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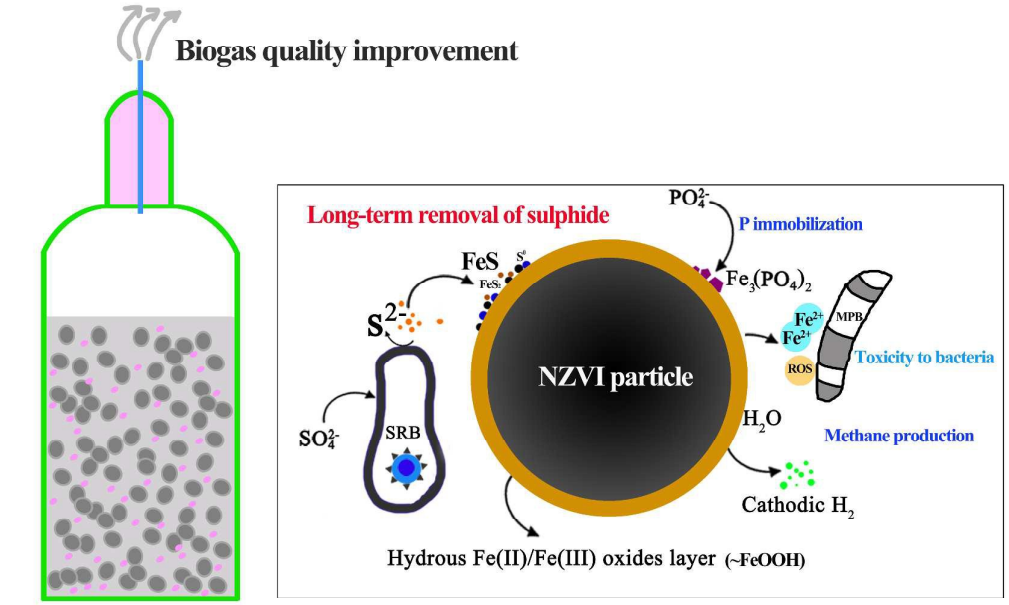
The long-term effects of NZVI on hydrogen sulphide removal in an anaerobic sludge digester were Investigated.



Anaerobic digestion Core-shell structure of NZVI

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Anaerobic digestion is one of the most widely-used technologies for waste activated sludge reduction and stabilization. Biogas is a versatile and clean renewable energy source. Burning of biogas containing hydrogen sulphide can shorten the life span of combustion engines and produce precursor of acid rain. The core-shell structure results in zero-valent iron nanoparticles with manifold functional properties. Our finding showed that supplying zero-valent iron nanoparticles to anaerobic sludge digesters can be an efficient method for the long-term removal of hydrogen sulphide in biogas. Meanwhile, the methane yield can be enhanced at the appropriate zero-valent iron nanoparticles dose.

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ARTICLE

The use of the core-shell structure of zero-valent iron nanoparticles (NZVI) for long-term removal of sulphide in sludge during anaerobic digestion

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The core-shell structure results in zero-valent iron nanoparticles (NZVI) with manifold functional properties. In this study, the long-term effects of NZVI on hydrogen sulphide removal in an anaerobic sludge digester were investigated. Within 20 days, the average hydrogen sulphide content in the biogas was successfully reduced from 300 (or 3620 of sulphate-rich sludge) mg/Nm^3 to 6.1 (121), 0.9 (3.3) and 0.5 (1.3) mg/Nm^3 in the presence of 0.05, 0.10 and 0.20% (wt) NZVI, respectively. The methane yield was enhanced at the low NZVI dose (0.05-0.10%) but decreased at the elevated dose (0.20%). Methane production and volatile solid degradation analyses implied that doses of 0.5-0.10% NZVI could accelerate sludge stabilization during anaerobic digestion. The phosphorus fractionation profile suggested that methane production could be inhibited at the elevated NZVI dose, partly due to the limited availability of soluble phosphorus due to the immobilization of bioavailable-P through the formation of vivianite. An analysis of the reducible inorganic sulphur species revealed that the elimination of hydrogen sulphide occurred via the reaction between hydrogen sulphide and the oxide shell of NZVI, which mainly formed FeS and some FeS_2 and S^0.

