Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Inhibitory effect of high phenol concentration in treating coal gasification wastewater in anaerobic biofilter

Yajie Li[1,2], *Salma Tabassum*[2], *Chunfeng Chu*[2], *Zhenjia Zhang*[2,*]

1. *School of Environmental Science and Engineering, Suzhou University of Science and Technology, Suzhou 215009, China*
2. *School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China*

ARTICLE INFO

Article history:
Received 11 March 2017
Revised 4 June 2017
Accepted 5 June 2017
Available online 15 June 2017

Keywords:
Inhibition
Phenol
Co-digestion
Anaerobic biofilters
Coal gasification wastewater

ABSTRACT

In this paper, the inhibition of methanogens by phenol in coal gasification wastewater (CGW) was investigated by both anaerobic toxicity tests and a lab-scale anaerobic biofilter reactor (AF). The anaerobic toxicity tests indicated that keeping the phenol concentration in the influent under 280 mg/L could maintain the methanogenic activity. In the AF treating CGW, the result showed that adding glucose solution as co-substrate could be beneficial for the quick start-up of the reactor. The effluent chemical oxygen demand (COD) and total phenol reached 1200 and 100 mg/L, respectively, and the methane production rate was 175 mL CH_4/g COD/day. However, if the concentration of phenol was increased, the inhibition of anaerobic micro-organisms was irreversible. The threshold of total phenol for AF operation was 200–250 mg/L. The extracellular polymeric substances (EPS) and particle size distribution of anaerobic granular sludge in the different stages were also examined, and the results indicated that the influence of toxicity in the system was more serious than its effect on flocculation of EPS. Moreover, the proportion of small size anaerobic granular sludge gradually increased from 10.2% to 34.6%. The results of high through-put sequencing indicated that the abundance of the *Chloroflexi* and *Planctomycetes* was inhibited by the toxicity of the CGW, and some shifts in the microbial community were observed at different stages.

Introduction

Nowadays, coal gasification technology is gaining more and more attention because of the current increase in the consumption of natural gas. However, a large quantity of coal gasification wastewater (CGW) is generated during the coal gasification processes, which contains a lot of toxic and refractory compounds such as phenolic compounds, cyanide, pyridine, and long-chain alkanes and so on (Cui et al., 2017; Xu et al., 2017). Although pretreatment by ammonia-stripping and solvent extraction is an effective method for cutting down ammonia and phenolic compounds (Li et al., 2016a), the residual refractory organic compounds in CGW remain high, of which phenolic compounds account for 40%–50% (Xu et al., 2014). Hence, it is necessary to explore an effective treatment method for the removal of phenols from CGW. The absorption of concentrated phenol solutions may cause severe pain, renal irritation, and shock in humans, and fatalities have been reported. A total dose of 1.5 g may be fatal. Phenols at high concentration do harm to most microorganisms and are also resistant to biodegradation (Hussain and Dubey, 2014).

Anaerobic treatment is by far the most widely applied and cost-effective process for wastewater treatment, improving the degradation rate for refractory organic compounds and enhancing the wastewater biodegradability (Li et al., 2016b; Park et al., 2008). Tests increasing phenol loads (from 100 to

* *Corresponding author*. E-mail: zjzhang@sjtu.edu.cn (Zhenjia Zhang).

http://dx.doi.org/10.1016/j.jes.2017.06.001

5000 mg/L) as the sole carbon source in a semi-continuous mesophilic anaerobic adaptation experiment were carried out, using an unadapted microbial community from a standard biogas plant. Phenol was completely reduced at starting concentrations of up to 2000 mg/L (Wirth et al., 2015). However, the large amount of granular activated carbon required as an absorber would be costly on an industrial scale (Ahmaruzzaman, 2008; Lin and Juang, 2009). Hence, there is a need for an advanced anaerobic reactor that is more practical in terms of investment costs and operability for improving the anaerobic biodegradation of CGW. The anaerobic biofilter (AF) is a kind of anaerobic reactor filled with fillers. Enriched functional bacteria grow on the surface of fillers and help in the degradation of toxic pollutants in CGW (Wang et al., 2013). Moreover, the mixed liquor suspended solids (MLSS) of an AF can reach to 30 g VSS/L (Wang et al., 2014a). The spatial distribution of microorganisms in the AF is conducive to reducing the effects of toxic pollutants on vulnerable bacteria. However, the start-up of anaerobic reactors always requires a long time, especially in the treatment of toxic and refractory wastewater. Nowadays, in order to reduce the startup time in anaerobic systems, anaerobic co-digestion has been employed for the disposal of refractory or toxic wastewater. Moreover, the anaerobic co-digestion of organic waste has the potential to make a significant contribution to the generation of renewable energy (Larsen et al., 2013).

Directly utilizing refractory pollutants as a carbon or energy source is difficult for anaerobic micro-organisms, but when another easily utilized carbon or energy source is added in the influent, the refractory substances can be degraded efficiently (Li et al., 2016a). Co-digestion using methanol addition to improve the biodegradation of CGW has previously been investigated. When a methanol concentration of 500 mg COD/L was added (organic loading rate 3.5 kg COD/m^3/day and phenol loading rate 0.6 kg/m^3/day), the corresponding maximum COD and phenol removal rates were 71% and 75%, respectively (Wang et al., 2010). An advanced anaerobic expanded granular sludge bed (AnaEG) was also developed recently by our research group for the treatment of CGW that adopted glucose as the substrate; it took about 87 days to start up the reactor (Li et al., 2014), which is faster than in previous reports.

In this study, anaerobic toxicity and recovery tests were performed, and then a laboratory-scale AF reactor was adopted to treat CGW. The aim was to explore the quick start-up of the reactor with the aid of co-digestion, and to determine the inhibitory effect of high phenol concentrations as well as the inhibition threshold of phenol. Finally, high-throughput sequencing was used to characterize the microbial communities during different stages of the AF operation. The abundance of microorganisms in the sludge in relation to the different stages of the operation was discussed.

