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Fermentation liquor of CaO_2 treated chemically enhanced primary sedimentation (CEPS) sludge for bioplastic biosynthesis

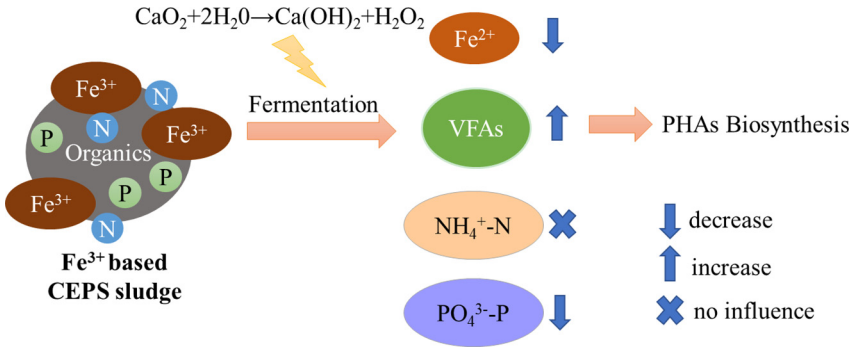
Juan Xu [a,b,*], Xiuyan Li [a,b], Lihong Gan [a], Xiaoyan Li [c,**]

[a] Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, School of Ecological and Environmental Sciences, East China Normal University, Shanghai, China
[b] Institute of Eco-Chongming, East China Normal University, Shanghai, China
[c] Environmental Engineering Research Centre, Department of Civil Engineering, The University of Hong Kong, Pokfulam, Hong Kong, China

HIGHLIGHTS

- Fe^{3+} based CEPS was applied for organics recovery from sewage.
- CaO_2 enhanced VFAs accumulation during fermentation of CEPS sludge.
- The highest VFAs yield of 455.8 mg COD/g VSS was obtained.
- PHAs was biosynthesized using CEPS sludge fermentation liquor as sole substrate.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemically enhanced primary sedimentation (CEPS) technology has been widely applied in Hong Kong, exhibiting excellent performance in contaminants removal from sewage. The generated CEPS sludge contains abundance of organics which could be recovered as volatile fatty acids (VFAs) by fermentation for further utilization. In this work, the effect of calcium peroxide (CaO_2) on the fermentation of $FeCl_3$ based CEPS sludge was investigated. The feasibility of utilizing the fermentation liquor as substrate for polyhydroxyalkanoates (PHAs) biosynthesis was also evaluated. Results demonstrated that CaO_2 addition facilitated the disintegration of CEPS sludge and enhanced VFAs production. The maximum VFAs yield of 455.8 mg COD/g VSS was obtained with the dosage of 0.1 g CaO_2/g SS, improving by 44.7% compared with the control sludge. Acetic and propionic acid were the predominant components of the VFAs. Microbial analysis indicated that CaO_2 induced microbial reduction of Fe(III), accelerating the initial disintegration of $FeCl_3$ based CEPS sludge. Microbial communities with hydrolysis and acidogenesis functions were enriched effectively. CaO_2 treatment had no significant influence on the release of ammonia nitrogen (NH_4^+-N), while reduced the concentration of orthophosphate (PO_4^{3-}-P) and ferrous (Fe^{2+}) in fermentation liquor, that was beneficial to the further utilization as substrate for PHAs biosynthesis. The VFA-rich fermentation liquor was proved to be a suitable substrate for PHAs biosynthesis. After cultivation, the PHAs content in activated sludge reached 22.3%, which was comparable to those obtained using waste materials as carbon source. This integrated technology could be a superior alternative of realizing sludge disposal and bioplastic production simultaneously.

* Correspondence to: J. Xu, Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, School of Ecological and Environmental Sciences, East China Normal University, Shanghai, China.
** Corresponding author.
E-mail addresses: jxu@des.ecnu.edu.cn (J. Xu), xlia@hku.hk (X. Li).

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1. Introduction

Chemically enhanced primary sedimentation (CEPS) refers to the wastewater treatment process by dosing chemicals (such as Fe^{3+} and polymers) for coagulation, flocculation and sedimentation to remove contaminants (Lin and Li, 2018). CEPS exhibits excellent performance in removing suspended solids, organics and nutrients in the wastewater, which are concentrated efficiently in the generated sludge from the water phase (Lin et al., 2018). Hong Kong has the largest CEPS plant in the world, i.e. Stonecutters Island Sewage Treatment Works, treating an average sewage flow of 1.7 million m^3/d by dosing $FeCl_3$ (10–20 mg/L Fe) to form more than 2000 tons/d of dewatered sludge. However, disposal of these CEPS sludge has become one of the most challenging environmental problems.

CEPS sludge contains abundance of organics which are valuable resources needed to be recovered rather than wasted. Fermentation is proved to be an efficient technology for extracting organics in the form of volatile fatty acids (VFAs) from excessive sludge, which are suitable carbon sources for biosynthesis (Huang et al., 2017; Zhang et al., 2018). The sludge fermentation process includes three steps of hydrolysis, acidification and methanogenesis. The VFAs production could be enhanced by accelerating the first two stages and inhibiting the last step (Li et al., 2015).

Calcium peroxide (CaO_2) has the capacity of slowly releasing H_2O_2, $Ca(OH)_2$ and oxygen in hydrous media, being considered as a solid form of H_2O_2 (Northup and Cassidy, 2008). CaO_2 could enhance the quantity and quality of short-chain fatty acids production from waste activated sludge (Li et al., 2015). Compared with the CEPS sludge primarily containing organics, the activated sludge is much more difficult for fermentation since it mainly consists of microbial cells that can be hardly disintegrated. Thus, it is possible that CaO_2 dosage can also enhance VFAs production during the fermentation of CEPS sludge.

Polyhydroxyalkanoates (PHAs) are considered as a superior alternative to traditional plastics due to their properties of biodegradability (Hand et al., 2016). Direct biosynthesis is the most cost-effective method to produce PHAs. VFAs are found to be a highly suitable substrate for PHAs biosynthesis (Verlinden et al., 2007). Currently, PHAs biosynthesis at industrial scale is conducted with pure cultures using define substrates as carbon sources (Salehizadeh and Van Loosdrecht, 2004). Economic evaluation indicates that the production expense of PHAs can be reduced over a half if utilizing renewable waste materials as substrates (Serafim et al., 2004). To reduce the production cost, efforts have been made to produce PHAs from waste materials such as food waste and excess activated sludge (Cai et al., 2009; Salehizadeh and Van Loosdrecht, 2004). However, information about CEPS sludge fermentation liquor for PHAs biosynthesis is rather limited.

In this work, $FeCl_3$ was used as the coagulant for domestic sewage treatment to concentrate organic contaminants into the CEPS sludge. The CEPS sludge was then processed by fermentation to convert the organics to VFAs for the following PHAs biosynthesis. CaO_2 was dosed to improve VFAs generation of CEPS sludge during fermentation. VFAs, soluble chemical oxygen demand (SCOD), sludge destruction and microbial community during fermentation were monitored to investigate the influences of CaO_2 on CEPS sludge fermentation and the properties of the generated fermentation liquor. To elucidate the mechanism of CaO_2 influencing the fermentation process, fermentation experiments dosing $Ca(OH)_2$ and H_2O_2 as additives were also conducted for comparison. The sequencing batch reactor (SBR) for PHAs biosynthesis was operated continuously to estimate the feasibility of utilizing the fermentation liquor of CEPS sludge as substrate. This work aims to develop a promising technology to recover resources from CEPS sludge, achieving sludge disposal together with the production of value-added products.

