

Research Article

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Effect of pretreatment with alkali on the anaerobic digestion characteristics of kitchen waste and analysis of microbial diversity

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Abstract: Kitchen waste contains high contents of organic matter and moisture, and it is prone to biodegrade and decompose to give odors. If not collected and transported promptly or treated improperly, it is highly likely to pollute the environment and spread diseases. Because the lipid content in kitchen waste is high and a portion of organic matter is not subject to hydrolysis, the development of anaerobic digestion technology has been greatly limited. Kitchen waste was pretreated with NaOH, KOH, and Ca(OH)₂ with different concentrations, and 50 days sequencing batch mesophilic anaerobic digestion experiments were conducted. This study sheds light on the pollution reduction and energy generation of kitchen waste. The results are as follows: (1) The lipid content of kitchen waste could be reduced, and the concentration of dissolved organic matter could be increased by pretreating with alkali. The degradation rate of kitchen waste lipid reached a maximum of 50.51%, if 3% NaOH was added, and the soluble chemical oxygen demand concentration was increased by 235.3%. (2) The cumulative methane (CH₄) output and biogas production efficiency were improved in the anaerobic digestion process with kitchen waste pretreated with alkali. The maximum daily gas output of kitchen waste pretreated with NaOH and KOH took place on the 11th to 12th day, with the biogas production efficiency of 40.4 and 45.2 mL·g-VS⁻¹. The cumulative CH₄ output was increased from 370.2 mL·g-VS⁻¹ (untreated) to 393.1 and 434.1 mL·g-VS⁻¹, respectively. In addition, the concentration of CH₄ in biogas was increased from 54.8%

(untreated) to 59.1% and 61.7%, respectively. (3) The Chao1 and Ace values of bacteria were increased first and then decreased. On the 10th day, the diversity of bacteria reached the highest value, and on the 20th day, the diversity of archaea reached its maximum. Therefore, it was verified that the improvement in the hydrolysis acidification efficiency and degree was crucial for the rapid and complete anaerobic digestion reactions.

Keywords: kitchen waste, pretreatment with alkali, anaerobic digestion, microbial diversity

1 Introduction

With the development of urbanization and level of national consumption, the output of kitchen waste increases continuously, and kitchen waste has become one of the most important components of urban living waste [1]. Kitchen waste contains high contents of organic matter and moisture, and it is prone to biodegrade and decompose to give odors. If not collected and transported promptly or treated improperly, it is highly likely to pollute the environment and to spread diseases. However, the traditional waste treatment methods, such as landfilling, incineration, and composting, have the disadvantages of occupying large area, high operating costs, or secondary pollution [2], so they are not ideal kitchen waste treatment methods. With the continuous improvement in traditional waste treatment technology and promotion of waste classification policy, the research on anaerobic digestion treatment of kitchen waste has drawn attention. However, because the lipid content in kitchen waste is high and a portion of organic matter is not subject to hydrolysis, the development of this technology has been greatly limited [3,4], so how to reduce the concentration of dissolved organic matter in kitchen waste is particularly important. Chemical pretreatment methods mainly include ozone oxidation pretreatment, acid hydrolysis pretreatment, alkali hydrolysis pretreatment, and so on. Chemical pretreatment

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methods have been adopted, and good performance has been delivered, by adding weak alkaline solution to reduce the concentration of dissolved organic matter in the produced waste, prevent the accumulation of acid in the process of anaerobic effect and inhibit the anaerobic digestion of gas production, and achieve good results. Acid and alkali pretreatment can be done by chemical reaction. It should promote the conversion of part of the insoluble organic matter in the substrate to dissolved organic matter by adjusting pH. The change of pH value makes fine cell wall dissolution, or by changing osmotic pressure, release of intracellular substances, has a certain hydrolysis effect [4]. Xin *et al.* [5] pretreated rice straws with 6% NaOH and 3% H₂SO₄, and the anaerobic-digestion biogas output was increased by 28.5% and 12.5%, respectively, compared to the control group. Based on these studies, we pretreated kitchen waste with NaOH, KOH, and Ca(OH)₂ with different concentrations, and the waste was treated in 50 days sequencing batch mesophilic anaerobic digestion experiments. Before and after the pretreatment, the contents of lipid and dissolved organic matter in the kitchen waste were detected, and the effects of pretreatment with alkali on CH₄ production rate, cumulative CH₄ output, pH, microbial diversity, and other parameters were analyzed. This work is beneficial for the energy generation from efficient anaerobic digestion of kitchen waste.

2 Materials and methods

2.1 Apparatus

As shown in Figure 1, a custom-made anaerobic digestion reactor was used in this experiment. The apparatus was composed of two jars (1 L) and a volumetric flask (1 L),

which functioned as the raw-material digestion tank, and biogas-collecting and effluent-collecting containers. These three containers were connected with anti-aging rubber tubes, so an intra-connected apparatus was assembled, and the air tightness was ensured.

2.2 Materials

2.2.1 Kitchen waste

The kitchen waste experimented was sampled from the cafeteria in the college. The waste was shredded and thoroughly homogenized by a blender. To ensure the consistency of the samples, 5 kg of kitchen waste was prepared at a time, and was stored at −20°C. Before the experiment, the sample was stored at 4°C for 12 h for thawing.

