INTRODUCTION

Kitchen waste is a biomass resource with high organic content but leading to severe environmental pollution if not properly treated before discharge.1,2 In China, urban kitchen waste accounts for 37%-62% of all household waste while the annual production of kitchen waste in major cities reached 97 million ton.3 In Beijing, Shanghai, and other major cities with a developed catering industry, the daily production of kitchen waste exceeds 2000 ton.4 Due to its high moisture and organic contents, the technology of anaerobic digestion (AD) is currently one of the most effective methods for treating kitchen waste.5,6 The process of AD can also produce renewable energy,7,8 whereby the digestion in a closed system avoids the emission of foul odor and reduces the environmental burden of kitchen waste.9,10 Using kitchen waste as raw material, methane production by anaerobic digestion is a good way to use waste resources efficiently. According to Zhang et al.,1 a high methane yield can be obtained when kitchen waste was treated by an anaerobic digestion. Due to the high salt concentration in the wastewater feed, and the possible accumulation of salt in the digestion sludge during the AD operation, the high concentration of NaCl can affect the diversity of the sludge, subsequently reducing the activity of methanogens11 and eventually leading to the collapse of the system. Although the composition of food waste is highly variable depending on its collection source, it usually contains a high salinity. Oh et al.12 reported that the NaCl-amended food waste contained 10-35 g L⁻¹ NaCl, while the nonwashed food waste contained 11.6 g L⁻¹ NaCl. Dai et al.13 collected food waste from canteens in Shanghai with NaCl concentration of 8.0 g L⁻¹. Wang et al.14 found that the NaCl concentration from food waste anaerobic digestion could reach 13.8 g L⁻¹. Other studies have shown that the concentration of Na⁺ was moderately inhibiting mesophilic methanogens at 3.5-5.5 g L⁻¹, but strongly inhibiting when the

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**RESEARCH ARTICLE**

Influence of ammonium acetate and betaine supplements on the anaerobic digestion under high salinity conditions

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Abstract
High salinity frequently causes inhibition and even failure in anaerobic digestion. To explore the impact of increasing NaCl concentrations on biogas production and reveal the role of repair factors in response to high salinity stress, the present research examined the effect of ammonium acetate and betaine as exogenous repair factors on the activity of microorganisms under high-salt conditions. High salinity wastewater with and without repair additives were compared. The cumulative methane production significantly increased and the methane concentration increased by approximately 30%. The results showed that the repair factors were efficient to relieve the effect of a high-salt environment on the anaerobic microbial flora, thus considerably reducing the salt-induced inhibition of anaerobic digestion.

KEYWORDS
ammonium acetate, anaerobic digestion, betaine, food waste, high salinity

1 | INTRODUCTION

Kitchen waste is a biomass resource with high organic content but leading to severe environmental pollution if not properly treated before discharge.1,2 In China, urban kitchen waste accounts for 37%-62% of all household waste while the annual production of kitchen waste in major cities reached 97 million ton.3 In Beijing, Shanghai, and other major cities with a developed catering industry, the daily production of kitchen waste exceeds 2000 ton.4 Due to its high moisture and organic contents, the technology of anaerobic digestion (AD) is currently one of the most effective methods for treating kitchen waste.5,6 The process of AD can also produce renewable energy,7,8 whereby the digestion in a closed system avoids the emission of foul odor and reduces the environmental burden of kitchen waste.9,10 Using kitchen waste as raw material, methane production by anaerobic digestion is a good way to use waste resources efficiently. According to Zhang et al.,1 a high methane yield can be obtained when kitchen waste was treated by an anaerobic digestion. Due to the high salt concentration in the wastewater feed, and the possible accumulation of salt in the digestion sludge during the AD operation, the high concentration of NaCl can affect the diversity of the sludge, subsequently reducing the activity of methanogens11 and eventually leading to the collapse of the system. Although the composition of food waste is highly variable depending on its collection source, it usually contains a high salinity. Oh et al.12 reported that the NaCl-amended food waste contained 10-35 g L⁻¹ NaCl, while the nonwashed food waste contained 11.6 g L⁻¹ NaCl. Dai et al.13 collected food waste from canteens in Shanghai with NaCl concentration of 8.0 g L⁻¹. Wang et al.14 found that the NaCl concentration from food waste anaerobic digestion could reach 13.8 g L⁻¹. Other studies have shown that the concentration of Na⁺ was moderately inhibiting mesophilic methanogens at 3.5-5.5 g L⁻¹, but strongly inhibiting when the
concentration of Na⁺ was 8 g L⁻¹. Zhao et al. found that low levels of NaCl improved the process of hydrolysis and acidification, but inhibited the production of methane, while high levels of NaCl inhibited both steps. The high salinity could moreover cause an imbalance of cell osmotic stress, resulting in plasmolysis or loss activity of cells, which would further cause inhibition or even failure of the anaerobic digestion process. High salt concentrations are therefore important for the activity of methanogens in AD sludge. Since a high-salt environment has a significant effect on the activity of the methanogenic flora, it can readily cause an excess formation of volatile fatty acids and then lead to an imbalance of the production and consumption of acid, resulting in the accumulation of VFAs. The pH of the system will diminish to values below the range of the optimum pH of the methanogens, leading to a disruption of the system. If the minimum value of the pH can be significantly increased during the early stage of digestion, the activity of the methanogens can be largely protected, so that the digestion can be carried out stably. Previous research indicated that ammonia nitrogen enhances the buffering capacity of anaerobic digestion systems by neutralizing volatile fatty acids produced during digestion. Research showed that the free radicals would increase under adverse conditions such as salt and drought, causing an enhanced membrane lipid peroxidation, a destruction of the membrane system, an increased content of the membrane lipid peroxidation product malonaldehyde (MDA), and an increased plasma membrane permeability. Exogenous addition of betaine can effectively scavenge free radicals.

