Freezing/thawing effect on sewage sludge degradation and electricity generation in microbial fuel cell

Yuejia Chen, Junqiu Jiang and Qingliang Zhao

ABSTRACT

The effect of sludge freezing/thawing on its disintegration and subsequent use as substrate in a microbial fuel cell (MFC) was investigated to enhance organic matter degradation and electricity generation. Experimental results indicated that long freezing time (more than 48 h) was effective in disintegrating the sludge collected from the secondary sedimentation tank of a wastewater treatment plant. Freezing/thawing pretreatment could enhance the degradation of total chemical oxygen demand (COD) and electricity generation in MFC due to the higher concentration of soluble COD and ammonium nitrogen available in the pretreated sludge. The removal efficiency of total COD was increased from 25.3% (raw sludge as substrate) to 66.2% and the maximum power output was increased from 8.9 (raw sludge as substrate) to 10.2 W/m^3 in MFC.

Key words | electricity generation, freezing/thawing pretreatment, microbial fuel cell, organic degradation, sewage sludge

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INTRODUCTION

Sewage sludge treatment has become increasingly important due to environmental, social and economic concerns. Conventionally, anaerobic digestion has been widely used for sludge treatment (Pilli *et al.* 2011). However, excessive heating requirement, long hydraulic retention time (>20 days), complicated operation, and sensitivity to shock loads and toxic materials limit the application of anaerobic digestion (Jiang *et al.* 2010).

Sewage sludge has been demonstrated as a good potential fuel for electricity generation in microbial fuel cells (MFCs) (Jiang *et al.* 2009; Xiao *et al.* 2011). Owing to their inherent advantages of high energy-conversion efficiency and mild reaction conditions, MFCs might be an alternative strategy for sludge degradation and energy recovery. Since only the soluble and easily biodegradable organic matters can be preferentially used by the microbe within MFC, various sludge pretreatment technologies have been studied to improve sludge hydrolysis (Jiang *et al.* 2010; Xiao *et al.* 2011; Yusoff *et al.* 2013), such as mechanical disintegration (Kim *et al.* 2008), thermal hydrolysis, alkaline addition, sonication (Jiang *et al.* 2010), and biological treatment.

Freezing/thawing treatment can promote solubilization of organic matter (Gao 2011) and enhance biogas yield during anaerobic sludge digestion (Montusiewicz *et al.* 2010). In cold regions such as Northeast China, parts of the USA and Canada, the freezing/thawing process occurs spontaneously in winter without extra energy input and chemical addition. Our previous study had indicated that alkalization and ultrasonication pretreatment of sludge could promote electricity generation and sludge organic matter degradation in MFCs (Jiang *et al.* 2010). If freezing/thawing pretreatment could be used instead of alkalination and ultrasonication, the material and energy consumptions would be substantially reduced. Thus, the freezing/thawing process might be an adequate substitute for alkalination or ultrasonication pretreatment of sludge in winter.

The objective of this study is to investigate the effect of sludge freezing/thawing on its disintegration and subsequent use as substrate in a MFC. Both the sludge organic matter degradation and electricity generation in the MFC were examined.

MATERIALS AND METHODS

Sludge and its characteristics

The raw sludge (RS) was collected from the secondary sedimentation tank in a municipal wastewater treatment plant

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(WWTP) of Harbin (China) and was stored at 4 °C before use. Anoxic/oxic biological processes are employed in the WWTP with an organic loading rate of 0.131 kilograms of 5-day biochemical oxygen demand per kilogram of mixed liquor suspended solids per day and sludge retention time of 17 d. All collected sludge samples were first gravitationally concentrated to a water content of about 97% (w/w). The characteristics of sludge were as follows: total chemical oxygen demand (TCOD) 26,000–28,000 mg/L, soluble COD (SCOD) 950–1,100 mg/L, pH 6.97–6.93, alkalinity 1,400–1,900 mg/L (as $CaCO_3$), volatile solids (VS) 19,000–19,460 mg/L, total solids (TS) 28,680–28,780 mg/L, and NH_4^+-N in sludge supernatant 68.8–72.3 mg/L.

