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Enhanced Methane Production from Sludge Anaerobic Digestion with the Addition of Potassium Permanganate

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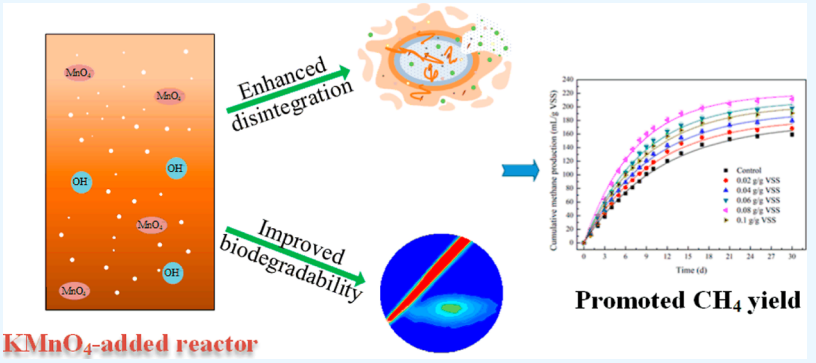

ABSTRACT: This work aims to reveal the effect of potassium permanganate ($KMnO_4$) on the sludge anaerobic digestion process, as well as the relevant mechanisms. Experimental data showed that the biomethane production was gradually increased from 159.3 ± 3.0 to 211.5 ± 5.1 mL/g VSS (volatile suspended solids) when the $KMnO_4$ content was increased from 0 to 0.08 g/g VSS, with an increasing rate of 32.8%. A further increase in the $KMnO_4$ dosage however resulted in the decline of the methane yield. First-order kinetic model analysis indicated that higher methane production potentials and hydrolysis rates were achieved in $KMnO_4$-added reactors than in the control. Mechanism analysis demonstrated that $KMnO_4$ not only efficiently disintegrated the sludge flocs, which resulted in the increased contents of dissolved organics, but also enhanced the proportion of biodegradable substances in the sludge liquor. Meanwhile, the biodegradabilities of recalcitrant humus and lignocellulose substances were found to be promoted by $KMnO_4$ treatment as higher methane yields were attained from $KMnO_4$-treated model substrates. 16S rRNA analysis illustrated that the functional microbes participated in anaerobic digestion were largely enriched in the $KMnO_4$-pretreated digestor. Furthermore, efficient inactivation of the fecal coliform was achieved by $KMnO_4$ pretreatment.

INTRODUCTION

As the main byproduct of sewage treatment plants (STPs), waste activated sludge (WAS) is hugely generated in daily operations. The total production of WAS in China in 2020 was estimated to be 60 million tons (80% water content), which posed great challenges to the STP operators.[1,2] Anaerobic digestion is a promising WAS treatment method as it possesses the abilities to simultaneously realize sludge minimization, stabilization, and resource recycle.[3,4] In WAS anaerobic digestion, the abundantly present organic substances are degraded, accompanied by the generation of methane-contained biogas, which means the concept of "waste to energy" is well-realized.[5,6] Sludge anaerobic digestion efficiency however is usually very low, which hinders its extensive applications in practical engineering.[7]

Disintegration is the first step of sludge anaerobic digestion, during which the organic substances in the solid phase are dissolved into sludge liquor.[8] For the existing extracellular polymeric substances (EPSs) and microbial cytoderm/cytomembrane, sludge disintegration is considered to be the rate-limiting step that restricts the overall reaction rate, and the pretreatment process is needed to facilitate the slow step for ultimately increasing the sludge anaerobic digestion performance.[9] In the last decade, chemical oxidation method has been widely adopted in sludge pretreatment due to the advantages of excellent treatment performance, high efficiency, low investment cost, and easy operation. The strong oxidants ozone (O_3), hydrogen peroxide (H_2O_2), potassium ferrate (K_2FeO_4), and calcium peroxide (CaO_2) are all effective for sludge pretreatment, but the high costs prevent their further applications.[10,11]

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Potassium permanganate ($KMnO_4$) is a commonly used oxidant in water and wastewater treatment.[12,13] For instance, it was found that the organic pollutant triclosan in water was efficiently degraded by 70−80% after 2.0 mg/L $KMnO_4$ treatment for 10 min[14] More recently, the applications of $KMnO_4$ in sludge treatment have raised increasing concerns. Wu et al.[15] adopted $KMnO_4$ to disintegrate sludge flocs and found that the SCOD (soluble chemical oxygen demand) content was promoted by 3473% after 500 mg/L $KMnO_4$ treatment. The research of Zheng et al.[16] also showed that the SCOD value was efficiently promoted by $KMnO_4$ treatment at 0.05 to 0.3 g/g total suspended solids (TSS) dosage range. Li et al.[17] adopted Na_2SO_3 coupled with $KMnO_4$ for sludge treatment and found that the SCOD of solo $KMnO_4$ and Na_2SO_3 + $KMnO_4$ treated samples were, respectively, 9.5-fold and 13.2-fold to that of the control, which means that the sludge disintegration extent was largely enhanced by both methods. The promoted disintegration by $KMnO_4$ pretreatment is undoubtedly beneficial to sludge anaerobic fermentation or digestion. Xu et al.[18] found that the sludge volatile suspended solids (VSS) content was largely reduced, while the SCOD content was increased by $KMnO_4$ pretreatment, causing a 7.69 times increase of the volatile fatty acids (VFAs) yield in the subsequent fermentation process. Based on the above discussion, we speculated that the methane generation from sludge anaerobic digestion could be enhanced when pretreated with $KMnO_4$ because a better disintegration effect and more digestion substrates were expected. This issue however has not been investigated to date.

The purpose of the work is therefore to find out the impact of $KMnO_4$ on the sludge anaerobic digestion system and explore the relevant mechanisms. The biomethane productions from the sludge in the presence of $KMnO_4$ at the concentration gradients of 0, 0.02, 0.04, 0.06, 0.08, and 0.1 g/g VSS were first investigated. Then, mechanism studies were conducted from the perspectives of EPS destruction, microbial cells leakage, biodegradability of sludge liquor, degradation of model substrates, and abundance of functional microbes. Finally, the inactivation of pathogens after anaerobic digestion by $KMnO_4$ treatment was studied. Compared with the previous literature, this work revealed the correlation between biomethane production and $KMnO_4$ dosages for the first time. For mechanism analysis, the previous studies always only focus on sludge disintegration as well as the generation of biodegradable organic substances. This work not only investigated the variation of disintegration efficiency by $KMnO_4$ treatment but also assessed the impacts of $KMnO_4$ on the biodegradability of recalcitrant organics. Furthermore, the conversion of digestion substrates to methane undergoes several biochemical processes, and how does $KMnO_4$ affect these processes has never been studied till now. In this work, the effects of $KMnO_4$ on these processes were evaluated using model substrates, by which the reaction kinetics of each process were revealed.

MATERIALS AND METHODS

WAS and $KMnO_4$. The WAS obtained from the Bailonggang STP (Shanghai, China) was first filtered using a 1.5 × 1.5 mm sieve before adopted for anaerobic digestion, whose primary characteristics are summarized in Table S1 (Supporting Information). $KMnO_4$ (99% purity) was provided by Aladdin company in China.

