Study on the effect of stepwise warming on two-phase anaerobic fermentation of cow manure

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Abstract. In order to explore the influence of different stepwise heating methods for the acid-producing phase on the gas production of the methane-producing phase, the stepwise heating method with the best acidification, the highest gas production and the best energy-saving effect was obtained. Using cow dung as the raw material, the acid-producing phase is used for stepwise warming, the temperature increase range is 20–35 °C, set three different step-by-step heating methods respectively: the first group is heated by 5 °C/2 d, and the second group is heated by 2.5 °C/d; the third group was heated by 2 °C, 8 °C and 5 °C respectively once every other day; constant temperature fermentation after heating to 35 °C; and a set of acid-producing phase constant temperature 35 °C fermentation; the methane-producing phase is always fermented at a constant temperature of 38 °C; analyze the changes of different parameters. The results show that, in the first group, the total mass concentration of volatile fatty acids is 9372.46 mg/L; the proportion of the total concentration of acetic acid butyric acid to the mass concentration of volatile fatty acids is 77%; the cumulative gas production volume is 6253 mL, and the cumulative methane production volume is 3741 mL, the total energy consumption is 638.32 kJ, and the heat generated by biogas combustion is 156.33 kJ; the difference between system energy consumption and production capacity is 481.99 kJ. As for the second group, the total mass concentration of volatile fatty acids is 7810.49 mg/L, and the proportion of the total concentration of acetic acid butyric acid to the mass concentration of volatile fatty acids is 74%. The cumulative gas production volume is 4583 mL, and the cumulative methane production volume is 2521 mL. The total energy consumption is 629.46 kJ, and the heat generated by biogas combustion is 114.58 kJ; the difference between system energy consumption and production capacity is 514.88 kJ. In the third group, the total mass concentration of volatile fatty acids is 7664.71 mg/L, the proportion of the total concentration of acetic acid butyric acid to the mass concentration of volatile fatty acids is 75%; the cumulative gas production volume is 4606 mL, the cumulative methane production volume is 2579 mL. The total energy consumption is 624.18 kJ, and the heat generated by biogas combustion is 115.15 kJ; the difference between system energy consumption and production capacity is 509.03 kJ. In the constant temperature 35 °C group, by contrast, the cumulative gas production volume is 6565 mL, the cumulative methane production volume is 3939 mL. The total energy consumption is 765.74 kJ, and the heat generated by biogas combustion is 164.13 kJ; the difference between system energy consumption and production capacity is 601.61 kJ. According to the comparative analysis of the three groups, the first group...
has the best acidification effect and highest gas production, and the difference in energy consumption compared with the constant temperature group is also lower, which is more economical. Therefore, the step-by-step heating method of 5 °C/2 d is the best.

1. Introduction

Anaerobic fermentation is an important way of resource recycling. Biogas fermentation can process environmental pollutants while producing clean fuels, thereby realizing waste reuse and promoting ecological and environmental balance[1]. Two-phase anaerobic fermentation can be divided into hydrolysis acid production and methanogenesis, the acidogenic phase and the methanogenic phase are artificially separated, so that acid-producing bacteria and methanogenic bacteria can grow and reproduce in the best growth environment, which can greatly improve fermentation efficiency[2-5]. As the most important factor affecting the fermentation process of biogas, temperature can affect the fermentation process by impacting the flow direction of the fermentation substrate as well as the formation of intermediate products. According to the fermentation temperature, biogas fermentation can be grouped into three categories: low temperature fermentation (15–20 °C), mesophilic fermentation (30–35 °C) and high temperature fermentation (50–55 °C). The mechanism of two-phase fermentation is greatly affected by temperature. Within a specific temperature range, every 10 °C reduction in the fermentation temperature lowers the growth and reproduction rate of fermenting bacteria by half. Therefore, to ensure the stable operation of biogas fermentation, it is necessary to control the fermentation temperature of the biogas system to maintain the heat balance of the biogas system, thereby improving the fermentation efficiency.

