Research Article

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Impact of nanoscale zerovalent iron on volatile fatty acid production from food waste: key enzymes and microbial community

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Abstract

BACKGROUND: The proper dosage of nanoscale zerovalent iron (NZVI) for volatile fatty acid (VFA) production was optimized and different kinds of organic acids and key enzymes during the process were analyzed to test the effect of NZVI on VFA fermentation from food waste.

RESULTS: Batch assays showed that proper NZVI dosage can promote VFA production, reaching the maximum accumulation of 22 250 mg COD L^{-1} at 10 g L^{-1} dosage, which is 1.92 times higher than that of the control group. Acetic acid accounted for the highest proportion (72%) of VFAs, and lactic acid was the main by-product of organic acids. The addition of NZVI provided a reducing power for the lactic acid fermentation in the system, which promoted the conversion of pyruvic acid to lactic acid. Moreover, the addition of NZVI not only greatly promoted the activity of ferredoxin oxidoreductase (0.35 IU g^{-1}) and acetyl-CoA (1.47 IU g^{-1}) but also caused changes in the microbial community structure in the fermentation system. After adding NZVI, besides *Lactobacillus*, *Olsenella* (12.69%) was another dominant bacterium which can rapidly convert the produced lactic acid into short-chain fatty acids (SCFAs), thereby affecting acid production.

CONCLUSION: These key enzymes resulted in the accumulation of VFAs. The fermentative bacteria responsible for SCFA production were also highly enriched in fermentation reactors with NZVI, which was in accordance with the accumulation of SCFAs.

Supporting information may be found in the online version of this article.

Keywords: nanoscale zerovalent iron (NZVI); volatile fatty acids (VFAs); ferredoxin oxidoreductase (FDXR); acetyl CoA (A-CoA); food waste

INTRODUCTION

With the continuous development of the economy, solid waste, in particular food waste, has become a concern. The amount of food waste is particularly huge, increasing greatly at an annual rate of around 8%,[1] and there is still 30% that has not entered the collection and transportation system.[2] In most cities in China, food waste is disposed through traditional incineration or dumped in landfills. These processes cannot realize the resource utilization of food waste and will also burden local finance. Therefore the resource utilization of food waste can not only reduce environmental problems but also recycle energy and achieve sustainable development.

Anaerobic fermentation is effective for the treatment of food waste and recycling of renewable resources. The inevitable products of anaerobic fermentation, volatile fatty acids (VFAs, which mainly comprise acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid), are often used as a carbon source for biological nitrogen and phosphorus removal.[3] In addition, VFAs can be used to produce biodegradable plastics, synthetic biogas and polyhydroxyalkanoates (PHAs)[4] and for electricity generation.[5]

At present, there are many ways to improve VFA production from waste activated sludge, such as calcium peroxide combined with free ammonia pretreatment,[6] sophorolipid addition,[7] free ammonia-assisted ultrasound pretreatment,[8] silver nanoparticles,[9–11] etc. However, in recent years, nanoscale zerovalent iron (NZVI) has become widely used in environmental remediation and industrial wastewater treatment, and it can be used to remove chlorinated pollutants, nitroaromatic compounds

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and heavy metals such as arsenic.[12] NZVI has a large specific surface area; according to estimates, the specific surface area of nano iron is 38.5 $m^2\ g^{-1}$, while that of ordinary iron powder is only 0.9 $m^2\ g^{-1}$. Zerovalent iron itself has reducibility and, owing to the surface effect of nano materials, its active center increases with the increase of surface area.[13] In addition, it may also affect the growth of microorganisms,[14] so it will have a certain impact on the anaerobic digestion process. The hydrolysis of organic matter is the rate-limiting phase of anaerobic fermentation. During the fermentation process, microorganisms only absorb soluble organic matter for utilization.[15] Therefore, if the hydrolysis of the substrate can be accelerated, it is bound to promote the accumulation of VFAs. In addition, NZVI can also optimize microbial population structure, change the type of hydrolysis fermentation and increase acetic acid production.[16]