Batch Methane Producing Experiment. This experiment was conducted in six serum bottles (V = 500 mL). Each one first received 150 mL of raw WAS. Then, these bottles were, respectively, added with 0, 0.0395, 0.079, 0.1186, 0.1581, and 0.1976 g of $KMnO_4$ to attain the $KMnO_4$ dosage gradients of 0, 0.02, 0.04, 0.06, 0.08, and 0.1 g/g VSS. After being mixed for 30 min using a magnetic stirrer (600 rpm), each bottle received 150 mL of inoculum, which was obtained from a pilot-scale sludge anaerobic digestor. Finally, all bottles were aerated with nitrogen for 5 min and immediately sealed for anaerobic digestion under a 35 °C environment in a shaker (150 rpm). The biogas production and methane content were, respectively, measured during anaerobic digestion, till the cumulative methane yield was not significantly enhanced after 30 days.

Assessing the Impacts of $KMnO_4$ on Sludge Disintegration and Biodegradability of Dissolved Organics. To reveal the impacts of $KMnO_4$ on sludge disintegration, the VSS, SCOD, soluble protein, and carbohydrate of sludge samples pretreated with different $KMnO_4$ dosages were detected. EPSs grab most organics in sludge, including lightly and tightly bound statuses that are named as LB-EPS and TB-EPS, respectively.[19] The two types of EPSs in the sludge with or without the pretreatment were then extracted using a heat method,[20] and the specific procedures are expounded in Text S1. Beside EPSs, microbial cells also contain abundant organic substrates. The damage of sludge microbes with the $KMnO_4$ treatment was reflected by DNA and lactate dehydrogenase (LDH) releases, which are both intracellular substances and could be liberated into sludge liquor when the microbial cells were broken. Furthermore, the live/dead cells photos of sludge with or without the pretreatment were captured using a fluorescence microscope (Nikon Eclipse 80i, Japan), from which the percentages of live or dead cells can both be calculated according to the procedures demonstrated in Text S2.

Besides biodegradable substrates, the sludge also contains abundant nonbiodegradable organics that are difficultly utilized by anaerobes. The excitation emission matrix (EEM) fluorescence technology was adopted to analyze the biodegradability of dissolved organics with or without the $KMnO_4$ treatment. In brief, each EEM spectrum contains five regions (Region I to V), among which Regions I and IV, respectively, refer to tyrosine- and polysaccharide-like substances, which are easily degraded by microbes, while Regions II, III, and V, respectively, represent tryptophan-, fulvic acid-, and humic acid-like substrates, which are difficult to biodegrade. The percent fluorescence response ($P_{i,n}$) values were used to assess the relative abundance of each region, as conducted in the literature.[21]

Assessing the Effects of $KMnO_4$ on the Biodegradability of Humus and Lignocellulose. As shown in Table S1, there are many recalcitrant organics detected in raw WAS, including humic acid, fulvic acid, lignin, cellulose, and hemicellulose. These substances are all difficultly utilized by the functional anaerobes. Their biodegradability however could be enhanced when pretreated by $KMnO_4$ because the structure and property could be varied in the $KMnO_4$ oxidization process. This experiment was conducted to evaluate whether $KMnO_4$ had some impacts on the biodegradability of recalcitrant organics using model substrates, including five groups (Groups I to V), with three serum bottles (V = 500 mL) serving as reactors in each group.

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Group I: First, each bottle received 150 mL of synthetic wastewater with humic acid serving as the only component, and the content of 1348 mg/L was in accordance with that measured in WAS. Then, 0, 0.079 or 0.1581 g of $KMnO_4$ was dosed into the three reactors to obtain the concentration gradients of 0, 0.04, and 0.08 g/g VSS. After being mixed for 30 min at 600 rpm, each reactor received 150 mL of inoculum and was then sealed for anaerobic digestion based on the procedures illustrated in the batch methane producing experiment. During 5 days of anaerobic digestion, the methane productions in different reactors were daily detected. The impacts of $KMnO_4$ on humic acid degradability were revealed through the variations of the methane yield with different $KMnO_4$ dosages.

Group II: All the operations were consistent with those performed in Group I, apart from 972 mg/L fulvic acid being substituted for humic acid as the model substrate.

Group III: This group was treated in accordance with the treatment in Group I, except that humic acid was substituted by 1035 mg/L lignin alkali.

Group IV: The experiment was conducted using the same procedures as those described in Group I, except that 1277 mg/L carboxymethylcellulose sodium was adopted as the solo model substrate.

Group V: All operations were the same as those conducted in Group I, except that 1148 mg/L xylan replaced humic acid to be the digestion substrate.

Analytical Methods. The contents of COD, TSS, and VSS were measured according to the standard methods.[22] Determinations of carbohydrate and protein were conducted using previously established methods.[23,24] EEM spectra were measured using a fluorescence spectrometer (Hitachi F-7000, Japan). DNA release after the $KMnO_4$ pretreatment was measured according to the literature,[25] and LDH contents were determined using the Cytotoxicity LDH Assay Kit-WST (Dojindo Laboratories, Japan) according to the manufacturer's instruction. Humus substrates were measured by the same method as that in the literature,[21] while lignocellulosic substrates were detected based on the previously reported method.[26] Methane contents in biogas were detected using a gas chromatograph (Agilent GC7890, USA). The impacts of $KMnO_4$ on the sludge methane production potential and hydrolysis rate were revealed through the first-order kinetic model,[27] with the operations detailed in Text S4. The impacts of $KMnO_4$ on the hydrolysis, acidogenesis, acetogenesis, and methanogenesis processes were revealed through several batch experiments using bovine serum albumin (BSA), glucose, butyrate, hydrogen, and acetate as the model substrate, respectively (Text S5). The structures of the microbial community were detected using the Illumina Miseq sequencing technology, with 515FmodF(5′-GTGYCAGCMGCCGCGGTAA-3′) and 806RmodR(5′-GGACTACNVGGGTWTCTAAT-3′) adopted as the primers, and the raw sequencing data were divided into many operational taxonomic units (OTUs) based on 97% similarity. The fecal coliform in different reactors after anaerobic digestion was enumerated using the Colilert-18 Test kit (IDEXX, USA), with the most probable number (MPN) being adopted as the unit (Text S6).