2.2.2 Activated sludge

The inoculum for anaerobic digestion in the experiment was obtained from the residual sludge of a neighboring sewage treatment plant. The sludge was transported to a large, sealed plastic container at about 20°C. After returning to the laboratory, the sludge was cultured and domesticated at 37°C as follows: 5 L of the sludge was placed in a 25 L sealed plastic container for cultivation at 37°C. Three days later, the sludge had undergone its adaptation period, and 2.5 kg of room-temperature kitchen waste was added for 10 days of cultivation. Then, 5 kg of room-temperature kitchen waste was domesticated for 10 days. The sludge could be used. The dry-matter weights of kitchen waste and inoculated sludge were measured after drying in an oven at 105°C for 24 h, and the organic matter content was measured after calcination with a

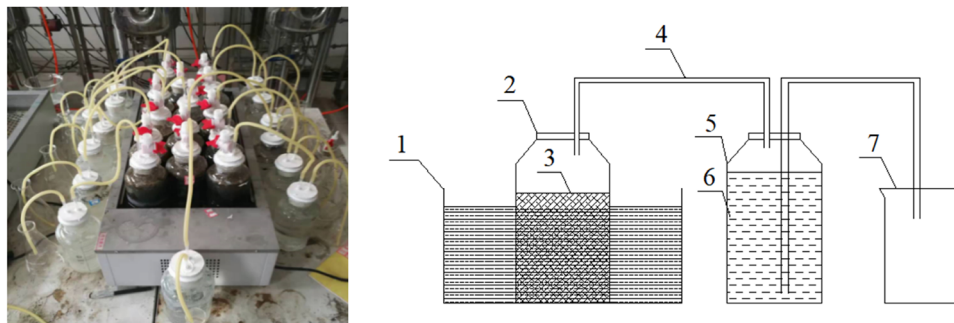


Figure 1: Physical and schematic illustration of the apparatus: (1) water bath, (2) digestion-reaction bottle, (3) mixture of kitchen waste and acclimated sludge, (4) air duct, (5) gas cylinder, (6) distilled water, and (7) beaker.

Table 1: Main parameters of wet ground state of kitchen waste and activated sludge

Parameter	TS (%)	VS (%)	pH	TC (%)	TN (%)
Kitchen waste	23.29	70.55	5.13	46.07	4.73
Activated sludge	9.32	37.73	7.41	—	—

muffle furnace at 550°C for 4 h. The primary parameters of the wet kitchen waste and activated sludge are shown in Table 1, and the contents of nutrients in the kitchen waste are shown in Table 2.

2.3 Experimental procedures

30 sets of digestion reactors (1 L) were used in the sequencing anaerobic digestion experiment of kitchen waste pretreated with alkali. A total of ten groups of experiments were conducted, and each experiment was repeated thrice. The mean value was taken as the real value. The experimental conditions are listed in Table 3. 1,000 g of kitchen waste was placed in a 1,000 mL jar, and 1, 2, and 3 wt% NaOH, KOH, and Ca(OH)₂ were separately added with stirring. The mixtures were stored at 4°C for 24 h. Then, the pH was adjusted to 6.2 ± 0.1 with 1 mol·L⁻¹ HCl. 100 g of the treated sample was placed in a 1 L jar in each experiment, and 300 mL of activated sludge was added. The mixture was diluted with water until the volume reached 1 L. These samples prepared were denoted as R_{Na1}, R_{Na2}, R_{Na3}, R_{K1}, R_{K2}, R_{K3}, R_{Ca1}, R_{Ca2}, and R_{Ca3}. And a control group containing kitchen waste without alkali was denoted as R₀. After sealing with wax, these reactors were placed in an electric thermostatic water bath, and the incubation was performed at 37°C for 50 days.

Table 2: Nutrient composition analysis of kitchen waste

Kitchen waste	TCOD (g·kg ⁻¹)	SCOD (g·kg ⁻¹)	Dissolved carbohydrate (g·kg ⁻¹)	Soluble protein (g·kg ⁻¹)	VFAs (g·kg ⁻¹)
[8]	238.5 ± 3.8	106.0 ± 5.3	81.7 ± 6.2	5.9 ± 1.4	7.3 ± 0.4
[9]	353.3 ± 1.7	162.5 ± 1.4	69.4 ± 1.7	6.8 ± 1.2	8.1 ± 1.6
The present work	311.5 ± 4.7	134.6 ± 2.4	74.4 ± 3.4	6.3 ± 2.1	7.4 ± 1.3

Table 3: Experimental conditions for the pretreatment of kitchen waste with alkali (m·m⁻¹, %)

	R _{Na1}	R _{Na2}	R _{Na3}	R _{K1}	R _{K2}	R _{K3}	R _{Ca1}	R _{Ca2}	R _{Ca3}	R ₀
Alkali type	NaOH	NaOH	NaOH	KOH	KOH	KOH	Ca(OH) ₂	Ca(OH) ₂	Ca(OH) ₂	—
Alkali concentration	1	2	3	1	2	3	1	2	3	—

2.4 Analysis methods

2.4.1 Analysis of physical and chemical properties

The kitchen waste and anaerobic digestion sludge were taken and homogenized by stirring or shaking manually. The physical and chemical properties of this sample, such as TS, VS, and lipid content, were measured. Additionally, this sample was centrifugated at a speed of 20,000 rpm for 10 min, and the supernatant was filtrated with a 0.45 μm membrane. The relevant physicochemical properties of soluble components, such as pH, total carbon (TC), total nitrogen (TN), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), soluble proteins, carbohydrates, lipids, and volatile organic acids (VFAs), in the filtrate were determined [6]. The measurement methods of these physical and chemical properties are shown in Table 4.

The biogas output was measured once a day by metering the water expelled by biogas. The cumulative composition of biogas was analyzed by gas chromatography under the following conditions: chromatographic column: stainless-steel column (TDX-01 packing, 2 m × 3 mm) produced by the National Chromatographic Research and Analysis Center of Dalian Institute of Chemical Physics, Chinese Academy of Sciences; detector: TCD; carrier gas: 20 mL·min⁻¹ He; current: 100 mA; attenuation: 1; detection temperature: 200°C; column temperature: 180°C; and injection temperature: 200°C.

