

Effect of Iron Supplementation On The Biogas Production and Microbial Community Distribution During Anaerobic Digestion of Food Waste Process

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Abstract

Iron as micronutrients is of great significance for forming a stable and efficient anaerobic digestion of food waste. Aim of this study was to examine the effect of iron supplementation on the mesophilic anaerobic digestion of food waste. Firstly, batch experiments were conducted with different iron concentration at a constant kitchen waste/inoculum ratio ($K/I = 1.0$), which indicated that the effect of iron on anaerobic digestion of was strictly dosage-dependent. Then, anaerobic digestion of food waste was conducted for 50 days in semi-continuous reactors with optimal iron concentration (2.0mg/L) under the same conditions. And the semi-continuous reactors obtained a good operation performance with low volatile fatty acids concentration, higher biogas production, high coenzyme F_{420} and dehydrogenase concentrations. Furthermore, two samples taken on 7th day and 50th day were analyzed by high-throughput sequencing, which illustrated that the composition anaerobe community was stable. However, the growth and activity of several syntrophic microbial groups (*Aminobacterium*, *Syntrophomonas*, *Anaerolineaceae*, *Methanosaeta*, *Methanosarcina*, *Methanobacterium* and *Methanospirillum*), were stimulated by iron supplementation. The shift of microbial community suggested that a high-efficiency microbial community for methane production from food waste was formed by iron supplementation.

1. Introduction

The annual generation of food waste (FW) is more than 1.4 billion tons globally, which accounts for approximately 33% of total annual global food production (Guo et al., 2019; Wong et al., 2018). And what's worse, the FW generation is expected to keep an ever-increasing owing to the rising living standards and population growth (Uçkun Kiran et al., 2014). FW is mainly composed of water, vegetables, fruit, meat, rice, bakery and dairy products, which easily spoil even at room temperature (Uçkun Kiran et al., 2014; Zhang et al., 2020b). Hence, huge FW not only inevitably causes a serious waste of resources, but also causes a series of environmental pollutions (Yue et al., 2020). Now FW management has been an urgent environmental, economic and social problem to be solved around the world (Gaby et al., 2017; Kaur et al., 2019).

Due to rich nutrition, FW is also considered as a typical "misplaced" renewable resource (Bedoić et al., 2020). High water content and high carbohydrate ratio make FW an ideal substrate for anaerobic digestion (AD) to produce methane (Yue et al., 2020; Zhang et al., 2020a). AD not only can realize the harmless and reduction of FW, but also can obtain biogas to realize the resource utilization of FW (Zhang et al., 2020a). Hence, AD has been one of the most suitable and efficient biological treatment for FW (Ren et al., 2018). Nevertheless, AD is a sensitive multi-stage bioprocess including hydrolysis, acidogenesis, acetogenesis and methanogenesis, which depends on different functional microorganism to degrade organic compounds into methane step by step (De Vrieze et al., 2012b). Hence, AD is easily affected by diverse environmental parameters such as pH, temperature, nutrition balance, ammonia, volatile fatty acids (VFAs) (Wang et al., 2018). The microbial population and activity are the core of the digesters to ensure long-term process stability and efficiency (Wang et al., 2018). Nutrition balance as one of environmental parameters is of great importance for the microbial growth during AD process (Choong et

al., 2016). Besides basic macronutrients such as carbon (C), nitrogen (N), phosphorus (P) and sulphur (S), trace elements (TE) such as nickel (Ni), iron (Fe) and cobalt (Co) play an irreplaceable role in enzymatic synthesis, in respiratory processes, cell structural stability and signal transduction of acidogenesis and methanogenesis(Choong et al., 2016; Eftaxias et al., 2018b; Hijazi et al., 2020; Thanh et al., 2016). In AD process, Fe not only is a component of the majority of metalloenzymes functioning to form the active site, cofactor and the structure itself, but also serves as a redox carrier to transfer electron(Harrop & Mascharak, 2005; Vintiloiu et al., 2013). Ni is an important component of coenzyme F₄₃₀ of methyl reductase which reduces methyl coenzyme M to methane in all methanogenic pathways(Friedmann et al., 1990; Thanh et al., 2016). It reported that Co is essential composition of corrinoid such as vitamin B12, which can bind to the coenzyme methylase to catalyze methane formation in both acetoclastic methanogens and hydrogenotrophic methanogens(Friedmann et al., 1990). Hence, adequate trace elements could support the microbial metabolism to maintain an effective AD process. However, one of remarkable characteristics of FW is low trace element, which could rigorously limit its AD(Hijazi et al., 2020; Zhang & Loh, 2019). Therefore, supplementation of trace elements is necessary and promising for efficient biogas production of FW.

In the literature, there are a number of reviews on the synergistic effect of trace metals supplementation in AD(Eftaxias et al., 2018a; Molaey et al., 2018; Schmidt et al., 2018; Wang et al., 2021). To our knowledge, no studies have examined how single iron addition effects the methane production and microbial community during AD of FW. This work reports on the influence of Fe addition on AD performance of FW. The results focus on the effect on biogas quantity, quality, coenzyme F₄₂₀, dehydrogenase concentration and microbial communities, especially methanogens.

