Nickel Foam Promotes Syntrophic Metabolism of Propionate and Butyrate in Anaerobic Digestion

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ABSTRACT: Enhanced interspecies electron transfer (IET) among symbiotic microorganisms is an effective method to increase the rate of methane (CH₄) production in anaerobic digestion. Direct interspecies electron transfer (DIET), which does not involve dissolved redox media, is considered an alternative and superior method to enhance methane production by interspecies hydrogen (H₂) transfer (IHT). In this study, nickel foam was built into a semicontinuous anaerobic reactor to investigate its effect on the metabolism of propionate and butyrate. Both increased the average yield of CH₄ in anaerobic digestion by 18.1 and 15.9%, respectively. Analysis of bacterial and archaeal communities showed that the addition of nickel foam could increase the relative abundance of microbial communities involved in DIET and could increase the diversity of microorganisms in the reactor. Moreover, the anaerobic digestion performance of the nickel foam reactor was good at high hydrogen partial pressure.

1. INTRODUCTION

Anaerobic digestion is a common process used to treat organic wastes and high-concentration wastewaters by converting and recovering energy from organic wastes.¹⁻³ Anaerobic digestion is the combined action of multiple microorganisms and is usually divided into successive stages of hydrolysis acidification, acetic acid production, and methanogenesis, which are performed collaboratively by hydrolytic bacteria, acid-producing microorganisms, and methanogens.⁴

In anaerobic digestion, propionate and butyrate are essential intermediate metabolites produced during the anaerobic digestion of organic compounds and can be used to study biological reactions.⁵⁻⁶ Anaerobic digestion of propionate and butyrate proceeds as follows: (1) propionate and butyrate are oxidized by propionate/butyrate-oxidizing bacteria to bicarbonate, acetate, and hydrogen or formic acid salt;⁷ (2) hydrothrophic methanogens use H₂ to reduce carbon dioxide (CO₂) to CH₄ and acetate-degrading methanogens convert acetic acid to methane (CH₄). This process is called interspecific hydrogen transfer (IHT).⁸⁻⁹ In IHT, H₂ transports electrons from alcohols or volatile fatty acids (VFAs) toward the transfer to CO₂.¹⁰⁻¹¹¹⁻¹³ The production of H₂ is thermodynamically infeasible under a standard state and is only feasible when H₂ is effectively removed by a nutritional companion (e.g., methanogens).⁶⁻¹² These conditions limit the rapid operation of anaerobic digestion.¹³

The direct interspecies electron transfer (DIET), which is considered to be a more efficient form of electron transfer, can occur under a certain condition.¹⁴⁻¹⁵ Since 2012, many studies have been conducted to improve methane production by supplementing anaerobic digestion reactors with conductive carbon materials.¹⁶⁻¹⁸ The mode of electron transfer in such studies has been shown to be mediated by the direct transfer of electrons to methanogenic archaea through conductive materials rather than by diffusion of electron-bearing intermediates (i.e., hydrogen or formate). Additional DIET based only on bioelectrical linkages was confirmed in cocultures of Geobacter spp. type-c cytochromes¹⁹ or conductive cilia can also work for interspecies electron transfer.²⁰

In recent years, several carbon-based materials, such as activated carbon,²¹ biochar,²² graphene,²³ graphite,²⁴ carbon cloth,²⁵,²⁶ and graphite felt,¹³ have been used to enhance methane-producing DIET. This is because carbon-based materials are typically highly conductive, biocompatible, chemically stable, and economically inexpensive.²⁷ Carbon-based materials can transfer electrons between species, reduce lag time and thermal energy, and promote the production of associated type-c cytochromes and electrically conductive pili.²⁸,²⁹