2. Materials and methods

2.1. CEPS sludge preparation

Raw domestic sewage was taken from the Stanley Sewage Treatment Works in Hong Kong. $FeCl_3$ was dosed into 40 L of sewage, making the final concentration of Fe^{3+} to 20 mg/L in the flocculation tank. The flocculation process was conducted by rapid mixing at 200 rpm for 1 min followed slow stirring at 30 rpm for 15 min. Then after sedimentation for 2 h, the supernatant was withdrawn and the residual sediment was collected as the Fe-based CEPS sludge. The sludge was filtered with a 1 mm × 1 mm screen and settled for 12 h at 4 °C for concentration before utilization. The characteristics of the prepared CEPS sludge were listed in Table 1.

2.2. Batch fermentation of CEPS sludge

500 mL CEPS sludge was filled in 550 mL glass serum bottles for fermentation. CaO_2 was added at 0.02, 0.05, and 0.1 g/g SS respectively in different bottles. Fermentation experiments dosing with H_2O_2 and $Ca(OH)_2$ were also conducted for comparison as H_2O_2 and $Ca(OH)_2$ could be released from CaO_2 during fermentation. Based on the stoichiometric calculation, 0.01, 0.02, 0.05 g H_2O_2, and 0.02, 0.05, 0.1 g $Ca(OH)_2$ would be produced from 0.02, 0.05, 0.1 g CaO_2 (Li et al., 2015). So the amounts of H_2O_2 and $Ca(OH)_2$ were dosed according to stoichiometric calculation. The control experiment was performed without dosing any chemical reagent. After filling with CEPS sludge, nitrogen gas was purged into the bottles to maintain an anaerobic condition. Then, different chemicals were added into the bottles immediately and sealed with rubber covers. The bottles were placed in a temperature-controlled air chamber (37 ± 1 °C) with magnetic stirring for mixing. The batch fermentation was conducted for 16 days and sludge samples were sampled at the designed time.

2.3. Microbial analysis

The CEPS sludge samples were collected from fermentation bottles at the end of the operation time. DNA of these samples was extracted using E.Z.N.A.® Water DNA Kit (OMEGA, USA). The quality and the quantity of DNA were examined by 0.8% agarose gel electrophoresis (DYY-6C, Beijing Liuhe, China) and spectrophotometrically quantified by UV spectrophotometer (NC 2000, Thermo Scientific, USA). For high-throughput sequencing, 16S rRNA genes were amplified using primers with the barcode. Sequencing was completed on Illumina Miseq platform. The resulting high quality sequences were processed to generate operational taxonomic units (OTUs) and allocated down to the kingdom, phylum, class, order, family, and genus level through Mothur program (Zhang et al., 2016).

Table 1
Characteristics of the CEPS sludge.

Parameters	Mean value
pH	6.61 ± 0.02
SS (suspended solid) (mg/L)	5710 ± 14
VSS (volatile suspended solid) (mg/L)	4750 ± 42
COD (chemical oxygen demand) (mg/L)	7000 ± 30
SCOD (soluble chemical oxygen demand) (mg/L)	350 ± 20
VFAs (volatile fatty acids in sludge supernatant) (mg/L)	151.3 ± 10.2
NH_4^+-N (ammonia in sludge supernatant) (mg/L)	17.8 ± 0.5
PO_4^{3-}-P (phosphorus in sludge supernatant) (mg/L)	0.1 ± 0.0
Carbohydrates in sludge supernatant (mg/L)	43.4 ± 0.6
Proteins in sludge supernatant (mg/L)	34.7 ± 0.9
Alkalinity (mg $CaCO_3$/L)	258.3 ± 15.7

2.4. SBR operation for PHAs biosynthesis

The fermentation liquor of 0.1 $gCaO_2$/g SS treated CEPS sludge was used as substrate for PHAs synthesis, diluting with tap water at a ratio of 1:0.6 without pH adjusting. Activated sludge of 5 g/L was inoculated into an aerobic SBR of 1 L. The operation cycle included feeding (15 min), aeration (4.5 h), settling (1 h), and decanting (15 min) of 0.33 L supernatant. In each cycle, the reactor was mixed with the magnetic stirrer except settling and decanting period. The sludge retention time (SRT) was maintained at 10 days. Air was purged into the SBR to maintain the dissolved oxygen (DO) concertation at 6 mg/L. The SBR was operated under the temperature of 21 °C.

2.5. Analytical methods

The sludge samples from the fermentation bottles were immediately centrifuged at 8000 rpm for 15 min to obtain the supernatants for further analysis. The suspended solid (SS), volatile suspended solid (VSS), chemical oxygen demand (COD), SCOD, ammonia (NH_4^+-N), phosphorus (PO_4^{3-}-P), ferrous (Fe^{2+}) and alkalinity were determined according to the standard methods (Federation and Association, 2005). VFAs including acetic acid (HAc), propionic acid (HPr), n-butyric acid (n-HBu), iso-butyric acid (iso-HBu), n-valeric acid (n-HVa), and iso-valeric acid (iso-HVa) were measured by GC (6890A, Agilent, USA) as described previously (Lin et al., 2017). The particle size distribution of the CEPS sludge was measured by a dynamic light scattering (DSL) particle size analyzer (LS 13320, Beckman Coulter, USA).

The activity of α-amylase and protease were measured as described in previous work (Li and Li, 2017). The relative enzyme activity of the control sludge was set as 1.0. All the enzyme activity was measured in triplicate.

For the PHAs determination, the sludge samples from the SBR for PHAs biosynthesis were certified and lyophilized. Approximately 20 mg of lyophilized sludge was added to 2 mL of chloroform and 2 mL of acidified methanol solution (containing 3% sulfuric acid by volume, as well as 1 mg/mL of benzoate acid as internal standard) (Oehmen et al., 2005), and the solution was kept in a thermotank at 100 °C for 2 h. After cooling, 1.0 mL pure water was added and the sample was transferred to 4 °C freezer for one night. The chloroform phase was collected for PHAs analysis by GC (6890A, Agilent, USA) equipped with HP-5MS column (30 m × 250 μm). The temperature program was set as: 2 min at 70 °C, rise of 20 °C/min to 230 °C. Helium was used as the carrier gas. A calibration curve was obtained by using the standards of poly-b-hydroxybutyrate-*co*-b-hydroxyvalerate (PHBV, 9% PHV) (Sigma-Aldrich, USA).

3. Results and discussion

3.1. Effect of different additives on pH evolution of CEPS sludge during fermentation

The pH evolution during the fermentation process of CEPS sludge with different additives was shown in Fig. 1. The pH increased with the addition of CaO_2, up to 10.3 with 0.1 g/g SS CaO_2 addition. The increasing pH was induced by $Ca(OH)_2$ liberating from CaO_2, that was verified by dosing $Ca(OH)_2$. The pH climb by CaO_2 was not as extensive as $Ca(OH)_2$ at the corresponding stoichiometric equivalent, that could be explained by H_2O_2 generation and the slow release of $Ca(OH)_2$. When H_2O_2 was solely dosed, the initial sludge pH decreased obviously induced by the weak disassociation of protons from H_2O_2. It was reported that alkaline conditions were more favorable for VFAs generation from excessive activated sludge by fermentation (Yuan et al., 2006). Thus, the continuous liberation of $Ca(OH)_2$ from CaO_2 dosed could help to maintain the CEPS sludge under alkaline condition, facilitating the VFAs production.

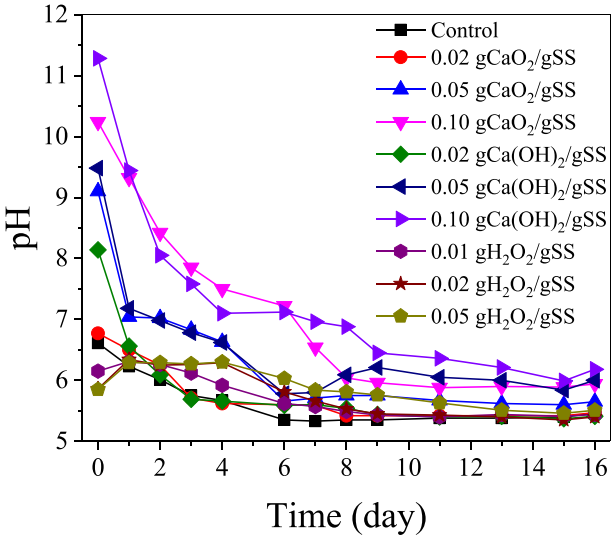

Fig. 1. pH evolution during fermentation of CEPS sludge with different additives.

