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Determination and abatement of methanogenic inhibition from oleic and palmitic acids

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ABSTRACT

Long chain fatty acids (LCFAs) are the intermediates from anaerobic digestion of lipid-rich waste, and limit the digestion even at millimolar concentrations. The digestion characteristics of unsaturated and saturated LCFAs, represented by oleic and palmitic acids, are investigated. Consequently, palmitic acid was far less inhibitory than oleic acid. Palmitic acid was almost not degraded before 20 d, and the subsequent methane was also produced at a lower rate, below a half for oleic acid. The heterogeneity between methanogenic sludge and palmitic acid was the likely reason. The abatement methods of soluble calcium addition and bentonite addition were synchronously compared to evaluate the effectiveness in upgrading the anaerobic performance of LCFAs. Limited improvement for palmitic digestion was observed. In contrast, calcium addition was more effective than bentonite addition in shortening lag-phase time, reducing reagent dosage, and decreasing solid concentration of digestate. Delaying the addition time of calcium salt had a negative effect on the abating the inhibition caused by oleic acid. The methanogenic activity decreased exponentially with the addition time of calcium salt, following the equation: Methanogenic activity (%) = $91.0e^{-t/942.5}+24.8e^{-t/6.2}-15.9$.

1. Introduction

Management and disposal of growing lipid-rich waste poses an enormous challenge, because of the hazards of the waste to the pipe clog, and pollution to the environment through deoxygenation of water, infiltration into soil sediments and aquifer (Sariaslani and Gadd, 2013; Wang et al., 2013; Iasmin et al., 2014). Anaerobic digestion is of great potential in lipid-rich waste treatment, due to the advantages in much higher methanogenic potential (Kim and Shin, 2010), waste reduction and stabilization, low carbon emission, and limited pollution (Molino et al., 2013; Wu et al., 2016). However, inhibition caused by the intermediates of lipid digestion, long chain fatty acids (LCFAs), is usually inevitable even when LCFAs are at millimolar concentrations. As a consequence, anaerobic digestion of lipid-rich waste is seriously influenced by biomass floatation, prolonged recovery time, and so on (Hwu et al., 1998; Shin et al., 2003; Kuang et al., 2006).

The major LCFAs in domestic wastewater and sludge are the saturated myristic, palmitic, and stearic acids, and the unsaturated oleic acid and linoleic acids, which comprise over 90% of the total LCFAs in waste streams (Novak and Carlson, 1970). Some investigations on LCFA components in lipid-rich waste or in nature showed that unsaturated linoleic, especially oleic acid, and saturated palmitic acid were more dominant in the major LCFAs (Carlier et al., 1991; Kobayashi et al., 2017). Furthermore, when oleic acid was fed into an anaerobic digester, palmitic acid became a key intermediate and accumulated onto the anaerobic sludge, even attaining more than 80% of all intermediates (Pereira et al., 2002, 2005). Thus, both oleic acid and palmitic acid are relatively significant components in the LCFA group.

Compared with palmitic acid, oleic acid was found more inhibitory on acetate degradation (Lalman and Bagley, 2001; Shin et al., 2003). However, oleic acid could be degraded more quickly than the saturated LCFAs containing the same or even less carbon (Shin et al., 2003). Another previous study revealed that when

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palmitic acid was fed into an anaerobic reactor, it was hardly degraded and precipitated between the sludge (Pereira et al., 2005). Hitherto, the reasons why there are different characteristics for anaerobic digestion of oleic and palmitic acid have not been figured out yet. Also, those previous studies recognized LCFAs as inhibitors only, and focused their attention on the effects of LCFAs on anaerobic digestion of acetate or propionate. The features of the digestion substrate for LCFAs are frequently ignored.

Adsorption of LCFAs onto the surface of the bacterial cells has been widely accepted as the main reason to cause toxicity by inducing the lysis of protoplasts and damaging the transport channel of bacterial cells (Petruy and Lettinga, 1997; Hwu et al., 1998; Nadais et al., 2003; Pereira et al., 2004; Kuang et al., 2006). Prevention strategies for inhibition have also been developed to abate the toxicity of LCFAs. Among those strategies, the measure of reagent addition, such as precipitation with soluble calcium (Ca) and adding adsorbent, is realized by diminishing the availability of adsorbed LCFAs (Hanaki et al., 1981; Angelidaki et al., 1990; Nordell et al., 2013; Kobayashi et al., 2014). Adding $CaCl_2$ is an earlier method, and its effectiveness was demonstrated in the previous studies (Hanaki et al., 1981; Kobayashi et al., 2014). Another effective and widely employed method is to add bentonite. The roles of bentonite were concluded as (1) adsorption of the inhibited substances on its surface to form flocs, which thereby lowers the effective oil concentration in the liquid phase, (2) release of the adsorbed biodegradable matter in the methanogenesis, and (3) a direct effect of the cations, such as calcium or magnesium found in bentonite (Angelidaki et al., 1990; Beccari et al., 2001; Palatsi et al., 2012). Those previous studies paid more attention to the feasibility of abatement methods, and little investigation has been done to compare different abatement methods. In terms of Ca addition, it is reported that it became inefficient after anaerobic culture had been exposed to LCFAs (Hanaki et al., 1981). Koster et al. (Koster, 1987) intensively studied the effects of the addition time of Ca salt on methanogenic inhibition from lauric acid. Nevertheless, the relevant investigation for widely existent oleic acid was hardly found.

