

Effect of influent pH on hydrolytic acidification performance and bacterial community structure in EGSB for pretreating crotonaldehyde manufacture wastewater after ozonation

Tao Liu, Zhiqiang Shen, Chunyu Zhang, Yudong Song, Jie Li, Zongpu Yang, Guangqing Song, Zhenfeng Han and Yuexi Zhou

ABSTRACT

The objective of this work was to evaluate the effect of influent pH on the hydrolytic acidification (HA) performance and microbial community structure in an expanded granular sludge bed (EGSB) pretreating crotonaldehyde manufacture wastewater (CMW) after ozonation. The results showed that higher chemical oxygen demand (COD) removal rate (40.1%) and acidification degree (27.6%) were obtained at pH 8.0 than those at pH 6.0 and pH 4.0. The concentration of extractable extracellular polymeric substance (EPS) in the sludge gradually decreased with the pH decreasing from 8.0 to 4.0. A similar change was also observed for the concentration of total volatile fatty acids (TVFA) in the effluent. The optimal detoxification efficiency by the HA process was obtained at pH 8.0, with higher removal efficiency (all higher than 90%) of the main toxic pollutants (crotonaldehyde, 5-formyl-6-methyl-4,5-dihydropyran, etc.) and higher anaerobic biodegradation rate (44.5%) in biochemical methane potential (BMP) assay. Among the predominant genera, the *Acinetobacter* and *Pseudomonas* were possibly related to biodegradation of pollutants, since their higher relative abundance also coincided with the better performance of the HA process at pH 8.0.

Key words | bacterial community structure, crotonaldehyde manufacture wastewater, detoxification, hydrolytic acidification, pH

Tao Liu
Jie Li
Zongpu Yang
School of Environmental and Municipal Engineering,
Lanzhou Jiaotong University,
Lanzhou 730000,
China

Tao Liu
Zhiqiang Shen
Yudong Song
Guangqing Song
Yuexi Zhou (corresponding author)
State Key Laboratory of Environmental Criteria and Risk Assessment,
Chinese Research Academy of Environmental Sciences,
Beijing 100012,
China
E-mail: zhouyuexi@263.net

Chunyu Zhang
Jilin Petrochemical Company, Ltd,
PetroChina, Jilin 132022,
China

Zhenfeng Han
College of Environmental Science and Engineering,
Hunan University and Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education,
Changsha, Hunan 410082,
China

INTRODUCTION

Crotonaldehyde is an important raw material in the manufacturing process of many chemical products, such as sorbic acid, butanol, butyraldehyde and butanoic acid, etc. Among them, sorbic acid is known as an efficient but low-toxicity food preservative, which has been the leading preservative in the global food sector over the past 30 years (Ohtsuki *et al.* 2012). According to the statistics of the China National Information Infrastructure (CNII), the annual production of sorbic acid has been up to 80,000 tons, which accounts for 86.2% of the global production in 2017. Accordingly, crotonaldehyde, as the main raw

material of sorbic acid, is also produced in large quantities. However, the crotonaldehyde manufacture wastewater (CMW, wastewater produced by the crotonaldehyde manufacture process) contains a high concentration of complex organics, including refractory and/or toxic pollutants. Song *et al.* (2017) indicated that CMW showed strong inhibition and toxicity to aerobic and anaerobic microorganisms, due to high-strength toxic components such as crotonaldehyde, (E,E)-2,4-hexadienal, 5-formyl-6-methyl-4,5-dihydropyran, etc. If discharged directly into the biological treatment units without pretreatment, CMW could adversely affect the microbial

activities. Therefore, it is of great necessity to study the pretreatment methods of CMW.

The pretreatment technologies for CMW have been scarcely reported in the literature. In China, incineration was applied to treat this type of wastewater, which was helpful for energy recovery, but also brought secondary pollution problems of waste gas (Zhao *et al.* 2016). Ozonation has been widely applied to pretreat the high-strength toxic organic wastewater because it can break the refractory organics effectively and causes no secondary pollution (Zhu *et al.* 2017; Shah *et al.* 2017). According to a previous report by our research group (published in a Chinese journal), the inhibition rate of specific methanogenic activity (SMA) of the CMW decreased from 82.0% to 47.3% after ozonation. It was clearly demonstrated that ozonation could effectively decrease the toxicity of CMW. However, the CMW after ozonation was still very toxic to microorganisms, which could be detrimental to the common biological processing units. Besides, it would cost too much to use ozonation individually to further improve the detoxification efficiency of CMW. Therefore, the hydrolytic acidification (HA) process was employed as the further pretreatment method following ozonation in this study.