The pH of the CEPS sludge decreased with the fermentation time due to the VFAs accumulation. After 16 days' fermentation, the pH values of CEPS sludge treated with CaO_2 and $Ca(OH)_2$ were a little higher than the control sludge. For H_2O_2 treated sludge (0.02 and 0.05 mg/g SS), pH kept stable during the first 4 days, indicating serious inhibition of the acidogenesis process. H_2O_2 was a strong chemical oxidant deteriorating the cell walls of microbes (Wang et al., 2009), leading to the inhibited activity of the microbes at the initial time. After 4 days, the pH decreased gradually, implying the adaption of the acidogenic bacteria and occurrence of acidogenesis for the H_2O_2 treated CEPS sludge.

3.2. Effect of different additives on hydrolysis of CEPS sludge during fermentation

The influence of CaO_2 dosage on the SCOD concentration was shown in Fig. 2a. Except the sludge treated with 0.1 g CaO_2/g SS, the SCOD evolution of the other three sludge were similar, increasing rapidly in the first 6 days, then slowly in the last 10 days. There was no significant difference in SCOD evolution profiles between the control sludge and the sludge treated with 0.02 g CaO_2/g SS. For sludge treated with 0.05 g CaO_2/g SS, SCOD generation was less than the control sludge for the first 4 days, while exceeded the control sludge at the later stage. For sludge treated with 0.1 g CaO_2/g SS, the SCOD was released significantly, much more than the control sludge for the first 3 days. However, SCOD concentration decreased suddenly during 4–7 days, then increased gradually to the highest value of 2506 mg/L on the 14th day. The destruction of VSS and particle size distribution of the CEPS sludge were measured after completing fermentation. As listed in Table 2, for 0, 0.02, 0.05 and 0.1 g CaO_2/g SS dosage, VSS destruction of CEPS sludge were 37.3%, 42.3%, 45.7% and 48.6%, respectively. With the increase of CaO_2 dosage, VSS destruction was enhanced efficiently. The particle size of CEPS sludge was also dependent on the CaO_2 dosage. As shown in Fig. S1, compared with the control sludge, the peak value at 25 μm had a weak shift to the left for sludge with 0.02 and 0.05 g CaO_2/g SS addition. For the sludge dosed with 0.1 g CaO_2/g SS, the peak value at 14 μm shifted to the left considerably, suggesting a smaller particle size of CEPS sludge than the others. The results indicated that particulate organics in CEPS sludge was hydrolyzed into soluble organics with the dosage of CaO_2, accelerating the disintegration and solubilization of CEPS sludge obviously. The accumulation of SCOD was determined by the production and consumption processes. SCOD was mainly generated by hydrolysis of particulate organic matters in CEPS sludge. Unlike the excessive activated sludge constituted of microbial cells (Neyens

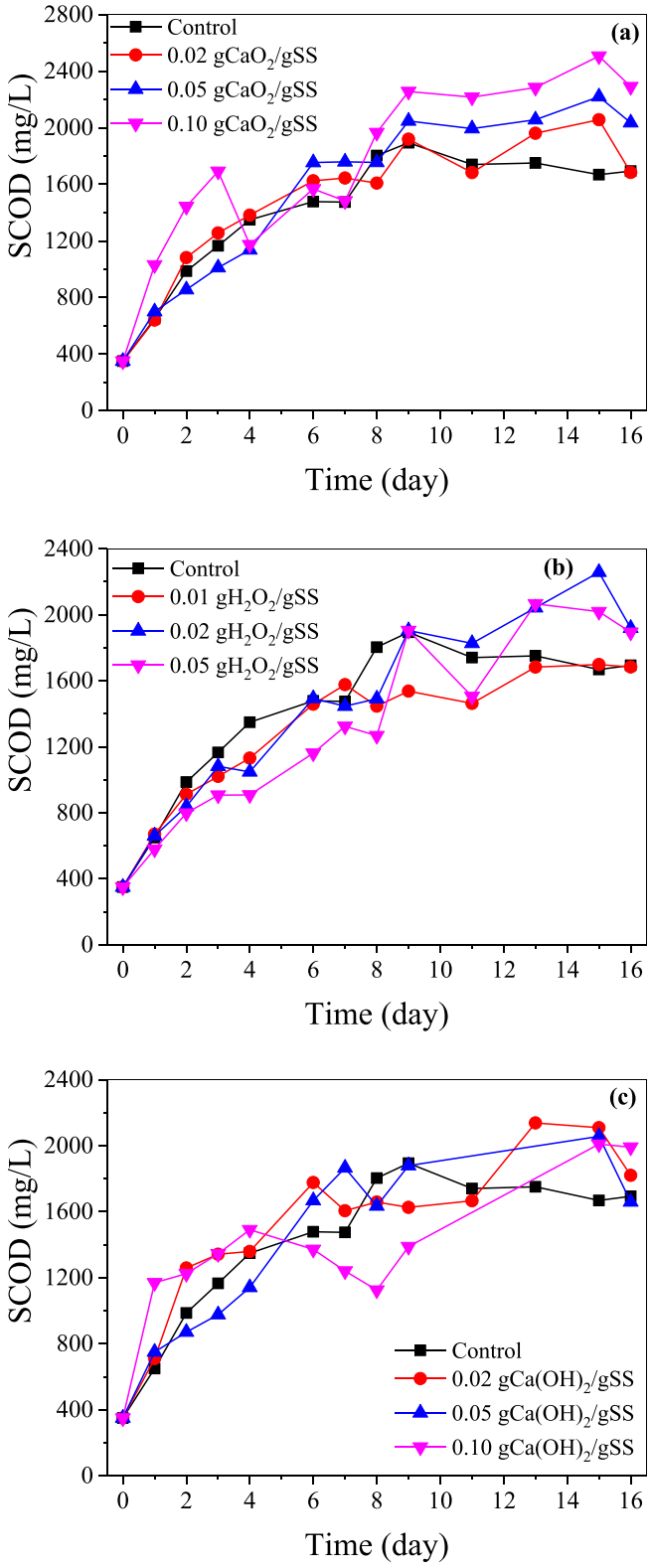

Fig. 2. SCOD evolution during fermentation of CEPS sludge with different additives.

et al., 2003), the hydrolysis of CEPS sludge primarily containing organics was much easier, making it more suitable for organics recovery. SCOD could be consumed by microbial metabolism during gasification, in addition to be oxidized by H_2O_2 released from CaO_2 (Li et al., 2015). The overall increased accumulation of SCOD induced by CaO_2 was mainly attributed to the enhanced hydrolysis of particulate organics.

Table 2
Recovery of VFAs and SCOD from CEPS sludge with different additives.