1. Introduction

Waste activated sludge (WAS) is a by-product of wastewater treatment plants (WWTPs). As a major and essential process used at modern WWTPs, anaerobic digestion can transform a large portion of the organic fraction of WAS into biogas, which is a versatile and renewable energy source that can be employed for producing heat, steam, electricity combined with heat, power (CHP) or vehicle fuel. In all cases, impurities such as hydrogen sulphide must be removed from the biogas before its use[1]. Sulphide in biogas can reduce the lifetime of metal pipes and shorten the life span of combustion engines due to corrosion[2]. The maximum acceptable hydrogen sulphide content is ca. 100-500 mg/Nm^3 when biogas is used for CHP, depending on the specific manufacturer, and the concentration should be further reduced to less than 5 mg/Nm^3 when used as fuel for vehicles[3]. Hydrogen sulphide also has an extremely low odour threshold (0.41 ppb) relative to most other odorous compounds and is highly toxic to humans at elevated concentrations[4].

Sulphate can act as an electron acceptor for sulphide for sulphate reducing bacteria (SRB) under anaerobic conditions. However, organic sulphur in the form of proteins or cell constituents is only partly reduced, and a large portion remains unchanged during anaerobic digestion[5]. The production range of hydrogen sulphide in biogas varies considerably depending on the sludge characterises (e.g., bioavailable-S derived from sewage) and the outcome of the competition between the SRB and methane-producing bacteria (MPB), which both compete for the same substrates, such as lactate and acetate[6, 7].

Although appropriate conditioning of WAS may decrease the sulphide content in biogas[8], further treatment is needed to meet the requirements of methane utilisation technologies[1]. Process-level control of sulphide emissions, such as oxygenation, sulphide precipitation or a combination of both, in an anaerobic bioreactor may be a feasible method for reducing sulphide contents in biogas and lowering sulphide toxicity to methane-producing bacteria (MPB)[5, 6]. Traditional oxidants hardly provide effective control for hydrogen sulphide in biogas or could significantly impair the anaerobic digestion environment. For example, regular nitrate doping during anaerobic sludge digestion increases the oxidation-reduction potential (ORP) values but does not reduce the sulphide concentrations in the biogas or digester liquor[5]. Recently, studies on the micro-aerobic removal of sulphide were conducted by continuously employing pure oxygen or air as an oxidant[1, 5, 9]. Attempts have been made to find an easy, long-

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term, and uncomplicated method for doping chemicals in anaerobic sludge digesters to remove sulphide in biogas.

Zero-valent iron nanoparticles (NZVI) have been widely used for treating hazardous and toxic wastes[10-12] due to their small size, large specific surface area, and elevated reactivity for rapid contaminant transformation. Yan et al. reported the inevitable reactions of NZVI with water (undergoing surface hydroxylation) and their effects on solution and surface chemistry[13]. Microscopic and spectroscopic studies have suggested that a thin surface layer (thickness of ca. 2~4 nm) mainly consisting of iron oxide (or amorphous oxide) will be formed at the agglomerate surface and between the individual particles[13, 14]. Two nano-constituents are present in the core–shell. Metallic iron acts as the electron source and reductant, and the oxide shell facilitates sorption and surface complexation while allowing electrons to pass into the metal core[13]. The core-shell structure provides NZVI with manifold functional properties[13]. Both positive and negative results have been reported regarding methane production in the presence of NZVI. Yang et al.[15] reported that NZVI inhibited methanogenic growth and methane production when present at concentrations of 1 mM or greater due to the disruption of cells. Our previous study showed that 0.10 wt% NZVI improved the biogas production by more than 30% from sewage sludge, with a solid content of approximately 15%[16]. Li et al. indicated that gaseous hydrogen sulphide was reduced effectively in the presence of iron nanoparticles and that X-ray photoelectron spectrometry (XPS) showed that gaseous hydrogen sulphide was immobilized as FeS and FeS_2[17]. Furthermore, Li et al. showed that the hydrogen sulphide in sludge was completely removed in less than 15 min when a dose of 0.5 g/L was used[17]. Yan et al. reported that doping NZVI into the solution resulted in rapid hydrogen sulphide uptake[13]. Although different studies have indicated that oxide shells possess the sorptive properties of sulphide, the long-term effectiveness of hydrogen sulphide removal in sludge anaerobic digestion remains unknown. The immobilized products of the NZVI-S(II) reaction in the sludge anaerobic digestion environment rather than the sulphide solution have not been clarified yet.

