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RESEARCH ARTICLE

Upgrading VFAs bioproduction from waste activated sludge via co-fermentation with soy sauce residue

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HIGHLIGHTS

- SSR addition upgraded VFAs production from WAS.
- Structure modification by pretreatments led to performance distinctions.
- Distinctions in microbial community was observed by pretreatments selection.
- Up to 0.49–0.65 billion €/year of market value potential was preliminary estimated.

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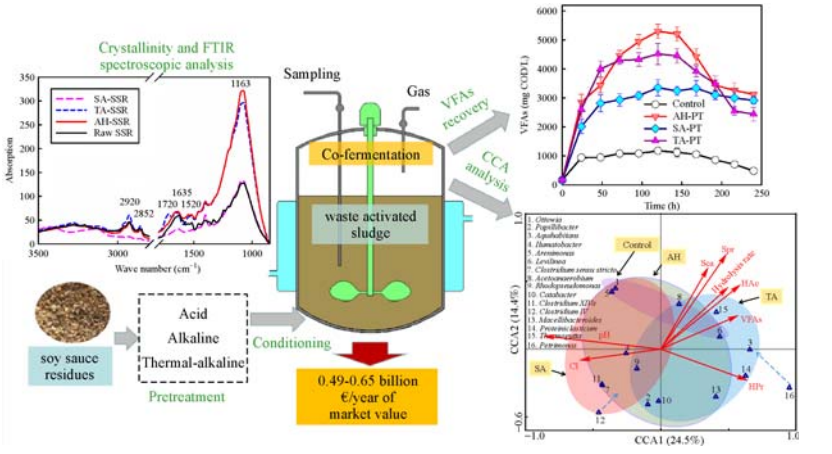
GRAPHIC ABSTRACT



ABSTRACT

Conditioning of extra carbon sources has been widely reported to facilitate fermentation of waste activated sludge (WAS). Soy sauce residue (SSR) was a relatively untapped carbon source for sludge conditioning. This batch study aimed to evaluate the possible implementation of SSR for volatile fatty acids (VFAs) production from WAS. To upgrade the bioavailability of feedstock, three typical pretreatment methods were conducted, i.e., ammonium hydroxide (AH), sulfuric acids (SA) and thermal assisted alkaline (TA). AH pretreated test (AH-PT) outperformed due to a relatively strong structure decomposition of cellulosic materials as revealed by infrared spectroscopic analysis and crystal index. As a result, performed a high hydrolysis rate of 4449 mg COD/d, 1.12-1.23-fold higher than that in TA and SA pretreated tests (TA-PT and SA-PT), and 7.8-fold higher than that in the Control test. Meanwhile, a volatile fatty acids (VFAs) contribution of 401.2 mg COD/g SSR·L and a maximum acidification rate of 3.59 d^{-1} was recorded, with a high sum proportion of mall molecular acetic and propionic 82.2%, 11% –70% increase over the other three tests. Besides, speciation process characterized with functional genus differentiation was identified by microbial diversity and distribution investigation and canonical correspondence analysis (CCA). Finally, a potential market value of 0.49–0.65 Billion €/year was preliminary estimated, showing promise of resource recovery from both WAS and SSR instead of extensive disposal.

1 Introduction

Waste activated sludge (WAS), being an inevitable by-product of current universal activated sludge processes, have been blamed for its high-pollution and energy consumption with no disposal or improper processing in

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voluminous amount (with approximately 80% moisture content). It has been a general realization that anaerobic digestion (AD) of WAS was one of the most cost-effective alternatives for sludge stabilization and bio-energy recovery. In the AD process, nontoxic organic carbon compounds (>60% in dry weight) can be converted to renewable energy and specific valuable products, simultaneously sludge reduction could be accomplished (Mills et al., 2014). The volatile fatty acids (VFAs), as one kind of the main intermediates during sludge fermentation, is an underlying carbon source with low cost to support biological processes such as enhanced biological nutrient removal (BNR) and PHAs bio-refinery (Lee et al., 2014). However, the sustainable production of VFAs is always constrained by unbalanced nutrient profiles. It was reported that conditioning by carbon-rich substrates was an efficient option to balance the carbon to nitrogen ratio (Rughoonundun et al., 2012). Agricultural based waste has been widely studied among these carbon sources. For example, Zhou reported a 12%–69% more VFAs production by corn straw addition to WAS (Zhou et al., 2013). And Jia used perennial rye-grass as a carbon source to adjust carbon to nitrogen ratio (C/N ratio) in WAS to enhance VFAs production (Jia et al., 2013). Besides, C/N ratio was successfully adjusted to promote VFAs production by co-fermentation of WAS and henna plant biomass (Huang et al., 2016).

