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Effect of pH on volatile fatty acids (VFAs) in rice wash

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ABSTRACT

The study investigated the characteristics of rice wash in fermentation, component and odd/even proportion of volatile fatty acids (VFAs), to contribute new knowledge by developing quantitative relationship between VFAs and polyhydroxyalkanoates. The fermentation experiments were conducted with and without controlling pH. Rice wash produces primarily even fatty acids in the system with uncontrolled pH. The change in VFAs in rice wash with pH was investigated. It was found that the maximum concentration of VFAs was 3,156.68 mg/L without controlling pH with concentrations of acetic acid, propionic acid, butyrate and valerate being 974.46; 361.97; 1,082.51, and 737.73 mg/L, respectively. Even fatty acids reached the maximum value of 2,056.97 mg/L. When pH was changed from 4 to 8, the dominant fatty acid changed from butyrate to propionic acid. The results showed that when pH was changed from acidic to alkaline, the proportion of odd fatty acids increased gradually. When pH was less than 4, 5, and 6, the ratios of odd to even fatty acids were 1:2.5, 1:2, and 1:3. When pH was 8, the proportion of odd to even fatty acids was 1:1. The study results can provide guidelines for comprehensive utilization of solid wastes and waste treatment.

Keywords: Rice wash; VFAs; pH; Odd/even number fatty acids

1. Introduction

Increased amount of food waste due to improved living standards and rapid population growth is a significant human and environmental health concern. Therefore, food waste disposal and resource utilization have gained increased research attention [1,2]. As an organic and rich renewable resource, food waste mainly consists of rice, flour, animal and vegetable oils, meat, and bones. Among them, rice wash can be used as a fertilizer for irrigation farmland. In addition, anaerobic fermentation of rice wash by microorganisms can produce a small organic acid-volatile fatty acid (VFA) molecule, which is often used as an additional carbon source for wastewater treatment at low C/N ratio [3] or synthetized substrate of polyhydroxyalkanoates (PHAs) [4,5]. However, anaerobic fermentation of rice wash has not been investigated in detail.

Utilizing VFAs produced from food waste [6] for generating energy can be useful in the context of current energy crisis and the environmental pollution [7]. Nevertheless, concentration of VFAs produced from food waste is affected by factors including C/N ratio, pH, and temperature [8–10]. The significant variation in VFAs concentration due to these factors restricts the development of techniques for utilizing VFAs for energy production. Among these factors, pH is a critical parameter that causes differences in the concentration and composition of VFAs, in addition to affecting microbial metabolism [11].

Several research studies have focused on VFAs production, including improving the hydrolyzing efficiency of organic solid waste [12], restraining activity of methanogenic bacteria [13], and optimizing the process of VFA production [14]. These studies found that different composition of VFAs produces different PHA component [15,16]. PHAs, which are biodegradable materials, consist of poly-β-hydroxybutyrate

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(PHB), polyhydroxyvalerate (PHV), polyhydroxybutyrate-hydroxyvalerate (PHBV), the physical characteristics of which can range between brittleness or thermal instability and soft or flexibility. PHAs dominated by PHB are completely water resistance and biodegradable compared with the traditional polystyrene, the crystal of which has higher hardness and brittleness [17]. The copolymer of PHB and PHV (PHBV) has a better flexibility, lower melting point and higher hardness compared with PHV [18]. In the last 20 years, only Akiyama et al. [19] have reported that even/odd numbers of VFAs are related to the generation of PHB and PHBV. However, the quantitative relationship between the monomer component of PHAs and the component of VFAs is rarely reported. Therefore, it is not clear how to regulate the direction of VFAs with the accumulation and monomer composition of PHAs.

Therefore, the aim of this study was to establish a quantitative relationship between VFAs and PHAs by studying odd/even number of VFAs at different pH values. The study also aimed to investigate anaerobic fermentation of rice water, and the composition and proportion of VFAs in rice wash at regulated pH.

2. Materials and methods

2.1. Anaerobic fermentation device

The anaerobic fermentation device is shown in Fig. 1. The device mainly consists of two 500 mL conical bottles, and the reactor temperature was automatically controlled by an incubator at 37°C ± 1°C. The gas produced during fermentation was collected by the drainage method.