Few previous studies have focused on both the area of long-term sulphide removal and its role in sludge stabilization (volatile solid (VS) degradation, methane production) in anaerobic digestion with NZVI doping. The changes in the distributions of phosphorus, iron and sulphur species during anaerobic digestion in the presence of NZVI have not been completely elucidated. The objectives of this study were to (1) evaluate the long-term potential of NZVI for removing sulphide in biogas from biodigesters; (2) investigate the effects of NZVI on VS degradation and methane production; and (3) clarify the profiles of phosphorus, iron and reduced inorganic sulphur (RIS) in NZVI-dosed sludge.

2. Materials and methods

2.1. Test materials

The WAS sample was collected directly from the end of the aeration basin of a local sewage treatment plant in Shanghai, China. The investigated treatment plant treats approximately 75,000 m^3/d of wastewater (93% domestic and 7% industrial sewage) using an anaerobic-anoxic-oxic process. The collected WAS sample was sent to the laboratory immediately and passed through a 0.15-mm sieve to remove grit. Table 1 shows the main characteristics of the sludge. NZVI were obtained from the Aladdin Chemistry Co., Ltd. (Shanghai, China). Scanning Electron Microscopy (SEM) images indicated that the NZVI was generally spherical and the particles ranged in size from 60 to 120 nm (Fig. 1) and were connected in chains, possibly due to chemical aggregation. The energy dispersive X-ray (EDX) spectrum for NZVI particles showed the presence of Fe and O (Fe: 89.78%, O: 10.22% (wt)) in this structure (Fig. 1), and the relatively high O content could partly result from the aging of the NZVI[18].

2.2. Experimental procedure

The experimental procedure is described schematically in Fig. 2. The WAS sample was rigorously mixed in the laboratory for 60 min to reduce the sulphide concentration by oxidation/stripping and to minimise sample variation. The sludge samples (350 mL each) were homogenized with 0, 0.175, 0.350 and 0.700 g of NZVI, respectively, and were transferred into 500-mL serum bottles (fifteen repetitions per dose, three for H_2S determination, three for pH analysis, three for biogas volume analysis, three for biogas composition analysis, three for other parameters (such as different species of Fe, RIS and P), XRD and VS) that were flushed with high purity N_2 to exclude oxygen and were sealed with a butyl rubber stopper. Anaerobic digestion was performed in the mesophilic range (32±1°C) for 20 days. Moreover, to investigate the potential of NZVI on sulphide reduction for sulphate-rich sludge, 0.35 g of sodium sulphate was added to 350 mL of sludge (three repetitions per dosage) to increase the amount of hydrogen sulphide generated.

The hydrogen sulphide concentration and pH were determined every two days. Methane production and volatile solid degradation were measured to determine the performance of the sludge anaerobic digestion. The fractionation profiles of iron, phosphorus and reduced inorganic sulphur (RIS) at the end of the experiment were evaluated to elucidate the detailed roles and mechanisms of

Table 1 Fundamental characteristics of the waste activated sludge used in this study

Parameter	Value	Elemental	Value (g/kg dry basis)
pH	6.50±0.02[c]	Fe	16.10
Total Solids (g/L)	10.14±0.06	Zn	1.08
Volatile Solids (g/L)	5.97±0.01	Pb	0.04
TSS (g/L)	6.62±0.24	Cu	0.14
VSS (g/L)	2.62±0.16	Cr	0.04
Total sulphur (%) [b]	1.02±0.04	Cd	n.d.