2. Material And Methods

2.1. Substrates and inoculum

Table 1
The characteristics of inoculum sludge and kitchen waste

	Inoculum sludge	Food waste
pH	7.20 ± 0.00	6.90 ± 0.01
TS (%)	3.86 ± 0.01	26.84 ± 0.01
VS (%)	1.44 ± 0.01	29.36 ± 0.01
The moisture content (%)	96.14 ± 0.01	73.16 ± 0.01
C/N	7.12 ± 0.01	20.46 ± 0.01

KW was collected from the Wuhan University cafeteria (Wuhan, Hubei province, China). First, indigestible substrates such as plastic and chopsticks were removed from FW before being homogenized, then the

homogenized KW substrates were kept in a -20°C fridge until required. The microbial inoculum (seed sludge) used was collected from an anaerobic digester in Municipal Engineering Ltd., Wuhan University, Wuhan. The seed sludge was first left undisturbed for 48h at 37°C to remove endogenous biogas production. Detailed characteristics of the substrate and seed sludge used in this study are presented in Table 1.

2.2. Batch trials for methane production from FW in the presence of different concentration of iron

Batch trials were conducted in a series of 2.0L identical serum bottles containing 0.2L of seed sludge and 0.8L of Milli-Q water to a final working volume of 1.0L with K/I ratio of 1.0 at 37°C. Then, different volume of iron supplement solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in deionized water was added into serum bottles to obtain different concentration of iron (0, 0.5, 1.0, 2.0, 4.0 and 6.0mg/L). All experiments were conducted in triplicate. The cumulative biogas production, methane yield, and coenzyme F_{420} and dehydrogenase were measured after 72h. No alkalinity or buffering agent was added into the system before the reaction, and the pH value was not adjusted during the process.

2.3. Biogas production and microbial community structure in semi-continuous AD experiment with optimal iron concentration

Semi-continuous reactors were operated to assess the effect of long-term supplementation of iron on methane production and microbial communities during AD of FW. First, 0.4L of seed sludge and 1.6L of Milli-Q water were added in each semi-continuous reactor to obtain a 2L working volume at 37°C. Second, each reactor was fed with K/I = 1.0 once a week to maintain a hydraulic retention time of 14 days. Meanwhile, a certain volume of supplement solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was added into each reactor to the optimal iron concentration obtained by batch trials at 2-day interval. Then reactors were manually shaken for 2min twice a day. Cumulative biogas production was measured every two days. The methane yield, coenzyme F_{420} and dehydrogenase concentrations were measured every week. Finally, to characterize the shifts in microbial communities in response to long-term supplementation of iron during AD of FW, samples taken on days 7 and 50 were analyzed via high-throughput sequencing. Three parallel reactors were conducted.

2.4. Analytical methods

The total volume of biogas production was measured via the drainage method at each time interval. Biogas samples were collected from the bottle headspaces using aluminum gas. The biogas composition in the biogas samples collected from the bottle headspaces using aluminum gas were quantified by gas chromatography (GC-2030, Tet Instrument, China) equipped with a thermal conductivity detector (TCD) and a TDX-01-21 column (1m×3mm). The operating temperatures of the injector, column, and detector were kept at 250, 200, and 110°C, respectively. High purity nitrogen was used as carrier gas at a flow rate of $500 \text{ mL} \cdot \text{min}^{-1}$.

Volatile fatty acids (VFAs) analysis was performed using a gas chromatography (GC-2030, Tet Instrument, China) equipped with a flame ionization detector (FID). First, the samples were harvested by centrifugation at $14,000 \times g$ for 15 min, after which the supernatants were filtrated. Then filtrated liquid digestate was mixed with 100mg/L of crotonic acid in a volume ratio of 9:1. Finally, 1 μL mixed liquid was injected onto an organic acid separation column (3m \times 3mm). The temperatures of the injection port, column, detector was 110, 200 and 280°C, respectively. High purity nitrogen was used as carrier gas (500 mL \cdot min⁻¹).

Dehydrogenase activity and coenzyme F₄₂₀ were measured via 2,3,5- triphenyltetrazoliumchloride spectrophotometry (Gabbita & Huang, 1984) and ultraviolet visible light spectrophotometry (Grochowski & White, 2010), respectively.

2.5. Metagenomic analysis

To study the effect of supplementation of iron on microbial communities during AD of FW, sludge samples were taken on days 7 and 50. The total DNA of each sample was extracted with the TIANamp Bacteria DNA Kit (TIANGEN Biotech Co., Ltd., Beijing, China) following the manufacturers' instructions. The DNA samples were then sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for high-throughput sequencing to elucidate the shifts in microbial communities. Microbial 16SrRNA sequences were amplified via polymerase chain reaction (PCR) using the forward ArBa515F (50-GTG CCA GCM GCC GCG GTA A -30) and reverse Arch806R (50- GGA CTA CVS GGG TAT CTA AT-30) primer pair (28). The complete genome of the sampled bacteria was sequenced in an Illumina MiSeq system (PE300, Illumina, San Diego, CA, USA) following the manufacturer's instructions.

