New Insight into Magnetic Enhanced Methane Production from Oily Sludge via Mesophilic Anaerobic Degradation Processes

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Abstract: Anaerobic digestion is a promising technology for treating and disposing of oily sludge, but the presence of oil in the sludge reduces methane production and sludge volume reduction. To overcome this limitation, this study creatively reports the use of magnetite to enhance methane production in oily sludge mesophilic anaerobic digestion and elucidates the underlying mechanism. Results show that the addition of magnetite increases methane production, with a 5% magnetite content leading to a 1.42-fold increase in cumulative methane output compared to the blank. Mechanistically, magnetite accelerates the release of organic matter, promotes oil degradation, increases volatile fatty acids (VFA) accumulation, and reduces the proportion of propionate. Additionally, magnetite alleviates pH decreases and increases the release of ammonia nitrogen and phosphate, resulting in effective sludge reduction, with volatile suspended solids (VSS) reduction ranging from 26.9% to 32.6%, higher than that of the blank. Moreover, magnetite accelerates electron transfer and increased the relative abundance of microorganisms associated with methane production, with the relative abundance of Methanosarcina increasing to 37.6~38.5% due to the presence of magnetite. This study provides a theoretical framework for effectively utilizing oily sludge through the application of magnetite.

Keywords: magnetite; oily sludge; volatile fatty acid; anaerobic digestion; microbial community

1. Introduction

Industries such as chemical, pharmaceutical, and coking often generate a large volume of oily sludge as a by-product during the biological treatment of wastewater. With the increasing demand for petroleum energy, the annual production of oily sludge worldwide is expected to reach \(2.2 \times 10^7\) t/a, and in China alone, it is projected to reach \(3.2 \times 10^6\) tons/year [1,2]. Oily sludge is considered hazardous waste, and if not disposed of properly, it could lead to serious ecological safety problems [3]. Landfilling, composting, and anaerobic digestion are among the methods used for oily sludge disposal [4,5]. Anaerobic digestion has gained significant attention due to its ability to recover energy gas and simultaneously achieve sludge reduction and harmless treatment [6].

Oily sludge differs significantly from traditional municipal sludge in terms of physical and chemical characteristics, which are evident in the following aspects. Firstly, oily sludge has a high oil content, which makes it easy to accumulate long-chain fatty acids (LCFA) during digestion and hinders the digestion process [7,8]. Secondly, oily sludge has high viscosity and emulsification, which are not conducive to microbial degradation [9]. Finally, the aging process in the production of oily sludge results in a low potential for methane production during anaerobic digestion. Therefore, improving the anaerobic digestion of oily sludge has become a topic of great interest for engineering practitioners and researchers [10].

Magnetite particles have been used as conductive materials in waste treatment processes. Magnetite is composed of iron oxides of different valence states of Fe (III) and Fe (II).
Magnetite shows specific mineral redox properties and electrical conductivity and is an ideal exogenous medium material to enhance the microbial extracellular electron transfer process. In the anaerobic digestion process, magnetite is considered beneficial to electron transfer and improves fermentation efficiency [11]. In addition, the presence of magnetite particles can react with the acid in the anaerobic reaction system to release hydrogen, thus improving the enrichment of hydrogen-consuming microorganisms and the conversion of propionic acid in the system [12,13]. Kang and Liu [14] evaluated the impact of magnetite particles on the anaerobic digestion and recovery of biogas from excess sludge and pointed out that increasing the concentration of magnetite in the range of 0–5.0 g/L was conducive to biogas generation. Zhang et al. [15] reported that magnetite nanomaterials increased the biogas production from sludge two-stage anaerobic digestion and reduced the waste sludge volume, and the yields of hydrogen and methane were as high as 11.9 mL/g and 109.8 mL/g, respectively. The particle size of magnetite nanomaterials can also significantly affect anaerobic digestibility. Hassanpourmoghadam et al. (2023) found that the addition of 120 mg/L of smaller nanoparticles had the greatest impact, with a 1.7-fold increase in methane production, a 26% increase in VSS damage, and a 35% increase in total chemical oxygen demand removal [16]. Magnetite can improve the biogas production of anaerobic co-digestion of pig manure and wheat straw, and 3.0 g/L was considered the optimal dose; the corresponding biogas production was 195 mL/g [16]. The mechanism analysis showed that the cellulase and dehydrogenase activities were significantly improved due to the presence of magnetite [17]. Although the above-mentioned magnetite enriches the resource utilization of organic matter, whether magnetite can alleviate the inhibition of oil on anaerobic digestion of sludge and improve digestion efficiency has not yet been investigated, and the relevant mechanisms involved, especially the microbiological mechanism, are still unclear.

