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Synthetic Effect of EDTA and Ni^{2+} on Methane Production and Microbial Communities in Anaerobic Digestion Process of Kitchen Wastes

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Abstract: Batch tests were carried out to study the effect of simultaneous addition of ethylenediaminetetraacetic acid and Ni^{2+} (EDTA-Ni) on anaerobic digestion (AD) performances of kitchen wastes (KWs). The results indicated that the cumulative biogas yield and methane content were enhanced to 563.82 mL/gVS and 63.7% by adding EDTA-Ni, respectively, which were almost 1.15 and 1.07-fold of that in the R2 with Ni^{2+} addition alone. At the same time, an obvious decrease of propionic acid was observed after EDTA-Ni addition. The speciation analysis of Ni showed that the percentages of water-soluble and exchangeable Ni were increased to 38.8% and 36.3% due to EDTA-Ni addition, respectively. Also, the high-throughput sequencing analysis revealed that the EDTA-Ni promoted the growth and metabolism of *Methanosarcina* and *Methanobacterium*, which might be the major reason for propionic acid degradation and methane production.

Keywords: EDTA; kitchen waste; methane; microbial community; Ni^{2+}

1. Introduction

Anaerobic digestion (AD) has been one of the most promising biological technologies for efficient treatment of various organic wastes such as kitchen wastes (KWs) [1]. The AD contains three steps: Hydrolysis, acidification and methanogenesis, and a large number of microorganisms were involved in the AD process. Among different microbial groups, methanogens are the most sensitive to trace metals (TMEs) deficiency because of their slow growth rates and stricter growth requirements [2].

It is well known that TMEs such as iron (Fe), cobalt (Co), and nickel (Ni) are essential components of cofactors and enzymes, and their roles in AD have been studied extensively [3,4]. Taking Ni as an example, it is an important element in carbon monoxide dehydrogenase and acetyl-coenzyme A synthase, participating in the acetogenic pathway [5,6]. Ni is also the central atom of cofactor F430, which provides an active site for methyl coenzyme M reductase [7,8]. Besides, the addition of Ni stimulated the growth of *Methanosarcina*, and promoted the degradation of acetate and propionate [9]. Therefore, the addition of TMEs was applied to create more favorable conditions for microorganisms and enhance AD performances [2,10].

Nevertheless, the added TMEs cannot be fully utilized by anaerobic microorganisms, because there are complex interactions, including precipitation, complexation, and adsorption, between TMEs, anions and biomass [11]. The requirement and dosing strategy for trace metals also depends on the portion of the supplemented trace metal that is bioavailable to the microorganisms for uptake [12]. To improve the bioavailable fraction of TMEs, chelating agents such as ethylenediaminetetraacetic acid (EDTA), ethylenediamine-N, N'-disuccinic acid (EDDS) and nitrilotriacetic acid (NTA) were added into AD systems [13–15]. EDTA is a reusable chelating agent that has been successfully used in enhancing

the bioavailability of TMEs by forming strong soluble complexes [16,17]. Although, the use of EDTA reduced the dosage of Ni^{2+}, maintained operational stability, and enhanced methane production in the AD process [14,17], methanogens involved in AD are critical for methane production. The reported researches were mainly focused on methane production, however, EDTA improves the bioavailability of Ni through microorganisms involved in methanogenesis. Thus, the synthetic effects of EDTA and Ni on methanogen still need to be further studied.

In this study, therefore, batch tests were conducted to investigate the synthetic effects of EDTA-Ni addition on methanogen involved in KWs AD. The biogas yield, methane content, and volatile fatty acids (VFAs) degradation were studied during the AD processes. Moreover, Ni speciation and methanogenic community structure were analyzed to understand the mechanism of methane production.

2. Materials and Methods

2.1. Experimental Materials

The inoculated sludge used in this study was taken from an anaerobic reactor in our laboratory. The sludge was settled at 4 °C for 12 h. The KWs were obtained from a student cafeteria of Nanjing Forestry University in China. The KWs were treated by a pulverizer (S2-A81, Joyoung, Jinan, China) and then filtered using a 2 mm sieve. The characteristics of the KWs and inoculated sludge are shown in Table 1.

Table 1. Characteristics of KWs and inoculated sludge.

Parameters	Unit	KWs	Inoculated Sludge
pH	-	6.7 ± 0.1	7.4 ± 0.1
Total solid (TS)	g/L	199.05 ± 0.2	11.5 ± 0.1
Volatile solid (VS)	g/L	168.98 ± 1.1	5.2 ± 0.3
Soluble chemical oxygen demand (SCOD)	g/L	19.67 ± 0.15	1.31 ± 0.02
Ash	% dw	15.1 ± 0.1	-
Water content	% ww	81.36 ± 0.42	98.67 ± 0.56
C	% dw	45.3 ± 0.1	-
H	% dw	6.4 ± 0.1	-
N	% dw	3.2 ± 0.1	-
Ni (total)	mg/kgTS	0.82 ± 0.1	28.54 ± 0.5
Ni (soluble)	mg/L	-	0.46 ± 0.03

Note: ww: wet weight, dw: dry weight.