Betaine has previously been used in plants related to high-salt soils. Studies have shown that glycine betaine (GB) was a natural nontoxic osmotic regulator, widely found in plants, animals, and microorganisms. Betaine is considered to be the most promising osmoprotectant and has received increasing attention in plant drought and salt tolerance studies. The results of Karabudak et al. showed that GB may induce the expression of tomato FAD7 (fatty acid desaturase 7) and LOX (lipoxygenase) genes, increasing the content of lipids and improving the stability of the membrane. Li et al. indicated that betaine increased the concentration of free calcium ions in tobacco cells and enhanced the expression of calmodulin (CAM) and heat shock transcription factor (HSF), which enhanced the expression of heat shock protein genes and improved both the heat tolerance and salt resistance. The application of betaine in anaerobic digestion has not been previously studied. Consequently, ammonium acetate and betaine were tested as exogenous repair factors to explore the microbial mechanism of anaerobic digestion of kitchen waste and its synthetic equivalent under high-salt environment. At the same time, a method to reduce or possibly eliminate the inhibition of an AD system by high-salt environment can be obtained.

2 EXPERIMENTAL MATERIALS AND METHODS

2.1 Experimental materials

Anaerobic digestion sludge, as inoculum, was obtained from a 100 m³ kitchen waste anaerobic digestion process from the Changping Pilot Base of the Beijing University of Chemical Technology. Experimental reagents such as soluble starch, peptone, sodium chloride, potassium chloride, acetone, ammonium acetate, and betaine were purchased from Beijing Chemical Factory.

2.2 Experimental layout

Several identical digesters were operated in parallel. Batch anaerobic digestion was carried out in a 1.2 L digester with an effective volume of 1 L (Figure 1). The temperature was kept at 35°C by using a thermostatic bath. The top of the digester was sealed with a rubber stopper, leaving sealed ports for the feed, the water sampling, and the biogas exhaust. The biogas was collected through a sealed airbag, and its produced volume was measured by a gas drainage method.

The main explored parameters of the experiments include pH, biogas volume, and methane content. The pH value was measured by a portable pH meter. A gas chromatograph GC-2014C was used to determine the biogas composition. A TCD detector and stainless steel-packed column TDX-0 were used. The carrier gas was argon, the column pressure was 0.3 MPa, the flow rate was 25 ml min⁻¹, the inlet temperature and the column temperature were 160°C, and the detector temperature was 180°C. Volatile solids (VS) were determined according to the APHA standard method.

![Figure 1](image-url)
Total Carbon Utilization was calculated as \( \frac{n(CH_4)}{n(\text{total carbon})} \) (with \( n \) as the amount of the respective substance).

