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Inhibition effects of pentachlorophenol (PCP) on anaerobic digestion system

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ABSTRACT

Pentachlorophenol (PCP), a kind of ionizable hydrophobic organic contaminant, had been extensively used in agricultural, industrial, and domestic applications as an important component of fungicides, bactericides, herbicides, insecticides, biocides, and wood preservatives, To investigate the effects of pentachlorophenol (PCP) on anaerobic digestion system, an experimental study was carried out in which simulated PCP-contaminated sewage water was treated using a batch reactor. The study revealed that the influences of PCP on acidogenic and methanogenic microorganisms. Affected by PCP, the fermentation type of system was transformed from butyric acid type to propionic acid type.Moreover, biogas production rate and methane content were less. PCP inhibited the activity of methane bacteria significantly: during the series of PCP addition (PCP concentration was 0, 14.9, 19.1, and 29.5 mg/L, respectively), the higher the PCP concentration was, the more serious the inhibition effect was. The removal rate of PCP was 94.6% when the PCP concentration was 14.9 mg/L, while it was only 54.8% when PCP concentration was raised to 29.5 mg/L. Acidogenic microorganism was more quickly adaptable for PCP than methanogenic microorganism.

Keywords: Pentachlorophenol; Inhibition; Anaerobic digestion system; Fermentation type; Microbial activity

1. Introduction

Since 1930s, pentachlorophenol (PCP), a wide spectrum biocide, had been extensively used in agricultural, industrial, and domestic applications as an important component of fungicides, bactericides, herbicides, insecticides, biocides, and wood preservatives [1–3]. It was reported that PCP is readily absorbed across the skin, lungs, and gastrointestinal lining. In the organs, PCP could disrupt the proton gradient across the membranes of cells, accumulate through the food chain and is considered to be mutagenic or at least co-mutagenic [4–6].

So far the PCP is usually treated by physical–chemical treatment and biological wastewater treatment [7–9]. However, biological wastewater treatment, i.e. anaerobic digestion process, is one of the preferred methods in removing PCP from wastewater [10]. However, PCP exhibits different degrees of toxicity

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for different variety of microorganisms. There are two kinds of microorganisms in an anaerobic digestion system: acidogenic microorganism and methanogenic microorganism. Therefore, in an anaerobic digestion system treating wastewater-containing PCP, studying its impacts on different microorganisms has great significance for maintaining system stability and improving PCP removing efficiency.

It is essential to understand the impacts of toxic organic pollutants on the anaerobic microbes to ensure successful anaerobic bio-treatment of them and to improve the treatment efficiency. Hence, experiments on PCP's effects on the metabolic activity of anaerobic microbes were carried out in this study to optimize the anaerobic treatment techniques for removing toxic organic pollutants.

2. Methods

2.1. Reactor setup

A batch reactor was built with transparent acrylic sheet (Fig. 1). The working volume of the reactor was about 10 L (height 80 cm and diameter 40 cm). The reactor was equipped with an inlet port and an outlet port for feeding and effluent discharge, respectively, and a vertical pipe for collecting samples. System temperature was maintained at the optimal mesophilic temperature range (35 ± 1 ℃).

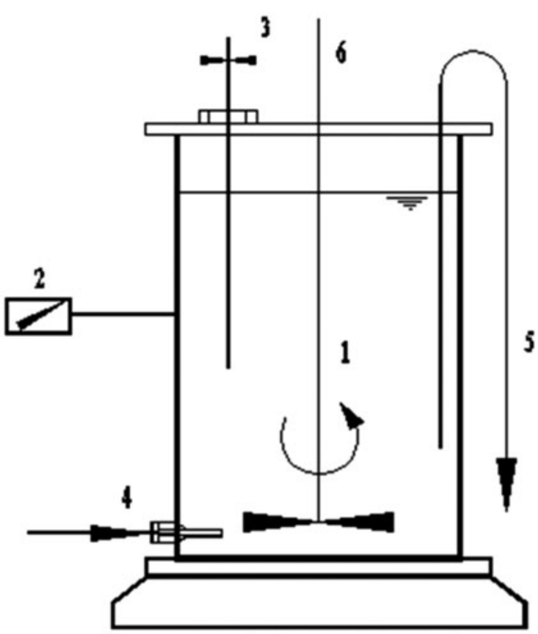

Fig. 1. Schematic diagram of the experimental system. (1) Batch reactor; (2) Temperature controller; (3) Sampling port; (4) Inlet port; (5) Outlet port; (6) Mixer.

