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Codigestion of Taihu blue algae with swine manure for biogas production

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ABSTRACT

Anaerobic digestion (AD) of Taihu blue algae and its codigestion with swine manure was evaluated at different inoculum substrate ratios (ISRs) from 0.5 to 3.0. Results showed that codigestion of blue algae with swine manure led to the highest methane (CH_4) production of 212.7 mL g^{-1} VS at ISR 2.0, while digestion of blue algae inoculated with granular sludge brought out the optimized CH_4 production of 73.5 mL g^{-1} VS at ISR 3.0. The values of pH, total ammonia nitrogen (TAN), free ammonia nitrogen (N-NH_3) and volatile fatty acids (VFAs) showed no significant difference between the digestion and codigestion, confirming the appropriate stability of the two batch anaerobic processes. Closer examination of VS removal rates and key enzymes variation proved codigestion had higher efficiencies in biodegradation and methanation, which demonstrated that AD of blue algae with swine manure was a promising technology for both solid wastes treatment and renewable-energy production.

1. Introduction

The bloom of cyanobacteria (commonly referred to as blue algae) derived from eutrophication of water bodies has resulted in the most pressing problem all over the world [1]. Especially in China, more than 60% of lakes such as Chaohu Lake, Taihu Lake and Dianchi Lake, have been eutrophicated and suffered from harmful algae blooms [2]. As a typical large, shallow inland lake located in the Yangtze River Delta, China, Taihu Lake has experienced increasingly massive cyanobacterial blooms during the past few years. To reduce Taihu Lake's eutrophication, refloatation of blue algae after blooming has been considered as one of the most efficient approaches to recover nitrogen and phosphorus from the lake [3]. Thousands of tons of blue algae was salvaged and collected every day in summer in Wuxi City since 2007 [4,5]. However, without further management, large amounts of salvaged blue algae will result in serious secondary environmental pollution.

Anaerobic digestion (AD), coupled with renewable-energy production in the form of biogas and waste treatment, has been responsible for degrading most of the carbonaceous material and regarded as a significant technology for the future [6]. In particular, AD of algae does not need advanced dewatering or further chemical extraction, which means a lower cost of operation than other methods such as incineration, composting and protein extraction. Researches on AD of algal biomass have been carried out worldwide since the first oil crises. Macroalgae such as *Macrocystis* [7], *Gracilaria* [8], *Hypnea*, *Ulva*, *Laminaria and Sargassum* [9] as well as microalgae such as *Microcystis* [10], *Scenedesmus* [11], *Spirulina* [12], *Euglena*, *Melosira and Oscillatoria* [13] have intrigued the interest to use these organisms for bioenergy generation. From these studies, it can be concluded that algae are good feedstock for the AD, because of high conversion rates and energy obtained efficiencies. For Taihu blue algae, limited investigations were carried out for biogas [10,14,15], biohydrogen and polyhydroxyalkanoate (PHA) production [16].

However, preliminary studies on AD of microalgae have shown low methane (CH_4) productivities compared with municipal solid waste or fruit and vegetable wastes [17,18]. The main reason has been attributed to the tough and protective cell walls of microalgae, which make them highly resistant to bacterial attack [19]. The low carbon/nitrogen (C/N) ratio of algae is also a serious problem to AD, which might result in elevated total ammonia nitrogen (TAN) in the digester. The ammonia toxicity due to accumulation of TAN (mainly from free ammonia, N-NH_3) was supposed to inhibit methanogens and further lead to volatile fatty acids (VFAs) accumulation [15,20]. Recalcitrant compounds like polyaromatics, heteropolysaccharides, algaenan, sporopollenin, silica, uronic acid and lignin as well as toxins (microcystins) were besides threatens to deterioration of AD [21]. Moreover, a seasonal provision (mainly from May to October, each year, depending on algae blooming) of Taihu blue algae could not meet the need of continuous operation of AD. Therefore, a codigestion technology should be

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developed to increase blue algae AD efficiency and achieve continuous operation without blue algae.

In this study, codigestion of Taihu blue algae with swine manure was evaluated at different inoculum substrate ratios (ISRs). For comparison, digestion of Taihu blue algae inoculated with granular sludge was additionally investigated. The objectives of this study were to (1) evaluate the algal biomass degradation efficiency and CH_4 yield at different ISRs; and (2) clarify the advantages of the codigestion through substrates variation and enzymatic characterizations during the AD processes.

2. Materials and methods

2.1. Substrates and inocula

The algal biomass used in the study was the mixture of algae bloom and lake water, which was freshly collected from Bogong Island, Taihu Lake (120° 23′ N, 31° 54′ E). The *Microcystis*, *Cyclotella*, *Cryptomonas* and *Scenedesmus* were the dominant species in the mixture, contributing 42.6%, 21.0%, 12.7% and 8.3% to the total biomass, respectively. Microalgae identification was carried out by microscopical examination (OLYMPUS IX70, Japan) according to the Phytoplankton Manual [22]. The mixture was cryopreserved at 4 °C before future use. Swine manure was a mixture of pig stool and urine freshly collected from Wuxi Nanyang Workstock Industry Co., Ltd. (China). Swine manure was served as both substrate and inoculum during the anaerobic codigestion in an attempt to propose an integrated system for residue reduction. The other anaerobic inoculum was selected as granular sludge from an internal circulation (IC) anaerobic digester (Wuxi, China) treating citric acid wastewater operating at 35 °C, with 6 h retention time. More specifically, Table 1 shows the chemical parameters of each individual waste and inoculum.

