Modeling anaerobic digestion of blue algae: Stoichiometric coefficients of amino acids acidogenesis and thermodynamics analysis

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ABSTRACT

In order to facilitate the application of Anaerobic Digestion Model No. 1 (ADM1), an approach for a detailed calculation of stoichiometric coefficients for amino acids acidogenesis during the anaerobic digestion of blue algae is presented. The simulation results obtained support the approach by good predictions of the dynamic behavior of cumulative methane production, pH values as well as the concentrations of acetate, propionate, butyrate, valerate and inorganic nitrogen. The sensitivity analysis based on Monte Carlo simulation showed that the stoichiometric coefficients for amino acids acidogenesis had high sensitivities to the outputs of the model. The model further indicated that the Gibbs free energies from the uptake of long-chain fatty acids (LCFA), valerate and butyrate were positive through the digestion, while the free energies for other components were negative. During the digestion, the cumulative heat productions from microbial activities and methane were 77.69 kJ and 185.76 kJ, respectively. This result suggested that proper heat preservation of anaerobic digesters could minimize the external heating needs due to the heat produced from microbial activities.

1. Introduction

The bloom of blue algae is a ubiquitous phenomenon in eutrophic lakes and reservoirs in many countries. The excessive growth of blue algae in lakes damages the natural functions of lakes and threats drinking water supply. Refloatation of algae from lakes is considered as the most efficient approach to reduce threatens of blue algae (Guo, 2007). However, without further management such large amounts of skimmed blue algae will result in a serious secondary pollution. Anaerobic digestion draws a growing concern due to the

potential of energy production and waste treatment (Cantrell et al., 2008). Previous studies demonstrated that anaerobic digestion of skimmed algae seems to be more practical compared to other recovery methods (Yan et al., 2010).

To increase the capacity of biogas production from blue algae, different technologies have been considered. Anaerobic co-digestion of blue algae with corn straw (Zhang et al., 2012) or sludge (Hu et al., 2009) is an efficient approach for improving process efficiency. Another approach is to co-generate high-valued products, such as poly-hydroxyalkanoates (Yan et al., 2010). Lab- and pilot-scale experiments, which are important tools to implement a new process design, require a long time to reach the steady state conditions. Furthermore, anaerobic digestion includes a series of interrelated reactions, and experimental assessment of the impacts of all involved variables on the process efficiency is time consuming and hardly possible. Therefore, the mathematical model is definitely useful to predict the behavior of an anaerobic system, optimize the production and prevent process failure.

In 2002, the International Water Association (IWA) Task Group for Mathematical Modeling of Anaerobic Digestion Processes developed a generic dynamic model known as ADM1 (Batstone et al., 2002) which has been widely used both for lab- and full-scale anaerobic reactors. Though several dynamic models on anaerobic digestion of micro-algae have been proposed (Mairet et al., 2012), the literature on ADM1 of micro-algae is limited. Cameron (2012) modeled the anaerobic digestion of *Chlorella vulgaris* based on ADM1 and Acidogenesis/Methanogenisis Model. Mairet et al. (2011) demonstrated the models on anaerobic digestion of *C. vulgaris* with ADM1 and a modified version (Contois kinetics for the hydrolysis). Their results showed that the modified ADM1 could adequately fit the experimental data for 140-days experiment with various flow rates and influent load. However, the stoichiometric coefficients of amino acids acidogenesis in ADM1 for above studies employed the recommended values from the technical report of IWA, which implied that all the products from amino acids acidogenesis have the same proportions, and therefore could not adequately predict experimental results, especially for the substrates with a high content of protein like blue algae. Moreover, the heat generated from microbial activities combined with ADM1 during anaerobic co-digestion of cattle manure and renewable energy crops was calculated by Lübken et al. (2007). Their results showed that the amount of heat from microbial activities, higher than the energy consumption of the stirrer, could not be neglected. However, the presented heat was only calculated in several separated days and the information of this heat as well as the thermodynamics during the whole digestion of blue algae were scarce.

The major objective of this study was to approach a detailed calculation of stoichiometric coefficients for amino acids acidogenesis to facilitate the application of ADM1. The dynamic behaviors of different components as well as the sensitivity analysis based on Monte Carlo simulation of the coefficients on the components were studied during the anaerobic digestion of blue algae. In addition, the changes of Gibbs free energy for components uptake and heats from methane and microbial activities were also explored.

2. Materials and methods

2.1. Experimental setup

The substrates used in the experiment were the mixtures of blue algae and lake water, collected from Meiliang Bay, Taihu Lake (120°30′E, 31°27′N). Meiliang Bay, one of the most eutrophied bays in the northern of Taihu Lake, has been frequently influenced by the blooms of blue algae (Zhong et al., 2010). The mixtures were stored at 4 °C before further use. The characteristics and components of the mixtures are shown in Table 1. Anaerobically digested dairy cattle manure slurry was filtered and used as the inoculums, which was collected from an 800-m³-size biogas plant (Qingdao, China) operating at 32 °C, with 25-days retention time. The total solid (TS) and volatile solid (VS) contents of the slurry are 21.50% and 63.93% TS, respectively.

A bench-top continuous stirred-tank reactor (CSTR) was fabricated from 10 mm glass sheets and equipped with a temperature-controlled water bath at 35 °C. The CSTR, with the working volume of 4.5 L, was connected to a wet-type gas flow meter and gas sampling ports using silicone tubes. The TS in CSTR was adjusted to 4.08% with algal mixture and inoculum. The reactor was then flushed with a mixture of 80% N_2 and 20% CO_2.