Being a fundamental flavor with a history of more than 2500 years in the diet of Chinese people, soy sauce has occupied an increasingly important position in the world (Fukushima, 1979). Currently, the annual output of soy sauce in mainland China is around 11 million tons, with a 10% annually market increase rate (Zhang et al., 2017). Soy sauce residue (SSR) is a by-production of soy sauce manufacturing process, with a production of 0.67 kg SSR/kg suspended solid (with 75% water content) as reported. It means that significant amounts of more than 7.37 million tons of SSR were produced every year. Even worse, it is difficult to storage and transport SSR because it is perishable with high humidity, and improperly treatment would lead to bad odor and cause environmental contamination. Notwithstanding, some wonderful traits were worth approving for SSR as a high-quality regulation carbon source. For example, SSR was mainly used in extensive methods like soy sauce fermentation, animal feed, organic fertilizer, oil squeezing, bioactive compounds extraction, bio-flocculant at present (Chen et al., 2014). Besides, plenty nutrient source such as 7%–18% crude lipids and 20%–30% crude proteins can be obtained as reported by Chen, providing far more resources for microbe metabolism than other cellulosic biomass.

Based on the above considerations, the main purpose of this project was to evaluate the feasibility of SSR as a conditioning substrate to promote both the hydrolysis and acidification of WAS. Since cellulose always arranges itself in a crystalline structure, creating a physical barrier for enzymes attack, pretreatment of SSR can result in an improved efficiency of hydrolysis of cellulose a promoted availability of carbon sources for subsequent anaerobic fermentation of WAS (Xiao et al., 2017). Therefore, three typical chemical pretreatments, including ammonium hydroxide (AH), sulfuric acids (SA) and thermal assisted alkaline (TA), were examined to promote bioavailability of SSR. Soluble organic matters, including soluble chemical oxygen demand (SCOD), soluble carbohydrates and soluble proteins, were monitored to help understand the hydrolysis in different pretreatment schemes. Afterwards, a clear insight of VFAs production and composition was obtained to access the acid-yield capacity of co-digests conditioned by different pretreated SSR. Besides, to acquire a clear knowledge of what happened in WAS and varied SSR co-fermentation sets, fourier transform infrared (FTIR) spectrometer was used to analyzed changes of functional groups. Meanwhile, the microbial community diversity were analyzed in phylum, class and genus level by high-throughput sequencing technology, and the interaction between functional genera and environmental parameters was explored by canonical correspondence analysis (CCA).

2 Materials and methods

2.1 Characteristics and pretreatment procedure of substrates

WAS was obtained from the secondary sedimentation tank of a municipal sewage treatment plant in Shanxi, China. After concentrated by settling for 24 h and dumping the supernatant first, it was kept at 4°C prior to use. Basic characteristics of WAS were listed as follows: pH 6.95±0.03, total suspended solids (TSS) 18072±1204 mg/L, volatile suspended solids (VSS) 12705±598 mg/L, total chemical oxygen demand (TCOD) 12705±598 mg/L, soluble chemical oxygen demand (SCOD) 448±6 mg/L, total protein 10792±291 mg COD/L, soluble protein 132±31 mg COD/L, total carbohydrate 1429±72 mg COD/L, soluble carbohydrate 47±1 mg COD/L and VFAs 165±10 mg COD/L. SSR was provided by Jinjia vinegar factory (Taiyuan, Shanxi, China). The collected SSR was first dried at 70°C till constant weight in oven, and then powdered to particle size < 180 mesh before use.

To increase the anaerobic digestibility of SSR, thermal and chemical pretreatment were used as the specific methods: The ammonium hydroxide (AH) pretreatment was performed by mixing with 21% ammonium hydroxide at a solid to liquid solid-to-liquid (S/L) ratio (g dry weight to mL) of 1:6 for 10 h at 70°C. The sulfuric acids (SA) pretreatment by 2.0% sulfuric acid (w/w) with S/L ratio of 1:10 was performed in an autoclave at 121°C for 90 min. And the thermal assisted alkaline (TA) pretreatment was conducted by 3% (w/w) sodium hydroxide at 85°C in a thermostatic water bath for one hour with S/L ratio of 1:10.

The main compositions of raw and pretreated VR are shown in Table 1. After pretreatment and cooling of the mixture, the residues were separated by centrifugation at 10000 rpm dried at 70°C till constant weight and finally milled to 1-2 mm before being used as co-feedstock of the WAS anaerobic fermentation.

2.2 Batch fermentation experiments

Batch-scale anaerobic fermentation experiments were carried out in a series of 500 mL anaerobic batch digesters, with a working volume of 300 mL. The feedstock of WAS and pretreated SSR were performed with a mass ratio (VSS/VS) of 2:1 (Zhou et al., 2013). Three co-fermentation sets (run in triplicates) were defined as ammonium hydroxide pretreated test (AH-PT), sulfuric acid pretreated test (SA-PT) and thermal assisted alkaline pretreated test (TA-PT). The Control test was fed with WAS alone. The pH values of the four fermenters after pretreatment were 7.00, 6.32, 8.51 and 6.95, respectively and were uncontrolled in the whole fermentation process. Before the digestion trial, the dissolved oxygen in the reactors was purged with nitrogen gas for 10 min to achieve strict anaerobic condition. Then, the bioreactors were placed in an air-bath shaker at temperature of 35±1°C with 100 rpm. The bioreactors were run for 10 days.