2.2. Batch laboratory AD tests

The AD study was carried out in Automatic Methane Potential Test System (AMPTS) II (Bioprocess, Sweden) with software of AMPTS v5.0. Process was according to AMPTS Operation and Maintenance Manual and Badshah's method [23]. Specifically, the experiments were performed in 500 mL reactors with mechanical agitators to provide skillful and gentle mixing for substrate and inoculum with various solid contents. Each reactor was kept at 35 °C in a temperature-controlled water-bath. Biogas produced was first passed through a scrubbing tubing filled with the alkali solution for CO_2 and H_2S removal, then transported to the gas flow meter. The data were recorded by the data-acquisition system.

Batch experiments were conducted in triplicate to determine the biogas production rates of Taihu blue algae, swine manure, granular sludge and their mixtures for 22 days, while another reactor was carried out under the same condition to study the process stability and parameters variation. Four different inoculum (swine manure or granular sludge) substrate (blue algae) ratios (based on VS ratio) in this study were 0.5, 1.0, 2.0 and 3.0, which were achieved by keeping a constant inoculum concentration (2 g VS L^{-1}) and varying the substrate concentration. By contrasting, biogas production rates of swine manure and granular sludge were tested to find out the background CH_4 production by inoculum as control. The working volumes in the reactors were adjusted to 400 mL with distilled water and flushed with nitrogen gas to ensure anaerobic conditions. Stirrers of all reactors were set to be 30 s on and 120 s off at 46 rpm during the whole experiment. Fig. S1 shows the schematic presentation of batch AD. CH_4 production potential (P_{max}), CH_4 production rate (R_{max}) and lag phase (λ) were modeled using the modified Gompertz equation [24]:

$$P = P_{max} \times \exp\left\{-\exp\left[\frac{R_{max} \times e}{P_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where P is the cumulative specific methane yield (mL g^{-1} VS) for a given time t; P_{max} is the maximum CH_4 potential (mL g^{-1} VS) at the end of digestion time; R_{max} is the CH_4 production rate (mL g^{-1} VS d^{-1}); λ is the lag phase (d); t is time (d) and e is exp (1), i.e. 2.71828.

Table 1
Characteristics of Taihu blue algae, swine manure and granular sludge.

Parameter	Blue algae	Swine manure	Granular sludge
Total solids, TS (%)	4.13 ± 0.18	23.58 ± 1.06	8.40 ± 0.91
Volatile solids, VS (% TS)	86.68 ± 1.47	89.86 ± 2.15	67.48 ± 2.24
Total carbon, TC (mg g^{-1} TS)	438.26 ± 9.65	371.50 ± 8.59	396.72 ± 15.32
Total nitrogen, TN (mg g^{-1} TS)	75.60 ± 3.58	43.32 ± 3.11	34.77 ± 1.46
Total phosphorous, TP (mg g^{-1} TS)	4.05 ± 0.52	1.15 ± 1.26	3.78 ± 0.39
Lipid (% TS)	8.26 ± 0.82	15.87 ± 1.35	NA
Protein (% TS)	59.87 ± 2.38	47.18 ± 1.34	NA
Carbohydrate (% TS)	18.41 ± 1.66	26.82 ± 2.48	NA

NA (no analysis). Data are the means of three measurements, and numbers in parentheses are the standard deviations.

2.3. Analytical methods

2.3.1. CH_4

CH_4 concentration in the biogas was analyzed using a gas chromatograph (GC 910, Kechuang, China) equipped with a thermal conductivity detector (TCD) and a stainless packed column (with Porapak N 60-80 as carrier, 1000 × 6 mm^2 I.D.). The injector, detector and oven temperatures were 100, 100 and 90 °C, respectively. Argon was used as the carrier gas at a flow rate of 15 mL min^{-1}, and the injection volume of sample was 0.1 mL [25].

2.3.2. Physicochemical analysis

The total solids (TS), volatile solids (VS), total carbon (TC), total nitrogen (TN), total phosphate (TP by ascorbic acid method) and TAN (by phenate method) were analyzed according to the APHA standard methods [26]. N-NH_3 concentration was calculated from the following formula [20]:

$$[\text{N-NH}_3] = \frac{[\text{TAN}] \times 10^{\text{pH}}}{e^{\left(\frac{6344}{273.15+T}\right)} + 10^{\text{pH}}} \quad (2)$$

where [N-NH_3] is the concentration of free ammonia nitrogen (mg L^{-1}); [TAN] is the total ammonia nitrogen concentration (mg L^{-1}) and T is the temperature (centigrade).

The pH was measured manually using a model Delta 320 pH Meter (Mettler-Toledo, Germany). The protein content was determined based on the total Kjeldahl nitrogen (TKN) measurement using the correction factor 6.25 [27]. The total lipid content was analyzed gravimetrically from the extract obtained with diethyl ether in a Soxtec System HT2 1045 extraction unit produced by Tecator [28]. Carbohydrate was estimated as the remaining fraction of VS after the determination of protein and lipid.

2.3.3. Organic acids

Organic acids were detected using HPLC (Agilent 1100, USA) equipped with a UV detector at the wavelength of 210 nm, with a ZORBAX SB-A column (300 × 7.8 mm^2 I.D., Biorad, USA) at the column temperature of 60 °C. Acetonitrile (0.5%) and 0.02 mol L^{-1} of KH_2PO_4 (99.5%) were used as the mobile phase with a flow rate of 0.5 mL min^{-1}. The pH of the samples was adjusted to 2.0–3.0 with H_3PO_4, and the injection volume was 0.01 mL [16]. The elution time for acetic acid, propionic acid and *n*-butyric acid were at about 12.7, 14.9 and 18.1 min, respectively.

2.3.4. Determination of activities of protease, acetate kinase (AK) and coenzyme F_{420}

The protease activity was analyzed by a Folin-phenol Reagent Method [29]. For determining AK's activity, digestion mixture was firstly washed and resuspended in 100 mM sodium phosphate buffer (pH 7.4). Resuspended mixture was sonicated at 20 kHz and 4 °C for 10 min to break down the cells of bacteria and afterwards centrifuged at 10,000 rpm and 4 °C for 15 min to remove the waste debris. The extracts were used for enzyme activity assay immediately [30]. Coenzyme F_{420} was assayed by adapting the classic procedure of spectrophotometric study [31]. Specific enzyme activities of protease and coenzyme F_{420} were defined as the unit of enzyme activity per milligram of VS, and AK activity was defined as the unit of enzyme activity per milligram of protein.