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The search for new materials that can promote anaerobic digestion has been a hot topic in recent years. Nickel foam is a metallic foam material that is often used to make supercapacitor materials, fuel cells, and catalytic materials due to its large porosity and good electrical conductivity and stability. Compared with nickel flakes of the same quality, nickel foam has a larger specific surface area for contact with microorganisms and its special internal foam structure is conducive to the attachment and growth of microorganisms. Compared to smaller particle size carbon-based materials such as biochar, graphene, and needle iron ore, as well as nickel powder and nickel nanoparticles, nickel foam can be integrated into the reactor at the setup stage with less loss and without frequent and repeated additions. The trace element nickel plays an important role in the growth of anaerobic digestion microorganisms, and the enzymatic activities of methyl-coenzyme M reductase, [Ni–Fe]-hydrogenase, acetyl-CoA synthase/decarbonyase, urease, carbon monoxide dehydrogenase, and superoxide dismutase all require the participation of nickel. Several researchers have demonstrated that the addition of different forms of Ni(II) to the reactor can increase methane production. However, heavy metal contamination may be induced if the subsequent sludge is not treated properly. Nickel foam is insoluble in water and tends to form a dense oxide film on the surface to prevent further oxidation of the main body. It is predicted that nickel foam may accelerate electron transfer during anaerobic digestion, thus promoting substrate degradation. Therefore, this study was conducted to investigate whether the degradation of propionate and butyrate could be promoted by adding nickel foam with ethanol as a specific substrate. Moreover, the changes in the microbial community in the reactor with nickel foam addition were analyzed. The effect of high hydrogen partial pressure on the reactor with or without nickel foam addition was also explored.

2. MATERIALS AND METHODS

2.1. Batch Fermentation Design. The semicontinuous methane production experiment was carried out in four anaerobic reactors with a working volume of 500 mL. A 3 L gas collection bag was attached to the top of each reactor to collect the generated gas. Each operating cycle of the semicontinuous reactor was 24 h. Each cycle proceeded as follows: first, 250 mL of manually configured fresh wastewater was replenished into the reactor; second, the reaction was allowed to proceed for 23 h in a 37 °C water bath pot; and finally, a water sample was extracted, and the wastewater was filtered out with gauze. The four reactors were P1 (propionate and nickel foam), B1 (butyrate and nickel foam), P0, and B0. The P0 and B0 reactors were used as controls (propionate and butyrate without adding nickel foam, respectively). At the beginning of the experiment, 250 mL of inoculated sludge was inoculated into each reactor. The P1 and B1 reactors were equipped with nickel foam (thickness 0.5 mm, pore size 110 PPI, purity 99.9%). The nickel foams were rinsed with ethanol and then cleaned three times with ultrasonication to wash away contaminants that may hinder the electron transfer rate.

2.2. Inoculated Sludge and Wastewater. The inoculated sludge was taken from the upflow anaerobic sludge bed of a sewage treatment plant (located in Shandong) and placed in a 4 °C refrigerator to maintain sludge activity. At the beginning of the experiment, each reactor was infused with 250 mL of sludge and the same amount of artificial wastewater. The total suspended sludge (TSS) content of the inoculated sludge was 60.9 ± 1.7 g/L (mean ± standard deviation, n = 3), and the volatile suspended sludge (VSS) content was 51.3 ± 3.6 g/L.

The composition (per liter) of artificial wastewater with ethanol as the initial carbon source was as follows: K2HPO4, 0.11 g; KH2PO4, 0.17 g; Na2SO4, 0.05 g; MgCl2·6H2O, 0.1 g; CaCl2·2H2O, 0.05 g; and trend element solution, 10 mL. The composition of the trace element solution (in g/L) was as follows: MnSO4·H2O, 0.5; FeSO4·7H2O, 0.1; NiCl2·6H2O, 0.04; CoCl2·6H2O, 0.048; ZnCl2, 0.13; CuSO4·5H2O, 0.01; AlK(SO4)2·12H2O, 0.01; H3BO3, 0.01; and Na2MoO4·2H2O, 0.025.

The effects of nickel foam in the startup phase were explored by configuring artificial wastewater with ethanol as the initial carbon source. After 30 days, sodium propionate was added to the artificial wastewater for the P1 and P0 reactors, gradually replacing ethanol as the organic nutrient source. The ratio of the two (in terms of chemical oxygen demand (COD)) in the artificial wastewater ranged from 1:3 to 2:2, 3:1, and 4:0, finally retaining sodium propionate as the only organic nutrient.

