

# Effect of Substrate Mixing Ratio and Zero-Valent Iron on Anaerobic Hydrolytic and Acidification of Kitchen Waste

Rufei Liu<sup>1, 2, a</sup>, Weijie Fan<sup>2, b</sup>, and Xiteng Chen<sup>2, c \*</sup>, and Song Yang<sup>1, d</sup>

<sup>1</sup> Cucde Environmental Technology Co., Ltd. Beijing, China;

<sup>2</sup> State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, China.

<sup>a</sup> lrf521125@126.com, <sup>b</sup> 2461089490@qq.com, <sup>c</sup> 1352848140@qq.com, <sup>d</sup> songyang@cucd.cn

**Abstract.** Based on the complex composition of kitchen waste, zero-valent iron addition and mixing substrate were proposed to enhance the hydrolysis and acidification of kitchen waste. The results showed that ethanol and acetic acid were mainly hydrolysis acidification products of kitchen waste, accounting for more than 90% of the total production. The experimental group with a mixing ratio of 50% had the highest hydrolysis and acidification efficiency during the acidification stage without ZVI addition. In general, the system with higher production of TVFA and ethanol was added higher dosage of ZVI. The production of ethanol and TVFA reached a higher level at the same time with 10 g/L ZVI. Compared with wet anaerobic fermentation, dry anaerobic fermentation obtained high TVFA production with low zero-valent iron dosage. ZVI addition avoided acidification and instability and promoted hydrolysis process. In addition, RDA analysis revealed that the addition of zero-valent iron was positively correlated with ethanol, acetic acid, and TVFA.

**Keywords:** Kitchen waste; ZVI; substrate mixing ratio; hydrolytic; acidification.

## 1. Introduction

Kitchen waste (KW) referred to kitchen garbage (KG) and food waste (FW), including fruits, vegetables, meat, baked goods, etc. KW has high organic matter content and high water content, which was prone to spoilage and deterioration [1]. In 2020, the amount of KW generated in China was approximately 121.03 to 145.24 million tons, accounting for about 51% to 61% of urban household waste. The components of KW are complex, containing certain amounts of cellulose, hemicellulose, and lignin. Due to their complex structure, they are difficult for microorganisms to rapidly utilize, resulting in a relatively slow hydrolysis and acidification process [2].

In recent years, many studies have proposed adding conductive materials to anaerobic fermentation reactors to improve the efficiency and stability of anaerobic fermentation, thereby enhancing electron transfer efficiency and acid production efficiency, accelerating the degradation of volatile fatty acids (VFAs) [3]. Zero-valent iron (ZVI) is an additive that can reduce the system redox potential and has strong reducing power. It can enhance microbial activity by promoting enzyme activity and can promote the conversion and utilization of certain recalcitrant chemical substances in acidic environments, thereby improving the biodegradability of organic matter [4]. Feng et al. [5] found that adding ZVI to an anaerobic sludge digestion system could promote acetate production, increase the degradation rate of proteins in sludge, and enhance methane yield. Cao et al. [6] discovered that adding ZVI stimulated the growth of related microorganisms and the activity of metabolic enzymes, thereby increasing the concentration of VFAs and thus enhancing the concentration of VFAs during the anaerobic fermentation of FW.

Therefore, this study used FW and KG as raw materials, adding ZVI in the hydrolysis and acidification phase with different substrate mixing ratios. It analyzed the enhancing effect of ZVI on substrate hydrolysis and acidification, and examined the impact of ZVI on the anaerobic fermentation performance of mixed waste.

## 2. Materials and Methods

### 2.1 Substrates

KG used in this research was collected from the school canteen with main components including vegetable leaves, fruit peels, eggshells, etc. FW also was collected from the canteen, mainly consisting of leftover rice and dishes. Packaging plastic bags, gravel, bones, and tissues were picked out. FW and KG were mashed and stored at 4°C for later use. The basic properties of the materials were shown in Table 1.