2.2. Materials

Rice wash was collected from the Guangzhou University canteen, and the supernatant was discharged after settling for 24 h. This same step was repeated for 5 d. When the total solid concentration (TS) was 5,000 ± 100 mg/L, the mixture was mixed evenly and poured into each anaerobic fermentation device to determine TS. The TS, volatile solid (VS), and dissolved chemical oxygen demand (SCOD) in the rice wash were 5,280 mg/L; 5,100 mg/L; and 3,500 mg/L, respectively. The concentration of VFAs was 531 mg/L, while pH was 4.20.

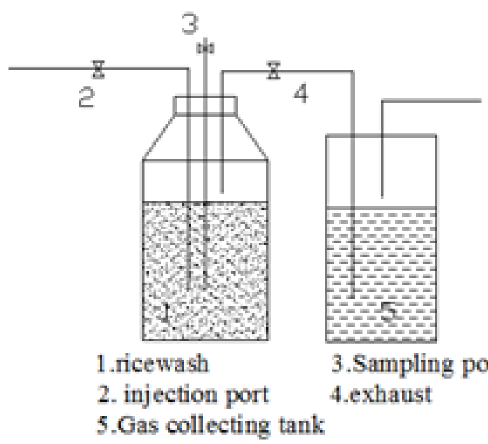

Fig. 1. Anaerobic fermentation device.

2.3. Analytical methods

TS and VS were measured using the gravimetric method [20], while pH was measured by the pHS 3C pH meter. VFA, and SCOD were measured after pretreatment by centrifuging the sample at 10,000 rpm in 10 min. The supernatant was filtered with membrane of 0.45 µm. Among them, SCOD was determined by potassium dichromate method [20].

The concentrations of VFAs (acetic acid, propionic acid, butyrate, and pentanoic acid) were determined using the gas chromatography (GC7900 (CNW, CD-WAX, FID), with the sample size of 1 µL. The temperature of the detector and the inlet were 250°C and 220°C, respectively. The column temperature was increased from 60°C to 150°C at the rate of 7°C min^{-1} in 5 min and then reached up to 230°C at the rate of 20°C min^{-1} in 10 min.

2.4. Experimental design

The rice wash with TS of 5,280 mg/L was poured into six 500 mL anaerobic fermentation devices labeled as I, II, III, IV, V, and VI. pH of II, III, IV, V, and VI were adjusted to 4, 5, 6, 7, and 8, respectively, using 5 mol/L NaOH and HCl, while pH of I was not adjusted. The resulting gas exited through the trachea. The system was stirred twice a day for 2 h at a time. Before testing analytical index, the stirred sample was filtered with membrane of 0.45 µm. There were no additional fermentation materials.

3. Data analysis

3.1. Changes in VFAs of raw rice wash acid at unadjusted pH

As shown in Fig. 2, during the process of anaerobic fermentation, the VFAs concentration of rice wash increased gradually and then decreased. In anaerobic fermentation for 15 d, even number fatty acids accounted for more than 60% overall. Among them, the total concentration of VFAs

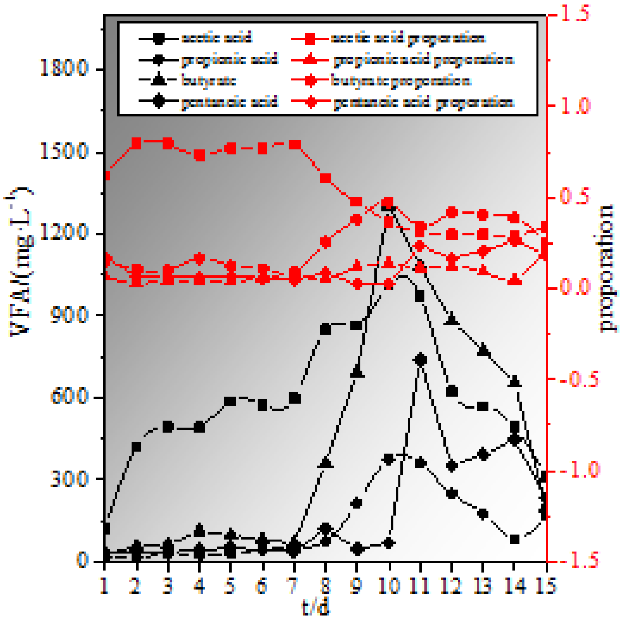

Fig. 2. Types variation of VFAs in rice wash in fermentation.