Similarly, sodium butyrate was added to the artificial wastewater of the P1 and P0 reactors and gradually replaced ethanol. The COD generated by the main carbon source of the artificial wastewater was maintained at 8000 mg/L.

The anaerobic digestion test was repeated three times, and the results were expressed as mean ± standard deviation.

2.3. Effect of Nickel Foam on the Degradation of Substrates at High Hydrogen Partial Pressure. To investigate the effect of a higher hydrogen pressure environment on the codification metabolism in the inner nickel foam reactor and the control reactor, the anaerobic sludge from the four reactors P1, P0, B1, and B0 was removed on the 60th day of the experiment as the inoculation sludge for the next step of the experiment. Four 250 mL serum vials were used to inoculate 50 mL of inoculation sludge into the corresponding reactor; 150 mL of artificial wastewater was added, and the resulting reactors were renamed p1, b1, p0, and b0. The COD of the artificial wastewater in p1, b1, p0, and b0 were maintained at 8000 mg/L. The carbon source of p1 and p0 is sodium propionate, and the carbon source of b1 and b0 is sodium butyrate. Nickel foam was divided into 1 × 1 cm2 blocks and placed in reactors p1 and b1 (10 blocks per bottle). The nickel foam was pretreated with ethanol and then ultrasonically cleaned with deionized water before being added to the reactors to remove impurities. The reactors were sealed with customized fermentation bottle caps and rinsed with nitrogen for 5 min. Before the test, 40 mL of hydrogen was added to the top space of all reactors. All tests were carried out in a 37 °C constant temperature water bath.

2.4. Analytical Methods. The volatile organic acid concentrations were detected by a gas chromatograph (GC-2010, Shimadzu, Japan) with a flame ionization detector (FID) and a KB-PLOTU column (30 m, 0.32 mm, 25 μm, Kromat). The column was heated from 100 to 180 °C at a rate of 20 °C/min. The carrier gas was 30 mL/min N2, the air flow was 400 mL/min, and the H2 flow was 50 mL/min. The deionized water used in this experiment was prepared by the high-purity water system in the laboratory.

Biogas composition was analyzed by gas chromatography (GC-2014C/TCD, Shimadzu, Japan). The mobile phase was 0.11 g; K2HPO4, 0.17 g; Na2SO4, 0.05 g; MgCl2·6H2O, 0.1 g; CaCl2·2H2O, 0.05 g; and trend element solution, 10 mL. The composition of the trace element solution (in g/L) was as follows: MnSO4·H2O, 0.5; FeSO4·7H2O, 0.1; NiCl2·6H2O, 0.04; CoCl2·6H2O, 0.048; ZnCl2, 0.13; CuSO4·5H2O, 0.01; AlK(SO4)2·12H2O, 0.01; H3BO3, 0.01; and Na2MoO4·2H2O, 0.025.
temperature was 50 °C. Methane production at standard pressure and temperature is converted using the following equation:

\[ V_{\text{CH}_4}(\text{STP} \ L) = V_{\text{CH}_4}(\text{at} \ T) \times \frac{273/(273 + T)}{(760 - W)/760} \]

where \( T \) is 25 °C and \( W \) is the water vapor pressure (mm Hg) at 25 °C.

The concentration of effluent nickel was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 7000DV, PerkinElmer, MA). The sludge samples were air-dried, sieved, and microwave-digested to measure the elemental nickel content in activated sludge by inductively coupled plasma-optical emission spectrometry (ICP-OES).