2.2. Experimental design

Activated sludge was collected from an IC reactor in a local brewery. The sludge was acclimated using nonPCP artificial wastewater until the anaerobic system was established, and the general parameters were relatively stable. The experiment was processed by the way of batch. In the batch reactor, MLSS concentration was 6,210 mg/L, and sludge loading was 0.48 kg/(kg d). Glucose was used as the carbon source (COD was about 3,000 mg/L) of the artificial wastewater, and nutrient salts and ammonium hydrogen phosphate were also added. Mineral salt composition of the feed is shown in Table 1. The pH of the system was maintained at 6.5–7.8 by Na_2CO_3 solution after every cycle.

Commonly, PCP concentration in municipal sewage is at the level of a few μg/L or less. But in order to studying the influence of PCP, the concentrations should be considered at the level of mg/L and were from low to high, which could indicate the removal rates of PCP in different concentrations. The highest concentration was considered as follows: In extreme conditions, influent PCP concentration could reach up to 12 mg/L [11]. In order to studying the evident influence of PCP for fermentation type of system, the concentration should be higher than 12 mg/L, so the highest concentration was 29.5 mg/L(about 30 mg/L).

From the above-mentioned details, PCP concentrations of 0, 14.9, 19.1, and 29.5 mg/L were used in this study. To investigate the effects of PCP on the anaerobic digestion system, batch experiments were conducted under PCP concentrations of 0.0 and 14.9 mg/L. To determine PCP concentration's effect on the transformation of fermentation type of anaerobic system, a series of PCP concentrations of 0, 14.9, 19.1, and 29.5 mg/L were used in the experiments.

2.3. Cycle of experiment and time of analysis established

In the absence of PCP, the cycle was 24 h, for the anaerobic process could be accomplished in each

Table 1
Mineral salt composition of the artificial wastewater [9]

Composition	Concentration (mg/L)
K_2HPO_4	1,730
KH_2PO_4	680
$(NH_4)_2SO_4$	1,000
$MgSO_4·7H_2O$	100
$FeSO_4·7H_2O$	20
$CaCl_2·2H_2O$	30
$MnSO_4·H_2O$	30

batch. In the presence of PCP, the cycle was 72 h, for the PCP influenced the microorganisms, the period of anaerobic process could be lengthened in each batch.

Studying the inhibition of PCP, the time of analysis is important. When PCP was inoculated, the microorganisms could adapt to the new situation. Therefore, in the new situation of PCP concentration, many cycle tests could be process to adapt for the PCP. When the system was stable operation (that to say, final indexes in each cycle were nearly the same), the result was obtained.

PCP is a kind of water-insoluble substance, so the stock PCP solution was prepared in 2 mol/L NaOH solution to convert PCP to the form of PCP-Na, so that it can dissolve in the artificial wastewater. The working solutions with different PCP concentrations were prepared by diluting the stock solution with 0.1 mol/L $NaNO_3$ solution.

2.4. Analytical methods

In this study, volatile fatty acid (VFA) was measured by gas chromatography method, and PCP was determined by high-performance liquid chromatography method (HPLC) [4,12]. The operating conditions of HPLC were as follows: mobile phase was 0.5% phosphoric acid/acetonitrile (15/85)%, flow rate was 1.0 mL/min, and the wave length of the UV detector was 280 nm. Before each analysis, all samples were centrifuged at the rotating speed of 3,000 rpm for 15 min, and the supernatant was filtered by 0.45-micron membrane.

Samples could be taken and analyzed, when the steadystate was established in the reactor. All chemicals used for the analyses were of analytical grade purity.

