Journal of Hazardous Materials 384 (2020) 121363

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

The effects of thiosulfinates on methane production from anaerobic co-digestion of waste activated sludge and food waste and mitigate method

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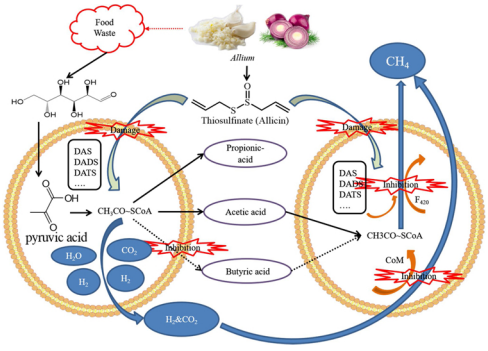
GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: R. Teresa

Keywords:
Thiosulfinates
Methane production
Anaerobic co-digestion
Food waste
Waste activated sludge
Pre-treatment method

ABSTRACT

Thiosulfinates, a natural antibiotic, existed in all parts of *Allium*, therefore might be accumulated in large amounts in food waste (FW). FW was often added into waste activated sludge (WAS) anaerobic digestion process as a kind of supplement for nutrition balance. However, the impact of thiosulfinates on methane production and the possible approach to mitigate its inhibition on the co-digestion process could be available in few literatures. This work was carried out in a series of batch experiment at pH 7.0 ± 0.2 and 35 ± 1.0 ℃ to promote the further understanding of this process. The experimental results showed that the methane accumulation decreased from 270.6 ± 13.4 to 16.7 ± 7.0 mL/g VSS (volatile suspended solids) when the initial concentration of thiosulfinates increased from 0 to 2.5 μg/g VSS. The activities of functional enzymes (F_{420} and CoM) were inhibited by 99.06% and 99.82% compared with control group when reactor contained 2.5 μg/g VSS thiosulfinates. Furthermore, different temperature, pH, and combination pretreat were applied to impair the inhibition of thiosulfinate. Compared with no pretreatment group, methane yield was increased by 2.26, 32.18 and 42.2-fold, respectively which group was under pretreatment method of heat (100 ℃), alkali (pH 9) and combination.

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https://doi.org/10.1016/j.jhazmat.2019.121363
Received 7 May 2018; Received in revised form 16 August 2018; Accepted 17 August 2018

1. Introduction

More than 1.3×10^{10} tonnes of food waste (FW) are generated each year worldwide, creating serious social, environmental and economic issues (FAOSTAT-FAO, 2017; Xu et al., 2018). FW is primarily disposed of by landfilling, incineration, or composting (Hagos et al., 2017). However, considering the negative environmental effects of those methods, anaerobic co-digestion is more desirable (Braguglia et al., 2018). Currently, anaerobic digestion is mainly applied in wastewater treatment plants to dispose of excess sludge, which is rich in microbial activity, and the primary products are hydrogen and methane (Wang et al., 2018a). The anaerobic digestion process main include hydrolysis stage, acidogenesis stage and methanogenesis stage, while, the methane produces from last stage and the yield rely the initial organic loading (Liu et al., 2019a; Zhao et al., 2019). A carbon source must be frequently added into the anaerobic digester to increase the organic loading and augment the product yield (Zhou et al., 2018). FW, which contains abundant organic matter and chemical energy, could be converted to biogas or high-value-added carbon-containing compounds through anaerobic co-digestion (Wainaina et al., 2018). Accordingly, many studies have evaluated FW as a cost-effective carbon source for addition in anaerobic digesters (Qin et al., 2019; Chen et al., 2015). FW is increasingly being treated via anaerobic co-digestion with waste activated sludge (WAS) with the goal of producing green energy and recycling carbon (Zhou et al., 2018; Liu et al., 2019b; Zhang et al., 2014). To promote sustainable development and produce more green energy, it is critical to optimise the efficiency of anaerobic co-digestion (Liu et al., 2019a; Zhang et al., 2014). Thus, many studies have explored the effects of a variety of factors on anaerobic co-digestion, included temperature, pH and inhibitors (Table S1). From previous study, the enhanced production of methane during anaerobic co-digestion could through use of adjustment temperature, pH, C/N ratio, pre-treatment and organic loading. Meanwhile, various inhibitors exist in real anaerobic co-digestion project that worth pay more focus.

Thiosulfinates, which are natural broad-spectrum antibiotics, are abundant in *Allium* species (e.g. onion, garlic and shallots), which are used widely in dishes worldwide and are considered as health foods (Putnik et al., 2019; Rose et al., 2018). Thiosulfinates accumulate in large amounts in FW with the measure of consumption of *Allium* augment over the world. In 2017, the total yield of onion, garlic and shallots reached 33.3 million metric tonnes worldwide (FAOSTAT-FAO, 2017). As *Allium* is used as a condiment in most dishes, most *Allium* from each dish will become FW. Meanwhile, the consumption of *Allium* was uncertainly from country to country and cuisine. Thus, the concentration of thiosulfinates in FW was hardly quantification exact, and the maximum containing possibility could reach 0.31 mg/g of FW (Table S2). Allicin (diallyl thiosulfinate) is an important thiosulfinate found in garlic (Focke et al., 1990; Lilia et al., 2017), and its maximum concentration is approximately 16.2 mg/g of fresh garlic (Putnik et al., 2019; Chen et al., 2017; Fratianni et al., 2016). Allicin forms when alliin and alliinase come into contact when the plant's cellular structure is damaged (Martins et al., 2016). Notably, allicin is detected in many *Allium* species, not just garlic, as shown in Table S2. The process of allicin production is shown in Fig. 1 (Gruhlke et al., 2010). Allicin plays roles in several key biochemical processes, including electron transfer and DNA transcription (Wills, 1956; Kubec et al., 1997; Rodrigues and Percival, 2019). As a result, many researchers have studied the medicinal value of thiosulfinates and the active ingredients of *Allium* species. However, few studies have reported the effects of thiosulfinates on methane production during the anaerobic co-digestion of FW and WAS. Thiosulfinates can be degraded into multiple active sulfur-containing compounds by heat or oxidation step by step such as diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) (Lilia et al., 2017), which exhibit broad-spectrum antibacterial effects as well (Borlinghaus et al., 2014). Thus, the effects of the degradation products of thiosulfinates on methane generation during the anaerobic co-digestion of FW and WAS should also be considered.

The purpose of this work was to evaluate the potential effects of allicin, a common thiosulfinate found in *Allium* species, on methane production during the anaerobic co-digestion of FW and WAS along with the mechanism of those effects. First, the effects of allicin on methane production in an anaerobic co-digestion system were explored by comparison to an allicin-free system during different stages of anaerobic co-digestion: solubilisation, hydrolysis, acidogenesis and acetogenesis. Next, the effects of thiosulfinates during the methanogenesis stage were evaluated by analysing the relative activities of enzymes involved in methane production, including coenzyme F420 (F_{420}), CoM and NADH. Meanwhile, the ability of thiosulfinates to damage the cellular structure was evaluated based on the concentrations of lactate dehydrogenase (LDH) and DNA in the fermentation liquor. Based on the results, the mechanism by which thiosulfinates affects methane yield from anaerobic fermentation was explored. Finally, we compared different thiosulfinate pre-treatment methods designed to weaken the inhibition of methane production by thiosulfinates during the anaerobic co-digestion of FW and WAS.

2. Materials and methods

2.1. The source and characteristics of WAS, FW and inocula

Raw sludge was obtained from a secondary sedimentation tank at a municipal wastewater treatment plant in Changsha, Hunan, China. The raw sludge was percolated through stainless-steel mesh (2.0 mm) and then allowed to settle for 24 h at 4 °C before being used in anaerobic fermentation experiments. The main characteristics of the concentrated WAS were as follows: pH = 6.8 ± 0.2; total suspended solids (TSS) = 22,450 ± 427 mg/L; VSS = 13,110 ± 164 mg/L; total chemical oxygen demand (TCOD) = 15,251 ± 227 mg/L; soluble chemical oxygen demand (SCOD) = 403 ± 9 mg/L; total protein = 8194 ± 319 mg COD/L; and total carbohydrate = 993 ± 107 mg COD/L.