2.2. Analytical methods

The daily biogas production was recorded by the gas flow meter. Samples from the digester were daily collected for measurements of pH, biogas components and volatile fatty acids (VFAs). The biogas components were analyzed by a gas chromatograph (SP 6890, Shandong Lunan Inc., China), equipped with Porapak Q stainless steel column (180-cm long, 3-mm outer diameter) and a thermal conductivity detector. The temperatures of the injector, detector and oven were 120 °C, 150 °C and 50 °C, respectively. VFAs were analyzed by a gas chromatograph (SP 6890, Shandong Lunan Inc., China), equipped with the Innowax column (30 m × Φ0.25 mm × 0.25 μm) and flame ionization detectors, after filtering samples through a 0.45 μm glass microfiber filter. The operating temperatures were 220 °C, 250 °C, and 150 °C, for the injection port, the detector and the oven, respectively. Total and soluble carbohydrates were measured according to the phenol–sulfuric method (Herbert et al., 1971) and anthrone colorimetric method (Raunkjær et al., 1994), respectively. A 2:1 mixture of petroleum ether and ethyl ether

Table 1 – The characteristics of the substrate.				
Component	Description	Symbol	Unit	Value
Soluble components	Total valerate	S_{va}	kgCOD/m³	0.013
	Total butyrate	S_{bu}	kgCOD/m³	0.307
	Total propionate	S_{pro}	kgCOD/m³	0.316
	Total acetate	S_{ac}	kgCOD/m³	0.657
Particulate components	Carbohydrates	$f_{ch,c}$	–	0.267
	Proteins	$f_{pr,c}$	–	0.517
	Lipids	$f_{li,c}$	–	0.095
	Particulate inerts	$f_{i,c}$	–	0.121

was used for lipid extraction from dry samples. Then, the lipid extraction was redissolved using hexane and methyl esterified for the GC–MS (Thermo LTQ-1100, USA) analysis (Lepage and Roy, 1984). The protein was analyzed through hydrolyzed amino acids according to the hypothesis in ADM1 that the mole content of protein was equal to the mole content of amino acids. The amino acids were measured by ion chromatography (ICS-5000, Dionex, USA) after the sample was hydrolyzed in 6 M HCl at 105 °C under vacuum for 24 h (Jia et al., 2001). The TS, VS and pH were determined according to the standard methods (SEPA, 2002).

2.3. Kinetic model assumptions

The ADM1 was used to simulate the major biochemical and physicochemical processes in the anaerobic digestion of blue algae. The stoichiometric coefficients of amino acids acidogenesis were obtained based on the following assumptions (Ramsay and Pullammanappallil, 2001). First, the protein hydrolysis was assumed much slower than the subsequent amino acids acidogenesis. Second, it was assumed that the pathways of amino acid acidogenesis remain constant and occur predominantly by only one pathway regardless of operation conditions. Table S1 shows the stoichiometric equations for the anaerobic degradation of different amino acids (Ramsay and Pullammanappallil, 2001). The chosen equation was based on the following steps. For the amino acids with only one known degradation pathway, their reactions were chosen as the dominant pathway. And the Stickland reaction was chosen as the dominant reaction for the degradation of amino acids involving more than one reaction. Furthermore, the Stickland oxidation reaction, where the produced ATP would be favorable for the common presence of hydrogenotrophic methanogens, was selected as the dominant reaction. In addition, tyrosine was assumed to be degraded to p-cresol due to that this reaction could produce less hydrogen with the same ATP yield compared to the other oxidation reaction. Consequently, the stoichiometric coefficients of amino acids acidogenesis to acids for the blue algae were determined in Table 2. The other organic components including aromatic components, methyl mercaptan and formamide, which were not included in ADM1 and also had low molar contents in the products, were ignored. Hence, the stoichiometric coefficients of amino acids acidogenesis for acetate, propionate, butyrate, valerate and hydrogen were 0.325, 0.041, 0.242, 0.293 and 0.099, respectively. In order to calculate the stoichiometric coefficients of LCFA to acid, the lipid compositions of blue algae were analyzed by GC–MS and performed in Fig. S1. The fatty acids C16:0, C18:0, C18:1 and C18:2 were found in blue algae. Palmitate and stearate, with an assumed molar ratio of 1:1, were chosen to be representative for LCFA. In methanogenic reactor systems, the degradation of LCFA proceeded via β-oxidation, yielding acetate and hydrogen. Hence, the stoichiometric coefficients of LCFA degradation for acetate and hydrogen were 0.717 and 0.283, respectively.

2.4. Model implementation and calibration

According to the biochemical conversion pathway of ADM1 (Fig. S2), the matrix of the model equations was established in

Table S2. The ADM1 was implemented using Aquasim 2.0 (Reichert, 1998) and values for initial conditions of equilibrium coefficients, kinetic parameters and stoichiometric coefficients of acidogenesis of monosaccharides were taken from the technical report of IWA (listed in Table S3). To achieve the best agreement between measured and simulated values, the parameters listed in Table 3 had to be adjusted.

2.5. Thermodynamics and heat analysis

Table 4 lists the involved anaerobic biochemical reactions and the change of Gibbs free energy (ΔG^0) at pH 7 under standard conditions (all solutes are at the concentration of 1 M, and gases have partial pressure of 1 atm) (Thauer et al., 1977). The actual ΔG of the reaction was calculated with Eq. (1).

$$\Delta G = \Delta G^0 + RT\ln \prod_i a_i^{\gamma_i} \tag{1}$$

where R is the universal gas constant, 8.314 J/K mol, T is the absolute temperature in K, a_i is the activity of the species S_i for a certain reaction with the stoichiometry $\Sigma\gamma_i S_i = 0$, which approximates molarities at low concentrations for aqueous species, while partial pressure in atm for gaseous ones.

The heat produced from microbial activities was calculated by Eq. (2).

$$E_{microbe} = \sum_j (\Delta G - \alpha_j E_{ATP})f_j V_{liq} \tag{2}$$

where $E_{microbe}$ is the heat from microbial activities (kJ/d), ΔG is the actual change of Gibbs free energy (kJ/mol), α_j is the ATP production of process j (mol ATP/mol reaction), E_{ATP} is the energy requirement for ATP formation (kJ/mol ATP), f_i is the daily molar concentration of the product of process j (mol/L d) and V_{liq} is the liquid volume (L). The synthesis of ATP as metabolic energy at equilibrium requires +32 kJ/mol under the standard condition in living cells and about +50 kJ/mol under the conditions assumed to prevail in an actively growing cell (Schink, 1997).

The heat produced from methane was calculated with Eq. (3).

$$E_{CH_4} = Q_{CH_4}H_{CH_4} \tag{3}$$

where E_{CH4} is the heat from methane (kJ/d), Q_{CH4} is the methane production (mol/d) and H_{CH4} is the calorific value of methane (kJ/mol).