The HA process was the first stage of the two-stage anaerobic digestion process, which involved the separate hydrolytic acidification stage and methanogenic stage in order to protect and/or enrich individual microbial communities in each stage to optimize their activities and thus to increase the stability of the anaerobic digestion process (Dareioti *et al.* 2014). It was reported that microorganisms of the HA stage had the ability to break refractory organic pollutants, and resolve the inhibiting and/or toxicity issue of contaminants for the following biological process (Genschow *et al.* 1996; Wu *et al.* 2016). Therefore, the HA process has been widely used for the pretreatment of wastewater, including pharmaceutical (Oktem *et al.* 2006), tannery (Wang *et al.* 2014), printing and dyeing (Wu *et al.* 2007) and especially petrochemical wastewater (Wu *et al.* 2016; Song *et al.* 2017).

There are many reports about the application of the HA process pretreating toxic substance-containing industrial wastewater; however, most of them focused on the effect of organic loading rate (OLR) (Wang *et al.* 2014; Song *et al.* 2017). Song *et al.* (2017) utilized EGSB to pretreat CMW and the optimum detoxification performance occurred at an OLR of 3.89 g COD/L·d. Wang *et al.* (2014) indicated that the average biochemical oxygen demand/chemical oxygen demand (BOD/COD) of the tannery wastewater increased from 0.38 to 0.56 after the HA treatment under optimal

OLRs. In fact, apart from the OLR, influent pH and temperature and so on are also key parameters influencing the operational efficiency of the HA process (Akao *et al.* 2007). Nevertheless, the effect of pH on the HA process pretreating highly toxic wastewater like CMW has been scarcely reported. It had been proved by various researches that transformation of influent pH not only affected COD removal efficiency, volatile fatty acids (VFA) yield and compositions, but also changed the abundance and diversity of microbial communities (Feng *et al.* 2009; Liu *et al.* 2012). Besides, different dominant bacteria had different abilities to resist and degrade toxic substances, thus pH might affect acidification and detoxification efficiency in the HA process (Meng *et al.* 2015; Yang *et al.* 2015). Thus, it is of great significance to evaluate the pretreatment effect of CMW at different pHs.

The purpose of this study was to evaluate the effect of influent pH on the acidification and detoxification efficiency of the HA process. In order to evaluate the reactor performance under different influent pHs, the changes of wastewater qualities (i.e. COD, VFA, specific pollutants, etc.), sludge characteristics (i.e. extracellular polymeric substance (EPS)) and reactor operation condition (i.e. oxidation reduction potential (ORP)) were monitored. Besides, characterization of organic compositions was undertaken and the biochemical methane potential (BMP) in the influent and effluent under different pHs was identified. Furthermore, differences of microbial communities in the HA process were characterized by Illumina MiSeq sequencing.

MATERIALS AND METHODS

Characteristics of the wastewater

The wastewater in this study was obtained from crotonaldehyde processing units in northern China, which was pretreated by ozonation due to containing organic matter that was highly concentrated and inhibitory to microorganisms. The characteristics of the CMW after ozonation are listed in Table 1.

Experimental setup

An expanded granular sludge bed (EGSB) was employed to cultivate anaerobic granular sludge, which was made of Plexiglas with an internal diameter of 35 mm and height of approximately 1,600 mm. The reactor was composed of a reaction zone (1.4 L) and sedimentation zone (0.6 L). The temperature was maintained at 25 ± 1 °C by a thermostatic

Table 1 | The characteristics of the CMW after ozonation

Parameter	pH	COD (mg/L)	TOC (mg/L)	TDS (mg/L)	SS (mg/L)
Value	2.42 ± 0.3	66,045 ± 3,212	27,064 ± 1,023	12,354 ± 246	95 ± 13

water bath and the hydraulic retention time (HRT) was 24 h. The up-flow velocity (V_{up}) was kept stable at 1.73 m/h by effluent recirculation with a peristaltic pump. The inoculated sludge was taken from the excess sludge of the Integrated Petrochemical wastewater treatment plant and cultivated by CMW with influent COD of 8,000 mg/L. The effect of influent pH on the HA process was investigated once the reactor had stabilized. The influent pH was adjusted using HCl or NaOH solutions at the following pH values: 4.0, 6.0 and 8.0. NH_4Cl and KH_2PO_4 were added in the influent as nitrogen and phosphorus, respectively, the nutrient ratio (COD: N: P) used was 200: 5: 1, and NaHCO_3 was added to set an influent alkalinity of 1,000 mg CaCO_3/L .

Analytical methods

The COD, total dissolved solids (TDS), suspended solids (SS) and volatile suspended solids (VSS) were measured according to the standard methods (SEPA 2002). Total organic carbon (TOC) was determined by a TOC analyzer (Shimadzu TOC-V_{CPH}, Japan). Oxidization reduction potential (ORP) and pH were measured by a portable meter (Mettler Toledo FE20, Switzerland).