2.3 DNA extraction and Illumina MiSeq sequencing

DNA from well-homogenized sludge samples were extracted according to a previous study (Zhou et al., 2015). Amplifications were performed with bacterial fused primers 341F and 805R. And the specific procedure of Polymerase chain reaction (PCR) reactions was conducted according to previous study (Zhou et al., 2015). The raw sequences were deposited with accession no. of SRR6179062 in NCBI database. A 97% identity threshold of operational taxonomic units (OTUs) was obtained based on the clustered sequences. Afterwards, rarefaction curves and alpha diversity with Shannon-index and Simpson indices included were compared to analyze difference in predominance bacterium. The CCA analysis was conducted by Canoco 4.5 to see correlations between 16 functional consortium and the 7 environmental variations including the pH corresponding to the maximum VFAs yield (expressed as pH), CI, Hydrolysis rate, the maximum VFA yield (expressed as VFAs), HAc, HPr, soluble carbohydrates (Sca) and soluble proteins (Spr) concentrations.

2.4 Analytical and calculation methods

Sludge fermentation samples collected were first centrifuged at 10000 rpm (9392 × g), and then filtered and stored at 4°C before analysis. A cellulose membrane filter of 0.45μm was used and the filtrate was used to analyze pH, soluble chemical oxygen demand (SCOD), VFAs, soluble carbohydrates and proteins as previously described (Zhou et al., 2015). Carbohydrates were determined by the phenol-sulfuric method and proteins were measured by the BCA protein assay kit (Sangon). The pH value was measured by a pH meter. The activity of α-glucosidase and protease was also measured according to (Rai et al., 2017). A Fiber Analyzer (ANKOM, USA) was used to measure cellulosic materials content in SSR, including cellulose, hemicelluloses and lignin. Biogas was analyzed by a GC (4890D, Agilent). The VFAs was analyzed by a GC (7890, Agilent) with a flame ionization detector (FID). VFAs production was calculated as the sum of the measured acetic (HAc), propionic (HPr), n-butyric (n-HBu), iso-butyric (iso-HBu), n-valeric (n-HVa) and iso-valeric (iso-HVa) acids. The conversion factors of COD are 1.07 g COD/g HAc, 1.51 g COD/g HPr, 1.82 g COD/g HBu, and 2.04 g COD/g HVa.

The hydrolysis rate of digesters was calculated as depicted in equation (Eq.) (1) (Jain et al., 2015).

$$\text{Hydrolysis rate} = \frac{SCOD_x - SCOD_0}{t}, \quad (1)$$

where $SCOD_x$ represents the total soluble organic matter concentration in hydrolyze of all co-fermentation sets (mg COD/L); $SCOD_0$ represents that in blank test (mg COD/L); t represents the reaction time period.

The contribution value of per g SSR addition to the improved VFAs production was computed as the followed Eq. (2) (Zhou et al., 2013).

Table 1 Characteristics of raw and pretreated soy sauce residue (SSRs) (% DM basis)

Component	Raw SSR	AH-pretreated SSR	TA-pretreated SSR	SA-pretreated SSR
VS (%)	76.8±0.8	60.4±1.4	67.5±0.9	70.3±0.3
Cellulose (%)	13.8±0.2	11.2±0.0	13.5±0.1	12.6±0.2
Hemicellulose (%)	15.2±0.1	10.8±0.5	12.8±0.1	13.3±0.2
Lignin (%)	5.7±0.0	4.1±0.2	4.5±0.1	5.0±0.3
Proteins (%)	22.6±1.2	20.3±0.8	19.8±1.4	19.2±1.2
Lipid and oil (%)	8.4±0.0	5.1±0.5	4.3±0.8	4.7±0.3
Ash (%)	23.2±0.5	39.6±0.8	34.2±0.6	28.7±0.7

Note: DM (dry matter).

$$Contribution\ \ value = \frac{VFA_{S_x} - VFA_{S_0}}{m_{SSR}}, \quad (2)$$

where $VFAs_x$ and $VFAs_0$ was the VFAs yield in different pretreated sets and the Control sets (mg COD/L), respectively; m_{SSR} was the SSR dosage in the co-fermentation sets (g/L).

To describe the kinetics of acidogenic process related to substrate utilization, biomass growth and product formation, an unstructured models are used (Lin and Li, 2018), as showed in Eq. (3).

$$C_{VFAs} = \frac{C_{\max}}{1 + \left(\frac{C_{\max}}{C_{VFAs_0}} - 1\right) e^{-k_{VFA}t}}, \quad (3)$$

where k_{VFA} is the apparent specific rate constant (1/d), C_{VFAs} is VFA concentration at time t and C_{VFAs0} and C_{max} is the initial and maximum VFA concentration.

Fourier transform infrared (FTIR) spectrometer was used to characterize lignocellulose structural change in SSR before and after pretreatments. Being freeze-dried and grinded to powder, 2–5 mg of the raw and pretreated SSR with 250 mg of dry potassium bromide was made into tablets respectively and placed on the sample holder. The PerkinElmer Spectrum One BFTIR spectrometer (Waltham, MA, USA) was used for infrared spectra scanning from 4000 cm^{-1} to 450 cm^{-1}. An empirical formula by Nelson and O'connor was used to evaluate the crystallinity changes of cellulose in soy sauce residue under different pretreatment schemes (Nelson and O'Connor. 1964).

$$CI = \frac{\alpha_{1372}\mathrm{cm}^{-1}}{\alpha_{2900}\mathrm{cm}^{-1}}, \quad (4)$$

where CI represents crystallinity of cellulose; α represents infrared spectral intensity determined at the specified wavenumber.

