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Study on the Effect of Inoculation of Mixed Inoculation on Hydrolysis and Acidification Performance of Kitchen Waste

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ABSTRACT

The effect of a mixture of different inoculants as inoculum on the hydrolytic and acidification process was investigated in order to improve the hydrolytic and acidification properties of kitchen waste. The results showed that the inoculation of Baijiu pit mud had the feasibility to improve the hydrolytic acidification performance during the hydrolytic acidification process of kitchen waste. At the end of the hydrolytic acidification process, the SCOD of the 1:0, 3:1,1:1, and 1;3 group increased by 55.97%, 48.67%, 30.82% and 7.03%, respectively, compared with that of the 1:0 group; the hydrolysis rate increased by 2.44%, 7.07%, 2.01% and 2.2% compared with that of the 0:1 group. The highest concentration of VFAs was found in the group with 1:3 ratio of Baijiu pit mud and anaerobic sludge, which was 11353.46 mg/L. The highest acidification rate was found in the 1:3 group, which was 68.57%, and 16.14% higher than that in the 1:0 group. The highest concentration of VFAs was found in the group with 1:3 ratio of Baijiu pit mud and anaerobic sludge, which was 11353.46 mg/L. The highest acidification rate was found in the 1:3 group, which was 68.57%, and 16.14% higher than that in the 1:0 group. Inoculation of Baijiu pit mud was able to change the fraction of VFAs during the hydrolytic acidification of kitchen waste. The fermentation type of the hydrolytic acidification system was ethanol-type fermentation, and the ethanol concentration was increased by 8.64, 8.59, 7.36, and 2.81 times in the groups with the ratio of Baijiu pit mud to anaerobic sludge of 1:0, 3:1, and 1:1, respectively. However, in the Baijiu pit mud and anaerobic sludge ratio of 1:3 and 0:1 group, the main type of fermentation was acetic acid type fermentation. The experimental groups inoculated with Baijiu pit mud.

KEYWORDS

Mixed inoculum, kitchen waste, hydrolysis acidification, ethanol.

1. INTRODUCTION

With the development of China's economy and society, an increasing amount of organic waste has been generated and there is a huge demand for resource utilisation and energy recovery [1]. Anaerobic digestion (AD) is a well-established organic waste treatment technology and one of the most promising options for sustainable waste management and renewable energy production. AD includes four steps: hydrolysis, acid production, acid and hydrogen production, and methane generation [2,3]. Most researchers have explored the factors affecting AD, including temperature conditions [4], raw material composition [5], and additives [6]. With the depth of the study, researchers realized that the inoculum plays an important role in the initiation and performance efficiency of the AD process [7]. The source of inoculum is crucial to the AD process, and different inoculum sources can provide different nutrients or mention different functional microorganisms [8]. In order to improve the AD process and increase methane production, it has been shown that mixing inoculums from different sources not only induces an increase in methane production, but also shows better pH stabilization [8,9].

The commonly used inoculums at this stage are (1) sludge from agricultural waste treatment plants, (2) sludge from biowastes, and so on. Firmicutes, Bacteroidetes, Chlorobacteria, and Proteobacteria are the bacterial communities with higher abundance in the AD process [17]. These microorganisms are mainly responsible for the catabolism of organic compounds such as polysaccharides, proteins and glycerol and metabolize substances such as acetic acid, hydrogen and carbon dioxide during the AD process [16]. The phylum Euryarchaeota is also a key microorganism in the AD process, among which microorganisms such as *Methanococcus* and *Methanobacteria* reduce CO_2 or decompose acetic acid to produce methane through H_2 reduction [18]. However, traditional anaerobic sludge lacks microorganisms of degrading cellulose, which may lead to poor hydrolysis and acidification performance of kitchen waste. Hydrolysis and acidification processes are the rate-limiting steps of anaerobic digestion, so it is necessary to add different inoculants to improve the hydrolysis and acidification performance of anaerobic sludge.