BMP assay of influent and effluent of different pH were measured in 250 ml culture bottles with distilled water as control (Owen *et al.* 1979). The biodegradation rate (R) of influent and effluent from different pH on the anaerobic granular sludge, which came from the methanogenic reactor of a brewery, was calculated by the following Equation (1).

$$R_i = \frac{S_i - S_0}{T} \times 100\% \quad (1)$$

where S_i and S_0 represent cumulative methane production of the experiment and control group, respectively, mL. T represent the theoretical maximum methane production.

The change of organic pollutants in the influent and effluent was characterized by gas chromatography with mass spectrometry (GC/MS) (Agilent 7890/5975c, USA) (Song *et al.* 2017). VFA were determined using gas chromatography equipped with a flame ionization detector (Agilent 7890, USA), and the acidification degree (AD) was calculated according to the description of Song *et al.* (2017). A heat extraction method was modified to extract extracellular polymeric substance (EPS) from the sludge

sample using a Ringer's solution as buffer salt solution (Kim *et al.* 1998). The EPS were analyzed for TOC, protein (PN), polysaccharide (PS) and deoxyribonucleic acid (DNA). The PN content was determined using a BCA protein kit (Sangon, China). The PS content was determined with the phenol-sulphuric acid method using glucose as the standard (Dubois *et al.* 1956). The DNA content was analyzed by diphenylamine method using calf thymus DNA as the standard (Giles & Myers 1965).

Microbial population analysis

The microbial communities of the sludge samples under different pH were investigated by Illumina high-throughput sequencing. Bacterial DNA was extracted from sludge samples using the E.Z.N.A.[®] soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. The V3-V4 region of the bacteria 16S ribosomal RNA (rRNA) gene were amplified by polymerase chain reaction (PCR) (95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) using primers 515 F (5'-GTGCCAGCMGCCGCGG-3') and 907 R (5'-CCGTCGAATTCMTTTRAGTTT-3'), where the barcode is an eight-base sequence unique to each sample. PCR reactions were performed in triplicate using 20 µL mixture containing 4 µL of 5× FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the Axy-Prep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2×250) on an Illumina MiSeq platform according to the standard protocols.

RESULTS AND DISCUSSION

Effects of influent pH on COD removal and ORP

In order to evaluate the effect of influent pH on the HA of CMW, influent pH was gradually decreased from 8.0 to 4.0,

while the influent COD was approximately 8,000 mg/L and hydraulic retention time (HRT) was 24 h. Figure 1(a) shows the changes in COD removal rate under different pH. When influent pH was 8.0, the COD removal rate was relatively stable with an average of 40.1%. However, when further decreasing the pH to 6.0 and 4.0, the COD of the effluent was unstable and the average COD removal rate was 30.3% and 30.8%, respectively. The optimum pH for COD removal was at pH 8.0 in this study, under which the VSS was 9.3 mg/L, which was higher than at pH 6.0 (7.1 mg/L) and pH 4.0 (6.3 mg/L). It revealed that microbial activity was superior to other conditions, so that more organics were used for anabolism and VFA production (Trisakti *et al.* 2015). Besides, there might exist the production of CH₄ and CO₂, which could contribute to the removal of COD (Lu *et al.* 2015).

Figure 1(b) shows the changes of ORP under different influent pHs. ORP increased gradually from -262 mV to 83 mV with influent pH decreasing from 8.0 to 4.0, possibly due to the inhibition of microorganisms, and affecting the metabolism of microorganisms. A similar result was found previously by Zhu *et al.* (2011), who reported that the ORP increased from -420 mV to -380 mV with the pH decreasing from 5 to 4.6. Blanc & Molof (1973) indicated

that an increasing ORP correlated to inhibited or decreasing levels of digester performance. Therefore, the ideal pH and ORP levels herein created a more advantageous circumstance for microorganisms. This provides some evidence that an alkaline condition created a more enabling environment for microorganisms and contributed to a higher COD removal rate.