Table S2 shows the differences in FW from different sources. Because the initial characteristics of real kitchen waste fluctuates greatly. In this study, the FW simulated kitchen waste to control the fermentation mixture various characteristics to approximate in the multi-batch test. The FW was obtained from a supermarket in Changsha, Hunan, China and compounded in the laboratory. The FW mainly contained rice, vegetables, and meat with the following composition (g/kg of FW): rice, 400; cabbage, 350; pork, 200; and tofu, 50. The FW was shredded and mixed in a blender and then stored at 4 °C before experimental use. The main characteristics of the experimental FW were as follows: pH = 5.38 ± 0.2; TSS = 160.9 ± 10 g/L; VSS = 116 ± 9 g/L; TCOD = 275 ± 5 g/L; total protein = 40.2 ± 3.1 g COD/L; and total carbohydrate = 126.8 ± 6.1 g COD/L.

Seed sludge (SD) from WAS was habituated in the laboratory, and the initial SD characteristics were consistent with the above characteristics of WAS. The main domestication process was as follows: Raw sludge (900 mL) was added to 1.0-L serum bottles, and the bottles were flushed with nitrogen gas for 10 min to remove oxygen. Next, all bottles were capped with rubber stoppers and sealed. Subsequently, SD was cultured in an air-bath shaker at 35 °C ± 1 °C with a stirring speed of 200 revolutions per minute (RPM). The SD in all bottles was then supplemented with FW (4 ± 0.1 g) every 48 h. The acclimation process was ended when methane production in the bottle was stable. The main characteristics of SD after acclimation were as follows: pH = 7.0 ± 0.2; TS = 68 ± 4.6 g/L; and VS 42.5 ± 2.3 g/L. The methanogens community main involved *Methanosarcina* and *Methanosaeta* (Wu et al., 2019; Liu et al., 2019c; Huang et al., 2019a). Finally, the SD was washed three times using ultrapure water to remove soluble organic matter before use.

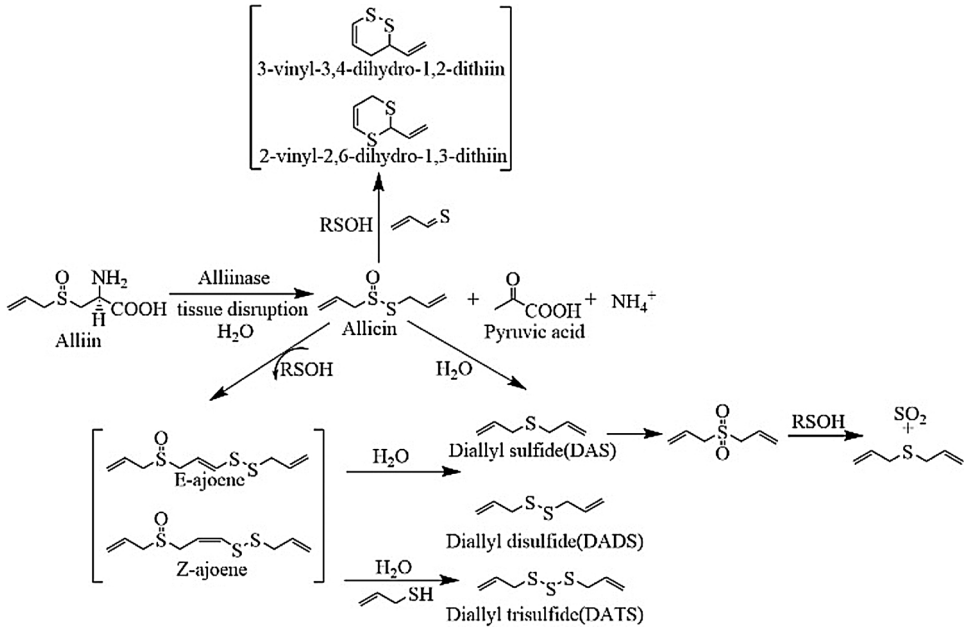

Fig. 1. Alliinase catalyze alliin to produce allicin and its degradation production.

2.2. Batch experiment for methane production from anaerobic co-digestion of FW and WAS in the presence of different dosage of thiosulfinates

Batch experiments were carried out in four identical serum bottles. The initial effective working volume of each bottle was 1000 mL. The purpose of the batch experiments was to assess the effect of thiosulfinates on methane production during the anaerobic co-digestion of FW and WAS. WAS, FW and thiosulfinates were added into the serum bottles and mixed. The initial characteristics of each reactor are shown in Table 2. Based on previous studies, the mass ratio of FW to WAS to SD in this work was 3:5:1 (Luo et al., 2018; Zhao et al., 2017). The total volume of the fermentation liquor was brought to 500 ml using ultrapure water. The design and mark method of amount dosage of thiosulfinates in this work is shown in Table 1. Meanwhile, thiosulfinates became into FW after pretreatment usually. Thus, this work set the mixture test group which simultaneous contained thiosulfinates of different pretreatment method so that closer to its real situation in FW. All reactors were flushed with nitrogen gas for 5 min to remove oxygen and guarantee anaerobic culturing conditions. Subsequently, all bottles were capped with rubber stoppers, sealed, and cultured in an air-bath shaker (35 °C ± 1 °C) at a stirring speed of 150 RPM for 15 d. The pH of each reactor during the experimental period was adjusted to 7.0 ± 0.2 with NaOH or HCl solution (4 M). All reactors were again flushed with nitrogen for 2 min after each sampling to maintain anaerobic conditions. The entire anaerobic co-digestion experiment lasted for 15 d to

Table 2
Effects of thiosulfinate addition on degradation rate of model compounds from co-digestion system on 3d[a].

Group	BSA (%)	Dextran (%)	Glucose (%)	Acetate (%)
CK	16.1 ± 0.7	27.1 ± 1.8	40.9 ± 2.3	51.2 ± 2.6
G1(F)	16.5 ± 0.9	27.8 ± 1.9	40.5 ± 2.1	49.5 ± 2.3
G2(F)	18.2 ± 0.6	30.5 ± 1.7	39.1 ± 1.8	42. 7 ± 2.4
G3(F)	26.6 ± 1.3	43.9 ± 2.1	31.5 ± 2.2	8.5 ± 0.1
G1(H)	16.2 ± 1.1	27.3 ± 1.9	40.8 ± 1.9	50.2 ± 1.2
G2(H)	16.7 ± 1.2	28.1 ± 2.0	40.3 ± 2.0	46.1 ± 1.6
G3(H)	19.3 ± 0.8	32.1 ± 2.1	38.1 ± 2.2	25.7 ± 0.8
G1(M)	19.5 ± 0.7	32.6 ± 2.2	37.8 ± 1.9	25.1 ± 1.3
G2(M)	20.7 ± 0.9	34.5 ± 2.2	36.7 ± 2.1	22.3 ± 1.1

[a] Results are the averages and their standard deviations of triplicate tests.

Table 1
The main characteristics of each test group at initial [a,b].