reached up to the highest value of 3,156 mg/L in day 10, and acetic acid, propionic acid, butyrate, and pentanoic acid were 974.46; 361.97; 1,082.5; and 737.7 mg/L, respectively. The even fatty acids also reached to a maximum value of 2,056.97 mg/L accounting for 65.16% of VFAs. The value of odd fatty acids began to increase gradually 10 d later. However, as shown in Fig. 3, the component of VFAs distributed uniformly on the 15th d of anaerobic fermentation, even numbered fatty acids accounted for 32%, while the odd fatty acids accounted for 68%. The odd to even fatty acids ratio was 1:2; however, each acid concentration was low. Acetic acid, propionic acid, butyrate, and pentanoic acid were 124.83, 79.83, 93.02, and 68.38 mg/L, respectively. The total concentration of VFAs reached only to 366.08 mg/L, which accounted for VFAs in 1/12.

With the rise in VFAs concentration, the concentration of the acid declined gradually except for acetic acid. Especially in the 5th d, the propionic acid was present in small amount. The proportion of propionic, butyrate, and pentanoic acids increased slightly in the 10th d, though their concentration declined, particularly butyrate concentration. In the 12th d, the proportion of butyrate accounted for 41.83%. After 12 d, the acids were evenly distributed.

3.2. Changes in VFAs composition with pH

As shown in Fig. 4, when pH was 4, the total amount of VFAs increased from 194.2 mg/L initially to 1,025.2 mg/L in day 4 then declined to 98.7 mg/L in day 7. The acids generally showed a declining trend after an initial increase. At the beginning of the reaction, the concentration of acetic acid was only 19.2 mg/L. Although acetic acid concentration increased a little, it declined to 21.1 mg/L in 5th d. The concentration of propionic acid increased gradually and reached the highest level of 112.0 mg/L in the 4th d, which was the lowest proportion of VFAs. At this time, butyrate was dominant.

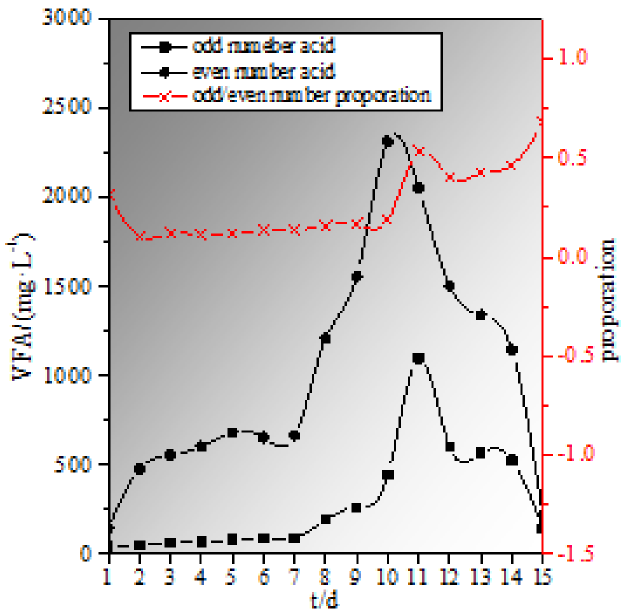

Fig. 3. Odd/even number proportion of VFAs in rice wash in fermentation.

Compared with Fig. 4, the propionic acid concentration in Fig. 5 increased slightly and reached to the peak value of 230.2 mg/L on the 4th d. Although butyrate accounted for large proportion initially, VFAs concentration increased sharply. The proportion of butyrate decreased from 58.62% on day 4 to 51.76% on day 5. The proportion of propionic acid in Fig. 5 is less than that in Fig. 4, in which the proportion of the propionic acid increased from 10.93% to 20.58%. Although the concentration of pentanoic acid increased, the trend was generally same as that of Fig. 5.

As shown in Fig. 6, the total VFAs content showed a declining trend after rising, and reached the peak value of 1,798.8 mg/L on the 8th d. Meanwhile, the concentration of acetic acid and propionic acid also reached their respective peak values of 849.5 and 437.9 mg/L, having the highest proportion of the total VFAs concentration (47.22% and 24.34%). Comparing VFAs in Figs. 4 and 5 when the fermentation days were prolonged, the VFAs content increased, though the proportion trend for acetic acid was not obvious. The proportions of propionic acid and butyrate were larger than those shown in Fig. 7. However, the proportion decreased slightly when compared with Fig. 6. The proportion of pentanoic acid