Effect of influent pH on VFA yields and compositions

The pH plays an important role in the VFA yields and compositions in the HA process (Liu *et al.* 2012). Figure 2(a) shows the change of total volatile fatty acids (TVFA) under different influent pHs. The concentration of TVFA in effluent decreased from 1,420.65 mg/L to 661.38 mg/L with the influent pH decreasing from 8.0 to 4.0. The average AD of the influent and effluent was 4.4%, 27.6% at pH 8.0, respectively, indicating that the AD of CMW could be improved obviously by the HA process. However, the AD of the effluent gradually dropped to 19.0% and 11.0% with influent pH dropping to 6.0 and 4.0, respectively. It should be concluded that more VFA was produced at pH 8.0. Similarly, Liu *et al.* (2012) reported that TVFA yield gradually increased with pH increasing from 3.0 to 9.0, while it

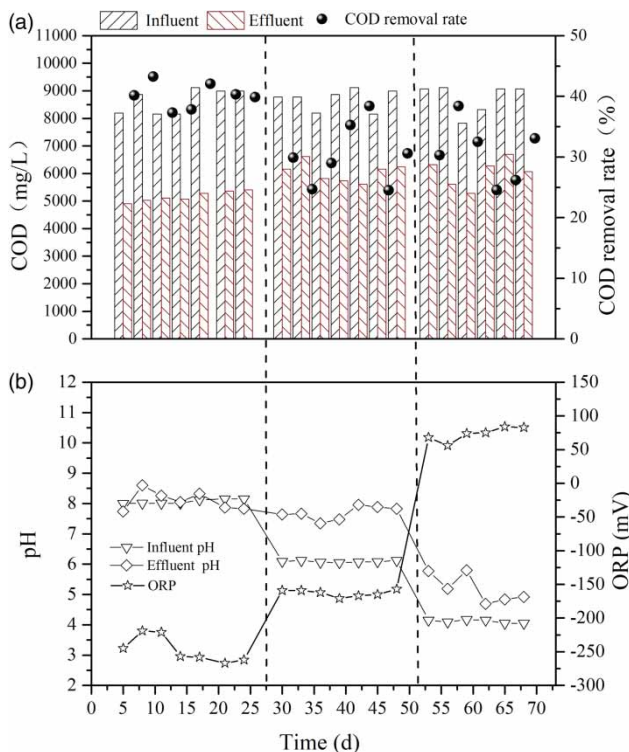


Figure 1 | Effect of influent pH on COD removal rate (a) and ORP (b).

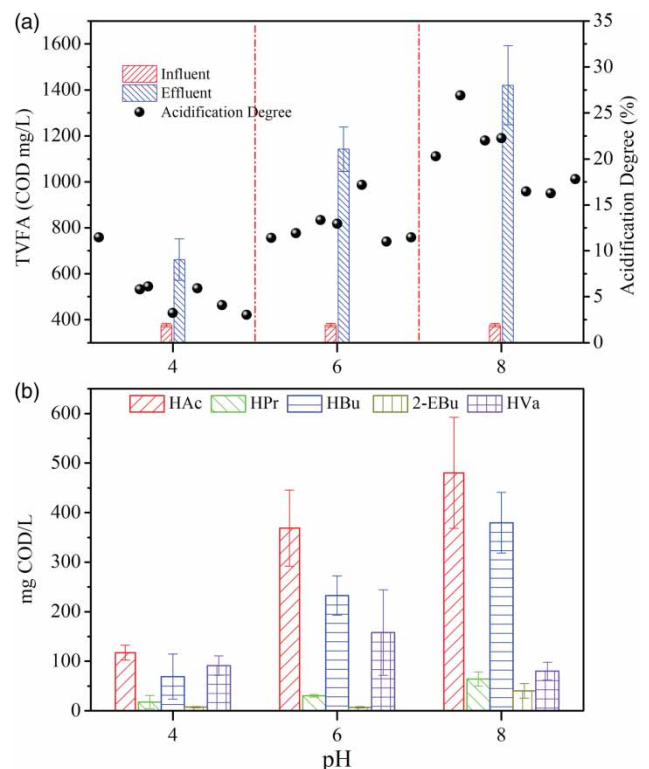


Figure 2 | Effect of influent pH on the VFA yield (a) and VFA compositions (b).

gradually decreased with the pH further increasing. This might be because microorganism activity was inhibited at too high or too low pH. Chen *et al.* (2007) also reported the VFA concentration under alkaline conditions was significantly higher than that under acidic and neutral conditions.

It has been reported that pH affected not only the yields of TVFA, but also the percentage of the single VFA (Feng *et al.* 2009). In this study, as seen in Figure 2(b), five VFA, including acetic (HAc), propionic (HPr), n-butyric (HBu), 2-ethyl butyric (2-EBu) and n-valeric (HVa), were observed in three conditions. At pH 8.0, the main VFA were HAc (42.6%) and HBu (36.3%), and there was little HPr (6.1%), 2-EBu (3.8%) and HVa (7.6%). Moreover, the percentage of HAc and HBu in TVFA gradually reduced to 25.27% and 24.3% with pH decreasing from 8.0 to 4.0. However, the concentration of HVa rose firstly and then fell, but its percentage in TVFA gradually rose from 7.8% at pH 8.0 to 32.06% at pH 4.0. Similarly, Liu *et al.* (2012) reported that the HAc and HBu percentage under alkaline conditions was higher than that under low pH conditions, but the HVa percentage decreased from 23.02% to 10.29% with pH increasing from 5.0 to 10.0. However, Yu & Fung (2003) found that the percentage of HAc and HBu gradually increased with the pH increasing, but found no significant change for the percentage of HVa. The difference in VFA composition under different pH between the aforementioned reports and this study was possibly ascribed to the different substrates, which were gelatin-rich wastewater in their reports and CMW in this study, respectively.