3. Results And Discussion

3.1. Batch trials for methane production from FW in the presence of different concentration of iron

Figure 1 presented biogas production and methane yield with different concentrations of iron. As shown in Fig. 2A, the biogas production was 1725, 1731, 1777, 1821, 1737 and 1543ml at 0.0, 0.5, 1.0, 2.0, 4.0 and 6.0mg /L of iron, respectively. Although the biogas production increased with the increasing of iron concentration between 0.5mg/L to 2.0mg/L, no significant increase was observed. However, when the iron concentration exceeded 2.0mg/L, the biogas production was reduced. Figure 1B illustrated that low concentrations of iron could enhance methane yield, whereas high concentrations of iron had an inhibitory effect on methane yield. And the maximum methane yield achieved at 2.0mg/L of iron was 137.5ml/g \cdot VS, which was approximately 1.17-fold of that in the control. The results are consistent with several previous studies (Choong et al., 2016; Facchin et al., 2013; Meng et al., 2013; Yu et al., 2015). Iron as an indispensable micronutrient could effectively enhance the activity of anaerobes and promote the growth of anaerobes at relatively lower concentration (Choong et al., 2016). However, high concentration of iron is easy to cause a toxicity on anaerobes, inhibit the process of methanogenesis (Choong et al.,

2016; Facchin et al., 2013). Therefore, the effect of iron on KW AD exhibited a hormetic trend with 2.0mg /L as the threshold.

3.2. Methane yield and biogas production with optimal iron concentrations in semi-continuous AD experiment

Figure 2 illustrated the time course of the cumulative biogas production and methane yield profiles with optimal iron concentrations in semi-continuous reactors. First of all, the cumulative biogas production rapidly increased at the beginning, and then gradually stabilized in each cycle. Notably, the total accumulative volume of biogas was about 4100ml in each cycle, which shown no significant impact on cumulative biogas production in semi-continuous reactors. However, as the reaction progresses, the time needed to reach the maximum biogas accumulation gradually decreased in each cycle. Meanwhile, methane yield gradually increased at the end of each cycle. Methane yield in 7th cycle for iron supplementation group was 168.68mL /g·VS. Compared to the first cycle, methane yield in 7th cycle increased by 18.6%. These phenomena demonstrated that supplementation of iron in semi-continuous reactors could enhanced methane content of biogas production. Iron could act as cofactors in enzymes that are required during methane fermentation(Yu et al., 2015; Zandvoort et al., 2006). Hence, for FW with low trace element content, supplementation of iron in AD system could enhanced methane yield.

3.3. Effect of optimal iron concentration on VFAs in semi-continuous AD experiment

VFAs are the main intermediate products that convert to methane(Tian et al., 2015; Velmurugan et al., 2010). Figure 3 showed the composition and variation of VFAs during AD of KW in semi-continuous AD experiment. As shown in Fig. 3, VFAs were composed of acetic acid, propionic acid and butyric acid. And the acetic acid content is the highest, followed by propionic acid. Besides, the variation of each VFAs was very similar. The concentration of each VFAs in each cycle firstly increased, and then decreased. Notably, as the reaction progresses, the maximum concentration of each VFAs in each cycle decreased. For example, the maximum concentration of acetic acid and propionic acid in last cycle was 0.25g/L and 0.06mg/L, which was about 0.38-fold of that in the first cycle, respectively. After 35 days, there was almost no butyric acid. Hence, the iron supplementation promoted VFAs degradation and prevented acid accumulation.

The bidirectional Wood-Ljungdahl (Reductive Acetyl-Coenzyme A) pathway is main route for many acetoclasts and acetogens to convert acetate to H₂ and CO₂. Enzymes carbon monoxide dehydrogenaseacetyl-coenzyme A synthase (CODH-ACS) and methyl-transferase-Ac Co-A are two important enzymes for this route, which need sufficient iron(Bender et al., 2011; Dobbek et al., 2001). Besides, it reported that reactions yield included into bidirectional Wood-Ljungdahl pathway was very little energy (e.g. acetate to bicarbonate: +25kJΔG⁰; propionate to acetate: +18kJ ΔG⁰)(Thauer et al., 1977), which makes the pathway easy to proceed. Therefore, relatively large amounts of iron are expected to be required for growth in acetoclastic/propionoclastic populations. So iron supplementation was beneficial to these enzymes synthesis.

3.4. Effect of optimal iron concentration on concentrations coenzyme F₄₂₀ and dehydrogenase concentrations in semi-continuous AD experiment

Dehydrogenase is an essential intracellular microbial enzyme (Salazar et al., 2011) and is widely used as an indicator of overall microbial activity occurred intracellularly in all living microbial cells. Moreover, coenzyme F₄₂₀ (7, 8-didemethyl-8-hydroxy-5-deazariboflavin derivative), a special coenzyme for electron transport, only presents in methane-producing microorganisms. Therefore, it is feasible to use coenzyme F₄₂₀ concentration as an indicator of methanogenic activity (Cheng et al., 2007; Whitmore et al., 1986).

