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Application of iron oxide (Fe_3O_4) nanoparticles during the two-stage anaerobic digestion with waste sludge: Impact on the biogas production and the substrate metabolism

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Fe_3O_4 nanoparticles (FNP) were implemented in two-stage anaerobic digestion to enhance the biogas production and waste sludge reduction in this study. The degradation and transformation of extracellular polymeric substances (EPS) and dissolved organic matters (DOM) under optimal FNP concentration during two-stage anaerobic process with heat pretreated waste sludge was investigated by analyzing the changes of soluble chemical oxygen demand (SCOD), carbohydrate and protein in EPS and DOM. FNP addition significantly enhanced volatile fatty acids (VFAs) production. The optimal dosage of FNP was 100 mg/L yielded 11.9 ml H_2/g VS hydrogen and 109.8 ml CH_4/g VS methane, which increased by 15.1% of hydrogen yield and 58.7% of methane yield compared with that of control. The maximum volatile suspended solids (VS) reduction was 46.9% with the addition of 100 mg/L FNP compared with that of control (34.3%). NH_4^+-N production was inhibited with 20, 50, and 100 mg/L FNP addition comparing with control.

1. Introduction

Waste sludge produced from municipal wastewater treatment plants (WWTPs) has become a critical environmental problem because of its huge quantity, potential secondary-pollution risk and high disposal cost [1,2]. Anaerobic digestion (AD) has been considered as the desirable technique for sludge treatment and disposal technology due to its efficient degradation and lower energy consumption [3,4]. As a concept, two-stage AD process combined hydrogen and methane production has been pointed out in recent years, and it has advantages of higher biogas yield, organic removal and shorter hydraulic digestion time, compared with traditional single-stage AD [5,6]. The system of two-stage anaerobic digestion can be divided into two phases: acidogenesis phase and methanogenesis phase [7]. This system not only satisfies the different growth rates and pH optima for the acidogenic microorganism and methanogenic microorganism, respectively, but also figures out the inhibition of the reactor acidification in the single-stage AD process [8]. Due to these advantages, two-stage AD, as an increasingly popular method, has been used for organic wastes disposal such as municipal solid waste [9], food waste [10] and palm oil mill effluent [11], and they all have got promising results.

Fe_3O_4 nanoparticle (FNP) has been applied in anaerobic digestion to enhance the methane production and the substrate degradation due to its characteristics of super paramagnetic, high coercivity, non-toxic, and biocompatible [12,13]. Anaerobic digesters have been shown to stimulate and stabilize the biogas process performance when FNP was added [13]. FNP is unstable and it can slowly dissolve and provide Fe^{2+} and Fe^{3+} ions. In fact, these iron ions are essential constituents of cofactors and enzymes, and their addition to anaerobic digesters can improve the activity of Archaea microorganisms - the most important methanogen microorganism [13,14]. However, addition of Fe^{2+} ions could only increase the biogas production in the first 24–48 h, and high concentration of iron ion has toxicity effect on the bacteria, which

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consequently decreased biogas production [15]. Thus, the FNP is good candidate to maintain optimal iron concentration for anaerobic digestion process because of its non-saturation and bioavailable. Simultaneously, several studies suggested that the supplementation with FNP could facilitate direct interspecies electron transfer (DIET) which is an alternative to the interspecies H_2/formate transfer in syntrophic methanogenesis [13,16].

It was reported that biogas production increased by 180% and methane production enhanced by 234% when FNP (7 nm, 100 ppm) was introduced into a mesophilic anaerobic waste digester [13]. Similar study was reported that the highest methane productivity with 20 mg/L FNP increased by 100.16% and the lag phase significantly decreased because of iron sustained-release from FNP to maintain optimal iron ion concentration in anaerobic digester treating cattle dung slurry [12]. Furthermore, as Fe_3O_4 was added into an anaerobic reactor, the methane production increased by 69.8% compared with control, and the potential DIET was a crucial reason for accelerating anaerobic digestion of waste sludge [17]. Until now, most researchers have focused on the optimization of gas production in methane production process, very few publications have discussed in detail the effect of FNP on hydrogen and methane production, and substrate metabolism during the two-stage anaerobic digestion.

Therefore, the main objective of this study was to determine the optimal FNP concentration for obtaining the highest hydrogen and methane production, and sludge reduction in the two-stage anaerobic digestion with waste sludge. Simultaneously, the concentration and quality of sludge EPS and DOM are critical factors in the assessment of operation performance and designing optimum conditions of sludge digestion reactors [18,19]. For this purpose, the main study can be further elaborated as follows: (1) evaluating the effect of different FNP concentrations on sludge substrate (EPS and DOM) changes in acidogenesis/methanogenesis step; (2) investigating the relationship between the biogas production and substrate metabolism under the FNP additional conditions. The results are beneficial in understanding the effect of FNP on degradation and transformation of the organic matters in waste sludge during the two-stage anaerobic digestion.

2. Materials and methods

2.1. Waste sludge and inoculum

Waste sludge used in this study was took from the secondary sedimentation tank and the inoculum was collected from the digestion tank of Tuandao municipal wastewater treatment plant located in the Qingdao, China. The collected raw waste sludge was storage at 4 °C before use. The characteristics of raw waste sludge and inoculum are shown in Table 1.

2.2. Biodegradability batch tests

In this study, sludge digestion was performed in 250 ml serum bottles with the working volume of 150 ml. 0, 20, 50, 100, 200 mg/L of FNP (diameter of 20–30 nm, purchased from Aladdin Industrial Corporation) was added into the five bottles. To disrupt the microbial cells and improve anaerobic digestion efficiency of waste sludge, the waste sludge was heated at 80 °C for 30 min in a water bath shaker, and then cooled down to room temperature. The pretreated waste sludge fully incorporated with FNP, which were substrate used for two-stage anaerobic digestion.

2.2.1. Hydrogen production process

Five bottles with pretreated waste sludge and FNP were put in reciprocal shaker at 36 ± 1 °C with stirring of 120 rpm, respectively. Before fermentation, all bottles were continuously sparged nitrogen to get anaerobic condition, and then capped with silica gel stoppers. This hydrogen production stage lasted for 1 day. The headspace gas was collected for hydrogen determination every 1 h and the samples were extracted every 6 h to measure the SCOD, carbohydrate, protein, pH and VFAs in EPS and DOM, and the SS, VS and FT-IR spectroscopy in DOM was also investigated.

2.2.2. Methane production process

After fermentation, 30 ml inoculum was added in the five bottles, respectively. The pH was adjusted to 7.0 using 1 mol/L NaOH. The bottles with mixed substrate were put in reciprocal shaker at 36 ± 1 °C with stirring of 120 rpm, after they were all continuously sparged with N_2 for 5 min to drive air and capped with silica gel stoppers. This stage lasted for 12 days. The headspace gas was collected for methane determination every day and the samples were measured every 2 d during the methanogenesis stage. Under identical condition, the sample measurements were all performed in triplicate.

2.3. Analytical methods

2.3.1. EPS and DOM extraction

EPS extraction: EPS were excreted by microorganisms and adsorbed organic matter accompanied by the growth of bacteria during the anaerobic digestion. The method of EPS extraction from the waste sludge was according to the thermal treatment method [20]. Firstly, the waste sludge was centrifuged at 5000 r/min for 10 min and removed the supernatant. The remaining waste sludge was washed 3 times and then put the washed waste sludge in a 80 °C thermostatic water bath for 10 min. After waste sludge cooled down to room temperature, it was centrifuged at 8000 r/min for 10 min with supernatant being collected. The sludge EPS was the collected supernatant filtered through a 0.45 μm cellulose acetate membrane.

DOM extraction: The samples were centrifuged at 8000 r/min for 20 min and the supernatant was collected. The collected supernatants were filtered through a 0.45 μm cellulose acetate membrane for DOM analysis [21].