[a] TSS: total suspended solids; VSS: volatile suspended solids; n.d.: not detected

[b] Total sulphur (dry basis) was determined by using an Elementar CNS analyser, model Vario EL III (Vario EL, Elementar Analyser system GmbH, Hanau, Germany)

[c] Standard deviation of three determinations

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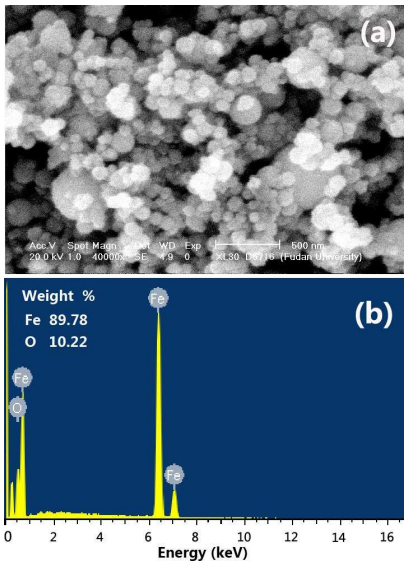

Fig. 1 SEM-EDS spectra of NZVI (40000×)

NZVI in anaerobic digestion. The determinations of the hydrogen sulphide concentration, biogas volume and biogas composition are depicted in Fig. S1.

2.3. Analytical methods

2.3.1 Fractionation profiles

Sludge samples were subjected to sequential extraction (SE) to determine the phosphorus and iron species after 20 d of anaerobic digestion, as described by Smith & Carliell-Marquet[19, 20]. Phosphorus was sequentially extracted using ultra-pure water (UPW), acetate buffer, NaOH, HCl and aqua regia. The portion of phosphorus that was extracted by UPW and acetate buffer was considered accessible to microorganisms and is referred to as bioavailable P in this study. Fe was sequential extracted using UPW, KNO_3, KF, Na_4P_2O, Disodium EDTA dehydrate, HNO_3 and aqua regia. The fraction of Fe extracted by UPW, KNO_3 and KF is considered accessible by microorganisms and is referred to as bioavailable Fe. The resultant fractions were analysed using a Varian 720-ES inductively coupled plasma-optical emission spectrometer (ICP-OES) (Agilent Technologies, Inc., CA, USA).

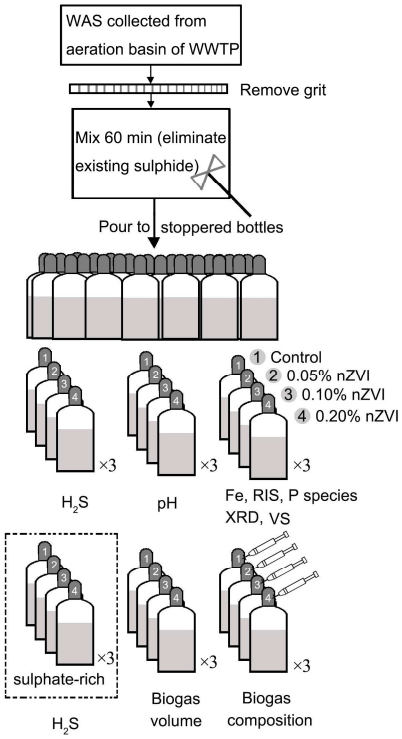

Fig. 2. Schematic description of the experimental laboratory procedure

X-ray diffraction (XRD) was used to characterize the transformation of P species in the sludge induced by the presence of NZVI after anaerobic digestion. For XRD analysis, an AD 8 Advance powder diffractometer (Bru-ker AXS Inc., Germany) was used with an accelerating voltage at 40 kV and a current at 40 mA. The samples (< 5μm) were examined at room temperature over a 2θ range of 10-90° using graphite monochromatic Cu Kα radiation. The step scan was 0.02° and the measuring time was 0.1 s/step. The diffractograms were obtained using Diff-plus and analysed using the MDI Jade 5.0 software. The portion of amorphous iron (hydr) oxides was estimated using an ascorbate extraction and is denoted as Fe-ASC[21]. This fraction was also tested to determine the concentrations of reactive iron oxides in the sludge after anaerobic digestion.