There have been some studies on the effect of temperature on two-phase anaerobic fermentation. Li et al., for example, studied the two-phase anaerobic fermentation of cow manure at different temperatures, and found that the gas production performance of the fermentation reaction system increased during the medium temperature fermentation at 35 °C, and the fermentation effect was improved[6]. The research of Zhao et al. and Jing Jie et al. showed that certain warming measures in the range of less than 37 °C could increase the hydrolysis rate and acidification rate, and the concentration of volatile fatty acids (VFA) in the acid generating phase reactor[7,8]. Liu et al. also carried out two-phase fermentation on cow dung at different temperatures, and the results showed that the gas production and fermentation effect of the fermentation reaction system increased with the rising temperature [9]. Shi et al. reported that during the anaerobic fermentation process at mesophilic fermentation, the increase in the temperature of acid-producing fermentation was beneficial to increase the production rate of volatile fatty acids [10]. Kozuchowska et al. (1995) found that acid-producing bacteria in the fermentation broth after warming treatment was conducive to the production of acetic acid, thereby improving the gas production efficiency [11]. In summary, the current studies on the temperature effect of two-phase anaerobic fermentation of cow dung mainly focus on the effect of constant temperature on fermentation during the fermentation cycle, few of which are on the temperature changes during the fermentation cycle. In the cold regions of China, the temperature is often below zero in winter, so the fermentation reaction system needs to consume more energy to maintain the system for long-term solid-temperature fermentation. Nevertheless, the system capacity may be lower than the energy consumption, in which case the factory will lose money. Thus, how to realize variable temperature control to reduce energy consumption becomes an urgent problem to be solved. In this study, cattle manure is used as the substrate and activated sludge as the inoculum to investigate the impacts of the acid-producing phase with different heating methods and the solid temperature of the methane-producing phase on the two-phase anaerobic fermentation of cattle manure during a fermentation cycle.
2. Materials and methods

2.1. Raw material composition

The raw material for fermentation was fresh cow dung, taken from a cattle farm on the outskirts of Hohhot, Inner Mongolia. After the dung was retrieved, the remaining undigested large fibers were removed first, and mixed with water to prepare a cow dung aqueous solution with a total solid content (TS) of 10%. The inoculum was obtained from the Xinxinban Sewage Treatment Plant in Hohhot. The properties and element content of cow manure and inoculated sludge are listed in Table 1.

| Nature and elements | pH   | Total solid content(%) | Volatile solid content(%) | N(%) | C(%) | H(%) | S(%) | C/N   |
|---------------------|------|------------------------|---------------------------|------|------|------|------|-------|
| Cattle manure       | 7.32 | 16.15                  | 10.76                     | 1.6  | 41.24| 5.44 | 0.35 | 25.88 |
| Inoculated sludge   | 7.48 | 4.53                   | 2.47                      | 3.08 | 24.24| 3.64 | 2.12 | 7.87  |

2.2. Test equipment and analytical instruments

2.2.1. Test device. Figure 1 shows the schematic diagram of experimental device.

1. Fermentation bottle  2. Bucket  3. Exhaust pipe  4. Water bath  5. Rubber stopper

a. Acid production phase reaction device

1. Fermentation bottle  2. Gas collecting cylinder  3. Water collecting measuring cylinder  4. Exhaust pipe  5. Drain pipe  6. Sampling port  7. Rubber stopper  8. Water bath

b. Methanogenic phase reactor

Figure 1. Diagram of experimental setup.
2.2.2. Main test equipment. T6 Xinyue Visible Spectrophotometer (Beijing Puxi General Instrument Co., Ltd.); GC9790II Gas Chromatograph (Zhejiang Fuli Analytical Instrument Co., Ltd.); HH-4 constant temperature water bath (Changzhou Jintan Honghua Instrument Factory); PHB-4 portable pH meter (Shanghai Leici Instrument Factory).

2.3. Experimental method

2.3.1. Experimental design. Control TS at 10 %, both the acid producing phase and the methane producing phase use 1L containers, add 10% fresh cow manure aqueous solution is added to the methanogenic phase with an inoculum that accounts for 30 % of the effective volume of the fermentation broth to start, no liquid in or out during startup; add 10 % fresh cow dung aqueous solution to acid producing phase acidify.

The single factor control variable method is adopted, with the temperature of the acid producing phase as a variable and other factors as constants. In the experiment, three groups of different step-by-step heating methods are selected, and the heating interval is 20–35 °C, the first group heats up 5 °C/2d; the second group heats up 2.5 °C/d; the third group is heated by 2 °C, 8 °C and 5 °C respectively once every other day; constant temperature fermentation after heating to 35 °C; and a set of acid-producing phase constant temperature 35 °C fermentation (only measure the average daily gas production); the methanogenic phase is always fermented at a constant temperature of 38 °C. The experiment set five days as the start-up period, and the hydraulic residence time of the methane-producing phase is 6 days.