2.3.2. Chemical analysis

The soluble COD (SCOD), total suspended solid (TS), volatile suspended solids (VS) and NH_4^+-N were measured by standard methods [22]. The pH was determined using a digital pH-meter (PHB-5, Aolilong, Hangzhou). The biogas (H_2, CH_4) composition was analyzed by a gas chromatograph (GC7900, Tianmei Shanghai, China) equipped with thermal conductivity detector (TCD), and the column was a packed column (PORAPAK Q 80–100, 2.0 m × 2.0 mm). The volume of biogas was determined by draining

Table 1
Characteristics of raw sludge (RS) and inoculum (IN).

	SCOD (mg/L)	Carbohydrate (mg/L)	Protein (mg/L)	VFAs (mg/L)	TS (g/L)	VS (g/L)	pH
RS	4007.6 ± 43.2	12.6 ± 0.9	196.8 ± 4.3	519.5 ± 5.2	11.0 ± 0.4	7.4 ± 0.3	6.7 ± 0.2
IN Ref.	1786.3 ± 31.8	20.8 ± 0.7	48.1 ± 3.2	32.3 ± 3.3	14.3 ± 0.2	7.1 ± 0.3	7.4 ± 0.1

saturated salt water. Gas chromatography (Shimadzu GC2010, Japan) equipped with a flame ionization detector (FID) was used to qualify volatile fatty acids (VFAs) production as previous method [4]. The protein and carbohydrate were detected by Lowry's method and phenol-sulfuric acid method, respectively [21].

2.3.3. Fourier transform infrared spectroscopy analysis

The composition of sludge DOM was analyzed using a Tensor 27 FTIR spectrometer (Bruker Optics, Ltd., Germany). Firstly, the DOM extraction was completely freeze-dried to powder by a vacuum freeze-drier (Labconco7670530, USA). The dried DOM powder was mixed with dried KBr (spectrometry grade) at a ratio of 1:100, and then homogenized in an agate grinder. Before measurement, the mixed powder was compressed to tablet by a tablet machine. The FTIR spectrum was recorded over the frequency range from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}.

3. Results and discussions

3.1. Hydrogen and methane production

Fig. 1 illustrates the hydrogen and methane yield, and the content of hydrogen and methane is shown in Table 2 during the acidogenesis and methanogenesis phase. In the acidogenesis phase, the hydrogen yield was improved with FNP addition in comparison with the control (Fig. 1a), and the FNP had a positive effect on the hydrogen production. The highest hydrogen yield was achieved with the addition of 50 mg/L FNP, which yielded 12.4 ml H_2/g VS, 1.2 times the hydrogen yield produced by the control. The hydrogen yield with 20, 100 and 200 mg/L were 11.4, 11.9 and 12.1 ml H_2/g VS, and the difference of the hydrogen yield was not distinct among groups of FNP addition. The main hydrogen was produced at the first 5 h in the acidogenesis phase. There was little difference in hydrogen production process between the groups of FNP addition and the control at the first 2 h of the acidogenesis stage. The biogas produced in the acidogenesis phase contained hydrogen and carbon dioxide without detectable other gases. The hydrogen contents with addition of FNP dosages were not significantly different and the highest hydrogen content was obtained with the addition of 50 mg/L FNP. However, the hydrogen content of 200 mg/L FNP addition (53.1%) was lower than that of control (54.3%).

In the methanogenesis phase, the addition of 20, 50, 100, 200 mg/L FNP yielded 75.0, 94.9, 109.8 and 91.4 mL CH_4/g VS methane (Fig. 1b), and increased the methane yield by 1.1, 1.4, 1.6 and 1.3 times the methane yield produced by the control, respectively. The composition of the biogas was methane, carbon dioxide and a little hydrogen without detectable other gases. The substrate treated with FNP was attained the higher methane content compared to the control except for the substrate with 200 mg/L FNP addition. Addition of 100 mg/L FNP attained the highest CH_4 content (65.5%), and the lowest CH_4 content (52.3%) was obtained with 200 mg/L FNP addition, which is lower than the CH_4 content of control (57.2%). Therefore, there was more carbon dioxide produced with the addition of 200 mg/L FNP. The remarkable increase in methane yield took place 5–8 d. At the first 3 days of methanogenesis phase, the cumulative methane yield with addition of different FNP dosages was not distinctly different, while the methane yield of the addition of 200 mg/L FNP had an obvious decrease compared with that of control. The results indicated that methanogen microorganism needed time to adapt the substrate with FNP addition, namely, the addition of FNP just had positive effect on methane production after 3 d, compared to the control. The addition of 200 mg/L FNP had inhibitory action on methane production process at the first 3 days. It was reported that high concentration of FNP addition has negative effect on the methanogen microorganism and anaerobic digestion process [23]. After 3 d, the bacteria had adapted the substrate with 200 mg/L FNP addition and shown positive effect on the methane production compared to the control.

The cumulative biogas production for above-mentioned FNP addition confirmed that the addition of 100 mg/L FNP attained the highest biogas yield (11.9 mL H_2/g VS and 109.8 mL CH_4/g VS) and the methane yield increased by 58.7% compared with control. It was reported that methane yielded at the dosage of 20 g/L Fe_3O_4 increased by 43.5% for single-stage anaerobic digestion [24]. When the substrate was manure, methane yield increased by 59.4% and the HRT was 30 days with 20 mg/L Fe_3O_4 nanoparticles addition [13]. In this study, the hydrogen yield of 11.9 mL H_2/g VS was also obtained, increased by 15.1% compared with control, and the whole two-stage anaerobic process just lasted for 12 days with addition of 100 mg/L FNP. The addition of FNP could promote the methanogenesis by the enhancement of syntrophic effect of methanogenic

Table 2
Effect of FNP dosages on hydrogen content (HC) and methane content (MC).

	0 mg/L	20 mg/L	50 mg/L	100 mg/L	200 mg/L
HC	54.3 ± 1.3%	54.9 ± 0.8%	56.2 ± 1.1%	55.1 ± 1.0%	53.1 ± 1.2%
MC	57.2 ± 0.7%	58.5 ± 1.2%	63.1 ± 0.7%	65.5 ± 0.7%	52.3 ± 0.6%

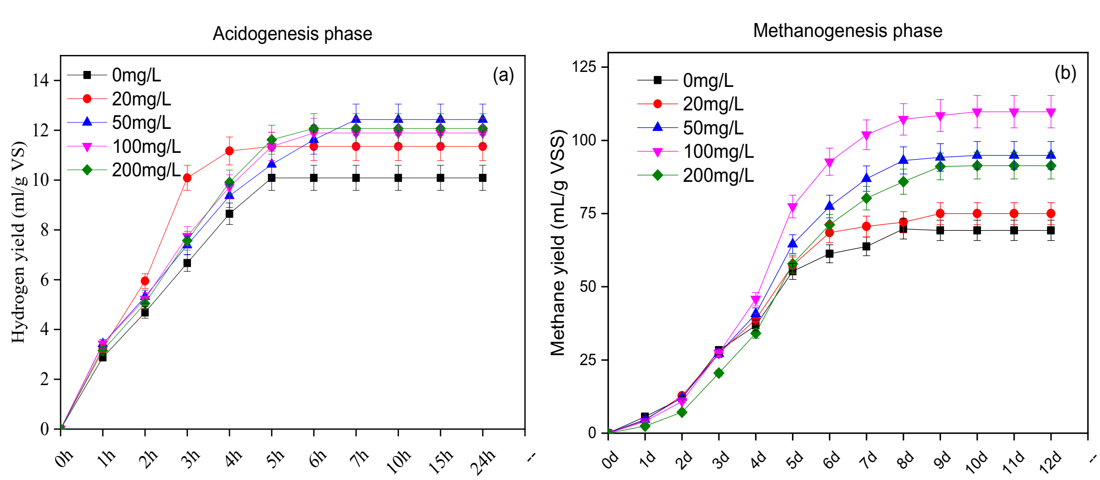

Fig. 1. Effect of FNP dosages on biogas yield: (a) hydrogen yield, (b) methane yield.

microorganisms through the electron transfer mediated [12]. Therefore, the application of FNP in the two-stage anaerobic digestion could further enhance the efficiency of methane production, and the sludge retention time would be shorter compared to single-stage anaerobic digestion.