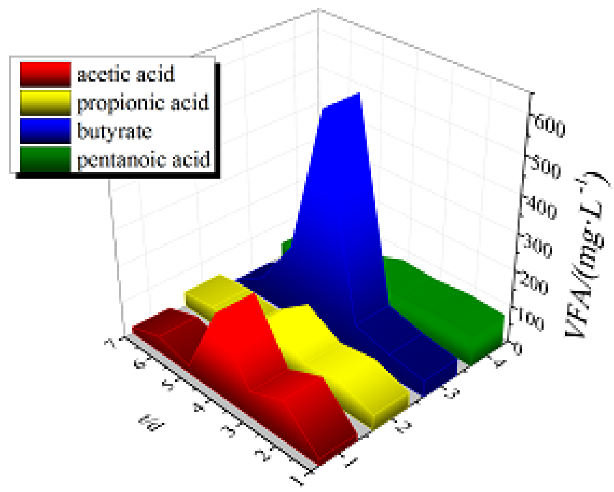

Fig. 4. Types variation of VFA at pH 4.

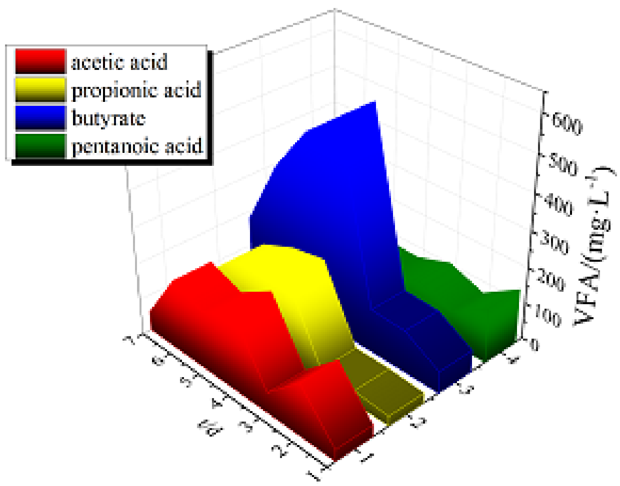

Fig. 5. Types variation of VFA at pH 5.

did not have a significant influence on the prophase. With increasing fermentation days, the proportion of pentanoic acid increased to 67% on the 12th d, and the concentration remained at 93.9 mg/L.

When pH was 7, the VFAs concentration reached the highest value of 3,406.0 mg/L on the 5th d. As shown in Fig. 7, the proportion of acetic acid increased initially and then declined with the highest proportion of acetic acid was 50.16%. Compared with pH 4, 5, and 6, acetic acid had a higher proportion and peak value at pH 7. At the initial stage of anaerobic fermentation, the capacity of acetic acid production increased with the concentration reaching 280.3 mg/L, while the proportion of propionic acid accounted for about 27% with the concentration reaching to the peak value of 891.2 mg/L on the 5th d. The proportion of butyrate accounted for 44.45% during the initial anaerobic fermentation, and the proportion fell to 20% with the growth of anaerobic fermentation time. The butyrate content also reached to 783.5 mg/L on the 5th d. The proportion of pentanoic acid accounted for no more than 10% though slightly increased at the later stage.

As shown in Fig. 8, the total content of VFAs decreased slightly compared with pH 7, though the proportion of each acid varied. As days of anaerobic fermentation increased, the proportion of acetic acid declined gradually and accounted for 22.70% on the 11th d. The proportion of propionic acid increased significantly and reached the highest value of 45.43% on the 6th d, and the peak value of 907.35 mg/L on the 5th d. In addition, the proportion of butyrate also decreased slightly to a minimum of 13.73% on the 5th d and then increased to 34.32%. Compared with Fig. 7, the proportion of pentanoic acid increased, though the content was smaller than one at pH 4 and 5, which reached the lowest value of 4.71% on the 5th d.

3.3. Changes in odd/even number proportion of VFAs with pH

At pH 4, the proportion of even fatty acids accounted for 76%, which was the largest proportion on the 4th d during anaerobic fermentation, and then decreased gradually. The odd fatty acids showed an opposite trend with proportions 4.6 times more than the even fatty acids on the 7th d. As shown in Fig. 9, when pH was 4, the concentration of the odd and even fatty acids had respective peaks of 319.78 and 798.83 mg/L on the 4th d. Even fatty acids did not decline significantly and were dominant in the later stage of anaerobic fermentation. At pH 6, the concentration of odd and even fatty acids increased and reached the peaks of 546.21 and 1,252.65 mg/L, respectively, in 8 d. Although the proportion of odd and even fatty acids did not change significantly in the anaerobic fermentation in 10 d, odd number fatty acid rose sharply on the 13th d, and was 2.11 times more than the proportion of even numbered fatty acids.