2.3 | Experimental design

All experiments were carried out in duplicate, and average values together with deviations (error bars) will be further reported. Gradient experiments were carried out on the quantity of starch while maintaining the same quantity of peptone (1.22 g). It was found that 6 g of starch achieved the highest methane yield, and hence 6 g of starch and 1.22 g of peptone were selected as substrates. With a total nitrogen content of 14.5 wt% in peptone, the C/N ratio is \( \frac{6 \times 72/162}{1.22 \times 0.145} = 15.1 \). This value is within the commonly applied optimum C/N range of AD, reported as 15-20.

The high-salt environment was created and controlled to a constant feed concentration of 20 g L\(^{-1}\) NaCl, within the range of commonly encountered salt concentrations in kitchen waste water,\(^{12-14}\) but selected to ensure that inhibition would normally take place, hence offering the possibility to study the effect and potential use of exogenous repair conditions. Blank controls were also operated under high-salt (20 g L\(^{-1}\)) and zero salt conditions. Digestions were performed at 35°C.

Ammonium acetate was added at 0, 1, 2, 4 g L\(^{-1}\) for a concentration of 20 g L\(^{-1}\) NaCl.

Alternatively, betaine was added at 0, 0.12, 0.6, 1.2 g L\(^{-1}\), again in an AD system with 20 g L\(^{-1}\) NaCl.

Finally, a dual addition of ammonium acetate and betaine, at their respective optimum concentration, was performed.

3 | RESULTS AND DISCUSSION

3.1 | Effect of ammonium acetate

3.1.1 | The methane yield and total carbon utilization

The methane yield and total carbon utilization rate when ammonium acetate was added under high-salt conditions were shown in Table 1. With the increase of the concentration of ammonium acetate, the yield of methane and the total carbon utilization both increased first and then decreased. Although the total carbon utilization rate at different ammonium acetate concentrations did not change significantly throughout the digestion process, the methane yield reached a maximum of 262.22 mL (g VS\(^{-1}\)) when the concentration of ammonium acetate was 1 g L\(^{-1}\), which was much higher than in other groups. The cumulative methane production reached 3060 mL. The concentration of 1 g L\(^{-1}\) ammonium acetate was hence selected as optimum dosage.

It should, however, be remembered that the acetate addition will also participate in the methane production according to the acetic acid type fermentation (2 CH\(_3\)COOH → 2 CH\(_4\) + 2 CO\(_2\)). The addition of 1 g of ammonium acetate adds 0.31 g of acetate, hence capable of producing 330 mL of methane for a 100% conversion. The net production of 3060 – 330 mL = 2730 mL still significantly exceeding the production in a salt-free environment (2232 mL only, as shown in Figure 2A).

3.1.2 | The change of methane content and pH

The cumulative production of methane in the anaerobic digestion was shown in Figure 2A. The cumulative yields of methane were 2232 mL and 862 mL in the salt-free environment and the high-salt control environment, respectively. The cumulative production of methane with ammonium added was 3060 mL. It can be seen that the inhibition of the production of methane by the salt was considerable. Ammonium acetate promoted the cumulative production of methane in the system.

The variation of methane concentration of different groups was shown in Figure 2B. The final stable value of the methane concentration of the high-salt control group fluctuated around 35%. The methane concentration in the salt-free group was stable in the range of 70%. With ammonium acetate added, the salt-free concentration level was nearly reached.

The pH of the system during the process of the digestion was monitored, and the pH curve of the system was plotted in Figure 2C. The pH curve mainly reflects the acidification of the system and the recovery after acidification. The acid stage of the control group without salt was up to 60 hours. The acidification stage of other groups under high salinity reached 150 hours, and then began to recover slowly. This

| TABLE 1 | Methane yield and total carbon utilization rate of ammonium acetate gradient under high-salt condition* |
|----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                | Ammonium acetate (0 g L\(^{-1}\)) | Ammonium acetate (1 g L\(^{-1}\)) | Ammonium acetate (2 g L\(^{-1}\)) | Ammonium acetate (4 g L\(^{-1}\)) |
| Methane yield mL (g VS\(^{-1}\)) | 232.61 ± 27.84 | 262.22 ± 26.74 | 256.32 ± 21.54 | 233.36 ± 24.54 |
| Total carbon utilization (%) | 29.66 ± 3.56 | 34.27 ± 3.16 | 33.94 ± 2.61 | 31.60 ± 3.33 |

*\(c(\text{NaCl}) = 20 \text{ g L}^{-1}.\)
result indicated that the high salinity environment caused a significant decrease in the rate of acidification. In addition, it is shown that the salt-free environment has stabilized at 300 hours, while the same situation requires approximately 500 hours under high salinity conditions: the high-salt environment severely impacts the system again.