2.4. Statistical analysis

All analytical results were conducted at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation. The standard deviations were analyzed by using Microsoft Excel 2003 for Windows.

3. Results and discussion

3.1. Substrates and inocula characterization

Chemical properties of the substrates and inocula were measured in terms of TS, VS, TC, TN and TP, as shown in Table 1. Results revealed the organic fraction contributed to the major part of the biomass, representing a VS content of 89.86% TS in swine manure, 86.68% TS in blue algae and 67.48% TS in granular sludge, respectively. As for C/N ratios, blue algae had the lowest ratio of 5.80, while swine manure (8.58) and granular sludge (11.41) exhibited relatively higher. The C/N ratios of all the different samples were, however, seem to under the range of 15–30 which was proposed to be most suitable for optimum operation of the AD [32,33]. Thus, in order to avoid the toxic accumulation of NH_3 levels in the bench-scale AD digester, the relatively low inoculum and substrate concentration in the range from 2.67 to 6 g VS L^{-1} were applied for Taihu blue algae AD.

Lipid, protein and carbohydrate contents were also determined in the substrates of blue algae and swine manure. Results showed that blue algae had a lipid composition of 8.26% TS, which is much lower than those recovered from algae cultured in the lab for biodiesel production [34]. Carbohydrate content in blue algae was 18.41% TS. The two compositions of lipid and carbohydrate in blue algae were significantly lower than those in swine manure. However, protein in blue algae occupied the main composition of 59.87% TS, which was higher than that in the swine manure of 47.18% TS. As it can be seen in Table 1, lipid, protein and carbohydrate contributed to the dominating composition in blue algae and swine manure, which means that blue algae and swine manure are suitable feedstocks for AD.

3.2. CH_4 production

The cumulative CH_4 yield of blue algae inoculated with swine manure or granular sludge, as a function of time under different ISRs, are shown in Fig. 1. The contribution of background CH_4 production by inoculum of swine manure or granular sludge was deducted from the entire cumulative CH_4 yield of codigestion. The cumulative CH_4 yield after 22 days was highest for codigestion of blue algae with swine manure at ISR of 2.0, with the value of 212.7 mL g^{-1} VS. As can be seen from Fig. 1, the cumulative CH_4 yield of blue algae during codigestion increased from 48.2 mL g^{-1} VS to 212.7 mL g^{-1} VS when the ISR increased from 0.5 to 2.0, representing a 341.3% increase in CH_4 conversion efficiency. However, when ISR reached 3.0, the volume of CH_4 produced decreased to 190.3 mL g^{-1} VS, showing the oversaturation of the inoculum. The same conclusion was previously achieved by other researchers using different substrates [35]. In order to evaluate the methanogens efficiency thoroughly, the experimental data obtained from the cumulative CH_4 production were fitted to the modified Gompertz equation with $R^2 > 0.97$. The regression results exhibited in Table 2 showed that P_{max}, R_{max} and λ were all dependent on the ISR. The P_{max} value predicted from the modified Gompertz equation seemed to be slightly higher than those of the experimental cumulative CH_4 yield. The P_{max} and R_{max} reached the highest level of 219.99 mL g^{-1} VS and 19.44 mL g^{-1} VS d^{-1} at ISR 2.0, demonstrating the optimized inoculum substrate ratio for the codigestion of blue algae with swine manure. Interestingly, the λ value showed the similar trend with P_{max}, with the highest value of 1.61 d at ISR 2.0. This might be because the microorganisms in the inoculum needed a period to adapt a new environment when they are transferred to the new condition [36].

Comparing to the codigestion of blue algae with swine manure, digestion of blue algae inoculated with granular sludge showed less effective in CH_4 production. The cumulative CH_4 yield of blue algae increased from 32.8 mL g^{-1} VS to 73.5 mL g^{-1} VS when the ISR increased from 0.5 to 3.0 during digestion. P_{max} had the also similar tendency as that obtained in the experiments. With the increase of ISR from 0.5 to 3.0, R_{max}, increased from 2.32 mL g^{-1} VS d^{-1} to 7.40 mL g^{-1} VS d^{-1}. The λ ranged from 0.04 d to 0.11 d with the shortest lag phase time at ISR 0.5. The lower CH_4 production rate might be due to the inferior hydrolysis efficiency derived from the barriers of algae cell, which will be discussed as follows.

3.3. Parameters variation during AD

To clarify the difference between AD of blue algae with swine manure (ISR 2.0) and granular sludge (ISR 3.0), inhibiting factors such as pH, TAN, N-NH_3 and VFAs were analyzed every 2 days during the whole processes. Fig. 2 shows that pH was fairly stable in the two digester, displaying a fluctuation from 7.18 to 7.65 during codigestion of blue algae with swine manure and change from 7.32 to 7.64 during digestion of blue algae inoculated with granular sludge, respectively. The pH values in the two digesters were generally maintained in the optimum pH range for high solid (4–10% TS) AD of 6.60–7.80, which is supportive of CH_4 production. TAN contents during digestion and codigestion processes showed the similar rising tendency, principally. At the initial fermentation stage of 14 days, microorganisms in the digesters mainly made use of carbohydrates for microbial growth and CH_4 production, which did not exert significant increase in TAN contents. At the later stage, because of the low carbohydrates soluted, microorganisms were forced to make more utilization of proteins, leading to the sudden rising of TAN contents to 579.14 mg L^{-1} and 510.08 mg L^{-1} in codigestion and digestion, respectively. Comparing to the inhibitory concentrations of TAN of 1700–5000 mg L^{-1} in mesophilic digesters [15], TAN in the two digesters during the processes are too little to inhibit the AD. Since N-NH_3 has been reported to be the main cause of methanogens inhibition, the concentration of N-NH_3 was calculated and presented in Fig. 2. As shown in Formula (2), the N-NH_3 concentration depends basically on three parameters: TAN concentration, temperature and pH. Due to the low concentration of TAN and stable pH, the N-NH_3 concentration changed from 4.71 mg L^{-1} to 28.19 mg L^{-1} in the co-digestion system and from 4.18 mg L^{-1} to 24.29 mg L^{-1} in the digestion system, which is significantly lower than the minimum concentration for methanogenic toxicity of 80–100 mg L^{-1} [37]. However, batch experiments could not factually give an index

