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Effect of pH on volatile fatty acid production from anaerobic digestion of potato peel waste

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ABSTRACT

In this study, potato peel waste was used as feedstock to produce volatile fatty acids (VFAs) by anaerobic digestion. The effects of different pH levels (pH 5.0, pH 7.0, pH 11.0, and uncontrolled pH) on VFA concentration and composition, intermediate products, and metabolic state were evaluated. The results showed that the highest total VFA production was achieved with pH 7.0 (41.9 g COD/L and 632.2 mg COD/g VS_{fed}), followed by that with uncontrolled pH. Butyric acid was the dominant product under acidic pH, whereas acetic acid dominated under alkaline pH. The type of acidogenic fermentation at pH 7.0 was the mixed-acid type. The change in NADH level in the mixed-acid type of fermentation consisted of small fluctuations, enhancing the stability and efficiency of fermentation. The enzymatic activities of acetate kinase and butyrate kinase were slightly inhibited at pH 5.0 and 11.0, resulting in relatively low VFAs production.

1. Introduction

Nearly one-third of global food production—from agricultural production to consumers—is lost or wasted annually (Food and Agriculture Organization of the United Nations, 2011). China has a small food waste per capita (55 kg annually), but owing to its large population base, total food waste is about 195 Mt annually (Braguglia et al., 2018). About 56% of food waste in China is attributed to vegetables and fruits, which are identified as high-potential sources for generating valuable compounds (Braguglia et al., 2018; Sindhu et al., 2019). With the strategy of developing potato as a staple food in China, potato as a major food crop has become an increasingly essential vegetable in the human diet. About one-third of potatoes are used for fresh consumption (Zhu et al., 2016). With regard to transport and storage, some decaying or germinating potatoes have to be discarded; with regard to daily consumption, most potatoes need to be peeled, resulting in a large amount of potato peel residue. Potato waste residue has high contents of moisture and starch, protein, non-starch polysaccharide, and other nutrients, rendering it ideal as a carbon source for fermentation (Liang et al., 2016; Sepelev and Galoburda, 2015). It can be used to produce lactic acid by mixed culture fermentation (Liang et al., 2015; Liang et al., 2016), as culture media for bacterial cellulose production (Abdelraof et al., 2019), as feedstock for biobutanol or bioethanol production (Chohan et al., 2020; Hijosa-Valsero et al., 2018; Khawla et al., 2014), as base materials for biodegradable films (Xie et al., 2020.), as a potential source for the extraction of steroidal alkaloids and starch (Hossain et al., 2014; Torres et al., 2020), as a precursor to synthesizing biochar (Sun et al., 2017; Yang, et al., 2018), and for biogas production by anaerobic digestion (AD) (Liang et al., 2015; Liang and Mcdonald, 2015).

An alternative approach to potato waste management is AD, which consists of a series of reactions: hydrolysis, acidogenesis, acetification, and methanogenesis. Notably, hydrolysis and acidogenesis of monophasic digesters for potato waste can be completed quickly, resulting in the rapid accumulation of volatile fatty acids (VFAs) and further inhibiting the activity of methanogenic microorganisms (Kaparaju and Rintala, 2005; Pistis et al., 2013). Compared with end products (methane), intermediate products — VFAs, can be more valuable substrates for some advanced products (Dai et al., 2020). Thus, potato peel waste (PPW) is an ideal feedstock for the production of VFAs by mixed culture fermentation. There are some advantages in this way, including ease of degradation, thus requiring no pretreatment, and the lack of need for an additional methanogenic inhibitor to stop the conversion of short-chain fatty acids to methane.

As potential and renewable carbon sources, VFAs can have wider applications, such as the biosynthesis of polyhydroxyalkanoates (PHAs) (Lee et al., 2014), biological removal of nutrients from wastewater (Zheng et al., 2010; Li et al., 2011), or bioenergy (Fei et al., 2011; Zong et al., 2009). Controlling the composition of VFAs affects the performance of downstream processing (Jankowska et al., 2015). Bengtsson

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et al. (2008) reported that acetate and butyrate tend to form hydroxybutyrate monomers in the polymer, and propionate tends to increase the amount of hydroxyvalerate. PHA with hydroxyvalerate shows increased elasticity and flexibility. Thus, when PHA is to be produced using the fermentation broth, the fermentation technique has to be regulated to increase the concentration of propionate. Zhou et al. (2018) summarized and reviewed the strategies for enhancing VFAs that can be used to adjust and control their distribution. Environmental conditions, including pH, organic loading time, and retention time, affect VFA moieties. Butyric acid production from food waste is dominant under treatment with pH 6.0–7.0, whereas acetic acid production is promoted under treatment with pH 5.0 or uncontrolled pH (Jiang et al., 2013). Yu and Fang (2002) indicated that propionate was favored during AD of dairy wastewater with pH 4.0–4.5, and acetic acid was the main product when pH was set at 5.0–6.5. Jankowska et al. (2017) reported that acetate and butyrate were the major products under acidic conditions, and acetate and propionate were the main products under alkaline conditions. The distinct research results suggest that the production of specific VFAs and distribution of VFAs depend not only on pH but on the type of substrates as well. Elucidating the effects of regulation on certain types of substrates can guide the selective production of VFAs and the application in downstream steps.

The effects of pH on the metabolic mechanism of VFAs during AD of PPW are rarely reported. In this study, the influence of pH adjustment on VFA composition and production during AD of PPW was systematically evaluated. Changes in VFA, NADH level, activities of key enzymes, and intermediate products during digestion were analyzed to determine the mechanism underlying the effect of pH on the efficiency of VFA production via AD of PPW.

2. Materials and methods

2.1. Inoculum and substrate

The inoculum came from the reacted active sludge of a mesophilic liquid anaerobic digester (Zibo, Shandong Province, China) fed with cattle dung. The inoculum was stored at 4 °C before use. PPW manually peeled from fresh products, was collected from a student cafeteria at Shandong University of Technology (Zibo, Shandong Province, China). The substrate was stored at 4 °C and beaten with a beater machine before use. The basic properties of the feedstock and inoculum are listed in Table 1.

2.2. Batch experiments

Batch tests were conducted at 37 °C in a continuous stirred-tank reactor with working a volume of 5 L. The inoculum and PPW were mixed in the inoculum size of 8% (based on volatile solids content (VS)). The total solid content (TS) of the digested material was adjusted to 10% by using tap water. The temperature was maintained at 37 °C ± 0.5 °C, and the stirring rate was set at 300 r/min. The 4 mol/L HCl and NaOH solution was used to adjust the pH automatically. The pH level was maintained at 5.0, 7.0, and 11.0 during AD, and treatment with uncontrolled pH was used for comparison. Each reactor was flushed with nitrogen gas (N_2) for 3 min before sealing. Samples were collected at the following time points: 4 h, 12 h, 2nd day, 3rd day, and 5th day.

Table 1
Characteristics of PPW and inoculum.