3.2. Effects of FNP on the changes of VFAs

As the main product in the acidification process of sludge, VFAs were not only useful indicators for monitoring the hydrogen production process but the easiest and important accessible fraction for methanogens [25]. The effect of different FNP dosages on VFAs changes are shown in Fig. 2. In the acidogenesis phase, the concentration of VFAs of raw sludge was 519.1 mg/L, and then had an increase in all groups. At the end of hydrogen production process, the concentration of VFAs with the addition of 20, 50, 100, 200 mg/L were 1847.4, 2220.4, 1843.5 and 1308.1 mg/L, which were 2.0, 2.4, 2.0 and 1.4 times that of control (940.1 mg/L), indicated that the FNP had the positive effect on VFAs production. The peak of VFAs value was 2250.5 mg/L at 18 h with the addition of 100 mg/L FNP. During the hydrogen production process, the concentration of VFAs showed a gradual increase with addition of 20 and 50 mg/L FNP, while it had the opposite tendency with addition of 0, 100, and

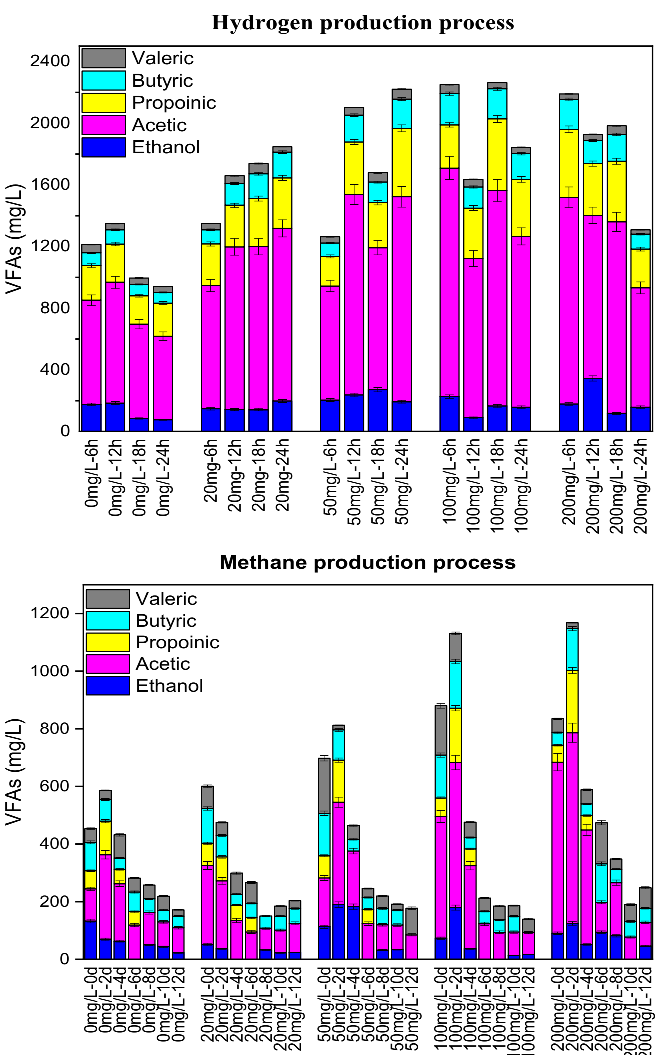

Fig. 2. Effect of different FNP dosages on VFAs changes during two-stage anaerobic process.

200 mg/L FNP after 6 h. Therefore, though the addition of 100 and 200 mg/L FNP had a higher VFAs production than the control, the relatively higher FNP dosages did not show obviously positive effect on VFAs production at the second half of acidogenesis phase. At the end of acidogenesis phase, acetic acid was the most prevalent VFAs for all groups, accounting for 57.6–60.6%. At the FNP dosage of 20, 50, 100, and 200, concentrations of acetic acid were 1119.4, 1329.8, 1107.5 and 774.0 mg/L, and the addition of 50 mg/L FNP achieved the maximum acetate acid production, 145.5% higher than that of control (541.9 mg/L). The concentrations of butyric acid with 20, 50, 100, and 200 mg/L FNP were 166.7, 190.2, 167.5 and 95.7 mg/L, which enhanced by 1.4, 1.8, 1.4 and 0.4 folds compared with control. Propionic acid concentration also increased by 52.5%, 106.4%, 72.9% and 17.3% with FNP addition. It was well-know that acetic acid could be directly degraded by methanogens to CH_4 and CO_2. High abundant of genera for syntrophic oxidation of butyrate to methane was obtained with conductive iron oxide addition sample, and interspecies electron transfer effect via the conductive iron oxide was the main mechanism that facilitated the electric syntrophy in butyrate oxidizing bacteria [16]. Therefore, it was also an important reason that addition of FNP could improve the biogas production, methane content and cumulative methane yield. Ethanol and valeric acid were also detected in all groups at the end of hydrogen fermentation. Mixed acid-type fermentation was the main fermenting pathway of the hydrogen production and addition of FNP did not change the fermenting pathway.

In the methanogenesis phase, VFAs showed an accumulation at the first 2 days except for the addition of 20 mg/L FNP, and the concentrations of VFAs were 1624.8, 2262.5 and 2336.2 mg/L with the addition of 50, 100 and 200 mg/L FNP, which were higher than that of control (1172.1 mg/L) at 2 d. It was implied that acid-producing bacteria still had a certain activity and the hydrogen fermentation process was existed at the first 2 days, which was in agreement with the conclusion that a little hydrogen was detective in the biogas production (Section 3.1). After 2 d, VFAs, which dramatically decreased at all groups, were adequately used by methanogens. During the methane production process, the concentration of VFAs decreased by 66.1%, 74.6%, 78.8% and 70.2% with the addition of 20, 50, 100 and 200 mg/L FNP, which was higher than that of control (62.1%). It was implied that the FNP improved the utilization of VFAs by methanogens. The remarkable decrease of VFAs took place 0–6 d with the addition of 20, 50, 100 and 200 mg/L FNP, while it took place throughout the methane production process with the control. The ethanol and propionic acid also decreased and the propionic acid was not detected at all groups. The changes of acetic acid were not obvious and it just decreased from 111.6 mg/L to 87.2 mg/L with the control. However, concentrations of acetic acid were 101.4, 84.9, 76.0 and 82.4 mg/L and decreased by 63.0%, 49.8%, 81.9% and 86.1% with the addition of 20, 50, 100 and 200 mg/L FNP, indicating acetic acid completely degraded compared with control. The butyric acid significantly decreased and was not detected with the addition of 50 and 100 mg/L FNP at the end of methanogenesis phase, while it just mildly decreased with control. As reported, addition of FNP could facilitate both VFAs formation and utilization, and obviously accelerate the interspecies electron transfer between acidogens, methanogens, and some synergistic microbes (e.g. acetate/butyrate oxidation bacteria) [26]. Therefore, the activity of syntrophic methanogenesis significantly improved, and the acetic and butyric acid were fully degraded and utilized with the addition of 50 and 100 mg/L FNP, indicating a close relationship between acetate/butyrate degradation and methane production during the methane production process.

3.3. Degradation and transformation of SCOD, carbohydrate and protein in EPS and DOM

Extracellular polymeric substances (EPS), obstacles to sludge hydrolysis, are mainly consisted of various kinds of organics such as polysaccharides, proteins, and lipids. As the nutrient was shortage in the dissolved substrate, EPS could be hydrolyzed to serve as carbon and energy source for bacteria during the anaerobic digestion process [27]. Dissolved organic matters (DOM), which were the easily usable carbon source for the microbes, could be directly utilized by microorganisms to produce biogas [28]. Simultaneously, DOM could greatly influence the microbial consortium and composition, representing the biodegradability of waste sludge. Therefore, it is important to investigate the transformation and utilization properties of organics in EPS and DOM during the two-stage anaerobic digestion process.