1. Materials and methods

1.1. Anaerobic biofilter reactor

The AF was made of cylindrical plexiglass and filled with soft fillers. The influent was pumped into the bottom of the reactor and the effluent flowed out from the top. The AF was operated at 35°C with the effective volume of 13.4 L. The hydraulic retention time (HRT) was controlled at 96 hr and the methane content was analyzed by passing the gas emitted through a 3 mol/L NaOH solution followed by collection in a gas collection bag. The volume of methane (CH_4) was monitored using a gas flow meter.

1.2. Inoculated sludge

The inoculated anaerobic activated sludge was taken from an expanded granular sludge bed (EGSB) treating starch wastewater. The inoculation volume was 20% of the effective volume of the reactor. The suspended solids (SS) and volatile suspended solids (VSS) in the reactor were 8.3 g/L and 4.8 g/L, respectively.

1.3. Coal gasification wastewater

Coal gasification wastewater was obtained from the Harbin Coal Chemical Industry Co. Ltd., Harbin, China. It was pretreated by phenol extraction and ammonia stripping. Some available substances can be recovered by pretreatment, and the toxicity of the CGW can be decreased for the subsequent bio-treatment. The characteristics of the CGW used in our study are shown in Table 1.

1.4. Methanogenic toxicity batch assays

Methanogenic toxicity batch tests were conducted in 250 mL sealed bottles, seeded with anaerobic sludge. Five sealed bottles, namely B1, B2, B3, B4 and B5, were prepared and some seeded sludge was added. A glucose solution (3 g COD/L) was used as the medium and $NaCO_3$ was used to adjust the pH to 6.8–7.2. The phenol concentrations were controlled at 0 (control), 280, 400, 550, and 800 mg/L, respectively, for the five samples. Moreover, a blank sample with only pure water and anaerobic granular sludge without the addition of glucose solution was also prepared. Methane production was measured by a gas displacement device filled with 3 mol/L NaOH solution. Nitrogen gas was bubbled up into each bottle to remove air and the assays were performed at 35 °C in an oscillating shaker at 120 r/min. All batch tests were repeated three times.

The relative activity (RA) was adopted to determine the degree of inhibition by phenol. RA can be calculated as follows:

$$RA = \frac{V_t - V_{blank}}{V_{control} - V_{blank}} \times 100\% \quad (1)$$

where, V_t is the cumulative methane production of the sample at a certain time; V_{blank} is the cumulative methane production of the blank sample at a certain time; $V_{control}$ is the cumulative methane production of the control sample at a certain time. RA of 75%–95% indicated slight inhibition; RA of 40%–75% indicated moderate inhibition; and RA < 40% indicated severe inhibition (Driessen et al., 1994).

1.5. The effect of phenol concentration on the treatment performance of the AF operation

The effect of phenol concentration on the treatment performance of the AF was investigated. The HRT was controlled at

Table 1 – Characteristics of the coal gasification wastewater.

Parameter	Concentration	Average value	Parameter	Concentration	Average value
COD (mg/L)	1500–2800	2500 ± 100	pH	6.8–8.2	7.5 ± 0.5
BOD_5 (mg/L)	10–23	11.5 ± 5	TC (mg/L)	720–860	822.5 ± 20
Total phenol (mg/L)	200–350	320 ± 20	IC (mg/L)	90–130	110 ± 10
NH_4^+-N (mg/L)	180–240	200 ± 30	TOC (mg/L)	650–745	700 ± 20

TC: total carbon; TOC: total organic carbon; IC: inorganic carbon; COD: chemical oxygen demand; BOD_5: biochemical oxygen demand.

96 hr and the temperature was kept at 35°C. The average influent total phenol concentration was gradually increased, and the operation was divided into 3 phases. As the Table 2 indicated, Phase I was the step involving quick start-up of the AF in co-digestion conditions. In phase I, glucose solution (COD = 2500 mg/L) was added to the influent as co-substrate. The volume ratios of glucose solution and CGW were 3:1, 1:1 and 0:1, in stages 1, 2 and 3, respectively. In phase II, the phenol concentration was increased to 250, 450 and 550 mg/L in stages 4, 5 and 6, respectively. In phase III the phenol concentration was decreased back to 150 mg/L in order to investigate the recovery of activity of the anaerobic micro-organisms after inhibition by high-concentration phenol.

1.6. Analytical methods

COD, biochemical oxygen demand (BOD_5), NH_4^+-N, pH and MLSS were analyzed according to the standard procedures (CEPB, 2003). Total organic carbon (TOC), inorganic carbon (IC) and total carbon (TC) were monitored by a total organic carbon analyzer (Shimadzu TOC-LCSH, Japan). The volume of biogas production was determined by a wet glass flow meter, and the methane content was analyzed using a gas displacement device filled with 3 mol/L NaOH solution. The concentration of total phenols was measured by the titration method (Makrigianni et al., 2015). The extracellular polymeric substances (EPS) of anaerobic granular sludge (AGS) used in the experiments consisted of protein and polysaccharide; protein was measured by the modified Lowry method (Frolund et al., 1996), and polysaccharide was measured by the phenol-sulfuric acid method (Geng et al., 2002).

1.7. High-throughput sequencing

The samples were subjected to deoxyribonucleic acid (DNA) extraction using E.Z.N.A. soil DNA kits (Omega Bio-Tek, Norcross, GA, USA). The concentration and purity of the extracted DNA were detected using a UV spectrophotometer and agarose gel electrophoresis. The V4 region of 16S rRNA was subjected to PCR amplification using the following primers: F 5′-AYTGGGYDTAAAGNG-3′ and R 5′-TACNVGGGTATCTAA TCC-3′. The amplification conditions were as follows: 98°C for 30 sec, 98°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec (27 cycles); 72°C for 5 min; and finally held at 4°C. The pure target PCR products were obtained by repeated excision of DNA bands from the 0.8% agarose gel electrophoresis, and quantified with a BioTek micro plate reader.