The reduced inorganic sulphur (RIS) species, including acid-volatile sulphide (AVS), Cr (II)-reducible sulphide (CRS) and elemental sulphur (ES), in the sludge samples were analysed using a modified cold diffusion SE method, as described in our previous study[22] and by Hsieh et al.[23, 24]. Analysis of the extractable reduced inorganic sulphur is shown in Fig S2. First, the sludge samples were frozen immediately after sampling and were stored frozen at -20 °C until analysis. Then, 30 mL of a deoxygenated 5% ZnAc solution was placed in a 500 mL flask containing 1 mL of the thawed sludge sample under a nitrogen atmosphere. Next, the flask was placed on a magnetic stirrer and stirred for 6 hrs at 600 rpm. For the AVS determination, 15 mL of concentrated HCl and 5 mL 1 mol/L ascorbic acid (to prevent Fe from forming Fe^{3+}) were introduced into the 500 mL flask. After AVS extraction, 15 mL of the Cr (II) solution was added to the remaining slurry and the captured sulphur (hydrogen sulphide) was determined as pyrite-S (FeS_2). Finally, 20 mL of N,N-dimethylformamide (DMF) was added to the slurry after the pyrite-S extraction to determine the ES (S^0). All of the captured hydrogen sulphide was analysed using the Cadmium Hydroxide-Methylene Blue method. The optimal extraction times of AVS, CRS and ES were 72, 48 and 48 h, respectively. All of the reactions occurred at ambient temperature.

2.3.2. Thermal gravity (TG) analysis

Thermal gravimetric and differential thermal gravimetric analyses (TGA/DTG) were performed using a SDT Q600 simultaneous thermal analyser (TA Instruments, USA). The samples were heated from 50 °C to 600 °C at a rate of 10 °C/min under nitrogen flow (100 mL/min).

2.3.3 Other methods

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The biogas volume was measured using the water displacement method[25]. To determine the biogas composition, approximately 10 mL of biogas was sampled in a glass syringe and was analysed using a gas chromatograph (GC) (Agilent Micro GC 3000) equipped with a TCD detector and a molecular sieve column. Each sample was measured in triplicate, and the average values of the triplicate measurements were used. The hydrogen sulphide content in the biogas was analysed according to the Cadmium Hydroxide-Methylene Blue method[22, 26]. Hydrogen sulphide was precipitated as cadmium sulphide by using an alkaline suspension of cadmium hydroxide to prevent the oxidation of sulphide. The collected sulphide was subsequently determined by using a UV spectrometer (T6, Beijing Puxi General Instrument Corp., China) to determine the methylene blue content produced from the reaction of the sulphide with a strongly acid solution of N, N-dimethyl- p-phenylenediamine and ferric chloride.

2.4. Statistical analyses

Repeated analyses of variance (ANOVAs) and chi-square tests were performed using the SPSS statistics software package version 19.0 (SPSS, Chicago, IL, USA). The objective of the statistical analysis was to identify significant differences among the parameters analysed for different treatments during anaerobic digestion. The correlations were considered statistically significant at the 95% confidence interval (p<0.05).

3. Results and discussion

3.1. Effects of NZVI on pH

Fig. 3a presents the pH trends of WAS in the presence of NZVI during anaerobic digestion. The NZVI-dosed sludge showed a steep increase in pH during the first 2 days. The pH increased from 6.5 in the control sludge to 7.0, 7.2 and 7.5 in the sludge treated with 0.05%, 0.10%, and 0.20% (wt) NZVI, respectively. In the subsequent period of 18 days, the pH continued to increase at a much slower rate and then gradually stabilized at the end of the experiment. The initial pH surge was attributed to rapid oxidation when NZVI entered the aqueous phase, as showed in Eq. (1)[27].

$$Fe^0(s)+2H_2O \rightarrow Fe^{2+}+H_2(g)+2OH^- \quad (1)$$

Next, as hydroxyl ions accumulated on the surfaces of the NZVI particles, the formation of a passivating oxyhydroxide coating (core-shell structure) slowed down the oxidation rate and increased the pH[13]. The pH increased from 6.50 to 7.58 gradually in the control sludge during 20 d of anaerobic digestion. The result is different from our previous study[22], in which pH showed a slight decrease at the beginning days. The phenomenon might be explained that the pH reduction is countered effectively by the alkalinity produced from methanogenic bacteria. Similar result was also found by Yang G. et al [28]. The changes in pH during anaerobic digestion could be divided into three steps based on the different pH values with and without NZVI treatment (Fig. 3b). Step 1 included the formation of the core-shell structure of NZVI in the sludge within 2 days. Step 2 implied that the pH