Parameter	CK	G1(F)	G1(H)	G1(M)	G2(F)	G2(H)	G2(M)	G3(F)	G3(H)
pH	7.0 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.9 ± 0.2	7.0 ± 0.2	7.0 ± 0.2	7.0 ± 0.2	6.9 ± 0.2	6.9 ± 0.2
TSS (g/L)	34.1 ± 2.3	34.2 ± 2.0	34.2 ± 2.4	34.3 ± 2.9	34.3 ± 3.8	34.3 ± 2.9	34.4 ± 3.1	34.4 ± 2.8	34.4 ± 3.8
VSS (g/L)	25.9 ± 1.1	26.0 ± 1.1	26.0 ± 1.2	26.3 ± 1.2	26.2 ± 1.3	26.2 ± 1.2	26.0 ± 1.5	26.3 ± 1.9	26.6 ± 1.9
TCOD (g/L)	115.7 ± 6.8	115.8 ± 6.2	115.8 ± 6.2	115.9 ± 7.4	115.8 ± 8.5	115.9 ± 7.6	116.1 ± 6.6	116.0 ± 7.7	116.2 ± 7.9
Total protein (g COD/L)	10.4 ± 2.0	10.5 ± 2.2	10.3 ± 2.1	11.3 ± 1.9	11.0 ± 1.2	11.2 ± 1.1	11.4 ± 2.1	11.6 ± 1.8	11.5 ± 2.1
Total carbohydrate (g COD/L)	46.1 ± 3.2	46.1 ± 3.7	46.2 ± 3.3	46.3 ± 3.1	46.2 ± 3.2	46.2 ± 3.1	46.3 ± 3.1	46.4 ± 2.9	46.4 ± 3.2
FA/TT [c]	NONE[d]	OF1[e]	OH1[e]	0.04	OF2[e]	OH2[e]	0.2	1	0
WAS/FW/SD	5/3/1								

[a] Results are the averages and their standard deviations of triplicate tests.
[b] The identifications of all experimental groups in this study where consist as shown in this table.
[c] FA: no pre-treatment, TT: total thiosulfinates (1.0 mg/L).
[d] NONE: none allicin added.
[e] OF1: only no pre-treatment allicin 0.1 mg/L, OH1: only heat pre-treatment allicin 0.1 mg/L, OF2: only none pre-treatment allicin 0.5 mg/L, OH2: only heat pre-treatment allicin 0.5 mg/L.

clearly understand the effect of thiosulfinates on methane generation from the anaerobic co-digestion of FW and WAS.

2.3. Assessing the effects of thiosulfinates on each stage of biochemical metabolic from anaerobic co-digestion

A batch experiment including nine identical serum bottles, each with a working volume of 1000 mL, was carried out to explore the effects of thiosulfinates during five different biochemical metabolic stages: hydrolysis, acidification, acetogenesis, homoacetogenesis and methanogenesis(Zhao et al., 2019; Xu et al., 2019). These metabolic processes generally occur together and are not readily differentiated in anaerobic co-digestion system (Zhao et al., 2019). Hence, in this work, simulated substrates were used to analyse the biochemical metabolic process of each stage of anaerobic co-digestion system in the presence of thiosulfinates. The effects of the thiosulfinates on the metabolic process was evaluated by comparing the degradation rates of the fermentation substrate and activities of functional enzymes in anaerobic co-digestion systems containing different levels of thiosulfinate (Wang et al., 2018b).

In the hydrolysis test, because of the complexity of the polysaccharide component of FW, FW was replaced by bovine serum albumin (BSA, a model protein compound) to simplify the chemical analysis. Dextran (9.8 g/L), SD (125 mL), and different levels of thiosulfinates (dosages listed in Table 2) were added to nine reactors. Ultrapure water was then added so that the final volume in all bottles was 500 mL. The pH value of the mixture in all bottles was adjusted to 7.0 ± 0.2 with NaOH or HCl solution (4 M). All reactors were flushed with nitrogen gas for 5 min to remove oxygen. All bottles were then capped with rubber stoppers, sealed then remain in an air-bath shaker (35 ± 1 ℃) at stirring speed of 150 RPM and sustained 5 day. Each group was sampled at a specific time to analysis TCOD, volatile fatty acids (VFAs), SCOD, total protein, methane production and the activities of key enzymes.

The acidification test was performed in the same way as the hydrolysis test except that the main organic loading (FW) in co-digestion system was replaced with anhydrous glucose (a model monosaccharide compound) at a concentration of 1547.2 mg COD/L.

The acetogenesis test was performed in the same way as the hydrolysis test except that the main organic loading (FW) in the co-digestion system was replaced by n-butyric acid (11 g/L).

The homoacetogenesis test assessed the effect of thiosulfinates on hydrogen consumption. Hydrogen is an important component in methane production. Methane can be produced from H_2 and CO_2 via homoacetogenesis (Liu and Chen, 2018; Wang et al., 2019a). The homoacetogenesis test was carried out using the same approach as the hydrolysis test. However, all reactors were flushed with mixed gas (hydrogen, carbon dioxide, and nitrogen in a ratio of 4:1:5) for 10 min to ensure the presence of abundant hydrogen in the anaerobic fermentation system.

The methanogenesis test was performed in the same way as the hydrolysis test except that the main organic loading (FW) in the co-digestion system was replaced by sodium acetate.

2.4. Analytic methods

Throughout all experiments, SCOD, total protein, allicin concentration, total carbohydrate, and VFA content in the liquid phase along with hydrogen and methane concentrations in the gas phase were measured each day.

The total gas volume was determined by releasing the pressure in the pedestal fermentation tank to equilibrate the tank pressure with the room pressure according to the method reported in Ref. (Oh et al. (2003)), and different yield of gas was used different volume bottle (100–500 ml) collection that it aimed to reduce the error in measure process. The cumulative of hydrogen and methane gas were calculated by the following equation (Wang et al., 2015).

$$V_{a,b} = V_{a,b-1} + V_{n,b} \times C_{n,b} - V_{n,b-1} \times C_{n,b-1} \quad (1)$$

where, $V_{a,b}$ mean the cumulative volumes of hydrogen gas in sampling day, $V_{a,b-1}$ mean the volume of collecting at before the day, $V_{n,b}$ and $V_{n,b-1}$ means total gas volume in the current and previous time intervals, $C_{n,b}$ and $C_{n,b-1}$ means respectively concentration of gas production detection by gas chromatography in the current and previous time intervals.

TSS and VSS were determined using the weight loss method (Wu et al., 2019), while pH was measured using a PHS-3C digital acidity metre (Zhao et al., 2018). Total protein and carbohydrate were determined using the Lowry-Folin method with BSA as the standard and the phenol-sulfuric acid method with glucose as the standard, respectively (Huang et al., 2019b; Chen et al., 2018). VFAs were determined using the method described in Ref. (Zhao et al. (2016)), and total VFAs were calculated as the sum of acetic, propionic, n-butyric, iso-butyric, n-valeric and iso-valeric acids. The methane and hydrogen fractions in the generated gas were determined using gas chromatography (GC112A, China) with the parameters used in previous studies (Wang et al., 2015; Wu et al., 2018).

The relative activities of functional enzymes (acetyl-CoA, butyryl-CoA, OAATC, F420, and CoM) involved in the biochemical metabolism of microorganisms during anaerobic co-digestion were determined using previously reported methods (Mu and Chen, 2011; Wang et al., 2018c; Li et al., 2015). Thiosulfinates were detected by high-performance liquid chromatography, as previously reported (Bocchini et al., 2001; Leontiev et al., 2018), and the detail of analytical conditions was shown in Text S1.

3. Results and discussion

3.1. Effect of different initial levels of thiosulfinates on methane yield from anaerobic co-digestion of WAS and FW

Fig. 2 shows the methane yield as a function of time for anaerobic co-digestion in the presence of different initial thiosulfinate concentrations. The methane yield of the reactor without thiosulfinates increased regularly with fermentation time from day 3 to 25; no significant increase in yield was observed between days 25 and 30 d ($p < 0.05$), in agreement with previous findings (Zhao et al., 2017). The lowest methane yield in this study was observe in reactor G3(F) with an initial thiosulfinate concentration of 2.5 μg/g VSS. In this reactor, almost no methane was detected before day 21, and the maximum total methane production was only 16.72 ± 7.0 ml/g VSS, 6.17% that of the control group. And the test of mark as G1(F) which was under same pretreatment as G3(F) and the initial concentration of thiosulfinates as 10% of G3(F) showed that the yield of methane was 157.3 ± 11.6 ml/g VSS, it was decreased by 41.9% that of control group, as shown in Fig. 2(A). Cumulative methane yield was inhibited with increasing thiosulfinate concentration, similar to the effect observed in other reactors with different pre-treatments, including heat treatment and mixing. For heat pre-treatment, the methane yield decreased from 249.4 ± 10.5 to 59.5 ± 10.3 ml/g VSS as the initial concentration of thiosulfinates increased from 0.05 to 2.5 μg/g VSS. Further chemical statistical analyses demonstrated that the maximal methane production was correlated with the initial thiosulfinate concentration in the anaerobic co-digestion system ($R^2 = 0.9005$), as shown in Fig. 2. The presence of allicin and its degradation products DAS, DADS and DATS inhibited methane production in the anaerobic co-digestion system. As shown in Fig. S2, the cumulative methane yields were only 138.3 ± 16.6, 127.7 ± 15.3, and 117.2 ± 14.1 ml/g VSS when the concentrations of DAS, DADS and DATS in the reactors were 0.25 μg/g, respectively. The methane yields from the reactors containing the lowest dosages (0.25 μg/g VSS) of allicin, DAS, DADS and DATS were only 58.3%, 51.2%, 47.3%, and 43.4% that of the reactor without any