When the pH was changed from acidic to neutral to weakly alkaline, the proportion of odd fatty acids increased gradually, while even fatty acids showed a downward trend, with odd fatty acids dominating gradually in VFAs. At pH 7, the concentration of odd fatty acid was 1.24 times more than that of even fatty acids on the 13th d. The concentration of odd fatty acid was 1.19 times more than that of even fatty acid at pH 8. The proportion curve shows an upward trend, and the proportion of odd to even fatty acids was more than 1:1.

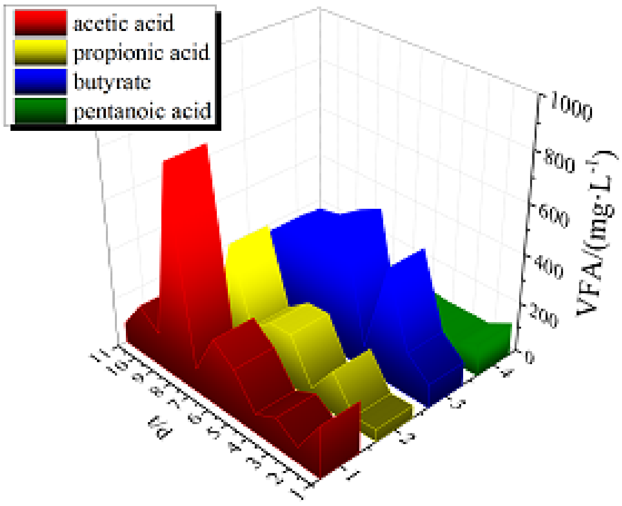

Fig. 6. Types variation of VFA at pH 6.

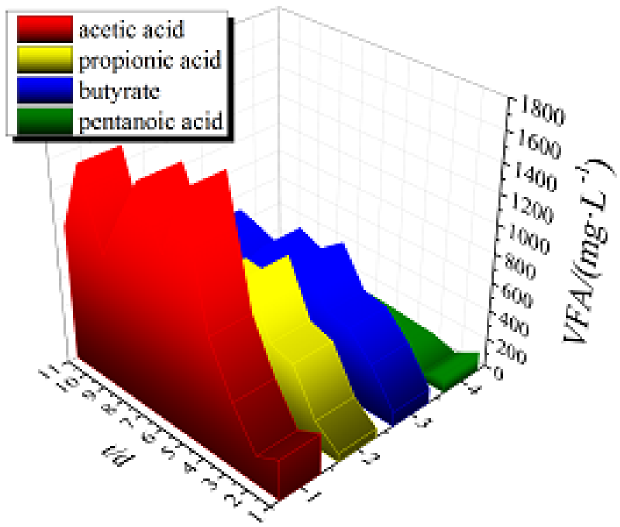

Fig. 7. Types variation of VFA at pH 7.

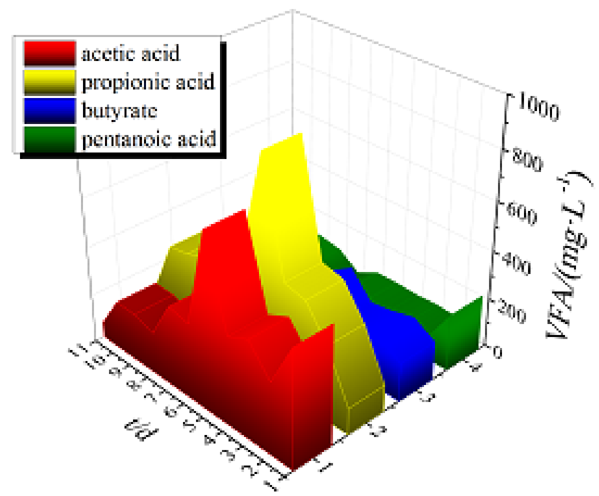

Fig. 8. Types variation of VFA at pH 8.