2.4.2 Microbial 16 S rRNA sequencing

The anaerobic digestive fluid was filtered through a 0.22 μm membrane, and the membrane was collected and stored at -20°C for testing. The high-throughput sequencing of 16 S rRNA was completed by Shanghai Meiji

Table 4: Primary physical and chemical characteristics and analytical methods

Physical and chemical characteristics	Analytical methods
TS	Drying [7]
VS	Calcination [8]
pH	Electrode method [8]
TC	Element analyzer [7]
TN	Element analyzer [8]
TOCD	Potassium-dichromate method [9]
SCOD	Potassium-dichromate method [10]
VFAs	Colorimetric method [11]
Protein	Protein content = (TN – ammonia nitrogen) × 6.25 [12]
Carbohydrate	Carbohydrate = VS – lipid – protein [13]
Lipid	Soxhlet extraction method [8]

Biomedical Technology Co., Ltd using the Illumina Miseq PE300 sequencing platform. The samples were extracted using the FastDNA® Spin Kit for lipid extraction kit according to the instructions for DNA extraction. The bacterial 16 S rRNA gene V3–V4 region was amplified using primers 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), while the archaeal 16 S rRNA gene V4–V5 region was amplified using primers 524F10extF (5'-TGYCAGCCGCCGCGG-TAA-3') and Arch958RmodR (5'-YCCGGCGTTGAVTCCAATT-3').

2.4.3 Data analysis

In the experiment, the physical and chemical indicators were measured in triplicate. The data were analyzed for significance and correlation using single-factor analysis of variance and multiple comparisons in SPSS v.18.0 software, evaluating the significant differences between each experimental treatment. The least significant difference method, ($\alpha = 0.05$) was used for multiple comparisons of the mean values. All data graphs were plotted using Origin-8.0.

3 Results and discussion

3.1 Effects of pretreatment with alkali on the organic components in kitchen waste

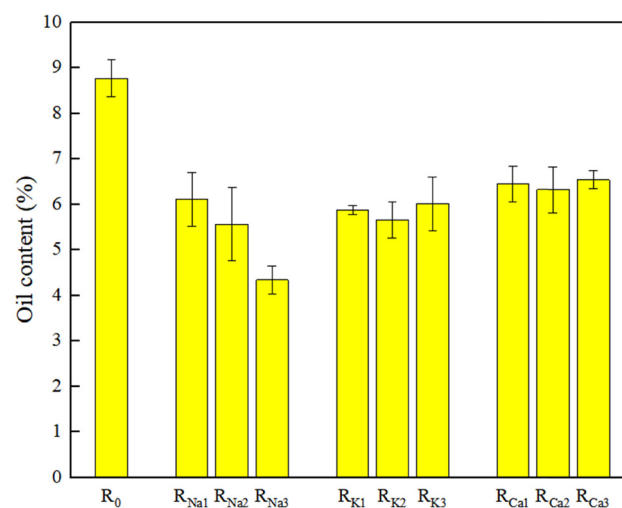
3.1.1 Lipid content

Figure 2 shows the effects of different pretreatments with alkali on the lipid content of kitchen waste. The lipid

content in the control group was 8.77%, while those in the R_{Na1} , R_{Na2} , and R_{Na3} groups were decreased to 6.11%, 5.56%, and 4.34%, respectively. The lipid contents in the R_{K1} , R_{K2} , and R_{K3} groups were decreased to 5.87%, 5.66%, and 6.01%, respectively, and those in the R_{Ca1} , R_{Ca2} , and R_{Ca3} groups were decreased to 6.45%, 6.32%, and 6.54%, respectively. The pretreatments with alkali could effectively promote the degradation of lipids in kitchen waste. In the experiment with R_{Na} group, within the concentration range of 1–3%, the lipid contents in kitchen waste decreased with the increase in NaOH concentration in the pretreatments. Within the concentration range of 1–3%, the pretreatments with KOH and $Ca(OH)_2$ could also promote the reduction in lipid contents of kitchen waste, but the decrease in lipid content was not linearly correlated to their dosages. The degradation rate of lipid in kitchen waste pretreated with 3% NaOH reached a maximum of 50.51%. Among the three alkalis, the degradation rate of lipid in kitchen waste pretreated with $Ca(OH)_2$ was the lowest, approximately 25.42–27.91%. In the pretreatments with strong alkalis, NaOH and KOH reacted with lipid *via* saponification reactions to give salts of fatty acids and glycerol. In contrast, in the pretreatment with $Ca(OH)_2$, a weakly alkaline environment was generated to promote the hydrolysis reactions of lipids in kitchen waste and to reduce the lipid content.

3.1.2 Content of dissolved organic matter

The pretreatment with alkali destroyed the chemical bonds of macromolecules in kitchen waste, thereby promoting

**Figure 2:** Lipid content of kitchen waste before and after alkali pretreatment.

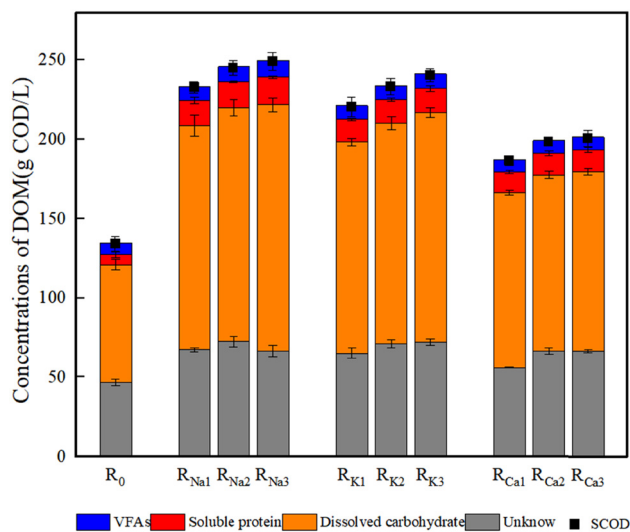


Figure 3: Dissolved organic matter concentrations before and after alkali pretreatment.