3. Results and discussion

3.1. The inhibition effect of PCP on anaerobic digestion system

The VFAs, including acetic acid, propionic acid, and butyric acid, were important intermediate products during anaerobic digestion, which could affect the stability of the anaerobic digestion system. VFAs cold also indicate the running status of the process. According to the literature, PCP's inhibition effect on the utilization of propionic acid was the largest and of acetic acid was the second largest [13]. The VFAs variation in the absence and in the presence of PCP are presented in Figs. 2 and 3, respectively. The result shown in Fig. 2 indicate the VFAs variation. The A, B, C, and D in Fig. 3 represents the different periods of VFAs variation in the PCP concentrations of 14.9 mg/L.

The comparison of Figs. 2 and 3 indicates that the VFAs' concentrations increased dramatically with the increasing of PCP concentration, and the transformation speed of VFAs was also affected.

Fig. 2 shows that the total VFA concentration increased from 538.4 to 805.6 mg/L in the first 6 h, then decreased to 330.2 mg/L during the following 14 h. While in the present of PCP, the VFAs' concentrations were determined every 2 h, and the results are shown in Fig. 3. As shown in the figure, the total VFA concentration slowly increased to 2020.8 mg/L in 34 h and then turned to decreased. It was obvious that in the presence of PCP, VFAs' concentrations were much higher than that in the absence of PCP, which indicates that PCP had strong inhibition effect on the consumption of VFAs. It could be attributed to that PCP was a strong electron acceptor which inhibited the activity of bacteria that utilize VFAs.

Similar to the toxic effect of PCP on the utilization of VFAs, PCP also affected the changing rate of VFAs. In the absence of PCP, the whole treatment process spent only 20 h, while in the presence of PCP; the process was prolonged to 72 h.

Fig. 2 shows the VFA production during a typical batch test. Butyric acid was the major VFA produced, so the fermentation of the system was defined as butyric acid type. Figs. 2 and 3 indicate that the fermentation type of the system was transformed from butyric acid type to propionic acid type after PCP was added into the system.

Table 2 lists variations of biogas production rate and methane content. The results also indicated the inhibition effect on anaerobic bacteria. In the absence

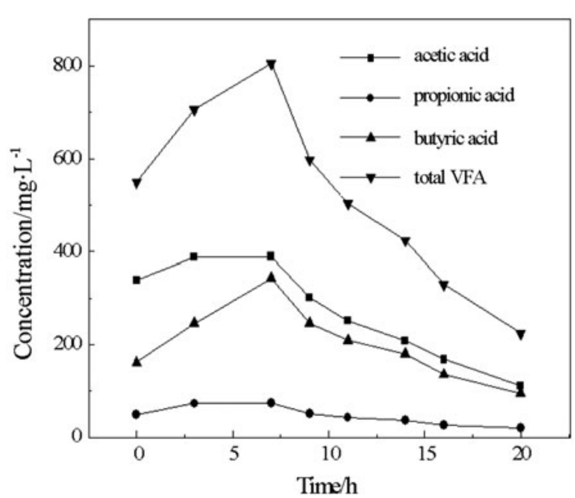

Fig. 2. Anaerobic digestion system VFAs variation in the absence of PCP.

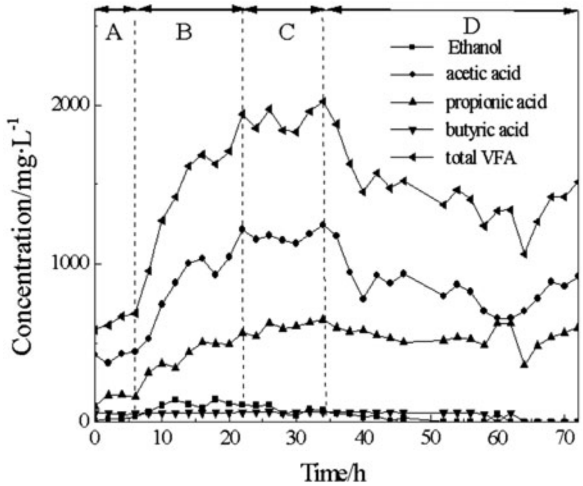

Fig. 3. Anaerobic digestion system VFAs variation in the presence of PCP.

of PCP, the biogas production rate was 5.51 m^3/(kgCOD.d) and methane content was 62.5%; While in the presence of PCP, the biogas production rate only was 0.73 m^3/(kgCOD.d) and methane content was only 34.8%.