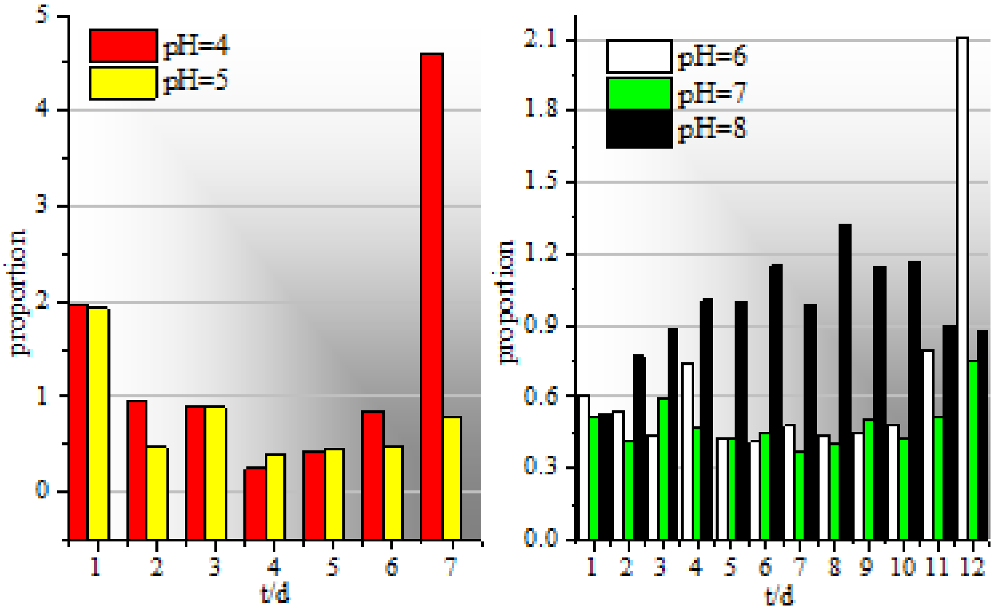

Fig. 9. Odd/even number proportion of VFAs.

4. Results and discussion

4.1. Influence of rice wash on VFAs in an uncontrolled pH system

As shown in Fig. 2, during anaerobic fermentation, the concentration of VFAs in the early stage did not significantly increase, mostly because organic solid in rice wash did not decompose completely. However, the concentration of VFAs in direct hydrolytic acidification was relatively small, and many non-degradable organics had not been effectively transformed. This is because majority of organics in the rice wash existed in the microbial cells, which released the organics by autolysis or hydrolysis, and then converted them into other substances under the effect of fermentative bacteria. However, the rigid structure of microbial cell walls can hinder the hydrolysis of easily degradable substances in cells. The lower pH of the rice wash during the initial stage of the anaerobic fermentation inhibited the activity of acid-producing bacteria (AB) and related enzymes. The microorganisms were further inhibited because of the increasing concentration of unionized organic acids, resulting in inhibition in the production of VFA and hindrance of the occurrence of subsequent acidification. With prolonged fermentation, the VFAs concentration increased, which can be because pH change from acidic to alkaline inhibited the activity of methanogen bacteria, stripped the extracellular polymers, and dissolved out the protein and glycogen. Consequently, the reproduction of AB was promoted to produce more VFAs. However, with the alkalinity in the fermentation broth increased gradually, the activity of AB was inhibited and the accumulation of VFAs showed a downward trend. Compared with acidic conditions, the effect of VFA accumulation was better in alkaline conditions [21], because the suitable pH for general microbial growth is 5–10, though extreme acidic or alkaline environment can also affect the activity of enzyme, thereby inhibiting the growth of microorganisms. Furthermore, because of high moisture content and low concentration of rice wash, alkalinity and microbial biomass provided were inadequate, causing excessive accumulation of white floc in the rice wash, and affecting the dissolution and hydrolysis of organic compounds.

Under alkaline condition, the main product in fermentation was acetic acid and propionic acid. As for the organic fertilization, accumulation of butyrate might be that the specific nature of rice wash can make activity of butyrate-degrading bacteria increased to promote the rise of butyrate concentration, or the activity of hydrogen-producing acetogens (HPA) was inhibited to effect activity of butyric acid bacteria under alkaline conditions [22], resulting in increased concentration of butyrate as shown in Fig. 2. Some studies have suggested that glycogen and protein can produce acetic acid directly, and HPA can degrade propionic acid, butyrate, two-carbon structured organic acids and alcohols to produce acetic acid. The reason might be that HPAs become the dominant bacteria under low pH. In the process of fermentation, there is no need to produce acids with the propionic acid fermentation bacteria, which leads to decreasing concentration of propionic acid.