3.3.1. Hydrogen production process

Fig. 3 illustrates the difference of SCOD, carbohydrate, protein in EPS between FNP addition and control during the hydrogen production process. In the raw waste sludge, the concentrations of SCOD, carbohydrate and protein in EPS were 5087.3, 146.7 and 621.3 mg/L, which significantly decreased in the hydrolysis and hydrogen production process. At the end of acidogenesis phase, concentrations of SCOD were 1294.1, 1065.2, 809.3 and 941.2 mg/L and decreased by 74.6%, 79.1%, 84.1% and 81.5% with the addition of 20, 50, 100 and 200 mg/L FNP, compared to 74.3% with the control. The main decrease of carbohydrate was obtained the first 6 h and then it had slightly increase with the addition of 50, 100 and 200 mg/L FNP, while the carbohydrate decreased throughout the acidogenesis phase with control and 20 mg/L FNP addition. The maximum decrease of carbohydrate (93.9%) occurred when the 50 mg/L FNP was added. The protein slightly decreased with FNP addition, which did not have remarkable difference compared to the control. As reported, the hydrolysis of waste sludge was affected by the assimilation of the microorganism, which could make the EPS release and accumulation [29]. Addition of Fe_3O_4 into digester could enhance decomposition of complex organics to improve hydrolysis efficiency [29,30]. Therefore, FNP addition had positive effect on the release of SCOD and carbohydrate compared to the control, and the optimum additional dosage was 50 mg/L FNP for the EPS release.

The concentrations of SCOD, carbohydrate, protein and NH_4^+-N in DOM were 4007.6, 12.6, 196.8 and 44.8 mg/L in the raw waste sludge, respectively. In the hydrogen production process, the concentration of SCOD increased between 0 h and 6 h, which indicated

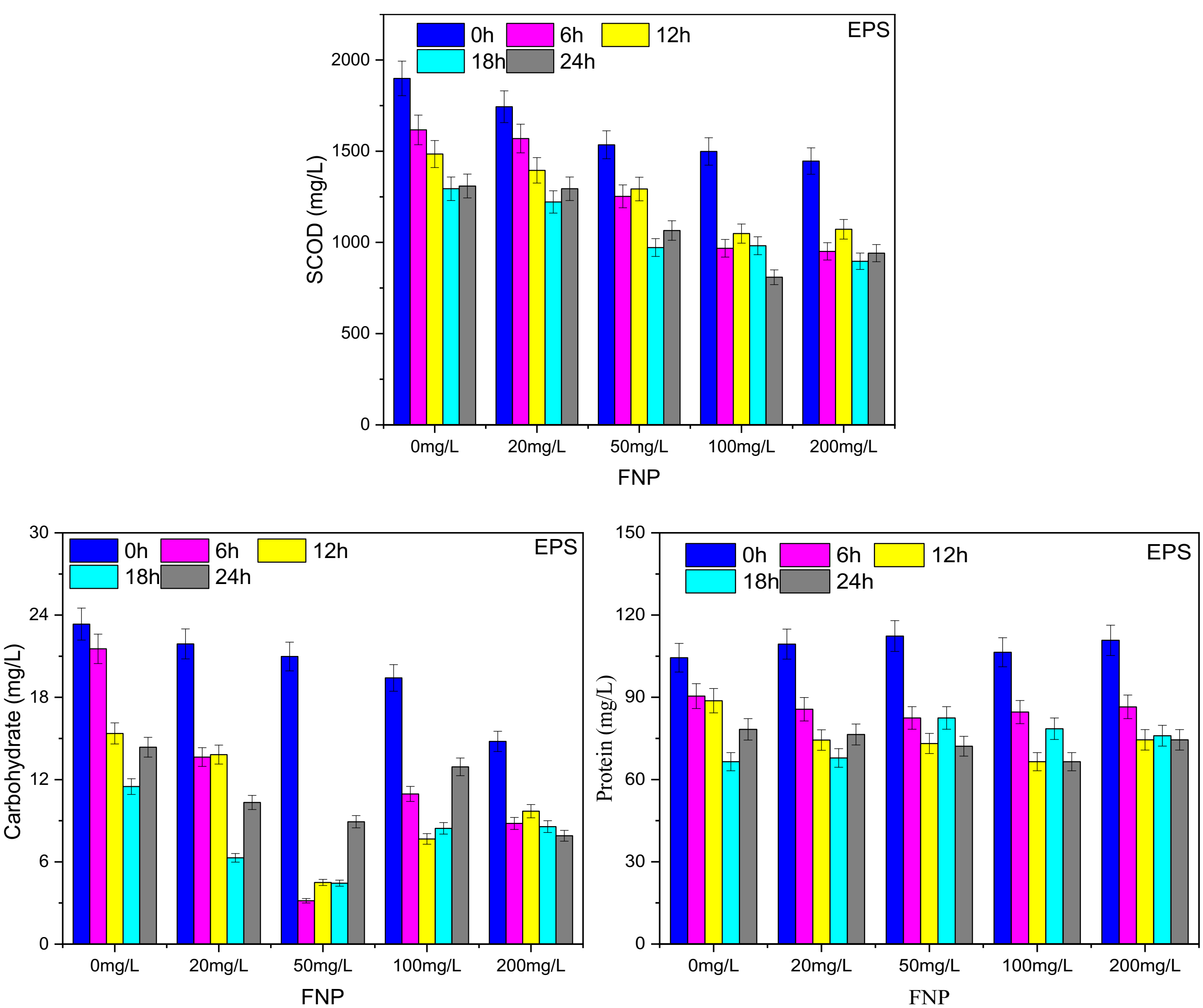

Fig. 3. Changes of SCOD, carbohydrate and protein in EPS during the hydrogen production process.

hydrolysis rate of waste sludge was higher than the utilization rate by microorganisms (Fig. 4). The peak values of SCOD were 8481.8, 11886.1, 10718.8 and 11442.2 mg/L with the addition of 20, 50, 100 and 200 mg/L FNP compared to the control (11107.9 mg/L) at 6 h, and then it decreased in the all groups except for 20 mg/L FNP addition, whose trends were opposite to the changes of EPS. The fluctuation of SCOD (decrease and timely increase) during the hydrogen production process could be explained that the solid particulate materials were converted into soluble organic compounds leading to an increase of SCOD, while the decrease of SCOD should be attributed to the utilization and digestion of available compounds by microorganisms [31]. The significant increase of SCOD was due to that the hydrolysis rate was higher than the utilization rate at the first 6 h. During the hydrogen production process, the concentration of carbohydrate and protein in DOM exhibited a progressive decline and the maximum reduction of carbohydrate (25.5%) and protein (37.8%) was obtained with the addition of 50 mg/L FNP, which was in agreement with conclusion that the highest hydrogen yield was obtained in this group. At the end of hydrogen production, the concentration of NH_4^+-N were 191.3, 213.6, 179.6, 187.8 and 176.3 mg/L with 0, 20, 50, 100 and 200 mg/L FNP addition, respectively. The increase of NH_4^+-N was related to the removal and utilization of protein in DOM.

3.3.2. Methane production process

Changes of SCOD, carbohydrate and protein in EPS with the addition of FNP compared with the control during the methane production process are illustrated in Fig. 5. The SCOD in EPS decreased at the first 4 days, and then increased in all groups. The lowest value of SCOD (2528.9 mg/L) was attained at 4 d with 100 mg/L FNP addition. The concentration of SCOD in EPS had a slight increase during the methane production process. At the end of methanogenesis phase, the concentration of protein in EPS were 48.4, 53.2, 46.8 and 43.6 mg/L with the addition of 20, 50, 100 and 200 mg/L FNP, which were higher than that of control (42.0 mg/L). The addition of FNP did not have obvious effect on the SCOD and protein hydrolysis in the methanogenesis phase. The concentration of carbohydrate in EPS decreased by 43.9%, 72.7% and 62.4% with the addition of 20, 100 and 200 mg/L FNP compared to that of control (27.6%). It was reported that the hydrolysis of waste sludge occurred throughout the anaerobic digestion process comprising the methane production process [32]. In this study, though there was a higher hydrolysis of carbohydrate in the methanogenesis phase, the main hydrolysis of waste sludge occurred in the hydrogen production process.