RESULTS AND DISCUSSION

Effects of $KMnO_4$ at Different Dosages on the Methane Yield from Sludge. Figure 1 depicts the cumulative methane productions from digestors pretreated by different doses of $KMnO_4$. It was observed that there was no significant increase in methane production from these reactors after 30 d of digestion ($P > 0.05$), indicating that the anaerobic reactions were completed and the maximum methane yields were realized. However, the specific productions from different reactors varied a lot. As the $KMnO_4$ content was increased from 0 to 0.08 g/g VSS, the cumulative methane generation was markedly enhanced from 159.3 ± 3.0 to 211.5 ± 5.1 mL/g VSS ($P < 0.01$), showing an increasing rate of 32.8%. When the $KMnO_4$ content was further increased to 0.1 g/g VSS, the cumulative methane production reduced to 190.8 ± 4.8 mL/g VSS, significantly lower than 211.5 ± 5.1 mL/g VSS, which was obtained from the 0.08 g/g VSS $KMnO_4$ reactor ($P < 0.05$). This may be attributed to the fact that the residual $KMnO_4$ exerted some negative effects on the inoculated methanogens, which are very sensitive to the change of the environment and could be severely disturbed at a high $KMnO_4$ dosage of 0.1 g/g VSS. Based on the above analysis, the biomethane yield was closely related to $KMnO_4$ dosages, which exhibited an increase followed by a decrease trend with the $KMnO_4$ content increasing from 0 to 0.1 g/g VSS, and the optimal $KMnO_4$ dosage for methane generation was 0.08 g/g VSS.

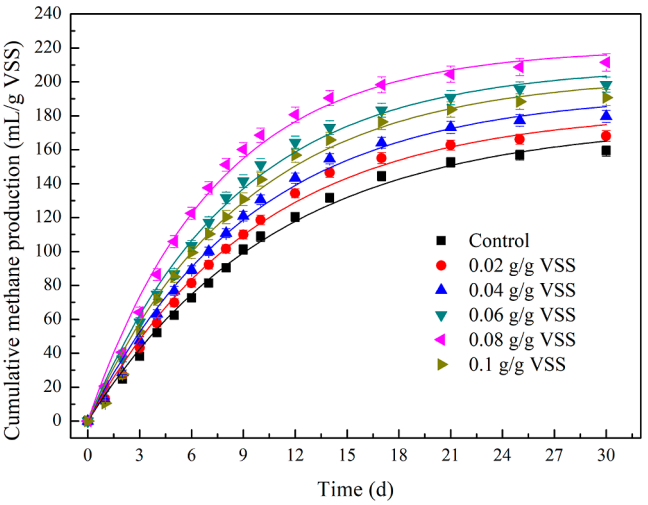

Figure 1. Cumulative methane production from the reactors with different $KMnO_4$ dosages. Symbols and lines refer to experimental data and the first-order kinetic model fit, respectively.

To more deeply understand how $KMnO_4$ affects sludge methane generation, the experimental results were then simulated using the first-order kinetic model (Figure 1). According to the simulative results presented in Table 1, the model captured well the experimental data as the R^2 values in all reactors surpassed 99%. Moreover, the parameters B_0 and k,

Table 1. Simulated Parameters of the First-Order Kinetic Model at Different $KMnO_4$ Dosages

$KMnO_4$ dosage (g/g VSS)	B_0(mL/g VSS)	k(d^{-1})	R^2
0	176.3 ± 3.6	0.092 ± 0.004	0.996
0.02	183.5 ± 3.9	0.101 ± 0.005	0.994
0.04	193.6 ± 3.8	0.105 ± 0.005	0.995
0.06	209.5 ± 3.6	0.118 ± 0.005	0.995
0.08	219.7 ± 3.9	0.136 ± 0.006	0.993
0.1	203.8 ± 4.7	0.112 ± 0.006	0.992

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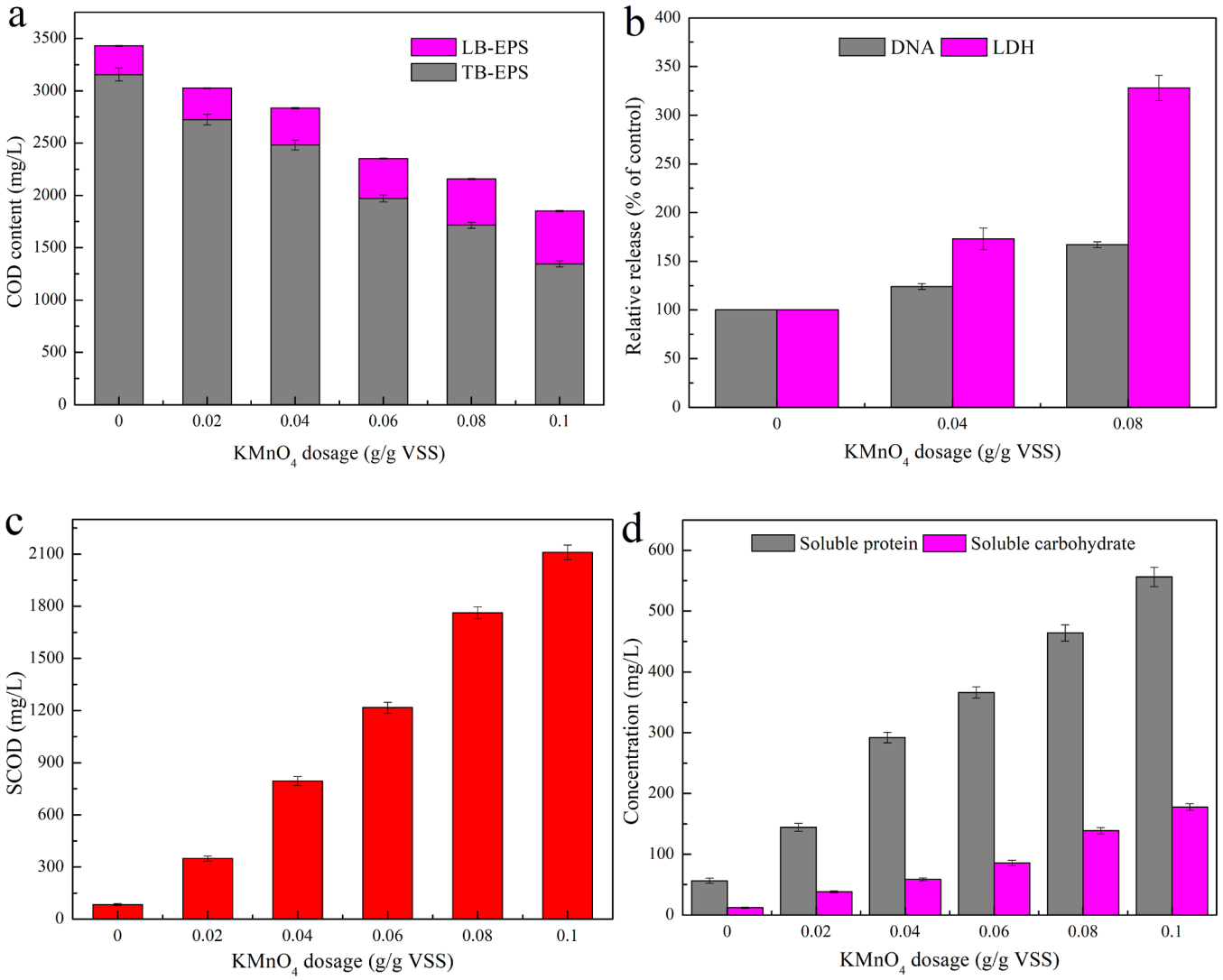