As an important trace metal in the metabolic process, iron plays an important role in hydrogenase, ferredoxin and other iron–sulfur proteins. [NiFe] and [FeFe] hydrogenases can catalyze the production of H_2 from protons and electrons, as well as the reverse reaction.[17] Pyruvate ferredoxin oxidoreductase (FDXR) is an enzyme located at the central fermentation pathway and closely related to the oxidative decarboxylation of pyruvate to acetyl CoA (A-CoA) during the production of acetic and butyric acids.[18]

It can be found that previous studies mainly used activated sludge for anaerobic fermentation to produce short-chain fatty acids (SCFAs). Compared with activated sludge, food waste has more abundant organic nutrients and is a better substrate for anaerobic fermentation to produce SCFAs. Moreover, NZVI is widely used in wastewater treatment, but there are few reports on the effect of adding NZVI as a substrate on food waste.[19,20] In this study, the effects of NZVI on volatile fatty acids (VFAs) during the fermentation of food waste were analyzed for the first time. The mechanism of NZVI in promoting VFAs in food waste and the effect on the microbial community will be discussed from the perspective of key enzymes.

MATERIALS AND METHODS

Materials

Anaerobic digestion substrate

Food waste was obtained from the cafeteria of the University of Science and Technology Beijing. It was mainly composed of rice, meat, vegetables, pasta snacks and vegetable soup. The food waste was manually selected from paper towels, bones, plastic garbage, etc., ground and mixed with a food processor and finally stored at −20 °C in a freezer.

Inoculation sludge

The anaerobic sludge used in this study was obtained from a sewage treatment plant in Beijing. Before the inoculation, hydrothermal treatment and acclimation of inoculated sludge were carried out to inhibit methanogens and active acid-producing bacteria, so the pH will decrease during acclimation. The sludge was hydrothermally treated at 105 °C for 2 h and then cooled down. Before the inoculation, the sludge was domesticated at 37 °C, and the pH value was reduced to approximately 6.5 and then stabilized for 2–3 days. The basic properties of the food waste and inoculated sludge in the laboratory are shown in Table 1.

Table 1. Basic properties of food waste and different inoculated sludges

Property	Food waste	Anaerobic sludge	Heated sludge
TS (%)	18.4 ± 1.1	9.9 ± 0.8	9.2 ± 0.6
VS (%)	17.3 ± 0.4	5.7 ± 0.6	5.7 ± 0.3
pH	6.3 ± 0.1	7.8 ± 0.1	4.0 ± 0.1
COD (mg L^{-1})	65 350 ± 233.8	–	–

TS and VS of food waste were measured after adding water at 2:1.

Other materials

Nano iron powder (≥99.9%, metal basis, 100 nm) was obtained from Aladdin (Shanghai, China). 2-Bromoethanesulfonic acid sodium salt (BES, ≥98%) was purchased from Adamas.

Methods

Experiment methods

Six conical flasks containing 300 g of food waste were prepared and sludge was inoculated at an inoculation ratio of 5% (Volatile Solid (VS ratio)). Subsequently, 50 mmol L^{-1} BES[21] and 0, 1, 3, 5, 8 and 10 g L^{-1} nano iron powder were added to the reactor. The reactor was then sealed and the whole process was operated in an anaerobic tank at constant temperature. The anaerobic environment was maintained during the whole fermentation process. The fermentation temperature was controlled at 37 ± 1 °C, the fermentation time was 120 h and samples were obtained every 6 h on the first day and every 24 h over the next 4 days.

Analysis methods

Before the analysis experiments, all samples were centrifuged for 10 min at 12 000 rpm and then filtered through a 0.45 μm membrane (Jinteng).