Chinese baijiu brewing has a history of more than 2,000 years, and strongly flavoured baijiu is the most popular baijiu product among Chinese baijiu. In the fermentation process of baijiu, grains are used as raw materials, the Baijiu cellars is used as the fermentation equipment and fermentation agent, and the BPM (BPM) is used as the fermentation agent [10]. BPM is a special layer of clay on the surface of the Baijiu cellars, which mainly plays the role of acid production and aroma production in the process of Baijiu production [11]. Butyric and hexanoic acids, which are extremely important in Baijiu production, are mainly synthesised by microorganisms in BPM [12]. BPM is the main habitat of anaerobic microorganisms during the fermentation of Baijiu, and at the phylum level, they are the dominant phyla of Firmicutes, Bacteroidetes, and Chlorobacteria [12,13]. Meanwhile, methanogenic archaea in BPM have an important role in maintaining pH stability in the cellar and promoting the production of flavor substances [14,15].

This experiment explored the feasibility of mixed inoculum of BPM and anaerobic sludge to promote the hydrolysis and acidification of kitchen waste. BPM and anaerobic sludge were mixed in different proportions as inoculum in the hydrolysis acidification process of kitchen waste. By detecting the physical and chemical indexes in the hydrolytic acidification reactor of kitchen waste, calculating the hydrolytic acidification rate in the hydrolytic acidification process, demonstrating the feasibility of inoculating BPM to promote the hydrolytic acidification process of kitchen waste, and providing a new choice of inoculum for the hydrolytic acidification process of kitchen waste.

2. MATERIALS AND METHODS

2.1. Inoculation and substrate

Baijiu cellar mud was taken from a Baijiu factory in Yibin, Sichuan. Due to the existence of a large number of large particles in Baijiu cellar mud, it needs to be pretreated. First, add proper amount of water to dissolve and stir the white wine cellar mud to prepare Baijiu cellar mud homogenate. Then the 20-mesh screen is used to screen the homogenate to remove the sand, stone, rice husk and other large particles in the BPM. Subsequently, the prepared homogenate was incubated at 35 ℃ for later use. Anaerobic sludge is taken from the anaerobic digested sludge in the sewage treatment workshop of the same white wine factory. After centrifugation at 3500r/min for 10min, the sludge with high solid content at the bottom is taken and incubated at 35 ℃ for use. Kitchen waste is taken from a university cafeteria in Sichuan. Impurities such as plastic, paper scraps, and bones are manually sorted out from the samples, and the waste is crushed multiple times with a grinder to form a homogeneous slurry. It is stored at 4 °C for later use. The basic characteristics of each material are shown in Table 1.

Table 1. Physicochemical properties of kitchen waste, PM and anaerobic sludge

	kitchen waste	BPM	sludge
TS (%)	32.52±0.76	31.74±0.07	8.28±0.22
VS (%)	31.12±0.57	2.69±0.07	4.36±0.10
VS/TS (%)	95.72	8.48	52.66
pH	4.23	3.29	8.16
Salinity (% wet basis)	1.72	0	0

2.2. Experimental equipment and methods

In this experiment, five different ratios of BPM and anaerobic sludge were used as inoculum during the hydrolysis and acidification of kitchen waste in CSTR reactor, and the ratios were VS BPM: VS anaerobic sludge=1:0, 3:1, 1:1, 1:3 and 0:1, and VS inoculum=2%. Then add kitchen waste, adjust the S/I ratio in the system to 2:1, and replenish water to 1.5L. In the experiment, medium temperature anaerobic was used, and the temperature of the water bath was controlled to (35 ± 1) °C. After feeding, nitrogen gas was blown for 2 minutes. Then let it ferment naturally. The experiment lasted for 5 days and physical and chemical indicators such as pH, VFAs, and SCOD were tested daily.

