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Enhancing swine wastewater hydrolysis with thermophilic bacteria and assisted pretreatments

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Abstract

Slow degradation rate of swine wastewater, which is mainly caused by particulate and refractory organic matters, is the main drawback of anaerobic digestion. Therefore, it is necessary to improve the hydrolysis of swine wastewater. In this study, different pretreatments were used to hydrolyze swine wastewater, including thermophilic bacteria (TB), alkali, acid, ultrasound (UL), and ultrasonic-combined thermophilic bacteria (UL-TB) pretreatment. The hydrolysis effect was investigated by analyzing the changes of pretreated soluble chemical oxygen demand (SCOD), soluble protein, and carbohydrate. The experimental results showed that effect of different pretreatments on swine wastewater hydrolysis had the following order: TB = alkali>UL-TB > UL>acid. Alkali pretreatment was effective for the release of protein from swine wastewater, and TB pretreatment was advantageous for carbohydrate release during hydrolysis. The results could provide valuable information for the disposition of swine wastewater as well as the application of TB-related pretreatments. © 2020 Water Environment Federation

Practitioner points

- TB and alkali pretreatment exhibited the highest hydrolysis ability.
- The release of carbohydrate by TB was higher than other pretreatments.
- Ultrasonic assistance generated inhibition on the hydrolysis of TB.

Key words

different pretreatments; solubilization; soluble organic matters; swine wastewater

Introduction

Rapid economic growth and urban expansion in developing countries have led to a large demand for pig farming (Lou et al., 2018). The high swine wastewater production, which accompanies with the conversion of pig farms from smaller size to concentrated animal feeding operations, has become a common environmental problem (Wang, Addy, et al., 2019). The main composition of swine wastewater are feces, urine, and washing water, which are characterized by high concentration of chemical oxygen demand (COD), protein, and carbohydrate (Ding, Cheng, Yu, & Huang, 2017; Liang, Yan, Lu, & Zhang, 2014). Consequently, swine wastewater needs to be treated before meeting emission standards. Anaerobic digestion (AD) is widely recognized as a favorable technique for treating swine wastewater (Cheng et al., 2018; Massé, Talbot, & Gilbert, 2011; Nasir, Mohd Ghazi, & Omar, 2012). This technique consisting of hydrolysis, acidogenesis, acetogenesis, and methanogenesis offers the possibility to reduce volatile suspended solids and odors, destroy pathogens, produce nutrients such as nitrogen and phosphorus, and generate energy in the form of methane gas (González-Fernández, León-Cofreces, & García-Encina, 2008; Isemin et al., 2019). Nevertheless, hydrolysis is determined to be the rate-limiting step throughout the AD process, where cell walls are disrupted so that intracellular organic matter can be available for biodegradation (Gao et al., 2019).

In order to improve the solubilization of organic matter and accelerate the hydrolysis, many pretreatment methods have been used in previous studies like thermal hydrolysis (Cristina, Cristina, & García-Encina, 2008; Tian et al., 2018), ultrasonication (Lee et al. 2009), alkaline (Lin et al., 2013, 2015), acidification (Xing, Li, Fan, & Hou, 2010), and flocculant agent (Campos, Almirall, Mtnez-Almela, Palatsi, & Flotats, 2008). However, due to high energy consumption, economic costs, and serious secondary pollution caused by these physical and chemical methods, biological pretreatment has attracted extensive attention.

In recent years, thermophilic bacteria (TB) have attracted increasing attention as an economical and promising biological pretreatment method. TB pretreatment has the advantages of high-solid phase reduction efficiency as well as simple control requirements (Carrère et al., 2010). Moreover, TB pretreatment can dissolve the cell wall and cell membrane of microorganisms by extracellular hydrolase secreted by thermophilic bacteria, disintegrate the floc structure in wastewater, and hydrolyze large complicated molecules to simpler ones (Yang et al., 2015). However, this effective pretreatment method was mainly used in the field of sludge hydrolysis, the application of TB pretreatment in accelerating the hydrolysis of swine wastewater has never been reported. It was reported that ultrasonic-combined thermophilic bacteria (UL-TB) pretreatment can further promote hydrolysis efficiency compared to TB pretreatment alone (Guo et al., 2016). In this study, UL-TB pretreatment was also used to accelerate the hydrolysis of swine wastewater.

Therefore, the purpose of this study was to investigate the effect of TB and assisted pretreatments on the hydrolysis ability of swine wastewater. Simultaneously, changing of soluble chemical oxygen demand (SCOD), protein, and carbohydrate concentrations in solution was analyzed to compare the differences between the five pretreatment methods. The results obtained in this study could provide valuable information for the disposition of swine wastewater as well as the application of TB-related pretreatments.