In the present study, LCFAs were more regarded as a substrate of anaerobic digestion, to investigate the effects of LCFA concentration variation on anaerobic sludge. A comparison between two typical abatement methods favors the acquisition of more appropriate prevention measure. Moreover, further optimization for the selected abatement method was also investigated.

2. Materials and methods

2.1. Digested sludge and reagents

The seed sludge was the mesophilic digested sludge for sewage sludge treatment, which was fetched from a wastewater treatment plant in Tsukuba, Ibaraki, Japan. The characteristics of the digested sludge are presented in Table 1. P values for data in the Table were controlled to less than 0.05. Oleic and palmitic acids were the representative LCFAs, used in this study. The regents of $CaCl_2 \cdot 2H_2O$ and bentonite were the additives to abate inhibition. Those chemical reagents involved were purchased from Wako Pure Chemical Industries, Ltd., Japan.

2.2. Experimental procedures

Three batch experiments were conducted (1) to investigate the digestion characteristics of oleic and palmitic acids (Experiment 1, E1), (2) to make a comparison for two reagent addition methods to abate inhibition (E2), and (3) to elucidate the effects of various Ca addition times on abatement of inhibition from oleic acid (E3).

For E1, 22 glass serum vials with a volume of 127 ml were prepared firstly. Subsequently, each vial was filled with 50 ml of digested sludge. A series of calculated volume or mass of oleic or palmitic acid was added to the corresponding vials, to ensure that the concentrations of individual oleic and palmitic acids were 2 mmol l^{-1}, 4 mmol l^{-1}, 6 mmol l^{-1}, 8 mmol l^{-1} and 10 mmol l^{-1}, respectively. A control test, without any LCFA added, was also set as a reference. Each test was arranged in duplicate. After those vials were flushed with nitrogen gas for at least 30 s to provide anaerobic conditions, the vial was sealed with a rubber stopper and aluminum cap. Whereafter the vials were transferred to an agitator in an incubator at 35 °C. During the operation period, biogas volume and composition from each vial were monitored and analyzed. The frequency of analysis depended on the estimated biogas production volume. The experiment ran until the biogas production stopped.

According to the results of E1 and previous research conclusions, 6 mmol l^{-1} of oleic acid, 6 mmol l^{-1} of palmitic acid, and 3 mmol l^{-1} of oleic acid+3 mmol l^{-1} of palmitic acid were chosen as the LCFA addition concentration in E2. The addition dosage of 10 g l^{-1} for bentonite was proved to be appropriate, based on the fact that methane was produced most at this dosage (Beccari et al., 2001; Wu et al., 2015). An investigation on the Ca addition for inhibition abatement has been conducted in a previous study, and appropriate dosage for $CaCl_2 \cdot 2H_2O$ addition was found to be 600 mg l^{-1} (Kobayashi et al., 2014). Thus, two groups of experiments were designed and initiated synchronously. The vials for the first group was filled with 10 g l^{-1} of bentonite addition, 50 ml of digested sludge, and determined LCFA dosages in each vial. The 600 mg l^{-1} of $CaCl_2 \cdot 2H_2O$ was substituted in the second group for 10 g l^{-1} of bentonite. Also, control tests for each group were set in E2. Control test for bentonite addition was carried out with 50 ml of digested sludge and 10 g l^{-1} of bentonite added, and control test for Ca addition was conducted with 50 ml of digested sludge and 600 mg l^{-1} of $CaCl_2 \cdot 2H_2O$ added. Each test was set in parallel. The following procedures referred to the described in E1.

Considering the effects of addition time on the abatement performance for Ca addition experiment, E3 was conducted to make a further investigation, with oleic acid at 6 mmol l^{-1} and various Ca addition ($CaCl_2 \cdot 2H_2O$, 600 mg l^{-1}) times. The procedure was the same as the second group in E2, except for the Ca addition time after full exposure between oleic acid and digested sludge. The

Table 1
Characteristics of the digested sludge used in the experiment and the tests at the end of the abatement experiment.

	Digested sludge	Bentonite addition				Ca addition			
		Control	Oleic 6 mmol l^{-1}	Palmitic 6 mmol l^{-1}	Oleic 3 mmol l^{-1} + Palmitic 3 mmol l^{-1}	Control	Oleic 6 mmol l^{-1}	Palmitic 6 mmol l^{-1}	Oleic 3 mmol l^{-1} + Palmitic 3 mmol l^{-1}
pH	7.19 ± 0.01	7.62 ± 0.04	7.13 ± 0.00	7.11 ± 0.01	7.12 ± 0.00	7.49 ± 0.05	7.26 ± 0.21	7.03 ± 0.02	7.06 ± 0.04
TS (%)	1.42 ± 0.01	1.85 ± 0.01	1.91 ± 0.08	2.00 ± 0.04	1.91 ± 0.05	0.96 ± 0.00	1.00 ± 0.02	1.11 ± 0.04	1.07 ± 0.04
VS (%)	1.05 ± 0.00	0.73 ± 0.01	0.63 ± 0.00	0.82 ± 0.04	0.73 ± 0.04	0.69 ± 0.00	0.68 ± 0.04	0.83 ± 0.05	0.65 ± 0.03
COD (g l^{-1})	16.3 ± 1.3	9.8 ± 0.1	9.3 ± 0.2	9.4 ± 0.1	9.5 ± 0.0	9.5 ± 0.0	9.5 ± 0.5	9.6 ± 0.2	9.4 ± 0.0