2.3. Physical and chemical analysis methods

The total solids (TS) and volatile solids (VS) content of solid samples were determined using the method determined by the State Environmental Protection Administration (2002). Measure pH using a pH/mV meter (online pH meter MIk pH 6.0, Asmik). After centrifugation at 8000rmp for 10 minutes, the sample was filtered and diluted using a 0.45 μ m microporous membrane. Measure soluble chemical oxygen demand (SCOD), ammonia nitrogen (AN), and volatile fatty acids (VFA) using diluted samples. SCOD was measured using a spectrophotometer (DR 1010 US Hash); The AN content was determined by Nessler's reagent spectrophotometry (HJ 535-2009); The concentration of VFAs was determined using gas chromatography (Trace1300, Thermo Fisher Scientific, detecting acetic acid, propionic acid, butyric acid, valeric acid, and hexanoic acid), with nitrogen as the carrier gas and a flow rate of 1 mL/min.

2.4. Calculation method

The COD conversion rate in the hydrolysis and acidification experiment of kitchen waste can be characterized by hydrolysis rate and acidification rate. The hydrolysis rate and acidification rate are calculated based on the reference literature for analysis and comparison.

$$\text{hydrolysis rate (\%)} = \text{SCOD}/\text{TCOD} \times 100\% \quad (2\text{-}1)$$

$$\text{acidification rate (\%)} = \text{TVFAs (Calculated by COD)}/\text{SCOD} \times 100\% \quad (2\text{-}2)$$

In equation (2-1) and (2-2), the COD equivalent concentration of VFAs is mainly calculated based on the sum of SCOD converted from various acids in the fermentation product. The conversion coefficients of ethanol, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid are 2.08, 1.066, 1.512, 1.816, 1.816, 2.037, and 2.037, respectively.

3. RESULTS AND DISCUSSION

3.1. The effect of mixed inoculants with different ratios on the hydrolysis and acidification of kitchen waste to produce VFAs

The changes in the concentration of VFAs during hydrolytic acidification of mixed inoculum of different ratios of Baijiu pit sludge and anaerobic sludge are shown in Figure 3-1. According to the experimental results, the concentration of VFAs in each experimental group rapidly increased within 1-2 days, and in dynamic equilibrium between 3-5 days. The highest concentrations of VFAs were found in the hydrolyzed acidified broth in the BPM to anaerobic sludge ratio of 1:3 group. At the end of the hydrolytic acidification of the kitchen waste, the concentrations of VFAs in the groups with ratios of 1:0, 3:1, 1:1, 1:3 and 0:1 were 6853.69 mg/L, 5306.80 mg/L, 6484.66 mg/L, 11353.46 mg/L and 8371.46 mg/L, respectively. Inoculation of a small amount of BPM was able to enhance the content of VFAs in the hydrolytic acidification process of kitchen waste. However, the content of VFAs was lower than that of the anaerobic sludge experimental group when the addition of BPM was higher. The inoculation of a small amount of BPM improves the acidification performance of kitchen waste in two main ways: (1) The addition of BPM enriches the diversity of hydrolytic acidification bacteria, resulting in a significant increase in hydrolytic acidification performance. (2) The addition of BPM may introduce certain special substances that may form a sensing during the process of hydrolytic acidification of kitchen waste, which is conducive to the enhancement of microbial activity, thus improving the hydrolytic acidification performance. However, the lower content of VFAs in the experimental group inoculated with a higher amount of BPM may be due to the following reasons: (1) The characteristics of high fat and salt in food waste may have an inhibitory effect on the microorganisms in BPM. (2) Anaerobic digestion is carried out under strictly anaerobic conditions, and the inoculation of BPM into the anaerobic digestion process may have a long adaptation period, resulting in poor hydrolytic acidification performance. (3) BPM itself still has nutrients, although after pretreatment, but there are still some difficult to degrade substances, which will lead to a slower rate of hydrolysis and digestion.