2.2. Experimental Design

Batch tests were performed in four identical serum bottles (marked as R1–R4, NewCare Medical Technology Co., Ltd., Suzhou, China) with a working volume of 200 mL. Both KWs (12.91 g/L) and inoculated sludge were added into each bottle, and their mass ratio (on basis of total solid (TS)) was determined to be 1:2. The R1 with no Ni^{2+} or EDTA (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) addition was set as a control test. The R2 was added with 3 mg Ni^{2+} per unit mass of volatile solid (g VS). The R3 was added with 15.0 mg/gVS of EDTA alone. In the R4, the EDTA and Ni^{2+} (EDTA-Ni) was added together. The pH values of R1-R4 were adjusted to be 7.5 using 1 M sodium hydroxide (NaOH) or 1 M hydrochloric acid (HCl). Then, all bottles were flushed for 3 min with nitrogen gas (N_2) to remove air and then sealed with rubber stoppers. Finally, all bottles were incubated in an air bath thermostat (SHZ-82, Changzhou Guohua Electric Appliance Co., Ltd., Changzhou, China), and the temperature and oscillation rate were controlled at 35 ± 1 °C and 80 rpm, respectively.

All experiments were done in triplicate, and the average and standard deviation were reported.

2.3. Chemicals

The Ni^{2+} is derived from $NiCl_2{\cdot}6H_2O$ (Nanjing Chemical Reagent Co., Ltd., Nanjing, China). The NaOH (Nanjing Chemical Reagent Co., Ltd., Nanjing, China), HCl (Nanjing Chemical Reagent Co., Ltd., Nanjing, China), HNO_3 (Laiyang Economic and Technological Development Zone Fine Chemical Plant, Laiyang, China), CH_3COOH (Nanjing Chemical Reagent Co., Ltd., Nanjing, China), CH_3COONH_4 (Nanjing Chemical Reagent Co., Ltd., Nanjing, China), and H_2O_2 (Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China) used in this study were analytical pure. The purity of nitrogen gas (N_2) was 99.999%.

2.4. Analytical Methods

2.4.1. Physical and Chemical Analysis

The total solid (TS), volatile solid (VS), soluble chemical oxygen demand (SCOD), and water content were analyzed according to the Standard Methods [18]. The pH was measured by a pH meter (PHS-3C, Leici, Shanghai, China). The C, H, and N were quantified by an elemental analyzer (Vario EL cube, Elementar, Hanau, Germany). The volume of biogas was collected by a liquid displacement device described in previous literature [19], and the bottle was filled with saturated $NaHCO_3$ solution. The composition of biogas was determined using gas chromatography (GC-2014, Shimadzu, Kyoto, Japan), and the operational parameters and procedures were described in the previous study [16].

The collected samples were centrifuged at 10,000 rpm for 10 min (TDZ4-WS, Xiangyi, Changsha, China), and then filtered through 0.45 μm cellulose acetate membranes. The filtrate was immediately analyzed for VFAs. The VFAs concentration including acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acids was measured by gas chromatography (Agilent 6890 N, Palo Alto, CA, USA) [3,20].

The speciation of Ni was assessed with the modified BCR sequential extraction method, and the detailed extraction steps were described in the references [15,21]. The Ni concentration from each extraction step was analyzed by an inductively coupled plasma emission spectrometer (iCAP 7200, Thermo Scientific, Waltham, MA, USA).

2.4.2. Microbial Analysis

The high-throughput sequencing technique was used for microbial analysis. The sludge samples collected on the 25th day were centrifuged at 10,000 rpm for 10 min. The pellets were pretreated twice with phosphate buffer refer to previous description [22]. The treated samples were stored at −80 °C before testing. Total genomic DNA was extracted by E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Winooski, VT, USA) according to the instruction manual. The extracted DNA was subjected to electrophoresis on a 1% agarose gel, and then was quantified using Qubit 2.0 kit (Life Technology, Carlsbad, CA, USA). The V3-V4 region of the 16S rRNA gene was amplified using primers: 349F (5′-CCCTACACGACGCTCTTCCGATCTN-3′) and 806R (5′-GACTGGAGTTCCTTGGCACCCGAGAATTCCAGGACTACVSGGGTATCTAAT-3′), respectively. The operating conditions and steps of PCR amplification followed the previous research [23]. Finally, the genomic samples were sequenced by the Miseq sequencing platform (Sangon Biotech (Shanghai) Co. Ltd., Shanghai, China). The sequencing results were analyzed with the MOTHUR program (http://www.mothur.org/).

2.5. Kinetics Model

The modified Gompertz equation was used to describe the kinetics of cumulative biogas production from KWs with different additives.

$$P = P_{\max} \exp\left\{-\exp\left[\frac{R_{\max} \times e}{P_{\max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

P is the product formed at fermentation time t; P_{max} is the potential maximum product formed; R_{max} is the maximum rate of product formed; λ is the lag time to exponential product formed; e is a constant (2.718).

In this experiment, the biogas yield was defined as the volume of biogas (mL) produced by unit mass of VS removal (gVS).

3. Results and Discussion

3.1. Effects of EDTA-Ni on Biogas Production and Composition

The changes of biogas yield and composition with different additives and AD time are shown in Figure 1. After an AD time of 25 days, the cumulative biogas yields in the R2 and R3 were 489.2 and 436.4 mL/gVS, respectively, which were almost 1.61 and 1.43 times of that in the R1 (304.4 mL/gVS). Simultaneously, the maximum value of cumulative biogas yield (563.8 mL/gVS) was observed in the R4, and it was increased by 15.2% compared with R2. Since the same amount of Ni^{2+} was added into the R2 and R4, respectively, the increase of biogas yield was mainly attributed to EDTA addition. As shown in Figure 1b, the peaks of daily biogas yield in the R1–R4 followed the order: R4 (136.05 mL/gVS) > R2 (103.19 mL/gVS) > R3 (98.37 mL/gVS) > R1 (46.47 mL/gVS). Furthermore, the R2 had the shortest time (6 days) to reach the peak of daily biogas yield, followed by the R3 and R4 (7 days), and the R1 (8 days). This result suggested that the addition of Ni^{2+} accelerated the biogas production, and the addition of EDTA also had positive effects on the anaerobic performance of KWs.