Freezing/thawing pretreatment

To examine the effect of freezing time on sludge disintegration, seven 120 mL samples of the concentrated sludge were transferred into 250-mL beakers and frozen at −20 °C for 12 h, 24 h, 36 h, 48 h, 60 h, 72 h or 84 h, respectively, and then thawed for another 12 h at room temperature (20 °C).

MFC and test

The MFC comprised two Plexiglas chambers and a proton exchange membrane (Nafion 117) located between the anode and cathode. The anode chamber was a cylinder (Φ12 cm × 10 cm) and the cathode chamber was a cuboid (6 cm × 6 cm × 10 cm). The effective volume of the anodic chamber and cathodic chamber was 600 mL and 360 mL, respectively. Both the anode and cathode electrodes consisted of a graphite fiber brush and titanium wire that collected electrons for the external circuit. The external resistor was 1,000 Ω. The anodic compartment of the MFC, in which magnetic stirring was used to mix the medium, was inoculated with sludge and repeatedly filled until bacteria colonized the electrode surface and started producing electricity. To compare the performance of the MFC using frozen/thawed sludge (FTS) as substrate with those of our previous studies (Jiang *et al*. 2010), ferricyanide ($K_3Fe(CN)_6$) was selected as the catholyte. Moreover, ferricyanide was used as the electron acceptor in the cathodic chamber as it readily accepted electrons (Logan *et al.* 2006). The catholyte was 50 mM $K_3Fe(CN)_6$ aqueous solution in a 100 mM KH_2PO_4 buffer with its pH adjusted to 7 using 1 M NaOH (Aelterman *et al.* 2006). Experiments were conducted in batch mode at room temperature (20 °C).

Analytical methods

Chemical analysis

The TCOD, SCOD, TS, VS, alkalinity, NH_4^+-N, and pH of sludge samples were measured according to the standard methods (APHA 1998). The amount of protein in the supernatant organics was determined using the modified Lowry method (Frølund *et al.* 1995) with bovine serum albumin as the standard. The carbohydrate content was measured using the anthrone method (Gaudy 1962) with glucose as the standard. The supernatant samples were obtained by centrifuging the sludge at 4,000 rev/min for 30 min. The contents of volatile fatty acids (VFA) were determined by gas chromatography (7890A GC-System, Agilent Technologies, USA) equipped with a flame ionization detector and a 15 m capillary column (Innowax; i.d. 0.53 mm; 19095N-121; Agilent Technologies).

The degree of sludge disintegration (DD_{COD}) was calculated as the SCOD increment compared with a reference measurement obtained by destroying all the bacterial cells by NaOH treatment (1 mol/L) for 24 h at 20 °C before the filtration step (Frølund *et al.* 1996; Gonze *et al.* 2003; Jiang *et al.* 2010):

$$DD_{COD} = \frac{(SCOD - SCOD_0)}{SCOD_{NaOH} - SCOD_0} \times 100\%$$

where SCOD and $SCOD_0$ values were the SCOD of the supernatant of the FTS and RS samples, and $SCOD_{NaOH}$ was that of the sample treated with NaOH, which was defined as the total SCOD after sludge disintegration.

Electrochemical analysis

Electrical voltage drop by the MFC was recorded using a multicenter voltage collection instrument (12-bit A/D-conversion chips US) connected to a personal computer via a universal serial bus (USB) (Intel, USA) interface and calibrated with a digital multimeter (Agilent HP 34970; Agilent, USA) before each test. The circuit consisted of a 10–9,999 Ω resistor. Except for those mentioned, the measurement of cathode working potential was performed by a reference electrode (Ag/AgCl, 195 mV vs. standard hydrogen electrode) connected to a multimeter (Agilent HP 34970). Voltage was converted to volume power density P_v (W/m^3) via the equation $P_v = UI/V_a$, where U is voltage (V), I is current (A), and V_a is the net liquid volume in the anodic chamber (m^3). The maximum power density and polarization curve were determined by adding fresh substrate to the MFC to get a constant power, and then by adjusting the external resistance to 10–9,999 Ω for recording the corresponding voltage drop.