As shown in Fig. 3, rice wash was focused on producing even number fatty acids. Furthermore, rice wash could increase the activity of butyric acid bacteria, so the concentration of butyrate was higher than acetic acid in the later stage. With the increase of the fermentation, the proportion of odd and even number fatty acids began to appear at a ratio of 1 to 1. Emergence of junction points between odd and even fatty acid was that the activity of AB especially for acetogenic bacteria and butyric acid bacteria. Methanogenic bacteria decomposed acetic acid to produce methane, while acetogenic bacteria became the disadvantaged group. Though, with the condition of decreasing organics and strengthening alkalinity, the activities of another AB were inhibited and appeared weak. The declined rate of the even number fatty acid is greater than that of the odd number fatty acid, so junction point between the even number fatty acid and the odd number fatty acid appeared at the ratio of 1 to 1. After the junction point, the concentration of odd number fatty acid was higher than that of even number fatty acid.

4.2. Influence of pH on the VFAs constitute

pH can affect the enzyme action in the process of fermentation. As evident in Figs. 3–7, pH can effectively change components and concentration of VFAs, since the environmental condition of microorganism was not the same. The growth of certain AB was promoted by controlling the pH [23], resulting in AB becoming the dominant species and a large number of specific products were produced. pH could also control the activity of enzymes during anaerobic fermentation, which can accelerate fermentation and the time of acid production.

As shown in Fig. 7, at pH 7, constitutes of each acid were generally high. Soluble organics in rice wash decomposed at pH 7. According to Figs. 4–8, the concentration of acetic acid increased initially and then decreased when pH increased from 4 to 8. This is because the acetogenic bacteria became the dominant species at pH 7–8, while they were vulnerable species at pH 4–6. It was found that the acetic acid was the main product of anaerobic fermentation. When pH was alkaline, the activity of Propionibacterium was better because of its tolerance to alkalinity at room temperature [24]. On the contrary, when pH was 4, concentration and proportion of butyrate were higher. In the high butyric acid condition of rice wash, butyric acid increased at acidic pH since butyric acid bacteria can tolerate acidic condition in comparison to other AB. When the fermenting liquid was acidic and methanogenic bacteria were inhibited, butyric acid bacteria became the dominant species competing with other AB.

4.3. Influence of pH on the proportion of odd/even number fatty acid

As shown in Fig. 9, when pH was 4 and 5, the odd number fatty acids were found in a large proportion. Since the growth rate of butyric acid bacteria was higher than the inhibition rate of acetogenic bacteria in the acidic conditions, even number fatty acid was dominant at all time. The later function of acetic acid and butyrate were consumed during methanation except for other HPA, resulting in the increase of odd number fatty acid. As can be seen in Fig. 9, at pH 6 and 7, propionic acid bacteria and acetogenic bacteria competed with nutrition, fluctuations of VFAs in a small range. Because acetic acid was the major fermentation product, the proportion of even number fatty acids was dominant. When pH ranged from 6 to 8, the proportion of odd number fatty acids gradually increased, and the ratio of odd/even number fatty acid was equal to or greater than 1. This was due to the advantage in Propionibacterium competing with acetogenic, leading to a rise in propionic acid concentration and odd number fatty acids. Therefore, the ratio of even to odd-numbered fermented VFAs could be controlled by pH [25].

5. Conclusions

The study investigated the fermentation of rice wash as the organic waste, discussed the composition of VFAs and the proportion of odd and even number fatty acids in rice wash at different pH. The following conclusions were derived in this study:

- Rice wash produces even number fatty acid during the fermentation process, and acetic acid and butyrate were found to be dominant.
- At pH 4, the proportion of butyrate was the largest, and the concentration of butyrate was relatively high.
- At pH 6, the proportion of pentanoic acid was the largest, and the concentration of butyrate was relatively higher.
- At pH 7, the proportion of acetic acid was the largest and the concentration of acetic acid was the highest.
- At pH 8, the proportion of propionic acid was the largest and the concentration of propionic acid was the highest.
- When pH changed from acidic to alkaline during anaerobic fermentation, the proportion of odd number fatty acids increased gradually, while the even number fatty acids gradually declined.
- When pH was 4, 5, and 6, the ratio of odd to even number fatty acid was 1:2.5, 1:2, and 1:3, while the proportion of odd numbers fatty acid appeared as 1:1 at pH 8.

Acknowledgments

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