2.5. Microbial Community Analysis. High-throughput pyrosequencing of the 16S rRNA gene was used to analyze the bacterial and archaeal community structure of the anaerobic sludge. After the methane production test, 15 mL of sludge was taken from P1, B1, P0, and B0 reactors, washed with configured phosphate buffer solution, and centrifuged at 11,000 rpm for 10 min. For bacteria, the V3–V4 region was amplified by polymerase chain reaction (PCR) using universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GACTACVSGGGTATCTAAT-3′). For archaea, the V4–V5 region of PCR was amplified using universal primers 524F10extF (5′-TGYCAGCCGCCGCGGTAA-3′) and Arch958RmodR (5′-YCCGGCGTTGAVTCCAATT-3′). PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA). After the individual quantification step, amplicons were pooled in equal amounts and pair-end 2250 bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3. Microbiome bioinformatics was performed with QIIME2 2019.4. Briefly, raw sequence data were demultiplexed using the demux plugin followed by primer cutting with the cutadapt plugin. Sequences were then quality filtered, denoised, merged, and chimera removed using the DADA2 plugin. Taxonomy was assigned to ASVs using the classify-sklearn naive Bayes taxonomy classifier in the feature-classifier plugin against the SILVA Release 132/UNITE Release 8.0 Database. The abundance table of ASV/OTU was leveled, and the leveling depth was set to 95% of the minimum sample sequence size. Visualization of the composition distribution of each sample at the phylum, genus, and classification levels using the feature table after removing the singleton.

All raw sequences were deposited in the NCBI Sequence Read Archive under accession number PRJNA746453.

3. RESULTS AND DISCUSSION

3.1. Impact of Nickel Foam on the Domestication Phase. For experimental preparation, nickel foam was placed in the P1 and B1 reactors as nickel foam reactors (P1 and B1), and the other two reactors without nickel foam were used as control reactors (P0 and B0).

During the initial phase of the experiment, all four reactors produced low yields of methane each day (Figure 1A). As the experiment progressed, the methane production rates of P1 and B1 increased rapidly from day 4 and stabilized at high levels of 746 and 755 mL/d by day 17, while the methane production of P0 and B0 reached a maximum after nearly 28 days. This indicates that the addition of nickel foam reactors can adapt to the substrate more quickly to stabilize methane production.
production at a higher level, resulting in an approximately 35% reduction in the cycle time to adapt to the substrate. The concentration of nickel ions in the effluent showed relatively low concentrations in all reactors (Figure 1B). Moreover, the content of elemental nickel in the sludge of the built-in nickel foam reactor and the sludge of the non-built-in nickel foam reactor were similar (Figure 1C), both of which complied with the control standard for agricultural sludge contaminants (GB4282−2018). Both sets of data indicated that nickel foam was more stable in the anaerobic system and provided almost no nickel ions to the system, and the built-in nickel foam did not cause potential heavy metal contamination. This faster adaptation to the substrate may be due to the function of the high electronic conductivity of nickel foam, which enhances anaerobic digestion.15,23

3.2. Methanogenesis of Sodium Propionate in a Built-In Nickel Foam Reactor. On day 31 of the experiment, a certain amount of ethanol in the feed began to be replaced by propionate, and the proportion of propionate increased step by step. Figure 2 shows the significant differences in methane production and volatile organic acids in the effluent water between the nickel foam reactor (P1) and the control reactor (P0). Each change in the proportion of propionate in the substrate caused large fluctuations in effluent organic acid concentrations. This effect is mainly manifested by changes in propionate and acetic acid in the effluent. This change is mainly due to the different anaerobic fermentation pathways for propionate and ethanol and the microorganisms have to adapt to the new ratio of substrates. From day 52 to day 58 of the reactor operation, when propionate completely replaced ethanol, the average daily methane production of the P0 reactor was 657 mL (Figure 2A). The average daily methane production of the P1 reactor was 776.2 mL, which is higher than that of the P0 reactor by about 18.1%.

Methane yield is the amount of methane produced to degrade 1 g of COD.13 Methane yield can be used to reflect the electron transfer rate of the substrate conversion process to methane.41 The methane yield in P0 was stable at 164.25 mL CH₄/g CODr, but the methane yield in P1 was increased to 194.05 mL CH₄/g CODr (Table 1). The addition of nickel foam promoted the methane yield, further indicating that the addition of nickel foam improved the electron transfer efficiency in cotrophic metabolism. The average effluent propionate concentration and average effluent acetate concen-