In Figure 1c, the biogas compositions were also changed by different additives. The contents of methane, hydrogen (H_2) and carbon dioxide (CO_2) in the R1 were 48.7%, 32.2%, and 19.1%, respectively. The addition of Ni^{2+}, EDTA, and EDTA-Ni improved the methane content by 21.7%, 16.8%, and 30.8% compared with the R1, respectively. The corresponding change trends of CO_2 and H_2 contents were opposite to that of methane. The change of CH_4 and CO_2 proportion might be due to the hydrogen-type methanogenesis pathway was enhanced by Ni addition.

The modified Gompertz equation was used to fit the biogas production of R1–R4, and the important parameters for the fitted equation were summarized in the Table 2. The correlation coefficients of R1–R4 were more than 98.96%, indicating that the modified Gompertz equation was able to describe the biogas formation from KWs. The potential maximum biogas production P_{max} and the maximum biogas production rate R_{max} in the R4 were higher than that in other reactors. The lag time λ in the R2 was shortest, followed by R4, R3 and R1. These results suggested that the AD process of KWs was enhanced by EDTA-Ni addition.

Table 2. Calculated results using modified Gompertz equation for biogas production.

Reactors	P_{max} (mL)	R_{max} (mL/d)	λ (d)	R^2
R1	312.95	45.16	4.20	0.9959
R2	489.18	79.03	2.94	0.9957
R3	436.4	79.30	3.85	0.9972
R4	563.8	88.15	3.38	0.9896

In summary, the separate addition of Ni^{2+} or EDTA enhanced the biogas yield and methane content; simultaneously, the addition of EDTA-Ni had a good synergistic effect on methane production from KWs. The similar results were founded by Vintiloiu [17] and Hu [14], who confirmed the chelating agents EDTA and NTA increased the bioavailability of Fe and Ni, respectively. In addition, the increase of the bioavailable fraction of Ni enhanced the methanogenic activity and the F430 concentration, leading to higher biogas yield and shorter lag phase [15,24]. Therefore, in this study, the improvement of the biogas yield and methane content with EDTA-Ni addition might be explained from two aspects. One reason was the fact that the addition of Ni^{2+} made up for the deficiency of Ni in KWs itself. The other reason was that the addition of EDTA further strengthened the bioavailability of Ni.

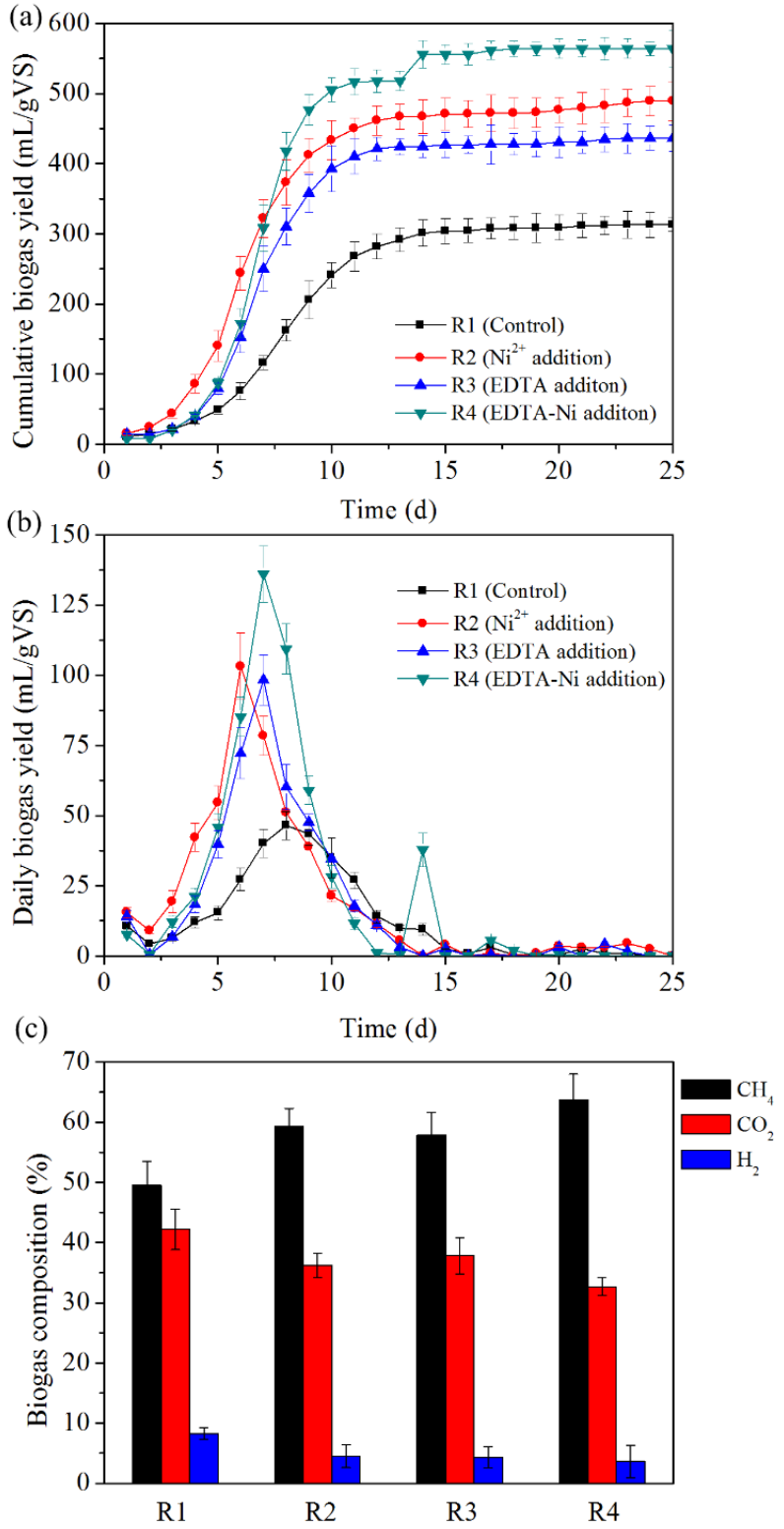

Figure 1. Effect of ethylenediaminetetraacetic acid (EDTA) and/or Ni on (**a**) cumulative biogas production and (**b**) daily biogas yield, and (**c**) biogas composition in the AD of KWs.