3 Results and discussion

3.1 Composition and structural characteristics of pretreated SSR

Table 1 elaborated changes of the lignocellulose composition in raw and pretreated SSRs. As recorded, a clear decomposition effect of lignocellulose was observed especially for hemicellulose and lignin. More than 28.95% of hemicellulose was removed after 10 h of AH pretreatment, while it reached 15.79% and 12.5% after TA and SA pretreatments, respectively. And the removed lignin was 28.07%, 21.05% and 13.46% under AH, TA and SA pretreatments, respectively. Besides, the observed VS reduction for AH, TA and SA pretreated SSR with 18.84%, 2.17% and 8.69% further suggested the effective dissolution promotion of organics such as carbohydrates and proteins. Moreover, proteins were released from the crystal structure of lignocellulose at equivalent level, while lipid and oil were partially washed out after the three pretreatment procedures.

A further proof of Fig. 1a with the spectra and absorption changes of functional groups of lignocellulose in untreated and pretreated SSRs was presented. As demonstrated, two strong absorption bands at 1163cm^{-1} and 1520 cm^{-1}, which can be ascribed to C-O-C asymmetric stretch vibration in cellulose and hemicellulose and stretching vibration of the benzene ring in lignin (Xiao et al., 2001; Pandey and Pitman, 2003), were clearly observed especially for AH-PT and TA-PT, suggesting a fundamental decomposition of lignocellulose. Besides, the maximum absorbance at 1635 cm^{-1}, corresponding to the carbon-carbon double bonds (-C = C-) vibrations of aromatic skeleton, was presented in AH-PT, also the band near 1720 cm^{-1}, which was most likely due to the acetyl and uronic ester groups in hemicelluloses, or the ester linkages of carboxylic group in lignin or hemicelluloses (Alemdar and Sain, 2008). Also the increased absorption at 2920 cm^{-1} for stretching vibration of-CH_2- and CH- in cellulose was observed after varied pretreatments (Zimmerley et al., 2010). Consequently, the clear change in functional groups of lignocellulose might support the improved organics dissolution in different co-fermenters.

Crystallinity can in some degree reflect the physical and chemical properties of the materials, and a decrease in crystallinity of lignocellulose means fairly hygroscopicity and high chemical reactivity (Velasquez et al., 2015), suggesting a higher hydrolysis efficiency of the co-fermenters. As seen in Fig. 1b, the crystallinity peaked in raw SSR with 0.97, suggesting the complex aggregation state of cellulose, and was corresponded with a lower hydrolysis rate of 570 mg COD/d only. AH-PT with a lowest crystallinity of 0.88, and TA-PT with a medium 0.90 were corresponded with the higher hydrolysis rate of 4449 mg COD/d and 3989 mg COD/d, respectively.

3.2 Changes in organic matter dissolution of co-fermenters

Since hydrolysis is the rate limiting step of AD (Pavlostathis and Gossett, 1986), assessment of soluble organics release was of great importance. Figure 2a is the time-course profile of SCOD release in varied co-fermenters. As depicted, a sharp increase was observed in all three sets with SSR addition compared to the Control test, suggesting a clearly upgrading effect of organics hydrolysis by SSR conditioning. For example, the maximum SCOD concentrations of 7857±244 mg COD/L, 6166±233 mg COD/L and 6094±274 mg COD/L at 120 h were obtained in the AH-PT, TA-PT and SA-PT tests, respectively, which were 3.77-4.86-folds higher than

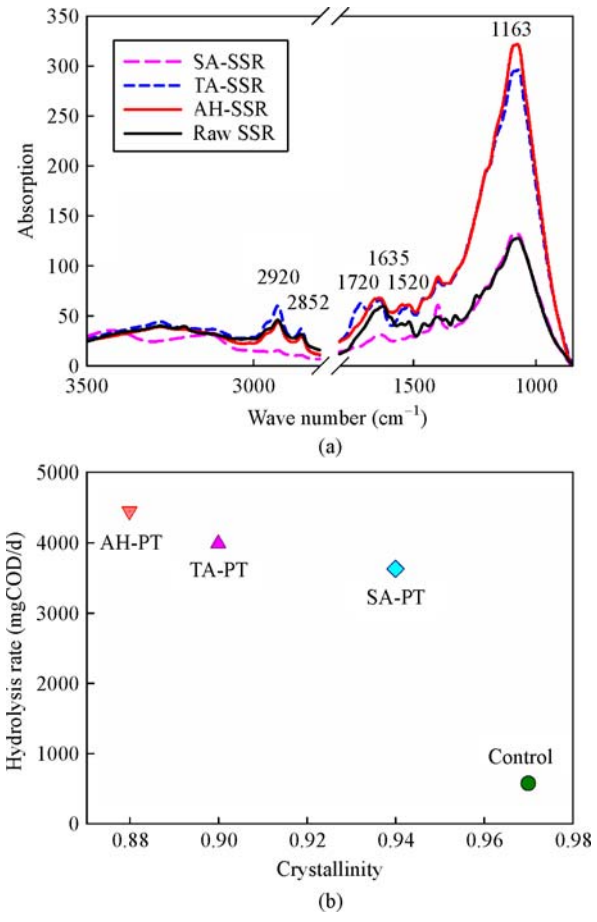

Fig. 1 Features of SSR structure changes before and after pretreated with AH, TA and SA: (a) for FTIR spectra characteristics, (b) for correlation between crystallinity and hydrolysis rate.

that obtained in the Control test (1615±112 mg COD). And a clear evidence was the enhanced activities of hydrolytic enzymes, a-glucosidase and protease (Fig. 2b), which was closely related with hydrolysis of carbohydrates and proteins.