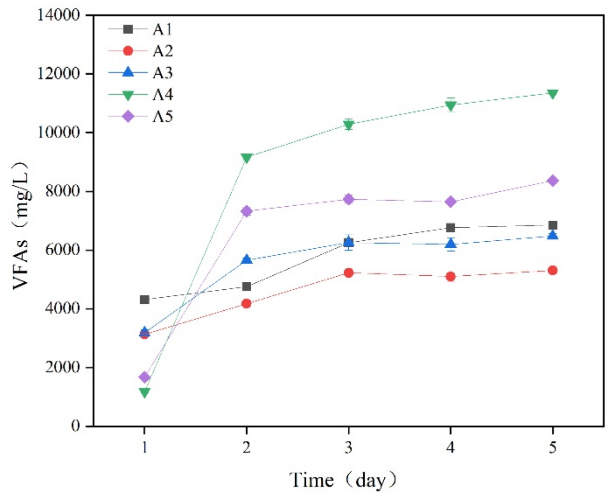

Figure 3-1. Changes in VFA concentration under different inoculum ratios

3.1.1. The effect of mixed inoculants with different ratios on the hydrolysis and acidification of VFAs components in kitchen waste

The carbons sources that methanogenic archaea can utilize are mainly small molecule substances such as ethanol and acetic acid, so it is necessary to pay attention to the composition of VFAs in the hydrolytic acidification broth of kitchen waste. The composition of VFAs in each experimental group are shown in the figure 3-2. It can be seen that after inoculation of BPM, the concentration of ethanol in hydrolytic acidification broth can be significantly increased. Ethanol is a neutral substance, and compared to other VFAs such as acetic acid and butyric acid, the generation of a large amount of ethanol is beneficial for the rapid utilization of methanogenic archaea in the future, as well as for the stability of pH in the system. At the end of fermentation, the ethanol concentrations in each experimental group were 3347.03mg/L, 3328.10mg/L, 2903.73mg/L, 1323.67mg/L, and 347.96mg/L, respectively. The higher concentration of ethanol in the BPM experiments may be due to the inclusion of yeast or ethanol-producing bacteria in the BPM, which results in higher concentrations of ethanol as the amount of BPM added increases. In contrast, ethanol concentrations were closer in the BPM cellar sludge ratio anaerobic sludge of 1:0 and 3:1 group. This may be due to the limited amount of nutrients within the anaerobic digestion system that can be directly utilized by the ethanol-producing bacteria, and hence the ethanol yields were similar in both. Hexanoic acid was also detected in the experimental group to which BPM was added. Hexanoic acid bacteria provided by BPM play a key role in the production of hexanoic acid, but hexanoic acid is not beneficial to the utilization of methanogenic archaea. Therefore, the content of hexanoic acid needs to be closely monitored during the use of BPM as an inoculum for anaerobic digestion. The type of fermentation in the hydrolytic acidification system was ethanol type fermentation in the BPM and anaerobic sludge ratios of 1:0, 3:1 and 1:1. However, in the BPM and anaerobic sludge ratios of 1:3 and 0:1 group, the main type of fermentation was acetic acid type, in which the content of ethanol was low and the content of acetic acid and butyric acid was high. This suggests that inoculation of BPM inoculated with white wine cellar sludge modifies the type of fermentation in the hydrolytic acidification of the food waste, thus changing the fraction of VFAs in the system.