Adapters were ligated to the PCR amplification products by enzyme digestion and enzyme reaction. The constructed DNA library was quantified using the PicoGreen dsDNA quantitation assay and the fluorescence spectrophotometer method. The DNA library (10 nmol/L) was progressively and quantitatively diluted to 4–5 pM and subjected to DNA sequencing on an Illumina MiSeq machine with 2 × 250 bp reads.

The raw data obtained from the Illumina MiSeq sequencing were saved as paired-end fastq sequences. The fastq sequences were firstly subjected to quality filtration. The required sequences were ≥150 bp long with an average base quality of ≥Q20 (*i.e.*, base accuracy was 99%) and had no N bases (fuzzy bases).

The filtered sequences were then connected using Flash software (http://www.genomics.jhu.edu/software/FLASH/index.shtml) with an overlap of ≥10 bp and without any base mismatches. The valid sequences were extracted according to the index information. Qiime (Caporaso et al., 2010) (version 1.7.0, http://qiime.org/) and Mothur (Schloss et al., 2009) (version 1.31.2, http://www.mothur.org/) software were used to further filter and remove chimeric sequences, and finally obtain high-quality sequences for subsequent analysis.

According to the sequence similarity, the high-quality sequences were classified to operational taxonomic units (OTUs) using Qiime software. The longest sequence in each OTU was selected as the representative sequence. The

Table 2 – The influent concentration of phenol at different stages.

Phase	I			II			III
Stage	1	2	3	4	5	6	7
Phenol concentration (mg/L)	50 ± 10	100 ± 12	150 ± 5	250 ± 8	450 ± 12	550 ± 15	150 ± 17

Stage 1: the volume ratio of glucose solution: CGW = 3:1.
Stage 2: the volume ratio of glucose solution: CGW = 1:1.
Stage 3: the volume ratio of glucose solution: CGW = 0:1.
CGW: coal gasification wastewater.

taxonomic information of each OTU was obtained by comparison with the sequence database using the BLAST method in Qiime (Altschul et al., 1990).

2. Results and discussion

2.1. Effect of phenol toxicity on anaerobic granular sludge

The batch tests, which included inhibition and recovery tests, were performed with different phenol concentrations. As shown in Fig. 1a, the cumulative methane production of the control sample (phenol concentration = 0 mg/L), the sample with phenol concentration = 280 mg/L and the sample with phenol concentration = 400 mg/L indicated little difference before the first 23 hr. However, after 23 hr the cumulative production of methane was gradually decreased with increasing phenol concentration in the samples. The inhibition due to phenol became more and more obvious when the phenol concentration was increased to 550 mg/L. Moreover, the activity of methanogens was completely restrained and no methane was produced.

As can be seen from Fig. 1b, when the phenol concentration was 280 mg/L, the RA exceeded 60% after 3 hr, and even reached 125% within 23 hr. Hence, the result indicated that phenol concentrations under 280 mg/L can stimulate the growth of methanogens to some extent. However, as the concentration was reduced, RA sharply decreased to 70% in 26 hr. All the samples were in a stable state within 44 hr. The result indicated that the phenol concentration of 280 mg/L caused moderate inhibition to the methanogens. The phenol concentrations of 400, 550 and 800 mg/L caused severe inhibition and had an adverse effect on the methanogenic activity.

After 70 hr, the sludge was taken out and washed several times with pure water, and then a glucose solution with 3 g COD/L was added, and the recovery of methanogen activity was investigated. As shown in Fig. 1c, the results indicated that with increasing phenol concentration in the system, the recoverability of methanogenic activity was gradually reduced. The inhibition of methanogens by low concentrations of phenol was temporary and reversible. However, the recovery of methanogen activity was greatly restrained by previous exposure to high concentrations of phenol. Hence, this suggests that the phenol concentration in influent should be controlled under 280 mg/L to ensure methanogenic activity in anaerobic granular sludge.

2.2. Performance of AF reactor

2.2.1. Phase I: quick start-up of the AF in co-digestion conditions

Operation of the AF was adopted to verify the results of the batch tests. The start-up of the AF was carried out adopting stepped organic loading to produce the most rapid biomass acclimation and development. Phenol loading was enhanced by increasing the influent phenol concentration upon the attainment of a pseudo-steady state. As shown in Fig. 2, when the ratio of glucose solution and CGW was 3:1 in stage 1, the influent concentrations of COD and total phenol were 2500 and 80 mg/L, respectively. Due to the short duration of stage 1,