RESULTS AND DISCUSSION

Effect of freezing/thawing on sludge disintegration

The effect of freezing time (from 12 to 84 h) on sludge disintegration was studied to determine the optimum freezing/thawing conditions. Experimental results indicated that DD_{COD} increased almost linearly with freezing time (0–36 h) (Figure 1(a)). It could be noted that DD_{COD} was 16.9% at $-20\,^{\circ}C$ for 36 h. During sludge freezing/thawing, SCOD increased sharply owing to sludge disintegration, then achieved stability until freezing for 60 h (DD_{COD} was 19.1%). There was a slight change of SCOD between 48 and 72 h. It could be noted that DD_{COD} was 18.9% at $-20\,^{\circ}C$ for 48 h, which achieved a similar solubilization of sewage sludge treated with 0.6 W/mL ultrasound for 15 min (Jiang *et al.* 2010).

A great increase of NH_4^+-N, soluble protein and soluble carbohydrates concentration was obtained (Figure 1(b)). It could be noted that soluble carbohydrates' concentration

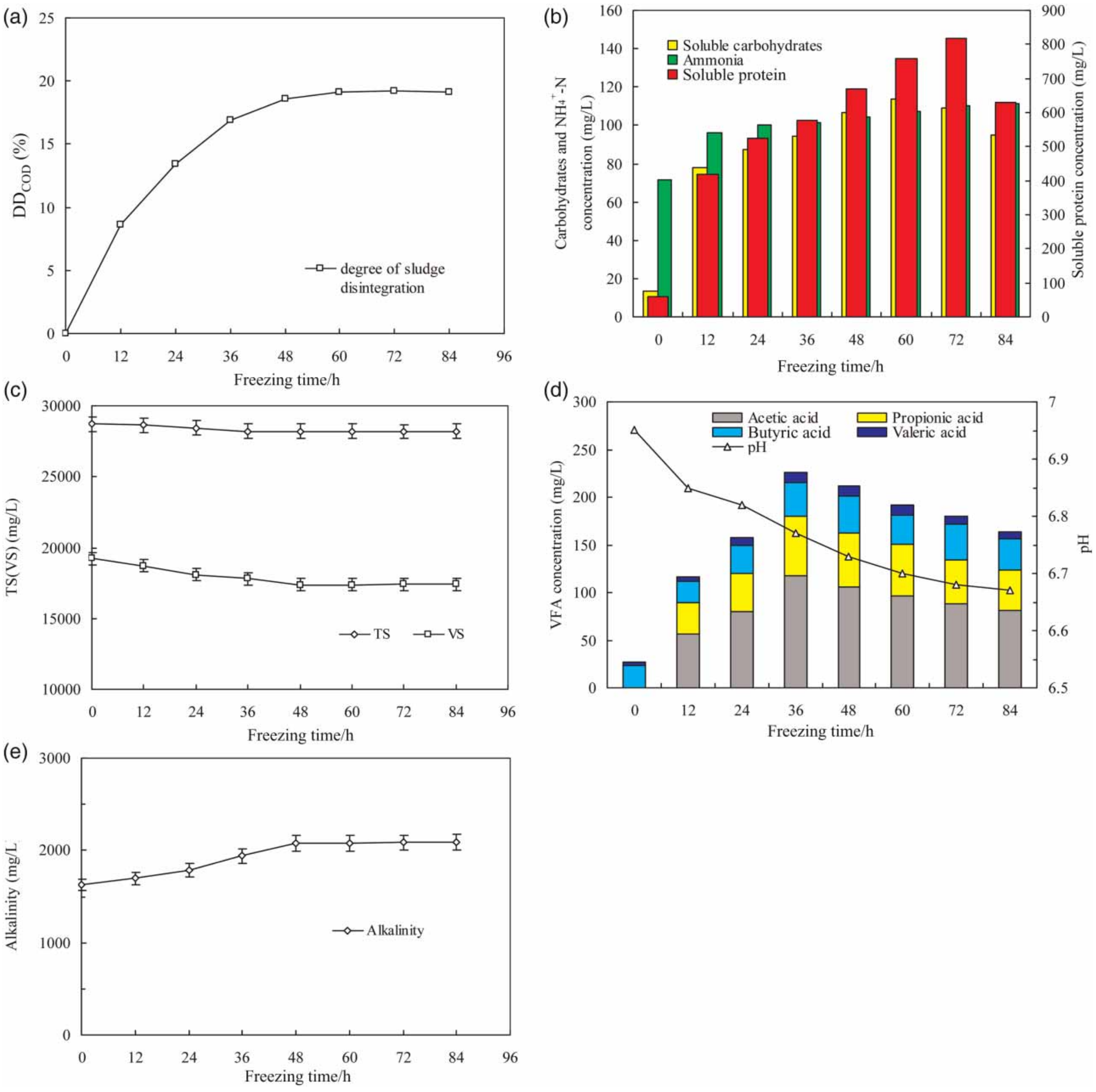

Figure 1 | Variation of sludge characteristics over freezing time during sludge freezing/thawing pretreatment: (a) DD_{COD}; (b) soluble carbohydrates, proteins and NH_4^+-N; (c) TS and VS; (d) VFAs and pH value; (e) alkalinity.

increased sharply from 13.3 to 77.8 mg/L in the first 12 h, and then followed a steady increase to 113.5 mg/L between 12 h and 60 h, which was nearly 10 times that of RS. The change of soluble protein concentration was similar to that of soluble carbohydrates. A sharp increase occurred in the first 12 h (from 59.5 mg/L to 419.4 mg/L), and then was followed by a steady increase between 12 and 72 h (from 419.4 to 816.2 mg/L). The increase of NH_4^+-N concentration was mainly due to the degradation of proteinous organic matter as its NH_4^+ ions were released (Carta *et al.* 2002). This observation was consistent with the studies by Örmeci & Vesilind (2001) using activated sludge and by Hu *et al.* (2011) using secondary sludge.