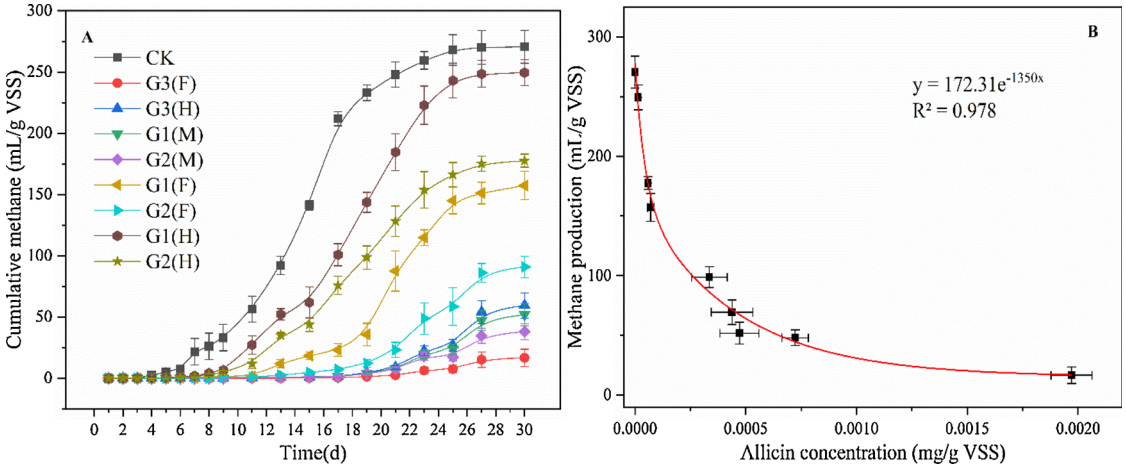

Fig. 2. Accumulated hydrogen production of anaerobic co-digestion system exists different conditions of thiosulfinate (A). The correlation between the different initial concentration of allicin (classical thiosulfinate in *Allium*) in fermentation substrate and the maximal methane production (B). Error bars represent standard deviation of triplicate tests.

thiosulfinates. These results demonstrate that the presence of thiosulfinates in anaerobic co-digestion systems can affect methane yield, and the effects are likely related to the biochemical properties of thiosulfinates during the metabolic processes of microorganisms. Therefore, we explored the effects of thiosulfinates on different microorganism metabolic stages during anaerobic co-digestion in the following.

3.2. Impacting of thiosulfinates on different metabolic stages during anaerobic co-digestion

Anaerobic co-digestion can be broken down into five stages. Although methane production occurs during the final stage (Wang et al., 2018a), the methane yield also depends on the first four stages. Thus, the methane yield is affected by the composition and yield of products in the first four steps (Qin et al., 2019). Therefore, this work evaluated the effect of thiosulfinates on each stage of the anaerobic co-digestion of FW and WAS.

The FW used in this study mainly contained carbohydrate, cellulose and protein, which consisted in the fermentation substrate as a granular state (Zhao et al., 2017). The effect of thiosulfinates on the solubilisation of substrates in the co-digestion system was attributed to the changes in soluble proteins and carbohydrates in the fermentation liquid in this study (Wang et al., 2018a). As shown in Fig. 3, the carbohydrate and protein contents in the fermentation liquid first increased and then decreased with fermentation time in all experimental groups. The maximum values of soluble carbohydrate and soluble protein in the control group were 11,976.9 ± 731.9 and 1693.4 ± 87.2 mg COD/L, respectively. These values were much greater in the systems with thiosulfinates in the fermentation liquid. The maximum concentrations of carbohydrate and soluble protein increased from 13,227.7 ± 892.7 and 1854.8 ± 135.2 mg COD/L to 19,703.1 ± 702.1 and 2795.1 ± 108.9 mg COD/L as the thiosulfinate dosage increased from 0.25 to 2.5 μg/g VSS, respectively. These results indicate that the release of organic substrates was enhanced by the presence of thiosulfinates.

Table 2, which shows the degradation rates of BSA, dextran, glucose, and sodium acetate, indicates that thiosulfinates facilitated BSA and dextran degradation. The experimental degradation rates of BSA and dextran were consistent with the results shown in Fig. 3 and showed that the degradation of organic substrates was facilitated by the presence of thiosulfinates. For example, the degradation rates of BSA and dextran in reactor G3(F), which contained the highest level of thiosulfinates (2.5 μg/g VSS), were enhanced by 65.2% and 62.0% compared to the control, respectively (Table 2). However, the opposite trend was observed for glucose and acetate degradation. The glucose degradation rates for content thiosulfinates group that it was 40.5%, 39.1%, and 31.5%, which was less than no addition group 0.9%, 4.4%, and 23%, respectively. Meanwhile, the acetate degradation rate was 49.5%, 42.7%, and 8.5% which was less than control group by 3%,

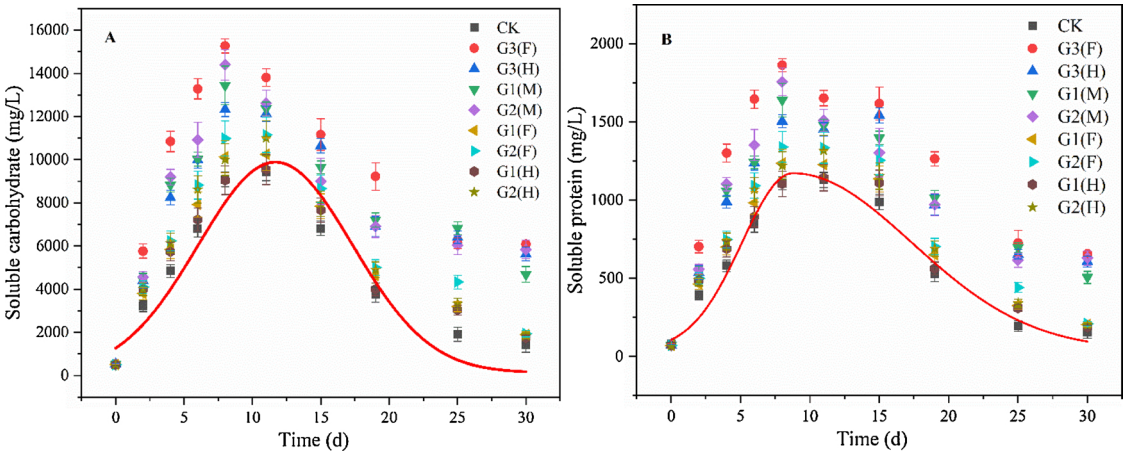

Fig. 3. Effect of thiosulfinate on the alteration of soluble carbohydrate (A) and protein (B) during whole experimental time. Error bars represent standard deviation of triplicate tests.