Additives (g/g SS)	VSS destruction (%)	VFA yield (mg COD/g VSS)	VFA recovery (%)	SCOD recovery (%)
Control	37.3	315.1	21.4	27.1
CaO_2 0.02	42.3	333.0	22.6	29.4
CaO_2 0.05	45.7	399.1	27.1	31.7
CaO_2 0.10	48.6	455.8	30.9	35.8
H_2O_2 0.01	36.4	327.6	22.2	24.3
H_2O_2 0.02	42.7	363.1	24.6	32.3
H_2O_2 0.05	43.5	349.2	23.7	29.5
$Ca(OH)_2$ 0.02	44.4	364.2	24.7	30.5
$Ca(OH)_2$ 0.05	44.8	335.9	22.8	29.4
$Ca(OH)_2$ 0.10	45.2	374.9	25.4	28.7

The influence of H_2O_2 treatment on the hydrolysis of CEPS sludge was displayed in Fig. 2b. There seemed to be negative effect on the sludge hydrolysis with addition of H_2O_2 during the first 11 days. After that, SCOD increased slightly for sludge treated with 0.02 and 0.05 g H_2O_2/g SS. Li et al. clarified that H_2O_2 facilitated the solubilization of excessive activated sludge, but the effect was statistically insignificant (Li et al., 2015). The reason might be the combination of positive roles and negative roles of H_2O_2 in sludge hydrolysis. As a strong chemical oxidant, H_2O_2 accelerated the solubilization of the organic particles into SCOD as well as the conversion of SCOD to CO_2. Thus, the SCOD could not be accumulated efficiently. In addition, H_2O_2 could destroy the cell walls of microorganisms (Wang et al., 2009). Although the chemical hydrolysis was enhanced, the biological hydrolysis was inhibited. This adverse effect would be more serious for CEPS sludge, which contained much less microbes than the excessive activated sludge. VSS destruction were 36.4%, 42.7%, 43.5% for sludge with 0.01, 0.02 and 0.05 g H_2O_2/g SS dosage. Compared with the CaO_2 treated sludge, the VSS destruction for H_2O_2 treated sludge was a little lower. The particle size of sludge treated with 0.05 g H_2O_2/g SS was smaller than the control sludge, which was shown in Fig. S1.

For $Ca(OH)_2$ treated CEPS sludge as shown in Fig. 2c, there was no obvious differences in SCOD production. The SCOD concentration of sludge treated with $Ca(OH)_2$ was a little higher than the control sludge. Previous studies reported that the hydrolysis of excessive activated sludge was remarkably improved under alkaline conditions (Neyens et al., 2003; Yuan et al., 2006). However, the roles of $Ca(OH)_2$ in CEPS sludge hydrolysis were not significant. This result could be explained by the components differences between the CEPS sludge and the excessive activated sludge. The alkali primarily took effects in activated sludge hydrolysis by interacting with the microbial cells to dissolute organics. For CEPS sludge, organics could be hydrolyzed and utilized directly without cell rupture process. Thus, the influence of alkali on the hydrolysis of CEPS sludge was not as important as activated sludge. In contrast, high dosage of $Ca(OH)_2$ led to the formation of some precipitates such as $Fe(OH)_3$ colloid with extremely low solubility, reflecting from the sludge size distribution displayed in Fig. S1. After fermentation for 16 days, VSS destruction were 44.4%, 44.8%, 45.2% for sludge with 0.02, 0.05 and 0.1 g $Ca(OH)_2$ dosage (Table 2). These values were comparable to the result of CaO_2 addition, which was the combing interactions of precipitants formation, improved chemical hydrolysis of sludge matrix (Yu et al., 2008) and enhanced microbial hydrolysis process discussed as follows.

Proteins and carbohydrates are the two predominant organic compounds in the CEPS sludge. The activity of two enzymes including α-amylase and protease were measured to evaluate the microbial activities for sludge hydrolysis. As shown in Table 3, the activities of α-amylase were improved with increasing CaO_2 dosage. In particular, the α-amylase activity of sludge with 0.1 g CaO_2/g SS addition was

nearly 2 times of the control sludge, suggesting the enhanced hydrolysis of carbohydrates by microorganisms. However, the hydrolysis of the proteins was not improved as much as carbohydrates with a relatively lower activity of protease. Li et al. found that proper addition of CaO_2 could improve the activities of hydrolytic enzymes including α-amylase and protease during the fermentation process of excessive activated sludge (Li et al., 2015). In this work, CaO_2 only promoted biological hydrolysis of carbohydrates in CEPS sludge. For H_2O_2 treated sludge, the activity of α-amylase was not influenced while protease activity was strengthened. For $Ca(OH)_2$ treated sludge, the activities of α-amylase and protease were both enhanced significantly. The influence of different additives on enzyme activities related with hydrolysis was complicated (Jin et al., 2018; Liu et al., 2018; Luo et al., 2011), needing to be further explored in the coming investigations.

3.3. Effect of different additives on VFAs generation of CEPS sludge during fermentation

The production of VFAs (calculated as COD) from CEPS sludge was influenced significantly with CaO_2 addition (Fig. 3a). For the first 8 days, the dosage of CaO_2 inhibited the VFAs generation to some extent, indicating that the acidogenesis process was adversely influenced by CaO_2. This was mainly attributed to the H_2O_2 liberated from CaO_2, which could deteriorate the cell walls of microorganisms and consequently prohibit microbial activity related to acidogenesis. After 8 days, VFAs accumulated rapidly for sludge treated with CaO_2 and exceeded the control sludge. The maximum VFAs yield was 455.8 mg COD/g VSS with the dosage of 0.1 g CaO_2/g SS on the 15th day (Table 2). The VFAs yield was improved by 44.7% compared with the control sludge, much higher than the VFAs yield of activated sludge listed in Table S1 (Huang et al., 2014; Li et al., 2015; Yuan et al., 2011). With the hydrolysis of the CEPS sludge, the substrates containing rich organics facilitated the growth of the acidogenic bacteria, contributing to the increased VFAs concentration.

The VFAs yield with H_2O_2 treatment was not enhanced as much as CaO_2 did (Fig. 3b). The maximum VFAs yield of 363.1 mg COD/g VSS was obtained with the dosage of 0.02 g H_2O_2/g SS on the 16th day. For the first 8 days, H_2O_2 had a negative influence on VFAs accumulation compared with the control sludge. The generation of VFAs decreased with the elevated H_2O_2 dosage, indicating a serious inhibition of the acidogenic bacteria by H_2O_2. With the prolonged fermentation time, VFAs concentration raised gradually for H_2O_2 treated sludge and exceeded the control sludge. This was due to the alleviated inhibition for the acidogenesis bacteria and more soluble organic substrates generated from H_2O_2 enhanced chemical hydrolysis process.

In the case of $Ca(OH)_2$ addition, a maximum VFAs yield of 374.9 mg COD/g VSS was obtained with the dosage of 0.1 g $Ca(OH)_2$/g SS on the 16th day (Fig. 3c). Compared with the control sludge, sludge treated with 0.02 g $Ca(OH)_2$/g SS showed better performance in VFAs production during the fermentation time. It was reported that alkaline fermentation facilitated VFAs production due to the increased bacteria related to sludge hydrolysis and acidification and the decreased bacteria involved in methanogenesis (Zheng et al., 2013). With the increasing dosage of $Ca(OH)_2$, the VFAs production was reduced initially, then exceeded the control set after about two weeks. It was attributed to the strong alkaline condition that depressed the activity of acidogenic bacteria (Chen et al., 2007). For CaO_2 treatment, as the $Ca(OH)_2$ was slowly released, the gentle increase of pH allowed the acidogenic bacteria to adapt to the increasing alkaline environment and alleviated microbial inhibition.

Table 3
The relative activity of key enzymes in CEPS sludge with different additives.