In DOM, the concentration of SCOD with the addition of 20, 50, 100, 200 mg/L FNP exhibited a gradual decline from 14068.4, 12858.6, 13928.6 and 12080.5 mg/L to 5656.9, 5217.3, 3217.4 and

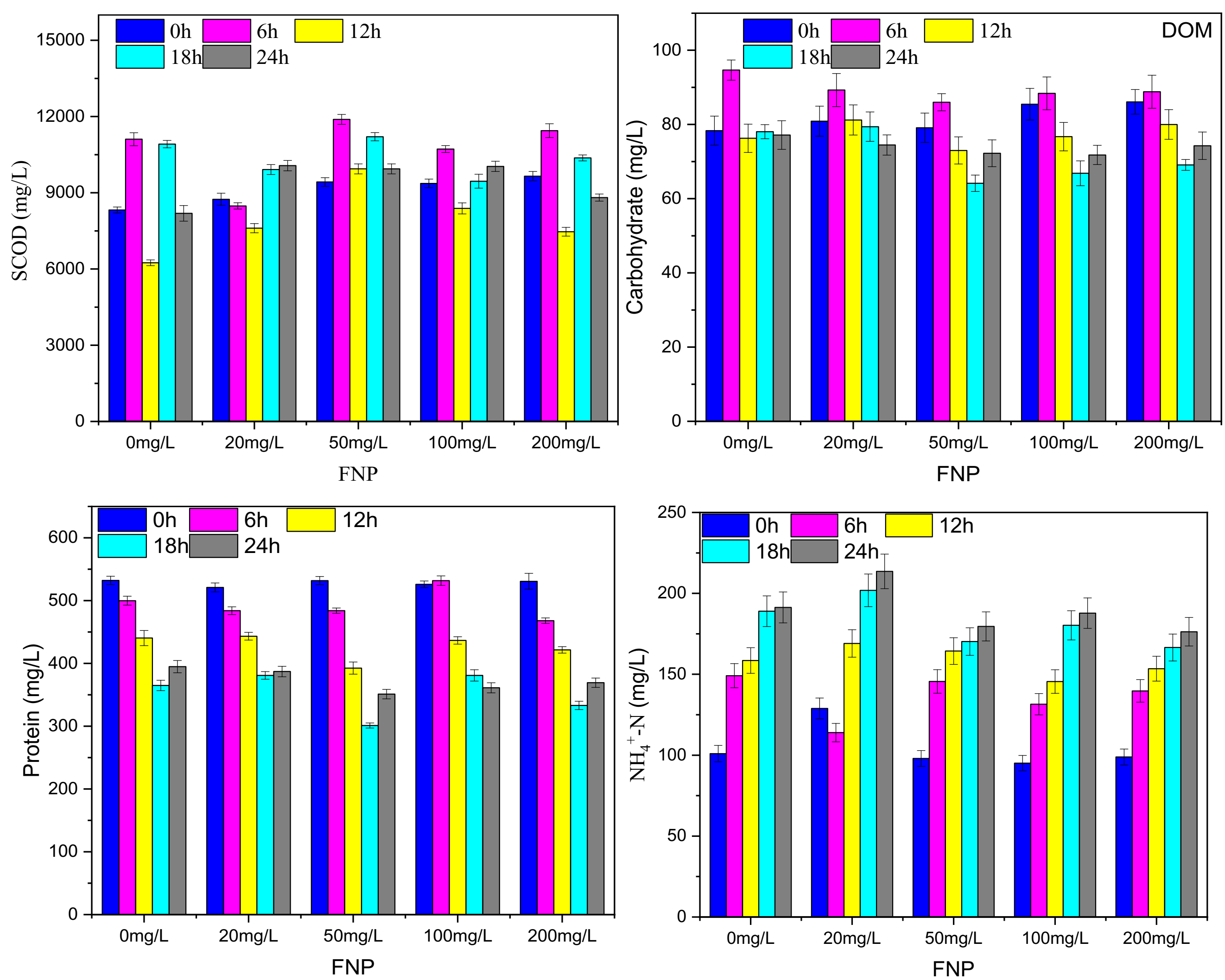

Fig. 4. Changes of SCOD, carbohydrate, protein and NH_4^+-N in DOM during the hydrogen production process.

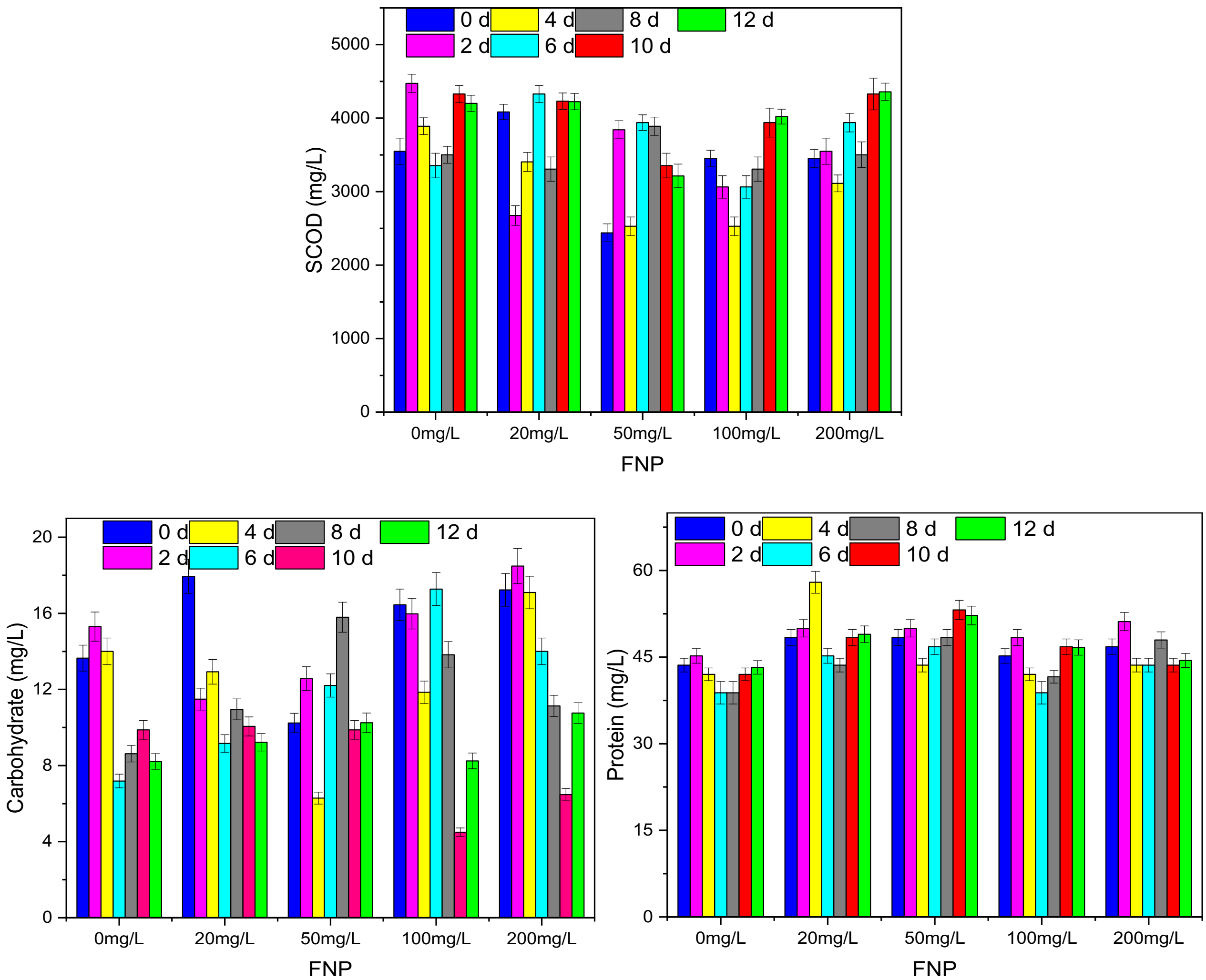

Fig. 5. Changes of SCOD, carbohydrate and protein in EPS during methane production process.

