



Evaluation of potassium as promoter on anaerobic digestion of saline organic wastewater

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ABSTRACT

In this work, the effect of potassium on mesophilic anaerobic digestion (AD) of saline organic wastewater, which consisted of simulated effluents obtained from heparin sodium production, was studied. The results showed that the addition of potassium chloride (KCl) to saline organic wastewater enhanced the AD efficiency. The optimal dosage was found to be 0.174% when the salt (NaCl) content was 2.0%. Under this condition, the chemical oxygen demand (COD) removal efficiency, dehydrogenase activities, and the viability of microorganisms reached 62.7%, 55.7 TF μL^{-1} , and 78.4%, respectively, which were 115.4%, 77.2%, and 20.3% higher than those without the addition of potassium chloride. The consumption of volatile fatty acids (VFAs) was enhanced during the AD process. Moreover, less humic-like and protein-like residues appeared in the wastewater after AD. Potassium could maintain the morphology of anaerobic microorganism under high salinity and showed a long-term effect.

1. Introduction

Many industrial sectors, including agrofood, petroleum, and leather industries generate saline organic wastewater (Lefebvre and Moletta, 2006). Except for high content of salt (> 1%), saline organic wastewater is usually rich in organic matter (chemical oxygen demand (COD) > 2000 mg L⁻¹) and toxic compounds (Mannina et al., 2016). With ever-tightening regulations for wastewater discharge, attention on saline wastewater treatment processes has increased rapidly over the past two decades (Shi et al., 2015). To solve the problem, the salt, organic components, and toxic compounds in the wastewater must be removed before discharge.

For removing salts from saline wastewater, physicochemical processes, including thermal techniques, coagulation–flocculation, ion exchange, and membrane techniques were usually utilized (Jang et al., 2013). However, organics in wastewater seriously affect the efficiency of physicochemical treatment. Therefore, pretreatments should be adopted properly to remove the organics. The removal of salts can be achieved efficiently by reverse osmosis (RO). Finally, the concentrate residue separated by RO could be evaporated and recycled, provided the purity is adequate (Lefebvre and Moletta, 2006). Therefore, effective removal of organic compounds from saline organic wastewater is of

great necessity.

Anaerobic treatment provides a method for reducing organic pollutants from agricultural and industrial sectors, while simultaneously offsetting the use of fossil fuels (Chen et al., 2008). However, high salinity strongly inhibits the metabolism of non-halophilic bacteria (Church et al., 2017) as high salt concentration (> 1%) usually causes the loss of cellular water (plasmolysis) or cytoplasm recession or even the disintegration of cells (Abou-Elela et al., 2010). Under high salinity conditions, the osmotic pressure difference across the cell wall usually leads to outflow of intracellular water or even the breakdown of the cell. Therefore, it is difficult to apply anaerobic biotechnology to the treatment of saline organic wastewater.

In order to solve this problem, much research have been conducted, such as screening halophilic organisms (Shi et al., 2015; Duan et al., 2015), acclimatizing microorganisms to saline wastewater (Mendez et al., 1995) or using marine microalgae (Shen et al., 2015). However, the sources of halophiles organisms are limited, and the non-halophilic microorganisms require long time periods to adapt to high salinity conditions. Mendez et al. (1995) reported that the time period was 719 days for non-halophilic anaerobic organisms to acclimatize when the salt content exceeded 17.0 g L⁻¹. Marine microalgae are efficient in removing nutrients, including phosphorus and nitrogen (Shen et al.,

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2015). However, they are inefficient in removing organic compounds in wastewater. Therefore, an improved approach, which was more flexible and economical to treat saline organic wastewater, is necessary.

One method is the addition of osmoprotectants to AD system for microbes to resist high salinity conditions. Then, the microbes could osmotically accumulate active compatible solutes to balance the external osmotic pressure (Martin et al., 1999). Usually, glycine betaine, choline, carnitine, and trehalose can be used as osmoprotectants (Oh et al., 2008). Zhang et al. (2016) reported that glycine betaine aids anaerobes to recover methanogenic activity following the inhibition of salinity. However, the compatible solutes were organic materials and could be consumed by organisms as carbon or nitrogen sources. Therefore, the addition of compatible solutes remains a critical issue in this field.

Another method is the “salt in” strategy, which was frequently adopted for extreme halophilic archaea and halotolerant bacteria. “Salt in” strategy means that inorganic ions are accumulated to balance the external osmotic pressure (Martin et al., 1999). In many hyper saline natural environments, the content of sodium ions is far greater than that of potassium ions and maintains a stable and high intracellular K^+ / Na^+ ratio, which is crucial for the survival of microbes (Plemenitaš et al., 2014). This enables the potassium to be used as a potential promoter for biological treatment of saline organic wastewater. However, to the best of our knowledge, the effect of potassium on AD of saline organic wastewater has still not been studied.