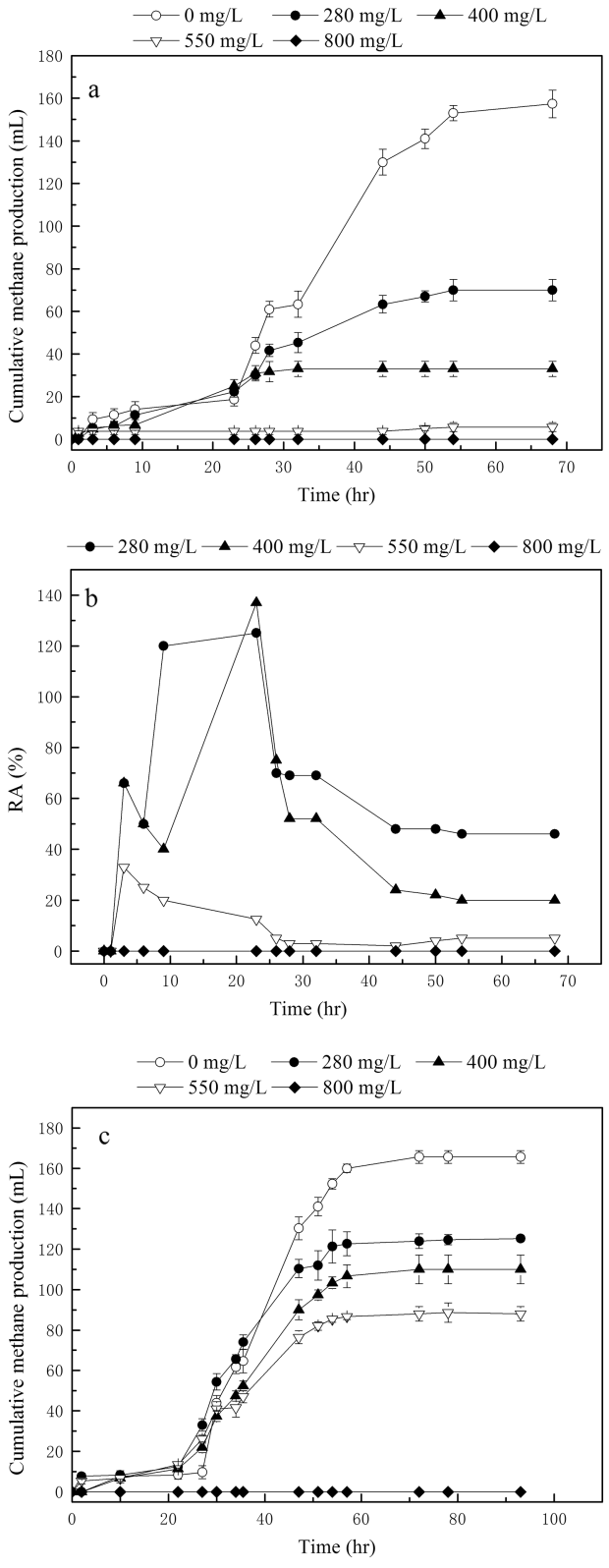

Fig. 1 – Anaerobic toxicity test performance: (a) Cumulative CH_4 production of anaerobic granular sludge in inhibition test; (b) The relative activity (RA) of anaerobic granular sludge in inhibition test; (c) Cumulative CH_4 production of anaerobic granular sludge in recovery test.

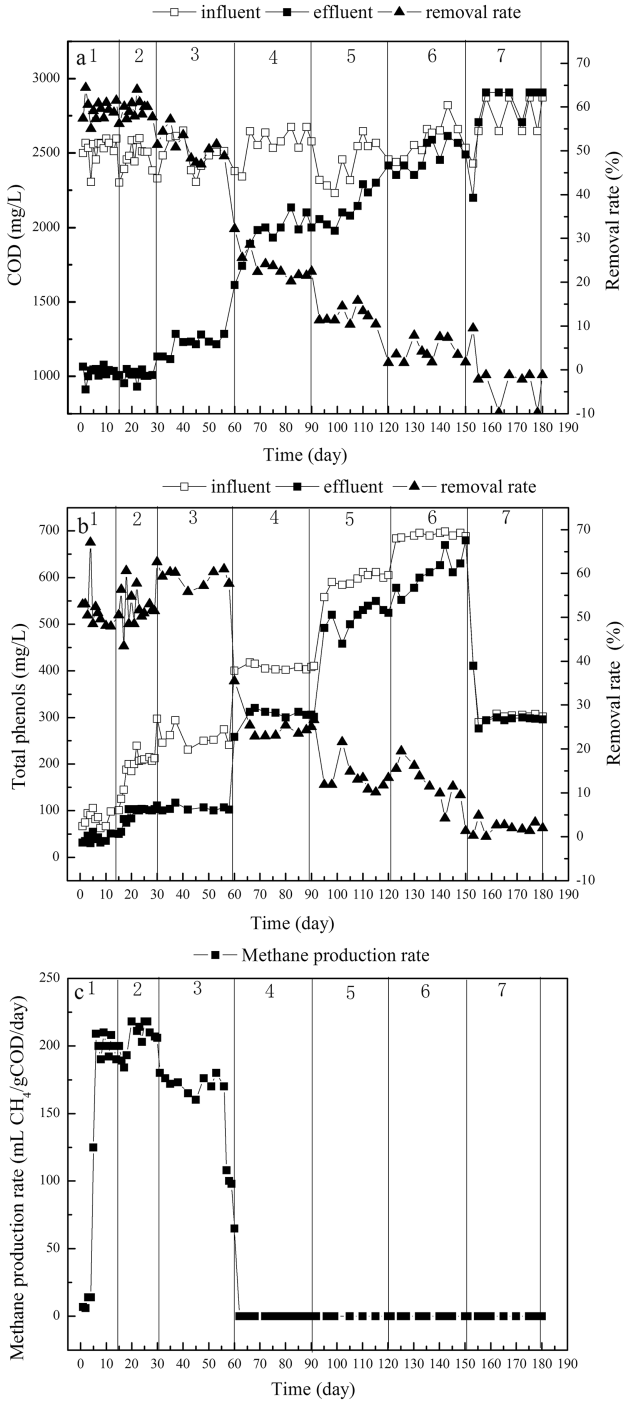

Fig. 2 – Performance of anaerobic biofilter reactor (AF) at different phenol concentrations: (a) The chemical oxygen demand (COD) removal; (b) total phenol removal; (c) methane production rate.

the effluent total phenol was in a state of instability, and the effluent COD and total phenol reached 1000 and 40 mg/L, with corresponding removal rates of 55% and 40%, respectively.

In stage 2, the effluent COD was still 1000 mg/L and the effluent total phenol attained a concentration of 100 mg/L. In stage 3, the effluent COD concentration increased slightly to 1200 mg/L and effluent total phenol was steady at 100 mg/L. The result indicated that adding glucose solution as co-substrate can induce the enzymes required for metabolism and produce enough energy to drive the initial transformation of the toxic matters. Hence, it can be helpful in establishing metabolic equilibrium for the improvement of the anaerobic biodegradation of CGW, because adding glucose solution can dilute the concentration of toxic pollutants to some extent and alleviate their inhibiting effect on the growth of methanogens. In addition, low concentrations of toxic pollutants can stimulate the proliferation of methanogens. Therefore, the methane production rate was more than 200 (mL CH_4/g COD/day) in stages 1 and 2. However, when the concentration of pollutants in influent was increased to the level in raw CGW in stage 3, the methane production rate was decreased to 175 (mL CH_4/g COD/day).