3.2. *Effects of EDTA-Ni on VFAs Concentration and Composition*

The VFAs are the intermediate products of AD, which are produced in the acidification process, and consumed by methanogens [25]. The changes of VFAs concentrations with different additives and

AD time are presented in Figure 2. It can be seen that acetic acid, propionic acid, and butyric acid were main VFAs components in all reactors, and the concentrations of total VFAs increased in the first few days, and then gradually decreased. For instance, in the R4, the total VFAs increased to 3298.7 mg/L on the 3rd day, and then decreased and maintained at around 650 mg/L on the 25th day. At the end of the reaction, the total VFAs concentrations in the R1, R2, and R3 were 1286.2, 834.7 and 1038.5 mg/L, respectively. Thus, it seemed that the addition of EDTA-Ni was more conducive to the conversion of VFAs to biogas than the addition of Ni or EDTA alone.

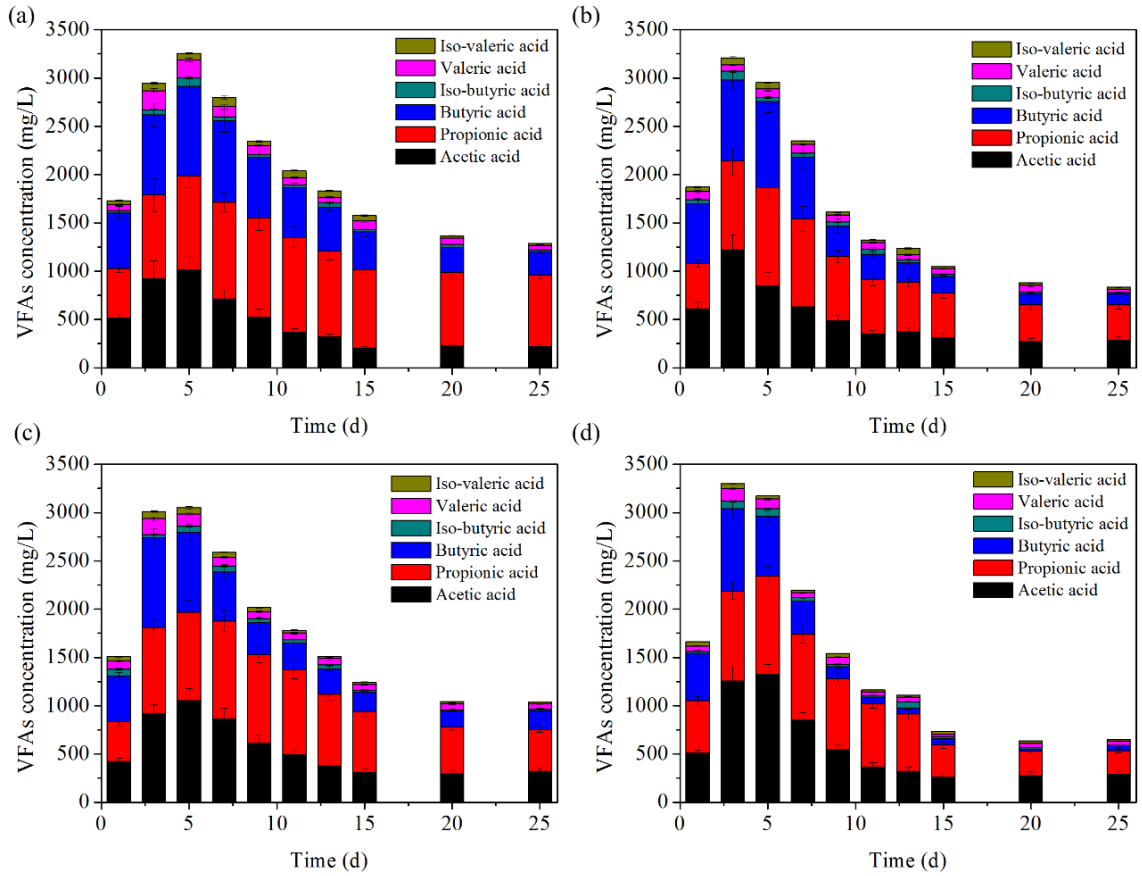

Figure 2. Changes of volatile fatty acids (VFAs) components in (**a**) R1 (Control test), (**b**) R2 (with Ni^{2+} addition), (**c**) R3 (with EDTA addition), (**d**) R4 (with EDTA-Ni addition).

Also, the different additives had an impact on VFAs components. The acetic acid concentration in the R4 reached the maximum value of 1324.9 mg/L on the 5th day and then decreased to 267.4 mg/L, corresponding concentrations of butyric acid also decreased from 854.3 to 78.9 mg/L. The propionic acid also reduced to 237.6 mg/L along with the decrease of acetic and butyric acids. The changes of acetic and butyric acids in the R1–R3 were similar to that in the R4. However, the degradation rate of propionic acid followed the order: R1 < R3 < R2 < R4, indicating that the addition of EDTA-Ni accelerated the degradation of propionic acid. In the previous investigation, the addition of Ni promoted the growth of methanogens in the AD of solid wastes [24]. The simultaneous addition of activated carbon and Ni^{2+} (AC-Ni) showed a significant increase in methane yield and reduction of propionic acid [26]. In this study, the faster degradation of VFAs, especially propionic acid, after EDTA-Ni addition, might be correlated to the enhanced effects of EDTA on Ni, which promoted the enrichment of methanogens.