VFA test. A DB-FFAP capillary column (Shimadzu, Japan) was used for gas chromatography. The temperature rise procedure was as follows: initial temperature of 60 °C for 2 min, increased to 100 °C at 20 °C min^{-1}, held for 1 min, increased to 170 °C at 10 °C min^{-1}, maintained for 1 min and raised to 210 °C at 20 °C min^{-1} for 2 min. The inlet (SO1 PL1) temperature, flame ionization detector (FID) temperature and injection volume were 220 °C, 240 °C and 0.4 μL respectively. The yield of VFAs was calculated in g chemical oxygen demand (COD) L^{-1}. The COD equivalents of acetic acid, propionic acid, isobutyric acid, *n*-butyric acid, isovaleric acid and *n*-valeric acid were 1.066, 1.512, 1.816, 1.816, 2.036 and 2.036 respectively.

Lactic acid test. Lactic acid and glucose were measured using a Shimadzu LC-20A high-performance liquid chromatography system and an SH1011 column. The mobile phase, flow rate, column temperature, UV detection wavelength and injection volume were 0.5 mmol L^{-1} H_2SO_4, 1 mL min^{-1}, 60 °C, 210 nm and 10 μL respectively.

Enzyme activity test. The levels of FDXR and A-CoA in samples were determined by the double antibody sandwich method using a Jiangsu Jingmei enzyme-free ferritin reductase and acetyl coenzyme A kit. The purified FDXR (A-CoA) antibody was coated with a microporous plate to prepare a solid phase antibody. FDXR

(A-CoA) was added into the microcapsule of the coated monoclonal antibody in turn and then combined with horseradish peroxidase (HRP)-labeled FDXR (A-CoA) antibody to form an antibody–antigen–enzyme labeled antibody complex. After thorough washing, the substrate tetramethylbenzidine (TMB) was added to color (more details in Appendix 2 in File S1).

Microbial community analysis. For a complete understanding of the microbial community evolution in fermentation systems due to NZVI addition, the 454 high-throughput pyrosequencing technique was applied. The relevant process is based on Shanghai Meiji Biomedical Technology Co., Ltd (Shanghai, China) (more details in Fig. S4 and Appendix 2 in File S1).

Other analytical methods. Soluble Chemical Oxygen Demand (SCOD) was measured using standard methods.[22] Total Solid (TS) was calculated from the dry organic waste from the filter after heating in an oven at 105 °C, and VS was measured after the dry organic waste was burned in a muffle furnace.

RESULTS AND DISCUSSION

Effects of NZVI on VFA production

The effects of different NZVI dosages on acid fermentation of food waste are shown in Fig. 1.

Figure 1 shows that the addition of a small amount of NZVI had no significant effect on the yield increase of VFAs compared with the control group. However, at 10 g L^{-1} dosage, the total yield of VFAs after 48 h was 22 250 mg COD L^{-1}, which was 1.92 times higher than that of the control group (11 570 mg COD L^{-1}). The acid production rate was significantly higher than that in the control group, indicating that the proper NZVI dosage contributes to the hydrolysis and acidification of the substrate. Luo *et al.*[20] carried out acidogenic fermentation with sludge as the substrate under experimental conditions of 40 mmol L^{-1} BES, 5 g L^{-1} NZVI dosage and 20 °C temperature for 4 days, obtaining 1307.8 mg COD L^{-1} acid yield. From the perspective of VFA production, the yield was much lower than that in our research.

There are several reasons worth analyzing. One might be the different experimental temperatures used in the studies. Temperature is an important factor affecting the metabolism of microorganisms. Hafid *et al.*[23] fermented food waste at pH 5 and 37 °C and found that the total organic acid concentration was very high. Zhao *et al.*[24] found that the microbial activity was highest at 37 °C and pH 6.5, followed by 55 °C and finally 20 °C. A large number of studies have shown that the effect of acid fermentation is improved at medium temperature.[25] Therefore fermentation temperature is considered to be an important factor. Low temperature results in low activity of the hydrolyzed acidified bacteria, thus affecting the production of VFAs. Second, the difference in substrates resulted in the variation of VFA production, given that food waste contains high sugar levels, whereas sludge mainly contains sludge. Moreover, the addition of iron leads to the reductive decomposition of the cell membrane after direct contact with NZVI, thereby inhibiting methane production during anaerobic digestion.[26] This inhibition is accompanied by the dissolution of NZVI and hydrogen generation, resulting in VFA accumulation.