Table 1. Average Parameters Related to Methane Generation and Each Volatile Acid from Day 54 to 58

| indicators                           | reactor | methane production rate (mL/d) | methane yield (mL CH₄/g CODr) | effluent propionate (mg COD/L) | effluent butyrate (mg COD/L) | effluent acetate (mg COD/L) |
|--------------------------------------|---------|-------------------------------|--------------------------------|--------------------------------|-----------------------------|----------------------------|
|                                      | P1      | 776.2 ± 6.3                   | 194.05 ± 1.6                   | 47.2 ± 1                        | 3.7 ± 0.7                   |
|                                      | P0      | 657 ± 9.6                     | 164.25 ± 2.4                   | 146.5 ± 3.7                     | 4.1 ± 1.6                   |
|                                      | B1      | 795.8 ± 12.9                  | 198.95 ± 3.2                   | 27.3 ± 5                        | 5.8 ± 0.4                   |
|                                      | B0      | 686.6 ± 8.9                   | 171.65 ± 2.2                   | 144.9 ± 1.3                     | 5.9 ± 0.8                   |

Figure 2. Daily methane production (A), acetate concentration in the effluent water (B), and propionate concentration in the effluent water (C) during the metabolism of propionate.
The concentration of the P0 reactor were 46.12 and 3.14 mg COD/L, respectively (Figure 2B,C), which were significantly higher than those in P1 (143.63 and 3.21 mg COD/L, respectively). To determine whether the addition of nickel foam had a statistically significant effect on promoting the anaerobic digestion process, one-way analysis of variance (ANOVA) was performed using SPSS 19.0 on data collected from different reactors during the stabilization period (days 54−58).

The results in Table 2 present the difference between the final exponential mean and the associated p-value for the same substrate from any two reactors. The default significance level of the system is 0.05, so when the p-value is less than the significance level, it can be assumed that a factor has a significant effect on the results. The p-values were less than 0.05 for all indicators except effluent acetate (Table 2). This indicates that there is a statistically significant difference in the results between P1 and P0. The p-value for effluent acetic acid concentration was 0.574, indicating a statistically insignificant difference in effluent acetic acid concentration between P1 and P0. In other words, placing nickel foam in a reactor with propionate as the only organic nutrient had a statistically significant effect on the results for methane production, methane yield, and effluent propionate. Overall, the placement of nickel foam was very beneficial for the anaerobic digestion of propionic acid. A comparison of the P1 and P0 reactors shows that the addition of nickel foam resulted in a higher methane yield and a lower propionate effluent concentration and that the effluents of P1 and P0 had a very low acetate concentration. This result suggests that nickel foam can act as an electron transfer channel between symbiotic microorganisms to facilitate the anaerobic digestion of propionate by enhancing the DIET pathway.

### 3.3. Methanogenesis Results and Analysis of Sodium Butyrate in a Built-In Nickel Foam Reactor

To evaluate the contribution of nickel foam to butyrate degradation, a certain percentage of carbon source in the reactor feed was replaced by sodium butyrate instead of ethanol starting from day 31. Moreover, the proportion of sodium butyrate was gradually increased until sodium butyrate became the only carbon source in the feed. Figure 3A shows that with each increase in butyrate in the influent, the B1 reactor was quickly adapted to the new substrate, and the B0 reactor took a relatively long time to adapt. When butyrate replaced ethanol as the sole organic nutrient source, the daily methane production of B1 was stable at 795.8 mL, which was about 15.9% higher than that of B0 (686.6 mL/d). This parameter can be used to reflect the electron transfer rate of the substrate conversion process to methane. The methane yield in B0 was

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**Table 2. One-way ANOVA for the Results of Each Item Tested during the Experiment**

| reactors | P\textsubscript{effluent butyrate} | P\textsubscript{methane production rate} | P\textsubscript{effluent acetate} | P\textsubscript{methane yield} | P\textsubscript{effluent propionate} |
|----------|--------------------------------|----------------------------------------|--------------------------------|--------------------------------|---------------------------------|
| P1       | 0.000                          | 0.574                                  | 0.000                          | 0.000                          | 0.000                           |
| P0       |                                |                                        |                                |                                |                                 |
| B1       | 0.000                          | 0.000                                  | 0.896                          | 0.000                          |                                 |
| B0       |                                |                                        |                                |                                |                                 |