Table 1. Basic Properties of Materials

| Basic Properties   | Kitchen garbage | Food waste |
|--------------------|-----------------|------------|
| TS (%)             | 13.16±0.62      | 27.70±1.12 |
| VS (%)             | 11.41±0.53      | 25.90±0.78 |
| pH                 | 5.33±0.01       | 3.71±0.01  |
| Crude fata (%)     | 2.78±1.02       | 27.52±1.24 |
| Crude proteina (%) | 15.60±0.88      | 17.18±0.97 |
| Total sugara (%)   | 7.93±0.51       | 11.85±0.43 |
| Lignina (%)        | 8.08±0.97       | 5.99±0.63  |
| Cellulosea (%)     | 22.12±0.15      | 9.50±0.41  |
| Hemicellulosea (%) | 16.38±0.34      | 22.94±0.53 |

a based on dry weight.

### 2.2 Experimental Setup

To study the effect of ZVI on anaerobic hydrolytic acidification, ZVI addition amount and raw material ratio were set as experimental factors, while ethanol and VFAs were response values. ZVI (Macklin Company, purity 98%, 0.15 mm) were used in this research. The anaerobic fermentation acidification phase was natural fermentation without inoculation. ZVI addition was set at 5 levels (0, 5, 10, 15, and 20 g/L). The substrate mixing ratio was designed on the mass fraction of FW in all fermentaiton substrates (0%, 25%, 50%, 75%, and 100%). TS level was set at 10% and 20%.

The hydrolysis and acidification phase was operated at 1 L batch anaerobic reactor with 700 mL effective working volume. KW and FW were mixed and thoroughly stirred evenly. Anaerobic reactors were placed in the 35°C water bath and continuously operated for 5 days. At the initial moment of the experiment and the end of fermentation, the total solid content (TS), volatile solid content (VS), and crude fat content of the effluent were measured. The pH, ethanol, and VFAs were measured every day. Anaerobic fermentation bottles were shaken well before taking the sample. All tests were set up with three parallel groups, and the results were the average of the three.

### 2.3 Analytical Methods

The pH was measured using a pH meter (Mettler Toledo, FiveEasy Plus FE28). The determination of TS and VS were in accordance with international standard methods [6]. The total sugar, crude fat, and, crude protein content was determined by standard method. The cellulose, hemicellulose, and lignin contents were determined by the fully automatic fiber analyzer (Hanon, ANKOM A2000). The ethanol and VFAs were detected using a gas chromatograph (Shimadzu, GC-2014) with a hydrogen flame ionization detector, and an HP-FFAP capillary column.

### 2.4 Statistical Analysis

Full factorial experiment design and data analysis were performed using Minitab 20 software. ZVI addition, substrate mixing ratio, and TS level were experimental factors, while ethanol and total volatile fatty acid (TVFA) yield were set as response values. Correlation analysis was conducted using Canoco 5.0.

### 3. Results and Discussions

#### 3.1 Raw Material Proportion

During the anaerobic hydrolysis and acidification process, complex organic matter is first converted into intermediate small molecular products and then metabolized into acetic acid, propionic acid, butyric acid, valeric acid, other short-chain organic acids, and ethanol. As shown in Fig 1., the concentration of ethanol and acetic acid was the highest among the hydrolysis acidification products of KG, accounting for more than 90% of the total production. At 10% TS level, the concentration of TVFA and ethanol with the 50% mixture ratio (50% FW and 50% KG) was the highest, and the result was the same in the 20% TS system. The crude protein, crude fat, and total sugar in FW were higher than that in KG, therefore, a higher yield could be obtained by mixing two materials.

However, the production of TVFA and ethanol was not positively correlated with the mixture proportion of FW. When there was only FW, the production of TVFA and ethanol was not higher than that of the mixture system. Taking the fermentation method by mixing KG and FW would effectively improve the hydrolysis and acidification efficiency of KG. By comparing the results of wet anaerobic fermentation and dry anaerobic fermentation, the mixture ratio of 50% was more appropriate for the hydrolysis and acidification of KW.