Parameter	Potato peel waste	Inoculum
pH	5.94	7.56
TS (%)	15.75	15.21
VS (%)	14.24	7.16
C (%)	35.36	10.95
N (%)	1.35	1.40
Carbohydrate (%)	8.68	2.10
Ash (%)	1.41	7.66

Note: All parameters are based on wet weight.

2.3. Analytical methods

To quantify the VFA, 10 mL of the collected samples was centrifuged at 12000 rpm for 15 min. The supernatant was filtered with 0.45 μm nylon syringe filters and 20% formic acid was used to adjust the pH < 3.0 for subsequent analysis. A gas chromatographer (GC6890, Agilent Technologies, Wilmington, USA) equipped with a flame ionization detector (FID) was used to measure the VFA. The Nukol free fatty acid-phase fused silica capillary column (DB-FFAP, 30 m × 0.53 mm × 1 μm) was used. The heating program was as follows: the temperature was maintained at 60 °C for 5 min, then increased to 200 °C at a rate of 8 °C/min, and held at 200 °C for 3 min. High-purity nitrogen (99.999%) was used as the carrier gas at a flow rate of 2.0 mL/min. The temperatures of the injector and the detector were set to 250 °C and 300 °C, respectively. The supernatant retained after centrifugation (12,000 rpm, 15 min), which was filtered using 0.45 μm nylon syringe filters, was used to determine soluble chemical oxygen demand (SCOD). A chemical oxygen demand (COD) analyzer (6B-3000A, Shengaohua Co., China) was used to measure the SCOD. The NADH level was determined using the method described in the study by Zhang et al. (2019). Glucose was analyzed using the dinitrosalicylic acid (DNS) colorimetric method (Saqib and Whitney, 2011). The reaction between pyruvic acid and 2,4-dinitrobenzene can produce pyruvate-2,4-dinitrophenylhydrazone, which is fuchsia–red in an alkaline solution. The mixed solution after the reaction was measured using an the Shimadzu UV-2550 spectrophotometer at 520 nm to determine the pyruvic acid. Specific steps were conducted as described in the study by Cooper et al. (2010), with slight modifications. Lactic acid was determined by p-hydroxybiphenol colorimetry as previously described (Wang et al., 2014). The principle is in accordance with the catalysis of copper ions, lactic acid reacts with concentrated sulfuric acid to form acetaldehyde, which can interact with p-hydroxybiphenyls to form a characteristic reddish purple substance at 565 nm. The enzymatic activity of acetate kinase (AK) and butyrate kinase (BK) was determined using ELISA test kits (Meimian Co., China), following the instructions provided by the manufacturer.

3. Results and discussion

3.1. Effect of pH on VFAs production

Some complex macromolecular organic materials in the PPW, such as starches and proteins, were converted to organic acids by hydrolytic bacterium and acidogenic microorganisms (Liang and McDonald, 2014). Changes in SCOD, VFA concentration, and VFA/SCOD are shown in Fig. 1. For the SCOD, it can be seen as an indication of hydrolysis performance (Hussain et al., 2017). The maximum SCOD concentration of 49.4 g/L was achieved with pH 11.0 after fermentation for 3 d and then decreased to 31.9 g/L. This reduction could be attributed to the lower production rate of the SCOD than that of its consumption rate. The SCOD fermented using pH 7.0 reached its maximum concentration on day 2, maintaining this level until the end of the fermentation process. These results agreed with the previous finding that the neutral conditions could improve the hydrolysis efficiency of food waste (Hussain et al., 2017). The maximum SCOD concentration achieved using uncontrolled pH was 32.6 g/L, and that achieved with pH 5.0 was 20.0 g/L. SCOD slightly changed when pH was set to 5.0 during fermentation. The aforementioned results reveal that neutral or alkaline conditions can potentially accelerate the solubilization of organic particles, whereas acidic conditions can inhibit the process. AD of kitchen waste reportedly shows higher efficiency in

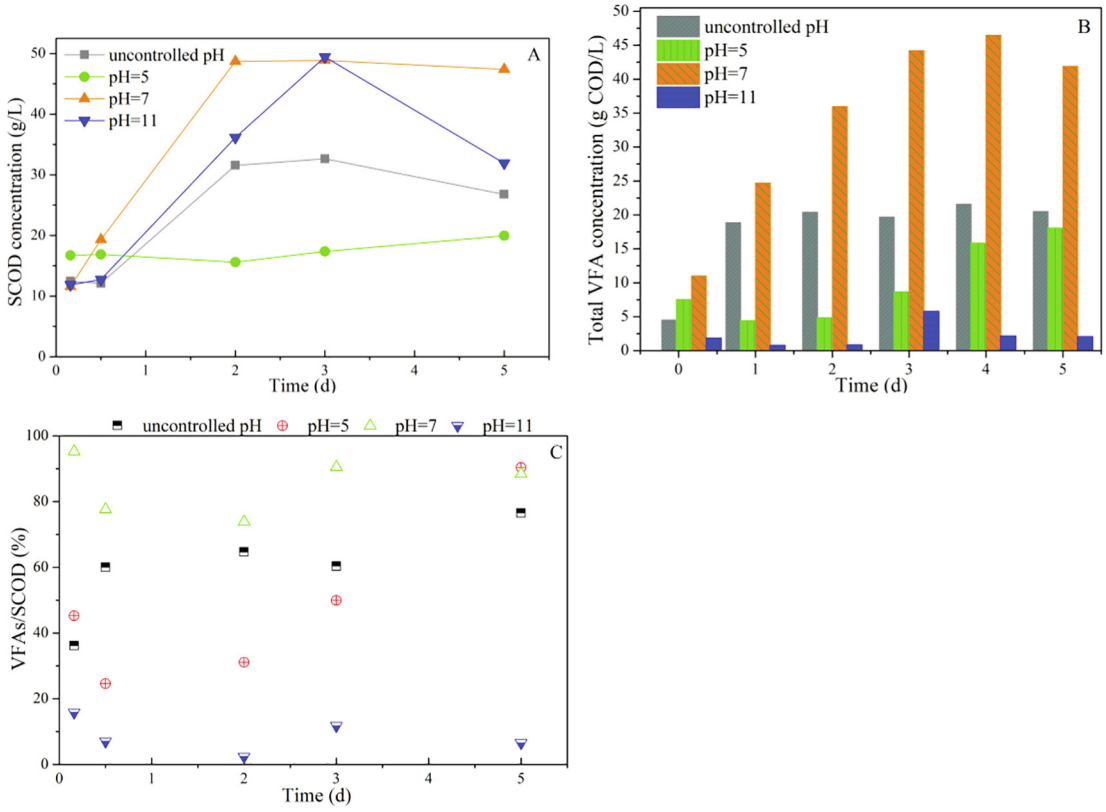

Fig. 1. Variation of SCOD concentration (A), total VFA concentration (B) and VFAs/SCOD (C) at different pH of PPW fermentation.

hydrolyzing carbohydrate, protein, and lipid at pH 7 than that at pH 5.0, 9.0, and 11.0 (Zhang et al., 2005). This finding contradicted Ma et al. (2019). Notably, among all treatments, the treatment with pH 7.0 and 11.0 achieved the highest hydrolysis performance in this study. The difference might be attributed to the abundance of protein in potato peel (Jeddou et al., 2016; Choi et al., 2016). That is, the enhancement of SCOD formation during fermentation with pH set to 11.0 was primarily caused by the hydrolysis and release of proteins under alkali conditions. Lin and Li (2018) reported that alkali pretreatment mainly influenced the hydrolysis and acidogenesis of protein. The low extent of hydrolysis when pH was set to 5.0 could be caused by the low pH, inhibiting the activity of hydrolytic bacteria. Several studies have reported that amylolytic microorganisms become inactive when pH was below 5.4 (Therion et al., 1982; Hussain et al., 2017).