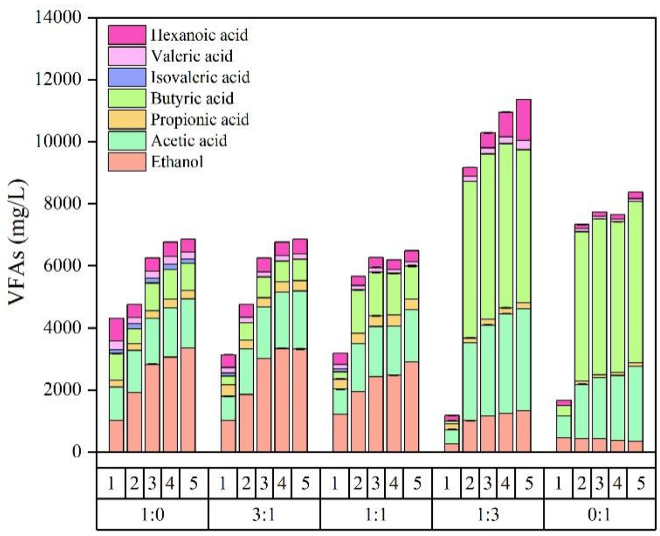

Figure 3-2. Changes in VFA concentration under different inoculum ratios

3.1.2. The effect of mixed inoculants with different ratios on the hydrolysis and acidification SCOD of kitchen waste

SCOD can be directly utilized by microorganisms and therefore SCOD concentration can directly reflect the level of organic matter concentration in the fermentation broth. The variation in concentration of SCOD is shown in Figure 3-3. During 1 day, the concentration of SCOD in the hydrolytic acidification broth was 14510.08 mg/L, 13861.60 mg/L, 12165.57 mg/L, 11018.25 mg/L, and 9471.87 mg/L in each experimental group, respectively. There was a difference in the initial SCOD concentration in each experimental group, which may be due to the inoculation of BPM, which introduced some SCOD, and the difference in the initial SCOD due to the different amounts of BPM added. At 2day, there was a minor decrease in the concentration of SCOD in each experimental group. This is probably due to the presence of methanogenic archaea in the inoculum, which will utilize the VFAs in the system for their methanogenic activities, resulting in a decrease in SCOD in the system. At 3day, a significant increase in SCOD was observed in all experimental groups, which could be attributed to the accumulation of VFAs in the system as the hydrolytic acidification fermentation proceeded, leading to a decrease in pH in the system to around 4.0, which inhibited the activity of the methanogenic archaea. At 3~5days, the SCOD of each experimental group was in dynamic equilibrium, and the SCOD contents of each experimental group were 28776.72 mg/L, 27429.87 mg/L, 24137.57 mg/L, 19747.83667 mg/L, and 18450.87 mg/L. At the end of the hydrolytic acidification, the experimental group inoculated with BPM mud its SCOD content increased significantly. This indicates that the inoculation of BPM has the effect of promoting the hydrolysis process of kitchen waste.