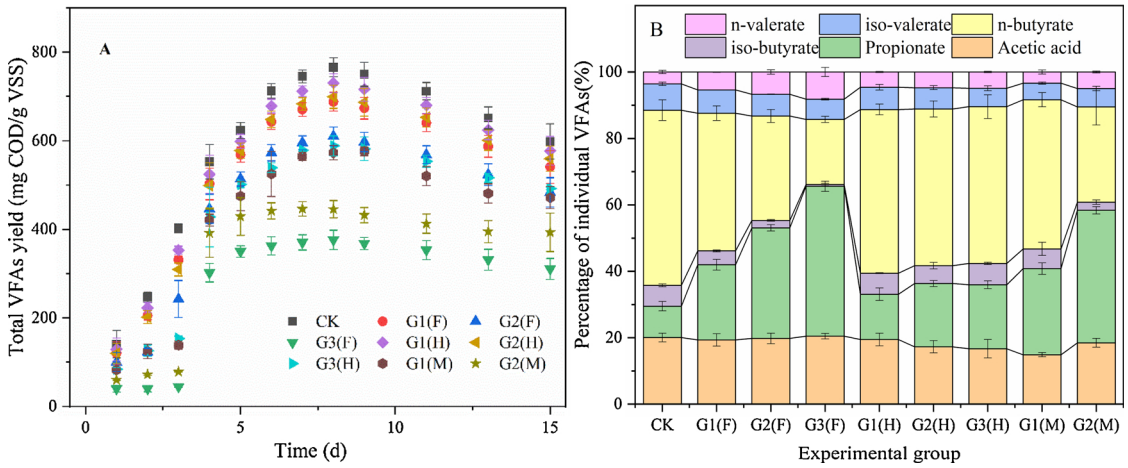

Fig. 4. Cumulative VFA production in each reactor during whole experimental period (A) and percentage of specific VFAs in each reactor (B). Error bar represents standard deviation of triplicate tests.

16.6%, and 83.4%, respectively.

In the anaerobic co-digestion of FW and WAS, VFAs was key intermediate products of methane generation (Wang et al., 2015). Fig. 4 shows the effects of different thiosulfinates concentrations on VFAs production. The decrease in VFA yield with fermentation time was greater in the reactors containing thiosulfinates compared to the control group. The VFA yield was affected to repress most when the thiosulfinates had not pre-treatment into anaerobic co-digestion system, for example group G3(F). Fig. 4(B) shows the different VFAs (acetic, propionic, iso-butyric, n-butyric, iso-valeric, and n-valeric acids) detected in each reactor. Acetic, butyric and propionic acids were the primary VFAs detected in the reactors containing thiosulfinates. Acetic, propionic, iso-butyric, and n-butyric acids may be formed directly from the fermentation of carbohydrates and proteins; in contrast, VFAs with higher molecular weights (e.g., valeric acids) are largely produced via protein digestion (Qin et al., 2019). In the FW used in this work, the carbohydrate content was higher than the protein content far away (Table 1). Thus, few valeric acids were produced in all reactors. Propionic and butyric acids are easily biodegraded to acetic acid under anaerobic conditions (Liu and Chen, 2018), as reflected by the results of this study. Notably, the proportion of propionic acid out of total VFAs increased from 9.4% to 45.1% as the thiosulfinate concentration increased from 0 to 2.5 μg/g VSS, while the proportion of butyrate acids (including iso-butyrate and n-butyrate) decreased from 59% to 20.1%. Meanwhile, the ramification of thiosulfinates (e.g. DAS, DADS, DATS) inhibited VFAs production as well (Fig. S2). These organic sulfides were mainly the primary metabolites of allicin, which inhibited VFA production even more strongly. In this study, DAS, DADS and DATS at the concentration of 1.0 mg/l decreased VFA yield from 598.5 ± 39.9 mg/g (control) to 251.8 ± 19.0, 117.7 ± 11.0, and 133.3 ± 21.6 mg/g VSS, respectively. Furthermore, COD balance analysis was showed in Table S3. It was detected the ratio of influent COD of the control and G3(F) reactors converted to methane by 61.1% and 0.06%. The results of chemical analysis showed the effects of thiosulfinates in methanogenesis stage on anaerobic co-digestion system that it appeared difference with the results of solubilisation stage.

3.3. Cellular integrity and functional enzyme activity during anaerobic co-digestion

According to previous studies, the structural integrity of digestive cells can be evaluated based on the evaluation of LDH and DNA in the supernatant of the fermentation reactor (Wang et al., 2018c). Higher LDH and DNA concentrations in the supernatant indicate a smaller number of intact cells with metabolic functions. Meanwhile, the produce of methane in the methanogenesis stage is primarily restricted by NADH, acetyl-CoA, F_{420}, and CoM (Liu and Chen, 2018; Wang et al., 2018c). NADH is an important compound in bacterial metabolism during anaerobic co-digestion that it joined almost all anaerobic fermentation metabolic processes of microorganisms (Zhang et al., 2019). Acetyl-CoA is a reference enzyme indicative of the acetic acid production ability of digestion bacteria; it is also a key precursor of methanogenesis (Qin et al. (2019); Wang et al. (2019b)). F_{420} and CoM are functional enzymes involved in methane production (Wang et al., 2018c). Thus, we estimated the effects of thiosulfinates on methane production during methanogenesis based on the relative activities of the above enzymes.

As shown in Fig. 5(A), significantly more LDH and DNA was released in the reactors containing thiosulfinates compared to the control. The LDH released in reactors G3(F) and G3(H) were 28% and 19.3% greater than in the control reactor, while the released DNA was enhanced by 21.7% and 16.3%, respectively. Because the detection time of functional enzymes was the day when thiosulfinates cannot be detected in fermentation liquor of all reactors. Thus, these experimental phenomena of functional enzymes possible explained the experimental results that the methanogens disabled the ability to restore methane production in a short period of the time that after the allicin entirely degradation in this study. Meanwhile, the degradation products of allicin after heat treatment also inhibited methane production during anaerobic co-digestion.

Fig. 5(B) shows the relative activities of the functional enzymes involved in methane production during the anaerobic co-digestion of FW and WAS. The experimental results demonstrated the relative activity of acetyl-CoA and NADH were inhibited by no pretreatment thiosulfinates during anaerobic co-digestion of FW and WAS. Nevertheless, the phenomenon of inhibition for acetyl-CoA activity was not signification when thiosulfinates after heat pretreatment, even appeared promotion in G1 group. Meanwhile, the relative activity of NADH was significantly inhibited in all reactors containing 2.5 μg/g VSS of thiosulfinates, regardless of pretreatment. The relative activities of acetyl-CoA and NADH were only 60.5% and 66.9% of those of the control group, respectively, when the thiosulfinates dosage was 2.5 μg/g VSS without pre-treatment. There was a highlight phenomenon worth more notable that relative activity of F_{420} and CoM was extremely inhibited in all reactors, that them were hardly detected during the experimental period. The strongest relative activity of F_{420} and CoM were still only 3.5% and 1% of control group from all test group, and the detection time was the day that allicin cannot be detected. This may be because the degradation products of allicin competed with F_{420} for electrons and directly reacted with the sulfhydryl groups of CoM (Foyer

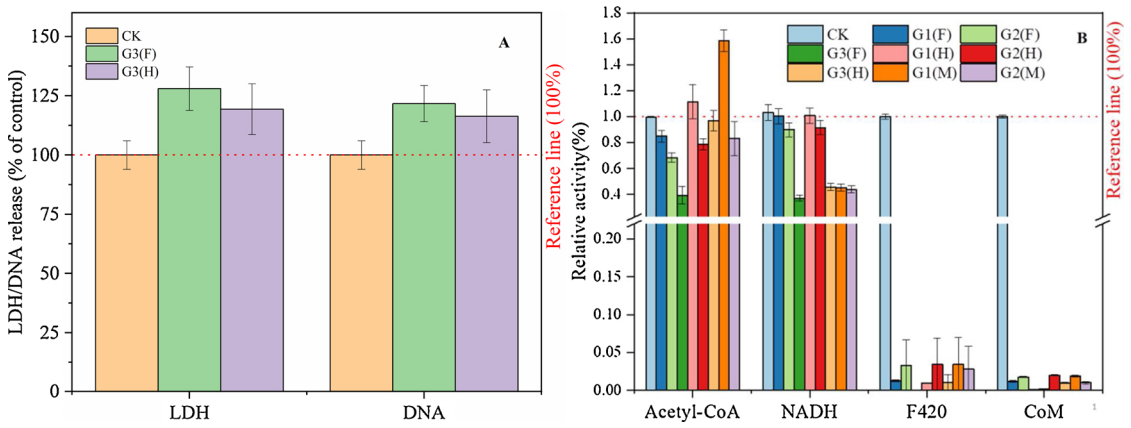

Fig. 5. Release of DNA&LDH in anaerobic co-digestion system under different pretreatment with addition same level dosage of thiosulfinate (2.5 μg/g VSS) (A), and relative activities of functional enzymes in all reactors (B). The detection time was after allicin degradation entirely. Error bar represents standard deviation of triplicate tests.

et al., 2002). Thus, the functional enzymes involved in methane production were inactivated by thiosulfinates, resulting in the reduction in methane generation.