Materials and Methods

Characteristic of swine wastewater, inoculation

Swine wastewater was obtained from a pig farm in Jimo district, Qingdao, China, with a stockpile of about 20 pigs, which was filtered through a 2-mm screen to remove large particles, and the appropriate dilution was made prior to use. The characteristics of swine wastewater are shown in Table 1.

Thermophilic bacteria strain (*Bacillus* sp. AT07-1, FJ231008) was isolated in the previous study (Guo, Li, Zeng, & Zhou, 2010). Before inoculation, the flasks and the components of the medium were sterilized at 121°C for 20 min. The strain was cultivated in a 250-mL flask containing 100 ml of Luria–Bertani (LB) liquid medium (10.0 g tryptone, 10.0 g NaCl, 5.0 g yeast extract, 1,000 ml distilled water), with shaking in an bath shaker (SHY-2, Yuecheng, Jiangsu, China) at 65°C, 140 rpm for 48 hr. Then bacteria suspension was collected and used as the inoculum for pretreatment.

Pretreatments of swine wastewater

Different pretreatments were applied to hydrolyze the swine wastewater.

1. Thermophilic bacteria (TB) pretreatment: Adding 200 ml swine wastewater in 250-mL Erlenmeyer flasks. Given the characteristics of TB pretreatment (Guo et al., 2014), bacteria suspension was inoculated in the swine wastewater in the ratio of 1:50 (v/v) at 65°C for 12 hr and adjusted to pH 7.0 ± 0.1. The lysis enzyme secreted by thermophilic bacteria was the principal theory of swine wastewater hydrolysis.
2. Acidic and alkaline pretreatment: The swine wastewater pH was adjusted to 2.0 by adding 6 mol/L hydrochloric acid (HCI) for acidic pretreatment, and the pH of swine wastewater was adjusted to 12.0 with 6 mol/L sodium hydroxide (NaOH) during alkaline pretreatment (González-Fernández et al., 2008). The wastewater was hydrolyzed for 12 hr with stirring of 120 rpm in water bath shaker (SHY-2, Yuecheng) at 25°C.
3. Ultrasonic (UL) pretreatment: The ultrasonic pretreatment was performed with the help of a cell-breaker (SCIENTZ-2D, Xinzhi, Zhejiang, China) at a frequency of 20 kHz (Wang, Wang, & Ji, 2005). Swine wastewater (200 ml) was added to a 250-mL Erlenmeyer flask. The ultrasonic probe was positioned 2 cm submerge the surface of the wastewater, and the swine wastewater pH was adjusted to 7.0 ± 0.1. The sonication time was 3 min (open 5s, off 2s) to release the insoluble organic matters at 4°C.
4. Ultrasonic-combined thermophilic bacteria (UL-TB) pretreatment: Ultrasonic pretreatment was first performed followed by TB pretreatment. The operation method was the same as described above.

All the experiments were performed in triplicate and conducted in 250-mL Erlenmeyer flasks with 200 ml swine wastewater. The supernatants after pretreatment were separated to measure SCOD, carbohydrates, and protein in dissolved organic matter (DOM).

Analytical methods

The samples were centrifuged at 8,000 rpm for 10 min, and centrifuged samples were filtered through a cellulose acetate membrane (0.45 μm pore size). SCOD and TSS were measured

Table 1. The characteristics of swine wastewater

SCOD (MG/L)	CARBOHYDRATE (MG/L)	PROTEIN (MG/L)	TSS (MG/L)	NH_4^+-*N* (MG/L)	PO_4^{3+}-P (MG/L)	PH
9,213.58 ± 460.68	136.38 ± 6.82	3,368.31 ± 168.42	14,270.00 ± 2,210.00	656.81 ± 32.84	84.84 ± 4.24	6.89 ± 0.34

according to our previous study (Gao et al., 2019). NH_4^+-N was measured by Nessler's reagent spectrophotometry, and PO_4^{3-}-P was determined by ammonium molybdate spectrophotometry (Shu et al., 2019). The pH was measured with a digital pH-meter (PHB-5, Aolilong, Hangzhou). The soluble protein was determined by the Lowry–Folin method with bovine serum albumin (BSA) as standard, and soluble carbohydrate was detected by the phenol-sulfuric acid method, using glucose as the standard (Gao et al., 2019).

Statistical analysis

The measurements above were conducted in triplicate. Statistical analysis for the effects of different pretreatments on SCOD, carbohydrate, and protein was conducted by the one-way analysis of variance (ANOVA), using the Statistical Package for Social Sciences (SPSS) software. $p < 0.05$ represents that there are significant differences between the values.