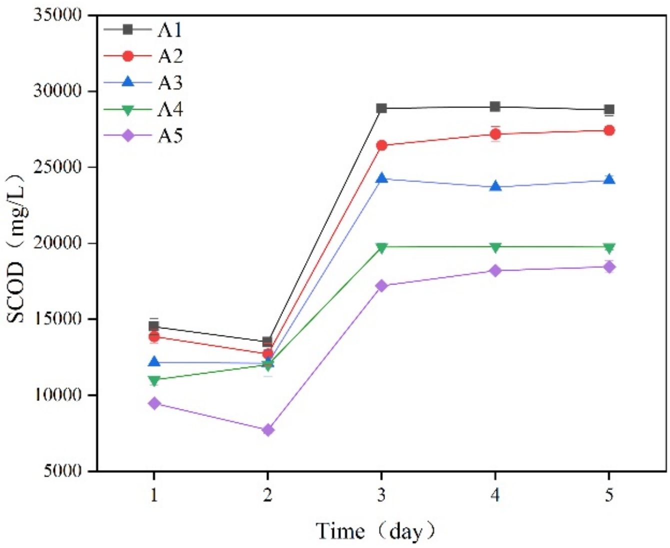

Figure 3-3. Changes in SCOD concentration under different inoculum ratios

3.1.3. The effect of mixed inoculants with different ratios on the hydrolysis and acidification rate of kitchen waste

In the process of hydrolytic acidification of kitchen waste, the hydrolysis rate can intuitively demonstrate the hydrolysis effect, and the acidification rate can effectively characterize the acidification performance, and the effect of the mixed inoculum on the performance of hydrolytic acidification of kitchen waste is shown in Figure 3-4. The hydrolysis rates of each experimental group were 63.94%, 68.57%, 63.51%, 63.70%, and 61.50%, respectively. It can be found that the hydrolysis rate of the experimental group added with inoculum of BPM has increased, which indicates that inoculating BPM can promote the hydrolysis of kitchen waste. However, the hydrolysis rate did not increase significantly, which may be due to the fact that some difficult to degrade SCOD carried by BPM during the addition process limited the hydrolysis performance, so there was little difference in the hydrolysis rate. The acidification rates of each experimental group were 27.32%, 30.11%, 29.86%, 61.30%, and 45.16%, respectively. Among them, the acidification rate of 1:0, 3:1 and 1:1 group is lower than that of 0:1 group, which may be because the refractory substances in the original BPM led to acidification difficulties and low acidification rate. At the same time, it may also be that the VFAs concentration in these three groups is high, which inhibits the activity of microorganisms, resulting in low acidification rate. The 1:3 group had the highest acidification rate, which was 16.14% higher than the 0-1 group. This shows that after mixing, the cellar mud and anaerobic sludge can be used as inoculum in the hydrolysis and acidification process of kitchen waste, which is conducive to improving its hydrolysis and acidification performance. At the same time, the Baijiu cellar mud can supplement and enhance the functions that anaerobic digestion sludge lacks.

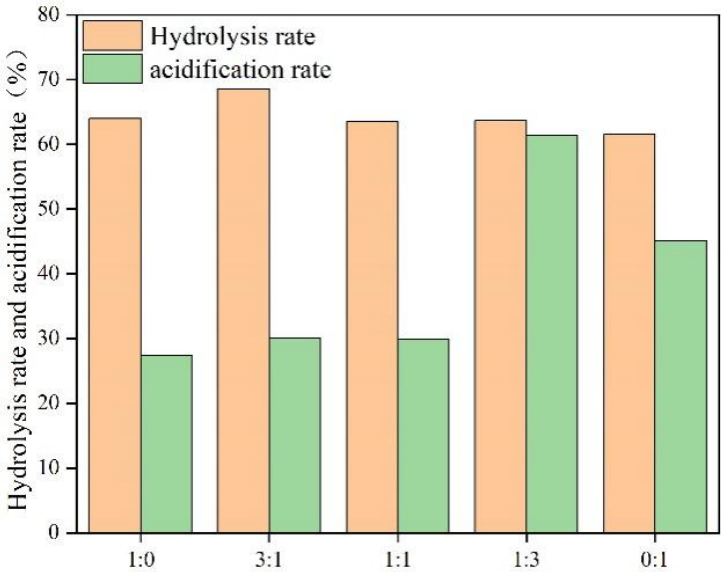

Figure 3-4. Hydrolysis and acidification rates of kitchen waste treated with mixed inoculants during the hydrolysis and acidification process

3.1.4. The effect of mixed inoculants with different ratios on the concentration of ammonia nitrogen in the hydrolysis and acidification of kitchen waste