Additives (g/g SS)	Relative activity of enzyme	
	α-Amylase	Protease
Control	1.00 ± 0.02	1.00 ± 0.02
CaO_2 0.02	1.43 ± 0.13	0.87 ± 0.02
CaO_2 0.05	1.75 ± 0.07	1.20 ± 0.05
CaO_2 0.10	1.91 ± 0.12	0.93 ± 0.06
H_2O_2 0.01	1.03 ± 0.04	1.13 ± 0.10
H_2O_2 0.02	0.75 ± 0.06	1.20 ± 0.07
H_2O_2 0.05	1.00 ± 0.02	1.40 ± 0.08
$Ca(OH)_2$ 0.02	1.36 ± 0.15	1.27 ± 0.15
$Ca(OH)_2$ 0.05	1.76 ± 0.13	1.13 ± 0.06
$Ca(OH)_2$ 0.10	1.97 ± 0.14	1.07 ± 0.05

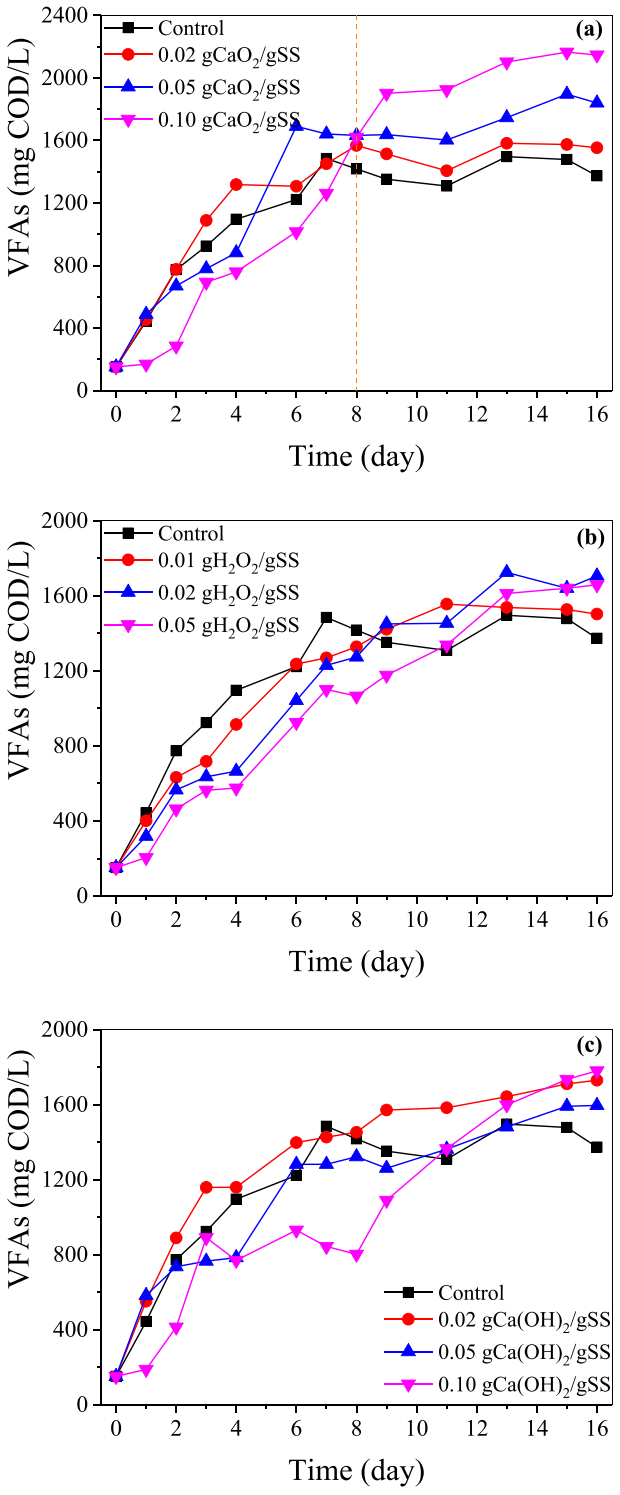

Fig. 3. VFAs evolution during fermentation of CEPS sludge with different additives.

The VFA components were displayed in Fig. 4. Acetic and propionic acids were the principle components of the generated VFAs for the CEPS sludge. However, the ratio of the individual VFA in the fermentation liquor was not identical. For the control sludge, acetic and propionic acids accounted for similar ratio of 43.2% and 40.0%. Iso-valeric acid contributed to 8.3% of the VFAs and the left was due to the other VFAs (Fig. 4a). The contribution of individual VFAs kept relatively stable during the whole fermentation process. For 0.1 g CaO_2/g SS treated sludge, the proportion of propionic acid increased with extended fermentation time to 48.1%, while acetic acid decreased to 36.3%. Iso-valeric acids and the other components accounted for about 15.6% of the VFAs (Fig. 4b). The accumulation of propionic acid was mainly due to the substrates changes induced by H_2O_2, as the evolution profiles of VFA components for CaO_2 and H_2O_2 treated sludge were quite similar. For 0.05 g H_2O_2/g SS treated sludge, the contribution of acetic and propionic acids were calculated to be 39.4% and 43.5% (Fig. 4c). For 0.1 g $Ca(OH)_2$/g SS treated sludge, the ratios of acetic and propionic acids were 43.2% and 41.2% (Fig. 4d). The results were in accordance with previous study, which found that $Ca(OH)_2$ addition showed no significant effect on the components of generated VFAs (Li et al., 2015).

3.4. Effect of different additives on microbial populations

Illumina high-throughput sequencing of 16S rRNA gene was employed to analyze the richness and diversity of microbial communities in CEPS sludge dosing different chemicals. The OTU numbers of sludge samples treated with CaO_2, H_2O_2 and $Ca(OH)_2$ were 2031, 2135 and 2175 respectively, while the value for the control sludge was 2145. CaO_2 addition reduced the richness and diversity of the microbial communities in CEPS sludge, indicating that the species with hydrolysis and acidogenesis functions were enriched effectively.

Fig. 5 showed the relative bacterial community abundance in CEPS sludge treated with different chemicals at the phylum level. *Proteobacteria* was the predominant phylum with sequence percentages ranging from 30.8% to 48.0%. *Bacteroidetes*, *Firmicutes*, and *Chloroflexi* were the subdominant groups, comprising 11.1% to 34.3%, 9.9% to 27.1%, and 2.3% to 9.9% of the detections, respectively. *Proteobacteria* and *Bacteroidetes* were the predominant microorganisms in anaerobic digestion systems, playing significant roles in hydrolysis and acidogenesis (Li et al., 2015). After dosing CaO_2, the proportions of *Bacteroidetes* increased slightly. CaO_2 addition significantly elevated the relative abundance of *Firmicutes*, which accelerated organics hydrolysis with the capacity of producing extracellular enzymes such as proteases, cellulases, and lipases (Lim et al., 2014). Similar results were observed for $Ca(OH)_2$ treated sludge and the increase tendency were more intensive. Thus, the alkaline conditions were more favorable for the growth of microbes with function of hydrolysis, accelerating the microbial hydrolysis and acidification process for VFAs production.

At the genus level as shown in Fig. 6, it was noticed that a special genus *Geothermobacter* with function of reducing Fe(III) appeared in CaO_2 treated CEPS sludge (Emerson, 2009). The hydrolysis of the CEPS sludge was initiated with disintegrating sludge flocculated by $FeCl_3$. The reduction of Fe(III) was a crucial step to solubilize the organics from the CEPS sludge. The presence of genus *Geothermobacter* indicated that CaO_2 treatment facilitated VFAs generation by enhancing microbial hydrolysis process. The relative abundance of *vadinBC27* increased significantly for CaO_2 and $Ca(OH)_2$ treated sludge. Limited information was available on the function of genus *vadinBC27*. It was reported that genus *vadinBC27* could greatly contribute to the degradation of recalcitrant organic contaminants such as landfill leachate (Xie et al., 2014). The increase of *vadinBC27* might be favorable for VFAs generation from refractory organics degradation.