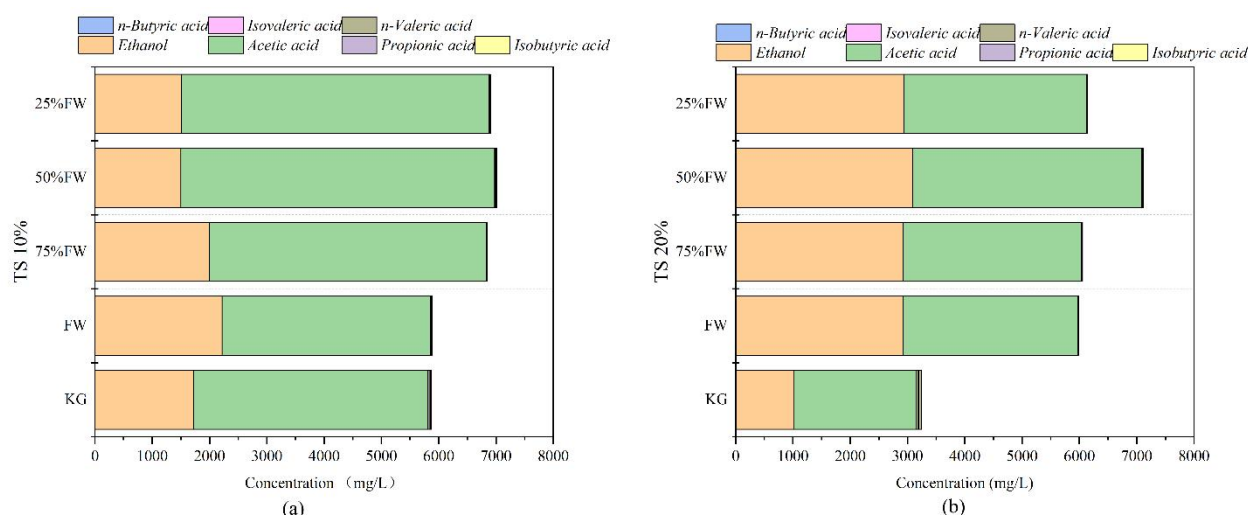


Fig. 1 Ethanol and TVFA concentrations at different substrate mixing ratios (a, TS 10%; b, TS 20%)

#### 3.2 Effects of ZVI on Hydrolysis and Acidification

As shown in Fig. 2, the equivalent regions of ethanol and TVFA concentrations under different ZVI addition and substrate mixing ratios showed different colors respectively. In general, the system with higher production of TVFA and ethanol was added a higher dosage of ZVI in the 10% TS fermentation system. When the dosage was 15 g/L, the TVFA and ethanol concentration of the system reached the maximum value. However, the mixing ratio to achieve the highest concentration of ethanol and TVFA was inconsistent. The mixing ratio was higher than 50% with high ethanol production, while lower than 50% with high TVFA production.

At the dry anaerobic fermentation system, the TVFA and ethanol concentration of the system could reach a high value at 5 g/L ZVI. Although the mixing ratios were not the same when reaching the concentration peak of ethanol and TVFA, the production of ethanol and TVFA reached a higher level at the same time at 10 g/L. Compared with wet anaerobic fermentation, dry anaerobic fermentation obtained high TVFA production with low ZVI dosage.