Therefore, in this work, pilot-scale mesophilic anaerobic digestion reactors were built to investigate the impact of magnetite on methane yield, organic matter conversion and removal, volatile fatty acid accumulation, digestion by-product release characteristics, and microbial community characteristics in the anaerobic digestion process of oily sludge. The mechanism of how magnetite enhances the anaerobic digestion of oily sludge was also analyzed to reveal the underlying process. The findings of this work provide valuable insights into the efficient utilization of oily sludge as a resource.

2. Materials and Methods

2.1. Source of Experimental Materials

The oily sludge was from the waste sludge produced by the anaerobic biological wastewater treatment plant containing waste oil. The recovered oily sludge was used on standby after removing large particle impurities through sedimentation. The main characteristics of oily sludge used in the experiment are presented in Table 1.

Table 1. Main characteristics of oily sludge and inoculum used in the work.

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>Oily Sludge</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>6.9 ± 0.1</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>Total suspended solids (TSS)</td>
<td>g/L</td>
<td>16.3 ± 0.3</td>
<td>8.2 ± 0.2</td>
</tr>
<tr>
<td>volatile suspended solids (VSS)</td>
<td>g/L</td>
<td>12.3 ± 0.1</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>Total chemical oxygen demand (TCOD)</td>
<td>g/L</td>
<td>23.6 ± 0.8</td>
<td>11.3 ± 0.5</td>
</tr>
<tr>
<td>Soluble chemical oxygen demand (SCOD)</td>
<td>mg/L</td>
<td>561 ± 25</td>
<td>320 ± 12</td>
</tr>
<tr>
<td>VFA</td>
<td>mg/L</td>
<td>185.6 ± 0.1</td>
<td>162.3 ± 8.9</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>mg/L</td>
<td>123 ± 5.6</td>
<td>52 ± 6</td>
</tr>
<tr>
<td>Soluble polysaccharides</td>
<td>mg/L</td>
<td>62 ± 0.6</td>
<td>/</td>
</tr>
<tr>
<td>Oil content</td>
<td>mg/L</td>
<td>834 ± 32</td>
<td>/</td>
</tr>
</tbody>
</table>

Note: the values presented are the average and standard deviation of three determinations.
The inoculum was taken from a continuous anaerobic digestion reactor in a laboratory, which mainly dealt with municipal sludge, and its organic matter removal efficiency was as high as 85%. The retrieved inoculum was filtered to remove impurities and then used for standby. The main characteristics of inoculated sludge are also displayed in Table 1.

The magnetite used in the experiment was purchased from Aladdin Pharmaceutical Company. The diameter of the magnetite powder was about 0.2 µm. The relative density of magnetite was 5.1. The Fe content was 72.1%, and the purity of magnetite exceeded 95%.

2.2. Effect of Magnetite on Oily Sludge Anaerobic Digestion

This work was carried out in five sets of cylindrical sequencing batch reactors with the same volume (effective working volume 5.0 L); each set of reactors contains three parallel reactors. The reactor was composed of plexiglass and equipped with a mechanical stirring device, and the rotating speed was controlled at approximately 100–200 rpm to ensure adequate mixing of the inoculum and digestion substrate during the operation. Then, each reactor was introduced into 2.0 L inoculum and 3.0 L oily sludge, and the volume ratio of inoculum to oily sludge was 2 to 3, which was slightly improved according to previous literature [18]. Subsequently, different levels of magnetite were added to the reactor and controlled at 0, 1%, 3%, 5%, and 7% (in terms of TS content). The above reactors were defined as R0–R4. In addition, another reactor was inoculated with only 2.0 L of inoculum and 3.0 L of distilled water to determine the contribution of the inoculum’s own anaerobic degradation to methane production. Finally, each reactor was filled with high-purity nitrogen (>99.99% purity) to remove oxygen to ensure a strictly anaerobic environment, and the above reactors were sealed and transferred to a constant temperature (35 ± 2 °C) air water bath shaker for mesophilic anaerobic digestion. During the period of anaerobic digestion, the changes in biogas production, chemical substances in digested liquid, and sludge characteristics were measured regularly to clarify the behavior characteristics of magnetite-enhanced anaerobic resource utilization of oily sludge.