Figure 4 shown the time course of coenzyme F₄₂₀ and dehydrogenase concentration with optimal iron concentration in semi-continuous AD reactors. The variations of coenzyme F₄₂₀ and dehydrogenase concentration were very similar. As the experiment went on, coenzyme F₄₂₀ and dehydrogenase concentration both increased, and obtained their maximum at the end 7th cycle. As shown in Fig. 4, the maximum dehydrogenase concentration was 119.71mg/L, which increased by 16.3%. The maximum coenzyme F₄₂₀ concentration in Fe supplementation groups were 0.24μmol/. And compared to the first cycle, coenzyme F₄₂₀ concentration in 7th cycle increased by 25.68%. As indispensable micronutrient, addition of iron in semi-continuous AD reactors could enhance the activity of anaerobe and promote the growth of anaerobe, especially methanogens (Choong et al., 2016; Eftaxias et al., 2018b; Molaey et al., 2018). These experimental results were consistent with the previous results of cumulative biogas production and methane yield in iron supplementation semi-continuous AD experiment. Increasing of the coenzyme F₄₂₀ and dehydrogenase concentration indicated a high activity of anaerobe, especially methanogens. Hence, the methane yield increased.

3.5. Shift of microbial community with optimal iron concentration in semi-continuous AD experiment

The AD operational conditions could change the microbial community structure, which determine methane production (Town et al., 2014). Two biomass samples collected at days 7 and 50 were analyzed via high-throughput sequencing, after which the resulting gene sequences of individual samples were assigned to different taxa levels (from phyla to genus). In this study, only the major groups (genera and phyla) with a relative abundance of no less than 0.1% were assigned to said samples. Changes of anaerobe community structure in semi-continuous reactor with optimal iron concentration shown in Fig. 5. As shown in Fig. 5A, the composition anaerobe community at phyla level was stable. The main anaerobe phyla were *Synergistetes*, *Firmicutes*, *Euryarchaeota*, *Bacteroidetes* and *Chloroflexi*, which are known to be involved in different phases of the AD process (Nelson et al., 2011). Other phyla such as *Proteobacteria*, *Actinobacteria* and *Armatimonadetes* were identified in minor proportions, which is consistent with previous studies (Ariesyady et al., 2007; Herson et al., 2006). In the early stage, *Synergistetes* (ca. 31.02%) was the most dominant anaerobe at the phylum level, followed by *Firmicutes* (ca. 23.94%), *Euryarchaeota* (ca. 16.44%), *Bacteroidetes* (ca. 16.44%) and *Chloroflexi* (ca. 4.19%).

However, after 50 days, *Euryarchaeota* grew to be the dominant anaerobe with relative abundances of 32.27%, while the relative abundances of *Firmicutes*, *Synergistetes*, *Bacteroidetes* and *Chloroflexi* were 22.67%, 16.70%, 16.25% and 6.68%, respectively. The result suggested that iron supplementation stimulated *Euryarchaeota* growth, which was beneficial to enhance methane yield. Conversely, *Synergistetes* was inhibited by the iron supplementation.

As shown in Fig. 5B, among the *Synergistetes* phyla, *Synergistaceae_uncultured* and *Aminobacterium* were the major genera during the entire process (42). As anaerobic reaction proceeded, the relative abundance of *Synergistaceae_uncultured* decreased from 25.49–7.5%, which was the main reason for the decreased of the relative abundance of *Synergistetes*. However, the relative abundance of *Aminobacterium* increased from 6.29–9.23%. Meanwhile, the relative abundance of *Anaerolineaceae_* (within the phylum of *Chloroflexi*) and *Syntrophomonas* (within the phylum of *Firmicutes*) increased from 3.76% and 1.43–5.19% and 7.05%, respectively. Notably, the enhancement of *Syntrophomonas* growth was strongest. *Aminobacterium* as a mesophilic amino-acid-degrading bacteria could grow in syntrophic interactions with the hydrogenotroph *Methanobacterium* by fermentation of a limited range of amino acids (Baena et al., 1998; Chertkov et al., 2010). Protein is an important component of KW; and it could be hydrolyzed to amino acids by hydrolytic bacteria. Hence, the enhancement of *Aminobacterium* growth stimulated by iron supplementation would guarantee an effectively syntrophic degradation of protein and improve the methane yield from FW. *Syntrophomonas*, as a syntrophic metabolizer with hydrogenotrophic methanogens, could convert fatty acids into acetate, H₂, formate and CH₄ (Zhang et al., 2020c). Therefore, the strongest enhancement of *Syntrophomonas* growth was beneficial to sustain efficient syntrophic communities of bacteria and archaea to avoid over accumulation of intermediate products such as H₂ and VFAs (Zhang et al., 2020c), which was in agreement with the change of VFAs shown in Fig. 4. Besides, it was reported that *Anaerolineaceae* can use H₂ as electron carrier to degrade alkane and long-chain fatty acid (LCFA) into methane by cocultivation with hydrogenotrophic methanogens (Liang et al., 2015; Liang et al., 2016). FW contains a certain amount of lipid, which has high methane yield potential. And lipid initially is hydrolyzed to LCFA by extracellular lipases that are excreted by the acidogenic bacteria and then glycerol, then is converted to acetate and hydrogen (H₂) through the β -oxidation pathway (syntrophic acetogenesis), and finally to methane (CH₄) by methanogenic archaea (Alves et al., 2009; Zhang et al., 2020b). Hence, the enhancement of *Anaerolineaceae* growth would be conducive to the degradation of lipid into methane.