Simultaneously, a slight decrease in TS and VS was observed in the first 36 h, and then concentrations remained stable during the next 48 h (Figure 1(c)). The TS and VS losses might be attributed to the reaction of the immobilizing exoenzymes present and the endoenzymes, released from the disrupted cell as an effect of the formation of extracellular and intracellular ice crystals. Furthermore, the biomass might still have activity during sludge freezing, and the microorganisms that survived could carry out the degradation. Proteins and fats, as a cryoprotectant, could protect the microorganisms from freezing; thus anaerobic biodegradation lasting for 12 h during the thawing period could occur (Montusiewicz *et al.* 2010). TS and VS were almost not removed when the freezing time was more than 36 h because anaerobic biodegradation only occurred during the thawing stage, which had a slight relationship with prolonged freezing time.

Concomitantly, a rapid rise in VFA concentration and decrease in pH was observed (Figure 1(d)), which confirmed the solubilization of organic matters and release of intracellular material. An increase of alkalinity was also noted (Figure 1(e)), consistent with the results obtained by Montusiewicz *et al.* (2010), who stated that the average alkalinity was increased from 1,100 mg/L (RS) to 1,400 mg/L (FTS) despite a decrease of pH from 6.39 to 5.82. A specific buffering condition could be existent in the process, resulting in the higher VFA increase (pH decrease) than alkalinity changes (Montusiewicz *et al.* 2010).