3.3. Changes of Ni Speciation

It is well known that the bioavailability of TMEs is closely related to their morphology [27,28]. Therefore, it is very important to study Ni speciation under different additives conditions for

understanding the enhancement of methane production. As seen in Figure 3, different additives showed great effects on the chemical speciation of Ni. In the R1 (control test), the Fe/Mn oxides and organic fraction were the predominant forms, accounting for 68.7%. While, the water-soluble fraction of Ni, directly utilized by anaerobic microorganisms [1,28], had the lowest percentage (5.2%) in the R1. The water-soluble and exchangeable fractions of Ni increased greatly from R1 to R4, corresponding Fe/Mn oxides and organic fraction decreased significantly. For example, the water-soluble fraction of Ni in the R4 with EDTA-Ni addition (38.8%) was 7.46, 3.35, and 1.82 times of that in the R1-R3, respectively. Also, the organic fraction of Ni in the R3 with EDTA addition was 18.7%, while it was 12.4% in the R2 with Ni^{2+} addition. After EDTA-Ni addition, the corresponding value of organic fraction Ni reduced to 9.8%. It is inferred from the data the addition of EDTA-Ni provided more bioavailable Ni for methanogens, thereby improving methane yield to 359.2 mL/gVS (shown in Figure 1). In the previous studies, Zhang [15] and Hu [14] confirmed that chelating agents EDDS and NTA had positive impacts on Ni bioavailability by increasing the dissolution of Ni, and further promoted methane production from food wastes and synthetic wastewater. Additionally, the water-soluble of Ni in the R3 was higher than that in the R2, while the change of methane yield was opposite to Ni speciation. This was because the absolute amount of Ni in the R3 was lower than that in the R2, even though the addition of EDTA enhanced the utilization of Ni in the inoculated sludge.

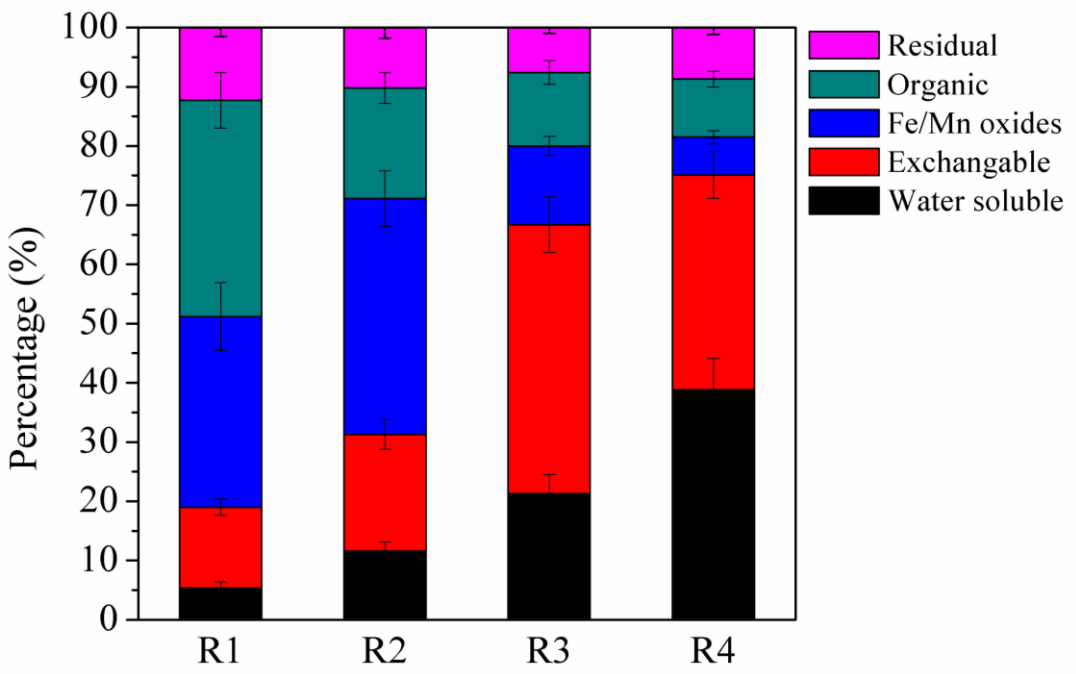

Figure 3. Distribution of different Ni fractions in the R1–R4 on the 20th day.

3.4. Microbial Community Structure and Diversity

In AD systems, methane is mainly produced by aceticlastic and hydrogenotrophic methanogens [2]. Therefore, it is very necessary to study the structure and diversity of methanogenic community. As shown in Figure 4, the different additives exhibited a significant change in the methanogenic community structure. In this study, *Methanosaeta* and *Methanosarcina* were dominant methanogens utilizing acetic acid for methane production [29–31]. The relative abundances of *Methanosaeta* were 34.45%, 22.01%, 25.62%, and 12.74% in the R1–R4, and the corresponding relative abundances of *Methanosarcina* were 10.86%, 29.60%, 23.34%, and 37.93%, respectively. It can be seen that the most predominant methanogen was changed from *Methanosaeta* to *Methanosarcina* due to the addition of EDTA-Ni. This phenomenon might be because the EDTA-Ni not only compensated for the deficiency of Ni in KWs itself, but also increased the fraction of water-soluble Ni, thereby meeting the growth and

metabolism needs of *Methanosarcina*. Zhang [9] demonstrated that the shortage of TMEs limited the growth of *Methanosarcina* even at high VFAs concentration. The change of aceticlastic methanogens in this study was in agreement with previous investigations [2,9]. They discovered that the addition of Ni increased methane yield and maintained operational stability of systems by promoting the enrichment of *Methanosarcina* in the AD of food wastes and maize, respectively.