The generation of VFAs may be caused by related microbial communities. The differences in the composition and properties of the substrates lead to differences in the microbial communities in the system. Therefore it is necessary to analyze the composition changes of various SCFAs after the addition of NZVI. Figure 2 shows the distribution of acid components in different groups at 48 h of fermentation. Acetic acid was the most important acid. In the absence of NZVI, acetic acid accounted for 52% of total VFAs. With the increase of NZVI addition, the proportion of acetic acid also increased. At 10 g L^{-1} dosage, acetic acid accounted for 72% of total VFAs. However, with the increase of iron, the yield of butyric acid gradually decreased.

By adding NZVI into the system, Fe^{2+}/Fe^{3+} was released to provide essential trace elements and electrons for H_2-producing acetogens, which caused the decomposition of butyric acid to produce acetic acid, thus leading to the accumulation of acetic acid. In order to elucidate the effects of NZVI on acetate production, acetate change is described in Fig. S1 in File S1.

Jia *et al.*[16] explored the effects of NZVI on biogas production from anaerobic digestion of sludge and found that the content of butyric acid was the highest, whereas the content of acetic acid decreased first (700 mg L^{-1}) in the whole fermentation system. This result probably occurred because no methane suppression measures were carried out in the system, and the acetic acid produced was further consumed by methanogens. In this study, acetic acid reached its maximum level at 48 h (16 000 mg COD L^{-1}) and then stabilized, indicating that the addition of NZVI can increase acetic acid production.

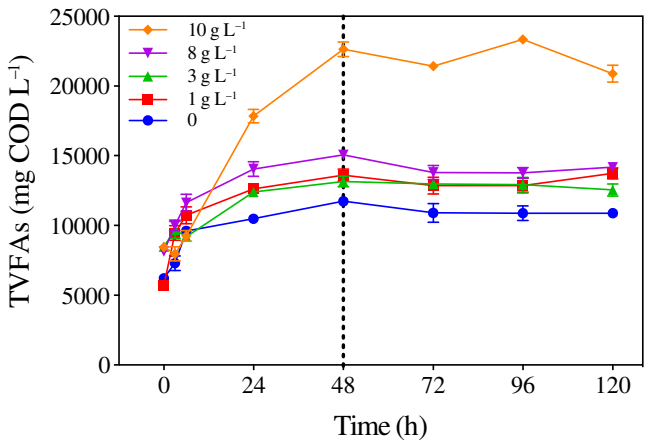

Figure 1. Effects of NZVI dosage on VFA production.

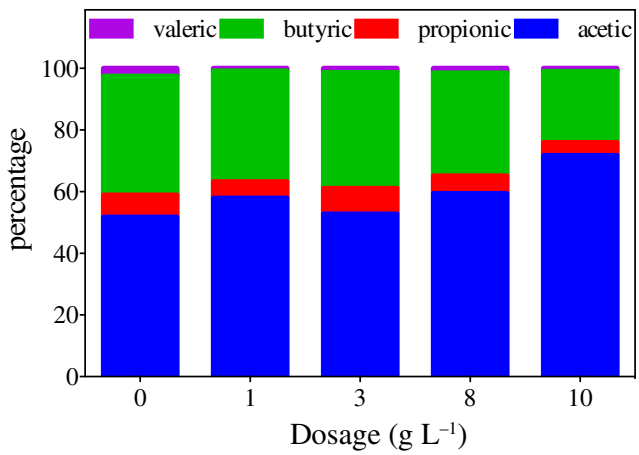

Figure 2. Effects of NZVI dosage on VFA composition.