Allicin can quickly penetrate into the cell membrane and bind to thiol-proteins in microorganisms, thereby inhibited metabolism. However, the inhibitory effects of the degradation products of allicin (DAS, DADS, and DATS) on the metabolism of digestion cells were stronger than that of allicin (Putnik et al., 2019; Rose et al., 2018). Thus, the relative activities of F_{420} and CoM were still observably inhibited by allicin in the groups subjected to heat treatment, as shown in Fig. 5(B).

3.4. Summary of the effects of thiosulfinates on anaerobic co-digestion

Fig. 6 shows the overall mechanism by which thiosulfinates inhibit methane production during anaerobic co-digestion. The mechanism is based on two primary observations. First, thiosulfinates decreased the relative activities of functional enzymes involved in methane metabolism. Allicin contains a pair of coupled sulfur atoms, which constitute a reactive group (-S (O) - S -), and thus exhibits broad-spectrum bacteriostatic function (Borlinghaus et al., 2014). The reactive group can bind sulfhydryl groups in several key coenzymes, leading to the inactivation of metabolic function (Oh et al., 2016). These coenzymes (NADH, F_{420}, and CoM) are pivotal catalysts in microbial metabolism for methane production (Li et al., 2019). Thus, methane production is entirely inhibited when the biochemical activities of functional enzymes is inhibited by thiosulfinates during anaerobic co-digestion. Those biochemical characters of thiosulfinates probably explained that the yield of methane almost could not detect during the experimental period when reactor of anaerobic co-digestion in presence of thiosulfinates at launch time. Second, thiosulfinates can inhibit spore germination and mycelia growth and even lead to cell dormancy or death in certain fungi (Viswanathan et al., 2014; Yu et al., 2010a). The release of LDH and DNA was enhanced by the presence of thiosulfinates in anaerobic co-digestion reactors, which mean the amount of digestion cell that still existed biochemical functional is reduced (described in chapter 3.3). Based on previous research for the effects of thiosulfinate on cell structure completely of microorganism, it was shown that allicin causes

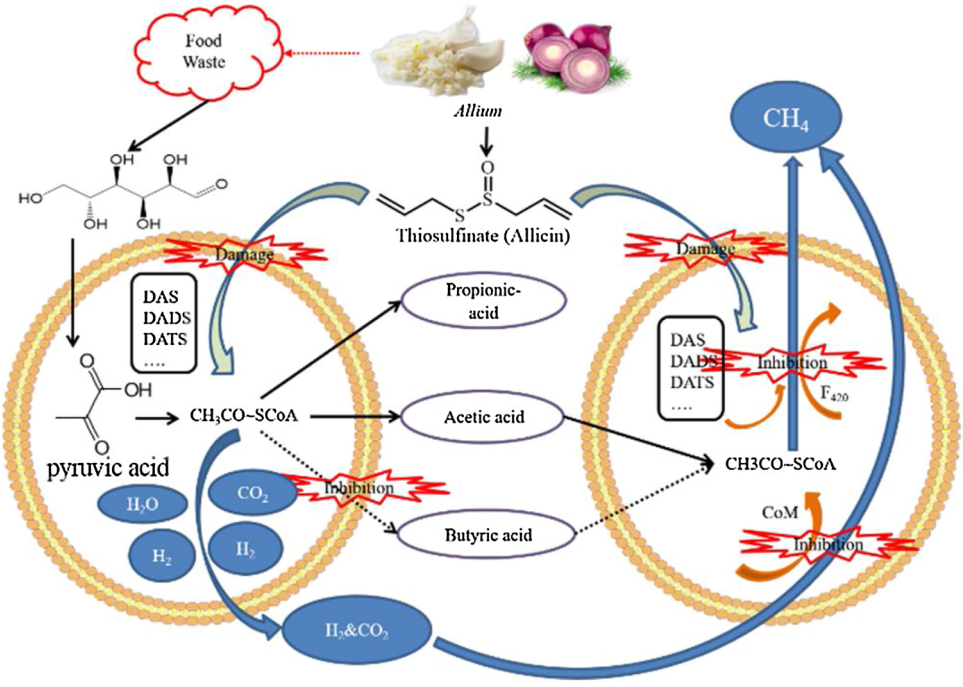

Fig. 6. The effects of thiosulfinates on the metabolic pathways for methane production during anaerobic co-digestion process.

Table 3
The test group setting for pre-treatment experiment.

Group label	Component	Pre-treatment
CK	1.0 mg TG [a] +75 ml FW +125 ml WAS +25 ml SD + ultrapure water [b]	None
H		Heat 100 ℃
A		Alkali pH = 9
M		Heat (100 ℃) + Alkali (pH = 9)

[a] Total thiosulfinates.
[b] Replenished ultrapure water to 300 ml.

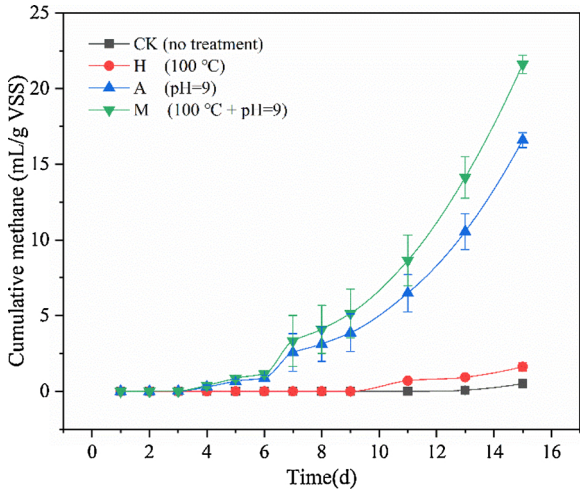

Fig. 7. Accumulative methane production in each different pre-treats reactor. Error bars represent the standard deviation of triplicate tests.

an oxidation of glutathione that results in a shift of the cellular redox-potential to a range correlating with the induction of apoptosis (Schafer and Buettner, 2001). Meanwhile, using some biochemical analysis methods had confirmed that allicin pushes microorganism cells into apoptosis via the "oxidative route" (Yu et al., 2010b).

3.5. Pretreatment reduces the inhibitory effects of thiosulfinates on methane generation during the anaerobic co-digestion of FW and WAS

As discussed in Section 3.1, thiosulfinates inhibited methane production during the anaerobic co-digestion of FW and WAS. Here, the effects of different pre-treatment methods on the inhibitory effect of thiosulfinates on methane generation were evaluated. The disability of biochemical activities of allicin may be caused by synthetic pathway blocked, reactive group damaged or physical loss of compounds (Chen et al., 2017). As many researchers had shown that temperature, time and alkali are the main factors involved in the catabolism of organic compounds (Liu et al., 2019c). Thus, based on the biochemical characteristics of allicin and its derivatives, two pre-treatment methods were selected: heat treatment and alkali treatment. These pre-treatment methods are expected to damage the structural stability of allicin (Méndez Lagunas and Castaigne, 2008). The experimental parameters are listed in Table 3.

As shown in Fig. 7, the methane production was restored to various degrees after pre-treatment compared with control group without treatment. Fig. 7 shows the cumulative methane production is increased by 125.6%, 198.4%, and 238.1% on the 15th day that compared the test group (H, A, M) with control group under same dosage of thiosulfinates. The chemical analysis showed that the optimal pre-treatment method was a combination of heat and alkali pretreatment. Using this optimal pre-treatment method, methane generation was restored to 21.6 ± 0.6 ml/g VSS. This experimental result was similar to batch experiment which was shown in Section 2.2. The possible reason was allicin that it treated with heat and alkali to produce ramification (DAS, DADS and DATS), and these ramifications also inhibit methane production from anaerobic co-digestion in this work. Furthermore, the methane production from anaerobic co-digestion of FW and WAS is increased from 0.5 ± 0.2 to 128.6 ± 3.5 ml/g VSS when thiosulfinates is pretreated by temperature augment from 0 to 200 ℃, as shown in Fig. S4(A). Meanwhile, the alkali treatment test is appeared similar experimental phenomenon with pH value increased, as shown in Fig. S4(B). These two kinds of pretreatment method could restore methane yield at 90% of no thiosulfinates group in this work, as Fig. S4 described. Based experimental results, heat (200 ℃) and alkali (pH = 20) treatment seems thoroughly damage chemical construct of thiosulfinates leading to its inhibition ability almost disable on methane production. These results indicate that the inhibitory effects of allicin and its derivatives were reduced by heat and alkali treatment. Alkali treatment had a greater effect than heat treatment, and the combination of heat and alkali treatments was the most effective method.