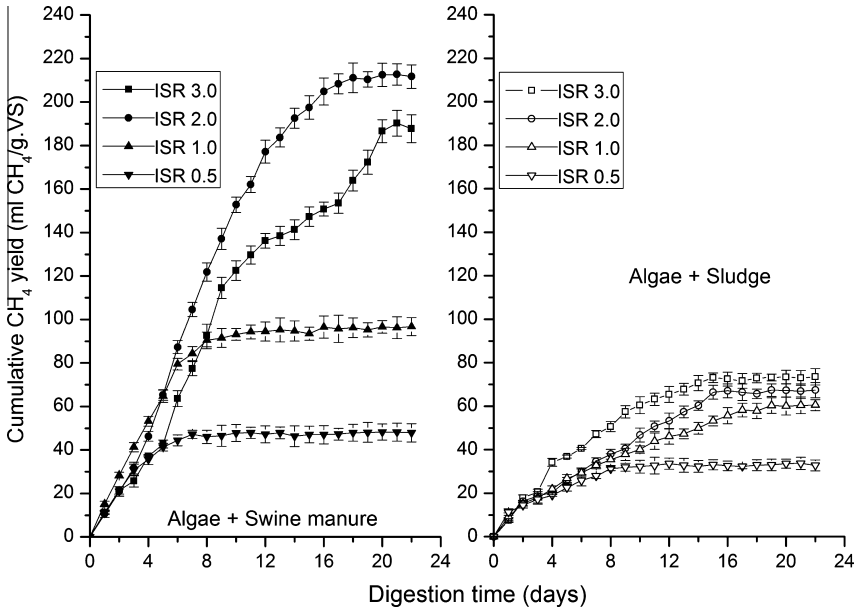

Fig. 1. Cumulative CH_4 production during batch AD of Taihu blue algae with swine manure and granular sludge at different ISRs.

Table 2
Summaries of estimated parameters from the Gompertz equation and experimental CH_4 yields for digestion and codigestion of Taihu blue algae.

ISRs	P_{max} (mL g^{-1} VS)		R_{max} (mL g^{-1} VS d^{-1})		λ (d)		R^2	
	Digestion	Codigestion	Digestion	Codigestion	Digestion	Codigestion	Digestion	Codigestion
3.0	74.55	190.90	7.40	13.17	0.11	1.15	0.992	0.985
2.0	73.12	219.99	5.17	19.44	0.11	1.61	0.987	0.999
1.0	65.02	96.23	4.29	15.65	0.07	0.34	0.986	0.996
0.5	33.31	47.59	2.32	10.81	0.04	0.10	0.972	0.990

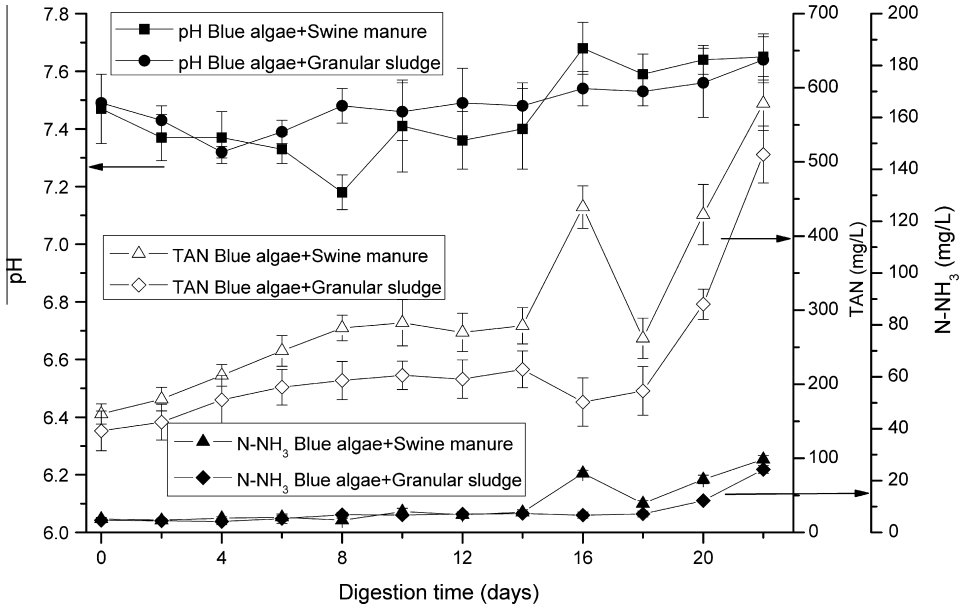

Fig. 2. pH, TAN and N-NH_3 changes during digestion and codigestion of Taihu blue algae.