3.5. Feasibility of producing PHAs with CaO_2 treated CEPS sludge fermentation liquor

The generation of VFAs was accompanied with N, P and Fe released from CEPS sludge, which would influence the following PHAs

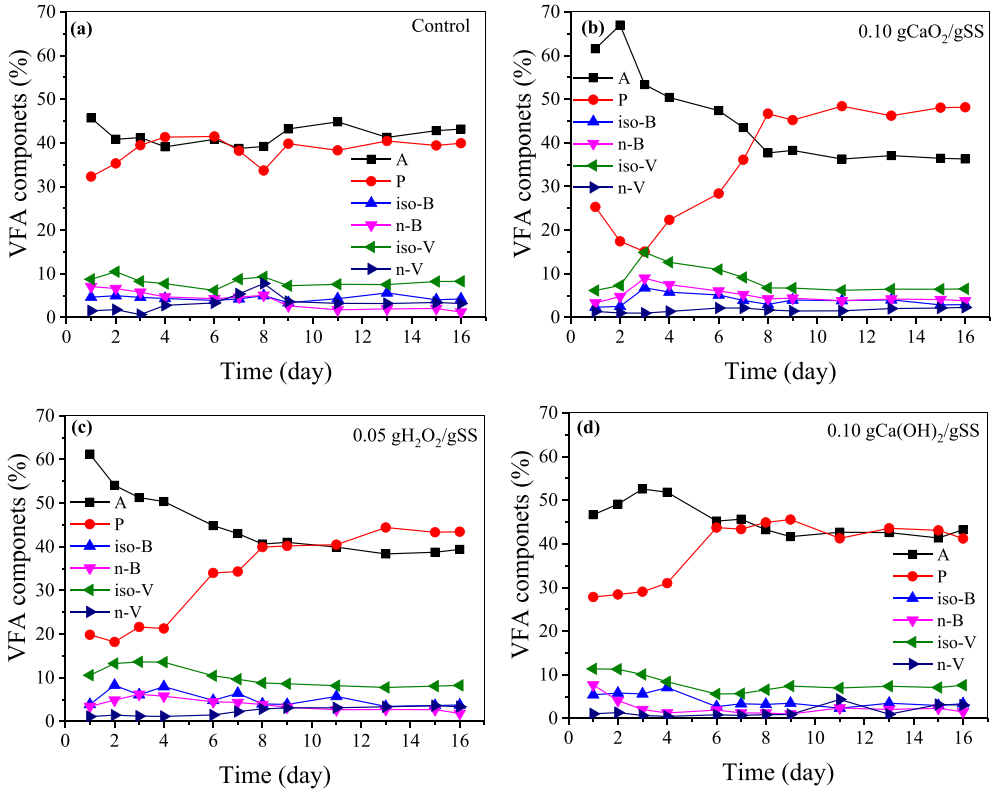

Fig. 4. Variation of VFA components (A: acetic; P: propionic; iso-B: iso-butyric; n-B: nbutyric; iso-V: iso-valeric; n-V: n-valeric) during fermentation of (a) control CEPS sludge, (b) sludge dosed with 0.1 g CaO_2/g SS, (c) sludge dosed with 0.05 g H_2O_2/g SS and (d) sludge dosed with 0.1 g $Ca(OH)_2$/g SS.

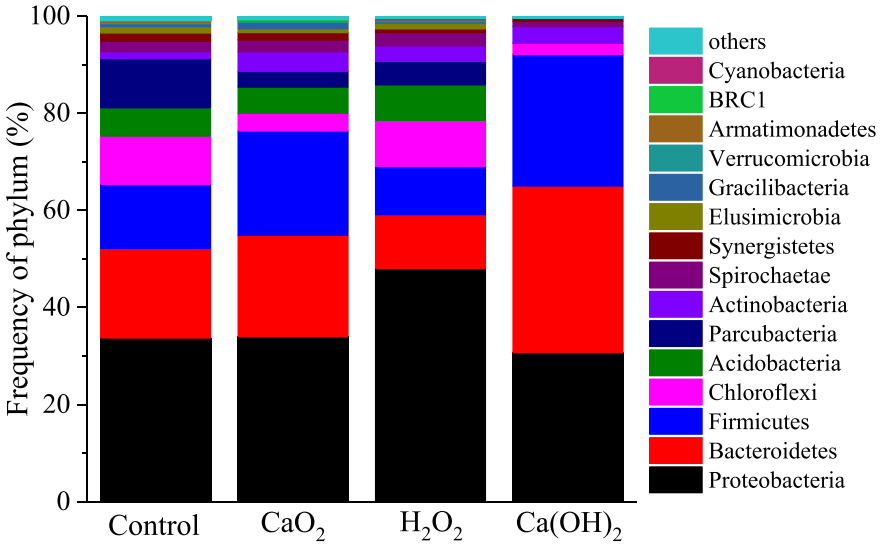

Fig. 5. Bacterial communities at phylum level (relative abundance over 0.1%).

production. N primarily existed as ammonium originated from hydrolysis of proteins. It was shown that the concentration of NH_4^+-N was not influenced by CaO_2 dosage obviously (Fig. 7a), implying a weak effect of CaO_2 on proteins hydrolysis. This result agreed with lower activity of protease listed in Table 3. The P and Fe, existed mainly in the form of chemical precipitates such as Fe-PO_4 minerals and FeOOH-adsorbed P (Wu et al., 2015). During fermentation process of pH dropping, P transferred from the sludge phase into the solution as PO_4^{3-}. Simultaneously, Fe in the sludge was reduced to Fe^{2+} by dissimilatory iron reduction (Li and Li, 2017). As shown in Fig. 7b, the release of P from sludge decreased with the increasing CaO_2, which was probably attributed to the precipitation of calcium phosphate. The CaO_2 addition also decreased Fe^{2+} concentration which was highly related with the solution pH (Fig. 7c). As Fe^{2+} was toxic for the microbes, CaO_2 dosage was

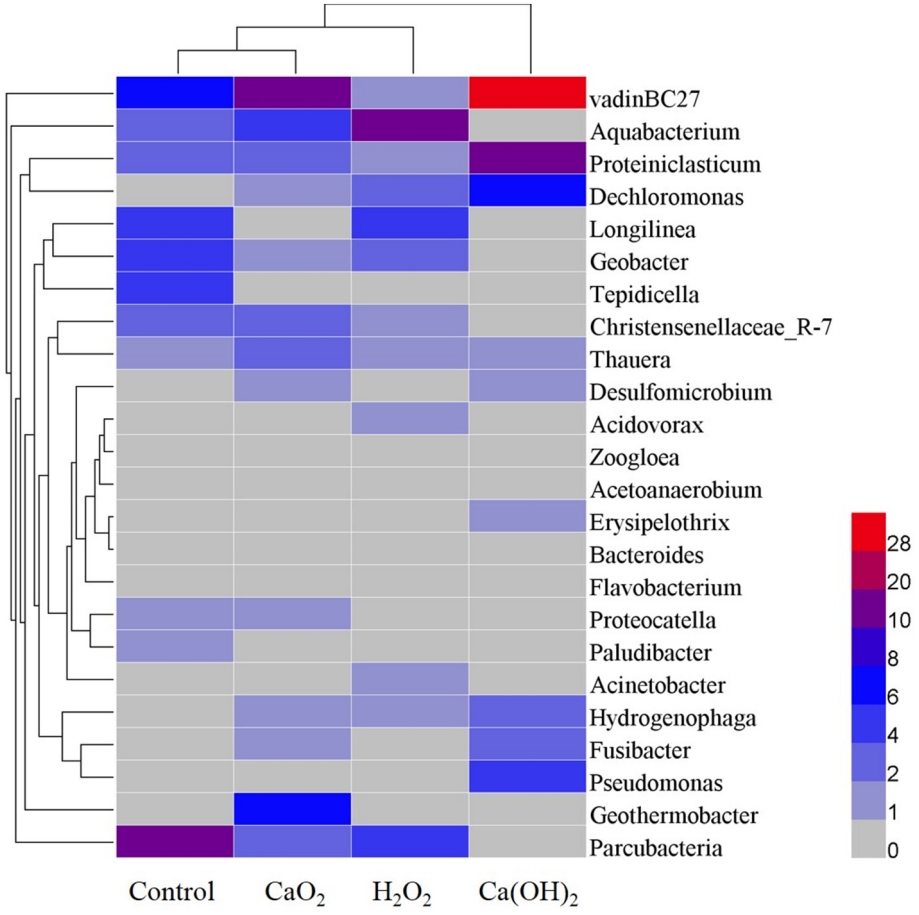

Fig. 6. Heat map of the most abundant genera in the four sludge samples. Only the top 24 genera were used to build the heat map, and the color intensity shows the relative abundance of genus in the sample.