Effect of freezing/thawing pretreatment on electricity generation in MFC

Since the microbe in MFC can only use soluble and easily-biodegradable organic matters and other small organic molecules as 'fuel', the DD_{COD} is defined as a measure of 'fuel' richness. As the DD_{COD} changed slightly after 48 h of freezing, the freezing times of 24, 36, and 48 h were investigated in the subsequent experiments.

After the successful start-up of the MFC reactor, both RS and FTS were introduced successively into the dual-chamber MFC with 100 mM $K_3Fe(CN)_6$ aqueous solution in the cathode chamber. After MFC operation for 5 h, the stable voltage output achieved 0.66 V with RS, 0.69 V with 24 h FTS, 0.70 V with 36 h FTS, and 0.73 V with 48 h FTS (Figure 2(a)). Zhang *et al.* (2012) had reported that the MFC reactor after start-up revealed a transient lag phase of 24 h, and then a low power production was noted from the second day. In comparison, the MFC using FTS as substrate with ferricyanide ($K_3Fe(CN)_6$) as cathodic electron acceptor had the advantage of quick start-up. As the sludge freezing time was prolonged, the power density increased (Figure 2(b)). A maximum power output of 10.2 W/m^{-3} was obtained with the 48 h FTS as anodic substrate. The higher power outputs with FTS than RS indicated that freezing/thawing pretreatment of sludge could accelerate electricity generation. Since sludge disintegration during freezing/thawing yielded more 'fuel' for the MFC, the power output increased as DD_{COD} increased.

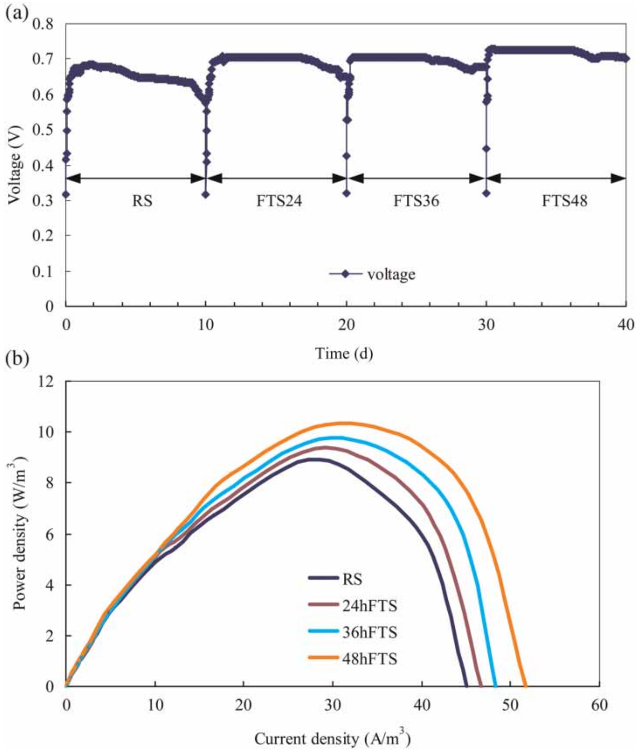

Figure 2 | Electricity generation in MFC with RS and FTS substrates: (a) cell voltage of MFC (external resistance of 1,000 Ω, potentials calculated vs. Ag/AgCl); (b) power output versus current density.

Effect of freezing/thawing pretreatment on organic matter degradation in MFC

As shown in Figure 3, more TCOD removal was obtained in the MFC with FTS as substrate than with RS during the 48 h degradation. The TCOD removal efficiency quickly reached 33.6% (frozen 24 h and thawed 12 h sludge) after MFC operation for 48 h, while it increased to 66.2% at the end of 240 h operation (frozen 48 h and thawed 12 h sludge). These results were close to those reported by Zhang *et al.* 2012 (TCOD removal 64 ± 8%, three-chamber biocathode MFC with sewage sludge as anodic substrate, 25 d operation), and were comparable to the results from sewage sludge pretreated with 1.5 W/mL ultrasound for 15 min (Jiang *et al.* 2010). This might be ascribed to the fact that the freezing/thawing pretreatment of sludge led to the release of soluble organic matters containing more protein and polysaccharide, and these matters could be easily degraded in the MFC (Rabaey *et al.* 2003, 2005). The results demonstrated that the freezing/thawing pretreatment of sludge was efficient in accelerating organic matter disintegration and enhancing organic degradation in the subsequent MFC.