The start-up phase was 60 days, which was faster than previous reports where the start-up phase for two-stage anaerobic digestion and two-phase anaerobic digestion of CGW was 210 days (Xu et al., 2014) and 170 days for a single-feed TUASB system (Wang et al., 2011). The explanation for the faster start-up is that the addition of glucose solution as a co-substrate provides a carbon source, nitrogen source and energy source for biodegradation. Shi (1999) carried out research on the addition of a carbon source as an easily degradable substance in a upflow anaerobic sludge bed (UASB), and found that it played a significant role in facilitating biodegradation of 2,4,6-trichlorophenol in the system.

Fu et al. (2001) discovered that Reactive Turquoise Blue (RTB) could not be the single carbon source for anaerobic microorganisms, but by adding 0.5 g/L glucose in the influent, the RTB could be degraded due to the co-digestion of glucose and RTB. Moreover, the addition of glucose solution can provide the necessary coenzyme NADH, vitamins and other nutrients needed for anaerobic microbial growth and reproduction. The use of anaerobic co-digestion is an important way to control the degradation of organic pollutants by key enzymes, and competitive competition between the matrix and refractory organics. The refractory organics and their degradation products are key factors that influence the anaerobic co-metabolism process. In the process of co-metabolism, there must be a suitable type of growth substrate, and the optimum concentration of the growth substrate is also determined by the type of matrix, the nature of the pollutants and the structure of the bacteria. A suitable co-substrate and concentration can not only promote the decomposition of refractory organics, but also reduce the concentration of metabolic intermediates. In order to adapt the co-metabolism environment, a suitable concentration of co-substrates can induce or stimulate microbial variation in the direction of refractory organic matter decomposition and accelerate domestication, as well as the structural adjustment of the microbial flora (Wang et al., 2014b).

2.2.2. *Phase II: increasing phenol loading*

The total phenol concentration in the influent was increased in a stepwise fashion while the COD concentration was kept at 2500 mg/L. When the concentration of total phenol was further increased in stages 4–6, the removal efficiency of COD dropped from 25% to 5%, and the removal efficiency of total phenol also declined from 25% to 10%. Even the methane

production rate declined to 0 (mL CH_4/g COD/day) in phase II. The result indicated that on one hand, a high concentration of phenol has an obvious inhibitory effect on anaerobic micro-organisms, especially methanogens, and inhibits the metabolic activity. On the other hand, not only phenol but also other toxic pollutants present in the CGW may also have an inhibitory effect on methanogens.

2.2.3. Phase III: recovery of phenol concentration

In the third phase, the removal rates of COD and total phenol trended to zero for the whole phase, which indicated that the inhibition of the anaerobic granular sludge due to phenol could not be recovered. Hence, the threshold of total phenol for AF operation was about 200–250 mg/L. Yan et al. (2008) also carried out a study on phenol inhibition and restoration of the bioactivity of anaerobic granular sludge. The result indicated that in cases where the phenol was removed after 24 hr of inhibition (phenol concentration of 200 and 300 mg/L, respectively), during the restoration process, the bioactivity of anaerobic granular sludge might be inhibited acutely from the 24th to 48th hr., and from the 48th to 72nd hr., the bioactivity of the anaerobic granular sludge could be partly recovered. Hence, different HRT and conditions would lead to different concentration thresholds for phenol-based inhibition in the system.

High concentrations of phenol were not beneficial for the growth of anaerobic micro-organisms. It is concluded that reducing the concentration of toxic pollutants in the influent, especially total phenol, may be the only way to solve the problems of low disposal efficiency and poor methane production.

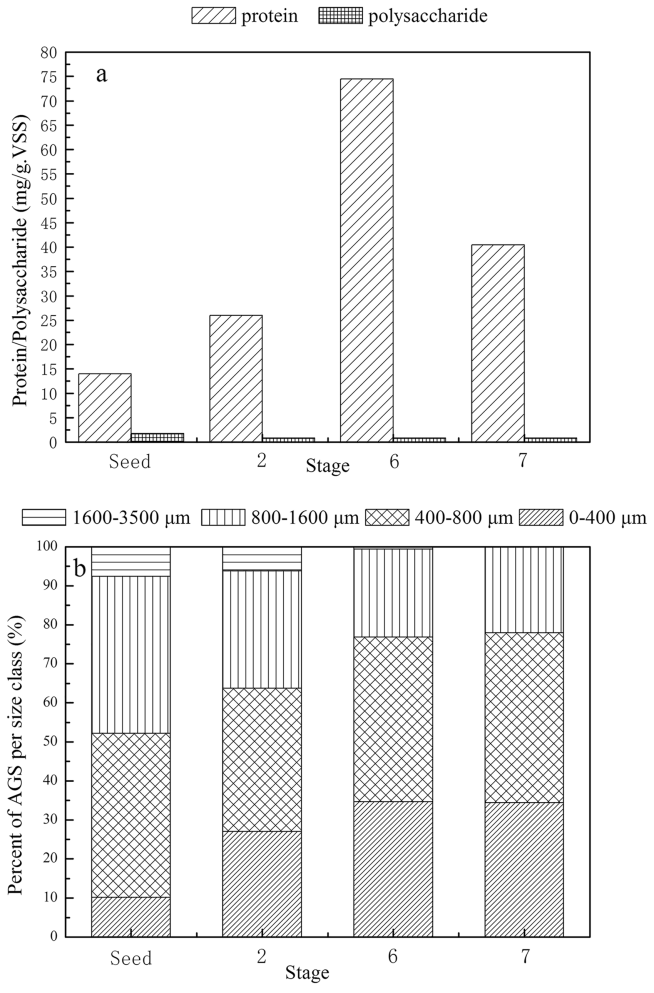

Fig. 3 – (a) Protein and polysaccharide variations and (b) particle size distribution of the anaerobic granular sludge in the different stages.

2.3. Anaerobic granular sludge

Proteins and polysaccharides were determined to be the main components of EPS, which is consistent with a previous report (McSwain et al., 2005). Polysaccharides are responsible for both adhesion and cohesion interactions, while proteins serve as a carbon and energy source (Voběrková et al., 2016).