Moreover, pH affects not only hydrolysis but acid production as well during AD. VFA production and its changing trend in each reactor were significantly different (Fig. 1B). The maximum VFA concentration (44.2 g COD/L) was achieved in the reactor with pH 7.0, followed by that with uncontrolled pH (21.6 g COD/L), pH 5.0 (18.1 g COD/L), and pH 11.0 (58.0 g COD/L). The highest yield of VFAs of 632.2 mg COD/g VS_{fed} at pH 7.0 was 1.3, 19.1, and 1.0 times higher than those at pH 5.0, pH 11, and uncontrolled pH (Table 2). Under treatments with uncontrolled pH and pH 5.0, the yield of butyric acid was the highest, reaching 157.2 and 128.1 mg COD/g VS_{fed} respectively; at pH 7.0, it mainly produced acetic acid, propionic acid and butyric acid were mainly produced, and the output levels were 289.5, 180.2, and 153.0 mg COD/g VS_{fed}, respectively; Under treatment with pH 11.0, the highest acetic acid yield was 28.4 mg COD/g VS_{fed}, which was considerably lower than those under other treatments. Notably, after fermentation for 4 h, acidification of the reactors with pH 5.0 and 11.0 were inhibited, whereas that of the reactors with pH 7.0 and uncontrolled pH increased steadily. This finding can be attributed to the adaptation of the microorganisms in the inoculum to the neutral conditions and a sudden change in pH, which slightly inhibited the activity of the microorganisms. Liu et al. (2012) found that the abundance of microorganisms under neutral conditions was richer than that under acidic or alkaline conditions. At the uncontrolled pH, VFA production was maintained at almost 20 g COD/L after 24 h; the pH was then reduced from 7.0 to 4.9. After fermentation for 2 d, acidogens were gradually recovered in the reactor with pH 5.0. VFA concentration was considerably higher during AD with pH 7.0. Acidification with pH 11.0 was almost fully suppressed, whereas hydrolysis was highly activated.

Table 2
Effects of pH on VFA production from PPW.

	Uncontrolled pH	pH = 5	pH = 7	pH = 11
VFA yield (mg COD/g VS_{fed})	309.5	272.6	632.2	31.4
Acetic acid yield (mg COD/g VS_{fed})	69.3	46.6	289.5	28.4
Propionic acid yield (mg COD/g VS_{fed})	3.7	6.8	180.2	1.9
Butyric acid yield (mg COD/g VS_{fed})	157.2	128.1	153.0	1.1
Hexavnoic acid yield (mg COD/g VS_{fed})	78.3	89.4	0	0
Ethanol (g COD/L)	2.7	1.2	1.1	1.0
SCOD (g/L)	26.8	20.0	47.4	31.9
VFAs/SCOD (%)	76.5	90.4	88.4	6.5

Lin and Li (2018) indicated that a higher pH led to higher hydrolase activity. This difference could be attributed to the high concentration of Na^+. Previous studies have indicated that excessive Na^+ impeded VFA production (Jin et al., 2016; Zhao et al., 2017). During the entire fermentation, no methane production was detected, and only a small amount of CO_2 was produced within the first 48 h of fermentation. This result indicated that the rapid hydrolysis and acidification of PPW inhibited the activity of methanogens, prevented the further conversion of VFA to methane, and improved the accumulation of VFA. Therefore, PPW was an ideal carbon source for VFA production.

The acidogenic efficiency could be calculated using the ratio VFA/SCOD (Jankowska et al., 2017; Jin et al., 2019). The variation in VFA/SCOD is presented in Fig. 1C. As shown in the figure, the acidogenic efficiency under treatment with pH 7.0 is significantly higher than at other pH levels. The average VFA/SCOD values at different pH levels were as follows: 59.6% with uncontrolled pH, 48.3% with pH 5.0, 8.7% with pH 11.0, and 85.1% with pH 7.0. The lowest conversion rate of SCOD into VFA was achieved with pH 11.0, indicating that a higher hydrolysis rate does not indicate a higher rate of acidogenesis. This result contradicted other studies (Liu et al., 2012; Jankowska et al., 2017). This inconsistency could be attributed to the high salt content and alkaline conditions that caused irreparable injury to microbes during the initial fermentation. The changes in VFA production, SCOD concentration, and VFA/SCOD under treatment with pH 5.0 indicated that the activity of the hydrolytic and acidogenic bacteria was inhibited by the acidic conditions (Farouk et al., 2019).

3.2. Effect of pH on VFAs composition

The type of VFA plays a significant role in its further application, rendering the analysis of VFA composition important (Jiang et al., 2011). The high percentage of acetate in waste-derived VFAs promotes electricity generation (Lee et al., 2014). Waste-derived VFA is one of the renewable resources for the production of mixed culture PHA. Acetate and propionate are precursors for the synthesis of polyhydroxybutyrate and polyhydroxyvalerate, respectively (Jiang et al., 2011). On the basis of the distribution of major products, AD can be divided into 6 types: the acetate–ethanol, propionate, butyrate, mixed, lactate, and the homoacetogenic pathway (Zhou et al., 2018). Fig. 2 shows the percentage of individual VFAs by using different pH levels, with acetic and butyric acid as the main products. Acetic acid was the primary product treated with pH 7.0 and 11.0, comprising 46%-77% and 74%-92% of the total VFA, respectively, whereas butyric acid comprised the main product with pH 5.0 and uncontrolled pH, comprising 11%-75% and 38%–82% of the VFA mix, respectively. Acetate–ethanol fermentation only occurred during the first 12 h under treatment with pH 5.0 and 7.0 and uncontrolled pH, whereas with pH 11.0, this type of fermentation was maintained throughout the process. Owing to the robustness of the acetate metabolic pathway under alkaline conditions (Huang et al., 2016), acetic acid was still produced even when fermentation was almost inhibited under treatment with pH 11.0. After 12 h, butyrate type fermentation was the main reaction under treatment with uncontrolled pH, and mixed-type metabolic pathway was the main reaction with pH 7.0. Moreover, BK exhibited increased activity in the first 2 d under treatment with uncontrolled pH, suggesting that the butyrate-type pathway was dominant (Fig. 5). The butyrate-type metabolic pathway was also favored under acidic conditions (Zhou et al., 2018). Under treatment with pH 7.0, the percentage of propionic acid increased as fermentation continued, whereas the content of acetic acid deceased. When the pH was set to 5.0, butyric acid was the dominant product, and the percentage of acetic acid decreased after 2 d. This result is consistent with the data on the activities of BK and AK (Fig. 5). Notably, with an increase in pH, the percentage of butyric acid decreased. Under treatment with pH 5.0 and uncontrolled pH, the hexanoic acid content comprised about 20% of total VFAs at the end of fermentation. This result could be attributed to the chain elongation reaction between acetate, butyrate, and ethanol (Agler et al., 2011). The ethanol concentration gradually decreased as the pH increased (Table 2). Under the treatment with uncontrolled pH, the highest concentration of ethanol was obtained—that is, more than twice as high as other groups. The VFA products from PPW were relatively simple and were beneficial for separation and purification.