2.3.2. Measurement method of experimental parameters. (1) pH: pH value is measured by a pH meter; (2) The ammonia nitrogen mass concentration: Nessler's spectrophotometric method (HJ 535-2009) [12]; (3) The mass concentration of volatile fatty acids: gas chromatography is used to determine the concentration of acetic acid, propionic acid and butyric acid. The capillary column model is DB-FFAP; hydrogen flame ionization; split ratio: 30:1; carrier gas: high purity nitrogen; column pressure: 0.4 KPa; method of temperature program: the initial temperature is set to 100 °C for 2 min, then increased to 130 °C at a rate of 6 °C/min for 1 min, and then increased to 190 °C at 10 °C/min and maintained for 2 min; the temperature of the injection port is 220 °C; the temperature of the detector is 230 °C; the sample volume is 1 μL[13]. (4) Cumulative gas production volume: use drainage gas collection method; the daily gas production is the volume of water in the water collecting cylinder measured regularly every day, and the cumulative gas production is the cumulative sum of the daily gas production. (5) The energy of two-phase anaerobic fermentation system is calculated by formula [14, 15]. (6) Cumulative methane production volume: gas chromatography; packed column GS-GASPRO113-4332; TCD detector; the carrier gas is H2; the detector and the column box are respectively 180 °C and 60 °C[16]. (7) SPSS software is used to perform variance analysis for each parameter. As the heating program starts on the 6th day, analysis of variance is performed on the data after the 5th day.

3. Results and discussion

3.1. The effect of different warming methods on the acid production phase of two-phase anaerobic fermentation of cow manure

3.1.1. Changes in pH. The pH value is an important factor in two-phase anaerobic fermentation [13]. When the environmental pH is 5.5–8.5, most acid-producing bacteria can grow normally, and even when the pH ≤5, some acid-producing bacteria can still survive [17-19]. The pH value not only affects the growth of acid-producing bacteria, but also the type of volatile fatty acid produced, that is, the type of anaerobic fermentation is determined by that of fermentation. Studies have shown that when the pH is over 6, the fermentation type is mostly butyric acid type.
Figure 2 shows the pH values of the initial solutions of the three groups are 7.29, and the corresponding pH value change trends are roughly the same. In the first 10 days, the three groups all dropped first, and then began to rise after the decline reached the lowest point, but their lowest points and the minimum values were different. This is because volatile fatty acids were produced at the beginning of fermentation, and the acid production rate increased on the 6th day and the pH declined, and the activity of ammonia-producing bacteria increased, so that the increase in ammonia-nitrogen content raised the pH [20]. The first group dropped to the lowest point on the 7th day of the reaction, and the pH was 6.19; the lowest point of the second group was reached on the 8th day, and the pH was 6.35; the lowest point of the third group was on the 7th day, and the pH was 6.30; This is similar to the results of two-phase anaerobic fermentation of livestock and poultry manure by Wu et al. [21]. After reaching the lowest value, the pH value change trends of the three groups all rose first, then fell, and finally fluctuated slightly. That is because with the gradually increase of temperature, the ammonia-nitrogen concentration and the acid production rate increase, but as the fermentation progresses, the system starts constant temperature fermentation and the volatile fatty acid content gradually accumulates. The whole experiment changed within the optimum pH range of acid-producing bacteria from the first day of fermentation. Results in Table 2 imply that the first group differ greatly from the second and third groups, yet there is no significant difference between the second and third groups. The first group has the smallest minimum pH value, and it also has a lower pH throughout the whole fermentation cycle.

![Figure 2. Effect of stepwise temperature increase on pH of acid-producing phase.](image)

**Table 2.** Analysis of the difference of pH changes under different step-by-step heating conditions.

|       | Group 1 | Group 2 | Group 3 |
|-------|---------|---------|---------|
| Group 1 |         |         |         |
| Group 2 | 0.037   |         |         |
| Group 3 | 0.002   | 0.185   |         |