Figure 2. COD content of LB-EPS and TB-EPS, that is, lightly and tightly bound EPS, respectively (a), relative release of DNA and LDH (b), SCOD contents (c), and soluble protein and carbohydrate contents (d) after $KMnO_4$ pretreatment at different dosages.

which, respectively, represent the methane production potential and hydrolysis rate, were both observed to be enhanced after $KMnO_4$ pretreatment. For instance, B_0 was enhanced from 176.3 ± 3.6 to 219.7 ± 3.9 mL/g VSS with the $KMnO_4$ dosage increasing from 0 to 0.08 g/g VSSS, which then decreased to 203.8 ± 4.7 mL/g VSS when the $KMnO_4$ content reached 0.1 g/g VSS. A similar variation trend was perceived in the k value, which was enhanced from 0.092 ± 0.004 to 0.136 ± 0.006 d^{-1} when the $KMnO_4$ content was increased from 0 to 0.08 g/g VSS and then declined to 0.112 ± 0.006 d^{-1} when the $KMnO_4$ dosage reached 0.1 g/g VSS. Though B_0 and k values both showed decreasing tendencies when the $KMnO_4$ content surpassed 0.08 g/g VSS, the data of 203.8 ± 4.7 mL/g VSS and 0.112 ± 0.006 d^{-1} were still notably higher than 176.3 ± 3.6 mL/g VSS and 0.092 ± 0.004 d^{-1} obtained from the control reactor. It is worth noting that the B_0 and k values were both positively linearly correlated with the increased $KMnO_4$ dosages at the range of 0 to 0.08 g/g VSS, and the R^2 values were 97.88 and 96.24%, respectively (Figure S1). The simulative results demonstrated that the sludge methane production potential and hydrolysis rate were both efficiently enhanced when $KMnO_4$ was added.

Effects of $KMnO_4$ on Sludge Disintegration and Biodegradability of Dissolved Organic Matters. The destruction of the sludge EPS by $KMnO_4$ treatment was first studied. According to the results presented in Figure 2a, the COD content of TB-EPS was gradually decreased from 3155 to 1345 mg/L with the $KMnO_4$ content enhancing from 0 to 0.1 g/g VSS, while the COD of LB-EPS was simultaneously promoted from 275 to 506 mg/L, suggesting that the sludge EPS was clearly disturbed when $KMnO_4$ was added. The total contents of TB-EPS and LB-EPS were calculated to be decreased from 3430 mg/L in the control to 3026, 2834, 2352, 2157, and 1851 mg/L by 0.02, 0.04, 0.06, 0.08, and 0.1 g/g VSS $KMnO_4$ pretreatment, respectively, suggesting that a considerable of organics in the EPS were liberated into sludge liquor when treated by $KMnO_4$. The results demonstrated that $KMnO_4$ efficiently destroyed the EPS structure of the sludge, and TB-EPS was partially transformed into LB-EPS and dissolved organics. The damage of microbial cells by $KMnO_4$ pretreatment was then explored. Figure 2b shows the release of DNA and LDH at different $KMnO_4$ dosages. When pretreated by 0.04 g/g VSS $KMnO_4$, the DNA and LDH releases were detected to be 124 and 173%, respectively, which then

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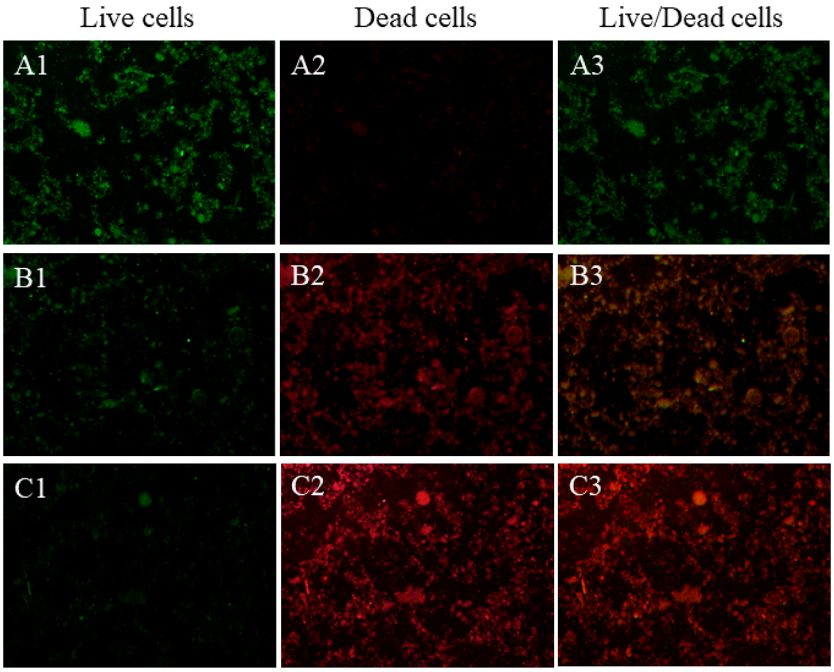

Figure 3. Fluorescence photos of live/dead cells in the sludge that pretreated by 0 g/g VSSS (A1-A3), 0.04 g/g VSSS (B1−B3) and 0.08 g/g VSSS (C1−C3) $KMnO_4$, respectively.

increased to 167 and 328%, respectively, with the $KMnO_4$ dosage increased to 0.08 g/g VSS, confirming that the sludge cells were largely destructed by $KMnO_4$ pretreatment, and higher dosages caused better effects on microbial cell destruction. To more visually display the destruction of sludge microbes, fluorescence photos of microbial cells with or without the $KMnO_4$ pretreatment were captured, as presented in Figure 3. The green fluorescence intensity, which represents live cells, was distinctly weakened with the increase of $KMnO_4$ dosages. Conversely, the red fluorescence intensity, which refers to dead cells, was increased when $KMnO_4$ was added. The percentages of live cells under different conditions were then calculated based on the specific fluorescence intensities, which decreased from 91.7% in the control to 38.6% when treated by 0.04 g/g VSS $KMnO_4$ and further reduced to 13.3% as $KMnO_4$ content reached 0.08 g/g VSS (Figure S2). The results above indicated that both EPS and sludge cells were effectively destroyed by $KMnO_4$, which means that efficient sludge disintegration was achieved.