3.3. Effect of pH on intermediate products

On the basis of the metabolic pathways of hydrolysis and acidogenesis, glucose, pyruvic acid, and lactic acid were the primary intermediate products. Starch in PPW is converted into glucose by hydrolase, glucose is converted to pyruvate by glycolysis, and pyruvate is converted into lactic acid, acetic acid, propionic acid, butyric acid, and so on (Chen et al., 2013). The variations in the concentration of glucose, pyruvic acid, and lactic acid during AD of PPW are presented in Fig. 3. It can be concluded that the glucose concentration was higher under acidic and alkaline conditions than under neutral conditions during the initial stage. Under treatment with pH 5.0 and 11.0, organic matter was more easily hydrolyzed because of the acid treatment or alkali treatment, resulting in increased glucose. Therefore, when the pH decreased to lower than 5 after 12 h, the glucose concentration at uncontrolled pH suddenly increased. After fermentation for 24 h, the glucose concentration in all treatments was below 1000 mg/L, indicating a balance between production and consumption of glucose. On the basis of the results of VFA production, the higher concentration of glucose did not coincide with the highly efficient VFA production. This lack of agreement shows that acidification, rather than hydrolysis, was the rate-limiting step for VFA production from PPW by AD.

Pyruvic acid is a critical control point in the metabolic network. A higher concentration of pyruvic acid was achieved with pH 11.0 than when other pH levels were applied, which could be attributable to acidogenic bacteria being suppressed by high salt concentrations and resulting in the accumulation of pyruvic acid. This result agreed well with VFAs production. The concentration of pyruvic acid increased at the beginning of the reaction (0–12 h) at uncontrolled pH and pH 7.0 and was then maintained at a relatively stable station. Compared with the production of VFAs and lactic acid, pyruvate was mainly converted into acetic acid, propionic acid and butyric acid after 12 h under treatment with pH 7.0. The concentration of pyruvic acid in the reactor with pH 5.0 was nearly constant after fermentation for 4 h. On day 2 of fermentation, pyruvate remained at a low concentration, however, the VFA concentration barely increased and the lactic acid concentration sharply increased, indicating that most of the pyruvate was converted to lactic acid by the action of lactate dehydrogenase at this stage (Phypers and Pierce, 2006).

Lactic acid is an intermediate in the production of propionic acid (Lee et al., 2008). The concentration of lactic acid under acidic conditions was generally higher than that under alkaline and neutral conditions during the entire fermentation process. The highest propionic acid production was achieved with pH 7.0, but the concentration of lactic acid was slightly lower than that at other pH levels. The reason could be that propionic acid was mainly produced by some acidogenic bacteria such as *Propionibacterium* and *Bifidobacterium* (Zhou et al., 2018).

3.4. Effect of pH on NADH level

NADH and NAD^+ are crucial in electron transfer in biological systems (Farabegoli et al., 2003). Some studies have suggested that NADH and NAD^+, as well as the energy metabolism, death, and various cellular functions in cells are closely interrelated (Ying, 2006). Various oxidation/reduction reactions in cells are facilitated by the electron transfer between the oxidized and reduced forms of this coenzyme (Zhang et al., 2019). When the fermentation is conducted under anaerobic conditions, a large amount of NADH produced by substrate

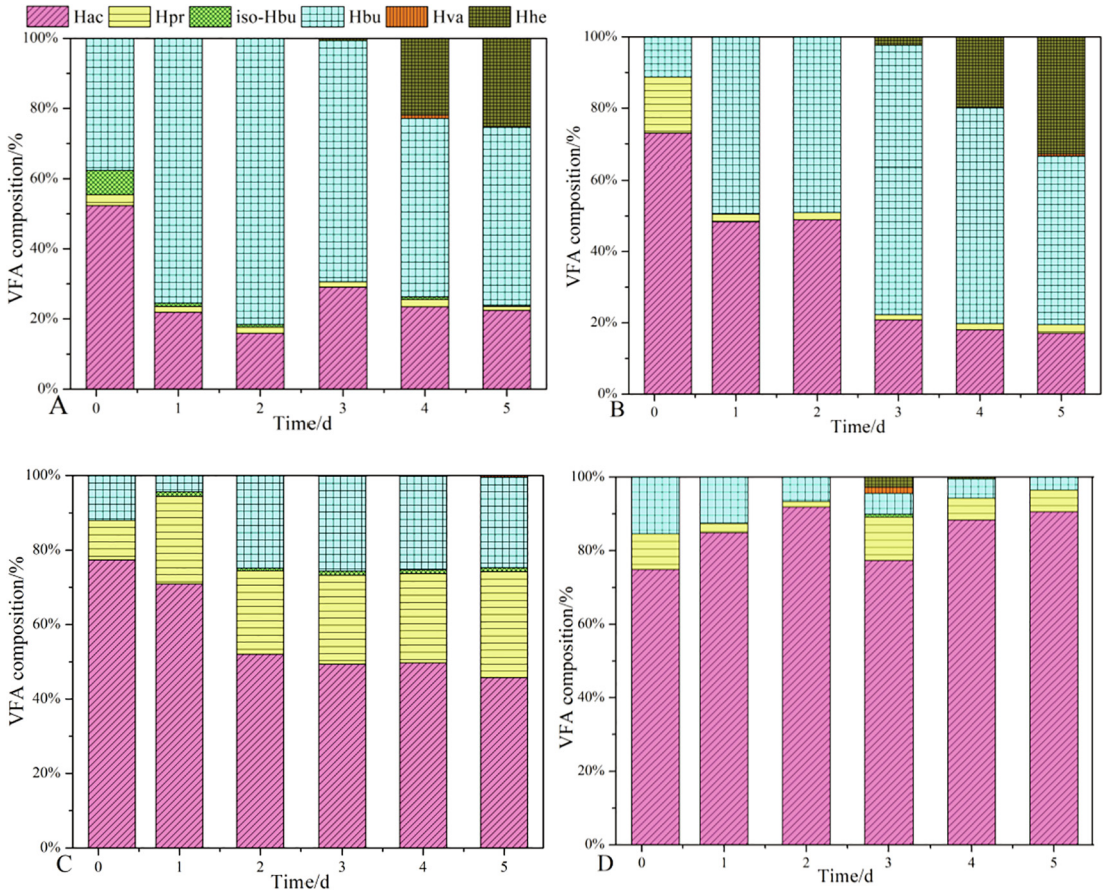

Fig. 2. Variation of VFA composition at different pH (A: uncontrolled pH; B: pH = 5; C: pH = 7; D: pH = 11) of PPW fermentation.