2.3. Analytical Method

TSS, VSS, phosphate, and COD were determined according to the international standard method [19]. pH was determined via pH analyzer (Leici, PHS-3S), and methane and VFA were determined via gas chromatography. Ammonia nitrogen was determined using Nessler’s reagent spectrophotometry. The total gas production and methane production were determined via drainage method. Before measuring the volume of methane, phenolphthalein was used as an indicator, and 2.0 mol/L NaOH was used to absorb acidic gases (such as carbon dioxide). The chromatograph was equipped with thermal conductivity detector and flame ionization detector for the determination of methane and VFA, respectively. The detailed determination steps of methane are described in the literature [20,21]. Protein and polysaccharide were determined via Folin phenol method and anthrone colorimetry, and bovine serum albumin and glucose were used as standard substrates, respectively.

The content of VFA was analyzed via gas chromatography (GC-7900P/FID) equipped with flame ionization detector (FID). Firstly, the sludge sample was centrifuged at 10,000 r, and the supernatant, after centrifugation, was passed through the membrane with a 10,000 µm filter membrane. The supernatant of the sludge after 10,000 mL was taken into the liquid phase vial, and then 3% formic acid was added for neutralization, and the accurate content of VFA was obtained via conversion using the standard curve. The conversion ratios of acetate, propionate, and butyrate to total COD were 1.07, 1.51, and 1.82 mg COD/L, respectively. The key enzymes associated with methane production in the experiment include protease, amylase, acetokinase (AK), Phosphotransferase (PTA), and F420, and the procedures for the determination of the above key enzymes were described in the literature [21]. Protease determination: the mixture was quickly and repeatedly washed 3 times with 0.1 M phosphate buffer (PBS) and then resuspended with PBS. The suspension was first treated in an ultrasonic cell crusher (20 kHz, 4 °C) for 10 min and then used at 10,000 r/min at 4 °C. Then, the mixture was centrifuged for 15 min, mixed 3.0 mL.
of pre-treatment supernatant with 1.0 mL of substrate, and incubated at 37 °C for 90 min. 2.0 mL of 10% trichloroacetic acid was added to terminate the reaction. The mixture was colorized at a wavelength of 400 nm. The pretreatment step before AK determination is the same as the pretreatment step of protease determination. A total of 140 µL of the pretreated mixture was taken, and then 160 µL cell extract was added. The initial absorbance value was determined at 340 nm wavelength, and the endpoint absorbance value was determined at the end of 5 min reaction. The pretreatment steps for F420 enzyme activity determination are as follows: 10 mL of the mixture discharged from different reactors were washed quickly and repeatedly twice with 0.9% sodium chloride solution 20 mL, respectively, and then the supernatant was discharged and re-suspended with 15 mL 0.9% sodium chloride solution. The suspension was heated at 95 °C for 20 min before cooling, and the supernatant was obtained by adding 2.5 times ethanol solution after cooling and centrifuging at 6000 r/min for 15 min. The liquid was divided into two parts, one was adjusted pH to 13.5 with 4.0 M sodium hydroxide solution as the sample to be tested, the other was adjusted pH to less than 3 with 6 m hydrochloric acid solution as reference sample, and the absorbance of the two samples was determined at 420 nm.