Table 2 shows the sludge particle size distribution under different conditions, in which the values of Dx(90), Dx(50), and Dx(10) were all found to be observably decreased after $KMnO_4$ pretreatment. For example, the Dx(90) value decreased from 101.36 ± 0.09 μm in the control to 94.53 ± 0.08 and 85.11 ± 0.08 μm, respectively, with 0.04 or 0.08 g/g VSS $KMnO_4$ pretreatment, which further confirmed that the sludge flocs were efficiently disintegrated in the $KMnO_4$ treatment process. As shown in eq 1, plenty of OH^- can be generated from the reaction of MnO_4^- and H_2O when $KMnO_4$ appeared in hydrous media. Previous studies reported that OH^- possesses the ability to destroy sludge flocs;[28,29] thus, the abundantly generated OH^- served as a crucial reason for $KMnO_4$ accelerating sludge disintegration. Meanwhile, $KMnO_4$ with strong oxidizability can directly disrupt the sludge flocs structure, which was also a crucial factor for the enhanced disintegration efficiency.

$$MnO_4^- + 2H_2O \rightarrow 4OH^- + MnO_2 \quad (1)$$

Table 2. Distribution of Sludge Particle Size With or Without the $KMnO_4$ Pretreatment

$KMnO_4$ dosages (g/g VSS)	particle size (μm)		
	Dx (10)	Dx (50)	Dx (90)
0	13.76 ± 0.02	38.83 ± 0.04	101.36 ± 0.09
0.04	12.04 ± 0.01	35.47 ± 0.04	94.53 ± 0.08
0.08	9.89 ± 0.01	30.75 ± 0.04	85.11 ± 0.08

Figure 2c depicts the changes of SCOD concentrations when pretreated by $KMnO_4$. With $KMnO_4$ content increasing from 0 to 0.1 g/g VSS, the sludge SCOD was clearly increased from 84 to 2110 mg/L, indicating that more dissolved organic substances were generated from the disintegration process by $KMnO_4$ pretreatment. The variations of soluble protein and carbohydrate concentrations by the $KMnO_4$ treatment were also studied, which were, respectively, increased from 56.5 and 11.9 mg/L to 556.2 and 177.7 mg/L with the $KMnO_4$ dosage increasing from 0 to 0.1 g/g VSS (Figure 2d). According to the above results, many biodegradable organics in the sludge were released into the liquid phase upon treatment with $KMnO_4$, providing more digestion substrates for methane production.

The variations of sludge liquor biodegradability by $KMnO_4$ pretreatment were then investigated using the EEM fluorescence technology (Figure 4). As seen from the EEM spectra, the percent fluorescence responses of Regions II, III, and V ($P_{II,n}$, $P_{III,n}$, and $P_{V,n}$) were all distinctly reduced when pretreated by $KMnO_4$, especially under the 0.08 g/g VSSS condition, illustrating that the proportion of non-biodegradable substances in dissolved organics was largely reduced. In contrast, the $P_{IV,n}$ value, which represents the easily biodegraded polysaccharide, was observably enhanced from 54.04% in the control to 62.65% and 71.31% after 0.04 and

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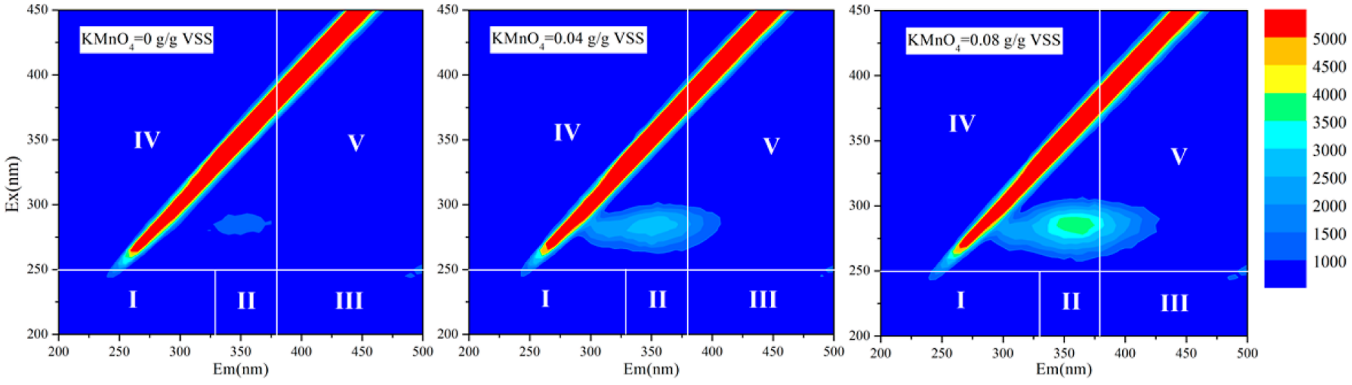

Region	$P_{i,n}$(%)		
	$KMnO_4$=0 g/g VSS	$KMnO_4$=0.04 g/g VSS	$KMnO_4$=0.08 g/g VSS
I	10.46	8.03	6.28
II	7.52	6.15	4.77
III	9.81	7.24	5.19
IV	54.04	62.65	71.31
V	18.17	15.93	12.45

Figure 4. EEM spectra and percentage of fluorescence response ($P_{i,n}$) of sludge liquor in the presence of $KMnO_4$ at different dosages.

0.08 g/g VSS $KMnO_4$ treatment, respectively. The experimental results above demonstrated that $KMnO_4$ efficiently promoted the biodegradability of soluble organics as the proportion of biodegradable organics was largely enhanced after $KMnO_4$ pretreatment.

As shown in Table S1, the non-biodegradable humus and lignocellulose were largely detected in the raw sludge. Several batch experiments using model substances were performed to further understand the promoted biodegradability of the sludge when $KMnO_4$ was added. Figure 5 shows the methane generation from different model substances at different $KMnO_4$ dosages, in which the daily methane yields of $KMnO_4$-pretreated samples were generally higher than that from 0 g/g VSS $KMnO_4$ reactors. For instance, the methane yield from the 0 g/g VSS $KMnO_4$-pretreated reactor in the humic acid group was only 7.01 mL on the 4th day of digestion, which then increased to, respectively, 8.75 and 10.17 mL in 0.04 and 0.08 g/g VSS $KMnO_4$-pretreated digestors (Figure 5a), indicating that the biodegradability of humic acid was enhanced by $KMnO_4$. Similar observations were found in other substances (Figures 5b−e), which further confirmed the stimulative efficacy of $KMnO_4$ on sludge organics biodegradability.

Effects of $KMnO_4$ on Each Biochemical Process in Sludge Anaerobic Digestion. After the disintegration, the largely produced dissolved organics are adopted for methane generation through several biochemical processes, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis.[30] The effects of $KMnO_4$ on them were reflected by comparing the degradations of model subtracts with or without the pretreatment. The results in Table 3 showed that except for acidogenesis, the other processes were all suppressed when $KMnO_4$ was added because the degradation rates of relevant model substrates were obviously decreased.

Furthermore, Table 4 demonstrates the specific degradation rate (SDR) of each model substrate, which can reflect the impacts of $KMnO_4$ on relevant microbial activities. The SDR of BSA in the control reactor was 26.89 mg/g VSS/h, which was regarded as the original activity of the hydrolytic microbes. When treated with 0.04 g/g VSS $KMnO_4$, the SDR of BSA decreased to 19.52 mg/g VSS/h, suggesting that the activity of hydrolytic microbes was reduced by 27.4%. As $KMnO_4$ content was increased to 0.08 g/g VSS, the activity of hydrolytic microbes was decreased to 13.18 mg/g VSS/h, with a suppression ratio of 51.0%. The activity of acidogenic microbes was not influenced by $KMnO_4$ because the SDR of glucose was not significantly decreased in the presence of $KMnO_4$ at both 0.04 and 0.08 g/g VSS dosages ($P > 0.05$). From the same calculation with hydrolytic microbes, the microbial activities related to acetogenesis, hydrogentrophic methanogenesis, and acetoclastic methanogenesis processes were, respectively, reduced by 25.7, 33.1, and 28.3% when pretreated by 0.04 g/g VSS $KMnO_4$, which were further decreased by, respectively, 39.8, 65.1, and 68.7% when the $KMnO_4$ dosage reached 0.08 g/g VSS. The results demonstrated that $KMnO_4$ exerted no significant impact on acidogenic microbe activity, while markedly suppressing the activities of all other anaerobes, and a relatively higher $KMnO_4$ dosage led to a more serious impact on each kind of anaerobes. This explained the observation from Figure 1 that the methane production was decreased when the $KMnO_4$ content was ultimately increased to 0.1 g/g VSSS, though higher $KMnO_4$ dosages brought about better disintegration effects (Figure 2).