degradation needs to be re-oxidized to NAD^+ during acidification to achieve NADH recycling to allow glycolysis and fermentation to continue (Chu et al., 1996; Zhou et al., 2018; Zhang et al, 2019). NADH is fluorescent, and its oxidized forms (NAD^+) exhibit no fluorescence. When the cellular redox state changes, the autofluorescence signal changes accordingly (Piston and Knobel, 1999). Thus, NADH levels can be regarded as an indicator of the metabolic state in the reactors (Zhang et al., 2019). Within NADH standards, NADH concentration is proportional to fluorescence intensity (Wos and Pollard, 2006). Fig. 4 shows that the fluorescence intensity of NADH ranges from 1.3 to 3.0 AU at different pH levels. The fluorescence intensity under treatment with pH 7.0 exhibited a trend similar to that under treatment with pH 11.0 and uncontrolled pH, but the value and margin of fluctuation of fluorescence intensity with pH 7.0 were significantly smaller than those under treatment with other pH levels. Wos and Pollard (2009) found that when the cell metabolism was active, the concentration of NADH was the lowest. As mentioned in previous sections, the markedly high SCOD and VFA concentration were also achieved with pH 7.0. These results suggest that when the NADH level achieves a relatively low and balanced state in the system, the fermentation process also reaches an appropriate balance (Chu et al., 1996), and VFA production is improved.

In the acidification stage of AD, two molecules of NADH are excess during acetate production, and generating butyrate can consume two molecules of NADH (Zhou et al., 2018). The reduction in the fluorescence intensity of NADH at uncontrolled pH during the first 2 d could be attributed to the consumption of NADH in the conversion of pyruvate to butyric acid. Under treatment with pH 7.0, the slight fluctuation of NADH in the whole fermentation might be caused by the different ratios of acetic acid, propionic acid and butyric acid production. Under treatment with pH 5.0, the fluorescence intensity of NADH decreased after 2 d, and the activity of BK increased simultaneously (Fig. 5). The reason could be that the butyric acid production rate was higher than the substrate degradation rate during this period, causing the total amount of NADH to decrease. This result was consistent with the dominance of the butyrate-type pathway in the late stages of fermentation with pH set to 5.0. Under alkaline conditions (pH = 11), the signal intensity of NADH decreased to 1.73 AU on day 2 and increased to 3.03 AU on day 3, which was higher than the signal intensity under other treatments. This difference could be attributable to the enzyme in the metabolic process of NADH consumption being inhibited to a certain extent by the strong alkaline condition in the initial stages. The activity of BK was considerably lower than that under other treatments. After 3 d, the high production of acetic acid resulted in a large surplus of NADH (Zhou et al., 2018; Chu et al., 1996).

3.5. Effect of pH on the activity of AK and BK

To further analyze the mechanism of different metabolic types at different pH levels, the key enzymes in the production of each organic acid were determined. In the metabolic pathway of pyruvate conversion to acetic acid, the key enzymes are AK and phosphotransacetylase (Bock et al., 1999). In the pathway of butyrate production, the key enzyme is BK and phosphotransbutyrylase (Zhu and Yang, 2004). Changes in key enzymatic activities in different types of acidogenic fermentation are shown in Fig. 5. With pH levels set to 5.0 and 11.0, the enzymatic activity of AK was higher than that of BK, and all enzymatic activities were lower than those at uncontrolled pH and pH 7.0. These findings are consistent with the data on total VFA production and VFA/SCOD. The results indicated that constant acidic and alkali conditions inhibited the activity of acidogenic bacteria to a certain extent. At uncontrolled pH and pH 7.0, the activities of AK and BK were consistent

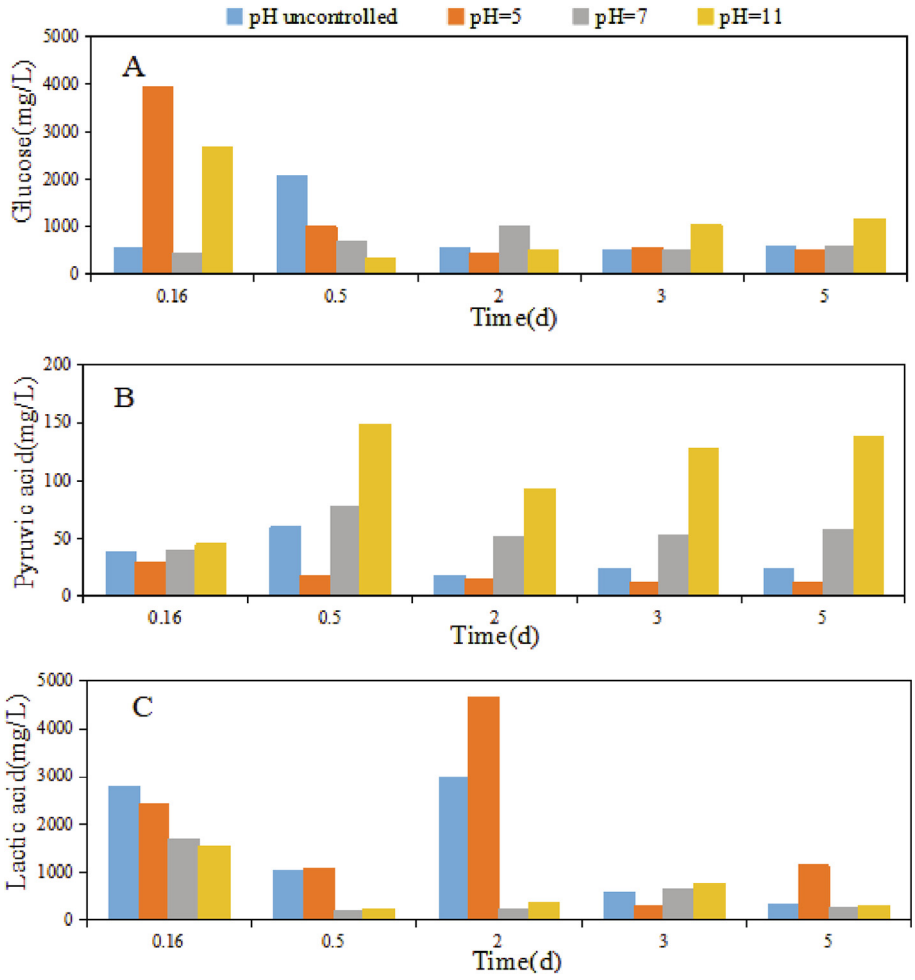

Fig. 3. The changes of intermediate products at different pH, A: Glucose; B: Pyruvic acid; C: Lactic acid during the fermentation of PPW.