the hydrolysis of insoluble macromolecular organic matter by breaking the chemical bonds. The pretreatment with alkali obviously promoted the dissolution of sugars and proteins in kitchen waste and generation of VFAs. The effect of pretreatment with alkali on the soluble organic matter in kitchen waste is illustrated in Figure 3. The contents of SCOD, soluble sugars, soluble proteins, and VFAs in the control group were 134.6 ± 2.4 , 74.4 ± 3.4 , 6.3 ± 2.1 , and $7.4 \pm 1.3 \text{ g} \cdot \text{L}^{-1}$, respectively. After the pretreatment with alkali, the solubility of organic matter in kitchen waste was improved. Among the R_{Na1}, R_{Na2}, and R_{Na3} groups, the R_{Na3} group showed better performance with the highest concentration of soluble organic matter. In detail, the SCOD content reached $249.8 \pm 3.4 \text{ g} \cdot \text{L}^{-1}$ and was increased by 235.3%. The contents of soluble sugars, soluble proteins, and VFAs were increased to 155.3 ± 4.1 , 17.4 ± 1.8 , and $8.6 \pm 1.1 \text{ g} \cdot \text{L}^{-1}$, respectively. Meanwhile, the contents of unknown organic compounds (including methanol, amino

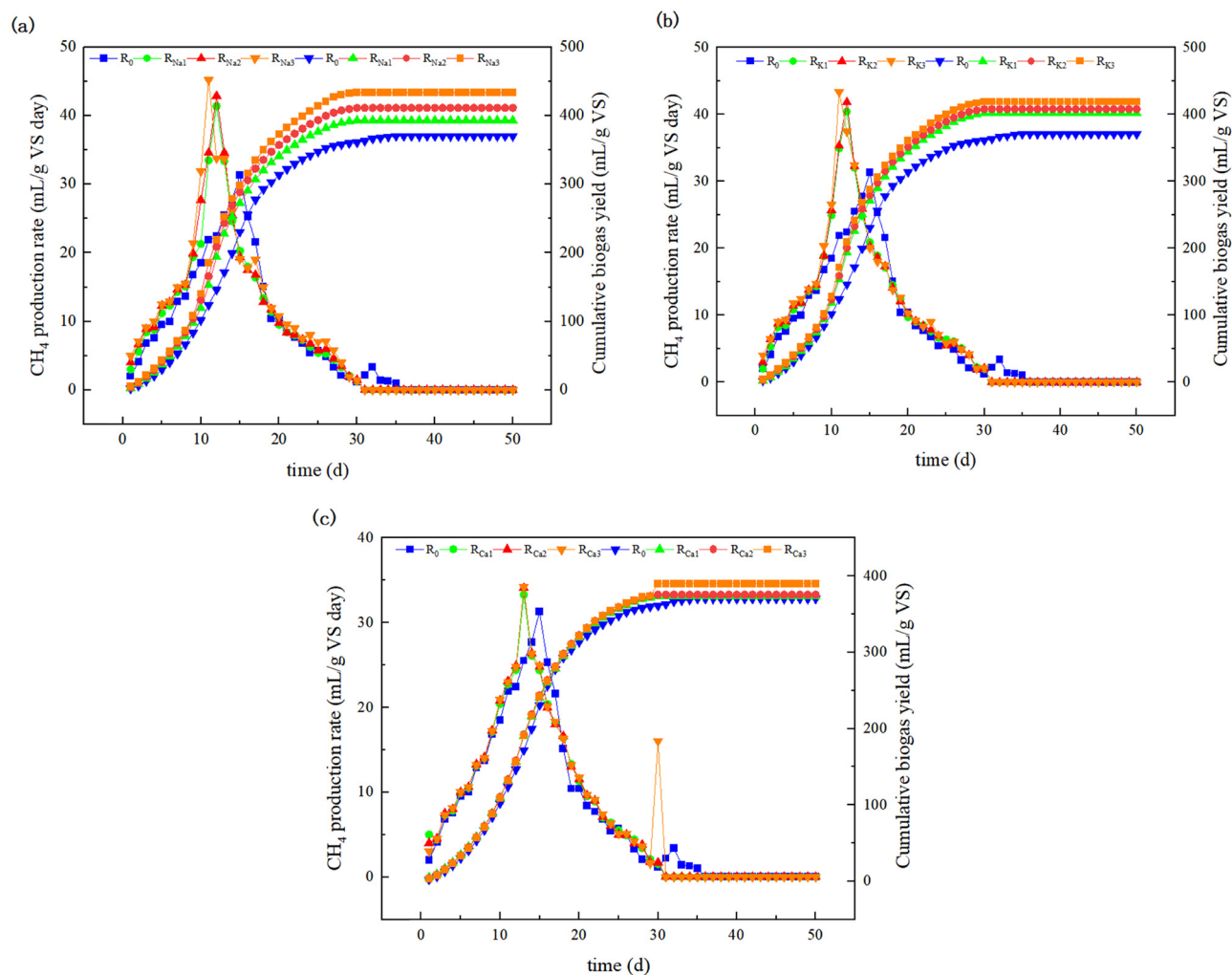


Figure 4: Accumulative biogas yield and CH₄ production rate during anaerobic digestion of kitchen waste pretreated with (a) NaOH, (b) KOH, and (c) Ca(OH)₂.

acids, long-chain fatty acids, etc.) were significantly increased to $66.57 \pm 3.7 \text{ g}\cdot\text{L}^{-1}$, accounting for 43.16% of the total SCOD.