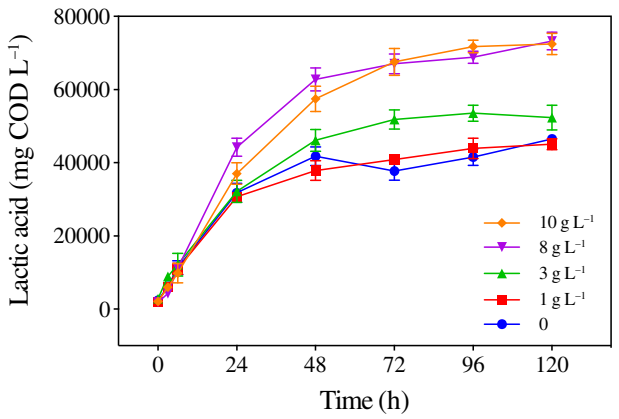

Figure 3. Effects of NZVI dosage on lactic acid production.

Effects of NZVI on lactic acid production

The composition of food waste is complicated and the accumulation of lactic acid also occurs during the preservation process because of the presence of lactic acid bacteria.[27] In the present study, open fermentation, i.e. non-sterile fermentation, was performed, so a certain number of lactic acid bacteria were present in the substrate. Thus lactic acid became the main by-product during the fermentation. Figure 3 shows the change in lactate production in the system with the addition of different gradient concentrations of NZVI.

The results showed that lactic acid concentration increased as the fermentation time was extended. At 0–24 h, the concentration of lactic acid significantly increased at different NZVI concentrations. During 24–48 h, the accumulation of lactic acid continued to increase, but the rate of production slowed down. After 48 h, the concentration of lactic acid increased slowly and then stabilized. The lactic acid yield was positively correlated with the dosage of NZVI. Lactic acid reached the maximum concentration (70 539.4 mg COD L^{-1}) with 10 g L^{-1} NZVI, which was 1.78 times higher than that of the control group (39 521.7 mg COD L^{-1}).

As a microbial nutrient element and a highly efficient catalyst, zerovalent iron promotes the hydrolysis of the fermentation substrate and is an activator of the enzyme.[28] In the anaerobic system, NADPH acts as a reducing agent to supply hydrogen, which helps to convert the final product (pyruvate) of the substrate to produce lactic acid, while the addition of a certain amount of NZVI could promote the conversion of pyruvate to lactic acid.

Relative activities of key enzymes related to VFA fermentation

Enzymes play an important role in the metabolic system of acidogenic fermentation, and their activity is directly related to VFA yield.[29] The metabolic pathway for VFA production is shown in Fig. S2 in File S1. FDXR is an important pathway in the metabolism of pyruvate and was therefore discussed in this thesis. The results are shown in Fig. 4(a).

FDXR activity in the control and 10 g L^{-1} groups increased rapidly during the first 48 h and reached the highest level at 48 h, followed by a slow decline. During the first 48 h, enzyme activity rapidly increased with 10 g L^{-1} NZVI. At 48 h, it was 1.22 times higher than that of the control group.

Luo *et al.*[20] also investigated some key enzyme activities in the fermentation process and found that the activity of FDXR in the NZVI reactor was 1.8 times higher than that of the control group,