The sequencing steps of microbial population characteristics are as follows: the digested oily sludge was centrifuged at 8000 rpm for 10 min, and the deposited solid samples were obtained and stored in the refrigerator at −20 °C. Microbial samples were analyzed by a biotech company in Shanghai using high-throughput sequencing technology. The bacterial sequencing primers were F:ACTCCTACGGGAGGCAGCA and R:GGACTACHVGGGTWTCTAAT, sequencing region was 16S_V3–V4, and bacterial sequencing reference sequence database was Silva database (http://www.arb-silva.de (accessed on 5 April 2022)). The primers of archaea community sequencing were F: TGYCAGCGCCGCGGTAA and R: YCCGGCGTTGAVTCCAATT, sequencing region was 16S_V4–V5 region, and reference sequence database was Silva database (http://www.arb-silva.de (accessed on 5 April 2022)).

3. Results and Discussion

3.1. Effect of Magnetite on Methane Production by Anaerobic Digestion of Oily Sludge

Cumulative and daily methane production are key parameters for assessing the performance of anaerobic digestion. The effect of magnetite on biogas yield and methane volume share during the anaerobic digestion of oily sludge is shown in Figure 1. The presence of magnetite can influence the cumulative methane yield and daily methane production. In the R0 reactor, the cumulative methane production was 138.2 mL/g VS, slightly lower than that during the anaerobic digestion of conventional municipal sludge [22]. This lower production in oily sludge can be attributed to the inhibition of anaerobic microbial metabolism by oil and grease. However, the presence of magnetite improved the anaerobic methane production from oily sludge, with the methane enhancement closely related to the magnetite content. When the magnetite content was increased from 1% to 5%, the cumulative maximum methane production increased from 168.9 mL/g to 196.5 mL/g VS, indicating that the presence of magnetite improved the anaerobic digestion of oily sludge. However, further increasing the magnetite content to 7% did not result in a significant increase in the cumulative methane yield compared to the 5% magnetite group (p > 0.05). Therefore, a 5% magnetite content is recommended for enhancing the anaerobic digestion of oily sludge.

Figure 1b illustrates the effect of magnetite on daily methane production. Initially, all reactors showed a high daily methane production for the first 5 days, owing to the presence of abundant organic matter at the beginning of the digestion and the efficient use of digestible organic matter by methanogenic archaea. As the digestion progressed, the pH decreased, and the ammonia concentration increased, leading to a slight reduction in the activity of methanogenic archaea and, consequently, a decrease in the daily methane production. However, on 12–15 d, the daily methane production increased again due to the increased metabolic activity of methanogenic archaea. The presence of magnetite
enhanced the daily methane production of oily sludge, with the highest daily methane production observed in the group with a magnetite content of 5%, consistent with the results in Figure 1a. For instance, on day 6, the daily methane production increased to 18.9 mL/g VS, within R3, which was higher than that in the other groups (12.3 mL/g VS–16.5 mL/g VS). During 12–15 d, the peak value of daily methane production appeared again in each group, and the peaks were 13.2 mL/g VS, 15.5 mL/g VS, 16.2 mL/g VS, 16.7 mL/g VS, and 16.5 mL/g VS, respectively. The experimental results showed that magnetite promoted the anaerobic digestion of oily sludge, and the optimal content of magnetite was 5%, corresponding to a methane yield of 196.5 mL/g, which was 1.42 times higher than that in R0. Previous studies suggest that magnetite participates in the synthesis of key enzymes in the methanation process and accelerates electron transfer during the organic matter conversion process, resulting in an increase in methane yield [23]. The addition of magnetite can also enhance electron transfer, which plays a critical role in methane production. As a type of iron oxide with high conductivity, magnetite can act as an electron transfer tunnel, enabling interspecific electron transfer between bacteria and methanogenic archaea, thereby promoting the accumulation of methane [24].

Figure 1. Effect of magnetite on methane production (a) and daily methane production (b) in oily sludge anaerobic digestion. The error bar represents the standard deviation of the three measurements.