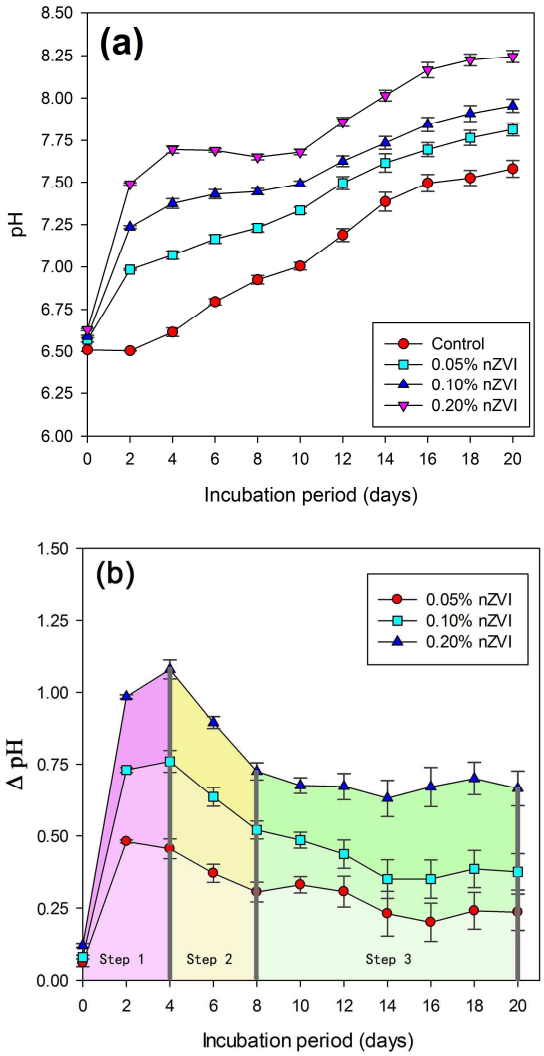

Fig. 3. The pH trends of sludge in the presence of NZVI during anaerobic digestion.

was buffered by organic acid anions, such as acetate derived from the decomposition of organic matter. Next, the changes in pH between the un-dosed and NZVI-dosed sludge were gradually stabilized.

3.2. The effects of NZVI on the hydrogen sulphide concentrations in biogas

The sludge without the NZVI treatment produced biogas with an average hydrogen sulphide concentration of 300 mg/Nm^3 during 20 d of anaerobic digestion. Because of the consumption of sulphate by SRB over time, the concentration of hydrogen sulphide in the biogas gradually decreased from 663 to 84 mg/Nm^3. NZVI doping significantly (P<0.005) reduced the average hydrogen sulphide concentration in the biogas to 6.1, 0.9 and 0.5 mg/Nm^3 in the treatments with NZVI concentrations of 0.05, 0.10 and 0.20% (wt) (Fig. 4a), respectively. The corresponding removal efficiencies were 98.0% (96.8~100%), 99.7% (98.3~100%) and 99.8% (98.3~100%), respectively.

In the sulphate-rich sludge without NZVI, the introduction of 1000 mg/L of sodium sulphate increased the average

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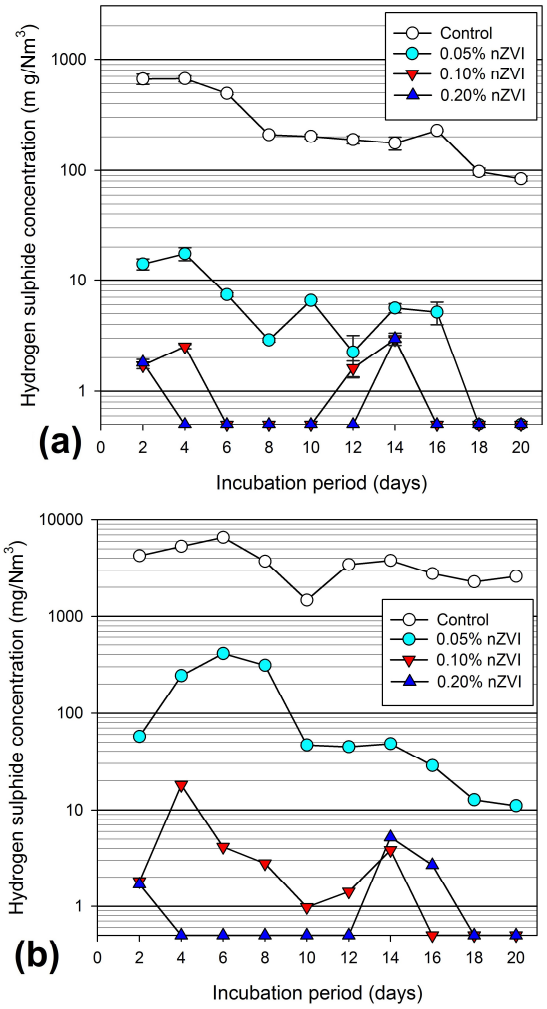