Figure 5B also shown that no significant change of archaeal community structure with iron supplementation in the semi-continuous AD reactors after 50 days. And the phylum *Euryarchaeota* was primarily represented by the genus *Methanosaeta*, *Methanosarcina* *Methanobacterium* and *Methanospirillum*. As anaerobic reaction proceeded, the relative abundance of *Methanosaeta*, *Methanosarcina*, *Methanobacterium* and *Methanospirillum* increased from 15.08%, 11.46%, 6.34% and 1.43–25.67%, 20.19%, 7.73% and 4.11%, respectively. Obviously, during the AD process, supplementation of iron enhanced these four methanogens growth. And the enhancement of *Methanosaeta* growth was strongest, followed by *Methanosarcina*. Notably, *Methanosaeta* always was the most dominant archaeal

genus in the semi-continuous AD reactors. *Methanosaeta*, an acetic-utilization methanogens, could converted acetate into methane, and its importance for methane production(Conklin et al., 2006; De Vrieze et al., 2012a; De Vrieze et al., 2015). This result is consistent with the observations of Zhang et al. (Zhang et al., 2020c). *Methanosarcina* as second dominant archaeal genus is a type of both acetoclastic and hydrogenotrophic methanogens. It could produce methane from acetate, methanol, monomethylamine, dimethylamine, trimethylamine, H₂/CO₂, and CO(Garcia et al., 2000). Thereby, the enhancement of *Methanosaeta* and *Methanosarcina* contributed to avoid over accumulation of VFAs. Besides, *Methanospirillum* and *Methanoculleus* detected in this process were both hydrogenotrophic methanogens. But the enchantment of them by iron supplementation was relatively low.

According to the results of the shift of microbial community, iron supplementation affect AD performance by forming a high-efficiency microbial community for methane production from KW. As an essential element for anaerobe, iron can synthesize and activate some key coenzymes involved in methanogenesis, such as such as formylmethanofuran dehydrogenase, hydrogenase, acetyl-coenzyme A synthetase, carbon monoxide dehydrogenase, coenzyme M methyltransferase, Ferricytochrome c, F₄₂₀H₂ dehydrogenase and heterodisulfide reductase (Baek et al., 2019; Qi et al., 2021; Schattauer et al., 2011), which are significant form the acid production to methane formation during AD process. For example, carbon monoxide dehydrogenase plays an important role in methane formation form acetate and acetate production from H₂, CO₂ and methanol(Schattauer et al., 2011). Hydrogenase consumes H₂ to offer electrons to CO₂ to produce methane by hydrogenotrophic methanogens (Choong et al., 2016). In short, the shift of microbial community suggested a dependence of methanogenic performance on iron.

4. Conclusions

The effect of iron on AD of KW presented a hormetic pattern. Iron supplementation with concentration of $\leq 2.0\text{mg/L}$ enhanced the methane yield, whereas higher iron concentration weakened methane yield. Digesters with optimal iron concentration maintained stable and efficient AD performance presented by high methane yield, low VFAs concentrations, high coenzyme F₄₂₀ concentrations and high dehydrogenase concentrations. Pyrosequencing results revealed that the phylum *Synergistetes* was the most dominant at the beginning, whereas *Euryarchaeota* became dominant phyla after 50 days. Iron supplementation improve the activity of bacteria (*Aminobacterium*, *Syntrophomonas* and *Anaerolineaceae*) and archaea (*Methanosaeta*, *Methanosarcina* *Methanobacterium* and *Methanospirillum*), which formed a high-efficiency microbial community for methane production from KW by AD.

Declarations

Authors' contributions Yue Xu and Jing Zhang: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing; Rongtang Zhang,

Liujie Sheng, Xinghua He, Haijun Lu and Na Wei: Writing - review & editing, Supervision. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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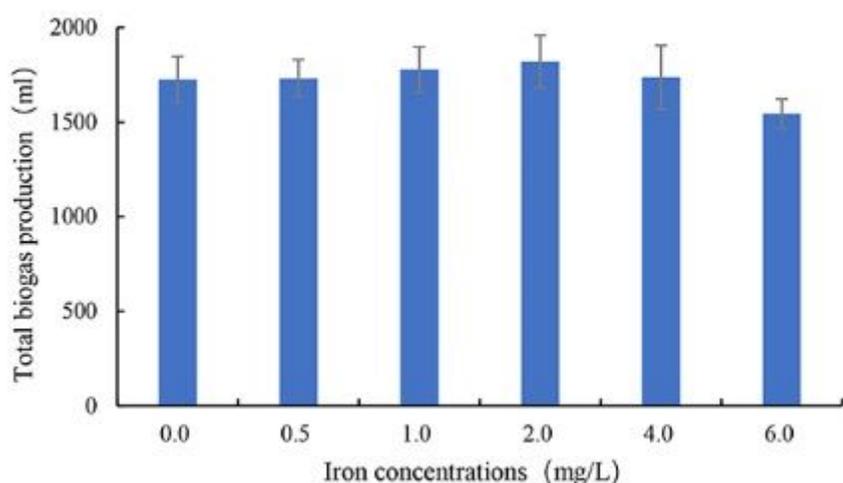
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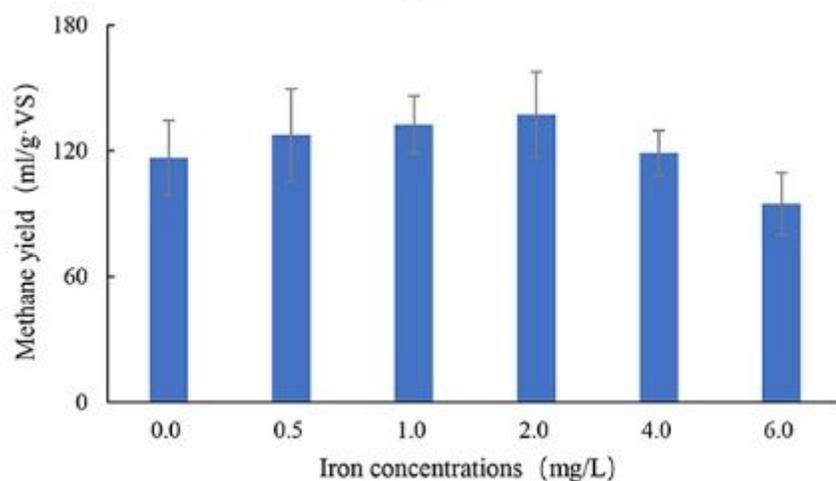
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Figures