4. Conclusion

This study is demonstrated that thiosulfinates, as a natural broad-spectrum antibiotic, inhibit methane production during anaerobic co-digestion in a dose-dependent manner, and it was diffusely presences in FW. The derivatives of allicin (DAS, DADS, and DATS) exhibited similar inhibitory effects to allicin. Thiosulfinates and its derivatives significantly reduced the production of butyric acid and hydrogen from glucose during the acidification stage (butyrate acid and acetic acid production) of anaerobic co-digestion and caused the fermentation to switch to propionic acid fermentation. Meanwhile, thiosulfinates greatly inhibited the relative activities of functional enzyme (F_{420} and CoM) involved in the methanogenesis stage of methane production. Thiosulfinates also damaged the cell membrane structure and induced apoptosis. Heat- and alkali-based pre-treatment was found to mitigate the inhibition of methane generation by thiosulfinates. With the tremendous amount of FW generated worldwide, this work provides new insights for improving methane production during anaerobic co-digestion as a strategy to reduce pollution.

Acknowledgements

This work was received completely support by the project of the National Natural Science Foundation of China (NSFC) (No. 51478170, 51779088, 51779089) and Hunan Provincial Science and Technology Department, China (No. 2017SK2352, 2017WK2091, 2017SK2420).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.121363.

References

Bocchini, P., Andalò, C., Pozzi, R., Galletti, G.C., Antonelli, A., 2001. Determination of diallyl thiosulfinate (allicin) in garlic (Allium sativum L.) by high-performance liquid chromatography with a post-column photochemical reactor. Anal. Chim. Acta 441, 37–43.
Borlinghaus, J., Albrecht, F., Gruhlke, M.C., Nwachukwu, I.D., Slusarenko, A.J., 2014. Allicin: chemistry and biological properties. Molecules 19, 12591–12618.
Braguglia, C.M., Gallipoli, A., Gianico, A., Pagliaccia, P., 2018. Anaerobic bioconversion of food waste into energy: a critical review. Bioresour. Technol. 248, 37–56.
Chen, X., Yuan, H., Zou, D., Liu, Y., Zhu, B., Chufo, A., Jaffar, M., Li, X., 2015. Improving biomethane yield by controlling fermentation type of acidogenic phase in two-phase anaerobic co-digestion of food waste and rice straw. Chem. Eng. J. 273, 254–260.
Chen, Z., Xu, M., Wang, C., Zhou, H., Fan, L., Huang, X., 2017. Thermolysis kinetics and thermal degradation compounds of alliin. Food Chem. 223, 25–30.
Chen, Y., Wu, Y., Wang, D., Li, H., Wang, Q., Liu, Y., Peng, L., Yang, Q., Li, X., Zeng, G., Chen, Y., 2018. Understanding the mechanisms of how poly aluminium chloride inhibits short-chain fatty acids production from anaerobic fermentation of waste activated sludge. Chem. Eng. J. 334, 1351–1360.
FAOSTAT-FAO, 2017. FAOSTAT-FAO.