to the accumulation of TAN and N-NH_3 for the long-term operation during the codigestion process, so a low organic loading rate (OLR) was recommended for continuous AD of Taihu blue algae [38]. As for the VFAs, Table 3 displays their variation during digestion and codigestion processes. Acetic acid, propionic acid and *n*-butyric acid were mainly formed during blue algae AD by anaerobic oxidation of carbohydrate, protein and even lipid. Acetic acid is considered to be the major precursor of CH_4 and can be converted to CH_4 directly [39], contributing to the most part of the VFAs in both digesters. The concentration of acetic acid increased significantly

in the first 8 days, and then reached the peak value of 264.17 mg L^{-1} during codigestion of blue algae with swine manure, while the concentration of acetic acid increased considerably in the initial 6 days, and afterwards reached the peak value of 172.98 mg L^{-1} during digestion of blue algae inoculated with granular sludge. After that, the concentration of acetic acid dropped slowly to under the detection limit until the end of the processes in both digesters. Propionic acid is supposed to cause greater inhibition on the activity of methanogens than other VFAs, contributing to a considerable part of the VFAs in both digesters. However, propionic acid could be degraded efficiently in the two digesters without accumulation after 18 days of digestion. *n*-Butyric acid was detected at the early stage of the process with the small amount in both digesters, which will not cause any inhibition to methanogenesis. The maximum total VFAs was calculated as 423.52 mg L^{-1} in the digester of blue algae with swine manure and 403.78 mg L^{-1} in the digester of blue algae inoculated with granular sludge, which also demonstrated that no methanogenesis inhibition was caused by the interact ion between NH_3 and pH. Therefore, inhibiting causes from pH, TAN, N-NH_3 and VFAs could not be responsible for the difference of CH_4 yield of blue algae between digestion and codigestion.

Further research shown in Fig. 3 declared the total VS removal rates changed from 18.72% to 35.44% with the different ISRs during codigestion of blue algae with swine manure, which is in accordance with the cumulative CH_4 yield. However, in the digester of blue algae inoculated with granular sludge, the total VS removal rates increased from 4.97% to 12.67% when the ISR increased from 0.5 to 3.0. Results showed that codigestion of blue algae with swine manure had higher efficiency in conversion from organic substrates into biogas. Specifically, carbohydrate and protein removal rates under different ISRs in the two digesters were also demonstrated in Fig. 3. Carbohydrate removal rate could reach 42.29% at ISR 2.0 during codigestion of blue algae with swine manure,

Table 3
VFAs variation during digestion and codigestion processes of Taihu blue algae.

Time (days)	Blue algae + Swine manure (mg L^{-1})			Blue algae + Granular sludge (mg L^{-1})		
	Acetic acid	Propionic acid	Butyric acid	Acetic acid	Propionic acid	Butyric acid
0	136.94 ± 7.38	142.63 ± 12.58	126.6 ± 16.13	121.42 ± 9.45	86.83 ± 11.24	95.63 ± 6.90
2	196.24 ± 12.46	138.93 ± 10.13	88.35 ± 5.83	192.25 ± 18.94	122.64 ± 10.23	88.89 ± 9.97
4	222.66 ± 19.73	111.91 ± 14.56	38.46 ± 4.24	159.72 ± 12.32	102.53 ± 6.55	63.45 ± 4.89
6	241.25 ± 17.45	103.26 ± 10.42	ND	172.98 ± 16.78	111.76 ± 8.85	69.71 ± 5.19
8	264.17 ± 14.68	171.41 ± 10.30	ND	161.10 ± 4.88	148.73 ± 10.27	50.08 ± 4.92
10	123.22 ± 9.24	267.7 ± 18.24	ND	140.57 ± 9.76	161.15 ± 8.26	52.13 ± 7.13
12	119.98 ± 18.45	237.16 ± 18.04	ND	38.19 ± 2.81	93.06 ± 4.18	21.84 ± 3.44
14	34.56 ± 6.89	200.85 ± 14.46	ND	7.85 ± 2.13	31.98 ± 1.97	ND
16	21.34 ± 2.46	171.22 ± 7.47	ND	8.32 ± 1.83	60.67 ± 5.51	ND
18	7.89 ± 1.36	90.32 ± 3.75	ND	12.68 ± 1.79	83.63 ± 1.93	ND
20	ND	ND	ND	95.02 ± 4.76	ND	ND
22	ND	ND	ND	ND	ND	ND

ND (not detected). Data are the means of three measurements, and numbers in parentheses are the standard deviations.