Characterization of organic compositions and BMP in influent and effluent

The influent and effluent of the HA process under different pH were analyzed by GC/MS. The influent consisted of a fairly complex composition, mainly including crotonaldehyde, 3-hydroxybutyraldehyde, 2,4-dimethyl-1,3-dioxane, 5-formyl-6-methyl-4,5-dihydropyran, 2-amino-1,3,4-thiadiazole and 4-isopropoxybutan-2-on. The removal rate for the organic matter identified by GC/MS at different pH is listed in Table 2. The crotonaldehyde removal rate had no obvious change at pH 8.0 and pH 6.0, but it decreased from 96% at pH 6.0 to 77% at pH 4.0. The removal rate of other main organics, such as 2,4-dimethyl-1,3-dioxane, 5-formyl-6-methyl-4,5-dihydropyran and 2-amino-1,3,4-thiadiazole, gradually decreased with decreasing pH from 8.0 to 4.0, which might result from the destruction of microbes at pH 4.0.

Table 2 | Removal rate for the compounds identified by GC/MS under different influent pHs

Number	Compound	Removal rate ^a (%)		
		pH 4.0	pH 6.0	pH 8.0
1	Crotonaldehyde	77	96	98
2	3-hydroxybutyraldehyde	90	>99 ^b	>99
3	2,4-dimethyl-1,3-dioxane	69	86	90
4	Pentane-1,4-diol	>99	>99	>99
5	(E,E)-2,4-hexadienal	>99	>99	>99
6	Isopropyl acetate	>99	>99	>99
7	5-ethyl-3-methyloxolan-2-one	>99	>99	>99
8	5-formyl-6-methyl-4,5-dihydropyran	64	87	>99
9	2-methyl-benzaldehyde	>99	>99	>99
10	6-methyl-2-heptanol	>99	>99	>99
11	Ethyl sorbate	>99	>99	>99
12	2-amino-1,3,4-thiadiazole	26	36	96
13	Methyl (R)-3-hydroxybutyrate	>99	>99	>99
14	2,4-dimethyl-3-pentanone	80	>99	>99
15	4-isopropoxybutan-2-on	77	>99	>99

^aWith respect to peak area.

^bThe organic matter was not detected in the effluent.

The BMP is a measurement of substrate biodegradability determined by monitoring cumulative methane production from a sample that is anaerobically incubated in a chemically defined medium (Owen *et al.* 1979). In this study, the BMP was applied to evaluate the detoxification efficiency of the HA process. Figure 3 shows the BMP of the influent and effluent under different pH. Methane production rate within 24 h at pH 8.0 was higher than in other conditions, which might be because more VFA were produced from the HA process and utilized by methanogens. In addition, the cumulative methane production of different groups was in the order of pH 8.0 > pH 6.0 > pH 4.0 > influent, and the biodegradation rate was 44.46%, 39.83%, 32.72%, 13.15%, respectively. The result of the BMP assay demonstrated that the HA process had a higher detoxification efficiency at pH 8.0, which created an enabling environment for microorganisms and was beneficial to degradation and transformation of toxicants in CMW.

Variations of EPS contents under different influent pHs

The effect of influent pH on EPS is shown in Figure 4. Extractable EPS (shown as TOC) gradually reduced from

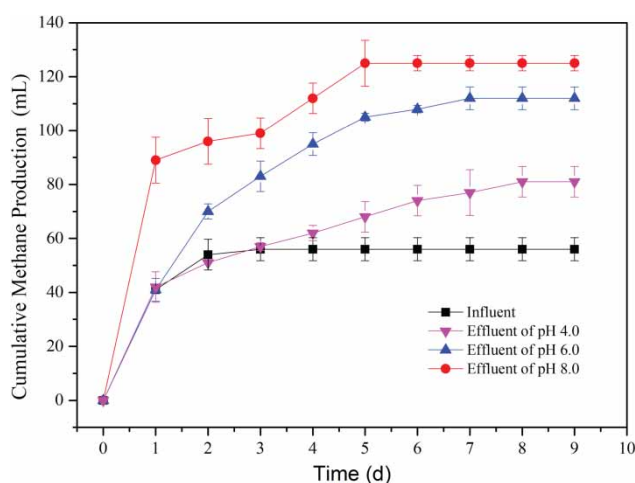


Figure 3 | The BMP of influent and effluent under different influent pHs.