2.6. Uncertainty and sensitivity analyses

The optimized values in Table 3, taken from different literatures, have no priori information available in the anaerobic digestion of blue algae. Hence, it is essential to analyze the influence of the parameters uncertainty on the outputs of the model. According to the approach of Sin et al. (2009), Monte Carlo simulation was chosen to analyze the uncertainty of the optimized parameters in Table 3. The upper and lower bounds of the parameters were based on technical report of IWA (Batstone et al., 2002). The parameter space was sampled by the Latin Hypercube Sampling (LHS) method (Iman and Conover, 1982). And 500 samples were used for Monte Carlo simulations, where parameter correlation was not considered

Table 2 – Calculations of stoichiometric coefficients for amino acids acid acidogenesis.

Amino acid (AA)	Mole content (%)	Equation selected	Acetate (mol/mol)	Propionate (mol/mol)	Butyrate (mol/mol)	Valerate (mol/mol)	Other carbon (mol/mol)	Ammonia (mol/mol)	CO$_2$ (mol/mol)	H$_2$ (mol/mol)	ATP (mol/mol)
Asp	9.33	22	1					1	2	2	2
Thr	5.41	21	1		0.5			1		−1	1
Ser	6.63	19	1					1	1	1	1
Glu	13.41	23	1		0.5			1	1		2
Gly	8.54	14	1					1		−1	
Ala	13.21	16	1					1	1	2	1
Cys	0.84	17	1					1	1	0.5	1
Val	7.15	4			1[b]			1	1	2	1
Met	0.7	18		1			1	1	1	1	1
Ile⁻	5.7	3				1[b]		1	1	2	1
Leu	9.29	1				1[b]		1	1	2	1
Tyr	3.08	10	1				1	1	1	1	1
Phe	3.48	5					1	1	1	2	1
Lys	3.61	30	1		1			2			1
His	1.1	25	1		0.5		1	2	1		2
Arg	4.71	28	0.5	0.5		0.5		4	1	−1	1
Pro	3.81	29	0.5	0.5		0.5		1		−1	
Total[a] (mol/ mol protein)			0.694	0.050	0.207	0.192	0.08	1.188	0.880	0.847	1.115
COD (kg/m^3)			0.302	0.038	0.226	0.272	–	–	–	0.092	–
Coefficient (kgCOD/ kgCOD)			0.325	0.041	0.242	0.293	-	–	–	0.099	–
Coefficient in ADM1 (kgCOD/kgCOD)			0.4	0.05	0.26	0.23	–	–	–	0.06	–

[a] Total equals the sum of amino acid content multiple organic acid stoichiometric coefficient.
[b] Represents isomer compounds.

(Sin et al., 2009). The model was simulated 500 times, and the outputs of time series were assessed in the mean and 90th and 10th percentiles of Monte Carlo outputs at each simulation time during 30 days.

The sensitivity analysis of the stoichiometric coefficients of amino acids acidogenesis on the outputs of the model was performed by linear regression of Monte Carlo simulations. The upper and lower bounds of the parameters were based on technical report of IWA (Batstone et al., 2002). The LHS method of was applied to draw 500 samples were drawn without considering the correlation of the parameters (Sin et al., 2011). The sensitivity analysis is obtained by performing linear regression for each of the model outputs of interest obtained from the Monte Carlo procedure. Since this approach requires scalar output, the mean of a time-series profile was used in this dynamic model outputs. A first order linear multivariate model was used to describe the relationship between model outputs (y) and the parameter values (θ_i) as following:

$$y_{\mathrm{reg}} = a + \sum_i b_i \cdot \theta_i \tag{4}$$

where y_{reg} is a vector of scalar values for the model output, a and b is a vector of coefficients and θ is a matrix of parameter values (the sampling matrix).

To obtain the standardized regression coefficients, the regression coefficients b_i are scaled using the standard deviations of model input and output of the Monte Carlo simulations:

$$\beta_i = \frac{\sigma_{\theta_i}}{\sigma_y} \cdot b_i \tag{5}$$

where β_i is the standardized regression coefficients, $\sigma_{\theta i}$ and σ_y is the standard deviations of model input and output, respectively.

3. Results

3.1. Simulation of reactor performance

Fig. 1 presents the simulated and experimental results of pH values, cumulative methane production and the concentrations of acetate, propionate, butyrate, valerate and inorganic nitrogen during anaerobic digestion of blue algae. As shown in Fig. 1(G), the simulation for cumulative production of methane matched the experimental data well. The cumulative production of methane significantly increased following a lag phase of 10 days, and ended at 5174.50 mL after 30 days. The overall methane content was 36.7%. Simulated and experimental pH values are shown in Fig. 1(E). The pH was fairly constant (6.8–7.6) during the whole digestion process, which was generally maintained at a level appropriate for substrate degradation and biogas production. The concentrations of acetate, butyrate and valerate were all increased significantly at the beginning, and then decreased after reaching the peak value both in the experiment and in the model. The predicted concentration decrease was more pronounced than the experimental observations for acetate, butyrate and valerate. However, the predictions for the concentrations of acetate, butyrate and valerate were reasonable, as shown in Fig. 1(A), (C) and (D), respectively. From Fig. 1(F), the simulated and experimental data of inorganic nitrogen had the same tendency in the reactor. In this work, no significant decrease in propionate concentration was observed and the concentration of propionate remained almost constant during the anaerobic process (shown in Fig. 1(B)).

3.2. Sensitivity analysis

The significance of the stoichiometric coefficients of amino acids acidogenesis on pH values, cumulative methane production and the concentrations of VFA and inorganic nitrogen as a function of absolute sensitivity measure (β_i) is shown in Fig. 2. From Fig. 2, it is clear that the stoichiometric coefficients of amino acids acidogenesis have different sensitivities on the outputs of the model. The absolute sensitivity measure of $f_{ac,aa}$ on the concentration of acetate was close to 1.0, which means that for the concentration of acetate, the significance of $f_{ac,aa}$ was the highest among all the parameters. Similarly, the $f_{bu,aa}$ was the most significant source for butyrate among the five parameters with the absolute sensitivity measure of 0.993. The results also showed that $f_{va,aa}$ could influence the pH value, cumulative methane production and the concentrations of acetate, propionate, valerate and inorganic nitrogen, which demonstrated the importance of $f_{va,aa}$ on the model. The concentrations of butyrate and valerate could only be significantly influenced by $f_{bu,aa}$ and $f_{va,aa}$, respectively. While more than two parameters could significantly influence the other components.