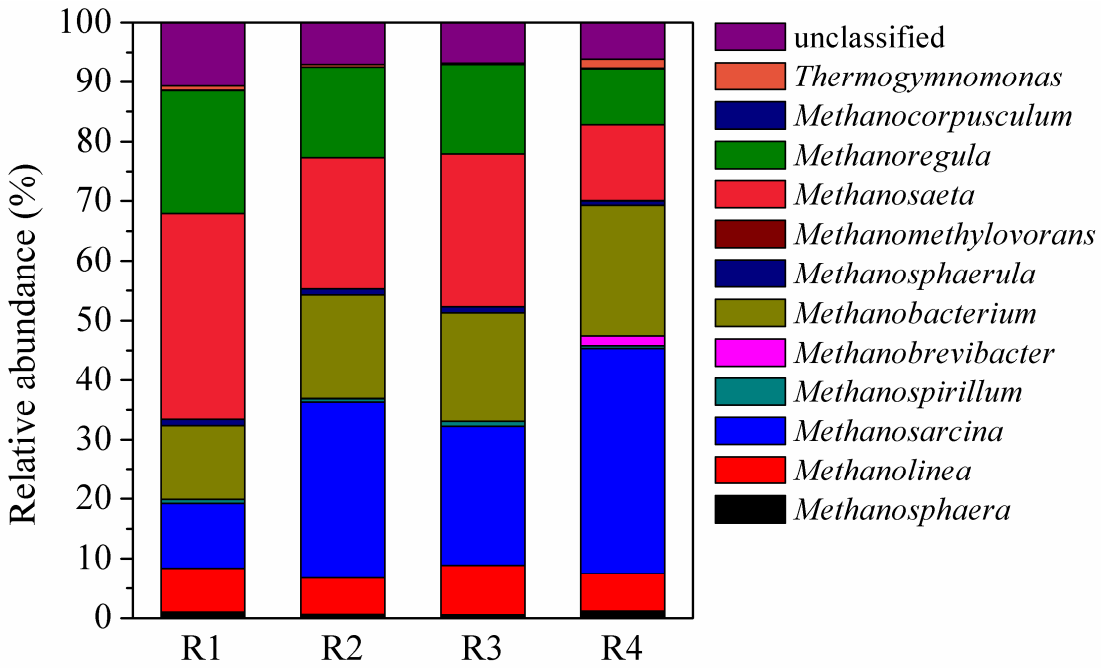

Figure 4. Changes of methanogenic community structure dynamics (at genus level) in R1–R4 reactors.

Methanobacterium, *Methanoregula*, *Methanomassiliicoccus*, and *Methanolinea* were classified as hydrogenotrophic methanogens, and they were very important for the AD systems [32,33]. After EDTA-Ni addition, the relative abundance of *Methanobacterium* was sharply enhanced to 21.83% from 12.45%. On the contrary, the relative abundances of *Methanoregula* decreased to 9.4% from 20.64%. Also, no obvious changes in other hydrogenotrophic methanogens were observed in the R1–R4. It was speculated that the increase of *Methanobacterium* might be owed to EDTA-Ni improved the production of H_2 by accelerating hydrolysis and acidification of KWs [34]. It is reported that the growth of *Methanoregula* required acetic acid [33], however, the concentration of acetic acid in the R4 was lowest on the 25th day.

Comprehensive analysis with Figures 1 and 2, it was found that the structure of the methanogenic community was closely related to high methane production and rapid VFAs degradation. It is reported that the degradation efficiency of *Methanosarcina* to acetate was 3–5 times of *Methanosaeta* because of its shorter generation time and faster growth rate [35]. At the same time, both *Methanosarcina* and *Methanobacterium* were able to produce methane using H_2 and CO_2. In the AD process, propioic acid was oxidized to be acetic acid, H_2 and CO_2. Therefore, a possible reason for propionic acid degradation in the R4 was that EDTA-Ni promoted the growth of *Methanosarcina* and *Methanobacterium*, resulting in the further utilization of acetic acid and H_2.

In this study, the Shannon index was used to analyze the effect of different additives on the diversity of methanogenic community. As is presented in Figure 5, the Shannon index of R4 with EDTA-Ni addition was highest (3.56), followed by R2 (Ni addition), R3 (EDTA addition), and R1 (control test) with Shannon index of 3.48, 3.29 and 3.04, respectively. The comparative results indicated that the addition of EDTA-Ni enhanced the diversity of methanogenic community, which was critical to maintaining high biogas yield in the R4 [9].

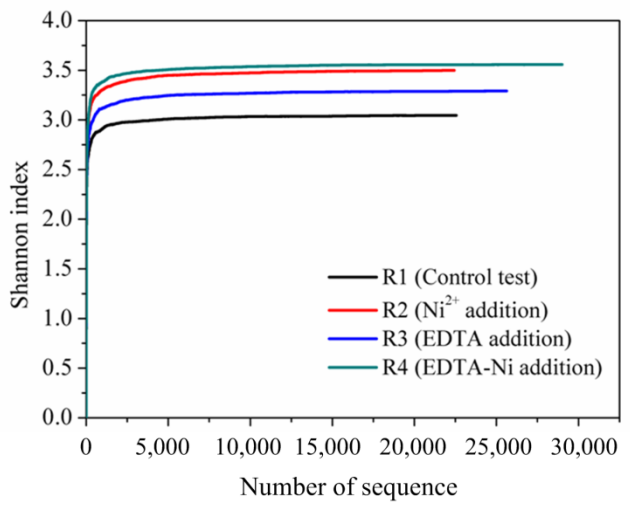

Figure 5. Shannon index of the methanogenic community in R1–R4 on the 25th day.