As is shown in Figure 2A-C, under high-salt conditions, the cumulative production of methane in the group with ammonium acetate added and the control group had little difference in the early stage (0-400 hours). In the later period (400-720 hours), the difference gradually appeared. According to Appels et al., the process of the anaerobic digestion of organic macromolecular substances can be divided into four stages: hydrolysis, acidification, acetic acidification, and methanation. In the methanation stage, acetate was used as substrates to form methane under the action of methanogens. Wang et al. found that $\text{NH}_4^+$ and small molecule acids form a buffer system, which in turn increased the methane yield. With the gradual consumption of acetate, the role of ammonium acetate can be exerted.

In summary, through the changes in methane production and pH, it can be seen that in the high-salt environment, the effect of ammonium acetate gradually manifested in the late stage of digestion (400-720 hours), mainly in the increase of the methane production and concentration. In the early and intermediate stages of digestion (0-400 hours), its effects are less outspoken since the yield of methane is reduced and the speed of acidification is generally slow.

### 3.2 Effect of betaine

#### 3.2.1 The methane yield and total carbon utilization

As shown in Table 2 and in Figure 3, the methane yield and the rate of total carbon utilization when adding a betaine gradient under high-salt conditions were almost the same during the digestion, although significantly exceeding the results of the control group. Thus combining results of methane yield and
of total carbon utilization, and in view of cost saving, the optimum addition amount of betaine was selected at 0.12 g L\(^{-1}\).

### 3.2.2 The change of methane content and pH

The groups with betaine added maintained a significantly higher methane production during the whole process. As was shown in Figure 3A, the cumulative production of methane with betaine added was 3147 mL, almost 3.7 times to that of the high-salt control group, and 915 mL higher than the salt-free control group. This was consistent with the expected role of betaine in anti-high salt before the experiment. It can be seen from Figure 3B that the methane concentration increased first and then tended to be stable throughout the anaerobic digestion process. The role of betaine appeared from the beginning. The methane concentration in the group with betaine added was stable at 200 hours; however, other groups required 300 hours. Figure 3C, illustrates that the acidification stage of the group with betaine added was also 60 hours, it was 90 hours shorter than the group with ammonium added. These results indicated that betaine can alleviate the inhibition of a high-salt environment but also accelerated the initiation of anaerobic digestion.

### TABLE 2 Methane yield and total carbon utilization rate of betaine gradient under high-salt condition\(^a\)

| Betaine (0 g L\(^{-1}\)) | Betaine (0.12 g L\(^{-1}\)) | Betaine (0.60 g L\(^{-1}\)) | Betaine (1.2 g L\(^{-1}\)) |
|--------------------------|---------------------------|---------------------------|---------------------------|
| Methane yield mL (g VS\(^{-1}\)) | 252.55 ± 26.67 | 277.23 ± 28.20 | 276.27 ± 29.67 | 283.06 ± 33.39 |
| Total carbon utilization (%) | 33.06 ± 3.49 | 35.97 ± 3.67 | 35.23 ± 3.78 | 35.16 ± 4.15 |

\(^a\)c(NaCl) = 20 g L\(^{-1}\).
Effect of the optimal addition of ammonium acetate and betaine

As is shown in Figure 4, during the process of the whole digestion, the cumulative yield of methane in the mixed optimal experimental group reached 4073 mL, almost twice that of the control group without salt and significantly better than the other experimental groups. The joint addition of ammonium acetate and betaine to the system significantly reduced the inhibition caused by high salt concentrations.

4 | CONCLUSIONS

In a high-salt environment, the digestion was severely inhibited.

Adding ammonium acetate improved the performance, especially at the later stages of digestion (400-720 hours). Betaine can significantly improve the methane production, the acidification hysteresis, and the stability of the system during the predigestion period (0-400 hours) in the high-salt environment. If both repair factors are jointly added, they significantly enhance the activity of methanogens and their methanogenic capacity.

The cumulative yield of methane increased by about 3.7 times, the concentration of methane increased by about 30%, and the stable methane concentration is achieved about 100 hours earlier. Ammonium acetate acts as exogenous buffer, while betaine can increase the stability of the intracellular environment, even under extreme conditions.

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