4256.8 mg/L, and decreased by 59.8%, 59.4%, 76.9% and 64.8%, respectively (Fig. 6). However, the decrease of SCOD with the control was just 49.1% and the concentration was 6342.0 mg/L at the end of methane production process. The carbohydrate was fully degraded, and decreased by 83.2%, 71.3%, 80.0% and 73.8% with the addition of 20, 50, 100 and 200 mg/L FNP, while it increased from 19.3 to 19.8 mg/L with the control. The remarkable decrease of SCOD took place at 4–8 d, but the significant decrease of carbohydrate occurred at the first 4 days with FNP addition. VFAs are the easiest fraction and the carbohydrate in waste sludge is more easily to degrade for biogas production than other fractions [32]. The methanogens preferentially used the VFAs and carbohydrate for methane production compared to SCOD, which was in agreement with our previous study [33]. The concentration of protein with the addition of 0, 20, 50, 100 and 200 mg/L FNP decreased by 79.1%, 82.8%, 83.8%, 81.5% and 84.8%, and there was not obvious difference between the groups of FNP addition and the control. Fe(III)-reducing microorganisms enriched by conductive Fe_3O_4 addition, could be capable of utilizing a variety of substrates and participating in the decomposition of complex organics via the dissimilatory iron reduction [34]. Therefore, the addition of FNP had improved the degradation and utilization of SCOD and carbohydrate, and the optimum dosage was 100 mg/L FNP. However, there was little effect on the protein degradation compared with control.

The NH_4^+-N exhibited a climb in all groups and its concentrations were 466.1, 487.2, 484.9 and 511.9 mg/L with the addition of 20, 50, 100 and 200 mg/L FNP, which were lower than that of control (525.9 mg/L) at the end of methanogenesis phase. Therefore, NH_4^+-N production was inhibited with 20, 50, and 100 mg/L FNP addition comparing with control.

3.4. FTIR spectra of DOM

Fourier transform infrared (FTIR) spectroscopy technique could not only be able to measure the degradation and transformation of functional groups of proteins, carbohydrates, phospholipids, and nucleic acids, but also provide information to monitor the performance of bioreactors [35]. In this study, spectra showed five predominant bands containing several characteristic functional groups: 3400-3440 cm^{-1}, 2875-2885 cm^{-1}, 1640-1655 cm^{-1}, 1095-1105 cm^{-1} and 940-950 cm^{-1}. The spectrum, which shows broad bands of absorption between 3408 and 3435 cm^{-1}, is attributed to stretching vibration of both hydroxyl (O–H) from the carbohydrates and amino (N–H) groups from the protein, and absorption bands near 2880 cm^{-1} are related to stretching of C–H bonds [36]. Two sharp peaks (around 1650 and 1100 cm^{-1}) are also observed in the spectrum, which are correlated with the protein secondary structure (C═O stretching vibration of β-sheets) and

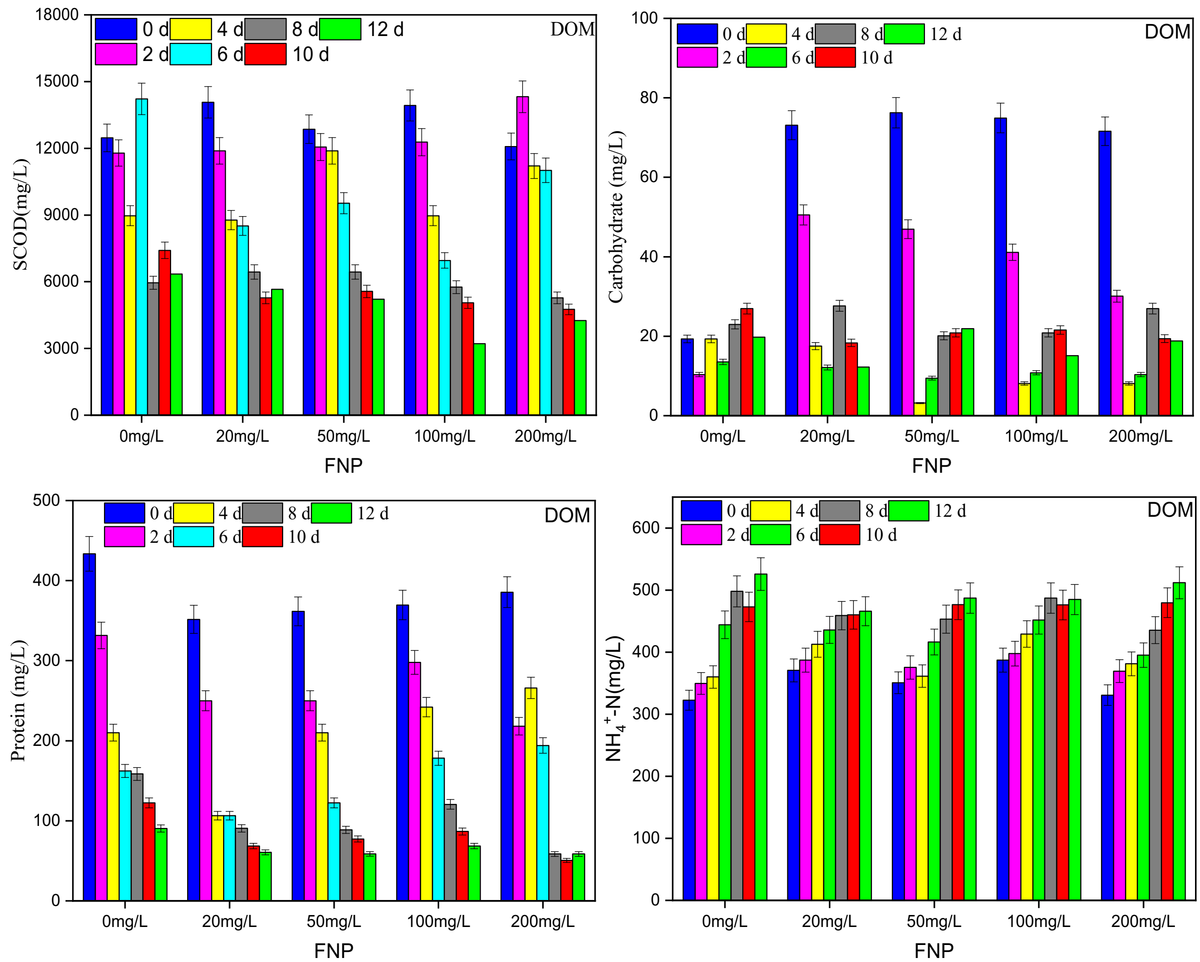

Fig. 6. Changes of SCOD, carbohydrate, protein and NH_4^+-N in DOM during the methane production process.

polysaccharide groups (O—C—O stretching vibrations) [37]. The band (<1000 cm^{-1}) belong to the fingerprint region and reflect the existence of unsaturated bonds in the sample. The spectra of DOM with additions of FNP during two-stage anaerobic digestion are shown in Fig. 7 and Fig. 8.