Table 3 – Initial and optimized values for parameters obtained in the model.						
Parameter	Description	Unit	Initial value	Optimized value	Source	Variation[a] (%)
$k_{m,c4}$	Maximum uptake rate of valerate and butyrate	kgCOD/kgCOD d	20	16.95	1	100
Y_{c4}	Valerate and butyrate-degrader yield	kgCOD/kgCOD	0.06	0.05	1	30
$k_{m,ac}$	Maximum uptake rate of valerate and butyrate	kgCOD/kgCOD d	8	5.64	2	100
$K_{s,ac}$	Half saturation coefficient of valerate and butyrate uptake	kgCOD/m³	0.15	0.12	2	100
Y_{su}	Sugar-degraders yield	kgCOD/kgCOD	0.1	0.06	3	30

1: Angelidaki and Ahring (1992); 2: the value was estimated based on experimental data; and 3: Romli et al. (1995).
[a] The variation was based on technical report of IWA (Batstone et al., 2002).

Table 4 – Changes of Gibbs free energies of the anaerobic biochemical reactions at pH 7 under the standard conditions.

Reaction	ΔG_f^0 (kJ/mol)
Glucose*	
$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2CO_2 + 4H_2 + 4ATP$	−215.50
$C_6H_{12}O_6 \rightarrow \frac{4}{3}CH_3CH_2COO^- + \frac{2}{3}CH_3COO^- + 2H^+ + \frac{2}{3}CO_2 + \frac{2}{3}H_2O + \frac{4}{9}ATP$	−311.10
$C_6H_{12}O_6 \rightarrow 4CH_3(CH_2)_2COO^- + 4H^+ + 2CO_2 + 2H_2 + 3ATP$	−264.00
Amino acids	
$HOOCCH_2CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + 2CO_2 + 2H_2 + 2ATP$	353.55
$CH_3CH(OH)CH(NH_3^+)COO^- + H_2 \rightarrow CH_3COO^- + \frac{1}{2}CH_3(CH_2)_2COO^- + \frac{1}{2}H^+ + NH_4^+ + ATP$	−143.42
$HOCH_2CH(NH_3^+)COO^- + H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + H_2 + ATP$	−87.13
$HOOC(CH_2)_2CH(NH_3^+)COO^- + H_2O \rightarrow CH_3COO^- + \frac{1}{2}CH_3(CH_2)_2COO^- + \frac{1}{2}H^+ + NH_4^+ + CO_2 + 2ATP$	−77.72
$CH(NH_3^+)COO^- + H_2 \rightarrow CH_3COO^- + NH_4^+$	−68.06
$CH_3CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + 2H_2 + ATP$	3.18
$HSCH_2CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + S^{2-} + 2H^+ + \frac{1}{2}H_2 + ATP$	−139.93
$CH_3CH(CH_3)CH(NH_3^+)COO^- + 2H_2O \rightarrow (CH_3)_2CHCOO^- + NH_4^+ + CO_2 + 2H_2 + ATP$	0.27
$CH_3S(CH_2)_2CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3CH_2COO^- + NH_4^+ + CO_2 + CH_4S + H_2 + ATP$	135.04
$CH_3CH_2CH(CH_3)CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3CH_2CH(CH_3)COO^- + NH_4^+ + CO_2 + 2H_2 + ATP$	−5.42
$C_6H_{13}O_2N + 2H_2O \rightarrow C_5H_{10}O_2 + NH_3 + CO_2 + 2H_2 + ATP$	3.83
$HO(C_6H_5)CH_2CH(NH_3^+)COO^- + 2H_2O \rightarrow C_6H_5O^- + CH_3COO^- + H^+ + NH_4^+ + CO_2 + H_2 + ATP$	−72.25
$C_6H_5CH_2CH(NH_3^+)COO^- + 2H_2O \rightarrow C_6H_5CH_2COO^- + NH_4^+ + CO_2 + 2H_2 + ATP$	−17.86
$C_6H_{14}O_2N_2 + 2H_2O \rightarrow C_2H_4O_2 + C_4H_8O_2 + 2NH_3 + ATP$	−53.76
$HNCHNCHCCH_2CH(NH_3^+)COO^- + 4H_2O + \frac{1}{2}H^+ \rightarrow CH_3ON + CH_3COO^- + \frac{1}{2}CH_3(CH_2)_2COO^- + 2NH_4^+ + CO_2 + 2ATP$	223.72
$HNC(NH_2)NH(CH_2)_3CH(NH_3^+)COO^- + 3H_2O + H_2 \rightarrow \frac{1}{2}CH_3COO^- + \frac{1}{2}CH_3CH_2COO^- + \frac{1}{2}CH_3(CH_2)_3COO^- + 4NH_4^+ + CO_2 + ATP$	−297.22
$(CH_2)_3NH_2^+CHCOO^- + H_2O + H_2 \rightarrow \frac{1}{2}CH_3COO^- + \frac{1}{2}CH_3CH_2COO^- + \frac{1}{2}CH_3(CH_2)_3COO^- + \frac{1}{2}H^+ + NH_4^+$	−91.43
LCFA	
$CH_3(CH_2)_{14}COO^- + 14H_2O \rightarrow 8CH_3COO^- + 7H^+ + 14H_2 + \frac{7}{3}ATP$	392.52
$CH_3(CH_2)_{16}COO^- + 16H_2O \rightarrow 9CH_3CCO^- + 8H^+ + 16H_2 + \frac{8}{3}ATP$	483.60

Reaction	ΔG_f^0 (kJ/mol)
Valerate	
$CH_3(CH_2)_3COO^- + 2H_2O \rightarrow CH_3CH_2COO^- + CH_3COO^- + H^+ + 2H_2 + \frac{7}{8}ATP$	48.717
Butyrate	
$CH_3(CH_2)_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2 + 2ATP$	48.50
Acetate	
$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 + \frac{1}{4}ATP$	−35.82
Hydrogen	
$H_2 + \frac{1}{4}CO_2 \rightarrow \frac{1}{4}CH_4 + \frac{1}{2}H_2O + \frac{1}{4}ATP$	−32.73