Shifts of the Microbial Community Structure by the $KMnO_4$ Pretreatment. The microbial community structures with or without the addition of $KMnO_4$ were investigated to reveal the microcosmic mechanisms for $KMnO_4$ affecting sludge methane production. The Chao index and Shannon index, which represent the richness and diversity, respectively, were correspondingly reduced from 2754.4 ± 68.1 and 6.18 ± 0.06 in the control to 2421.7 ± 63.4 and 5.73 ± 0.05 in 0.08 g/g VSS $KMnO_4$-pretreated reactor, indicating that the richness and diversity of the microbial community were both decreased by $KMnO_4$ pretreatment (Table S2). In contrast, the microbial

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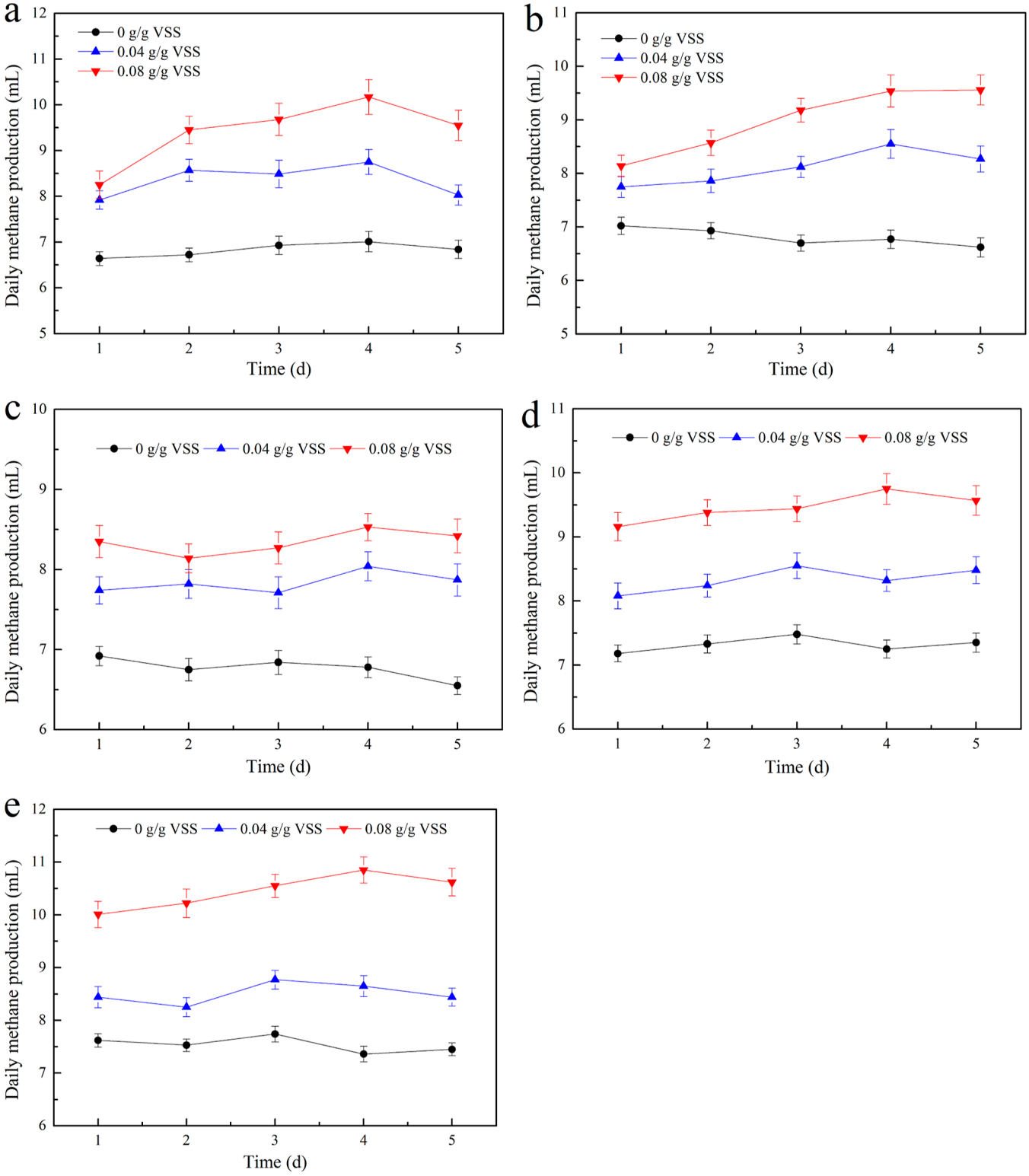

Figure 5. Daily methane production from humic acid (a), fulvic acid (b), lignin (c), cellulose (d), and hemicellulose (e) with or without the $KMnO_4$ pretreatment.

evenness was enhanced in the $KMnO_4$-pretreated reactor because the Simpson index was increased from 0.0055 ± 0.0007 to 0.0121 ± 0.0010 when $KMnO_4$ was added (Table S2). Venn analysis of microbial OTUs between the two reactors with or without the $KMnO_4$ pretreatment was then conducted, as shown in Figure S3. The total OTUs were, respectively, 2149 and 1833 in the control and $KMnO_4$-pretreated reactors, containing 1707 of common OTUs, which further proved the decreased microbial diversity by $KMnO_4$ treatment. The data are in agreement with that observed from Figure 3 as sludge microbes were largely killed when treated with $KMnO_4$.