The spectra of DOM showed variations between samples with additions of FNP and the control during hydrogen production process. The peaks around 3420 cm^{-1}, 2880 cm^{-1}, 1640 cm^{-1} and 1100 cm^{-1} demonstrated the presence of protein, carbohydrate and C—H bonds in DOM at the hydrogen production process. The peak near 3420 cm^{-1} slightly shifted in DOM during the hydrogen production process. However, it was difficult to determine which group caused this shift, because of the presence of protein and carbohydrate in this region. The peak value of band near 3420 cm $^{-1}$ with 20, 50, 100, and 200 mg/L FNP addition were 46.7%, 37.3%, 38.2%, and 48.2%, which were all lower than that of control (55.2%) at the end of acidogenesis phase. Compared with control, the intensity of band near 3420 cm^{-1} exhibited a climb with FNP addition especially with the addition of 50 mg/L FNP, which was because more organic matters in DOM were hydrolyzed from the EPS. This result was consistent with the change of SCOD in DOM (section 3.3.1). The intensity of band near 2880 cm^{-1} and 1100 cm^{-1} with the addition of 50 and 100 mg/L FNP slightly decreased, whereas that of control and 20 mg/L FNP addition exhibited obvious increase at the first 12 h. Therefore, the addition of 50 and 100 mg/L FNP had positive effect on the decrease of methyl and polysaccharide, which was consistent with the change of carbohydrate in DOM during the acidogenesis phase. The change of intensity at peak near 1640 cm^{-1} had little difference between additions of FNP and the control, and there was little influence on the utilization of protein in DOM with the addition of FNP. The band near 945 cm^{-1} and 916 cm^{-1} was indicated the presence of extracellular nucleic acids, which could represent microbial population and biomass in waste sludge to some extent. However, their functions had not been clearly identified.

In the methane production process, DOM were also contained carbohydrates, protein, lipids and C—H bonds based the bands near 3420 cm^{-1}, 2880 cm^{-1}, 1640 cm^{-1}, 1384 cm^{-1} and 1100 cm^{-1}. The peak near 3420 cm^{-1} significantly shifted and the intensity exhibited a slightly increase. There were little changes between addition of FNP and the control at peak 2880 cm^{-1}. The peak at 2358 cm^{-1} was due to the interference of carbon dioxide and the intensity remarkably decreased especially with the addition of 20 and 200 mg/L FNP. The peak near 1640 cm^{-1} exhibited slight blue-shift with additions of 20, 50 and 100 mg/L FNP compared with control. It was reported that the peak between 1648 and 1657 cm^{-1} was specifically assigned to α-helix of protein and the peak between 1630 and 1640 cm^{-1} was attributed to β-sheets of protein [27]. The structure of α-helix needed less strength and energy dissipation capacity compared to β-sheets at deformation [36]. Therefore, additions of 20, 50 and 100 mg/L FNP was resulted the more α-helix and the less β-sheets in the protein, and had positive effect on the protein degradation during the methanogenesis phase. The peak near 1384 cm^{-1} was characteristics of the C—H

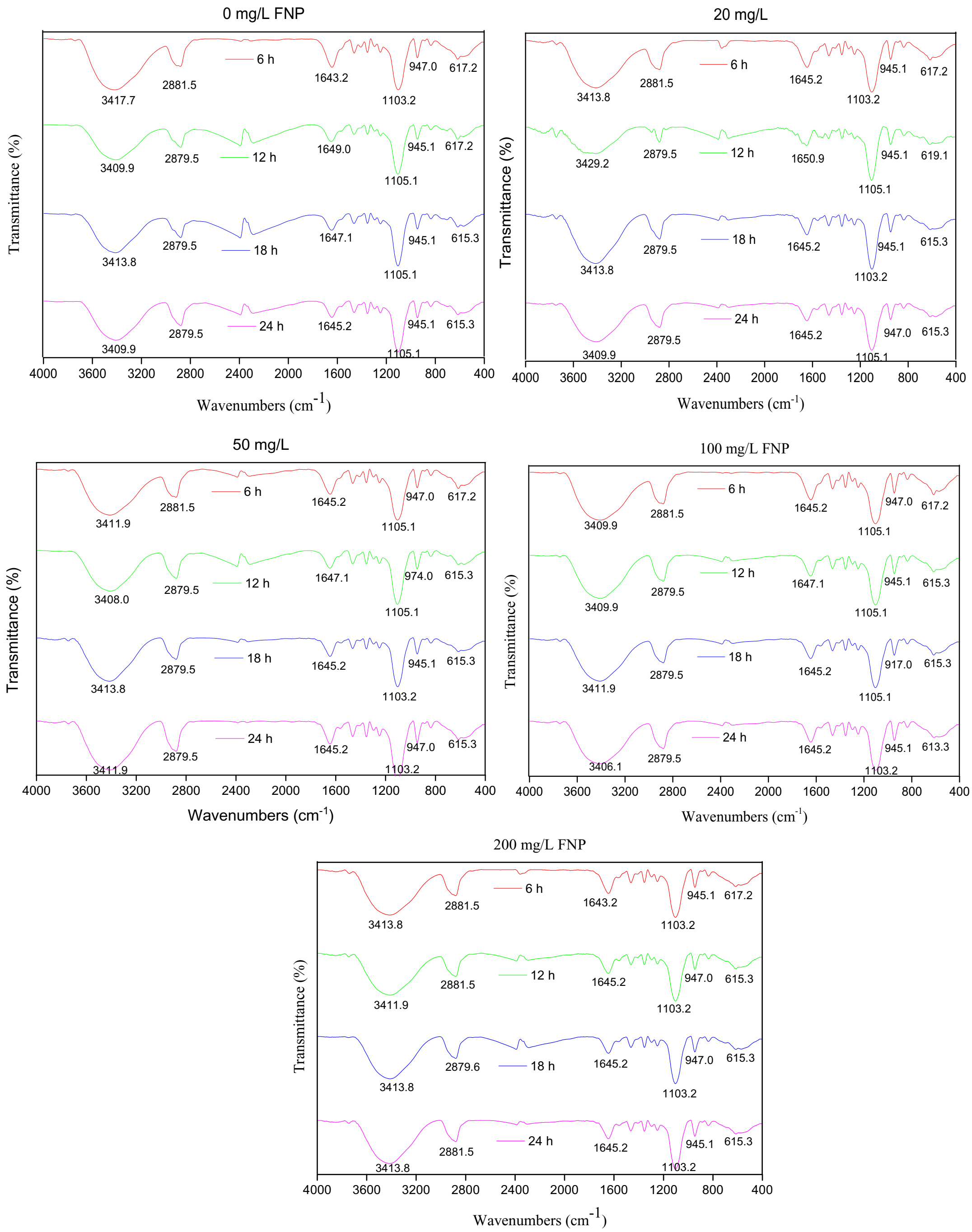

Fig. 7. FTIR spectra of DOM with FNP addition during the hydrogen production process.

vibrations in the methyl. The peak near 1100 cm^{-1} slightly shifted with the FNP addition compared with the control. It was implied that the FNP addition had significant effect on the functional groups of polysaccharide, which was in agreement with the change of carbohydrate in DOM.

3.5. The reduction of waste sludge TS and VS

The objective of anaerobic digestion was not only the biogas production but also the waste sludge reduction. Therefore, changes of TS and VS were important parameter for evaluating the performance of anaerobic digestion. Fig. 9 shows the effect of FNP addition on sludge reduction during the two-stage anaerobic process. The concentrations of TS and VS were 11.0 and 7.4 g/L in the raw waste sludge. After heat pretreatment, the concentration of TS decreased to 10.7, 10.5, 10.6 and 10.8 g/L, and that of VS decreased by 6.8%, 10.8%, 9.5% and 9.3% with the addition of 20, 50, 100 and 200 mg/L, respectively. However, the decrease of TS was just 1.8% with the control. In the hydrogen production process, there was not obvious decrease of TS and VS in all groups and change of TS with the addition of FNP did not have significant difference compared with that of control. The maximum decrease of VS (13.6%) was obtained with addition of 50 mg/L FNP. In the methane production process, the concentration of TS decreased by 13.4%, 6.6%, 19.2% and