The profiles of SCOD concentrations in kitchen waste pretreated with different alkalis showed different trends. The R_{Na1} , R_{Na2} , and R_{Na3} groups showed better kitchen waste treatment performance. With the increase in NaOH concentration, the content of dissolved organic matter was also increased. The SCOD contents in these three groups were 233.5 ± 6.7 , 245.9 ± 5.4 , and $249.8 \pm 3.4 \text{ g}\cdot\text{L}^{-1}$, respectively. After the pretreatment with KOH, the SCOD contents in kitchen waste increased to $241.3\text{--}221.3 \text{ g}\cdot\text{L}^{-1}$. Similar to the trend of profile regarding NaOH, the concentration of dissolved organic matter was also increased with the increase in KOH concentration. After the pretreatment with $\text{Ca}(\text{OH})_2$, the SCOD concentration was slightly increased to $187.4\text{--}201.4 \text{ g}\cdot\text{L}^{-1}$. The trend was similar to those of profiles regarding NaOH and KOH, with the increase in $\text{Ca}(\text{OH})_2$ concentration, the content of dissolved organic matter was also increased. However, the performance of pretreatment with $\text{Ca}(\text{OH})_2$ was poor. Therefore, strong alkaline substances, such as NaOH and KOH, could effectively decompose the organic macromolecules in kitchen waste and transform the insoluble organic matter into dissolved organic matter, increasing the concentration of dissolved organic matter. The stronger the alkalinity is, the better the pretreatment effect is. This principle is consistent with the results reported in the study by Elbeshbishy *et al.* [14] on the fermentation of pretreated kitchen waste for the production of hydrogen. After their pretreatment of kitchen waste with NaOH ($\text{pH} = 11.0$), the SCOD concentration of kitchen waste was also increased, and the concentrations of soluble sugars and proteins were increased by 21% and 26%, respectively. Similarly, after different pretreatments with alkalis in the present work, the concentrations of soluble sugars, soluble proteins, and

VFAs in kitchen waste were increased. Compared to NaOH and KOH, the performance of $\text{Ca}(\text{OH})_2$ was poorer. The main reason is that slightly soluble salts would be generated from Ca^{2+} under alkaline conditions, inhibiting the dissolution of a proportion of organic matter. However, the pH and alkalinity of the substrate could be regulated by Ca^{2+} , promoting the generation of VFAs [15]. In summary, the pretreatment with alkali improves the solubility of organic matter in kitchen waste, shortens the liquefaction time of organic matter such as proteins and sugars during the hydrolysis course, and improves the degradation efficiency.

3.2 Effect of pretreatment with alkali on the anaerobic digestion of kitchen waste

3.2.1 Biogas production characteristics

Figure 4 and Table 5 show the variation trends of CH_4 production efficiency, cumulative output of biogas and CH_4 , and biogas concentration after pretreatment with alkali. The cumulative biogas output and cumulative CH_4 output of the control group R_0 were 675.5 ± 6.5 and $370.2 \pm 3.1 \text{ mL}\cdot\text{g}\cdot\text{VS}^{-1}$, respectively. The concentrations of CH_4 , carbon dioxide, and other gases in the biogas of group R_0 were $54.8\% \pm 4.7\%$, $39.6\% \pm 1.5\%$, and $5.6\% \pm 0.4\%$, respectively.

Figure 4a shows that the maximum daily biogas output of untreated kitchen waste took place on the 15th day, with biogas production efficiency of $31.35 \text{ mL}\cdot\text{g}\cdot\text{VS}^{-1}$. The cumulative CH_4 output and biogas production efficiency of anaerobic digestion kitchen waste pretreated with NaOH were improved. The maximum daily biogas output of R_{Na1} , R_{Na2} , and R_{Na3} groups took place on the 12th, 12th, and 11th day,

Table 5: Production and proportion of biogas, CH_4 , and CO_2 during anaerobic digestion

Group	Cumulative biogas yield ($\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$)	Cumulative CH_4 yield ($\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$)	Concentration of CH_4 (%)	Concentration of carbon dioxide (%)	Concentration of other gases (%)
R_0	675.5 ± 6.5	370.2 ± 3.1	54.8 ± 4.7	39.6 ± 1.5	5.6 ± 0.4
R_{Na1}	650.8 ± 7.8	393.1 ± 0.9	60.4 ± 3.6	31.8 ± 1.6	7.8 ± 0.8
R_{Na2}	671.0 ± 4.5	411.3 ± 2.5	61.3 ± 0.9	30.6 ± 1.7	8.1 ± 0.3
R_{Na3}	703.6 ± 5.3	434.1 ± 1.7	61.7 ± 2.1	30.0 ± 2.1	8.3 ± 0.6
R_{K1}	679.4 ± 4.2	401.5 ± 3.3	59.1 ± 3.7	33.2 ± 3.5	7.7 ± 0.2
R_{K2}	680.8 ± 3.1	407.8 ± 3.1	59.9 ± 3.4	32.5 ± 2.7	7.6 ± 0.3
R_{K3}	692.4 ± 4.1	418.9 ± 0.2	60.5 ± 3.3	31.6 ± 2.6	7.9 ± 0.6
R_{Ca1}	670.6 ± 0.5	373.5 ± 4.6	55.7 ± 4.5	37.9 ± 1.7	6.4 ± 0.4
R_{Ca2}	657.6 ± 3.3	375.5 ± 6.3	57.1 ± 5.1	36.2 ± 1.9	6.7 ± 0.4
R_{Ca3}	678.9 ± 2.1	389.7 ± 4.3	57.4 ± 2.9	35.2 ± 2.0	7.4 ± 0.5