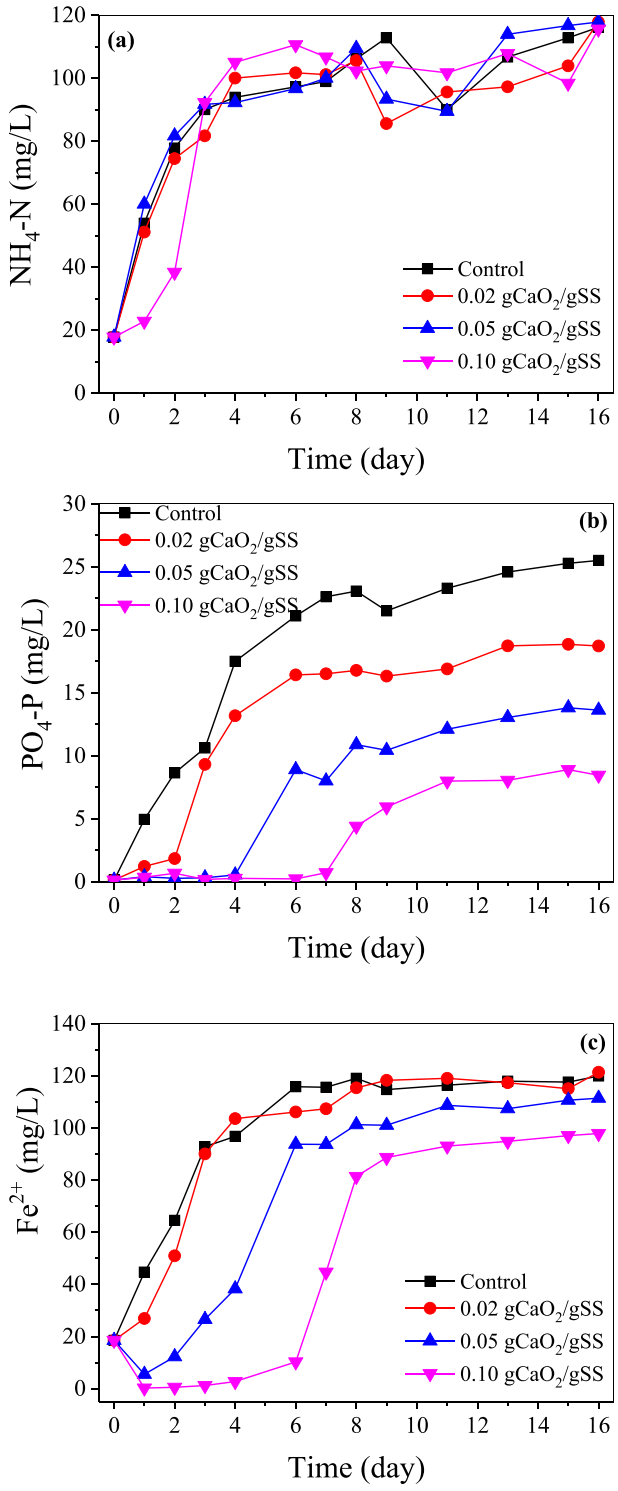

Fig. 7. The evolution of (a) NH_4-N (b) PO_4-P and (c) Fe^{2+} during the fermentation of CEPS sludge treated with CaO_2.

beneficial to the further utilization of fermentation liquor as substrate for biosynthesis due to the reduced concentration of Fe^{2+}. Thus, the final COD:N:P of the CEPS sludge fermentation liquor was 269:13.5:1 with 0.1 g CaO_2/g SS addition.

The fermentation liquor of CaO_2 treated CEPS sludge was used as substrate for PHAs biosynthesis in the SBR. After cultivation for 35 days, the PHAs content in activated sludge reached the highest value of 22.3% (Fig. 8a). PHAs synthesis and degradation had a simultaneous existence during the operation cycle. The highest PHAs content in sludge presented at about 20 min after feeding. After that, the PHAs was consumed due to metabolism of microbes (Fig. 8b). The PHAs content achieved in this study using CEPS fermentation liquor as sole substrate was comparable to those studies using waste materials as carbon source listed in Table S2 (Jia et al., 2014; Liu et al., 2008). Results showed that fermentation liquor of CaO_2 treated Fe-CEPS sludge could be a suitable substrate for PHAs biosynthesis.

In summary, CaO_2 played positive roles in sludge hydrolysis, VFAs generation and PHAs biosynthesis: (1) CaO_2 induced microbial reduction of Fe(III), facilitating the initial sludge disintegration; (2) H_2O_2 and hydroxyl radicals released from CaO_2 decomposed recalcitrant organics into simple substrates for microbial utilization, increasing available carbon sources; (3) Continuous liberation of $Ca(OH)_2$ from CaO_2 helped to maintain the alkaline condition, which promoted chemical hydrolysis of particulate organics and microbial hydrolysis of carbohydrates; (4) H_2O_2 released from CaO_2 induced accumulation of propionic acid, which was the main component of the generated VFAs; (5) CaO_2 reduced PO_4^{3-}-P and Fe^{2+} concentration in the fermentation liquor, that was beneficial to utilized as substrate for PHAs production.

4. Conclusions

This work demonstrated that CaO_2 enhanced the disintegration of Fe-CEPS sludge and improved VFAs yield during fermentation. The maximum VFAs yield of 455.8 mg COD/g VSS was achieved with the dosage of 0.1 g CaO_2/g SS, that was improved by 44.7% compared with the

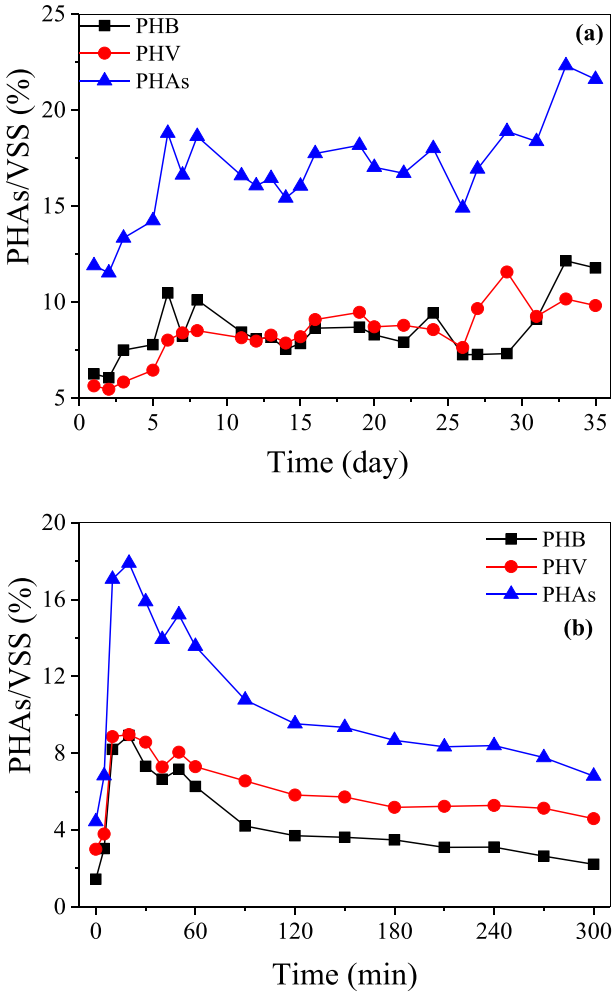

Fig. 8. (a) PHAs content during cultivation time of 35 days, and (b) evolution of PHAs content during a batch cycle of 300 min.

control sludge. After cultivation for 35 days, the PHAs content in activated sludge reached 22.3% using CEPS sludge fermentation liquor as sole carbon source. This integrated technology could be a superior alternative of realizing sludge disposal and bioplastic production simultaneously.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.392.