In the process of kitchen waste hydrolytic acidification, the nitrogen element in the system conforms to the law of conservation, and most of the nitrogen is converted into ammonia nitrogen in the fermentation solution through microbial degradation and digestion, and a small portion is converted into microbial cells. The concentration of NH_4^+-N can have a significant effect on the anaerobic digestion process, promoting hydrolytic acidification efficiency to some extent when the concentration is below 200 mg/L. Whereas, the concentration of NH_4^+-N exceeding 1500 mg/L can inhibit the system. The change in the concentration of NH_4^+-N in hydrolytic acidification in each experimental group is shown in Figure 3-5. The ammonia NH_4^+-N content in the hydrolytic acidification solution of BPM and anaerobic sludge with mixing ratios of 1:0, 3:1 and 1:1 showed an increase. At the end of fermentation, the NH_4^+-N content in each experimental group was 1344.99 mg/L, 1289.18 mg/L and 1233.36 mg/L, respectively, which was much higher than that in the inoculated anaerobic sludge, although it did not reach the concentration of inhibiting methanogenic archaea. This suggests that inoculation of BPM cellar mud accelerates the rate of conversion of proteinaceous material to simple nitrogen-containing basics, such as amino acids, with an increase in the production of NH_4^+-N as a secondary product. This may be due to the fact that inoculation with BPM mud increased the diversity and abundance of protein-degrading microorganisms in the system. The concentration of NH_4^+-N in the hydrolytic acidification broths with ratios of 3:1 and 0:1 showed a decrease followed by an increase. This may be due to the fact that the microorganisms within the hydrolytic acidification system utilizes NH_4^+-N for their biological activities. At the end of fermentation, the ammonia NH_4^+-N contents of these two were 758.44 mg/L and 797.79 mg/L, respectively, which did not reach the inhibition situation of ammonia NH_4^+-N on the system. The above results show that inoculation of BPM does not affect the stability of the system.

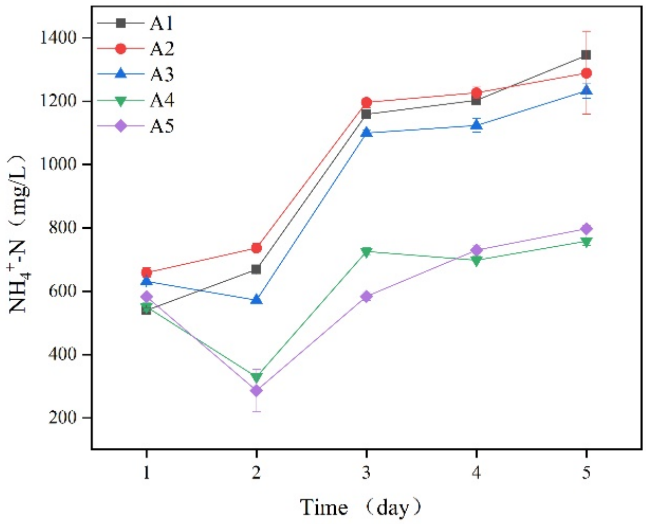

Figure 3-5. Changes in Ammonia nitrogen concentration under different inoculum ratios

4. CONCLUSION

Explored the use of mixed inoculums from different sources to improve the hydrolytic and acidification properties of kitchen waste. At the end of the hydrolytic acidification process, the SCOD of the 1:0, 3:1,1:1, and 1;3 group increased by 55.97%, 48.67%, 30.82% and 7.03%, respectively, compared with that of the 1:0 group; the hydrolysis rate increased by 2.44%, 7.07%, 2.01% and 2.2% compared with that of the 0:1 group. The highest concentration of VFAs was found in the group with 1:3 ratio of Baijiu pit mud and anaerobic sludge, which was 11353.46 mg/L. The highest acidification rate was found in the 1:3 group, which was 68.57%, and 16.14% higher than that in the 1:0 group. The highest concentration of VFAs was found in the group with 1:3 ratio of Baijiu pit mud and anaerobic sludge, which was 11353.46 mg/L. The highest acidification rate was found in the 1:3 group, which was 68.57%, and 16.14% higher than that in the 1:0 group. Inoculation of Baijiu pit mud was able to change the fraction of VFAs during the hydrolytic acidification of kitchen waste. The fermentation type of the hydrolytic acidification system was ethanol-type fermentation, and the ethanol concentration was increased by 8.64, 8.59, 7.36, and 2.81 times in the groups with the ratio of Baijiu pit mud to anaerobic sludge of 1:0, 3:1, and 1:1, respectively. However, in the Baijiu pit mud and anaerobic sludge ratio of 1:3 and 0:1 group, the main type of fermentation was acetic acid type fermentation. The experimental groups inoculated with Baijiu pit mud.

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