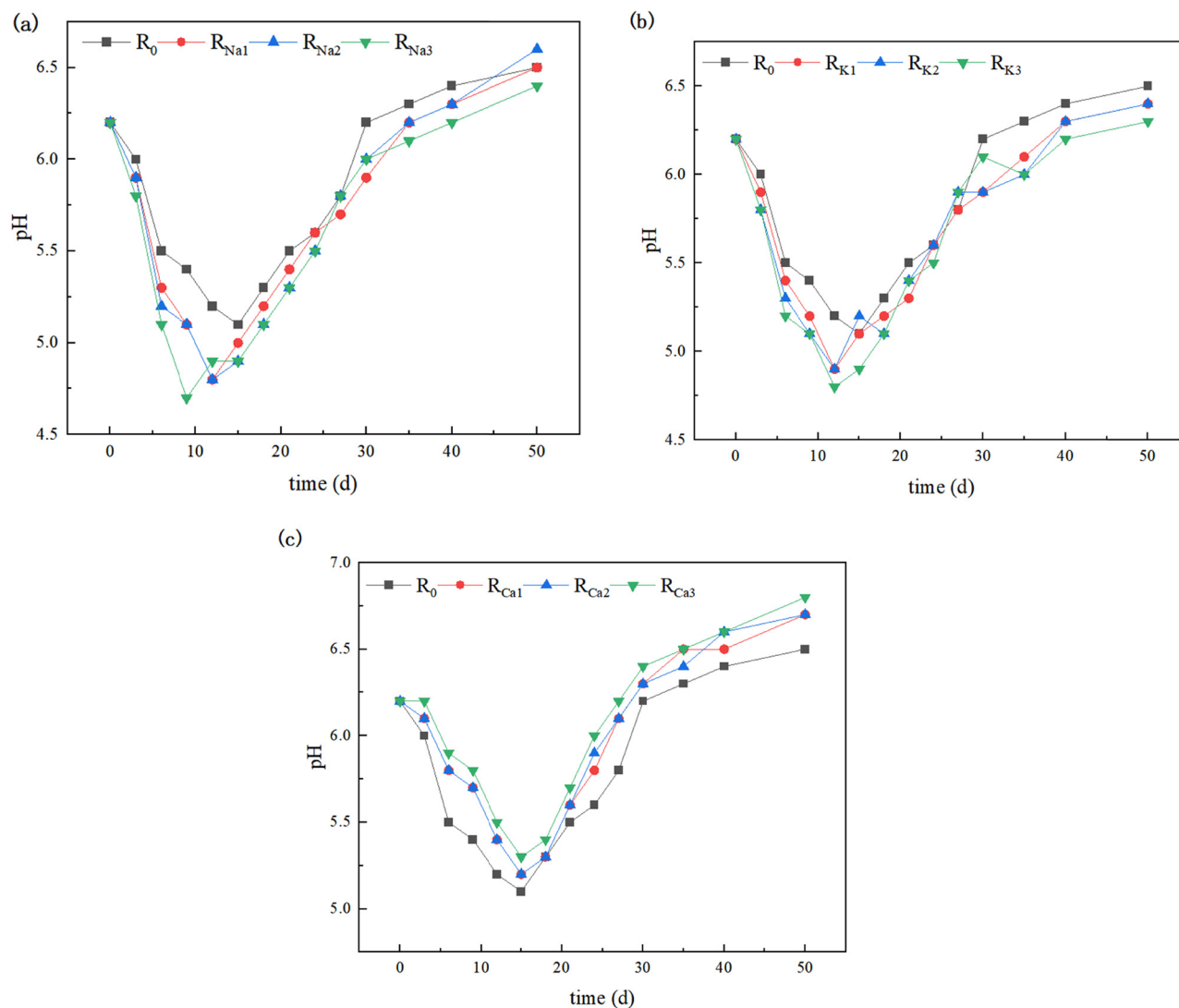


Figure 5: The change in pH during anaerobic digestion of kitchen waste with alkali pretreatment by (a) NaOH, (b) KOH, and (c) $Ca(OH)_2$.

earlier than that of untreated kitchen waste, with biogas production efficiency of 41.4, 42.9, and 45.2 $\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$, respectively. The cumulative CH_4 output was increased from 370.2 $\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$ (untreated) to 393.1, 411.3, and 434.1 $\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$, respectively, and the CH_4 concentration in biogas was also increased from 54.8% (untreated) to 60.4%, 61.3%, and 61.7%, respectively. Similarly, the kitchen waste pretreated with KOH also showed improved cumulative CH_4 output and biogas production efficiency in the anaerobic digestion process. The maximum daily biogas output of R_{K1} , R_{K2} , and R_{K3} groups took place on the 12th, 12th, and 11th day, with the biogas production efficiency of 40.4, 41.8, and 43.3 $\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$ and improved cumulative CH_4 output of 401.5, 407.8, and 418.9 $\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$, respectively. The CH_4 concentrations in biogas were increased to 59.1%, 59.9%, and 60.5%, respectively. Compared to NaOH and

KOH, the cumulative CH_4 output and biogas production efficiency in the anaerobic digestion of kitchen waste pretreated with $Ca(OH)_2$ were less improved. The maximum daily biogas output of R_{Ca1} , R_{Ca2} , and R_{Ca3} groups took place on the 13th day, with the improved cumulative CH_4 output of 33.3, 34.1, and 34.2 $\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$, improved cumulative CH_4 output of 373.5, 375.5, and 389.7 $\text{mL}\cdot\text{g}\cdot\text{VS}^{-1}$, and improved CH_4 concentrations of 55.7%, 57.1%, and 57.4%, respectively.