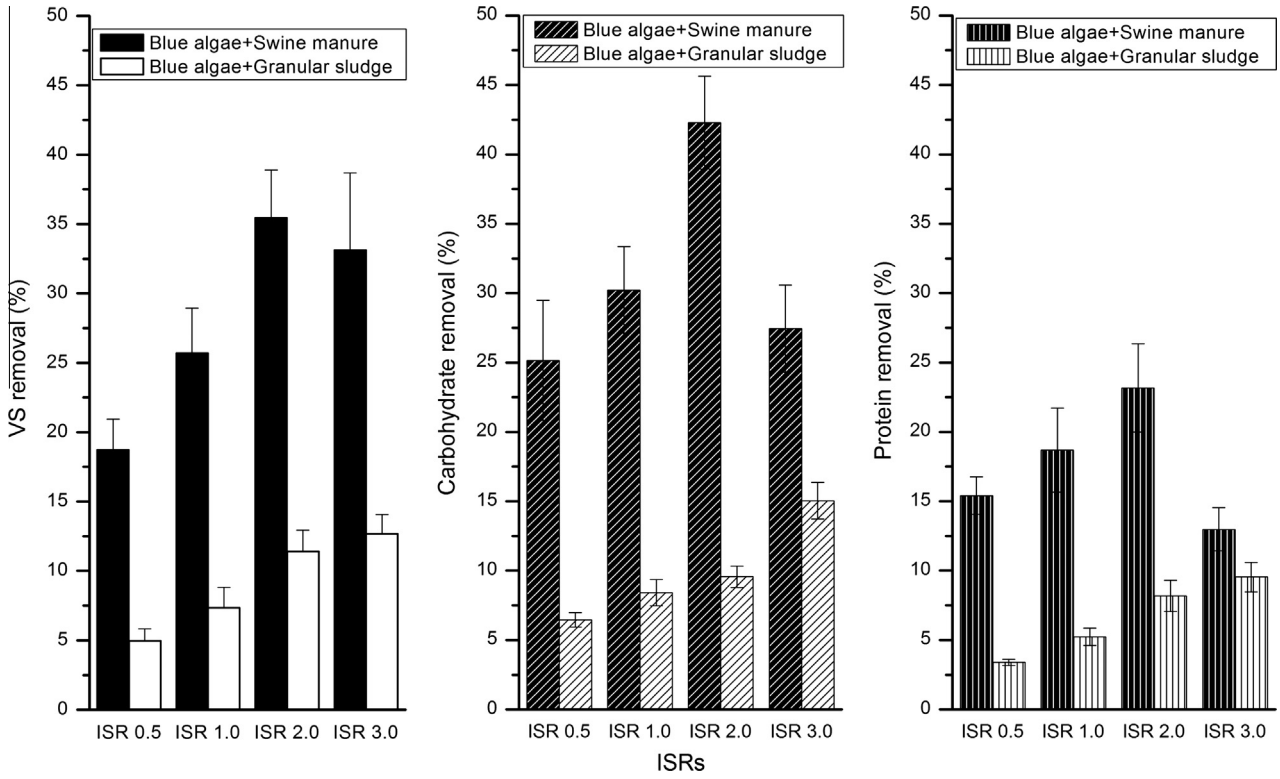

Fig. 3. VS, carbohydrate and protein removal rates after digestion and codigestion of Taihu blue algae at different ISRs.

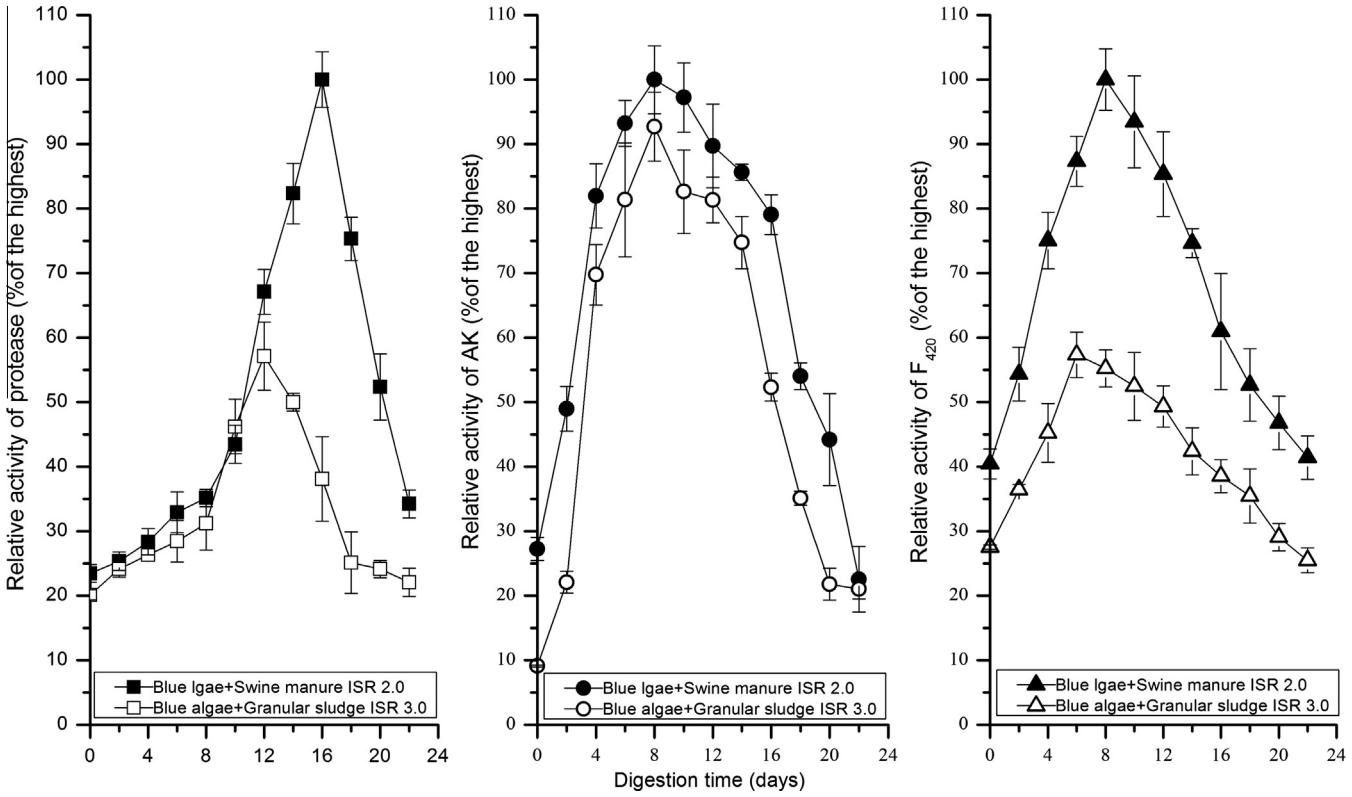

Fig. 4. Changes in enzymes activities of protease, AK and coenzyme F_{420} during AD processes.

while the highest carbohydrate removal rate was 15.04% at ISR 3.0 during digestion of blue algae inoculated with granular sludge. Protein removal rate was the most efficient with the number of 23.17% and 9.53% at ISR 2.0 during codigestion of blue algae with swine manure and at ISR 3.0 during digestion of blue algae inoculated with granular sludge respectively, which was relative lower than that of carbohydrate.