4. Conclusions

In the present study, the effects and mechanisms of EDTA-Ni addition on the AD performances of KWs were investigated. The results revealed that the addition of EDTA-Ni enhanced the methane yield and promoted the degradation of VFAs. Also, EDTA-Ni stimulated the growth and metabolism of methanogens by enhancing the fraction of water-soluble Ni, simultaneously, changed the structure and diversity of methanogenic communities significantly. The aceticlastic methanogen *Methanosarcina* and hydrogenotrophic methanogen *Methanobacterium* were dominant microorganisms after EDTA-Ni addition, which might be a major reason for propionic acid degradation and methane production.

Author Contributions: Y.L. and X.K. conceived and designed the experiments; Y.L. and T.Z. performed the experiments; X.K. and T.Z. analyzed the results; and Y.L. and X.K. wrote and revised the paper.

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References

1. Braguglia, C.M.; Gallipoli, A.; Gianico, A.; Pagliaccia, P. Anaerobic bioconversion of food waste into energy: A critical review. *Bioresour. Technol.* **2018**, *248*, 37–56. [CrossRef] [PubMed]
2. Evranos, B.; Demirel, B. The impact of Ni, Co and Mo supplementation on methane yield from anaerobic mono-digestion of maize silage. *Environ. Technol.* **2015**, *36*, 1556–1562. [CrossRef] [PubMed]
3. Menon, A.; Wang, J.Y.; Giannis, A. Optimization of micronutrient supplement for enhancing biogas production from food waste in two-phase thermophilic anaerobic digestion. *Waste Manag.* **2017**, *59*, 465–475. [CrossRef] [PubMed]
4. Schmidt, T.; Nelles, M.; Scholwin, F.; Pröter, J. Trace element supplementation in the biogas production from wheat stillage—Optimization of metal dosing. *Bioresour. Technol.* **2014**, *168*, 80–85. [CrossRef] [PubMed]
5. Harro, P.; Todd, C.; Maschara, K.; Pradip, K. Structural and spectroscopic models of the A-cluster of acetyl coenzyme a synthase/carbon monoxide dehydrogenase: Nature's Monsanto acetic acid catalyst. *Coordin. Chem. Rev.* **2005**, *249*, 3007–3024. [CrossRef]
6. Shima, S.; Warkentin, E.; Thauer, R.K.; Ermler, U. Structure and function of enzymes involved in the methanogenic pathway utilizing carbon dioxide and molecular hydrogen. *J. Biosci. Bioeng.* **2002**, *93*, 519–530. [CrossRef]
7. Ferry, J.G. The chemical biology of methanogenesis. *Plane Space Sci.* **2010**, *58*, 1775–1783. [CrossRef]

