mol/L during the whole

stage of fermentation. After the simulation, the curve can fit the experimental data very well.

The R^2 values were also calculated to show goodness of fit between the simulated data and the experimental data of different VFAs, total nitrogen, and ammonia (Fig. 3) in the full-scale fermentation process. The R^2 values of acetate, propionate, butyrate and valerate were 0.9552, 0.9678, 0.9851 and 0.9871, respectively. The R^2 results for total nitrogen and ammonia were 0.9896 and 0.9921, respectively. The relative errors for full scale fermentation were all about 0.05, suggesting that there is a reasonably good agreement between the model outputs and the experimental results.

By using standard ADM1 variables and kinetics for the modified FA inhibition forms calibrated based on batch fermentation, the modified ADM1 models were able to successfully predict the reactor performance under full-scale scenarios. Predicted acetate accumulation generally complied with the experimental observations, with deviations attributed to less accurate predicted inhibitory processes *etc*. Many studies have investigated the impacts of FA inhibition on the VFA accumulation from waste activated sludge (Gallert et al., 1998), however, there is little information about the modification of ADM1 for VFA generation in the condition of high FA-based inhibition. It has been confirmed that the alkaline condition was the optimal condition for VFA accumulation in sludge fermentation.

Finally, it is important that the data simulated by modified ADM1 models can fit the experimental data in full-scale sludge fermentation well, indicating that the improved ADM1 was verified to be applicable for batch, semi-continuous and full-scale sludge fermentation systems.

3. Conclusions

In this study, three kinds of FA inhibition forms, *e.g*., simple inhibition, Monod inhibition or non-inhibition forms, were integrated into the ADM1 model, respectively, and the simulation results were tested with the experimental data in batch, semi-continuous and full-scale sludge fermentation. In the modified ADM1, the K_S of acetate is 0.025, the $k_{m,ac}$ is 12.51, and the $K_{I\text{-}NH3}$ is 0.02, respectively. The statistical R^2 values showed that the simple inhibition form can simulate the VFA generation better than the other two forms in a batch reactor, while the Monod kinetics form was more appropriate to simulate the VFA production in semi-continuous fermentation. Finally, the modified ADM1 model was successfully developed to describe the VFA generation and ammonia accumulation in a 30 m^3 full-scale sludge fermentation reactor, indicating that the model developed in this study was applicable for different high-solid sludge fermentation systems.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.03.004.

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