3.2. Effect of Magnetite on the Release of Soluble Organic Matter during Oily Sludge Anaerobic Digestion

The extracellular polymeric substances (EPS) and rigid cell walls that encase the sludge are crucial factors that limit the release of intracellular organic matter. This leads to hydrolysis being the rate-limiting step in anaerobic digestion [25,26]. Figure 2 shows the effect of magnetite on the hydrolysis process of anaerobic digestion of oily sludge. It can be observed that the concentration of soluble chemical oxygen demand (SCOD) within each group first increased and then gradually decreased with fermentation time. The presence of magnetite increased the concentration of SCOD, and the higher the magnetite content, the more significant the increase in SCOD. In R0, the maximum value of SCOD was 2614 mg/L.
When the magnetite content was increased to 7%, the concentration of SCOD increased to 3845 mg/L, which was about 1.47 times higher than that in R0. Similar experimental results were also found in other magnetite presence groups. These experimental results confirm that magnetite stimulated the release of organic matter from the oily sludge, thereby increasing the concentration of SCOD. The primary organic matter in oily sludge is protein and polysaccharides, and it was necessary to investigate the effect of magnetite on the concentration of soluble protein and polysaccharides. Figure 2b,c show that the changing trend of soluble protein and polysaccharide was roughly consistent with that of SCOD. This indicated that magnetite increased the concentration of soluble protein and polysaccharide, especially when the magnetite content was 7%. The concentration of soluble protein and polysaccharide increased to 945 mg/L and 402 mg/L, respectively, which was significantly higher than that in R0 (481 mg/L and 204 mg/L). The presence of magnetite increased the concentration of organic matter in oily sludge anaerobic digestion, providing material support for the metabolism of methanogenic microorganisms.

Figure 2. Effect of magnetite on release characteristics of dissolved organic matter during oily sludge anaerobic digestion ((a) SCOD; (b) soluble protein; (c) soluble polysaccharides; (d) total oil concentration). The error bar represents the standard deviation of the three measurements.

Oil is also an important degradable organic matter contained within the sludge, the concentration of oil within the fermentation broth gradually decreased, and the presence of magnetite accelerated the oil degradation. At the end of digestion, the concentration of oil in the magnetite presence group was significantly lower than that in the blank, e.g., in the 5% magnetite group, the concentration of oil and grease was 120 mg/L, whereas, in R0, the oil concentration was as high as 251 mg/L. It was confirmed that oil was converted to long-chain fatty acids during anaerobic digestion, and the accumulation of LCFA reduced the metabolic activity of methanogenic bacteria, thereby reducing methane production [27,28]. Magnetite accelerated the hydrolysis process of organic matter within the oily sludge and promoted the degradation of oil, thus increasing methane production.
3.3. Effect of Magnetite on VFA Content and Composition during Oily Sludge Anaerobic Digestion

VFA is an important value product within organic matter anaerobic digestion, which can be consumed as a digestive substrate by methanogenic archaea and then biotransformed to methane. It was confirmed that methane conversion through the acetate pathway accounts for approximately 70% of total methane production, while hydrogen consumption accounts for approximately 30% of methane production [29]. However, excessive VFA accumulation can lead to system acidification, which, in turn, reduces methane production. Figure 3a shows the variation of VFA accumulation in the oily sludge anaerobic digestion with magnetite, which showed a sharp increase and then a slow decrease in VFA, but the presence of magnetite increased the accumulation of VFA. On 5 d, the order of VFA concentration was as follows: R4 > R3 > R2 > R1 > R0. The concentration of VFA in R4 was as high as 1064 mg/L, while the concentration of VFA in R0 was only 451 mg/L, which was much lower than that of VFA in the presence of magnetite. Magnetite increased the accumulation of VFA and provided sufficient substrates for the methanogenic process.
were 38.5% and 33.5%, respectively. In R3, the percentage of acetate increased to 42.6%, but the percentage of propionate decreased to 21.6%. It was reported that magnetite affects organic matter hydrolysis and acidification processes that may be related to the process of anisotropic Fe reduction [14]. As iron oxide, magnetite can be enriched with Fe(III)-reducing microorganisms that are able to participate in the decomposition of complex organic substrates through anisotropic iron reduction [30]. In addition, the increase of Fe$^{2+}$ concentration was also found in the digestive system, which was related to the reduction of Fe$^{3+}$. In R4, the concentration of Fe$^{2+}$ was 1.52 g/L on 10 d, while the concentration of Fe$^{2+}$ on 15 d increased to 2.05 g/L, while the concentration of Fe$^{3+}$ decreased from 3.12 g/L to 2.54 g/L. A large number of electrons produced by dissimilatory iron reduction may accelerate the hydrolysis of organic compounds. The main reason for the degradation of propionate is that magnetite strengthens the direct interspecific electron transfer because of its good conductivity [14].