3.1.2. Changes in ammonia nitrogen mass concentration. The mass concentration of ammonia nitrogen is an important parameter with two sides in the anaerobic fermentation process. The growth and metabolism of microorganisms require nitrogen and carbon sources. Ammonia nitrogen can not only provide a nitrogen source, but also adjust the pH of the system to a certain extent and prevent acid accumulation. Nevertheless, if the ammonia nitrogen mass concentration exceeds the appropriate range, methane synthase will be inactivated and the normal operation of anaerobic fermentation will be hindered. Some studies believe that ammonia nitrogen mass concentration exceeding 1500 mg/L will partly inhibit the anaerobic digestion system [22].
Figure 3 shows the ammonia nitrogen mass concentration of the three groups changed less than 1500 mg/L throughout the fermentation period, which was suitable for the growth of acid-producing bacteria. The greater numerical fluctuation is resulted from the acid-generating phase transition temperature rather than the constant temperature. As shown in Table 3, the first group differs insignificantly from the second group, but significantly from the third group. There is a significant difference between the second group and the third group. Moreover, the third group has the largest numerical fluctuation, followed by the second group and the first group. This is because the temperature change of the third group of heating methods is the most complicated and not equal, while the second group has the smallest temperature change gradient and equal changes. This is consistent with the change of pH in Figure 2. The higher the fermentation temperature, the greater the ammonia nitrogen mass concentration.

![Figure 3. Effect of stepwise temperature increase on ammonia nitrogen mass concentration in acid-producing phase.](image)

Table 3. Differential analysis of ammonia nitrogen mass concentration changes under different gradual heating conditions.

|                | Group 1 | Group 2 | Group 3 |
|----------------|---------|---------|---------|
| Group 1        |         |         |         |
| Group 2        | 0.382   |         |         |
| Group 3        | 0.001   | 0.000   |         |

3.1.3. Changes in the mass concentration of volatile fatty acids. In the two-phase fermentation of solid materials, volatile fatty acids (VFAs) are one of the important indicators for evaluating the state of the acid producing phase. One of the main functions of the hydrolysis acid-producing phase is to convert the raw material waste into volatile fatty acids and other substances, which can be used and decomposed by the next methane-producing phase to provide a reaction substrate for the methanogenic phase. Too high mass concentration of volatile fatty acids will cause the pH of the fermentation broth to be too low, affect the metabolic efficiency of acid-producing bacteria, and inhibit the production of methane gas.

Figure 4 shows that the variation trends of mass concentration of volatile fatty acids in the three groups are roughly consistent, contrary to that of pH value. Results in Table 4 imply that the first group and the second group, the first group and the third group are significantly different, and there is no significant difference between the second group and the third group. In the first ten days, the three
groups all rose first, and then began to fall after reaching the highest point. Specifically, the first group reached the highest point on the 7th day, with a maximum value of 890.25 mg/L; the second group reached the highest point on the 8th day, and the maximum value was 670.04 mg/L; the third group reached the highest point on the 8th day, and the maximum value was 808.29 mg/L. From the 11th day on, the three groups all rose significantly, reached a peak again, and then declined with small fluctuations. At the beginning of the experiment, acid-producing bacteria could use more easily degradable substances, so the mass concentration of volatile fatty acids rose rapidly, as the fermentation progressed, the easily degradable substances decreased and the mass concentration of volatile fatty acids began to decline after reaching the highest point. The temperature also had a crucial effect on the content of volatile fatty acids. To be specific, the acid production rate increased with the rising temperature, and the mass concentration of volatile fatty acids accumulated as the fermentation progressed, resulting in two peaks. The time and maximum value for the three groups to reach the highest point again are that the first group reached the highest point on the 11th day, and the maximum was 901.11 mg/L; the second group reached the highest point on the 13th day with a maximum value of 751.26 mg/L; the third group reached the highest point on the 12th day with a maximum value of 820.66 mg/L. The second peak after the 10th day is the highest mass concentration of volatile fatty acids accumulated in the whole fermentation cycle. In terms of the mass concentration of accumulated volatile fatty acids in the three groups, the order of the maximum value is: group 1>group 3>group 2; the average values are 669.46 mg/L in group 1, 557.89 mg/L in group 2, and 547.47 mg/L in group 3, and the corresponding standard deviations are 180.2 mg/L, 144.97 mg/L, and 167.17 mg/L. The order of the average values is: group 1>group 2>group 3, but the order of standard deviation is: group 1> group 3> group 2. It can be seen that although the acidification effect of the first group is better, the changes are the most unstable. In addition, second group has the highest stability though its acidification effect is intermediate.

![Figure 4](image_url)

**Figure 4.** Effect of stepwise temperature increase on the mass concentration of volatile fatty acids in the acid-producing phase.