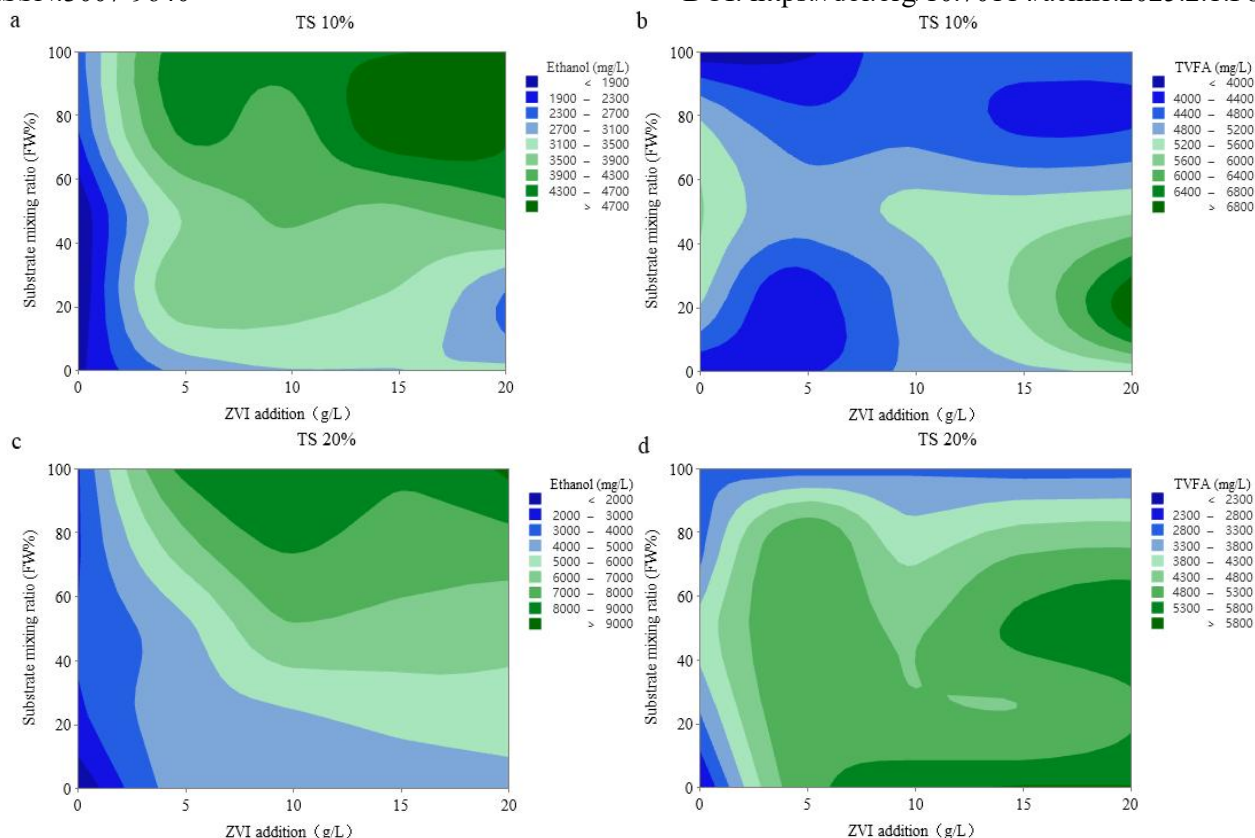


Fig. 2 Contour map of ethanol and TVFA concentration at different ZVI addition and substrate mixing ratios (a,b TS 10%; c,d TS 20%).

### 3.3 Effects of System Solid Content on Hydrolysis and Acidification

In the fermentation system with 10 g/L ZVI and substrate mixing ratio of 50%, the production of TVFA and ethanol in dry anaerobic fermentation was higher than that in wet anaerobic fermentation. On the fifth day of anaerobic fermentation, the total production of TVFA and ethanol in dry anaerobic fermentation was up to 11500 mg/L, while only 9354 mg/L in the wet anaerobic fermentation system. It was worth noting that the total TVFA and ethanol production of the wet anaerobic fermentation system was relatively higher than that of dry fermentation system without adding ZVI. After adding ZVI, the yield of acidizing products in dry fermentation system was increased. Dry anaerobic fermentation system was more prone to acidification and instability, while ZVI improved this situation and promoted the hydrolysis process [4].

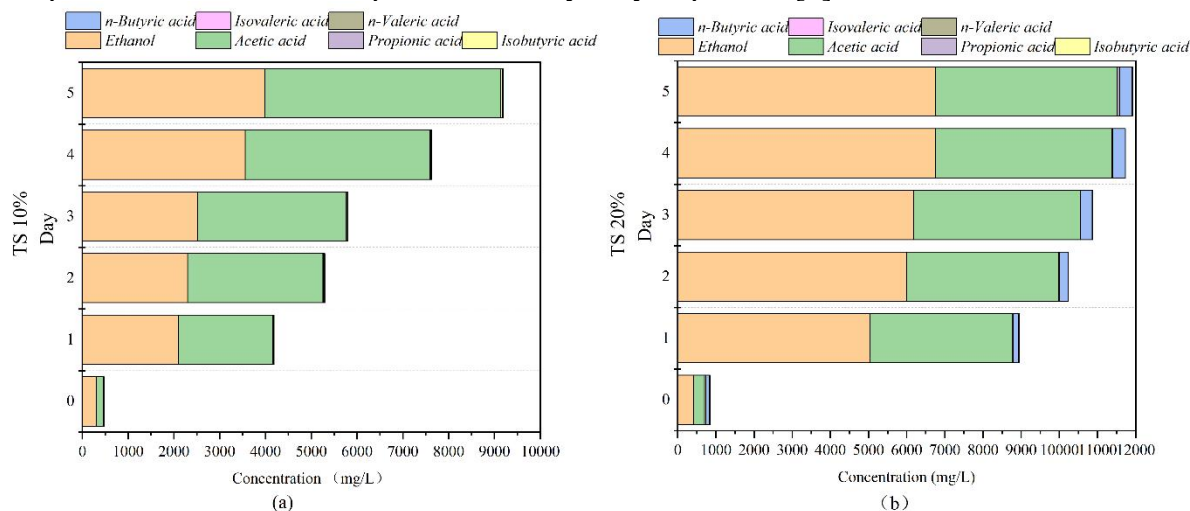


Fig. 3 Ethanol and TVFA concentration at different system solid contents (a, TS 10%; b TS 20%)