3.4. Effect of Magnetite on Operation Parameters of Oily Sludge Anaerobic Digestion

pH is a key parameter in the process of anaerobic digestion. Because pH is not controlled in this work, the existence of magnetite will affect the change in pH. As shown in Figure 4a, pH decreased at first and then increased in all groups. In R0, pH decreased rapidly and decreased to the lowest value of 4.2 on 4 d. The presence of magnetite can alleviate the decrease of pH to some extent, thus reducing the inhibitory effect of pH on the methanogenic process. When the content of magnetite was 1%, the lowest pH in the whole digestion process was 5.8, which occurred on 10 d. When the content of magnetite increased to 5%, the lowest pH increased to 6.16, and the corresponding digestion time was 6 d. Similar experimental results were found in other magnetite exposure groups. The above experimental results show that magnetite can effectively alleviate the decrease in pH in the oily sludge anaerobic digestion, thus avoiding the inhibition of methanogenic microorganisms by an acidic digestion environment. Magnetite can effectively accelerate the biotransformation of VFA and avoid excessive accumulation of VFA. Although the production of VFA in the magnetite group was higher than that in the blank group, the accumulated VFA did not reach the threshold to inhibit the activity of anaerobic microorganisms.

![Figure 4](image-url)

**Figure 4.** Effect of magnetite on pH (a) and VSS reduction (b) in anaerobic digestion of oily sludge. The error bar represents the standard deviation of the three measurements.

In addition to methane production, sludge reduction is also an important parameter in the process of anaerobic digestion. The effect of magnetite on VSS reduction during the oily sludge anaerobic digestion is shown in Figure 4b. It can be seen that magnetite promoted VSS reduction, and the higher the magnetite content was, the more significant the VSS reduction was. In R0, the VSS reduction was 21.3%, while the VSS reduction rate in
the magnetite presence group increased to 26.9–32.6%. The VSS reduction is mainly due to the fact that the organic matter in oily sludge was consumed by microorganisms and transformed into biogas, VFA, and other valuable products [31,32]. Magnetite accelerates the electron transfer in the process of biotransformation of organic matter, improves the utilization efficiency of organic matter, and thus strengthens the reduction of VSS [11,33]. The enhancement of VSS reduction within the magnetite presence group is consistent with the enhanced biogas production results in Figure 1. The increase in the VSS reduction rate can reduce the cost of the follow-up treatment of digested sludge and has certain economic benefits.

The decomposition of proteins in the process of anaerobic digestion will produce ammonia nitrogen, while the process of anaerobic digestion is accompanied by the release of phosphate. The release of ammonia nitrogen and phosphate from oily sludge during anaerobic digestion by magnetite was also investigated. As shown in Figure 5, in R0, the concentrations of ammonia nitrogen and phosphate in the fermentation liquid were 197.6 mg/L and 126.2 mg/L, respectively, while in the presence group of magnetite, the concentrations of ammonia nitrogen and phosphate increased to 218.3–264.6 mg/L and 134.6–149.2 mg/L, respectively, indicating that magnetite increased the release of ammonia nitrogen and phosphate, by-products of anaerobic digestion of oily sludge. It should be noted that the higher the concentration of magnetite, the higher the content of ammonia nitrogen and phosphate. The increase of ammonia nitrogen and phosphate concentration in the fermentation broth indicated that magnetite improved the hydrolysis process of oily sludge, which was consistent with the results in Figure 2. Magnetite was reported to accelerate the digestion process of highly concentrated anaerobic wastewater and to increase biogas production [34]. In kitchen waste treatment, magnetite enhanced the hydrolysis and acidification processes and improved the anaerobic digestion efficiency [35]. The presence of magnetite provided favorable conditions for the anaerobic hydrolysis and acidification processes of oily sludge, thus enhancing the release of ammonia nitrogen and phosphate as by-products of the digestion process. The ammonia nitrogen and phosphate released in this study did not reach the threshold of inhibition of anaerobic microorganisms [31].