**Table 4.** The difference analysis of the mass concentration of volatile fatty acids under different gradual heating conditions.

|                | Group 1 | Group 2 | Group 3 |
|----------------|---------|---------|---------|
| Group 1        |         | 0.004   |         |
| Group 2        | 0.003   |         | 0.890   |
| Group 3        |         |         |         |
3.1.4. Change in the percentage of the total concentration of acetic acid butyric acid in the mass concentration of volatile fatty acids. The most common components of volatile fatty acids are formic acid, acetic acid, n-propionic acid, and n-butyric acid [23]. Acetic acid is the most effective fatty acid used by methanogens. When the bacteria undergo anaerobic fermentation, nearly 70% of the methane gas is derived from the decomposition of acetic acid. It is not difficult to see the importance of acetic acid content for biogas fermentation. Both propionic acid and butyric acid need to be converted into acetic acid before they can be used in the methanogenic phase to generate methane. Of the two, butyric acid is more easily converted into acetic acid [24]. Therefore, the higher the concentration of acetic acid produced by the acid-producing phase fermentation, the higher the efficiency of the acid-producing phase in the fermentation system [6].

As shown in Figure 5, the variation trends of these three groups are roughly the same as those of volatile fatty acids: each has two peaks. The content of acetic acid butyric acid is above 60%, thus the three groups are all deemed butyric acid type fermentation. The first group reached its peak values of 89% and 87% on the 7th and 11th day respectively; the second group hit its peak values of 83% and 84% on the 7th and 12th day respectively; the third group peaked at 85% and 87% on the 8th and 12th day, respectively. The averages of the three groups were 77%, 74%, and 75%, the standard deviations were 10%, 8%, and 10%. Results in Table 5 indicate that there were no significant differences between the three groups. Therefore, changes in temperature did not impact the total concentration of butyrate acetate, that is, the type of fermentation was not affected by temperature variation.

![Figure 5](image.png)

**Figure 5.** Effect of stepwise temperature increase on the the percentage of the total concentration of acetic acid butyric acid in the mass concentration of volatile fatty acids in the acid-producing phase.

| Group  | Group 2 | Group 3 |
|--------|---------|---------|
| Group 1 | 0.116   |         |
| Group 2 |         | 0.603   |

**Table 5.** The difference analysis of the percentage of the total concentration of acetic acid butyric acid in the mass concentration of volatile fatty acids in the acid-producing phase under different gradual heating conditions.
3.2. The effect of different warming methods on the methane production phase of cow manure two-phase anaerobic fermentation

3.2.1. Changes in cumulative gas production volume and cumulative methane production volume.

Figure 6. Effect of stepwise temperature increase of acid producing phase on cumulative gas production volume.

Figure 7. Effect of stepwise temperature increase of acid-producing phase on cumulative methane production volume.

Table 6. Under different gradual heating conditions of the acid-producing phase, the difference between the cumulative gas production volume of the methane-producing phase and the cumulative production volume of methane is analyzed.

|          | A_1 | A_2 | A_3 | A_4 | B_1 | B_2 | B_3 | B_4 |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|
| A_1      |     |     |     |     | -   | -   | -   | -   |
| A_2      | 0.025 |     |     |     | -   | -   | -   | -   |
| A_3      | 0.70 | 0.641 |     |     | -   | -   | -   | -   |
| A_4      | 0.016 | 0.003 | 0.002 |     | -   | -   | -   | -   |
| B_1      | -   | -   | -   | -   | 0.008 |     | -   | -   |
| B_2      | -   | -   | -   | -   |     | 0.03 | 0.602 |     |
| B_3      | -   | -   | -   | -   |     |     |     | 0.004 |
| B_4      | -   | -   | -   | -   |     |     |     | -   |

A: Cumulative gas production volume.
B: Cumulative methane production volume.
The subscript numbers 1, 2, 3, 4 represent the first group, the second group, the third group and the constant temperature 35 °C group respectively.

Figures 6 and 7 show the cumulative gas production volume and cumulative methane production volume changes for the three groups. Results in Table 6 imply that in the cumulative gas production volume, only the first and second groups have significant differences, and there is no significant difference between the other groups. For the cumulative methane production volume, there is no significant difference between the second group and the third group, but the other groups have significant differences. The second group and the third group tended to be close at the end of the experiment. The cumulative gas production volume of the first group is the highest and increases
evenly. The cumulative gas production volume and cumulative methane production volume of the three groups are respectively: are 6235 mL and 3741 mL for the first group, 4583 mL and 2521 mL for the second group, and 4606 mL and 2579 mL for the third group; the sorts are: group 1>group 3>group 2.