As shown in Fig. 3a, the protein was increased with increasing total phenol concentrations. Moreover, the concentration of exocellular proteins was greater than the exocellular polysaccharide concentration. Similar results were reported in a previous study (Higgins and Novak, 1997a). The presence of protein in the exocellular fraction suggests that it might play a role in flocculation (Voběrková et al., 2016). The amount of protein in EPS also influences sludge dewaterability (Higgins and Novak, 1997a, 1997b; Houghton et al., 2001). The ratio of protein to polysaccharide (P/C) is a crucial factor to determine the surface properties of sludge, such as hydrophobicity and surface charge (Shin et al., 2000; Liu and Fang, 2003; Hoa et al., 2003).

In our study, the P/C in the seed stage and stages 2, 6, and 7 were 8, 33.3, 50.6, and 96, respectively. Some reports indicated that an increased P/C leads to a decrease in the negative charge of the sludge surface and increased surface hydrophobicity. Hence, it could reduce the electrostatic repulsion between the sludge cells, enhance the hydrophobicity and affinity, and then promote sludge particle cohesion and stabilization (Zheng et al., 2005).

As shown in Fig. 3b, from the seed stage to stage 6, the proportion of small size (0–400 μm) anaerobic granular sludge gradually increased from 10.2% to 34.6%; on the contrary, the proportion of 800–1600 um size anaerobic granular sludge was decreased from 40.2% to 22.6%, due to the inhibition caused by phenol in the system. On the one hand, a high concentration of phenol facilitated the production of protein in EPS, which made the anaerobic granular sludge compact. On the other hand, it resulted in destruction and fracture of the anaerobic granular sludge. Moreover, it was observed that the influence on the disintegration of anaerobic granular sludge in the system was more serious than the effect on the flocculation of EPS (Jiang, 2007) also came to the same conclusion in treating nitrophenol wastewater in an upflow anaerobic sludge bed reactor. Before the experiment, the large size granular sludge particles exceeding 0.5 mm accounted for 85% of sludge particles, but after the degradation of 3-nitrophenol and 2,6-nitrophenol, the granular sludge was affected by the nitrophenol toxicity and suffered disintegration. Then the particle size exceeding 0.5 mm accounted for only 40%, and the activity of methanogens was inhibited by nitrophenol.

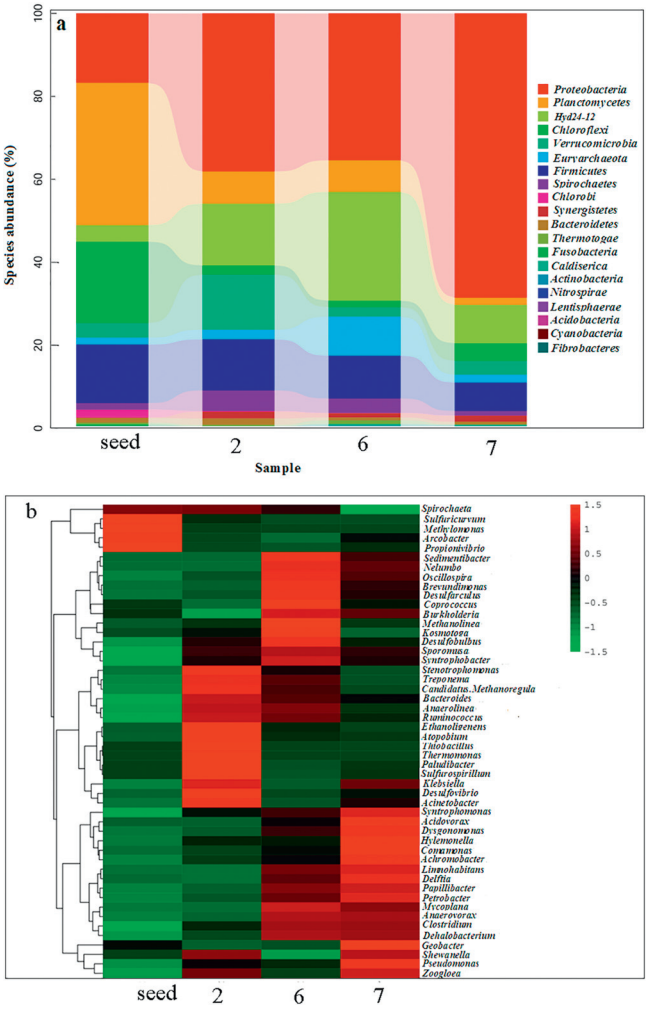

Fig. 4 – Abundance of the major bacterial groups in samples at different stages in anaerobic biofilter (AF) operation.

2.4. Bacterial community shift

Sludge from the seed stage and stages 2, 6 and 7 was taken from the reactor and analyzed by high-throughput sequencing. Analytical results indicated that the proliferation of bacteria from the phyla of *Chloroflexi* and *Planctomycetes* were inhibited by the toxicity of the CGW, and the proportion of the *Chloroflexi* was decreased from 8.7% in seed to 2.1% in stage 2, while the proportion of *Planctomycetes* was decreased from 15.2% in seed to 7.4% in stage 2.

On the contrary, the abundance of some microorganisms was increased in stage 2, but with further increases in the concentration of the phenol, the bacterial abundance decreased. However, with the decrease in the concentration of total phenol in the system, the abundance of the microorganisms could rebound. Fig. 4 indicates that the abundance of *Proteobacteria* was increased from 7.4% in seed to 36% in stage 2, but in stage 6, when total phenol reached 800 mg/L, it was slightly decreased to 33%; when total phenol was restored to 300 mg/L, it was increased again to 67%. The same conclusion applied to the phyla of *Verrucomicrobia* and *Synergistetes*. Many species belonging to *Proteobacteria* are acetic acid bacteria (Augimeri et al., 2015), which have strong tolerance to toxic substances. Genus *Klebsiella*, which has some relationship with hydrolysis acid fermentation (Yong et al., 2015), showed increased abundance in stage 2: it reached 15.58% and then decreased to 2.23% in stage 6, when total phenol reached 700 mg/L in the influent. However, it was increased to 11.13% when influent total phenol returned to 300 mg/L. Bacterial genus *Desulfovibrio*, which belongs to the group of anaerobic sulfate-reducing bacteria (Fievet et al., 2015), was accounted for 0.33% in the seed stage and increased to 7% in stage 2, but decreased to 0.94% in stage 6; then the abundance recovered a little in stage 7, with the proportion reaching 2.17%.