### 3.4 Relevance Analysis

Redundancy Analysis (RDA) was performed on the experimental parameters (highlighted in red) and response value parameters (highlighted in blue), and the results are shown in Fig. 4. RDA1 and RDA2 were the correlation coefficients of the first and second ordination axes, respectively. The scale of the axis represented the positive or negative correlation. The cosine value of the angle between two vectors indicated the correlation coefficient. And length of a vector projected onto another vector was proportional to the importance of the factor. RDA Axis 1 and Axis 2 jointly explained 81.74% of all variables. From Fig. 4, it can be seen that the vectors of acetic acid and TVFA were close. In the experimental group, acetic acid accounts for more than 95% of the TVFA mass fraction.

The addition of ZVI was positively correlated with ethanol, acetic acid, and TVFA. It proved that ZVI could promote ethanol and acetic acid fermentation of KW. However, the positive correlation of substrate mixing ratios (FW percentage) was lower than that of ZVI. It was noteworthy that the substrate mixing ratios were negatively correlated with propionic acid, n-Butyric acid, isovaleric acid, and n-Valeric acid. In addition, TS was positively correlated with propionic acid. Previous studies had shown that a dry anaerobic fermentation system with high solid content was prone to acidification and instability due to excessive accumulation of propionic acid.

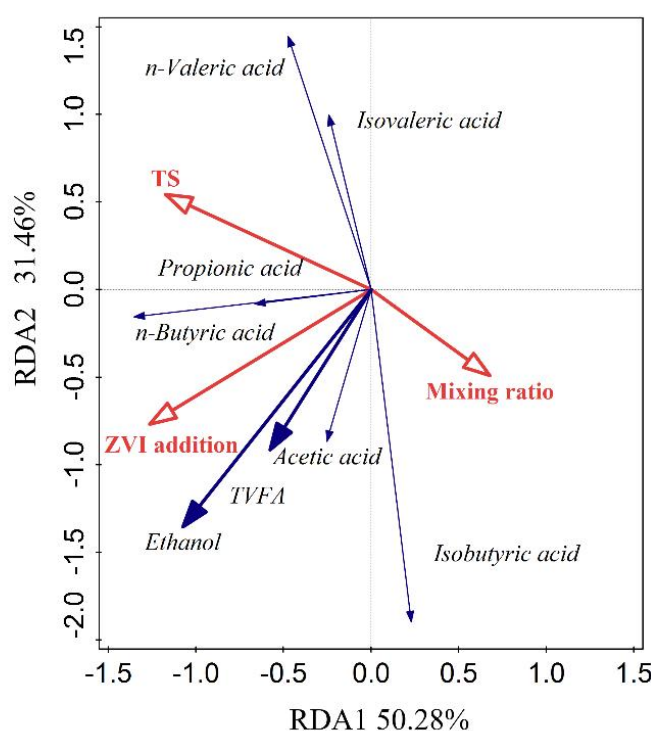


Fig. 4 RDA correlation analysis

## 4. Conclusion

ZVI addition and substrate mixing were proposed to enhance the anaerobic fermentation hydrolysis and acidification of KW. The results showed that the concentration of ethanol and acetic acid was the highest among the hydrolysis acidification products of KW, accounting for more than 90% of the total production. In addition, the concentration of TVFA and ethanol with the 50% mixture ratio (50% FW and 50% KG) was the highest in both 10% and 20% TS systems. The addition of ZVI promoted the increase of TVFA and ethanol yield. TVFA yield with low ZVI addition in dry anaerobic fermentation was higher than that in wet anaerobic fermentation. ZVI addition avoided acidification, and promoted the hydrolysis process. ZVI addition was positively correlated with ethanol, acetic acid, and TVFA from RDA analysis.

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