Besides, it was worth noting that AH-PT was ranked first in SCOD concentration curve, indicating a better performance in organics dissolution than TA-PT and SA-PT co-fermenters. And the calculated hydrolysis rate in 24h shed more light on the pretreatment effects (Table 2). AH-PT performed a high hydrolysis rate of 4449 mg COD/d, 1.12-1.23-fold higher than that in TA and SA pretreated tests, and 7.8-fold higher than that in the Control test. TA-PT and SA-PT did not perform well, although a strong acidic or alkaline attack was more powerful in hydrolysis promotion as reported in literatures (Xiao et al., 2017). The inferior performance of TA in hydrolysis was consistence with the decrease of both carbohydrates and proteins 24 onwards as recorded in Fig. S1 (see it in supplementary material). It is most likely that the dramatic change in ecological environment in particular the sharp pH decline from 8.51 to 5.75 (Fig. S2) influenced the activity of hydrolytic microbes.

3.3 VFAs production and composition from co-fermenters

The VFAs production and composition in varied co-fermenters were shown in Fig. 3. The total VFAs concentration sharply climbed till a plateau at 120 h fermentation time. AH-PT reached the height of 5300±244 mg COD/L, ranking first among four groups, whereas TA-PT and SA-PT acquired a relatively lower summit, with 4517±367 mg COD/L and 3350±279 mg COD/L, respectively, 2.8–4.5 times compared with the Control test with 1178±22 mg COD/L only (Fig. 3c). It is clear that the VFAs production of the co-fermenters was greatly upgraded by SSR addition. And AH performed best among the three co-fermenters, which might benefited from efficient organics dissolution and sufficient hydrolysis as

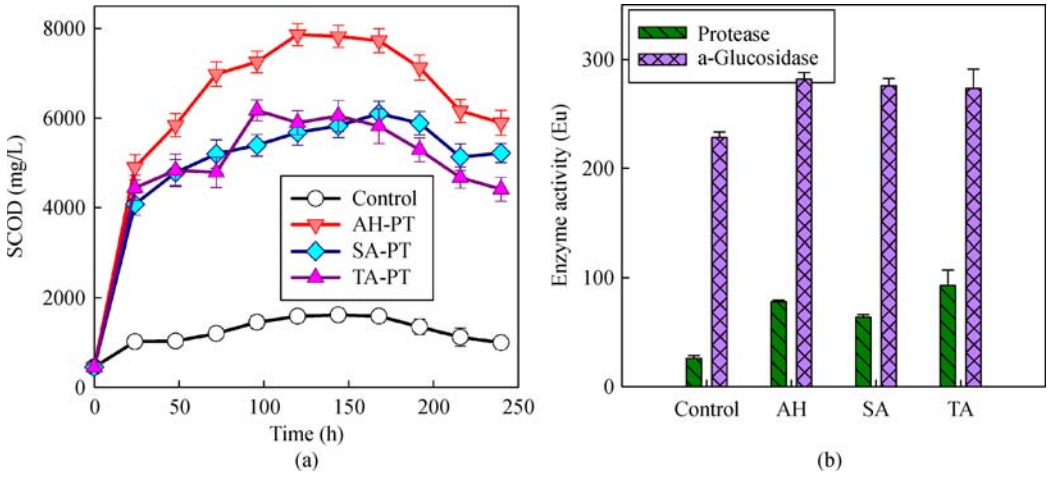

Fig. 2 Hydrolysis performance of WAS co-fermentation with different pretreated. SSR: (a) for SCOD release, (b) for enzyme activity at 120 h (Note: error bars represent standard deviation).

discussed before than the other strategies to promote VFAs production from the mixtures. For example, the highest VFAs contribution rate appeared in AH-PT with 401.2 mg COD/g SSR·L compared with only 340.2 mg COD/g SSR·L and 248.8 mg COD/g SSR·L in TA-PT and SA-PT, respectively (Table 2). However, the calculated similar k_{VFA} for AH-PT and TA-PT with 3.59 d^{-1} and 3.58 d^{-1}, respectively, suggested great potential of TA-PT in acidification promotion than in hydrolysis improvement of the co-fermenters (Fig. S3). After the peak, a decline due to consumption of VFAs to methane production was observed. And the changes in AH-PT and TA-PT were remarkable than that in SA-PT, which was consistence with the higher methane yield (Fig. S4).

And this promotion was generally attributed to the enhancement of the small molecular VFAs (C2 and C3). As depicted in Fig. 3b, both summit concentration of HAc and HPr in AH-PT ranked the first (with 2761±22 mg COD/L and 1656±12 mg COD/L, respectively). And the sum proportion of was 82.24%, 73.70% and 73.40% in AH-PT, TA-PT and SA-PT, respectively, 1.5–1.7 times enhancement than that in Control test. HAc was consistently most abundant as detailed in Fig. 3c, in line with results in WAS digestion alone and in other co-fermentation groups, such as mixed sludge + bagasse (Rughoonundun et al., 2012) and WAS + corn straw (Zhou et al., 2013).