8. Ermler, U.; Grabarse, W.; Shima, S.; Goubeaud, M.; Thauer, R.K. Crystal Structure of Methyl-Coenzyme M Reductase: The Key Enzyme of Biological Methane Formation. *Science* **1997**, *278*, 1457–1462. [CrossRef]
9. Zhang, W.; Chen, B.; Li, A.; Zhang, L.; Li, R.; Yang, T.; Xing, W. Mechanism of process imbalance of long-term anaerobic digestion of food waste and role of trace elements in maintaining anaerobic process stability. *Bioresour. Technol.* **2019**, *275*, 172–182. [CrossRef]
10. Fermoso, F.G.; Collins, G.; Bartacek, J.; O'Flaherty, V.; Lens, P. Role of nickel in high rate methanol degradation in anaerobic granular sludge bioreactors. *Biodegradation* **2008**, *19*, 725–737. [CrossRef]
11. Thanh, P.M.; Ketheesan, B.; Yan, Z.; Stuckey, D.C. Trace metal speciation and bioavailability in anaerobic digestion: A review. *Biotechnol. Adv.* **2016**, *34*, 122–136. [CrossRef] [PubMed]
12. Zandvoort, M.H.; Hullebusch, E.D.V.; Fermoso, F.G.; Lens, P.N.L. Trace Metals in Anaerobic Granular Sludge Reactors: Bioavailability and Dosing Strategies. *Eng. Life Sci.* **2006**, *6*, 293–301. [CrossRef]
13. Cai, Y.; Hu, K.; Zheng, Z.; Zhang, Y.; Guo, S.; Zhao, X.; Cui, Z.; Wang, X. Effects of adding EDTA and Fe2+ on the performance of reactor and microbial community structure in two simulated phases of anaerobic digestion. *Bioresour. Technol.* **2019**, *275*, 183–191. [CrossRef] [PubMed]
14. Hu, Q.H.; Li, X.F.; Du, G.C.; Chen, J. Effect of nitrilotriacetic acid on bioavailability of nickel during methane fermentation. *Chem. Eng. J.* **2008**, *143*, 111–116. [CrossRef]
15. Zhang, W.; Lei, Z.; Li, A. Enhanced anaerobic digestion of food waste by trace metal elements supplementation and reduced metals dosage by green chelating agent [S, S]-EDDS via improving metals bioavailability. *Water Res.* **2015**, *84*, 266–277. [CrossRef] [PubMed]
16. Cai, Y.; Zhao, X.; Zhao, Y.; Wang, H.; Yuan, X.; Zhu, W.; Cui, Z.; Wang, X. Optimization of Fe^{2+} supplement in anaerobic digestion accounting for the Fe-bioavailability. *Bioresour. Technol.* **2018**, *250*, 163–170. [CrossRef]
17. Vintiloiu, A.; Boxriker, M.; Lemmer, A.; Oechsner, H.; Jungbluth, T.; Mathies, E.; Ramhold, D. Effect of ethylenediaminetetraacetic acid (EDTA) on the bioavailability of trace elements during anaerobic digestion. *Chem. Eng. J.* **2013**, *223*, 436–441. [CrossRef]
18. APHA; AWWA; WEF. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
19. Barbara, T.; Agata, G.; Andrea, G.; Daniele, M.; Pamela, P.; Marco, D.C.; Simona, R.; Braguglia, C.M. Long-term anaerobic digestion of food waste at semi-pilot scale: Relationship between microbial community structure and process performances. *Biomass Bioenergy* **2018**, *11*, 55–64.
20. Hu, Y.; Jing, Z.; Sudo, Y.; Niu, Q.; Du, J.; Wu, J.; Li, Y.Y. Effect of influent COD/SO_4^{2-} ratios on UASB treatment of a synthetic sulfate-containing wastewater. *Chemosphere* **2015**, *130*, 24–33. [CrossRef]
21. Rauret, G.; López-Sánchez, J.F.; Sahuquillo, A.; Rubio, R.; Davidson, C.; Ure, A.; Quevauviller, P. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J. Environ. Monit.* **1999**, *1*, 57–61. [CrossRef]
22. Wang, G.; Li, Q.; Gao, X.; Wang, X.C. Synergetic promotion of syntrophic methane production from anaerobic digestion of complex organic wastes by biochar: Performance and associated mechanisms. *Bioresour. Technol.* **2018**, *250*, 812–820. [CrossRef] [PubMed]
23. Yang, X.; Wan, C.; Lee, D.J.; Du, M.; Pan, X. Continuous volatile fatty acid production from waste activated sludge hydrolyzed at pH 12. *Bioresour. Technol.* **2014**, *168*, 173–179. [CrossRef] [PubMed]
24. Moestedt, J.; Nordell, E.; Shakeri Yekta, S.; Lundgren, J.; Martí, M.; Sundberg, C.; Ejlertsson, J.; Svensson, B.H.; Björn, A. Effects of trace element addition on process stability during anaerobic co-digestion of OFMSW and slaughterhouse waste. *Waste Manag.* **2016**, *47*, 11–20. [CrossRef] [PubMed]
25. Kang, X.R.; Zhang, G.M.; Chen, L.; Dong, W.Y.; Tian, W.D. Effect of Initial pH Adjustment on Hydrolysis and Acidification of Sludge by Ultrasonic Pretreatment. *Ind. Eng. Chem. Res.* **2011**, *50*, 12372–12378. [CrossRef]
26. Ko, J.H.; Wang, N.; Yuan, T.; Lu, F.; He, P.; Xu, Q. Effect of nickel-containing activated carbon on food waste anaerobic digestion. *Bioresour. Technol.* **2018**, *266*, 516–523. [CrossRef] [PubMed]
27. Thanh, P.M.; Ketheesan, B.; Stuckey, D.C.; Zhou, Y. Dosing of Ethylenediamine-N,N'-disuccinic acid (EDDS) to improve the bioavailability of Fe^{2+} in the presence of sulfide in a submerged anaerobic membrane bioreactor. *Chem. Eng. J.* **2017**, *330*, 175–182. [CrossRef]
28. Gustavsson, J.; Yekta, S.S.; Karlsson, A.; Skyllberg, U.; Svensson, B.H. Potential bioavailability and chemical forms of Co and Ni in the biogas process—An evaluation based on sequential and acid volatile sulfide extractions. *Eng. Life Sci.* **2013**, *13*, 572–579. [CrossRef]

29. Liu, Y.; Whitman, W.B. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Ann. N. Y. Acad. Sci.* **2010**, *1125*, 171–189. [CrossRef]
30. Niu, Q.; Kubota, K.; Wei, Q.; Jing, Z.; Zhang, Y.; Li, Y.Y. Effect of ammonia inhibition on microbial community dynamic and process functional resilience in mesophilic methane fermentation of chicken manure. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 2161–2169. [CrossRef]
31. Du, J.; Hu, Y.; Qi, W.; Zhang, Y.; Jing, Z.; Norton, M.; Li, Y.Y. Influence of four antimicrobials on methane-producing archaea and sulfate-reducing bacteria in anaerobic granular sludge. *Chemosphere* **2015**, *140*, 184–190. [CrossRef]
32. Leclerc, M.; Delgènes, J.P.; Godon, J.J. Diversity of the archaeal community in 44 anaerobic digesters as determined by single strand conformation polymorphism analysis and 16S rDNA sequencing. *Environ. Microbiol.* **2004**, *6*, 809–819. [CrossRef] [PubMed]
33. Brauer, S.L.; Hinsby, C.Q.; Ward, R.J.; Yavitt, J.B.; Zinder, S.H. Methanoregula boonei gen. nov. sp. nov. an acidiphilic methanogen isolated from an acidic peat bog. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 45–52. [CrossRef] [PubMed]
34. Yin, Q.; Yang, S.; Wang, Z.; Xing, L.; Wu, G. Clarifying electron transfer and metagenomic analysis of microbial community in the methane production process with the addition of ferroferric oxide. *Chem. Eng. J.* **2018**, *333*, 216–225. [CrossRef]
35. Mer, J.L.; Roger, P. Production, oxidation, emission and consumption of methane by soils: A review. *Eur. J. Soil Biol.* **2001**, *37*, 25–50. [CrossRef]

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