Results and Discussion

Change of SCOD concentration

Chemical oxygen demand is generally considered to be an important parameter for the concentration of organic compounds, while SCOD reflects the soluble organic level (Zhen et al., 2015). The pretreatment effect can be expressed by the change in the concentration of SCOD (Lin et al., 2013). Figure 1 shows the hydrolysis effect of different pretreatment methods on SCOD in swine wastewater. It was obvious that five different pretreatment methods all increased the SCOD concentration, which indicated that more and more particulate organics in wastewater became soluble substrates (Tong & Chen, 2007). As can be seen, the SCOD with TB pretreatment increased from 9,213.58 to 12,579.70 mg/L, which was higher than other pretreatments. It indicated that TB pretreatment had a positive effect on the hydrolysis of swine wastewater. For alkaline pretreatment, the range of pH 8–12 was beneficial for SCOD release of swine wastewater, particularly being treated within short period of time (Lin et al., 2013). In this study, SCOD after alkaline pretreatment reached 12,577.60 mg/L and p-value between TB pretreatment was 0.112 (Table S1). It implied that the effects of alkaline and TB pretreatment on releasing of SCOD were not significant. The SCOD with acid pretreatment increased to 10,559.38 mg/L, which was the lowest of all pretreatments. It could be that the self-protection mechanism of microorganisms resists strong acid environment conditions by producing acidic exopolysaccharide (Wang, Salem, & Sani, 2019), which results in the unsatisfactory release of SCOD. The content of SCOD after UL and UL-TB pretreatment increased from 9,213.58 to 11,274.06 and 11,608.20 mg/L, respectively, with little difference. However, SCOD with UL-TB pretreatment was lower than that of TB pretreatment alone ($p < .05$), which was attributed to inhibition of intracellular metabolism when ultrasound was exposed for more than 15 s (Guo et al., 2016). The results indicated that the releasing of SCOD was more efficient at TB and alkaline pretreatment, and UL had an inhibitory effect on TB pretreatment instead.

Soluble protein and carbohydrate solubilization

The proteins, carbohydrates, and lipids in SCOD released after pretreatment can be converted into soluble substances such as volatile fatty acids, among which the composition of lipid was comparatively low in swine wastewater, less than 1% lipid on the basis of SCOD (Lin et al., 2015). Therefore, the hydrolysis effects of different pretreatments on swine wastewater can be better compared by studying changes in soluble protein and carbohydrate concentration.

Change of soluble protein concentration. The change of soluble protein in swine wastewater is shown in Figure 2. The variation of protein is a composite result of dynamic equilibrium of dissolution-hydrolysis (Liu, Xin, Kang, Yuan, & Du, 2014): On the one hand, some extracellular enzymes (protease, etc.) can destroy microbial flocs to release proteins into the liquid phase; on the other hand, proteins are constantly hydrolyzed into small molecules such as amino acids, and consumed by microorganisms subsequently. In this study, the concentration of protein using alkaline, TB, UL-TB, acidic, and UL pretreatment increased from 3,368.31 to 7,379.12, 5,874.28, 5,759.89, 4,337.41, and 3,438.58 mg/L, which enhanced by 1.20-, 0.74-, 0.71-, 0.29-, and 0.02-folds, respectively. As can be

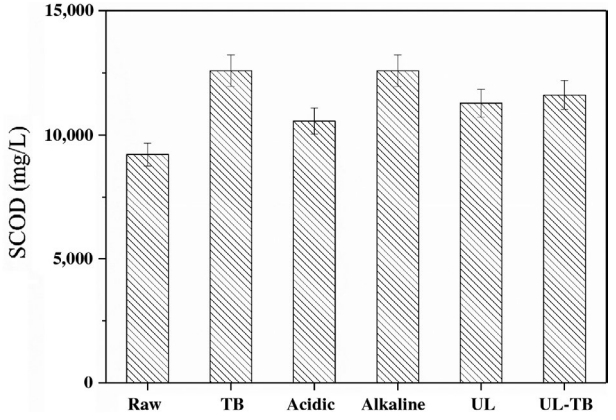

Figure 1. The hydrolysis effect of different pretreatment methods on SCOD in swine wastewater.

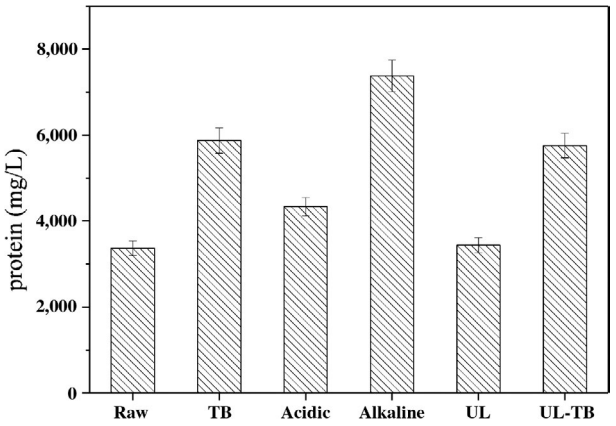

Figure 2. The hydrolysis effect of different pretreatment methods on protein in swine wastewater.