VS are important organic substrates for anaerobic fermentation, and almost all precursors of CH_4 come from VS during AD [40]. The difference in VS removal rates could account for the difference in CH_4 production between the two AD processes. Codigestion of blue algae with swine manure had higher cumulative CH_4 yield with greater VS removal rate. Besides, removal rates of intracellular organics such as protein were lower than that could be secreted from the cells (carbohydrate) throughout the experiments. Thus, cell wall barrier may be an important factor exerting the negative effects on AD. Pretreatment of blue algae for cell disruption was recommended to improve the CH_4 production [41]. In conclusion, higher cumulative CH_4 yield from codigestion of blue algae and swine manure than that of blue algae inoculated with granular sludge might be due to higher activities of hydrolytic and methanogenic microorganism from swine manure [42].

3.4. Enzymatic characterizations in AD

Hydrolysis and biodegradation of the substrates in an AD are mainly promoted by various enzymes excreted from microorganism, and enzymatic activities might give additional information about the difference between digestion and codigestion processes. Although substantial numbers of enzymes took part in CH_4 production during AD, in this study only three key enzymes responsible correspondingly for substrate hydrolysis (protease), acidification (AK) and methanation (coenzyme F_{420}) were analyzed. Codigestion with the highest cumulative CH_4 yield by codigestion of blue algae with swine manure at ISR 2.0 and digestion of blue algae inoculated with granular sludge at ISR 3.0 were investigated to illustrate the microbial activity difference.

The activities of three key enzymes were each semi-quantitatively determined by using the highest UV–VIS absorbance (representing the highest enzymatic activity) of the samples as 100%, and relative activities of the other samples were expressed as percent of the highest. As indicated in Fig. 4, the activities of protease, AK and coenzyme F_{420} had the same tendencies in the different digesters, showing an increasing trend during the first 12–16 d, 8 d and 6–8 d respectively and dropping rapidly until the end of digestion. The high enzymatic activities were well agreed with the high CH_4 production rates in Fig. 1. Comparing the highest enzymatic activities in the digestion and codigestion processes, AK activity had no significant difference between each other. To the protease and coenzyme F_{420}, the highest values during the digestion of blue algae inoculated with granular sludge were only 57.13% and 57.36% respectively of those in the codigestion of blue algae with swine manure. Obviously, not only the hydrolysis of soluble protein but the transformation activity of electron donors of the redox-driven proton translocation in methanogenic Archaea (expressed by coenzyme F_{420} [43]) was significantly lower during codigestion of blue algae with sludge. All these consisted perfectly with the above observed experimental results.

4. Conclusion

Codigestion of Taihu blue algae with swine manure resulted in improved CH_4 yield of 212.7 mL g^{-1} VS at ISR 2.0, while digestion of blue algae simply led to optimized CH_4 production of 73.5 mL g^{-1} VS at ISR 3.0. During the digestion and codigestion processes, pH, TAN and VFAs corroborated the appropriate stability of the anaerobic processes and showed no significant difference. VS removal and key enzymes variation manifested codigestion had

higher efficiencies in biodegradation and methanation, which confirmed AD of blue algae with swine manure is a promising technology for both solid wastes treatment and renewable-energy production.

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Appendix A. Supplementary material