The main reason for the much higher cumulative CH_4 output, biogas production efficiency, and CH_4 concentration of the kitchen waste pretreated by NaOH and KOH in the anaerobic digestion process is that the strong alkalinity of NaOH and KOH destroyed the structures of macromolecules in kitchen waste, thereby promoting the bond-breaking and hydrolysis of insoluble macromolecular organic matter and increasing the concentrations of

dissolved organic matter such as sugars, proteins, and VFAs. The hydrolysis was promoted to provide preferable early-stage conditions for the transformation of hydrolysates into CH_4 by archaea in the later stage. The poor performance of waste pretreated with $\text{Ca}(\text{OH})_2$ is ascribed to the weak alkalinity of $\text{Ca}(\text{OH})_2$, of which the ability to destroy the chemical bonds in organic matter in kitchen waste was weak. Meanwhile, under alkaline conditions, the Ca^{2+} in $\text{Ca}(\text{OH})_2$ would be transformed into slightly soluble salts, which would inhibit the dissolution of a portion of organic matter. Nevertheless, the pH and alkalinity of the substrate could be adjusted with Ca^{2+} , promoting the generation of VFAs and certain hydrolysis reactions. This is beneficial for the methanation in the later stage. This result is consistent with the study of Cui *et al.* [16], who reported that NaOH could break the ester bonds in the lignin-carbohydrate composite and release lignin-encapsulated cellulose. Thanks to these effects, the kitchen waste could be hydrolyzed and acidified by anaerobic microorganisms, by which the decomposition could be promoted. As a result, the cumulative biogas output and biogas production efficiency in the anaerobic digestion course could be improved.

3.2.2 Variation trend of pH

Figure 5 shows the variation trend of pH during the anaerobic digestion process of kitchen waste pretreated with alkali. The pH of all the groups at their initial stages was in the range of 6.2 ± 0.1 . During the 50 days anaerobic digestion process, the pH profiles of the control group R_0 and kitchen waste pretreated with alkali exhibited a concave shape. Among them, the pH of the control group R_0 reached its minimum of 5.1 on the 15th day, while that of $R_{\text{Na}1}$, $R_{\text{Na}2}$, and $R_{\text{Na}3}$ groups reached their minimums of 4.8, 4.8, and 4.7 on the 12th, 12th, and 9th day, respectively. At the end of the reactions, the final pH was maintained in the range of 6.4–6.6. The pH of $R_{\text{K}1}$, $R_{\text{K}2}$, and $R_{\text{K}3}$ groups reached their minimums of 4.9, 4.9, and 4.8, respectively, on the 12th day. At the end of anaerobic digestion, the final pH was maintained in the range of 6.3–6.4. The pH of $R_{\text{Ca}1}$, $R_{\text{Ca}2}$, and $R_{\text{Ca}3}$

Table 7: Abundance and diversity analysis of bacterium

Sample	OTU	Chao1	Ace	Shannon	Simpson	Coverage
$R_{\text{Na}3}^1$	78	74.9	75.3	1.921	0.371	0.9996
$R_{\text{Na}3}^{10}$	79	75.0	75.4	1.975	0.365	0.9994
$R_{\text{Na}3}^{20}$	83	78.9	79.2	2.076	0.312	0.9997
$R_{\text{Na}3}^{30}$	79	75.4	76.1	1.971	0.354	0.9998

groups reached their minimums of 5.2, 5.2, and 5.3, respectively, on the 12th day, and the final pH was maintained in the range of 6.7–6.8.

The pH of the control group R_0 and those pretreated with alkali decreased and then increased during the 50-days anaerobic digestion process. The main reason is that the organic matter in the kitchen waste was decomposed by hydrolytic microorganisms to produce small-molecule organic acids, leading to the decrease in pH in the reaction systems, and then these small-molecule organic acids were converted by archaea into CH_4 , resulting in the increase in pH. The pH of R_{Na} and R_{K} groups was lower than that of the control group R_0 , indicating that NaOH and KOH had a positive effect on the hydrolysis of kitchen waste. The chemical bonds in kitchen waste were destroyed by strong alkalis, thereby promoting the hydrolysis and acidification of organic matter, and transforming insoluble organic matter into soluble organic matter. As a result, the hydrolysis efficiency was improved. The hydrolysis process is the limiting factor determining the rate of anaerobic digestion and CH_4 production. With a higher hydrolysis rate and better acidification effect, the methanation in the later stage will be carried out to a higher extent. Therefore, the pH of kitchen waste pretreated with NaOH and KOH was low, and the biogas production efficiency was high. The main reason for the relatively high pH of $\text{Ca}(\text{OH})_2$ -pretreated kitchen waste in the anaerobic digestion process is that the weak alkalinity of $\text{Ca}(\text{OH})_2$ endowed it with weak ability to destroy the chemical bonds. In addition, the presence of Ca^{2+} increased the alkalinity of the digestion system, so the decrease in pH was hindered [17].