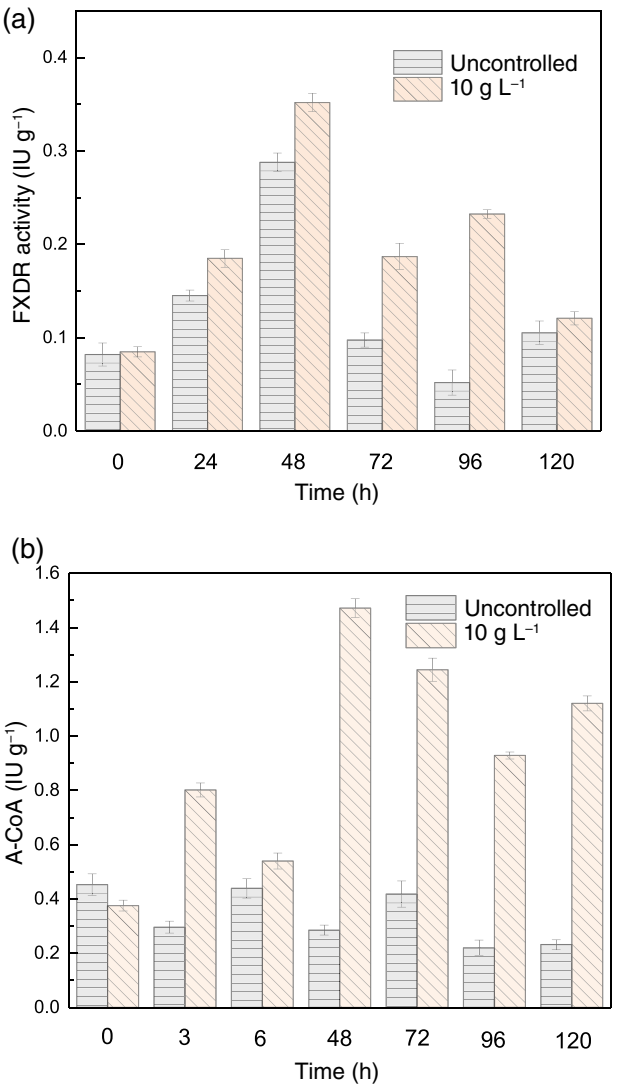

Figure 4. Effects of NZVI on (a) FDXR activity and (b) A-CoA activity.

which was similar to this study. Zerovalent iron is a reductant. In the interior of the anaerobic system, it can release electrons through microelectrolysis and neutralization of organic acids, turning itself into Fe^{2+} and stimulating the improvement of anaerobic microbial enzyme activity. At the same time, carbon macromolecular organic compounds and zerovalent iron constitute a microelectrolytic cell, which promotes the transformation of macromolecular organic compounds into medium and low molecules and facilitates the use of microorganisms.[30] Thus the addition of NZVI can strongly stimulate the FDXR activity in the system and is conducive to the full utilization of substrate nutrients.

Apart from FDXR, A-CoA is another key enzyme in anaerobic digestion. A-CoA is an important intermediate metabolite of energy substance metabolism. Acetone acid is oxidized and decarboxylated to form A-CoA, which is also a precursor of synthetic fatty acids. Therefore the activity of A-CoA was investigated in this study and the results are shown in Fig. 4(b).

The activity of A-CoA in the control group slightly fluctuated during the fermentation process, whereas the activity of A-CoA in the NZVI group reached the maximum at 48 h of fermentation, which was 2.92 times higher than that at the beginning and 4.17 times higher than that of the control group. This result was similar

to the trend of the FDXR, indicating that NZVI can not only improve the activity of FDXR but also that of A-CoA.

In some microorganisms, FDXR also catalyzes the same reaction of pyruvate dehydrogenase complex, catalyzing the conversion of pyruvate into A-CoA.[31] In this study, the activities of FDXR and A-CoA were significantly increased with 10 g L^{-1} NZVI compared with the control. Therefore A-CoA activity can be produced efficiently by the FDXR.

Table 2. Sample information for bacterial community structure analysis

Sample	Sample source	Sampling time (h)
X1	Beginning of fermentation	0
X2	Control (no NZVI added)	48
X3	10 g L^{-1} NZVI	48

Microbial community analysis

The characteristics of acid production in the reactor are closely related to the microbial community structure. Therefore it is necessary to further analyze the microbial community structure changes in the anaerobic reactor. Sample information for bacterial community structure analysis is shown in Table 2.