3.4 Microbial community structure and function analysis

Fig. S5 showed the microbial community structure at phylum level. As depicted, three dominated phyla, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* shared a high proportion of the total bacterial sequences (69.8%–77.2%) in all four communities, similar with findings obtained in previous studies (Zhou et al., 2016). Besides, Clear changes were observed at the class level (Fig. S5b). For example, the relative abundance of *Clostridia*, a knowable major group of hydrolyzing and fermentative bacteria, increased notably after co-fermentation with SSR. As recorded, it increased by 239%–328% in SSR conditioning tests than that of the Control test with 8.6% only, and AH-PT held the highest record with 36.75%.

Figure 4a elaborated the relative bacteria abundance at genus level. As depicted, besides the microbial consortia (Ottowia (5.92%) and Levilinea (3.11%)) dominated in the Control test, which were common in wastewater treatment, a clear microbial diversity was enhanced in co-fermenters. For example, *Papillibacter* (Class *Clostridia*), which was known as a cellulolytic microbe (Defnoun et al., 2000), took up the largest proportion in AH-PT (3.53%), 1.52 to 1.98-folds higher than that in the TA-PT and SA-PT, but not be detected in the Control test. The abundance therefore provided evidence to the efficient lignocellulose decomposition of the AH-PT and was corresponded with its remarkable performance in hydrolysis. The dominated characteristic genera in the AH-PT were *Clostridium* in Class *Clostridia* including *Clostridium IV*, *Clostridium XIV and Clostridium sensu stricto* with a sum abundance of 9.85%, which was capable of waste decomposition and use glucose and glycerol to produce VFAs (Udaondo et al., 2017). *Rhodopseudomonas* (Shi and Yu, 2006), capable of VFAs and hydrogen production from glucose, was also abundant in AH-PT with 2.03%. Other hydrolytic and VFAs-producing bacteria were also detected such as *Catabacter* (Lau et al., 2007), Ilumatobacter (Matsumoto et al., 2013) and *Macellibacteroides* (Rout et al., 2017) with abundance of 2.28%, 2.1% and1.32%, respectively. *Clostridium* also peaked in SA-PT especially for *Clostridium sensu stricto* with a high abundance of 5.35%. The other dominated genus was *Rhodopseudomonas* with a proportion of 2.8%. Besides, acidophilic genus *Oscillibacter* (Table S1) was detected with a higher proportion of 2.72% than the other sets (Iino et al., 2007). A subtle difference was observed in TA-PT. For example, the activity of *Clostridium* was much lower than that in AH-PT and SA-PT, with only 1.16%, while the proteins hydrolysis related genus *Aquihabitans* in class *Clostridium* (Zhou et al., 2017) peaked in TA-PT with abundance of 4.17%, followed by *Acetoanaerobium* (3.08%) specialized in amino-acid-biodegradation. The other fermentation bacteria included *Macellibacteroides* (2.02%), *Proteiniclasticum* (1.88%), *Rhodopseudomonas* (1.17%) and *Petrimonas* (1.99%), which can form HAc, HPr, traces of iso-HVa and n-HBu with the addition of yeast extract, peptone and glucose (Hahnke et al., 2016). It seems that less genera related with organics dissolution (*Clostridium* and *Papillibacter* in particular) and more acid producing genera based on protein degradation (like *Acetoanaerobium, Proteiniclasticum and Petrimonas*) were collected

Table 2 Summary of performance parameters in WAS and pretreated SSR co-fermentation sets

Pretreatment Methods	SCOD		VFAs			
	Hydrolysis Rate (mg COD/d)	Peak Value (mg COD)	Peak Value (mg COD)	Contribution Value (mg COD/g·L)	k_{VFAmax} (1/d)	HAc + HPr proportion (%)
Control	570	1615±112[a]	1178±22	79.2	3.17	18.0 + 31.4
AH	4449	7857±244	5300±244	401.2	3.59	52.1 + 30.2
TA	3989	6041±349	4517±367	340.0	3.58	65.7 + 8.0
SA	3626	6094±274	3350±279	248.8	3.37	48.0 + 25.2

Note: a, Error bars represent standard deviation.