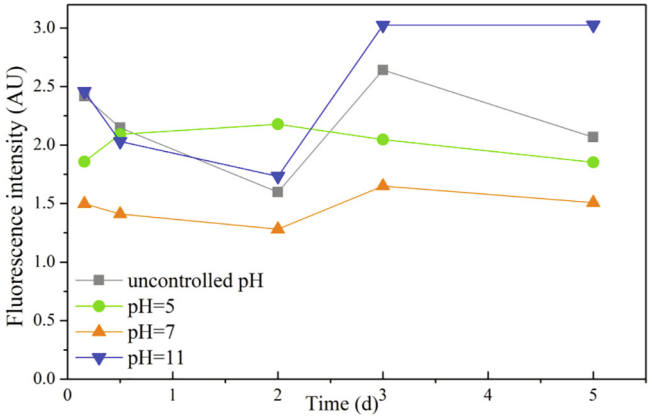

Fig. 4. Fluorescence intensity of NADH of PPW fermentation at different pH.

with the production of acetic and butyric acid, suggesting that the accumulation of acetic and butyric acid at those pH levels were mainly derived from the pyruvate produced by glycolysis. The NADH results revealed that the change in NADH was consistent with the enzymatic activity. When the activity of BK was higher, the fluorescence intensity of NADH decreased; when the activity of AK was higher, a certain amount of NADH was released, and the fluorescence intensity of NADH increased accordingly. The activity of AK and BK when pH was set to 7.0 was higher than when other pH levels were used, which was consistent with the VFA production (Fig. 1B). With pH set to 5.0, the activity of AK decreased gradually, and the activity of BK decreased first and then increased. The behavior of the butyric acid production suggests that the glycolytic pyruvate pathway is the main metabolic pathway for the formation of butyric acid. The activity of AK was considerably higher than that of BK with pH set to 11, which was consistent with the results for NADH (Fig. 4). This result indicated that the dominant acetic acid was converted from pyruvate. At the end of the fermentation process, the activity of AK increased when pH was set to 11 and was even higher with other pH levels. As discussed in previous sections, VFA production increased in the reactor with pH 11.0 at the end of fermentation. These results were consistent.

4. Conclusion

In this study, the highest VFA production of PPW by AD was achieved under the treatment with pH 7.0, followed by that with uncontrolled pH, pH 5.0, and pH 11.0. Methane production was suppressed by rapid VFA accumulation. Under treatments with uncontrolled pH and pH 5.0, butyric acid was the main VFA product. The fermentation types at pH 11.0 and 7.0 were acetate and mixed-acid types, respectively. A stable and low NADH level improved VFA production. The enzymatic activities of AK and BK at pH 5.0 and 11.0 were lower than those at pH 7.0 and uncontrolled pH.

CRediT authorship contribution statement

Yu Lu: Data curation, Writing - original draft. **Qi Zhang:** Investigation. **Xiangyou Wang:** Supervision. **Xiaonan Zhou:**

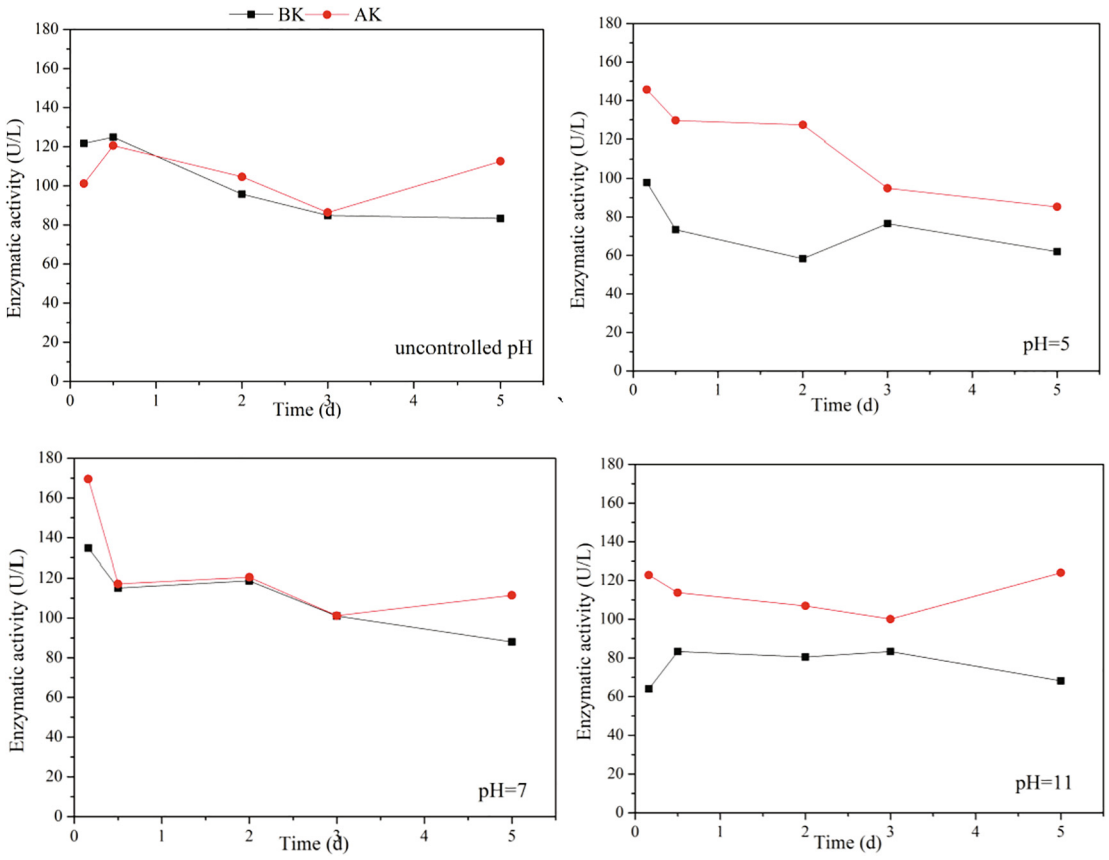

Fig. 5. Key enzyme activities (AK and BK) of PPW fermentation with time at different pH.