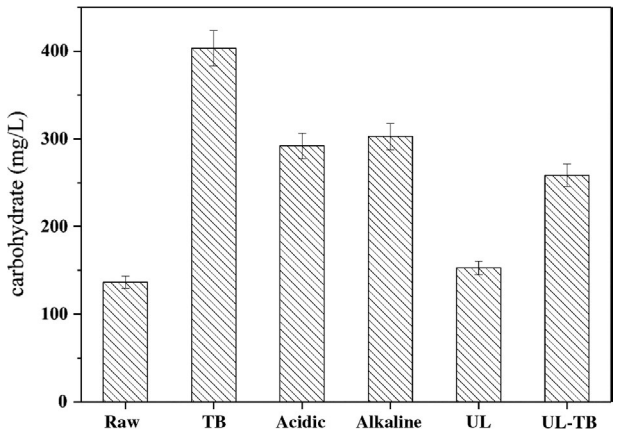

Figure 3. The hydrolysis effect of different pretreatment methods on carbohydrate in swine wastewater.

seen, the protein with alkaline pretreatment was higher than other pretreatments significantly (Table S2). This result was related to the research that alkaline treatment was more beneficial to the release of soluble protein (Yuan, Huang, Lei, Zhao, & Zhang, 2017). The protein release of the UL-TB pretreatment was lower than that of the TB pretreatment alone, which could be attributed to the reduction of soluble protein concentration caused by oxidation of free radicals under 20 kHz ultrasound pretreatment (Liu et al., 2017).

Change of soluble carbohydrate concentration. Figure 3 shows the hydrolysis effect of different pretreatment methods on soluble carbohydrate in swine wastewater. The concentration of carbohydrate with TB pretreatment increased from 136.38 to 403.38 mg/L under the function of extracellular enzymes secreted by TB (Guo, Zhao, She, Lu, & Zong, 2012), which was higher than other pretreatments significantly (Table S3). Similar results were also observed in the previous study, which found that the effect of TB pretreatment on carbohydrate release was better (Liang, Lu, Li, Zhang, & She, 2015). Compared with TB pretreatment, a lower content of carbohydrate was observed at UL, UL-TB, acidic, and alkaline pretreatment, and the concentration of carbohydrate reached to 152.65, 258.36, 291.83, and 302.63 mg/L, respectively. It indicated that the releasing of carbohydrate after UL-TB pretreatment was lower than that of TB pretreatment, and TB pretreatment was more beneficial for the increase of carbohydrate.

Based on above results, TB and alkali pretreatment were more effective for the hydrolysis of swine wastewater, which can release more organic matter compared with other pretreatments. The dosage of chemical reagent (NaOH) is an important factor affecting the effect of alkaline pretreatment. Although strong alkali condition could improve the hydrolysis efficiency of swine wastewater, it would also produce higher economic costs due to the increase of chemical reagent dosage, and extreme pH conditions after pretreatment could inhibit the microbial activity involved in acidogenic fermentation (Lin et al., 2015), which makes subsequent treatment difficult. Compared to alkaline pretreatment, thermophilic bacteria inoculated into the reaction system can quickly become the dominant population, and other anaerobic or facultative microbes also can co-exist in a digestion process, thus maintaining the microbial activity in the reaction system (Liu, Zhu, Li, & Yuan, 2011). Therefore, the method when hydrolyzing swine wastewater by TB pretreatment in practice is more appropriate considering the easy viability of thermophilic bacteria and subsequent fermentation processes. In addition, to better clarify the efficiency of swine wastewater hydrolysis, the compositional and structural characteristics of extracellular polymeric substances (EPS) and dissolved organic matter (DOM) also deserve further study.

Conclusions

This study first reported the hydrolysis effect of thermophilic bacteria and assisted pretreatment on swine wastewater. TB and alkali pretreatment exhibited the highest hydrolysis performance, implying its potential application in swine wastewater treatment. TB pretreatment was found to enhance the solubilization of carbohydrate (up to 1.20-fold) efficiently, while inhibited protein release compared to alkali pretreatment. The assistance of UL brought about inhibition on the hydrolysis of TB instead.

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Data Availability Statement

I and co-authors promise that the data in the article are available.

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