![Figure 5. Effect of magnetite on the release characteristics of ammonia nitrogen (a) and phosphate (b) in anaerobic digestion of oily sludge. The error bar represents the standard deviation of the three measurements.](image-url)
3.5. Effects of Magnetite on Key Enzymes and Microbial Community Characteristics in Oily Sludge Anaerobic Digestion

In order to further understand the effects of magnetite on the activities of hydrolytic bacteria and methanogenic bacteria, the effects of magnetite on the activities of protease, amylase, AK, PTA, and coenzyme F420 involved in the anaerobic digestion of oily sludge were investigated (Table 2). It can be clearly found that the presence of magnetite significantly increased the activities of key enzymes in the hydrolysis and acidification processes. For example, within the R3, the relative activities of protease and amylase were 124% and 115%, respectively, which were significantly higher than those in R0. The increase in hydrolytic enzyme activities was consistent with the release characteristics of dissolved organic matter within Figure 3. The activities of AK and PTA, the key enzymes associated with the acidification process, were also affected by magnetite, and the activities of AK and PTA increased to 112% and 114%, respectively, in R3. As for the methanogenesis process, F420 played an important role in the biotransformation of acetate. Magnetite also increased the activity of F420; in particular, when the magnetite content was increased to 7%, the relative activity of F420 increased to 125.3%, which was significantly higher than the other groups in the experiment. Similar experimental results were also found within other magnetite presence groups. Kang and Liu [14] also found that magnetite can effectively increase the activities of coenzyme F420 and protease, which is consistent with the results of this work. Magnetite accelerates the transfer of electrons, which, in turn, enhances the contact and utilization of key enzymes with digestive substrates and intensifies the activity of key enzymes. The increase in the activity of key enzymes in the process of hydrolysis, acidification, and methanation of magnetite is also one of the reasons for the increase in methane production.

Table 2. Effect of magnetite on the activities of key enzymes in anaerobic digestion of oily sludge.

<table>
<thead>
<tr>
<th>Key Enzyme Types</th>
<th>R0</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease</td>
<td>100</td>
<td>105 ± 3.2</td>
<td>108.2 ± 4.2</td>
<td>109.1 ± 2.7</td>
<td>112.3 ± 3.7</td>
</tr>
<tr>
<td>Amylase</td>
<td>100</td>
<td>108.2 ± 1.5</td>
<td>111.2 ± 5.2</td>
<td>116.5 ± 2.4</td>
<td>121.3 ± 3.5</td>
</tr>
<tr>
<td>AK</td>
<td>100</td>
<td>103.5 ± 2.2</td>
<td>112.6 ± 3.8</td>
<td>116.8 ± 4.1</td>
<td>119.5 ± 4.8</td>
</tr>
<tr>
<td>PTA</td>
<td>100</td>
<td>104.1 ± 3.1</td>
<td>109.5 ± 7.1</td>
<td>119.3 ± 3.8</td>
<td>124.1 ± 4.6</td>
</tr>
<tr>
<td>F420</td>
<td>100</td>
<td>107.3 ± 1.8</td>
<td>113.7 ± 3.9</td>
<td>117.2 ± 1.6</td>
<td>126.4 ± 5.8</td>
</tr>
</tbody>
</table>

Note: The data in the table are the mean and standard deviation.

Microbes are important participants in the decomposition and consumption of organic matter during anaerobic digestion, and their species richness and population characteristics can well reflect the performance of anaerobic digestion [36,37]. In order to further demonstrate the impact of magnetite on the microbial abundance information and community composition of anaerobic digestion of oily sludge, samples were taken during the stable period of each group for microbial diversity sequencing.