References

Cai, M.M., Chua, H., Zhao, Q.L., Shirley, S.N., Ren, J., 2009. Optimal production of polyhydroxyalkanoates (PHA) in activated sludge fed by volatile fatty acids (VFAs) generated from alkaline excess sludge fermentation. Bioresour. Technol. 100, 1399–1405.

Chen, Y.G., Jiang, S., Yuan, H.Y., Zhou, Q., Gu, G.W., 2007. Hydrolysis and acidification of waste activated sludge at different pHs. Water Res. 41, 683–689.

Emerson, D., 2009. Potential for iron-reduction and iron-cycling in iron oxyhydroxide-rich microbial mats at loihi seamount. Geomicrobiol J. 26, 639–647.

Federation WE, Association APH, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA), Washington, DC, USA.

Hand, S., Gill, J., Chu, K.H., 2016. Phage-based extraction of polyhydroxybutyrate (PHB) produced from synthetic crude glycerol. Sci. Total Environ. 557, 317–321.

Huang, L., Chen, B., Pistolozzi, M., Wu, Z.Q., Wang, J.F., 2014. Inoculation and alkali coeffect in volatile fatty acids production and microbial community shift in the anaerobic fermentation of waste activated sludge. Bioresour. Technol. 153, 87–94.

Huang, H.N., Zheng, X., Chen, Y.G., Liu, H., Wan, R., Su, Y.L., 2017. Alkaline fermentation of waste sludge causes a significant reduction of antibiotic resistance genes in anaerobic reactors. Sci. Total Environ. 580, 380–387.

Jia, Q.Q., Xiong, H.L., Wang, H., Shi, H.C., Sheng, X.Y., Sun, R., et al., 2014. Production of polyhydroxyalkanoates (PHA) by bacterial consortium from excess sludge fermentation liquid at laboratory and pilot scales. Bioresour. Technol. 171, 159–167.

Jin, B., Niu, J., Dai, J., Li, N., Zhou, P., Niu, J., et al., 2018. New insights into the enhancement of biochemical degradation potential from waste activated sludge with low organic content by Potassium Monopersulfate treatment. Bioresour. Technol. 265, 8–16.

Li, R.H., Li, X.Y., 2017. Recovery of phosphorus and volatile fatty acids from wastewater and food waste with an iron-flocculation sequencing batch reactor and acidogenic co-fermentation. Bioresour. Technol. 245, 615–624.

Li, Y.M., Wang, J., Zhang, A., Wang, L., 2015. Enhancing the quantity and quality of short-chain fatty acids production from waste activated sludge using CaO_2 as an additive. Water Res. 83, 84–93.

Lim, J.W., Chiam, J.A., Wang, J.Y., 2014. Microbial community structure reveals how microaeration improves fermentation during anaerobic co-digestion of brown water and food waste. Bioresour. Technol. 171, 132–138.

Lin, L., Li, X.Y., 2018. Acidogenic fermentation of iron-enhanced primary sedimentation sludge under different pH conditions for production of volatile fatty acids. Chemosphere 194, 692–700.

Lin, L., Li, R.H., Li, Y., Xu, J., Li, X.Y., 2017. Recovery of organic carbon and phosphorus from wastewater by Fe-enhanced primary sedimentation and sludge fermentation. Process Biochem. 54, 135–139.

Lin, L., Li, R.H., Li, X.Y., 2018. Recovery of organic resources from sewage sludge of Al-enhanced primary sedimentation by alkali pretreatment and acidogenic fermentation. J. Clean. Prod. 172, 3334–3341.

Liu, H.Y., Hall, P.V., Darby, J.L., Coats, E.R., Green, P.G., Thompson, D.E., et al., 2008. Production of polyhydroxyalkanoate during treatment of tomato cannery wastewater. Water Environ. Res. 80, 367–372.

Liu, Y., Zhao, J., Li, X., Wang, D., Yang, Q., Zeng, G., 2018. Synergistic effect of free nitrite acid integrated with biosurfactant alkyl polyglucose on sludge anaerobic fermentation. Waste Manag. 78, 310–317.

Luo, K., Yang, Q., Yu, J., Li, X.M., Yang, G.J., Xie, B.X., et al., 2011. Combined effect of sodium dodecyl sulfate and enzyme on waste activated sludge hydrolysis and acidification. Bioresour. Technol. 102, 7103–7110.

Neyens, E., Baeyens, J., Creemers, C., 2003. Alkaline thermal sludge hydrolysis. J. Hazard. Mater. 97, 295–314.

Northup, A., Cassidy, D., 2008. Calcium peroxide (CaO_2)for use in modified Fenton chemistry. J. Hazard. Mater. 152, 1164–1170.

Oehmen, A., Kellerlehmann, B., Zeng, R.J., Yuan, Z., Keller, J., 2005. Optimisation of poly-beta-hydroxyalkanoate analysis using gas chromatography for enhanced biological phosphorus removal systems. J. Chromatogr. A 1070, 131–136.

Salehizadeh, H., Van Loosdrecht, M.C.M., 2004. Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance. Biotechnol. Adv. 22, 261–279.

Serafim, L.S., Lemos, P.C., Oliveira, R., Reis, M.A.M., 2004. Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. Biotechnol. Bioeng. 87, 145–160.

Verlinden, R.A.J., Hill, D.J., Kenward, M.A., Williams, C.D., Radecka, I., 2007. Bacterial synthesis of biodegradable polyhydroxyalkanoates. J. Appl. Microbiol. 102, 1437–1449.

Wang, Y.W., Wei, Y.S., Liu, J.X., 2009. Effect of H_2O_2 dosing strategy on sludge pretreatment by microwave-H_2O_2 advanced oxidation process. J. Hazard. Mater. 169, 680–684.

Wu, H., Ikeda-Ohno, A., Wang, Y., Waite, T.D., 2015. Iron and phosphorus speciation in Fe-conditioned membrane bioreactor activated sludge. Water Res. 76, 213–226.

Xie, Z.F., Wang, Z.W., Wang, Q.Y., Zhu, C.W., Wu, Z.C., 2014. An anaerobic dynamic membrane bioreactor (AnDMBR) for landfill leachate treatment: performance and microbial community identification. Bioresour. Technol. 161, 29–39.

Yu, G.H., He, P.J., Shao, L.M., He, P.P., 2008. Toward understanding the mechanism of improving the production of volatile fatty acids from activated sludge at pH 10.0. Water Res. 42, 4637–4644.

Yuan, H.Y., Chen, Y.G., Zhang, H.X., Jiang, S., Zhou, Q., Gu, G.W., 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. Environ. Sci. Technol. 40, 2025–2029.

Yuan, Q., Sparling, R., Oleszkiewicz, J.A., 2011. VFA generation from waste activated sludge: effect of temperature and mixing. Chemosphere 82, 603–607.

Zhang, F., Yang, J.H., Dai, K., Chen, Y., Li, Q.R., Gao, F.M., et al., 2016. Characterization of microbial compositions in a thermophilic chemostat of mixed culture fermentation. Appl. Microbiol. Biotechnol. 100, 1511–1521.

Zhang, W., Alvarez-Gaitan, J., Dastyar, W., Saint, C., Zhao, M., Short, M., 2018. Value-added products derived from waste activated sludge: a biorefinery perspective. Water 10, 545.

Zheng, X., Su, Y.L., Li, X., Xiao, N.D., Wang, D.B., Chen, Y.G., 2013. Pyrosequencing reveals the key microorganisms involved in sludge alkaline fermentation for efficient short-chain fatty acids production. Environ. Sci. Technol. 47, 4262–4268.