The phylum level analysis is shown in Fig. S3 in File S1. In X1, X2 and X3 samples, the most abundant bacteria were Firmicutes, accounting for 58.75, 96.44 and 80.9% respectively. Firmicutes are dominant hydrolyzed bacteria which are capable of degrading sugars, fats and proteins for acid production.[32] In X1 samples,

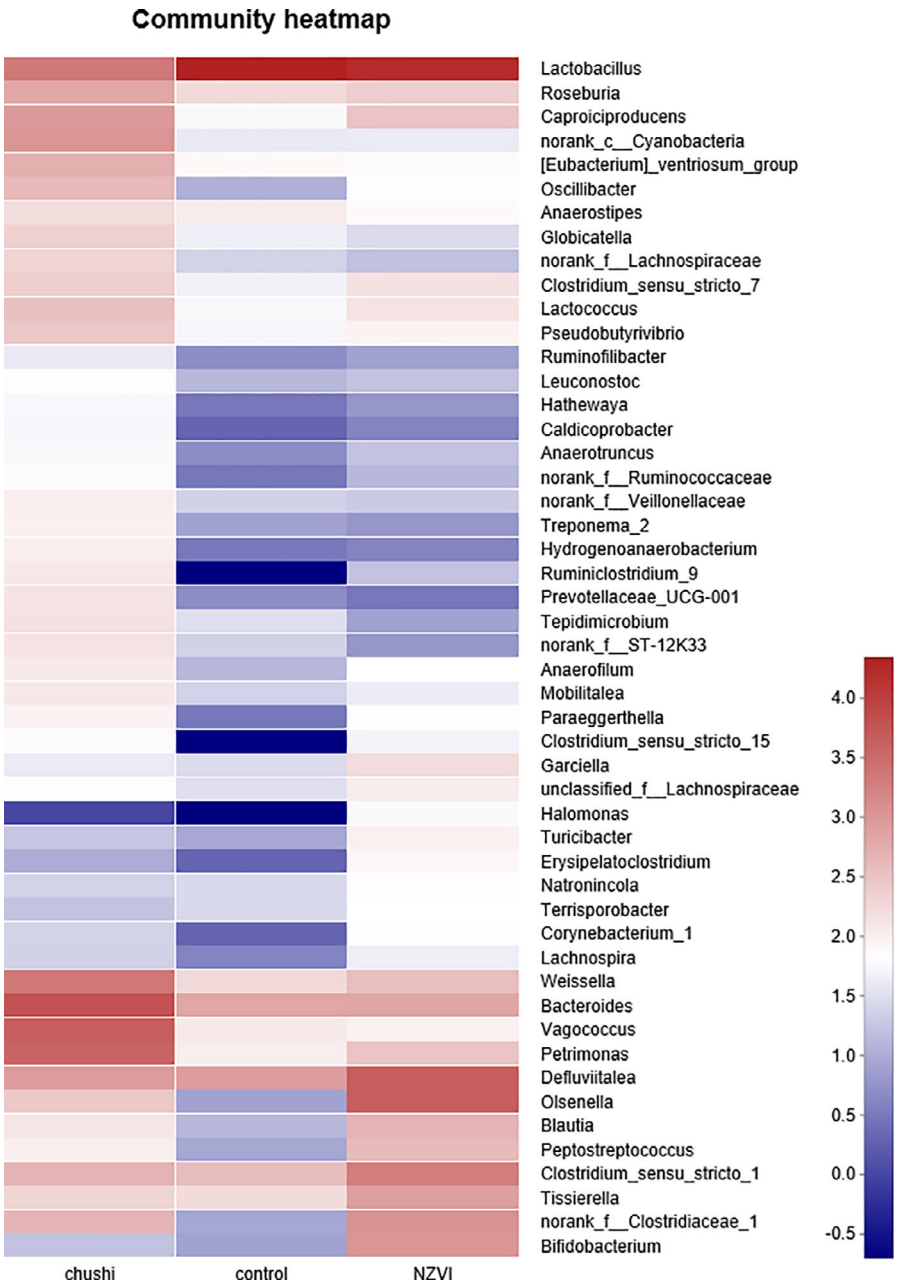

Figure 5. Community heatmap at genus level.