Investigation. **Jiying Zhu:** Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdelraof, M., Hasanin, M.S., El-Saied, H., 2019. Ecofriendly green conversion of potato peel wastes to high productivity bacterial cellulose. Carbohydr. Polym. 211, 75–83.
Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T., 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol. 29, 70–78.
Bengtsson, S., Hallquist, J., Werker, A., Welander, T., 2008. Acidogenic fermentation of industrial wastewaters: effects of chemostat retention time and pH on volatile fatty acids production. Biochem. Eng. J. 40 (3), 492–499.
Bock, A.K., Glasemacher, J., Schmidt, R., Schönheit, P., 1999. Purification and characterization of two extremely thermostable enzymes, phosphate acetyltransferase and acetate kinase, from the hyperthermophilic eubacterium thermotoga maritima. J. Bacteriol. 181 (6), 1861–1867.
Braguglia, C.M., Gallipoli, A., Gianico, A., Pagliaccia, P., 2018. Anaerobic bioconversion of food waste into energy: a critical review. Bioresour. Technol. 248, 37–56.
Chen, H., Meng, H., Nie, Z., Zhang, M., 2013. Polyhydroxyalkanoate production from fermented volatile fatty acids: effect of pH and feeding regimes. Bioresour. Technol. 128, 533–538.
Chohan, N.A., Aruwajoye, G.S., Sewsynker-Sukai, Y., Kana, E.B.G., 2020. Valorisation of potato peel wastes for bioethanol production using simultaneous saccharification and fermentation: process optimization and kinetic assessment. Renewable Energy 146, 1031–1040.
Choi, S.H., Kozukue, N., Kim, H.J., Friedman, M., 2016. Analysis of protein amino acids, non-protein amino acids and metabolites, dietary protein, glucose, fructose, sucrose, phenolic, and flavonoid content and antioxidative properties of potato tubers, peels, and cortexes (pulps). J. Food Compos. Anal. 50, 77–87.
Chu, A., Mavinic, D.S., Ramey, W.D., Kelly, H.G., 1996. A biochemical model describing volatile fatty acid metabolism in thermophilic aerobic digestion of wastewater sludge. Water Res. 30 (8), 1759–1770.
Cooper, A.J.L., Krasnikov, B.F., Pinto, J.T., Bruschi, S.A., 2010. Measurement of cysteine S-conjugate β-lyase activity. Curr. Protoc. Toxicol. 4.36, 1–18.
Dai, K., Zhang, W., Zeng, R.J., Zhang, F., 2020. Production of chemicals in thermophilic mixed culture fermentation: mechanism and strategy. Crit. Rev. Environ. Sci. Technol. 50 (1), 1–30.
Farabegoli, G., Hellinga, C., Heijnen, J.J., Loosdrecht, M.C.M.V., 2003. Study on the use of NADH fluorescence measurements for monitoring wastewater treatment systems. Water Res. 37 (11), 2732–2738.
Farouk, R.Y., Li, L., Wang, Y., Li, Y., Melak, S., 2019. Influence of pretreatment and pH on the enhancement of hydrogen and volatile fatty acids production from food waste in the semicontinuously running reactor. Int. J. Hydrogen Energy 46 (4), 3729–3738.
Fei, Q., Chang, H.N., Shang, L., Choi, J.D., Kim, N., Kang, J., 2011. The effect of volatile fatty acids as a sole carbon source on lipid accumulation by Cryptococcus albidus for biodiesel production. Bioresour. Technol. 102 (3), 2695–2701.
Food and Agriculture Organization of the United Nations (FAO), 2011. Global food losses and food waste - extent, causes and prevention.
Hijosa-Valsero, M., Paniagua-García, A.I., Díez-Antolínez, R., 2018. Industrial potato peel as a feedstock for biobutanol production. New Biotechnol. 46, 54–60.
Hossain, M.B., Tiwari, B.K., Gangopadhyay, N., O'Donnell, C.P., Brunton, N.P., Rai, D.K., 2014. Ultrasonic extraction of steroidal alkaloids from potato peel waste. Ultrason. Sonochem. 21 (4), 1470–1476.
Huang, W., Huang, W., Yuan, T., Zhao, Z., Cai, W., Zhang, Z., et al., 2016. Volatile fatty acids (VFAs) production from swine manure through short-term dry anaerobic digestion and its separation from nitrogen and phosphorus resources in the digestate. Water Res. 90 (1), 344–353.
Hussain, A., Filiatrault, M., Guiot, S.R., 2017. Acidogenic digestion of food waste in a thermophilic leach bed reactor: effect of pH and leachate recirculation rate on hydrolysis and volatile fatty acid production. Bioresour. Technol. 245, 1–9.
Jankowska, E., Chwiałkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2015. Effect of pH and retention time on volatile fatty acids production during mixed culture fermentation. Bioresour. Technol. 190, 274–280.
Jankowska, E., Chwialkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2017. Volatile