Table 4 – (continued)

* The glucose degraded via the first, second and third reaction is 50%, 35% and 15%, respectively.
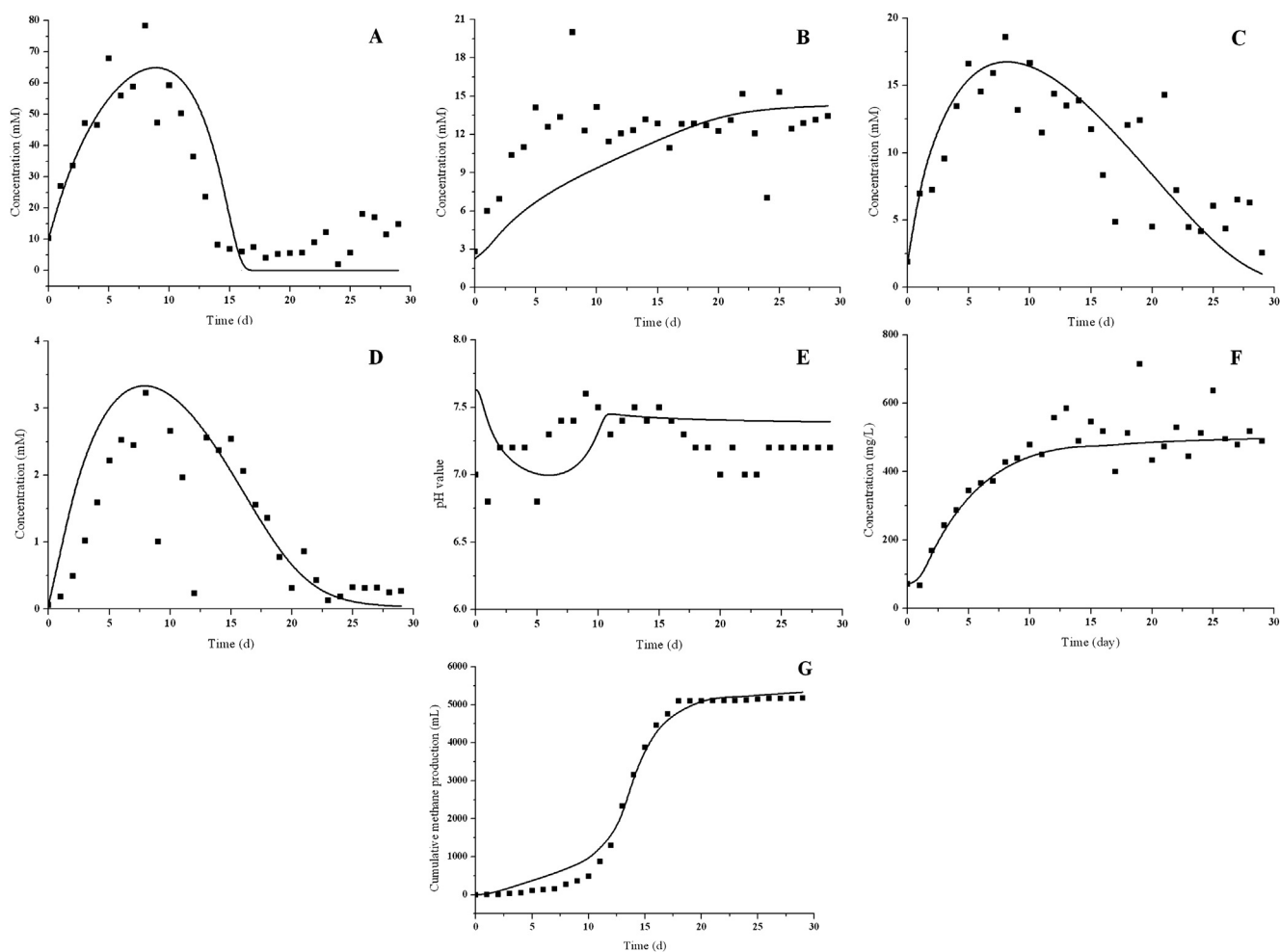
Fig. 1 – Comparisons of simulated (—) with experimental (■) data for (A) acetate concentration; (B) propionate concentration; (C) butyrate concentration; (D) valerate concentration; (E) pH values; (F) inorganic nitrogen concentration and (G) cumulative methane production.
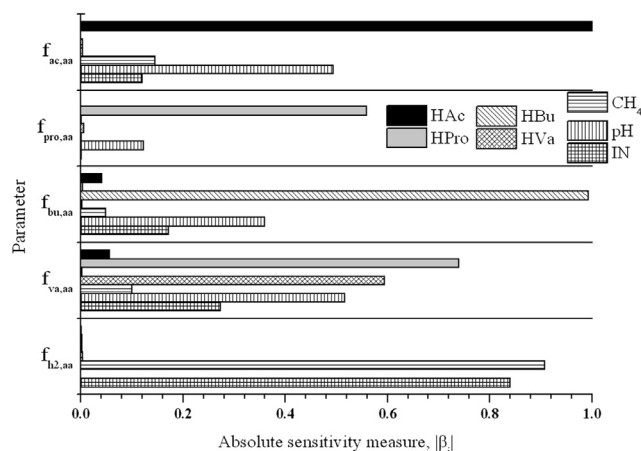
Fig. 2 – Sensitivities of the stoichiometric coefficients for amino acids acidogenesis on the outputs of the model.

To indicate the uncertainty of optimized parameters in Table 3, the mean, the 10th percentile, and the 90th percentile of Monte Carlo simulations are shown in Fig. S3. It was clear that the uncertainties of propionate, valerate and inorganic nitrogen concentrations were minimal. And the uncertainties of acetate and butyrate concentrations were larger than other outputs of this model. It agreed with the fact that the optimized parameters were mainly associated with the uptake process of acetate and butyrate. According to ADM1, the pH value and methane production could be significantly influenced by the concentrations of acetate and butyrate. Hence, the uncertainties of both outputs of this model were also observed.