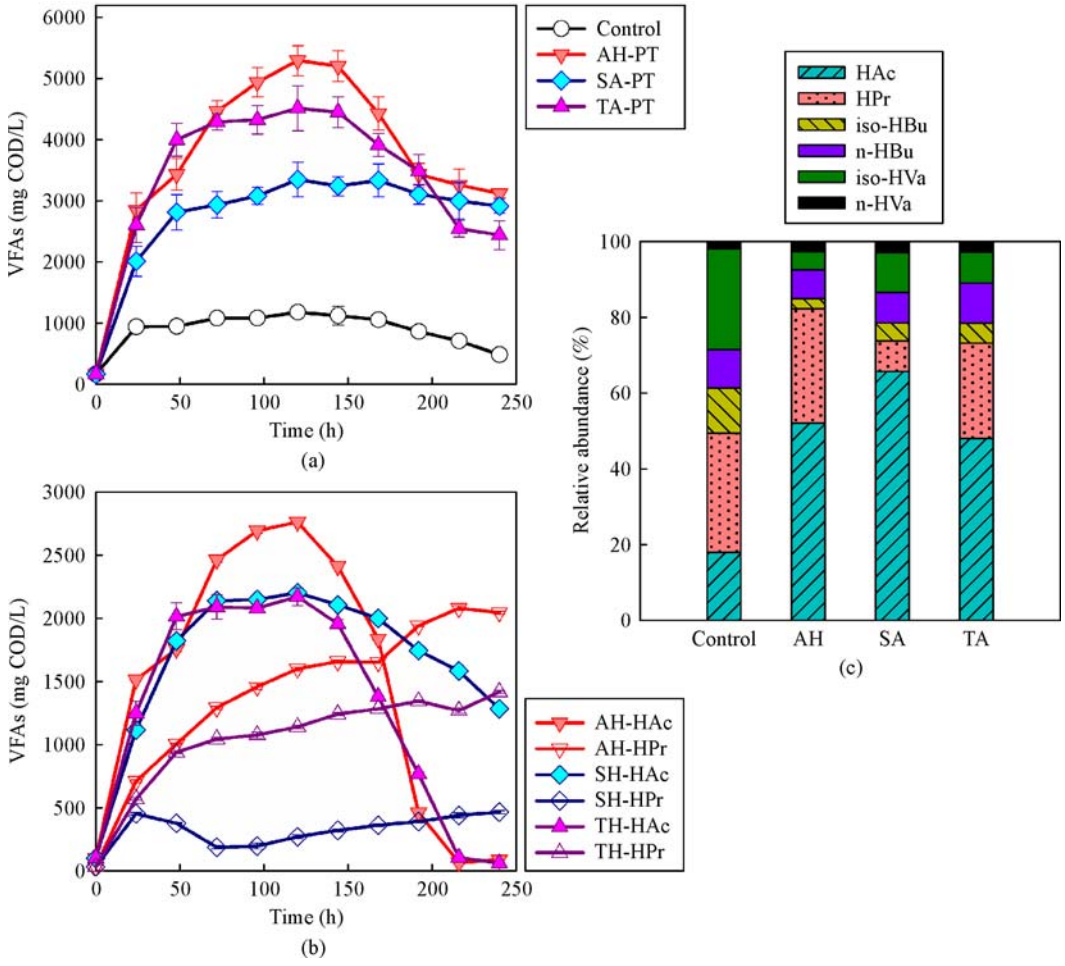

Fig. 3 Acidification performance of WAS co-fermentation with different pretreated SSR: (a) for VFAs production, (b) for HAc and HPr concentration variation, (c) for individual VFA percentage at 120 h (Note: error bars represent standard deviation).

in TA-PT. Consequently, it is a reasonable assumption that the insufficient performance of TA-PT was most likely rooted in inhibitory effects of the dramatic change in ecological environment especially for the pH value on genera related with organics dissolution and hydrolysis, which reduced the overall hydrolysis performance of TA-PT co-fermenters, and resulted in substrate insufficient for the subsequent acid production.

3.5 Interaction between environmental variables and microbial community

Figure 4b showed the relationship between functional microbial groups and environmental measurements. All contents except CI, pH were positively correlated with axis 1 with a 28.3% variance, and similarly, only the contents of CI showed positive interrelations with axis 2 (explaining 27.8% of the variance of the genera distribution). As the length of an arrow-line means the strength of relationship between the environmental variable and microbial community, Sca followed by Spr, hydrolysis rate and pH, with a longer and similar vector length, were proved to show equivalent intensity of influence on microbial community distribution. The same situation was with VFAs and HAc based on the proximate vector length.

From the results of the CCA, we can find that, a diversity of microbial community was obtained after co-fermentation with different pretreatments compared to a single species component in Control test, especially for AH-PT. Besides, correlations between typical measurements and functional genera were detected. For example, CI was co-related with several genera including *Clostridium IV*, *Clostridium XIV, Clostridium sensu stricto* and *Papillibacter*, which were all reported with capacity of organics decomposition and were enriched mainly in AH-PT. This was consistent with the observed results of structural characteristics and hydrolysis performance. Besides, VFA production showed a high positive correlation with several bacteria genera, such as *Macellibacteroides, Proteiniclasticum*, *Acetoanaerobium*, *Aquihabitans, Thermogutta* and *Petrimonas*, which were all active in AH-PT and TA-PT. While the organics dissolution related genera *Clostridium*