122.3 to 77.6 mg/(g-VSS) with influent pH decreasing from 8.0 to 4.0. However, the content of PN, PS and DNA in EPS were similar under pH 8.0 and pH 6.0, which were both lower than that under pH 4.0. The EPS in the activated sludge come from the natural secretions of bacteria, cell surface material shedding, cell autolysis and hydrolysis products from wastewater (Wilén *et al.* 2003). It has been reported that EPS play a significant role in protecting cells against environmental changes in pH, water quality, salt content, hydraulic pressure and toxic compounds (Guibaud *et al.* 2005; Henriques & Love 2007). In this study, the content of EPS was also positively correlated with that of TVFA, which was similar to that reported by Li *et al.* (2018). Besides, some studies have demonstrated that a hostile environment might affect the bacterial metabolic

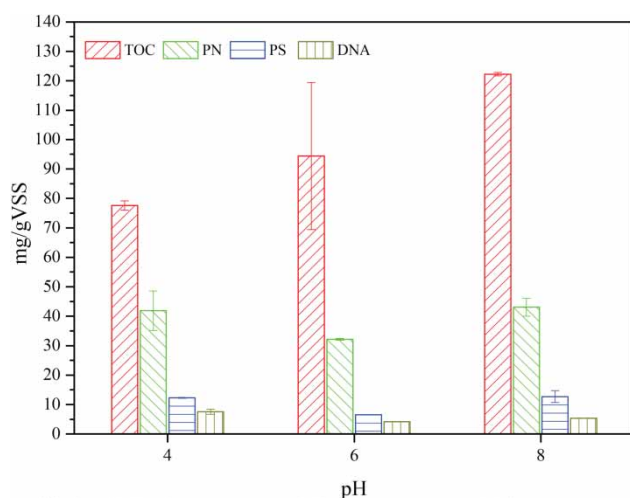


Figure 4 | Variations of EPS in the activated sludge under different influent pHs.

activity and then reduce the EPS production (Hsieh *et al.* 1994). It deserves to be noticed that the content of PN was higher in EPS production, revealing that PN played a more significant role in protecting cells from the harsh external environment. The observation was also well reported by another report (Sheng *et al.* 2005), in which PN was the major component in EPS protecting cells against the harmful effects by limiting diffusion and/or by chemical binding. According to Ramirez-Nuñez & Romeromedrano (2011), cell autolysis occurred at an acidic pH (5.4). Therefore, the higher content of DNA in EPS at pH 4.0 might be due to more serious cell autolysis, which caused the release of DNA under a hostile environment.

Microbial community analysis

Illumina MiSeq sequencing was used to reveal the differences in microbial community structure among sludge samples of different pH. The effective reads at pH 4.0, 6.0 and 8.0 were 45,402, 50,850 and 45,309, respectively. The parameters related to the abundance and diversity of microbial communities are shown in Table 3. The coverage of sludge samples were all above 0.99, which could well reflect the real profile of microorganisms (Song *et al.* 2017). The OTUs in the sample of pH 8.0, pH 6.0 and pH 4.0 were 323, 339 and 295 respectively. The Shannon indexes at pH 8.0, pH 6.0 and pH 4.0 were 3.20, 3.32 and 3.36, respectively. The phylum classifications of sequences from three sludge samples are shown in Figure 5(a). It was shown that *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Thermotogae* were the dominant phyla in three sludge samples, accounting for more than 69.24% of total bacteria. *Bacteroidetes* existed widely in the anaerobic digester reactor, which was able to degrade a variety of complex organic macromolecules, including proteins and carbohydrates, in the fermentation system (Kratat *et al.* 2011). The proportion of *Bacteroidetes* reached the maximum (44.72%) at pH 6.0, and was only 13.04% at pH 4.0. Similarly, Zheng *et al.* (2013) indicated that the optimum pH of *Bacteroidetes* was 6.5–8.5. The phylum *Firmicutes* and *Proteobacteria* had all been found previously in

Table 3 | Different indices of community diversity for different samples

Sample	Reads	OTUs	Chao 1	Shannon	Coverage
pH 4.0	45,402	295	334.81	3.36	0.9989
pH 6.0	50,850	339	342.90	3.32	0.9997
pH 8.0	45,319	323	349.45	3.20	0.9990