The microbial community structures in the two reactors at the genus level are depicted in Figure 6. Some species

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Table 3. Effects of $KMnO_4$ at Different Dosages on the Biochemical Processes in Sludge Anaerobic Digestion

time (d)	$KMnO_4$ dosages (g/g VSS)	hydrolysis	acidogenesis	acetogenesis	hydrogenotrophic methonogenesis	acetoclastic methanogenesis
		BSA degradation (%)	glucose degradation (%)	butyrate degradation (%)	hydrogen degradation (%)	acetate degradation (%)
1	0	27.5 ± 2.0	87.5 ± 2.7	22.7 ± 1.8	25.9 ± 1.1	12.2 ± 0.8
	0.04	20.8 ± 1.7	84.7 ± 2.2	16.6 ± 1.4	12.6 ± 0.9	8.2 ± 0.7
	0.08	15.3 ± 1.1	80.4 ± 2.1	12.4 ± 1.1	5.2 ± 0.5	3.3 ± 0.4
3	0	70.4 ± 2.5	96.2 ± 3.0	47.5 ± 2.3	57.4 ± 2.8	42.8 ± 2.2
	0.04	51.1 ± 2.1	96.0 ± 2.8	35.3 ± 2.1	38.4 ± 2.3	30.7 ± 1.5
	0.08	34.5 ± 1.8	95.7 ± 2.8	28.6 ± 1.8	20.1 ± 1.0	13.4 ± 1.2

Table 4. SDR of BSA, Glucose, Butyrate, Hydrogen, and Acetate With the Addition of $KMnO_4$ at Different Dosages[a]

	$KMnO_4$ dosages (g/g VSS)		
substrate	0	0.04	0.08
BSA	26.89 ± 0.95	19.52 ± 0.80	13.18 ± 0.69
glucose	8.40 ± 0.26	8.38 ± 0.24	8.36 ± 0.24
butyrate	10.37 ± 0.50	7.71 ± 0.46	6.24 ± 0.39
hydrogen	0.166 ± 0.008	0.111 ± 0.007	0.058 ± 0.003
acetate	4.67 ± 0.24	3.35 ± 0.16	1.46 ± 0.13

[a]The unit of the SDR is milligrams per gram of VSS per hour.

participating in anaerobic digestion were detected in both the reactors. However, their abundances varied greatly when pretreated by $KMnO_4$. For example, the total abundance of three typical hydrolytic species norank_f__Caldilineaceae, norank_f__Bacteroidetes_vadinHA17, and Leptolinea was increased from 3.81% in the control to 6.14% in the $KMnO_4$-treated digestor. The species Romboutsia, norank_f__Anaerolineaceae, Exilispira, Longilinea, norank_f__Cloacimonadaceae, and unclassifed_f__Anaerolineaceae are all the functional microbes participating in the acidogenesis or acetogenesis process. Among them, Romboutsia was reported to be a VFA producer by using glucose as the substrate,[31] and the abundance was increased from 0.96% to 1.43% by $KMnO_4$ treatment. The species norank_f__Anaerolineaceae and Longilinea can degrade various carbohydrates for VFA production[32] and were, respectively, enriched from the abundances of 1.28% and 0.87 to 1.64% and 1.55% by the $KMnO_4$ pretreatment. The abundance of Exilispira, a species that was reported to be connected with the medium chain fatty acid (MCFA) generation in anaerobic digestion and to possess the ability to degrade recalcitrant dicamba,[33,34] was increased from 0.52 to 1.08% when pretreated by $KMnO_4$. The abundance of norank_f__Cloacimonadaceae, an acetogenic bacterium that can convert propionate into acetic acid,[35,36] was increased from 1.16 to 1.78% after the $KMnO_4$ pretreatment. The species unclassifed_f__Anaerolineaceae can utilize hydrocarbons to produce VFAs,[37] and the abundance was increased from 0.82 to 1.29% by the $KMnO_4$ pretreatment. Further calculation indicated that the total abundances of acidogenic and acetogenic microbes were, respectively, 5.61 and 8.77% in the control and $KMnO_4$-treated reactors. Beyond this, two methanogenic genera Methanosaeta and Methanolinea, which are acetoclastic and hydrogenotrophic methanogens, respectively, were found in the two reactors. As presented in Figure 6, the abundances of Methanosaeta and Methanolinea were, respectively, 0.94 and 0.55% in the control, which were enhanced to, respectively, 1.53 and 1.69% in the $KMnO_4$-treated reactor, suggesting that the two kinds of methanogens were both enriched by the $KMnO_4$ treatment. Beyond this, the abundance of Methanosaeta was higher than that of Methanolinea in the control, which was reversed when treated by $KMnO_4$ (Methanolinea surpassed Methanosaeta), indicating that the major methanogenesis pathway was changed from acetoclastic to hydrogenotrophic after the treatment. In terms of the above analysis, different kinds of functional microbes were all enriched in the $KMnO_4$-pretreated reactor, which was a crucial reason for the promotion of methane generation by the $KMnO_4$ pretreatment (Figure 1).

Effects of $KMnO_4$ on the Removal of the Fecal Coliform. WAS typically contains many pathogens, such as the fecal coliform, and could result in high health risks to humans without proper disposal. $KMnO_4$ was reported to possess the ability to kill various pathogens;[38] thus, the elimination of the fecal coliform by $KMnO_4$ pretreatment was studied in this work, with the results depicted in Figure S4. After anaerobic digestion, the number of fecal coliform bacteria was 6372 MPN/g TSS in the control reactor, which observably reduced to 1138 MPN/g TSS in the presence of $KMnO_4$ at 0.04 g/g VSS. When the $KMnO_4$ content was increased to 0.08 g/g VSS, the fecal coliform was continuously killed to 272 MPN/g TSS, lower than the Class A level (1000 MPN/g TSS) of the EPA protocol. This result demonstrated that the environmental risks of the digested sludge were largely reduced by the $KMnO_4$ treatment.

Overall Understanding and Implication. This study found that the anaerobic digestion performance of the sludge was effectively enhanced by the $KMnO_4$ pretreatment. The methane generation first increased from 159.3 ± 3.0 to 211.5 ± 5.1 mL/g VSS with the $KMnO_4$ content increas from 0 to 0.08 g/g VSS and then declined to 190.8 ± 4.8 mL/g VSS as the $KMnO_4$ content was further increased to 0.1 g/g VSS. The increasing rate of the methane yield was 32.8% by 0.08 g/g VSS $KMnO_4$ treatment, which was observably higher than the values of 22, 25.1, and 10.3% by H_2O_2, K_2FeO_4, and $Ca(ClO)_2$ treatments, respectively, but lower than that by CaO_2 treatment (Table 5). The disintegration efficiency of the sludge was sufficiently facilitated when $KMnO_4$ was added, showing a positive correlation with the increased $KMnO_4$ content in the 0 to 0.1 g/g VSS range. Moreover, the biodegradability of sludge organics was remarkably enhanced when pretreated by $KMnO_4$. On one hand, the proportion of easily biodegraded substances in the liquid phase was increased when $KMnO_4$ was added. On the other hand, the methane production potentials of recalcitrant humus and lignocellulose substances were promoted when pretreated by $KMnO_4$. The biochemical reaction kinetics analysis revealed that except for acidogenic microbes, the activities of hydrolytic, acetogenic, and methanogenic microbes were all suppressed by $KMnO_4$,