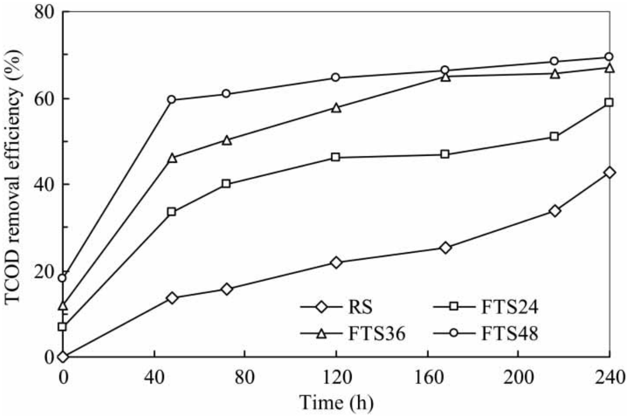

Figure 3 | TCOD removal efficiency during MFC treatment of RS and FTS substrates.

The characteristics of RS and FTS after MFC treatment are shown in Table 1. Both alkalinity and NH_4^+-N increased in all four MFCs. After 2 d treatment in MFC, alkalinity was increased by 56.2% (frozen 24 h), 47.1% (frozen 36 h), and 48.8% (frozen 48 h), while NH_4^+-N was increased by 44.5% (frozen 24 h), 56.8% (frozen 36 h), and 69.1% (frozen 48 h). NH_4^+-N concentration of anodic solution was maximized at 192.1 mg/L as a hydrolysis product of sludge after 6 d treatment for the FTS frozen for 48 h, which was consistent with the maximum power density (Figure 2). Higher ammonium nitrogen (less than 500 mg/L) could improve the performance of the MFC (Nam *et al.* 2010a) because of the increasing anolyte conductivity with the presence of excess NH_4^+-N and protons, which reduced ohmic resistance and increased power output in MFCs (Ishii *et al.* 2008; Mohan & Das 2009; Nam *et al.* 2010b).

The above results indicated that freezing/thawing pretreatment of sludge could enhance organic matter degradation and electricity generation in the MFC, among which the FTS substrate (frozen 48 h/thawed 12 h) demonstrated the best performance.

CONCLUSIONS

This study demonstrated an economic and efficient way for sewage sludge disintegration. Freezing/thawing pretreatment (freezing from 12 h to 84 h) led to more than two-fold increases in SCOD.

Freezing/thawing pretreatment of sludge could enhance organic matter removal and electricity generation in the MFC, and the FTS substrate (frozen 48 h/thawed 12 h) demonstrated the best performance due to the higher

Table 1 | Characteristics of RS and FTS after MFC treatment

Parameter	Unit	Operation time (d)	RS	FTS (frozen 24 h)	FTS (frozen 36 h)	FTS (frozen 48 h)
pH	–	0	6.95	6.82	6.77	6.73
		2	6.85	6.70	6.68	6.66
		6	6.78	6.68	6.65	6.63
Alkalinity	mg/L	0	1,626.6	1,938.2	2,389.9	2,577.6
		2	2,865.5	3,027.7	3,516.3	3,853.9
		6	3,065.6	3,827.8	4,047.8	4,517.0
NH_4^+-N	mg/L	0	71.5	99.9	101.1	104.1
		2	136.6	144.4	158.5	176.0
		6	142.8	159.5	185.7	192.1

concentration of SCOD and ammonium nitrogen. TCOD was removed by 66.2% and a maximum power output of 10.2 W/m^3 was obtained with the 48 h FTS as anodic substrate after MFC operation for 6 d.

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