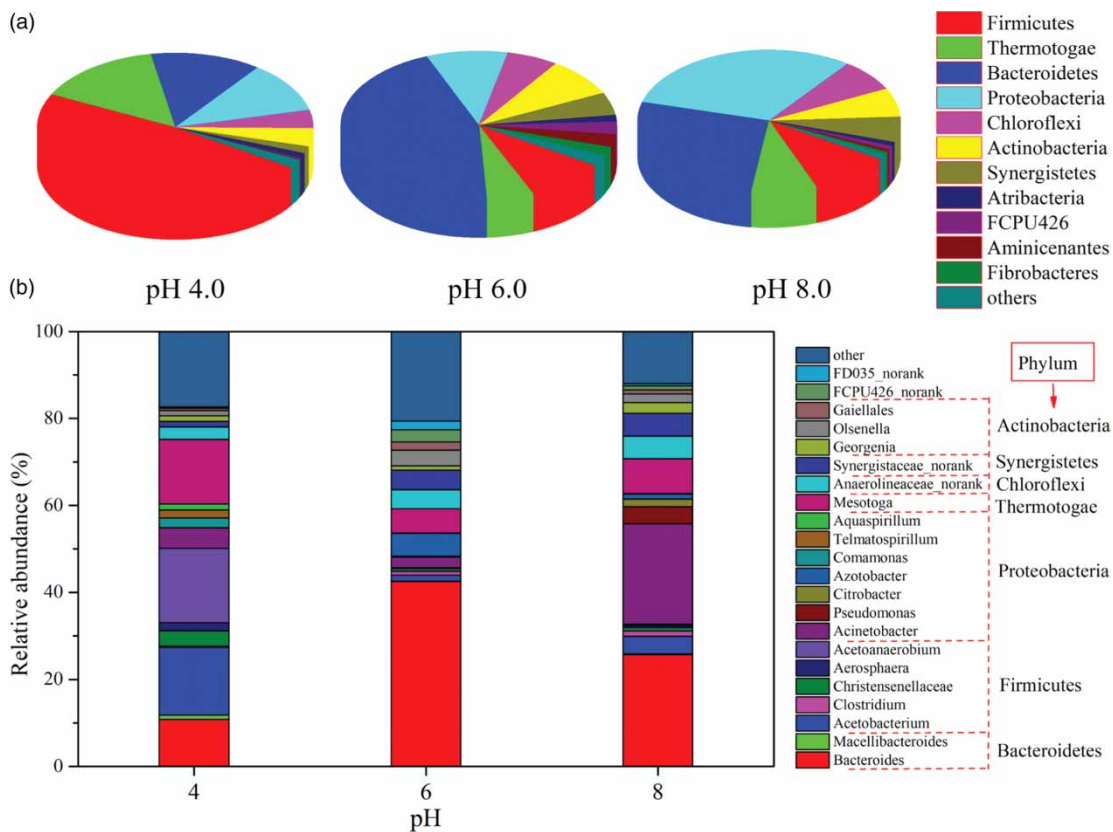


Figure 5 | The relative abundance of major bacterial sequences (>1% in any sample) from three samples at the (a) phylum level and (b) genus level.

highly efficient anaerobic digestion reactors, and were also reported to be dominant phyla in the actual petrochemical wastewater treatment systems (Hasan *et al.* 2006; Zheng *et al.* 2013; Yang *et al.* 2015). In this study, the proportion of *Firmicutes* reached the maximum (48.16%) at pH 4.0 and was much higher than that under other conditions. Hasan *et al.* (2006) revealed that *Firmicutes* could produce extracellular enzymes to survive in the toxic environment, which is important for hydrolysis and utilization of the refractory pollutants in petrochemical wastewater. The proportion of *Proteobacteria* reached the maximum (30.96%) at pH 8.0, and decreased to 9.58% and 11.09% at pH 6.0 and 4.0, respectively. Feng *et al.* (2009) reported that *Proteobacteria* formed a dominant community structure when a mixture of waste sludge and carbohydrate was fermented at pH 8.0. *Thermotogae* are a kind of heterotrophic anaerobes bacteria, which would use carbohydrates (Nesbo *et al.* 2006). The proportion of *Thermotogae* reached the maximum (14.81%) at pH 4.0, and was 8.00% and 5.55% at pH 8.0 and 6.0, respectively. Meng *et al.* (2015) indicated that *Thermotogae* had the ability to survive in an amoxicillin manufacture wastewater treatment system.