3.3. Thermodynamics and heat analysis

The changes of Gibbs free energy for uptake of glucose, LCFA, amino acids, valerate, butyrate, propionate, acetate and
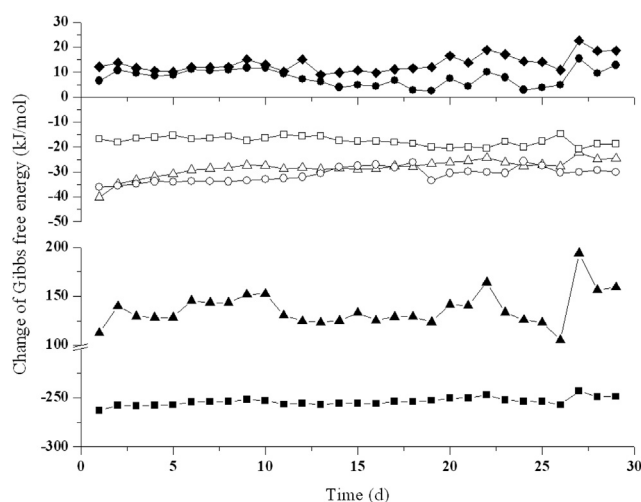

Fig. 3 – Changes of Gibbs free energy for uptake of glucose (■), LCFA (▲), valerate (◆), butyrate (●), amino acids (△), acetate (○) and hydrogen (□) during anaerobic digestion.

hydrogen during anaerobic digestion are shown in Fig. 3. Just as observed, the free energies of LCFA, valerate and butyrate were positive through the digestion, by contrast, the other parameter's free energies were negative. The free energies of glucose and amino acids increased from −262.89 kJ/mol to −248.51 kJ/mol and from −40.12 kJ/mol to −24.64 kJ/mol, respectively. And the free energies of acetate and hydrogen changed slightly, from −35.99 kJ/mol to −25.79 kJ/mol and from −20.80 kJ/mol to −14.67 kJ/mol, respectively. The free energy of LCFA fluctuated most of the time, which was between 112.21 kJ/mol and 193.99 kJ/mol.

The heat produced from microbial activities and methane is shown in Fig. 4. The daily heat production of methane reached the peak value of 37.38 kJ/d at the 13th day, and then decreased gradually. However, the daily heat production of microbial activities, from 0.19 kJ/d to 5.97 kJ/d, changed more slightly compared to that from methane. The peak value of 5.97 kJ/d from microbial activity appeared at the 14th day, which was later than the peak heat from methane. The cumulative heat from methane increased sharply after an obvious lag phase, and almost ceased after 18 d. By contrast, the cumulative heat from microbial activities increased gradually. The cumulative heats from microbial activity and methane were 77.69 kJ and 185.76 kJ, respectively.

4. Discussion

Hydrolysis, defined as the breakdown of organic substrates into smaller products which can be subsequently uptake and degraded by bacteria, is often the rate-limiting step for the anaerobic digestion of organic solids. The particulate components determine the rates and mechanisms of hydrolysis in waste and wastewater treatment (Morgenroth et al., 2002). In anaerobic digestion, proteins are first hydrolyzed into peptides and individual amino acids by proteolytic enzymes. The peptides and amino acids are then acidified into VFA, hydrogen, ammonium, and reduced sulfur. The VFA are further converted into acetate and H_2/CO_2 by acetogens. Finally, acetate and H_2/CO_2 are converted to methane by acetoclastic and
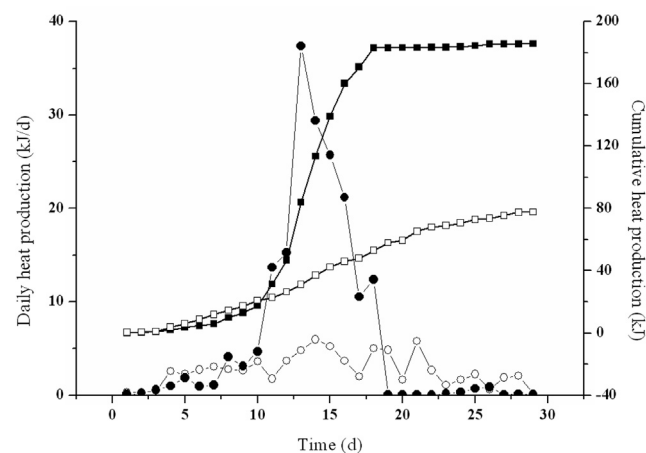

Fig. 4 – Daily (cycle) and cumulative (box) heat production from microbial activities (hollow) and methane (solid).

hydrogenotrophic methanogens, respectively. High concentration of amino acids could lead to process inhibition by ammonia. However, this system was not inhibited through the calculation of free ammonia (shown in Fig. S4). The stoichiometric coefficients for amino acids acidogenesis are crucial for the model of anaerobic digestion, due to the fact that it is used to determine the biomass yields, reactant consumption and production formation in the model. In addition, it could help to better understand the range of process operability and the detention time, especially for the high-protein content substrate. This information together with the kinetics of reaction was used to calculate the mass balance equations (Ramsay and Pullammanappallil, 2001). However, the stoichiometric coefficients for amino acids acidogenesis were usually derived empirically through an overall catabolic reaction of an amino acid developed from an average formula of this amino acid (Aldin et al., 2011). This type of coefficients has obviously limited applications in the model of anaerobic digestion of high-protein content matter. The approach for calculating stoichiometric coefficients in this study has been approved to fit well to experimental results under different feed conditions according to Ramsay and Pullammanappallil (2001). The discrepancy in the prediction of propionate in their model was not observed here. For a further study, it is necessary to verify the feasibility in continues reactors both in unsteady state and steady one (Schoen et al., 2009).

The stoichiometric coefficients were generally fixed in the model of anaerobic digestion and taken from the technical report by IWA because it was suggested that the coefficients had low sensitivities. However, Jeong et al. (2005) showed that the yield of products on the substrate had high sensitivities to model components during the anaerobic digestion of glucose. They also indicated that $f_{bu,su}$ and $f_{pro,su}$ were the most sensitive for all the ranges examined. The sensitivity analysis results in this study showed that the outputs of this model were affected significantly by the stoichiometric coefficients of amino acids acidogenesis. From Fig. 2, the sensitivities of stoichiometric coefficients on components are mainly dependent on the processes. For example, the concentration of valerate was mainly dependent on the fraction of valerate from amino acids during the amino acids acidogenesis. This means that, as shown in Fig. 2, $f_{va,aa}$ was more sensitive to the concentration of valerate than other coefficients. The concentration of butyrate was slightly dependent on $f_{va,aa}$, in contrast with other outputs. The pH value was related to most of the processes and was very sensitive to almost all coefficients excluding $f_{h2,aa}$. The cumulative production of methane should be interlinked to all processes in a general model. However, as shown in Fig. 1(B), the propionate was not degraded in the process. Hence, $f_{pro,aa}$ has low sensitivity to the cumulative production of methane. Along with the propionate concentration being affected by $f_{va,aa}$ and $f_{pro,aa}$, this indicated that the output was not only sensitive to its own stoichiometric coefficients but also the coefficients of its precursor and consumer.