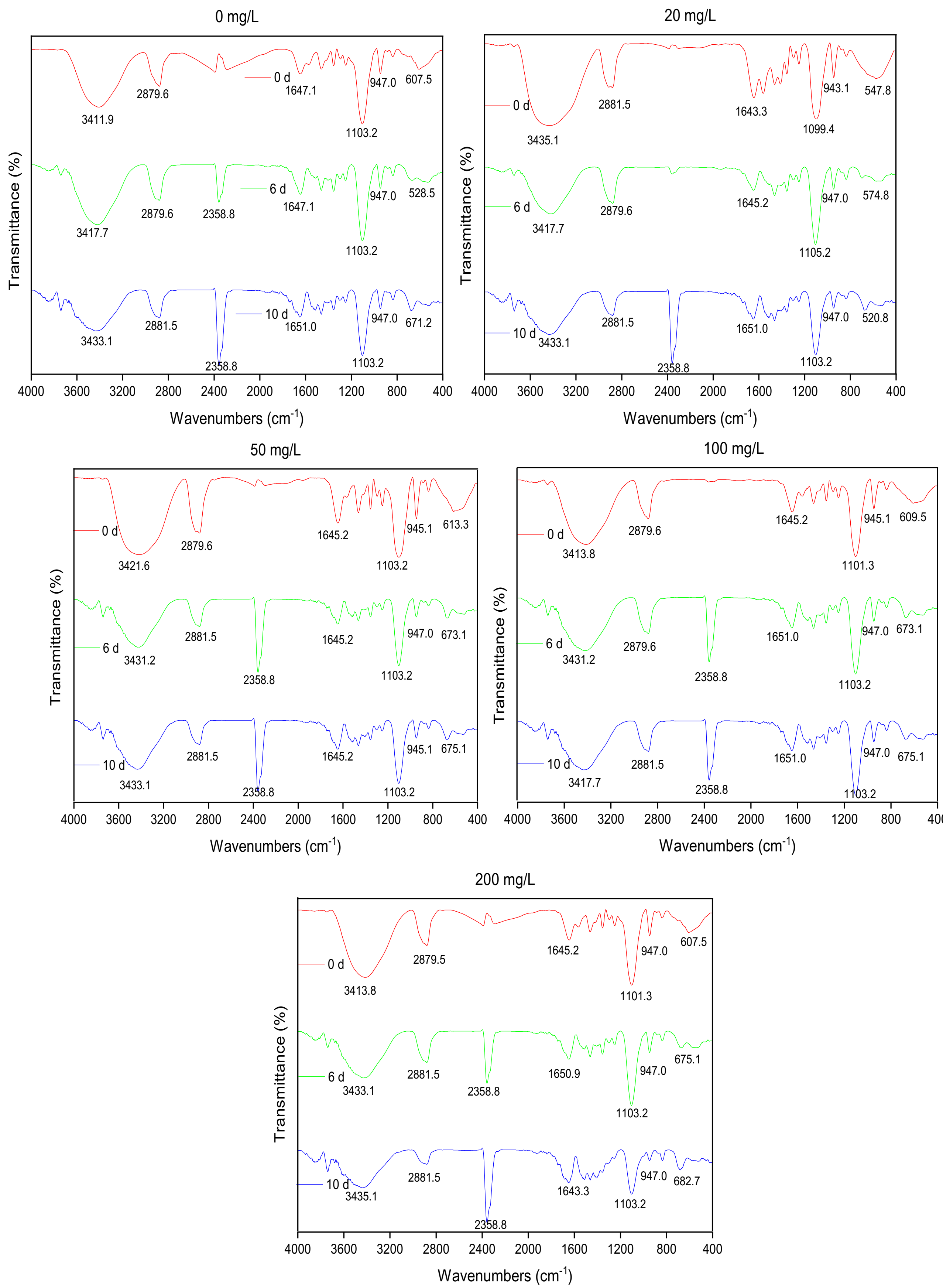

Fig. 8. FTIR spectra of DOM with FNP addition during methane production process.

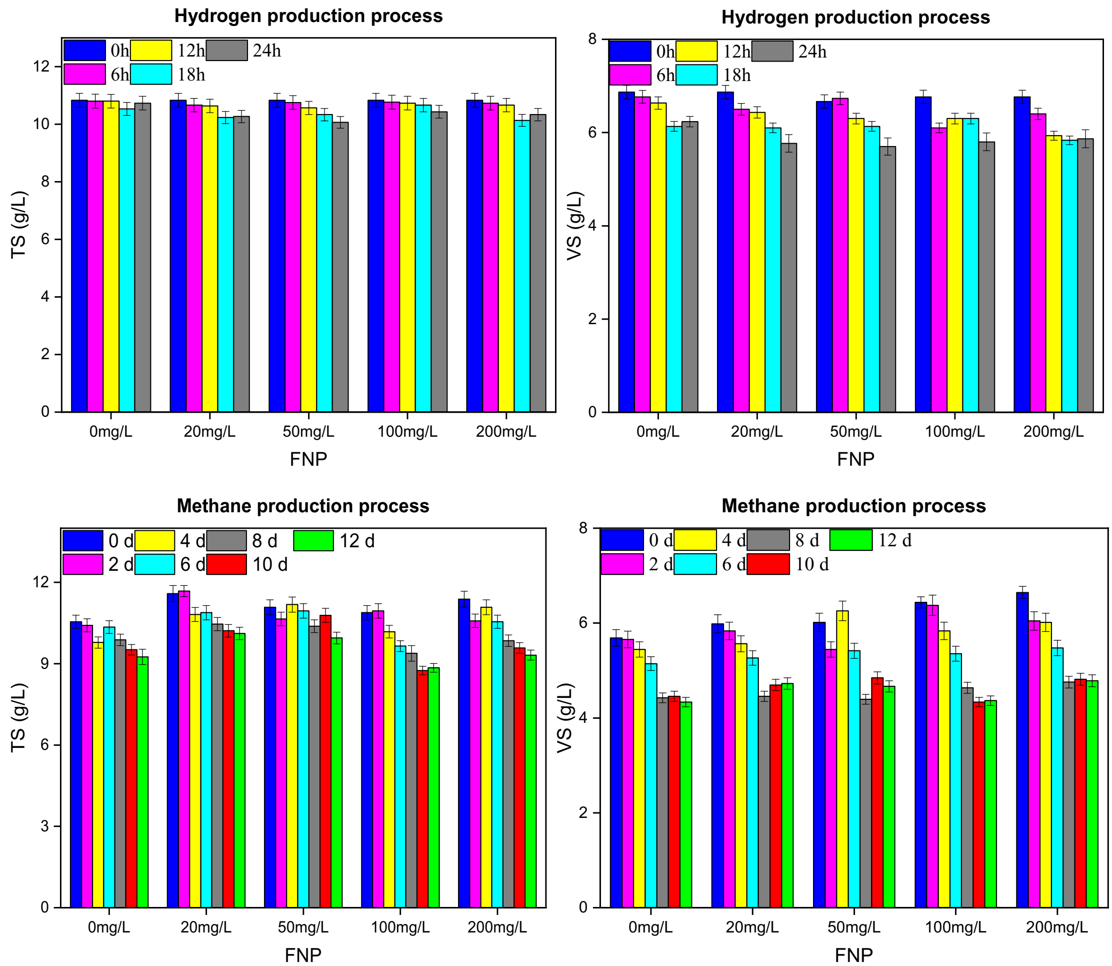

Fig. 9. Changes of TS and VS during the two-stage anaerobic digestion.

12.0% with the addition of 20, 50, 100 and 200 mg/L FNP, compared with that of control (11.2%). At the end of methane production process, concentrations of VS were 4.72, 4.67, 4.36 and 4.79 g/L and decreased by 21.1%, 22.4%, 32.2% and 28.0% with 20, 50, 100 and 200 mg/L FNP, and it was 23.7% with that of control. The TS and VS reduction of 26.5% and 46.9% with 100 mg/L addition was achieved compared to that of 16.4% and 34.3% with the control. Therefore, the main TS and VS reduction took place in the methane production process and the optimum dosage of FNP was 100 mg/L for the sludge reduction.

4. Conclusion

Addition of FNP improved the efficiency of two-stage anaerobic digestion. The production of VFAs enhanced by 2.5 times with the addition of 100 mg/L FNP during the acidogenesis phase, and the efficiency of acetoclastic methanogenesis was enhanced with FNP addition. The addition of FNP had positive effect on hydrolysis of SCOD and carbohydrate in EPS. FNP addition could improve the degradation of SCOD and carbohydrate in DOM, whereas there was little effect on the protein degradation compared with control. FTIR spectra indicated that the addition of FNP had distinct effects on the functional groups of polysaccharide and protein.

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