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References

[1] Wu ZL, Shen HF, Ondruschka B, Zhang YC, Wang WM, Bremner DH. Removal of blue–green algae using the hybrid method of hydrodynamic cavitation and ozonation. J Hazard Mater 2012;235–236:152–8.
[2] Liu W, Qiu R. Water eutrophication in China and the combating strategies. J Chem Technol Biotechnol 2007;82:781–6.
[3] Yan Q, Li YC, Huang B, Wang AJ, Zou H, Miao HF, et al. Proteomic profiling of the acid tolerance response (ATR) during the enhanced biomethanation process from Taihu blue algae with butyrate stress on anaerobic sludge. J Hazard Mater 2012;235–236:286–90.
[4] Zhang XJ, Chen C, Ding JQ, Hou AX, Li Y, Niu ZB, et al. Water crisis in Wuxi, China: analysis of the origin. J Hazard Mater 2007;182(2010):130–5.
[5] Yan Q, Wang AJ, Yu CF, Ren NQ, Zhang YB, Zhang GS. Enzymatic characterization of acid tolerance response (ATR) during the enhanced biohydrogen production process from Taihu cyanobacteria via anaerobic digestion. Int J Hydrogen Energy 2011;36:405–10.
[6] Di Maria F, Sordi A, Micale C. Energy production from mechanical biological treatment and composting plants exploiting solid anaerobic digestion batch: an Italian case study. Energy Convers Manage 2012;56:112–20.
[7] Demirbas A. Use of algae as biofuel sources. Energy Convers Manage 2010;51:2738–49.
[8] Costa JC, Goncalves PR, Nobrel A, Alves MM. Biomethanation potential of macroalgae *Ulva* spp. and *Gracilaria* spp. and in co-digestion with waste activated sludge. Bioresour Technol 2012;114:320–6.
[9] Chynoweth DP. Renewable biomethane from land and ocean energy crops and organic wastes. Hortscience 2005;40:283–6.
[10] Zeng SJ, Yuan XZ, Shi XS, Qiu YL. Effect of inoculum/substrate ratio on methane yield and orthophosphate release from anaerobic digestion of *Microcystis* spp. J Hazard Mater 2010;178:89–93.
[11] Hodaifa G, Martinez ME, Sanchez S. Use of industrial wastewater from olive-oil extraction for biomass production of *Scenedesmus obliquus*. Bioresour Technol 2008;99:1111–7.
[12] Inglesby AE, Fisher AC. Enhanced methane yields from anaerobic digestion of Arthrospira maxima biomass in an advanced flow-through reactor with an integrated recirculation loop microbial fuel cell. Energy Environ Sci 2012;5:7996–8006.
[13] Alzate ME, Munoz R, Rogalla F, Fdz-Polanco F, Perez-Elvira SI. Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. Bioresour Technol 2012;123:488–94.
[14] Yuan XZ, Shi XS, Zhang DL, Qiu YL, Guo RB, Wang LS. Biogas production and microcystin biodegradation in anaerobic digestion of blue algae. Energy Environ Sci 2011;4:1511–5.
[15] Zhong WZ, Zhang ZZ, Luo YJ, Qiao W, Xiao M, Zhang M. Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. Bioresour Technol 2012;114:281–6.
[16] Yan Q, Zhao MX, Miao HF, Ruan WQ, Song RT. Coupling of the hydrogen and polyhydroxyalkanoates (PHA) production through anaerobic digestion from Taihu blue algae. Bioresour Technol 2010;101:4508–12.
[17] Yangin-Gomec C, Ozturk I. Effect of maize silage addition on biomethane recovery from mesophilic co-digestion of chicken and cattle manure to suppress ammonia inhibition. Energy Convers Manage 2013;71:92–100.
[18] Frijns J, Hofman J, Nederlof M. The potential of (waste) water as energy carrier. Energy Convers Manage 2013;65:357–63.
[19] Mussgnug JH, Klassen V, Schluter A, Kruse O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J Biotechnol 2010;150:51–6.
[20] Astals S, Nolla-Ardèvol V, Mata-Alvarez J. Thermophilic co-digestion of pig manure and crude glycerol: process performance and digestate stability. J Biotechnol 2013;166:97–104.
[21] Gonzalez-Fernandez C, Sialve B, Bernet N, Steyer JP. Comparison of ultrasound and thermal pretreatment of *Scenedesmus* biomass on methane production. Bioresour Technol 2012;110:610–6.
[22] Sournia A. Phytoplankton Manual, United Nations Educational Scientific and Cultural Organization (UNESCO); 1978.
[23] Badshah M, Lam DM, Liu J, Mattiasson B. Use of an Automatic Methane Potential Test System for evaluating the biomethane potential of sugarcane bagasse after different treatments. Bioresour Technol 2012;114:262–9.
[24] Kafle GK, Kim SH. Anaerobic treatment of apple waste with swine manure for biogas production: batch and continuous operation. Appl Energy 2013;103:61–72.
[25] Yan Q, Yu D, Wang ZL, Zou H, Ruan WQ. Phenol inhibition and restoration of the bioactivity of anaerobic granular sludge. Appl Biochem Biotechnol 2008;150:259–65.
[26] Eaton AD, Clesceri LS, Greenberg AE. Standard methods for the examination of water and wastewater. 21st ed. Washington DC, USA: American Public Health Association/American Water Works Association/Water Environment Federation; 2005.
[27] Msuya F, Neori A. Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed *Ulva* lactuca cultured in seawater tanks. J Appl Phycol 2008;20:1021–31.
[28] Zhao MX, Ruan WQ. Biogas performance from co-digestion of Taihu algae and kitchen wastes. Energy Convers Manage 2013;75:21–4.
[29] Ledoux M, Lamy F. Determination of proteins and sulfobetaine with the folin-phenol reagent. Anal Biochem 1986;157:28–31.
[30] Mu H, Chen YG. Long-term effect of ZnO nanoparticles on waste activated sludge anaerobic digestion. Water Res 2011;45:5612–20.
[31] Wang JY, Liu XY, Kao JCM, Stabnikova L. Digestion of pre-treated food waste in a hybrid anaerobic solid–liquid (HASL) system. J Chem Technol Biotechnol 2006;81:345–51.
[32] Xu FQ, Shi J, Lv W, Yu ZT, Li YB. Comparison of different liquid anaerobic digestion effluents as inocula and nitrogen sources for solid-state batch anaerobic digestion of corn stover. Waste Manage 2013;33:26–32.
[33] Wang XJ, Yang GH, Feng YZ, Ren GX, Han XH. Optimizing feeding composition and carbon-nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour Technol 2012;120:78–83.
[34] Williams PJlB, Laurens LML. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy Environ Sci 2010;3:554–90.
[35] Raposo F, Fernandez-Cegri V, De la Rubia MA, Borja R, Beline F, Cavinato C, et al. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. J Chem Technol Biotechnol 2011;86:1088–98.
[36] Mu Y, Yu HQ, Wang G. Evaluation of three methods for enriching H_2-producing cultures from anaerobic sludge. Enzyme Microbial Technol 2007;40:947–53.
[37] Nielsen HB, Angelidaki I. Strategies for optimizing recovery of the biogas process following ammonia inhibition. Bioresour Technol 2008;99:7995–8001.
[38] Eldyasti A, Chowdhury N, Nakhla G, Zhu J. Biological nutrient removal from leachate using a pilot liquid-solid circulating fluidized bed bioreactor (LSCFB). J Hazard Mater 2010;181:289–97.
[39] Dreher TM, Mott HV, Lupo CD, Oswald AS, Clay SA, Stone JJ. Effects of chlortetracycline amended feed on anaerobic sequencing batch reactor performance of swine manure digestion. Bioresour Technol 2012;125:65–74.
[40] Lin YQ, Wang DH, Wu SQ, Wang CM. Alkali pretreatment enhances biogas production in the anaerobic digestion of pulp and paper sludge. J Hazard Mater 2009;170:366–73.
[41] Gonzalez-Fernandez C, Sialve B, Bernet N, Steyer JP. Thermal pretreatment to improve methane production of *Scenedesmus* biomass. Biomass Bioenergy 2012;40:105–11.
[42] Snell-Castro R, Godon JJ, Delgenes JP, Dabert P. Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis. FEMS Microbiol Ecol 2005;52:229–42.
[43] Deppenmeier U. Redox-driven proton translocation in methanogenic Archaea. Cell Mol Life Sci 2002;59:1513–33.