Secondly, as shown in Fig. 2, in stage 2 the effluent total phenol was 100 mg/L with a removal rate reaching 60%. In stage 6, the high concentration of total phenol inhibited the growth and proliferation of some micro-organisms, which led to a decrease in the total phenol removal rate to 10%. In stage 7, even though the influent total phenol recovered to 300 mg/L, the corresponding removal rate could not be improved, and the removal rate of COD indicated the same trend in our research. The variation of total phenol in the system may also affect the abundance of some microorganisms. The Fig. 4 shows that some species belonging to *Bacteroidetes* and *Firmicutes* are hydrolytic fermenting bacteria or acidogens, which play an important part in degradation of toxic pollutants and are beneficial for the conversion of phenol to benzoate, because the conversion of phenol to benzoate has been demonstrated to be the rate-limiting step in the anaerobic degradation of phenol (Veeresh et al., 2005). As shown in Fig. 4, the proportions of *Bacteroidetes* and *Firmicutes* reached 0.49% and 6.2%, respectively, in seed, then increased to 1.4% and 11.6% in stage 2, but they gradually declined in stages 6 and 7, and finally reached 0.61% and 6.7%, respectively. The *Spirochaetes*, typically, which have a helical coiled morphology and grow chemoheterotrophically, have metabolic activities including acetate, ethanol, and lactate fermentation from glucose (Godon et al., 1997) as well as acetate oxidation (Lee et al., 2015). The proportion of *Spirochaetes* was about 4.6% in stage 2, but it gradually decreased to 1.0% in stage 7.

3. Conclusions

This study showed that a phenol concentration under 280 mg/L in batch tests can facilitate the growth of methanogens. Moreover, the performance of the AF indicated that adding glucose solution as co-substrate enabled a quick start-up of the AF, with a start-up time of only 60 days, and the effluent COD and total phenol reached 1200 and 100 mg/L, respectively. The methane production rate was about 175 (mL CH_4/g COD/day). The threshold of total phenol for AF operation was about 200–250 mg/L. As the phenol concentration increased, the concentration of exocellular protein was increased and the particle size distribution of the anaerobic granular sludge accumulated in the small size range. Additionally, high through-put sequencing results demonstrated that the pollutant removal had some relationship with shifts in the main microbial community.

Acknowledgments

The authors express their gratitude to the School of Environmental Science and Engineering, Shanghai Jiao Tong University for providing the research facilities and Prof. Fan Lü from State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, Shanghai, China. Dr. Tabassum was financially supported by the Talented Young Scientist Program supported by the Ministry of Science and Technology, P.R. China as an Assistant Researcher (IND-15-003) at the School of Environmental Science and Engineering, Shanghai Jiao Tong University, China.

REFERENCES

Ahmaruzzaman, M., 2008. Adsorption of phenolic compounds on low-cost adsorbents: a review. Adv. Colloid Interface Sci. 143, 48–67.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Augimeri, R.V., Varley, A.J., Strap, J.L., 2015. The phytohormone ethylene enhances cellulose production, regulates CRP/FNRKx transcription and causes differential Gene expression within the bacterial cellulose synthesis operon of Komagataeibacter (Gluconacetobacter) xylinus ATCC 53582. Front. Microbiol. 6, 1–27.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

CEPB, 2003. Standard Methods for Water and Wastewater Analysis. Environmental Science Publishing House, Beijing.

Cui, P.Z., Mai, Z.H., Yang, S.Y., Qian, Y., 2017. Integrated treatment processes for coal-gasification wastewater with high concentration of phenol and ammonia. J. Clean. Prod. 142, 2218–2226.

Driessen, W.J.B.M., Tielbaard, M.H., Vereijken, T.L.F.M., 1994. Experience on anaerobic treatment of distillery effluent with the UASB process. Water Sci. Technol. 20 (12), 193–301.

Fievet, A., Ducret, A., Mignot, T., Valette, O., Robert, L., Pardoux, R., Dolla, A., Aubert, C., 2015. Single-cell analysis of growth and cell division of the anaerobe *Desulfovibrio vulgaris* Hildenborough. Front. Microbiol. 6. http://dx.doi.org/10.3389/fmicb.2015.01378.

Frolund, B., Palmgren, R., Keiding, K., 1996. Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Res. 30, 1749–1758.

Fu, L.Y., Wen, X.H., Qian, Y., 2001. Study on characteristic of anaerobic biodegradation of reactive turquoise blue (RTB). Shanghai Environ Sci. 20 (6), 274–276.

Geng, X., Liang, B., Liang, Y., 2002. Phenol-sulfuric acid derivative spectrum method of rapid determination of polysaccharide in Chinese medicine research. J. Sichuan Univ. 34, 62–64.

Godon, J.J., Zumstein, E., Dabert, P., Habouzit, F., Moletta, R., 1997. Molecular microbial diversity of an anaerobic digestor as determined by small-subunit rDNA sequence analysis. Appl. Microbiol. Biotechnol. 63, 2802–2813.

Higgins, M.J., Novak, J.T., 1997a. Characterization of exocellular protein and its role in bioflocculation. J. Environ. Eng. 123, 479–485.

Higgins, M.J., Novak, J.T., 1997b. The effect of cations on the settling and dewatering of activated sludges: laboratory results. Water Environ. Res. 12, 215–224.

Hoa, P.T., Nair, L., Visvanathan, C., 2003. The effect of nutrients on extracellular polymeric substance production and its influence on sludge properties. Water SA 29, 437–442.

Houghton, J.I., Quarmby, J., Stephenson, T., 2001. Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer. Water Sci. Technol. 44, 373–379.

Hussain, A., Dubey, S.K., 2014. Specific methanogenic activity test for anaerobic treatment of phenolic wastewater. Desalin. Water Treat. 52, 7015–7025.