Figure 6a demonstrates the effect of magnetite on microorganisms at the phylum level within the oily sludge anaerobic digestion, and it can be seen that Firmicutes, Bacteroidota, Patescibacteria, and Chloroflexi were the major phylum-level microorganisms with a high percentage of 65.0–76.7%. Magnetite produced large differences in the relative abundance of individual phylum-level microorganisms. The relative abundance of Firmicutes within the R0 was 35.6%, while within the magnetite presence group, the relative abundance of Firmicutes increased to 38.5–40.3%. In particular, the relative abundance of Firmicutes increased to 40.3% within R4, which was significantly higher than the other groups. Firmicutes are one of the most common bacterial clades in the anaerobic digestion of organic matter and play an important role in the hydrolysis and acidification of complex organic matter [38,39]. In addition, it is worth noting that the Proteobacteria phylum increases slightly with the addition of magnetite. The relative abundance of the Proteobacteria phylum increased to 4.15~4.36% within the magnetite presence group, which was higher...
than the 3.15% in R0. It was confirmed that many electroactive bacterial genera, including *Schwannia*, *Pseudomonas*, and *Desulfovibrio*, belong to the Proteobacteria phylum [40]. It can be inferred that the presence of magnetite improves the interspecies electron transfer within the anaerobic digestion of oily sludge, which contributes to methane production and thus improves the resource utilization of oily sludge.

Figure 6. Effect of magnetite on the relative abundance of microorganisms at the phylum level (a) and the archaeal microorganisms at the genus level (b) within anaerobic digestion of oily sludge.

Figure 6b illustrates the relative abundance of archaeal microorganisms at the genus level within the anaerobic digestion of oily sludge by magnetite. It can be seen that *Methanosarcina*, *Methanosaeta*, and *Candidatus Methanofastidiosum* are the top three archaebacteria at the genus level, and the sum of the relative abundance of the three within each group is about 59.0–67.6%. The relative abundance of *Methanosarcina* was only 35.6% in the R0, but it was increased to 37.6–38.5% in the presence of magnetite. *Methanosarcina* is an acetic acid trophic methanogenic bacterium and is important for improving methane production and preventing system acidification [41]. Magnetite attached to the conductive flagellum can act as an electronic channel to Sarcina, and magnetite can enable strains lacking the *omcS* functional gene to have the ability of extracellular electrons, stimulate the transfer of extracellular electrons, and directly transfer the electrons generated after the oxidation of acetic acid through the conductive channel formed by conductive biochar to promote the production of CO₂ to methane to improve the process of acetic acid oxidation and methane production [34,42].

3.6. Implementation Significance

The effective treatment of oily sludge is crucial for environmental and ecological security. This study demonstrates the use of magnetite to enhance the anaerobic digestion of oily sludge. Results show that 5% magnetite is the optimum dosage, leading to methane production as high as 196.5 mL/g VS. Furthermore, VSS reduction in the magnetite presence group increased to 26.9–32.6%, which was higher than that in the blank. Although previous studies mainly applied magnetite to high-concentration organic wastewater [43,44], there has been little research on the anaerobic digestion of oily sludge by magnetite until now. This study fills this gap and proves the economic feasibility of using magnetite to enhance methane production from oily sludge.
Magnetite accelerates the biotransformation of organic matter within the oily sludge, increases the activity of key enzymes associated with methane production, and establishes a DIET pathway between synthetically electroactive bacteria and methanogenic organisms. Moreover, the gene abundance of enzymes associated with carbon dioxide reduction during hydrogen methane production increases significantly with the addition of magnetite [45]. Magnetite not only improves the degradation of oil and grease within the oily sludge but also helps avoid the presence of oil and grease that could be harmful to subsequent sludge disposal. In addition, there are normalized tests by ISO and OECD, which also help to understand the anaerobic metabolism of organic matter [46–48]. Therefore, the application of magnetite to enhance oily sludge has significant potential for resource utilization and reduction of oily sludge.

4. Conclusions

This work represents the first report on the use of magnetite for enhancing methane production from mesophilic anaerobic digestion of oily sludge. The results demonstrate that the addition of 5% magnetite significantly increased methane production, reaching 196.5 mL/g VS, which was 1.42 times higher than the blank. This enhancement is attributed to several mechanisms, including the acceleration of biotransformation of organic matter within the oily sludge, the avoidance of excessive pH reduction, the promotion of the activity of key enzymes responsible for hydrolysis, acidification, and F420, and the promotion of the relative abundance of microorganisms involved in methane production, particularly Methanosarcina and Methanobacterium. These findings expand the application of magnetite and provide useful insights for the effective resource utilization of oily sludge.

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