3.2.2. Cumulative gas production and methane production volume of the methane-producing phase during fermentation at a constant temperature of 35 °C for the acid-producing phase. Figure 8 shows the cumulative gas production volume in the constant temperature group was 6565 mL, and the cumulative methane production volume was 3939 mL. Results in Table 6 imply that regarding the cumulative gas production volume and cumulative methane production volume, there are significant differences between this group and the other three groups.

![Figure 8. Cumulative gas production volume and methane production volume of methane-producing phase at a constant temperature of 35 °C for acid-producing phase](image)

3.3. A subsection cow manure two-phase anaerobic fermentation system consumes energy and produces heat

Table 7. Energy consumption and production capacity of a two-phase anaerobic fermentation system with step-by-step heating and solidification of the acid-producing phase at 35 °C.

| Three different stepwise warming methods | The energy required to raise the temperature of two-phase anaerobic fermentation system Q/J | Heat loss in two-phase anaerobic fermentation system Q/J | Total energy consumed by the two phases of the fermentation system Q/J | Heat generated by the combustion of biogas Q/J | Total energy consumption of two-phase anaerobic fermentation system Q/J | Difference between energy consumption and production capacity of two-phase anaerobic fermentation system ΔQ/J |
|----------------------------------------|-------------------------------------|------------------------------------------------------|------------------------------------------------------|-----------------------------------------------|------------------------------------------------------|------------------------------------------------------|
| First group                            | 28.86                               | 127.42                                               | 156.28                                               | 156.33                                        | 638.32                                               | 481.99                                               |
| Second group                           | 28.86                               | 48.18                                                | 118.56                                               | 436.86                                        | 114.58                                               | 629.46                                               | 514.88                                               |
| Third group                            | 28.86                               | 113.28                                               | 147.42                                               | 482.04                                        | 115.15                                               | 624.18                                               | 509.03                                               |
| Constant temperature 35 °C            | 28.86                               | 254.84                                               | 283.7                                                | 164.13                                        | 765.74                                               | 601.61                                               |
3.3.1. Comparison of the energy of the cow manure two-phase anaerobic fermentation system with different warming methods. The energy consumption of the three groups by different warming methods is given in Table 7: constant temperature 35 °C> first group> second group> third group. The generated heat is: constant temperature 35 °C> first group> third group> second group; the energy difference is: constant temperature 35 °C> group 2 > group 3 > group 1. It can be seen that the first group produces the most heat energy and has the smallest difference between energy consumption and production capacity, and has the best economic benefits.

4. Conclusions
Two-phase anaerobic fermentation technology has the advantages of good fermentation system stability, high system fermentation efficiency and high gas production efficiency. The temperature is more important for the anaerobic fermentation system, and it will individually affect the biochemical reaction rate of the anaerobic fermentation process[25]. Furthermore, the microorganisms involved in anaerobic fermentation can adapt to the temperature range of 10–65 °C [26]. This experiment verifies that the acid-producing phase hydrolysis acidification effect is better when using 5 °C/2d stepwise heating, the maximum mass concentration of volatile fatty acids is 9372.46 mg/L, the total concentration of acetic acid butyric acid accounts for 77% of the mass concentration of volatile fatty acids, the cumulative gas production volume is 6253 mL, and the cumulative methane production volume is 3741 mL. Through analysis of variance, except for the percentages of the total concentration of acetic acid butyric acid to the mass concentration of volatile fatty acids and to the concentration of ammonia nitrogen, there are significant differences between the first group and other groups (including the constant temperature group) (p<0.05). It can be seen from the experimental results that the first group has the best value for each parameter, and the fermentation efficiency is higher. Therefore, the best stepwise warming method in this experiment is 5 °C/2d. By comparing the energy consumption of the acid production phase in the two fermentation methods of constant temperature and stepwise warming, it can be known that the energy consumption and energy difference of constant temperature fermentation are much higher than those of stepwise warming fermentation. In the cold regions of China, the temperature in winter is relatively low, and long-term constant temperature fermentation consumes a considerable amount of energy. Conclusion can thus be drawn that, compared with constant temperature fermentation, stepwise warming fermentation is more suitable for winter factories in cold regions due to its better energy saving effect and economic benefits.

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