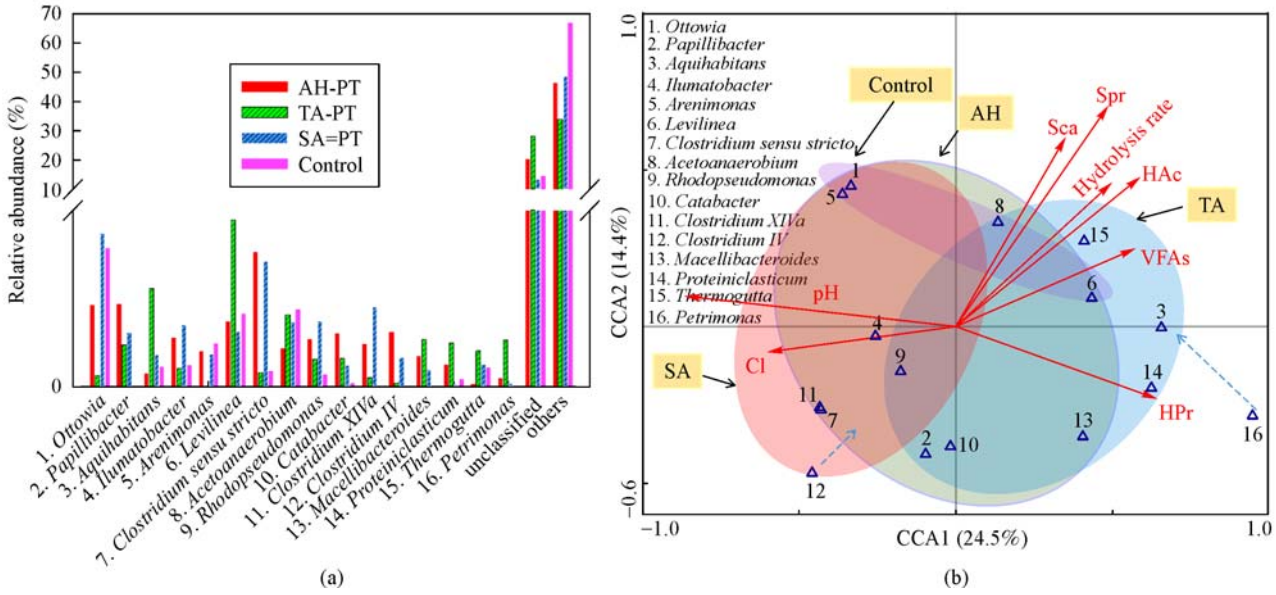

Fig. 4 Interaction mechanism of microorganism community and environmental conditions in co-fermentation sets of WAS with different pretreated SSR: (a) for taxonomic classification of pyrosequences from bacterial communities at the genus level; (b) for canonical correspondence analysis (CCA) between enriched genera and environmental variables [VFAs, acetic acid (HAc), propionic acid (HPr), crystal index (CI), hydrolysis rate, soluble proteins (Spr) and carbohydrates (Sca)].

and *Papillibacter* was enriched in AH-PT and SA-PT, but less in TA-PT. This, therefore, further proved the inference that TA-PT was indeed inferior in organics decomposition in hydrolysis phase but turned better in VFAs production in terms of functional microbial distribution. Instead, AH-PT, benefited from a mild initial habitat, a diversity of microbes covering both typical hydrolysis and acidification genera was cultivated, which laid solid foundation for its high VFAs yield. As for SA-PT, a medium level of characteristic genera abundance resulted in ordinary performance.

3.6 Significances and potential implementation

The more extensive use of soy sauce to cook in various countries certainly brings increasing production of SSR. As reported by an annual output of around 11 million tons with 0.67 kg SSR/ kg suspended solid, around 7.37 million tons SSR per year were urgent to be adequately managed (Zhang et al., 2017). In this study, the co-fermentation of SSR-WAS was confirmed to be a suitable solution of achieving both solid waste disposal and valuable resource reclamation. As recorded by the experimental data, it can produce up to 401.2 kg COD_{VFA}/t feedstock, equivalent to 2.96 million tons COD_{VFA}/year. Moreover, considering the observed individual VFA composition, where acetate and propionate take up 52.09% and 30.15%, it represents 1.54 and 0.89 million tons COD_{VFA}, respectively. It was reported that price of acetate and propionate as high as 0.45 and 1.01 €/kg respectively (Crutchik et al., 2018). Hence, allowing for a 60%– 70% discount due to insufficient purity of the produced acids, a price of 0.27–0.32 and 0.60–0.70 €/kg for acetate and propionate was reasonable. In this sense, 995.1–1114.3 million € was evaluated by acids recovery. Furthermore, based on the S/L ratio of 1:6 and the market price for 21% ammonium hydroxide of 0.11 €/kg, about 467.8 million for pretreatment should be deducted. Consequently, the acidogenic route an increase the market value potential up to 0.49–0.65 billion€/year instead of land occupation and potential environmental contamination of traditional extensive management of both WAS and SSR.

4 Conclusions

VFAs production was efficiently upgraded from WAS by co-fermentation with pretreated SSR, especially for the AH-PT test. The VFAs concentration and contribution peaked at 4517±367 mg COD/L and 401.2 mg COD /g SSR·L in the AH-PT test, respectively 4.49 times and 5.07 times higher than the sole WAS (Control) test. The promotion was attributed to strong lignocellulose decomposition in SSR (as depicted by fiber analyzer and FTIR) and the moderate pH change for functional consortium growth (as demonstrated by microbial community analysis and CCA results), which laid solid foundation for both hydrolysis and acidification performance of the co-fermenter. By comparison, the TA was inferior in the VFAs yield, which might be rooted in the dramatic change in the pH value during early hydrolysis phase, which

resulted in poor organics dissolution and inadequate SCOD release. According to the findings of this study, it is possible to estimate a potential market value of 0.49–0.65 billion €/year, showing promise of resource recovery from both WAS and SSR.

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