another dominant group was Bacteroidetes (35.63%). In X2 samples, the abundance of Firmicutes increased significantly, while Bacteroidetes decreased. In X3 samples, Bacteroidetes decreased and another dominant phylum, Actinobacteria (16.07%), which degrade and produce acid during anaerobic digestion with organic matter, appeared; there is a close relationship.[33]

Bacteroidetes was also a dominant bacterium especially in the process of treating cow dung with a continuously stirred tank reactor (CSTR) anaerobic digestion system, mixing methane with cow dung and straw, and anaerobic digestion of some organic wastes rich in sugars, such as food waste.[34] In this study, the abundance of Bacteroidetes in X2 and X3 samples was reduced, possibly because X2 and X3 were sampled at 48 h of fermentation, and the methanation inhibition treatment was carried out, resulting in a decrease in abundance.

After adding NZVI, a new phylum was introduced: Actinobacteria appeared in X3 which were not found in X1 and X2. As zerovalent iron oxidizes into Fe^{3+}, it can promote the hydrolysis of cellulose and the decomposition of glucose,[35] while the organic matter such as lignin and cellulose in the matrix is a good nutrient for microorganisms such as Actinobacteria.[36] The increase in the abundance of Actinobacteria also indicates that the decomposition of organic matter in the substrate by adding NZVI can better promote the acid production process.

In order to further demonstrate the functional evolution of the bacterial microbial community, the bacteria were analyzed at genus level, and the heatmap is shown in Fig. 5.

It can be seen from Fig. 5 that the diversity of bacteria was greatest in the initial samples of fermentation, with no obvious special dominant bacteria. However, with the difference of fermentation time and conditions, the diversity of bacterial structure became more and more obvious, with the most obvious difference being *Lactobacillus*. In the samples fermented for 0 h, *Lactobacillus* only accounted for 7.67%, but in the control group, *Lactobacillus* became the dominant bacterial group at 48 h, accounting for 48.06%, while the *Lactobacillus* abundance with 10 g L^{-1} NZVI was 65.74%, far higher than that in the control. *Lactobacillus* is resistant to low pH value and high ethanol concentration, which can promote the substrate production of lactic acid and acetic acid, inhibit the growth of yeast and compete with yeast for nutrition,[37] which is also an important reason for the rapid increase of lactic acid and acetic acid after adding NZVI. In the initial sample, the relative abundance of *Bacteroides* was 21.16%, while after 48 h of fermentation, the *Bacteroides* abundance in the control and NZVI groups rapidly decreased to 1.91 and 2.64% respectively.

The relative abundance of *Defluviitalea* increased from 3.07% (0 h) to 12.64% (48 h) with NZVI, while there was no significant change in the control group. In addition, the relative abundance of *Olsenella* increased from 0.96% (0 h) to 12.69% (48 h) in the system with the addition of NZVI, becoming another dominant bacterium. The genus *Olsenella* was first identified in 2001 as a microorganism in human dental caries and the rumen of cattle. It was characterized by drug resistance and can produce lactic acid/acetic acid anaerobically. Besides, *Olsenella* microbes can rapidly convert lactic acid into SCFAs,[38] which is also an important reason for the accumulation of SCFAs after the addition of NZVI.

CONCLUSIONS

The highest VFA level of 22 250 mg COD L^{-1} occurred at 10 g L^{-1} NZVI with a fermentation time of 48 h, 1.92-fold higher than that of the control, wherein the maximum proportion of acetic acid reached up to 72%. The addition of NZVI provided a reducing power in the system, which improved the yield of VFAs and lactic acid. The activity of key enzymes was stimulated by NZVI. At 10 g L^{-1} dosage, the FDXR and A-CoA activities were respectively 1.22 and 5.17 times higher than those of the control at 48 h. These key enzymes resulted in the accumulation of VFAs. The fermentative bacteria responsible for SCFA production were also highly enriched in fermentation reactors with NZVI, which was in accordance with the accumulation of SCFAs.

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Supporting Information

Supporting information may be found in the online version of this article.

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