3.2. Removal rates of PCP under different PCP concentrations

Different PCP concentrations' effects on PCP anaerobic removal are shown in Fig. 4. As a preparing stage, before the experiment started, the sludge was acclimated with low-PCP artificial wastewater. In this stage, the sorption removal of PCP was accomplished and the sludge was saturated with PCP, which means that no more sorption removal of PCP could happen during the following experiment.

As shown in Fig. 4, when PCP concentration was 14.9 mg/L, the removal rate of PCP was 94.6%, but it decreased to only 54.8% when PCP concentration was raised to 29.5 mg/L. It shows that PCP's toxic effect on anaerobic system was different for different concentrations. Higher PCP concentration would cause more serious toxic effect on anaerobic system.

Table 2
Variations of biogas production and methane content in the absence and in the presence of PCP

	In the absence of PCP	In the presence of PCP
Methane production rate (m^3/(kgCOD d))	5.51	0.73
Methane content (%)	62.5	34.8

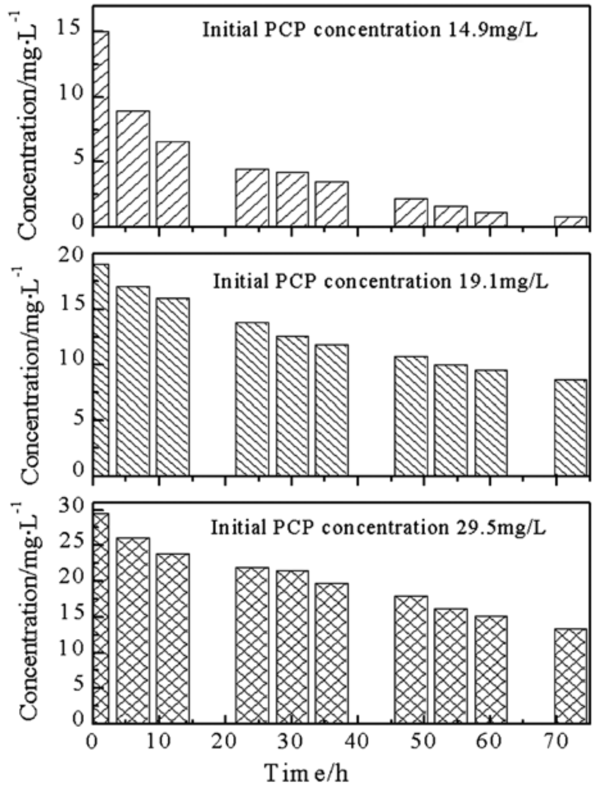

Fig. 4. PCP removal efficiencies under different PCP concentrations.

3.3. VFAs production metabolism in different periods

Anaerobic treatment has two separated stages (acidogenic stage and methanogenic stage). In the acidogenic stage, VFAs were generated by acidogenic microorganisms and their concentrations increased. While in the methanogenic stage, methanogenic bacteria's metabolic activity consumed VFAs and their concentration turned to decrease. The VFAs' variations in different periods under PCP concentration of 14.9 mg/L were presented in Fig. 5, and I, II, III, and IV were four periods of the treatment process. PCP was added into the system in I period, and the II, III periods were the phase of system to adapt the new situation with PCP. At last, the system reached a stable operation in IV period.

As shown in Fig. 5(I), total VFA continuously increased to 1705.7 mg/L in 30 h, and then, their concentrations stayed almost unchanged, which indicated that the system only existed acidogenic phase. From Fig. 5(II and III), the system appeared more pronounced two phases: acidogenic phase and methanogenic phase. In Fig. 5(IV), total VFA increased from 382.1 to 2087.4 mg/L in 36 h and then decreased to