A



B

Figure 1

Total biogas production (A) and methane yield (B) under different Iron concentrations

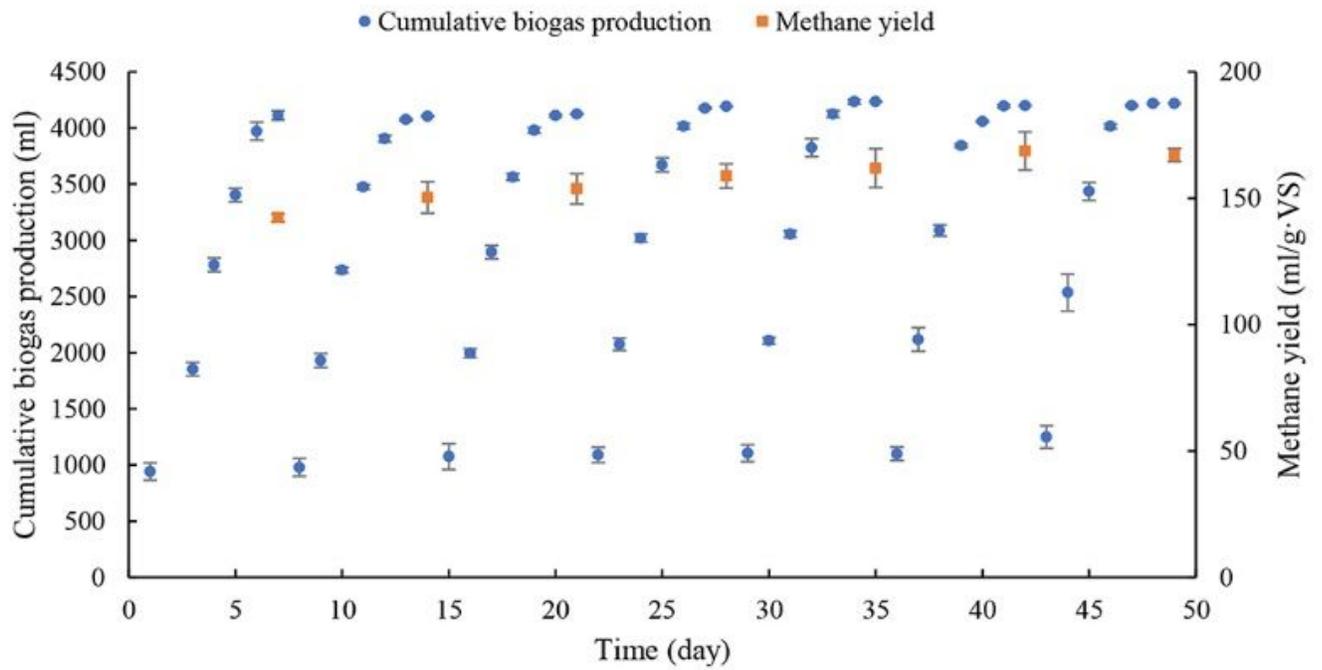


Figure 2

Changes in cumulative biogas production and methane yield profiles with optimal iron concentrations in semi-continuous reactor