fatty acids production during mixed culture fermentation - the impact of substrate complexity and pH. Chem. Eng. J. 326 (15), 901–910.
Jeddou, K.B., Bouaziz, F., Zouari-Ellouzi, S., Chaari, F., Nouri-Ellouz, O., et al., 2016. Improvement of texture and sensory properties of cakes by addition of potato peel powder with high level of dietary fiber and protein. Food Chem. 217 (15), 668–677.
Jin, B., Wang, S., Xing, L., Li, B., Peng, Y., 2016. The effect of salinity on waste activated sludge alkaline fermentation and kinetic analysis. J. Environ. Sci. 43, 80–90.
Jin, Y., Lin, Y., Wang, P., Jin, R., Gao, M., Wang, Q., et al., 2019. Volatile fatty acids production from saccharification residue from food waste ethanol fermentation: Effect of pH and microbial community. Bioresour. Technol. 292, 121957.
Jiang, Y., Hebly, M., Kleerebezem, R., Muyzer, G., Loosdrecht, M.C.M., 2011. Metabolic modeling of mixed substrate uptake for polyhydroxyalkanoate (PHA) production. Water Res. 45 (3), 1309–1321.
Jiang, J., Zhang, Y., Li, K., Wang, Q., Gong, C., Li, M., 2013. Volatile fatty acids production from food waste: effects of pH, temperature, and organic loading rate. Bioresour. Technol. 143, 525–530.
Kaparaju, P., Rintala, J., 2005. Anaerobic co-digestion of potato tuber and its industrial by-products with pig manure. Resour. Conservat. Recycl. 43(2), 175–188.
Lee, H.S., Salerno, M.B., Rittmann, B.E., 2008. Thermodynamic evaluation on H2 production in glucose fermentation. Environ. Sci. Technol. 42 (7), 2401-2407.
Khawla, B.J., Sameh, M., Imen, G., Donyes, F., Dhouha, G., Raoudha, E.G., et al., 2014. Potato peel as feedstock for bioethanol production: a comparison of acidic and enzymatic hydrolysis. Ind. Crops Prod. 52, 144–149.
Lee, W.S., Chua, A.S.M., Yeoh, H.K., Ngoh, G.C., 2014. A review of the production and applications of waste-derived volatile fatty acids. Chem. Eng. J. 235, 83–99.
Li, X., Chen, H., Hu, L., Yu, L., Chen, Y., Gu, G., 2011. Pilot-scale waste activated sludge alkaline fermentation, fermentation liquid separation, and application of fermentation liquid to improve biological nutrient removal. Environ. Sci. Technol. 45 (5), 1834–1839.
Liang, S., Mcdonald, A.G., 2014. Chemical and thermal characterization of potato peel waste and its fermentation residue as potential resources for biofuel and bioproducts production. J. Agric. Food. Chem. 62 (33), 8421–8429.
Liang, S., Mcdonald, A.G., 2015. Anaerobic digestion of pre-fermented potato peel wastes for methane production. Waste Manage. 46, 197–200.
Liang, S., Mcdonald, A.G., Coats, E.R., 2015. Lactic acid production from potato peel waste by anaerobic sequencing batch fermentation using undefined mixed culture. Waste Manage. 45, 51–56.
Liang, S., Gliniewicz, K., Gerritsen, A.T., Mcdonald, A.G., 2016. Analysis of microbial community variation during the mixed culture fermentation of agricultural peel wastes to produce lactic acid. Bioresour. Technol. 208, 7–12.
Lin, L., Li, X., 2018. Effects of pH adjustment on the hydrolysis of al-enhanced primary sedimentation sludge for volatile fatty acid production. Chem. Eng. J. 346 (15), 50–56.
Liu, H., Wang, J., Liu, X., Fu, B., Chen, J., Yu, H., 2012. Acidogenic fermentation of proteinaceous sewage sludge: effect of pH. Water Res. 46 (3), 799–807.
Ma, J., Xie, S., Yu, L., Zhen, Y., Zhao, Q., Frear, C., et al., 2019. pH shaped kinetic characteristics and microbial community of food waste hydrolysis and acidification. Biochem. Eng. J. 146 (15), 52–59.
Pistis, A., Asquer, C., Scano, E.A., 2013. Anaerobic digestion of potato industry by-products on a pilot-scale plant under thermophilic conditions. Environ. Eng. Manage. J. 12 (S11), 93–96.
Phypers, B., Pierce, J.T., 2006. Lactate physiology in health and disease. Continuing Education in Anaesthesia Critical Care & Pain. 6 (3), 128–132.
Piston, D.W., Knobel, S.M., 1999. Real-time analysis of glucose metabolism by microscopy. Trends Endocrinol. Metab. 10 (10), 413–417.
Saqib, A.A.N., Whitney, P.J., 2011. Differential behaviour of the dinitrosalicylic acid (DNS) reagent towards mono- and di-saccharide sugars. Biomass Bioenergy 35 (11), 4748–4750.
Sepelev, I., Galoburda, R., 2015. Industrial potato peels waste application in food production: a review. Res. Rural Dev. Int. Scient. Conf. 1, 130–136.
Sindhu, R., Gnansounou, E., Rebello, S., Binod, P., Varjani, S., Thakur, I.S., et al., 2019. Conversion of food and kitchen waste to value-added products. J. Environ. Manage. 241, 619–630.
Sun, Y., Yang, G., Zhang, L., Sun, Z., 2017. Preparation of high performance H_2S removal biochar by direct fluidized bed carbonization using potato peel waste. Process Saf. Environ. Prot. 107, 281–288.
Therion, J.J., Kistner, A., Kornelius, J.H., 1982. Effect of pH on growth rates of rumen amylolytic and lactilytic bacteria. Appl. Environ. Microbiol. 44 (2), 428–434.
Torres, M.D., Fradinho, P., Rodríguez, P., Falqué, E., Santos, V., Domínguez, H., 2020. Biorefinery concept for discarded potatoes: recovery of starch and bioactive compounds. J. Food Eng. 275, 109886.
Wang, K., Yin, J., Shen, D., Li, N., 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect of pH. Bioresour. Technol. 161, 395–401.
Wos, M., Pollard, P., 2006. Sensitive and meaningful measures of bacterial metabolic activity using NADH fluorescence. Water Res. 40 (10), 2084–2092.
Wos, M., Pollard, P., 2009. Cellular nicotinamide adenine dinucleotide (NADH) as an indicator of bacterial metabolic activity dynamics in activated sludge. Water Sci. Technol. 60 (3), 783–791.
Xie, Y., Niu, X., Yang, J., Fan, R., Shi, J., Ullah, N., et al., 2020. Active biodegradable films based on the whole potato peel incorporated with bacterial cellulose and curcumin. Int. J. Biol. Macromol. 150, 480–491.
Yang, X., Kwon, E.E., Dou, X., Zhang, M., Kim, K.H., Tsang, D.C.W., et al., 2018. Fabrication of spherical biochar by a two-step thermal process from waste potato peel. Sci. Total Environ. 626, 478–485.
Ying, W., 2006. NAD^+ and NADH in cellular functions and cell death. Frontiers in Bioscience. 11 (1), 3129.
Yu, H.Q., Fang, H.H.P., 2002. Acidogenesis of dairy wastewater at various pH levels. Water Sci. Technol. 45 (10), 201–206.
Zhang, B., Zhang, L., Zhang, S., Shi, H., Cai, W., 2005. The influence of pH on hydrolysis and acidogenesis of kitchen wastes in two-phase anaerobic digestion. Environ. Technol. 26 (3), 329–340.
Zhang, M., Zhang, Y., Li, Z., Zhang, C., Tan, X., Liu, X., et al., 2019. Anaerobic co-digestion of food waste/excess sludge: substrates - products transformation and role of NADH as an indicator. J. Environ. Manage. 232 (15), 197–206.
Zhao, J., Liu, Y., Wang, D., Chen, F., Li, X., Zeng, G., et al., 2017. Potential impact of salinity on methane production from food waste anaerobic digestion. Waste Manage. 67, 308–314.
Zheng, X., Chen, Y., Liu, C., 2010. Waste activated sludge alkaline fermentation liquid as carbon source for biological nutrients removal in anaerobic followed by alternating aerobic-anoxic sequencing batch reactors. Chin. J. Chem. Eng. 18 (3), 478–485.
Zhou, M., Yan, B., Wong, J.W.C., Zhang, Y., 2018. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: a mini-review focusing on acidogenic metabolic pathways. Bioresour. Technol. 248, 68–78.
Zhu, Y., Yang, S., 2004. Effect of pH on metabolic pathway shift in fermentation of xylose by *Clostridium tyrobutyricum*. J. Biotechnol. 110 (2), 143–157.
Zhu, X., Cheng, Y., Chen, P., Peng, P., Liu, S., Li, D., et al., 2016. Effect of alkaline and high-pressure homogenization on the extraction of phenolic acids from potato peels. Innovative Food Sci. Emerg. Technol. 37, 91–97.
Zong, W., Yu, R., Zhang, P., Fan, M., Zhou, Z., 2009. Efficient hydrogen gas production from cassava and food waste by a two-step process of dark fermentation and photofermentation. Biomass Bioenergy 33 (10), 1458–1463.