Figure 3. Daily methane production (A), acetate concentration in the effluent water (B), and butyrate concentration in the effluent water (C) during butyrate metabolism.
stable at 171.65 mL CH₄/g CODr but increased to 198.95 mL CH₄/g CODr in B1 (Table 2). This result suggests that the placement of nickel foam enhances the electron transfer in the methane conversion. As can be seen from Figure 3, the effluent acetic acid concentrations of both B1 and B0 were low (Figure 3B), while the butyrate effluent concentration of the B0 reactor was much higher than that of B1 (Figure 3C). This result means that reactor B1 degrades butyrate more completely. The p-values were less than 0.05 for all indicators except effluent acetate (Table 2). The result suggests that in the reactor where butyrate was the only carbon source, the placement of nickel foam had a significant effect on anaerobic methanogenesis. Overall, the addition of nickel foam is very beneficial for the anaerobic digestion of butyrate. A comparison of the B1 and B0 reactors shows that the methane yield is higher and the butyrate effluent concentration is lower with the addition of nickel foam. This result suggests that nickel foam can act as an electron transfer channel between symbiotic microorganisms to facilitate anaerobic digestion of butyrate by enhancing the DIET pathway.

3.4. Microbial Community Analysis. Efficient anaerobic digestion depends on the biological activity of electron/acetic acid-producing and electron-consuming/methanogens of the digestion process in question. After 58 days of the experiment, high-resolution 16S rRNA pyrophosphate sequencing of anaerobic sludge in the four reactors was performed, leading to microbial diversity analysis of bacteria and archaea in each reactor. Figure 4A shows the genus-level bacterial community structure of anaerobic digestion of propionate/butyrate. In this analysis, bacteria accounted for 53320−93854 validated sequences with Shannon indices ranging from 7.37 to 8.11 (Table 3). Higher Shannon index/Simpson index values indicate higher community diversity. The Shannon index is more applicable to complex communities, while the Simpson index is more sensitive to evenness and dominant ASVs/OTU in the community and is more applicable to simple communities.

The main genera in the community are listed in Figure 4, and the genera with large proportions in the four reactors are Desulfovibrio, Blvii28_wastewater-sludge_group, Bacteroidetes_vadinHA17, Syntrophobacter, and Syntrophomonas. Among them, Syntrophomonas is a conutrient butyrate-oxidizing bacterium that can oxidize butyrate to acetate by coculture with hydrogen-consuming microorganisms. The substrate of

![Figure 4. Bacterial community structure at the genus level (A) and archaebacterial community structure at the genus level (B) for anaerobic digestion of propionate/butyrate.](https://doi.org/10.1021/acsomega.1c02682)
reactors B0 and B1 was butyrate, so the proportion of *Syntrophomonas* in reactors B0 and B1 was much higher than that in reactors P0 and P1. In contrast, *Desulfovibrio* (sulfate-reducing bacteria) and *Syntrophobacter*, a hydrogen- and acetic acid-producing bacteria that oxidizes propionate, accounted for a much higher proportion in reactors P0 and P1 than that in B0 and B1 groups. It is hypothesized that this result is determined by the carbon source in the artificially prepared water. In addition, the percentage of *Syntrophobacter* in the P1 reactor (5.35%) was higher than that in the P0 reactor (4.16%). This result may be due to the addition of nickel foam stimulating the growth of *Syntrophobacter*.

*Geobacter* species are the first bacteria shown to act as electron-providing partners for DIET. *Geobacter* species were present in relatively low proportions in all four reactor sludge samples, but the relative abundance of P1 (0.21%) was higher than that of P0 (0.12%) and the relative abundance of B1 (0.09%) was higher than that of B0 (0.04%). Recent articles have indicated that there may be many kinds of microorganisms involved in DIET. The Shannon index results in Figure 4 and Table 3 show that the reactors with nickel foam addition have high biodiversity. Microorganisms involved in DIET may exist among the increased number of microbial species.