According to the second thermodynamic law, a reaction can occur only if ΔG is negative. Hence, from Fig. 3, the degradations of glucose, amino acids, acetate and hydrogen are spontaneous, and the degradations of LCFA, valerate and butyrate are non-spontaneous. However, the activities of the

hydrogenotrophic methanogens could remove hydrogen to make the metabolism of VFA-degrading bacteria thermodynamically possible. The relationship between VFA-degrading bacteria and the hydrogenotrophic methanogens is called interspecies hydrogen transfer (Ahring, 2003). The ubiquity of interspecies hydrogen transfer in anaerobic digestion is required for the complete degradation of complex organic matter (Schink, 1997). In this study, the hydrogen content (shown in Fig. S5) increased significantly in the first 8 days, and then decreased slowly due to the fact that the abundance of the hydrogenotrophic methanogens was low at the beginning of the digestion, causing the accumulation of the hydrogen. And the hydrogen content was in the range of 10^{-3} to 10^{-6} bar, which could maintain the negative of Gibbs free energy (Batstone et al., 2006). For biological reaction, the absolute value of ΔG should be larger than 2 kJ/mol, which is the minimum amount of Gibbs free energy required to sustain microbial growth and/or conversion of a substrate. The lowest absolute ΔG of 2.49 kJ/mol during the process appeared in butyrate at the 19th day. Hickey and Switzenbaum (1991) as well as Dwyer et al. (1998) also observed the minimum absolute Gibbs free energy in the butyrate oxidation during the anaerobic digestion. Dwyer further postulated that this behavior could be described by the model of Powell (1984).

The heat from microbial activities was generally neglected in the calculation of energy balance for anaerobic digesters. However, Lübken et al. (2007) calculated the heat from microbial degradation of organics based on ADM1 in the pilot-scale digester with co-digestion of cattle manure and renewable energy crops. According to their model, this heat could compensate for about 11% of the energy necessary for substrate heating. From Fig. 4, the daily heat production from microbial activities was higher than that of methane produced at the beginning and end of the digestion. Even in the maximum methane production day, the heat from microbial activities was 12.9% of the heat from methane. This result implies that the anaerobic metabolism is much more exothermic than suggested in most literatures. Lindorfer et al. (2005) described the actual heat in anaerobic systems and reported that using energy crops in mono-fermentation could lead to a self-heating of the digester. They further concluded that the cause for the self-heating of the digester was associated with the heat enthalpy due to the bacterial metabolism. During the anaerobic digestion of blue algae, the cumulative heat from microbial activities was 41.8% of cumulative heat from methane. Gallert and Winter (2005) mentioned that a self-heating potential of 3.1 °C was released from biochemical conversion in the anaerobic digestion with 36 g/L organic dry matter content and 50% biodegradability, 0.9 g surplus sludge and 17.1 g biogas. The cumulative heat from microbial activities in this study could supply a self-heating potential of 4.1 °C for the anaerobic digester.

5. Conclusion

In order to facilitate the application of Anaerobic Digestion Model No. 1 (ADM1), an approach for a detailed calculation of stoichiometric coefficients for amino acids acidogenesis was presented. The model was used to simulate the biochemical conversion and calculate composition production during the

anaerobic digestion of blue algae. The simulation results obtained in this study support the approach by providing good predictions of the dynamics in this anaerobic digester. In addition, the model predicts the dynamic behavior of not only cumulative production of methane but also pH values, the concentrations of acetate, propionate, butyrate and valerate. The Monte Carlo simulation was used for the sensitivity analysis. The results showed that the stoichiometric coefficients for amino acids acidogenesis had high sensitivities to the components. And the outputs of the model were not only sensitive to their own stoichiometric coefficients but also to the coefficients of their precursor and consumer.

The Gibbs free energy for uptake of LCFA, valerate and butyrate, just as expected, was positive through the digestion, while the free energies of other components were negative. However, the interspecies hydrogen transfer could remove the produced hydrogen to make the metabolism of VFA-degrading bacteria thermodynamically possible. The cumulative heat production from methane increased sharply after an obvious lag phases, and almost ceased after 18 d. And the cumulative heat from microbial activities increased gradually. During the anaerobic digestion of blue algae, the cumulative heat from microbial activities accounted for 41.8% of cumulative heat from methane. The cumulative heat from microbial activities in this study could supply a self-heating potential of 4.1 °C for this anaerobic digester. Hence, proper heat preservation of anaerobic reactors can minimize the external heating of the reactor due to the heat produced from microbial activities.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2013.11.015.

R E F E R E N C E S

Angelidaki, I., Ahring, B., 1992. Effects of free long-chain fatty acids on thermophilic anaerobic digestion. Appl. Microbiol. Biotechnol. 37 (6), 808–812.

Ahring, B.K., 2003. Perspectives for anaerobic digestion. Adv. Biochem. Eng. Biotechnol. 81, 1–30.

Aldin, S., Nakhla, G., Ray, M.B., 2011. Modeling the influence of particulate protein size on hydrolysis in anaerobic digestion. Ind. Eng. Chem. Res. 50 (18), 10843–10849.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H.,

Vavilin, V.A., 2002. Anaerobic Digestion Model No. 1 (ADM1), IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes. IWA Publishing, London.

Batstone, D.J., Picioreanu, C., van Loosdrecht, M.C.M., 2006. Multidimensional modelling to investigate interspecies hydrogen transfer in anaerobic biofilms. Water Res. 40 (16), 3099–3108.

Cameron, E.T., 2012. Anaerobic Digestion of Microalgae: Modeling and Identification for Optimization and Control. Open Access Dissertations and Theses http://digitalcommons.mcmaster. ca/opendissertations/6843.