3.2.3 Analysis of effects of pretreatment with alkali on microbial abundance and diversity

The alpha diversity reflects the abundance and diversity of species in a single sample. The abundance of species is the number of species, which can be evaluated by the Chao1 and Ace indexes [18]. The larger the Chao1 and Ace indices are, the more abundant the species are. The diversity of species is affected by the abundance and evenness of

Table 6: Abundance and diversity analysis of bacterium

Sample	OTU	Chao1	Ace	Shannon	Simpson	Coverage
$R_{\text{Na}3}^1$	315	334	337	3.521	0.089	0.9998
$R_{\text{Na}3}^{10}$	321	347	351	3.645	0.084	0.9996
$R_{\text{Na}3}^{20}$	294	343	341	3.456	0.086	0.9997
$R_{\text{Na}3}^{30}$	277	324	326	3.319	0.090	0.9995

species in the community of sample. With the same species abundance, the greater the evenness of each species in the community is, the higher the community diversity is. The diversity of species can be assessed by the Shannon and Simpson indexes. The larger a Shannon index is, the smaller the corresponding Simpson index is, and a small Simpson index indicates high microbial species diversity of the sample. A high-throughput sequencing technology was adopted to select and analyze the microbial communities in the kitchen waste group R_{Na3} pretreated with 3% NaOH on the 1st, 10th, 20th, and 30th day. These communities were labeled as R_{Na3}^1 , R_{Na3}^{10} , R_{Na3}^{20} , and R_{Na3}^{30} , respectively. The analysis results of relative abundance and diversity of bacteria and archaea during the anaerobic digestion process are shown in Tables 6 and 7. The Coverage values in both tables denote the OTU coverage rate. The higher the value is, the higher the probability of species being detected is. This parameter can reflect whether the sequencing results represent the real situation of microorganisms in the sample.

Table 6 shows that the coverage values of bacterial abundance and diversity in all the samples exceed 0.9990, and the sequencing results can reflect the real situation of microorganisms. The Chao1 and Ace values of R_{Na3}^1 , R_{Na3}^{10} , R_{Na3}^{20} , and R_{Na3}^{30} bacteria showed volcanic trends, indicating that the bacterial abundance in the anaerobic digestion system increased in the first place. The maximum value of microbial diversity took place on the 10th day. The large number of microbial species was beneficial to the decomposition of organic matter in kitchen waste and promoted the transformation of insoluble organic matter into soluble organic matter. The organic acids generated reduced the pH of the system, consistent with the lowest pH at this stage. The Shannon index of R_{Na3}^{10} was increased by 0.124 and Simpson index was decreased by 0.005, indicating that the bacterial diversity reached a maximum on the 10th day. In this stage, the reaction substrate in the anaerobic digestion system was sufficient, and the chemical bonds in the kitchen waste could be destroyed by alkali in the pretreatment step. Thereby, the hydrolysis and acidification of the substrate were promoted, and the pH of the system was reduced. Later, due to the decomposition of the reaction substrate and lack of nutrients utilized by microorganisms, the number of microbial species was decreased, resulting in the decrease in Shannon index and increase in Simpson index. On the 30th day, the Shannon and Simpson indexes were 3.319 and 0.090, respectively.

Table 7 shows that the coverage values of abundance and diversity of archaea in all the samples also exceed 0.9990, and the sequencing results can represent the real

situation of microorganisms. The Chao1 and Ace values of archaea in R_{Na3}^1 , R_{Na3}^{10} , R_{Na3}^{20} , and R_{Na3}^{30} increased to their maximum on the 20th day, and then declined. Different from the bacterial diversity (maximum on the 10th day), the diversity of archaea reached its maximum on the 20th day, indicating that the kitchen waste pretreated with alkali in the system was hydrolyzed and acidified by bacteria to produce organic acids in the first place, providing nutrients for archaea microorganisms for methanation. Compared to R_{Na3}^1 , the Shannon index of R_{Na3}^{20} was increased by 0.155 and Simpson index was decreased by 0.059, indicating that the diversity of archaea was the most abundant on the 20th day. At this stage, the reaction substrate in the anaerobic digestion system was sufficient. After the pretreatment with alkali, the chemical bonds in the kitchen waste were destroyed to promote the hydrolysis and acidification of the substrate. After the acidification reactions, the organic acids were utilized by archaea microorganisms to produce CH_4 , proving that the improving of efficiency and degree of hydrolysis and acidification is critical for rapid and thorough anaerobic digestion.

4 Conclusion

1. The pretreatment with alkali reduced the lipid content in the kitchen waste and increased the content of dissolved organic matter, which can be done by chemical reaction, and promoted the conversion of part of the insoluble organic matter in the substrate to dissolved organic matter. After the pretreatment with 3% NaOH, the degradation rate of kitchen waste lipid reached its maximum of 50.51%. The SCOD content was increased by 235.3%, and the contents of soluble sugars, soluble proteins, and VFAs were increased to 155.3 ± 4.1 , 17.4 ± 1.8 , and $8.6 \pm 1.1 \text{ g} \cdot \text{L}^{-1}$, respectively.
2. The cumulative CH_4 output and biogas production efficiency of kitchen waste pretreated with alkali in the anaerobic digestion process were improved. The maximum daily biogas output of kitchen waste pretreated with NaOH and KOH took place on the 11th to 12th day, earlier than the day when the maximum daily biogas output occurred in the control group. The biogas production efficiency was 40.4 and $45.2 \text{ mL} \cdot \text{g} \cdot \text{VS}^{-1}$, respectively. The maximum daily biogas output of R_{Ca1} , R_{Ca2} , and R_{Ca3} groups took place on the 13th day, and the cumulative CH_4 output was increased to 373.5, 375.5, and $389.7 \text{ mL} \cdot \text{g} \cdot \text{VS}^{-1}$, respectively.
3. The coverage values of bacterial and archaea abundance and diversity in all the samples were higher

than 0.9990, and the Chao1 and Ace values of bacteria were increased in the first place and then decreased. On the 10th day, the microbial diversity reached its maximum. The result indicates that increasing of efficiency and degree of hydrolysis and acidification is a crucial factor determining the rapid and thorough anaerobic digestion.

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