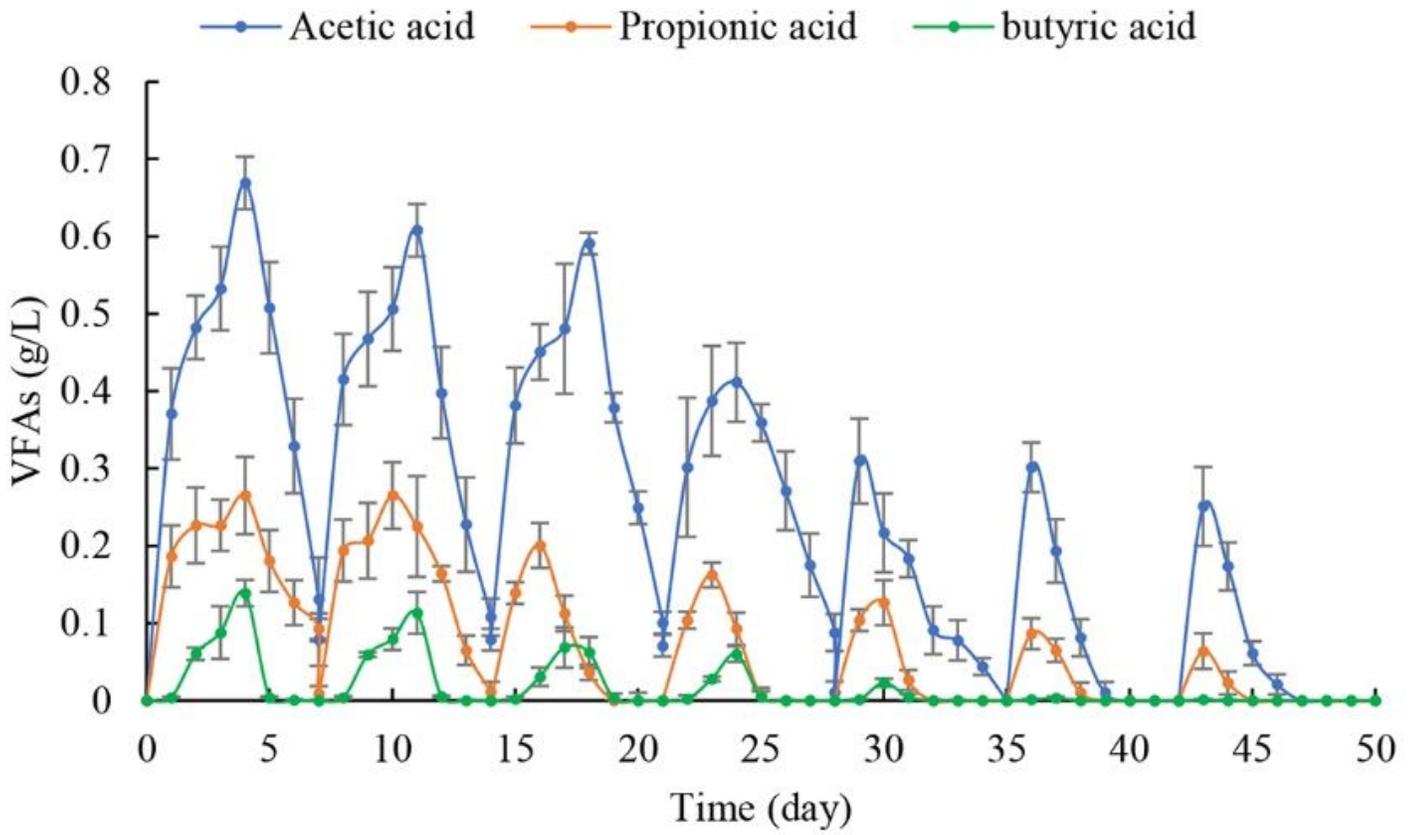


Figure 3

The composition and variation of VFAs during AD of KW in semi-continuous AD experiment