Cantrell, K.B., Ducey, T., Ro, K.S., Hunt, P.G., 2008. Livestock waste-to-bioenergy generation opportunities. Bioresour. Technol. 99 (17), 7941–7953.

Dwyer, D.F., Weeg-Aerssens, E., Shelton, D.R., Tiedje, J.M., 1998. Bioenergetics conditions of butyrate metabolism by a syntropic, anaerobic bacterium in coculture with hydrogen-oxidizing methanogenic and sulfidogenic bacteria. Appl. Environ. Microbiol. 54 (6), 1354–1359.

Gallert, C., Winter, J., 2005. Bacterial metabolism in wastewater treatment systems. In: Jördening, Hans-Joachim, Winter, Josef (Eds.), Environmental Biotechnology, Concepts and Applications.

Guo, L., 2007. Dong battle with the green monster of Taihu Lake. Science 317 (5842), 1166.

Herbert, D., Philipps, P.J., Strange, R.E., 1971. Carbohydrate analysis. Methods Enzymol. 5B (2), 265–277.

Hickey, R.F., Switzenbaum, M.S., 1991. Thermodynamics of volatile fatty acid accumulation in anaerobic digesters subject to increases in hydraulic and organic loading. Res. J. Water Pollut. Control Fed. 63 (2), 141–144.

Hu, P., Yan, Q., Song, R., Ruan, W., 2009. Biogas production through anaerobic digestion from the mixture of blue algae and sludge. Chin. J. Environ. Eng. 3 (3), 559–563.

Iman, L.R., Conover, W.J., 1982. A distribution-free approach to inducing rank correlation among input variables. Commun. Stat. Simul. Comput. 11 (3), 311–334.

Jeong, H.S., Suh, C.W., Lim, J.L., Lee, S.H., Shin, H.S., 2005. Analysis and application of ADM1 for anaerobic methane production. Bioprocess Biosyst. Eng. 27 (2), 81–89.

Jia, M., Keutgen, N., Matsuhashi, S., Mitzuniwa, C., Ito, T., Fujimura, T., Hashinot, S., 2001. Ion chromatographic analysis of selected free amino acids and cations to investigate the change of nitrogen metabolism by herbicide stress in soybean (Glycine max). J. Agric. Food Chem 49 (1), 276–280.

Lindorfer, H., Kirchmayr, R., Braun, R., 2005. Self-heating of anaerobic digesters using energy crops. Water Sci. Technol. 53 (8), 159–166.

Lepage, G., Roy, C.C., 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. J. Lipid Res. 25 (12), 1391–1396.

Lübken, M., Wicherna, M., Schlattmann, M., Gronauer, A., Horn, H., 2007. Modelling the energy balance of an anaerobic digester fed with cattle manure and renewable energy crops. Water Res. 41 (18), 4085–4096.

Mairet, F., Bernard, O., Cameron, E., Ras, M., Lardon, L., Steyer, J.P., Chachuat, B., 2012. Three-reaction model for the anaerobic digestion of microalgae. Biotechnol. Bioeng. 109 (2), 415–425.

Mairet, F., Bernard, O., Ras, M., Lardon, L., Steyer, J.P., 2011. Modeling anaerobic digestion of microalgae using ADM1. Bioresour. Technol. 102 (13), 6823–6829.

Morgenroth, E., Kommedal, R., Harremoës, P., 2002. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment – a review. Water Sci. Technol. 45 (6), 25–40.

Powell, G.E., 1984. Equalization of specific growth rates for syntropic associations in batch culture. J. Chem. Technol. Biotechnol. 34B (2), 97–100.

Ramsay, I.R., Pullammanappallil, P.C., 2001. Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry. Biodegradation 12 (4), 247−257.

Raunkjær, K., Hvitved-Jacobsen, T., Nielsen, P.H., 1994. Measurement of pools of protein, carbohydrate and lipid in domestic wastewater. Water Res. 28 (2), 251−262.

Reichert, P., 1998. AQUASIM 2.0-User Manual, Computer Program for the Identification and Simulation of Aquatic Systems. Swiss Federal Institute for Environmental Science and Technology (EAWAG), Dubendorf, Switzerland.

Romli, M., Keller, J.P., Lee, P.L., Greenfield, P.F., 1995. Model prediction and verification of a two-stage high-rate anaerobic wastewater treatment system subjected to shock loads. Process Saf. Environ. Protect. 73, 151−154.

Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiol. Mol. Biol. Rev. 61 (2), 262−280.

Schoen, M.A., Sperl, D., Gadermaier, M., Goberna, M., Franke-Whittle, I., Insam, H., Ablinger, J., Wett, B., 2009. Population dynamics at digester overload conditions. Bioresour. Technol. 100, 5648−5655.

SEPA (State Environmental Protection Administration of China), 2002. Monitor and Analysis Method of Water and Wastewater. Chinese Environmental Science Publication House, Beijing.

Sin, G., Gernaey, K.V., Neumann, M.B., van Loosdrecht, M.C.M., Gujer, W., 2009. Uncertainty analysis in WWTP model applications: a critical discussion using an example from design. Water Res. 43 (11), 2894−2906.

Sin, G., Gernaey, K.V., Neumann, M.B., van Loosdrecht, M.C.M., Gujer, W., 2011. Global sensitivity analysis in wastewater treatment plant model applications: prioritizing sources of uncertainty. Water Res. 45 (2), 639−651.

Thauer, R.K., Jungerman, K., Decker, K., 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41 (1), 100−180.

Yan, Q., Zhao, M., Miao, H., Ruan, W., Song, R., 2010. Coupling of the hydrogen and polyhydroxyalkanoates (PHA) production through anaerobic digestion from Taihu blue algae. Bioresour. Technol. 101 (12), 4508−4512.

Zhang, W., Zhang, Z., Luo, Y., Qiao, W., Xiao, M., Zhang, M., 2012. Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. Bioresour. Technol. 114, 281−286.

Zhong, J., Fan, C., Liu, G., Zhang, L., Shang, J., Gu, X., 2010. Seasonal variation of potential denitrification rates of surface sediment from Meiliang Bay, Taihu Lake, China. J. Environ. Sci. 22 (7), 961−967.