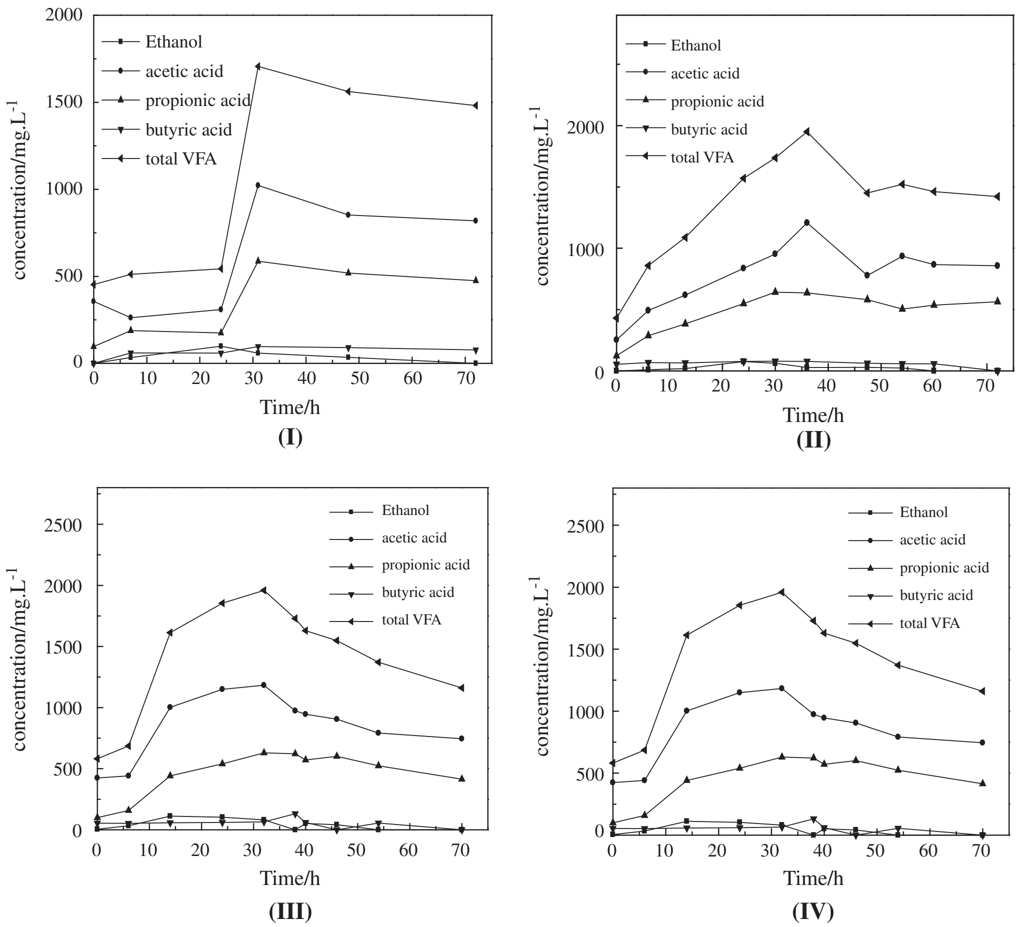

Fig. 5. Comparison of VFAs variation in four different periods. In this figure (I)–(IV) were four periods of the treatment process under PCP concentration of 14.9 mg/L.

651.3 mg/L. The obvious increase and declination of VFAs' concentrations indicated that the system reached a stable two-stage status (acidogenic stage and methanogenic stage).

The different variation trends of VFAs show that PCP had different impacts on different microorganisms. Acidogenic microorganism was more quickly adaptable for PCP than methanogenic microorganism. One hypothesis was that methanogenic bacteria were more sensitive to PCP than other microorganisms. It was reported that dechlorination under fermentative condition had been shown to be more favorable compared with the methanogenic condition [1–14,15].

4. Conclusion

In this study, PCP's impact on anaerobic digestion system was evaluated. In the presence of PCP, VFAs' concentrations in the system were much higher than that in the absence of PCP. This comparison showed that PCP had great inhibition effect on the utilization of VFAs. In addition, the fermentation type of the system was transformed from butyric acid type to propionic acid type, and the whole treatment process was obviously prolonged after PCP was added.

PCP anaerobic removal experiments were carried out under different concentrations of PCP. With the increasing of PCP concentrations, PCP removal efficiency declined obviously from 94.6 to 54.8%. These experimental results indicated that the higher the PCP concentration was, the more serious its toxic effect on anaerobic system would be.

The comparison of VFAs' variations in four different periods of anaerobic treatment process showed that PCP had different impacts on different microorganisms. Experimental results indicated that acidogenic microorganism were more quickly adaptable for PCP than methanogenic microorganism.

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