Figure 4B shows the structure of the genus-level archaeal community for anaerobic digestion of propionate/butyrate. In all four reactors, *Methanothrix* and *Methanobacterium* are the two archaeal genera that account for a high proportion of the total. *Methanothrix* and *Methanobacterium* are the most readily detectable archaea in anaerobic digestion. *Methanothrix* is an acetic acid-consuming methanogenic bacterium that cannot utilize hydrogen or formate. *Methanobacterium* is a hydrogenotrophic methanogenic bacterium. The *Methanobacterium* abundance was relatively high in the reactors with the addition of nickel foam. By 16S rRNA high-throughput sequencing analysis, the addition of nickel foam resulted in increased microbial diversity in the reactor and an increase in microorganisms that may be involved in direct interspecies electron transfer.

### Table 3. Microbial Diversity Index of the Flora

| microbial sample | Shannon index | Simpson index |
|------------------|---------------|---------------|
| bacteria         |               |               |
| B1 100           | 3.7124        | 0.973568      |
| B0 65            | 5.757457      | 0.955945      |
| P1 70            | 8.1132        | 0.980968      |
| P0 100           | 7.54866       | 0.975439      |
| archaea          |               |               |
| B1 122           | 1.71418       | 0.56201       |
| B0 118           | 1.52492       | 0.521744      |
| P1 137           | 2.08196       | 0.597693      |
| P0 121           | 1.74171       | 0.565508      |

### 3.5. Effect of Nickel Foam on the Degradation of Substrates at High H2 Partial Pressure

At standard temperature and pressure, the degradation of propionic and butyric acids to produce H2 is an endothermic process that is not thermodynamically feasible. This process is only feasible if H2 is efficiently removed by methanogens. Therefore, H2 partial pressure is a relatively important factor affecting the metabolism of propionate and butyrate by IHT. When DIET is present and replaces H2 as the main pathway of IET, it may enable anaerobic digestion at high H2 partial pressures and also maintain anabolic function. To determine whether the addition of nickel foam enabled the reactors to maintain anabolic functioning at high H2 partial pressures, tests were performed.

Figure 5. Cumulative methane yield (A) and volatile acid concentration (C) in a reactor with propionate as the substrate at a higher hydrogen partial pressure, and cumulative methane yield (B) and volatile acid concentration (D) in a reactor with butyrate as the substrate.
performed in an anaerobic fermenter inoculated separately with granular sludge from a semicontinuous digestion reactor (Figure 5). The addition of nickel foam to the two reactors of p1 and b1 resulted in a cumulative methane yield of 277 mL (p1) and 292 mL (b1) at the 24th hour. The methane production of the control reactors (p0 and b0) stagnated at the 20th hour, and the cumulative methane production was 182 mL (p0) and 194 mL (b0). In the control reactors (p0 and b0) without nickel foam, the coprophilic metabolism of propionate/butyrate was more significantly inhibited by high H2 partial pressures. In p0, the degradation of propionate had nearly stopped by the 24th hour, and in b0, the degradation of butyrate had nearly stopped by the 20th hour. It can be inferred that IHT is the predominant extracellular electron transport pathway in p0 and b0. At high hydrogen partial pressures, the co-colonial metabolism of propionate or butyrate in the nickel foam reactors (p1 and b1) was hardly inhibited. This indicates the appearance of other pathways of electron transfer in the reactor with the addition of nickel foam to complete the metabolism of propionate and butyrate.

The concentration of all types of volatile acids in the reactor was decreased to low levels after 24 h. This result may be due to the enhanced DIET pathway in the p1 and b1 reactors as a result of the addition of nickel foam. The synergistic mechanism of nickel foam-based DIET can successfully avoid the adverse effects of higher hydrogen partial pressure and maintain the normal conversion of propionate and butyrate to CH4. The results verify that nickel foam can act as an electron transfer channel between VFA-oxidizing bacteria and methanogens in coculture systems, allowing substrates to be metabolized through the less energy-consuming DIET pathway.

4. CONCLUSIONS

This trial showed that nickel foam could effectively improve the degradation of butyrate and propionate by promoting DIET, with an average methane productivity increase of 18.1 and 15.9%, respectively. At the same time, the diversity of DIET, with an average methane productivity increase of 18.1

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