The objective of the present study is to examine the potential of potassium as a promoter for AD for saline organic wastewater by (1) investigating the effects of potassium on the performance of AD; (2) determining the optimal dosage of potassium during the treatment of saline organic wastewater containing salt (NaCl) content of 2.0%; (3) analyzing the effect of addition of potassium on the change of viability and morphology of anaerobic microorganisms under different potassium concentrations. This study could provide a new strategy for enhancing the anaerobic treatment of saline organic wastewater in practice.

2. Materials and methods

2.1. Wastewater and seed sludge

The wastewater used in this study was a basic synthetic wastewater (BSW) which simulated casing wastewater. Casing wastewater is mainly produced in the production of heparin sodium. The salt concentration and COD of casing wastewater are (0.9–2.5%) and 1000–5000 $mg L^{-1}$, respectively. The main composition is listed in Table 1. The metal trace solutions for an optimum anaerobic microbial growth were prepared according to the work of Zhang et al. (2014). The initial mean COD of BSW was 4240 $mg L^{-1}$ (COD/N/P = 200/4.7/1). The seed sludge used in this study was collected from a mesophilic UASB reactor treating starch wastewater (Wuxi, China). It was then washed twice with distilled water to remove dissolved organic matter and sieved to remove sand and other particles. Table 2 presents the characteristics of seed sludge.

Table 1
Composition of the synthetic wastewater.

Composition	Concentration ($g L^{-1}$)	Trace metals	Concentration ($g L^{-1}$)
$C_6H_{12}O_6$	4.00	$MnCl_2 \cdot 4H_2O$	0.55
KH_2PO_4	0.15	$CaCl_2 \cdot 2H_2O$	0.05
NH_4Cl	0.16	$ZnCl_2$	0.07
$NaNO_2$	0.20	$CoCl_2 \cdot 6H_2O$	0.12
		$NiCl_2 \cdot 6H_2O$	0.12
		$FeCl_2$	0.40

Table 2
Main physico-chemical characteristics of the seed sludge.

Parameters	Unit	Value
Total solids	$g L^{-1}$	25.2 ± 0.3
Volatile solids	$g L^{-1}$	19.7 ± 0.2
VS/TS	%	0.78
Average particle size	μm	391
Conductivity	$mS cm^{-1}$	3.8 ± 0.2
Salinity	$g L^{-1}$	2.3
Sodium	$g L^{-1}$	0.14
Sulfate	$g L^{-1}$	0.36
Ammonium	$g L^{-1}$	1.05
Density	$g cm^{-3}$	1.01 ± 0.02

2.2. Biomethane potential test

In order to determine the optimal dosage of potassium in wastewater with salt (NaCl) content of 2.0%, biomethane potential (BMP) test was performed in triplicates with Automated Methane Potential Test System (AMPTS; Bioprocess Control AB, Lund, Sweden). The methane production was determined using water displacement method after removing CO_2 with 3 M NaOH. Eight groups of AMPTS bottles were used for BMP test. Each bottle contained 400 mL of BSW and 8.0 g of NaCl, which were then mixed with seed sludge. The seed sludge content (TS) was $13.75 g L^{-1}$ in each bottle. The food-to-microorganism ratio was $0.397 g COD (g VS)^{-1}$. Then, 0.00%, 0.017%, 0.035%, 0.087%, 0.174%, 0.348%, 0.870%, and 2.262% of KCl were added separately to different AMPTS bottles, which were denoted as R0, R1, R2, ..., R7, respectively. Another group of AMPTS bottles containing 400 mL of BSW and seed sludge mixture without NaCl and KCl was set as the control and named as R8. The bottles were then flushed with N_2 gas for 10 min. The experiment was performed under the temperature of 35 °C.

2.3. Long-term batch test

The results from BMP test showed that the addition of 0.174% and 0.348% of KCl could significantly promote the methane production. Based on this preliminary result, long-term batch tests were conducted in triplicates to further study the promoting effect of potassium. The experiments were conducted in 5000 mL cultivation bottles, which were sealed with a rubber bung. Two 4-mm diameter syringe needles penetrated through the rubber bung. One was deep into the liquid level for sampling, and the other was set over the liquid level for gas collection. The KCl contents of the three different bottles were designated to be 0.174% (D-1), 0.348% (D-2) and 0.000% (Control), respectively. The bottles were flushed for 5 min with N_2 . Then, the cultivation bottles were incubated in a water-bath at 35 °C and shaken manually before sampling. Mixed samples of 5 mL were collected after shaking. Then, 5 mL of BSW was added into the bottle. The experiments lasted for 308 h. Finally, the cultivation bottles were incubated under static conditions for two months to examine the long-term effect of potassium on bacterial morphology.

2.4. Analytical methods

COD, VS and TS were determined according to the APHA Standard Methods (APHA, 2012). In order to eliminate the interference of chloride, $HgSO_4$ was added as a shielding reagent, and the sample was diluted by 20 times before COD measurement. Additionally, before the refluxing procedure, methane was collected using water displacement method after removing CO_2 with 3 M NaOH. Ethanol and VFAs were determined using gas chromatography (GC-2010 Plus; Shimadzu, Japan). Dehydrogenase activities in the digesters were determined according to the method reported by Zhang et al., (2011). The

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