Focke, M., Feld, A., Lichtenthaler, K., 1990. Allicin, a naturally occurring antibiotic from garlic, specifically inhibits acetyl-CoA synthetase. FEBS Lett. 261, 2.
Foyer, C.H., Noctor, G., Vanacker, H., Gomez, L., 2002. Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. J. Exp. Bot. 53, 1283–1304.
Fratianni, F., Riccardi, R., Spigno, P., Ombra, M.N., Cozzolino, A., Tremonte, P., Coppola, R., Nazzaro, F., 2016. Biochemical characterization and antimicrobial and antifungal activity of two endemic varieties of garlic (Allium sativum L.) of the Campania Region, Southern Italy. J. Med. Food 19, 686–691.
Gruhlke, M.C., Portz, D., Stitz, M., Anwar, A., Schneider, T., Jacob, C., Schlaich, N.L., Slusarenko, A.J., 2010. Allicin disrupts the cell's electrochemical potential and induces apoptosis in yeast. Free Radic. Biol. Med. 49, 1916–1924.
Hagos, K., Zong, J., Li, D., Liu, C., Lu, X., 2017. Anaerobic co-digestion process for biogas production: progress, challenges and perspectives. Renew. Sustain. Energy Rev. 76, 1485–1496.
Huang, X., Xu, Q., Wu, Y., Wang, D., Yang, Q., Chen, F., Wu, Y., Pi, Z., Chen, Z., Li, X., Zhong, Q., 2019a. Effect of clarithromycin on the production of volatile fatty acids from waste activated sludge anaerobic fermentation. Bioresour. Technol. 288 121598-121598.
Huang, X., Zhao, J., Xu, Q., Li, X., Wang, D., Yang, Q., Liu, Y., Tao, Z., 2019b. Enhanced volatile fatty acids production from waste activated sludge anaerobic fermentation by adding tofu residue. Bioresour. Technol. 274, 430–438.
Kubec, R., Velíšek, J., Doležal, M., Kubelka, V., 1997. Sulfur-containing volatiles arising by thermal degradation of alliin and deoxyalliin. J. Agric. Food Chem. 45, 3580–3585.
Leontiev, R., Hohaus, N., Jacob, C., Gruhlke, M.C.H., Slusarenko, A.J., 2018. A comparison of the antibacterial and antifungal activities of thiosulfinate analogues of allicin. Sci. Rep. 8, 6763.
Li, X., Chen, Y., Zhao, S., Chen, H., Zheng, X., Luo, J., Liu, Y., 2015. Efficient production of optically pure L-lactic acid from food waste at ambient temperature by regulating key enzyme activity. Water Res. 70, 148–157.
Li, X., Liu, Y., Xu, Q., Liu, X., Huang, X., Yang, J., Wang, D., Wang, Q., Liu, Y., Yang, Q., 2019. Enhanced methane production from waste activated sludge by combining calcium peroxide with ultrasonic: performance, mechanism, and implication. Bioresour. Technol. 279, 108–116.
Lilia, M.-L., Juan, R.-R., David, R.-V., Anabel, L.-O., 2017. Changes in physical properties and relations with allicin degradation during convective drying of garlic. J. Food Meas. Charact. 11, 1227–1232.
Liu, H., Chen, Y., 2018. Enhanced methane production from food waste using cysteine to increase biotransformation of l-monosaccharide, volatile fatty acids, and biohydrogen. Environ. Sci. Technol. 52, 3777–3785.
Liu, X., Xu, Q., Wang, D., Wu, Y., Yang, Q., Liu, Y., Wang, Q., Li, X., Li, H., Zeng, G., Yang, G., 2019a. Unveiling the mechanisms of how cationic polyacrylamide affects short-chain fatty acids accumulation during long-term anaerobic fermentation of waste activated sludge. Water Res. 155, 142–151.
Liu, Y., Feng, K., Li, H., 2019b. Rapid conversion from food waste to electricity by combining anaerobic fermentation and liquid catalytic fuel cell. Appl. Energy 233–234, 395–402.
Liu, X., Xu, Q., Wang, D., Yang, Q., Wu, Y., Li, Y., Fu, Q., Yang, F., Liu, Y., Ni, B.-J., Wang, Q., Li, X., 2019c. Thermal-alkaline pretreatment of polyacrylamide flocculated waste activated sludge: Process optimization and effects on anaerobic digestion and polyacrylamide degradation. Bioresour. Technol. 281, 158–167.
Luo, K., Xie, X., Yang, Q., Chen, F., Zhong, Y., Xie, P., Wang, G., 2018. Multi-hydrolytic enzyme accumulation and microbial community structure of anaerobic co-digestion of food waste and waste-activated sludge. Environ. Technol. 1–10.
Martins, N., Petropoulos, S., Ferreira, I.C., 2016. Chemical composition and bioactive compounds of garlic (Allium sativum L.) as affected by pre- and post-harvest conditions: a review. Food Chem. 211, 41–50.
Méndez Lagunas, L.L., Castaigne, F., 2008. Effect of temperature cycling on allinase activity in garlic. Food Chem. 111, 56–60.
Mu, H., Chen, Y., 2011. Long-term effect of ZnO nanoparticles on waste activated sludge anaerobic digestion. Water Res. 45, 5612–5620.
Oh, S.-E., Van Ginkel, S., Logan, B.E., 2003. The relative effectiveness of pH control and heat treatment for enhancing biohydrogen gas production. Environ. Sci. Technol. 37, 5186–5190.
Oh, D.-G., Jang, Y.K., Woo, J.E., Kim, J.-S., Lee, C.H., 2016. Metabolomics reveals the effect of garlic on antioxidant- and protease-activities during Cheonggukjang (fermented soybean paste) fermentation. Food Res. Int. 82, 86–94.
Putnik, P., Gabrić, D., Roohinejad, S., Barba, F.J., Granato, D., Mallikarjunan, K., Lorenzo, J.M., Bursać Kovačević, D., 2019. An overview of organosulfur compounds from Allium spp.: from processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. Food Chem. 276, 680–691.
Qin, Y., Li, L., Wu, J., Xiao, B., Hojo, T., Kubota, K., Cheng, J., Li, Y.Y., 2019. Co-production of biohydrogen and biomethane from food waste and paper waste via recirculated two-phase anaerobic digestion process: bioenergy yields and metabolic distribution. Bioresour. Technol. 276, 325–334.
Rodrigues, C., Percival, S.S., 2019. Immunomodulatory effects of glutathione, garlic derivatives, and hydrogen sulfide. Nutrients 11.
Rose, P., Moore, P.K., Zhu, Y.Z., 2018. Garlic and gaseous mediators. Trends Pharmacol. Sci. 39, 624–634.
Schafer, F.Q., Buettner, G.R., 2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. 1191–1212.
Viswanathan, V., Phadatare, A.G., Mukne, A., 2014. Antimycobacterial and Antibacterial Activity of Allium sativum Bulbs. Indian J. Pharm. Sci. 76, 256–261.
Wainaina, S., Horvath, I.S., Taherzadeh, M.J., 2018. Biochemicals from food waste and recalcitrant biomass via syngas fermentation: a review. Bioresour. Technol. 248, 113–121.
Wang, D., Zhao, J., Zeng, G., Chen, Y., Bond, P.L., Li, X., 2015. How does poly(hydroxyalkanoate) affect methane production from the anaerobic digestion of waste-activated sludge? Environ. Sci. Technol. 49, 12253–12262.
Wang, D., Liu, B., Liu, X., Xu, Q., Yang, Q., Liu, Y., Zeng, G., Li, X., Ni, B.J., 2018a. How does free ammonia-based sludge pretreatment improve methane production from anaerobic digestion of waste activated sludge. Chemosphere 206, 491–501.
Wang, Y., Zhao, J., Wang, D., Liu, Y., Wang, Q., Ni, B.J., Chen, F., Yang, Q., Li, X., Zeng, G., Yuan, Z., 2018b. Free nitrous acid promotes hydrogen production from dark fermentation of waste activated sludge. Water Res. 145, 113–124.
Wang, D., Duan, Y., Yang, Q., Liu, Y., Ni, B.J., Wang, Q., Zeng, G., Li, X., Yuan, Z., 2018c. Free ammonia enhances dark fermentative hydrogen production from waste activated sludge. Water Res. 133, 272–281.
Wang, Y., Wang, D., Chen, F., Yang, Q., Li, Y., Li, X., Zeng, G., 2019a. Effect of triclocarban on hydrogen production from dark fermentation of waste activated sludge. Bioresour. Technol. 279, 307–316.
Wang, D., He, D., Liu, X., Xu, Q., Yang, Q., Li, X., Liu, Y., Wang, Q., Ni, B.J., Li, H., 2019b. The underlying mechanism of calcium peroxide pretreatment enhancing methane production from anaerobic digestion of waste activated sludge. Water Res. 164, 114934.
Wills, E.D., 1956. Enzyme inhibition by allicin, the active principle of garlic. Biochem. J. 63, 514–520.
Wu, J., Yang, Q., Luo, W., Sun, J., Xu, Q., Chen, F., Zhao, J., Yi, K., Wang, X., Wang, D., Li, X., Zeng, G., 2018. Role of free nitrous acid in the pretreatment of waste activated sludge: extracellular polymeric substances disruption or cells lysis? Chem. Eng. J. 336, 28–37.
Wu, Y., Wang, D., Liu, X., Xu, Q., Chen, Y., Yang, Q., Li, H., Ni, B., 2019. Effect of poly aluminum chloride on dark fermentative hydrogen accumulation from waste activated sludge. Water Res. 153, 217–228.
Xu, F., Li, Y., Ge, X., Yang, L., Li, Y., 2018. Anaerobic digestion of food waste – Challenges and opportunities. Bioresour. Technol. 247, 1047–1058.
Xu, Q., Liu, X., Wang, D., Liu, Y., Wang, Q., Ni, B.-J., Li, X., Yang, Q., Li, H., 2019. Enhanced short-chain fatty acids production from waste activated sludge by sophorolipid: performance, mechanism, and implication. Bioresour. Technol. 284, 456–465.
Yu, L., Guo, N., Meng, R., Liu, B., Tang, X., Jin, J., Cui, Y., Deng, X., 2010a. Allicin-induced global gene expression profile of Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol. 88, 219–229.
Yu, L., Guo, N., Meng, R., Liu, B., Tang, X., Jin, J., Cui, Y., Deng, X., 2010b. Allicin-induced global gene expression profile of Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol. 88, 219–229.
Zhang, C., Su, H., Baeyens, J., Tan, T., 2014. Reviewing the anaerobic digestion of food waste for biogas production. Renew. Sustain. Energy Rev. 38, 383–392.
Zhang, M., Zhang, Y., Li, Z., Zhang, C., Tan, X., Liu, X., Wan, C., Yang, X., Lee, D.J., 2019. Anaerobic co-digestion of food waste/excess sludge: substrates – products transformation and role of NADH as an indicator. J. Environ. Manage. 232, 197–206.
Zhao, J., Zhang, C., Wang, D., Li, X., An, H., Xie, T., Chen, F., Xu, Q., Sun, Y., Zeng, G., Yang, Q., 2016. Revealing the underlying mechanisms of how sodium chloride affects short-chain fatty acid production from the cofermentation of waste activated sludge and food waste. ACS Sustain. Chem. Eng. 4, 4675–4684.
Zhao, J., Liu, Y., Wang, D., Chen, F., Li, X., Zeng, G., Yang, Q., 2017. Potential impact of salinity on methane production from food waste anaerobic digestion. Waste Manag. 67, 308–314.
Zhao, J., Liu, Y., Wang, Y., Lian, Y., Wang, Q., Yang, Q., Wang, D., Xie, G.-J., Zeng, G., Sun, Y., Li, X., Ni, B.-J., 2018. Clarifying the role of free ammonia in the production of short-chain fatty acids from waste activated sludge anaerobic fermentation. ACS Sustain. Chem. Eng. 6, 14104–14113.
Zhao, J., Jing, Y., Zhang, J., Sun, Y., Wang, Y., Wang, H., Bi, X., 2019. Aged refuse enhances anaerobic fermentation of food waste to produce short-chain fatty acids. Bioresour. Technol. 289, 121547.
Zhou, M., Yan, B., Wong, J.W.C., Zhang, Y., 2018. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: a mini-review focusing on acidogenic metabolic pathways. Bioresour. Technol. 248, 68–78.