Therefore, the different adaptive ability of *Firmicutes* and *Proteobacteria* in a complex environment affected their abundance at different pH. Besides, *Actinobacteria*, *Chloroflexi*, and *Synergistetes* were also important phyla in the HA process of petrochemical wastewater, and their relative abundance at pH 4.0 was less than at pH 6.0 and 8.0. Phylum *Actinobacteria*, a kind of strict anaerobes, are known for their ability to tackle aromatic compounds (Kratat *et al.* 2011), and it might be related to degradation of aromatic compounds in CMW. Phylum *Chloroflexi* had the ability to degrade carbohydrates, starch, amino acids, and even the recalcitrant microcrystalline cellulose to acetate and other short-chain fatty acids. (Yamada *et al.* 2005). In addition, Maspolim *et al.* (2015) indicated that the optimum pH of *Chloroflexi* was 6.0–7.2. Phylum *Synergistetes* were previously reported in various anaerobic environments, and could oxidize saturated fatty acids with 4–10 carbon atoms (e.g. iso-butryate) to acetate (Rivière *et al.* 2009).

Standing on the genus level allows us to further infer the functions of the community. As shown in Figure 5(b), the dominant bacteria communities were *Bacteroides*, *Acinetobacter*, *Mesotoga*, *Acetobacterium* and *Acetoanaerobium* in

three samples. In recently research, it was reported that *Bacteroides* were hydrolysis/fermentative bacterium, and most of them produced acetic acid, propionate acid as the major end-products (Grabowski *et al.* 2005; Hatamoto *et al.* 2014). The proportion of *Bacteroides* was 26.0% at pH 8.0 and 43.0% at pH 6.0, but decreased to 11.0% at pH 4.0. Maspolim *et al.* (2015) indicated that optimum pH range of *Bacteroides* was 7.0–8.5. Ma *et al.* (2015) reported that *Acinetobacter* was capable of degrading lipid-containing wastewater. Moreover, various studies have reported that *Acinetobacter* has the potential ability to degrade toxic substances, such as 3, 5, 6-trichloro-2-pyridinol, azo dyes, etc. (Zhang *et al.* 2018; Zhu *et al.* 2018). In this study, the proportion of *Acinetobacter* at pH 8.0 was 23.1% and was well above other conditions. There were more substances utilized by the microorganism with the degradation of toxicants, which possibly explained the better detoxification at pH 8.0. The most dominant genera at pH 4.0 were *Bacteroides* (10.8%), *Mesotoga* (15.00%), *Acetobacterium* (16.00%) and *Acetoanaerobium* (17.00%). *Mesotoga*, belonging to *Thermotogae*, played a crucial ecological role in ecosystems polluted by aromatic compounds under anaerobiosis, including polychlorinated biphenyl, chlorophenols, polycyclic aromatic hydrocarbons, etc. (Hania *et al.* 2011). Both *Acetobacterium* and *Acetoanaerobium*, belonging to *Firmicutes* phylum, had the ability of acetate production from H₂ and CO₂ (Liu *et al.* 2015; Omar *et al.* 2018). Savant *et al.* (2006) also reported that *Acetobacterium* was capable of dechlorinating C1 and C2 compounds, such as dichloromethane, trichloroethane and trichloroethene, etc. The stronger ability to survive in adverse environment made higher abundance of *Mesotoga*, *Acetobacterium* and *Acetoanaerobium* at pH 4.0. Moreover, *Anaerolineaceae_norank*, *Olsenella*, *Azotobacter* and *Pseudomonas* were also important genera in EGSB reactor, where their proportion at pH 8.0 was more than at other conditions. *Anaerolineaceae_norank* (belonging to *Anaerolineaceae* family and *Chloroflexi* phylum) have the ability to utilize carbohydrates and proteinaceous carbon sources under anaerobic conditions (Yamada *et al.* 2005). Most of *Azotobacter*, belonging to *Proteobacteria* phylum, was adversely affected with the pH decreasing, and neutral pH has no inhibitory effect on their growth, and 80.8% of the isolates were tolerant to pH 10.0 (Aleem *et al.* 2003). It is worth mentioning that *Pseudomonas*, belonging to *Proteobacteria*, survived at pH 8.0 and had the ability to degrade refractory cyanide and aromatic compounds (Liu *et al.* 2015; Dong *et al.* 2016), which is beneficial to the degradation of aromatic compounds in CMW.

CONCLUSION

The influent pH could obviously influence the performance of the HA process pretreating CMW. The average AD of the effluent decreased from 27.6% to 11.0% with the influent pH decreasing from 8.0 to 4.0, and the concentration of extractable EPS in sludge also decreased with the decrease of influent pH. The removal rate of toxic pollutants and the result of the BMP assay under different pHs showed that better detoxification efficiency was obtained at pH 8.0. Among the dominant genera, the *Acinetobacter* and *Pseudomonas* were possibly related to biodegradation of toxicants, and the higher relative abundance of them also coincided with the higher performance of HA process at pH 8.0.

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