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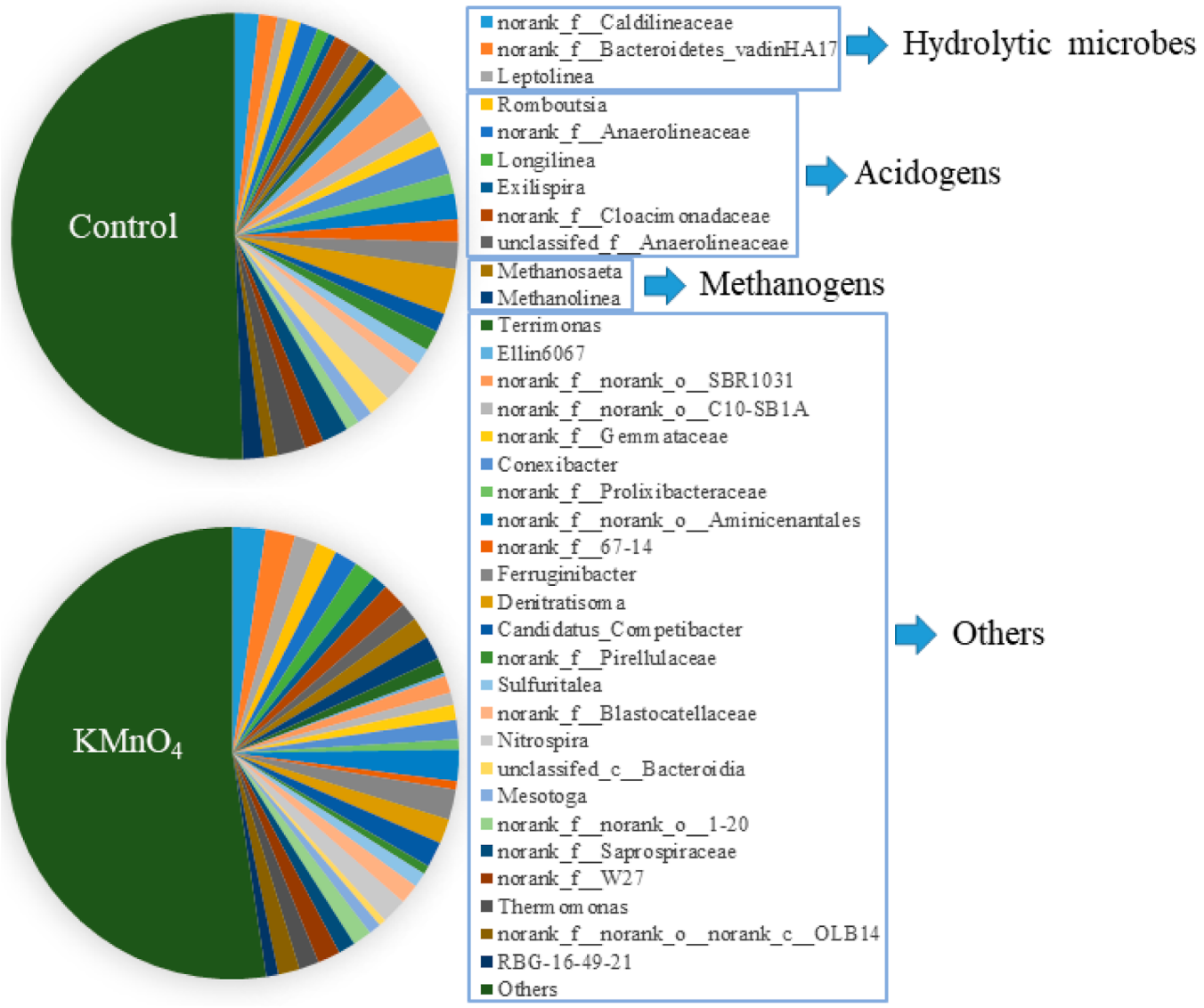

Figure 6. Structures of the microbial community in the control and 0.08 g/g VSS $KMnO_4$-pretreated reactors at the genus level on the 12th day of digestion.

Table 5. Comparison of $KMnO_4$ and Other Chemical Oxidants for Anaerobic Methane Production From Sludge

		methane yield			
oxidant	dosage	raw sludge	treated sludge	increasing rate	reference
H_2O_2	50 mg/g TS			22%	Zhang et al.[39]
K_2FeO_4	56 mg Fe/g TSS	154.28 mL/g VSS	193.03 mL/g VSS	25.1%	He et al.[40]
$Ca(ClO)_2$	0.1 g/g TSS	106.15 mg COD	117.07 mg COD	10.3%	Yu et al.[41]
CaO_2	0.14 g/g VSS	146.3 mL/g VSS	215.9 mL/g VSS	47.6%	Wang et al.[21]
$KMnO_4$	0.08 g/g VSS	159.3 mL/g VSS	211.5 mL/g VSS	32.8%	This study

and a higher dosage caused more severe inhibiting effects. The suppression effect of 0.1 g/g VSS $KMnO_4$ on these biochemical processes neutralized the benefits from the enhanced sludge disintegration and organics biodegradability, which then resulted in the decrease of methane production. Microbial community analysis illustrated that all the hydrolytic, acidogenic, acetogenic, and methanogenic microbes were enriched in the $KMnO_4$-pretreated reactor.

The results herein reported effectively filled the knowledge gaps of $KMnO_4$ affecting the sludge anaerobic digestion system. However, it should be emphasized that the methane production results in this work were obtained only using batch experiments, and semi-continuous or continuous experiments are needed to further verify the optimum $KMnO_4$ dosage and amend other parameters before scaling up this $KMnO_4$-based technology. In addition, to avoid the adverse impact of $KMnO_4$ on methanogens for further increasing the biomethane generation, the two-phase sludge anaerobic digestion process can be taken into consideration. The literature showed that the inhibition of rhamnolipid on methanogens in the methanogenic phase was relieved compared with that in the acidogenic phase,[42] which means that the suppressive effect of $KMnO_4$ on functional microbes could be alleviated with the two-phase sludge anaerobic digestion process applied.

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CONCLUSIONS

The impact of $KMnO_4$ on sludge anaerobic digestion, especially for biomethane generation, was studied in this work. The methane yield first increased and then decreased when the $KMnO_4$ content was increased from 0 to 0.1 g/g VSS, with the maximum value of 211.5 ± 5.1 mLg VSS attained at the $KMnO_4$ content of 0.08 g/g VSS, 1.328 times that of the control. Mechanism investigation revealed that both the disintegration extent and biodegradability of the sludge were promoted by $KMnO_4$. Microbial analysis demonstrated that the structure of the microbial community in the $KMnO_4$-treated digestor was more beneficial to methane generation than the control as the functional microbes (e.g., Methanosaeta and Methanolinea) were observably enriched by the $KMnO_4$ treatment. After anaerobic digestion, the number of fecal coliform bacteria was largely reduced in the $KMnO_4$-pretreated reactor.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c04132.

Analytical methods, linear fittings of B_0 and k values to $KMnO_4$ dosages in the range of 0 to 0.08 g/g VSS, fraction of live cells to total cells in the sludge with different $KMnO_4$ dosages, Venn diagram of sludge microbial OTUs between the control and 0.08 g/g VSS $KMnO_4$-pretreated reactors, number of fecal coliform bacteria in the control and $KMnO_4$-pretreated reactors after 30 d of anaerobic digestion, main characteristics of WAS and inoculum used in this study, and variations of several microbial ecological diversity indicators with the addition of 0.08 g/g VSS $KMnO_4$ (PDF)

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Notes

The authors declare no competing financial interest.

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