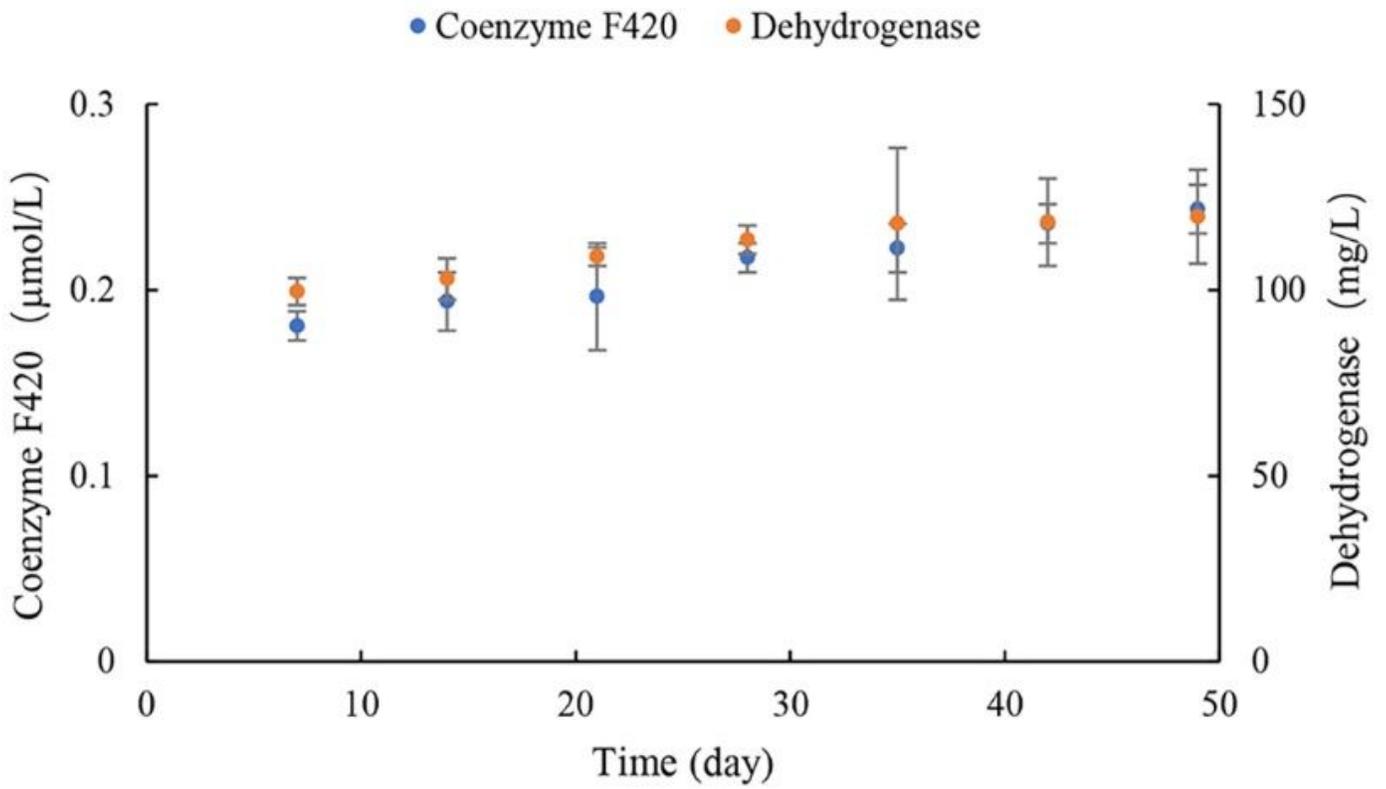


Figure 4

The variation of Coenzyme F420 and dehydrogenase concentrations during AD of KW in semi-continuous AD experiment

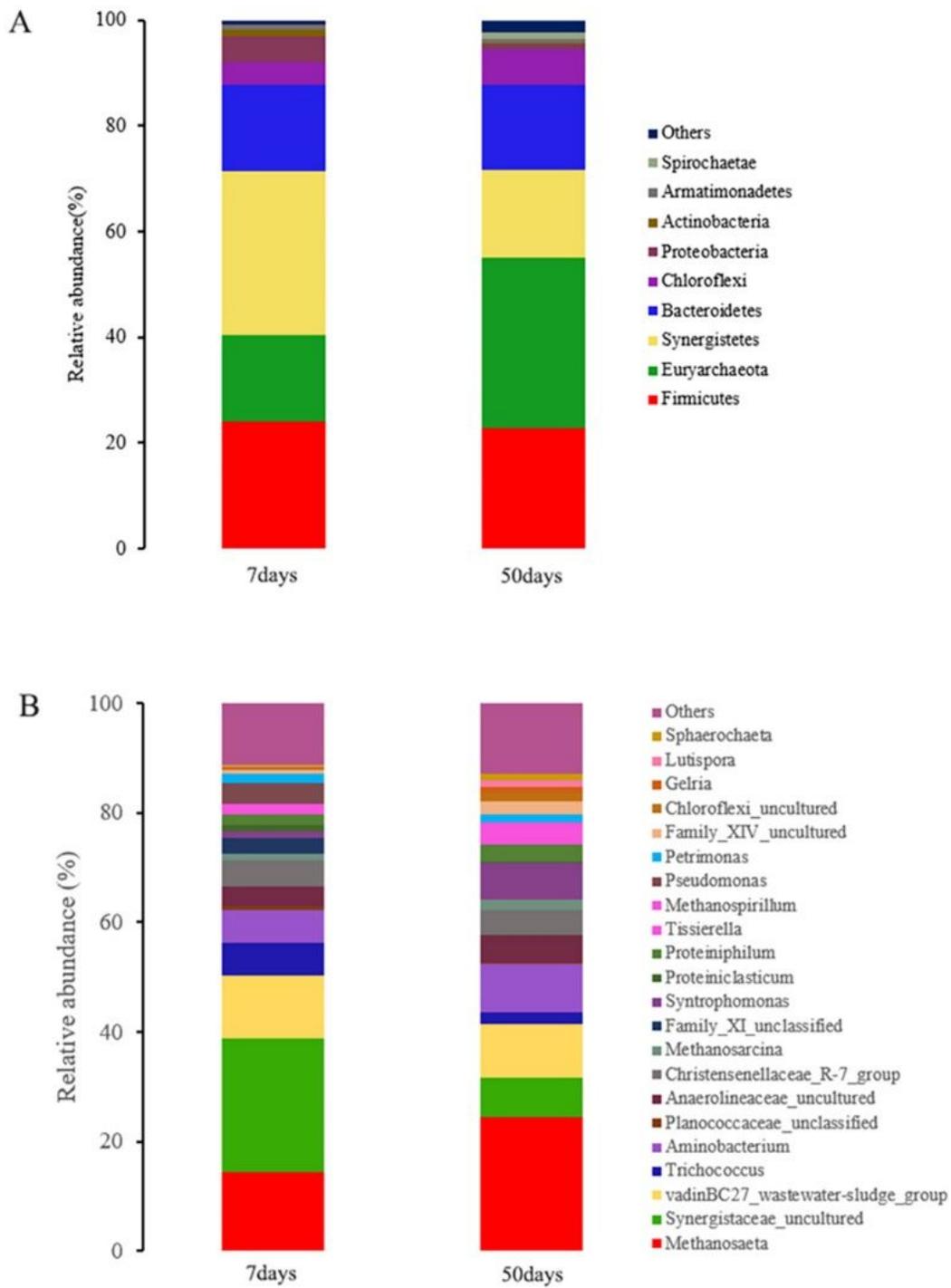


Figure 5

The shift of microbial community at phylum-level (A) and genus-level (B)