Jiang, L.N., 2007. Effects of Different Co-substrates on Degradation of Nitrophenols Using Upflow Anaerobic Sludge Bed (UASB)Reactors. The Ocean University of China, QingDao.

Larsen, A.C., Gomes, B.M., Gomes, S.D., Zenatti, D.C., Torres, D.G.B., 2013. Anaerobic co-digestion of crude glycerin and starch industry effluent. Eng. Agríc. Jaboticabal 33, 341–352.

Lee, S.H., Park, J.H., Kim, S.H., Yu, B.J., Yoon, J.J., Park, H.D., 2015. Evidence of syntrophic acetate oxidation by Spirochaetes during anaerobic methane production. Bioresour. Technol. 190, 543–549.

Li, C., Tabassum, S., Zhang, Z., 2014. An advanced anaerobic expanded granular sludge bed (AnaEG) for the treatment of coal gasification wastewater. RSC Adv. 4, 57580–57586.

Li, Y., Tabassum, S., Yu, Z., Wu, X., Zhang, X., Song, Y., Chu, C., Zhang, Z., 2016a. Effect of effluent recirculation rate on the performance of anaerobic bio-filter treating coal gasification wastewater under co-digestion conditions. RSC Adv. 6, 87926–87934.

Li, Y., Tabassum, S., Zhang, Z., 2016b. An advanced anaerobic biofilter with effluent recirculation for phenol removal and methane production in treatment of coal gasification wastewater. J. Environ. Sci. China 47, 23–33.

Lin, S.H., Juang, R.S., 2009. Adsorption of phenol and its derivatives from water using synthetic resins and low-cost natural adsorbents: a review. J. Environ. Manag. 90, 1336–1349.

Liu, Y., Fang, H.H.P., 2003. Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge. Crit. Rev. Environ. Sci. Technol. 33, 237–273.

Makrigianni, V., Giannakas, A., Deligiannakis, Y., Konstantinou, I., 2015. Adsorption of phenol and methylene blue from aqueous solutions by pyrolytic tire char: equilibrium and kinetic studies. J. Environ. Chem. Eng. 3, 574–582.

McSwain, B.S., Irvine, R.L., Hausner, M., Wilderer, P.A., 2005. Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. Appl. Environ. Microbiol. 71, 1051–1057.

Park, Y., Hong, F., Cheon, J., Hidaka, T., Tsuno, H., 2008. Comparison of thermophilic anaerobic digestion characteristics between single-phase and two-phase systems for kitchen garbage treatment. J. Biosci. Bioeng. 105, 48–54.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al., 2009. Introducing mother: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.

Shi, H.C., 1999. Influence of a supplemental carbon source on anaerobic biological treatment of 2, 4, 6-TCP. Environ. Sci. 20 (5), 11–15.

Shin, H.S., Kang, S.T., Nam, S.Y., 2000. Effect of carbohydrates to protein in EPS on sludge settling characteristics. Biotechnol. Bioprocess Eng. 5, 460–464.

Veeresh, G., Kumar, P., Mehrotra, I., 2005. Treatment of phenol and cresols in upflow anaerobic sludge blanket (UASB) process: a review. Water Res. 39, 154–170.

Voběrková, S., Hermanová, Soňa, Hrubanová, Kamila, Krzyžánek, Vladislav, 2016. Biofilm formation and extracellular polymeric substances (EPS) production by *Bacillus subtilis* depending on nutritional conditions in the presence of polyester film. Folia Microbiol. 61, 91–100.

Wang, W., Han, H., Yuan, M., Li, H., 2010. Enhanced anaerobic biodegradability of real coal gasification wastewater with methanol addition. J. Environ. Sci. 22, 1868–1874.
Wang, W., Han, H., Yuan, M., Li, H., Fang, F., Wang, K., 2011. Treatment of coal gasification wastewater by a two-continuous UASB system with step-feed for COD and phenols removal. Bioresour. Technol. 102, 5454–5460.
Wang, Z., Xu, X., Chen, J., Yang, F., 2013. Treatment of Lurgi coal gasification wastewater in pre-denitrification anaerobic and aerobic biofilm process. J Environ Chem Eng. 1, 899–905.
Wang, W., Zhang, J., Wang, S., Shen, J., Pan, S., 2014a. Oxygen-limited aeration for relieving the impact of phenolic compounds in anaerobic treatment of coal gasification wastewater. Int. Biodeterior. Biodegrad. 95, 110–116.
Wang, W., Pan, S., Zhang, J., Wang, S., Shen, J., 2014b. Progress in anaerobic co-metabolism of refractory organics. Environ. Sci. Technol. 37 (6), 78–82.
Wirth, B., Krebs, M., Andert, J., 2015. Anaerobic degradation of increased phenol concentrations in batch assays. Environ. Sci. Pollut. Res. 22, 19048–19059.
Xu, P., Han, H., Hou, B., Jia, S., Zhao, Q., 2014. Treatment of coal gasification wastewater by a two-phase anaerobic digestion. Desalin. Water Treat. 12, 1–11.
Xu, P., Ma, W.C., Hou, B.L., Shi, Z., 2017. A novel integration of microwave catalytic oxidation and MBBR process and its application in advanced treatment of biologically pretreated Lurgi coal gasification wastewater. Sep. Purif. Technol. 177, 233–238.
Yan, Q., Yu, D., Wang, Z.L., Zou, H., Ruan, W.Q., 2008. Phenol inhibition and restoration of the bioactivity of anaerobic granular sludge. Appl. Biochem. Biotechnol. 150, 259–265.
Yong, J.K., Hyun, W.J., Juyi, P., 2015. Production of 2, 3-butanediol by *Klebsiella oxytoca* from various sugars in microalgal hydrolysate. Biotechnol. Prog. 31, 1669–1675.
Zheng, Y.M., Yu, H.Q., Sheng, G.P., 2005. Physical and chemical characteristics of granular activated sludge from a sequencing barch airlift reactor. Process Biochem. 40, 645–650.