Fig. 4. Effect of NZVI doping on hydrogen sulphide concentration in biogas (a: without sulphate addition, b: with the addition of 1000 mg/L sodium sulphate).

hydrogen sulphide concentration from 312 to 3620 mg/Nm^3 during anaerobic digestion (Fig. 4b). With the addition of sodium sulphate, the NZVI treatments resulted in significantly (P<0.001) lower hydrogen sulphide concentrations in the biogas relative to the treatments with 121, 3.3 and 1.3 mg/Nm^3 doses of NZVI (0.05, 0.10 and 0.20% (wt), respectively). The corresponding removal efficiencies reached 97.2% (91.7~99.6%), 99.9% (99.7~100%) and nearly 100%, and the removal efficiencies were positively correlated with the NZVI dosages. Generally, the addition of 0.05% NZVI results in an average hydrogen sulphide concentration of less than 10 mg/Nm^3 (or 150 mg/Nm^3 of sulphate-rich sludge), which was lower than most of the manufacturers' specifications for power generation. Furthermore, a dose of 0.10% NZVI can decrease the average hydrogen sulphide concentration to less than 1 mg/Nm^3 (or 5 mg/Nm^3 of sulphate-rich sludge), which could meet the requirements of other uses (e.g., fuel for vehicles).

When situated in aqueous environments, an oxide layer will be formed on the surface of the NZVI. The chemical composition results indicate that the oxide layer consists of two phases, a mixed phase of Fe(II)/Fe(III) near the metallic core and a phase of predominantly Fe(III) oxide near the oxide/water interface[18, 29]. The surface of the oxide invariantly contains hydroxide groups after exposure to water, resulting in an apparent surface stoichiometry that is similar to that of FeOOH[30, 31]. The characterization of the oxide layer was similar to that of ferric hydroxide (FeOOH), which was an effective and long-lasting reagent for removing hydrogen sulphide from sewage sludge in anaerobic environments according to our previous study[22].

3.3. Effects of NZVI on methane production and volatile solid degradation

Fig. 5 shows the cumulative volumes of methane produced during 20 d of anaerobic digestion. The volume of methane is a useful measure of sludge treatment because it is directly related to the combustion capability in electric power plants or CHP plants[20]. The methane production in the presence of different doses of NZVI varied. With respect to methane production, NZVI dosages of 0.05 and 0.10% increased the methane production by 9.8 and 4.6%. However, the methane production decreased by 8.8% at the elevated NZVI dose (0.20%). ZVI can produce hydrogen gas and stimulate sulphate reducers and methanogenic populations. Carpenter et al indicated that NZVI enhance methanogenic activity and improve biogas by decreasing the amount of CO_2 released[32]. The metallic iron core could act as a slow-release electron donor. Enhancement of the proportion of acetate conversion[33], acidogenesis,[34, 35] under anaerobic conditions by adding iron powder has been reported. However, the bio-toxicity of NZVI was also recorded due to cell membrane disruption when small nanoparticles enter the cell[36]. S. Karri et al. reported that the dissolution of NZVI in sludge resulted in the generation of reactive oxygen species (ROS) and a high concentration of soluble Fe^{2+}, which could be harmful to methanogens[37]. Moreover, the bioavailable phosphorus immobilization also affected the methane yield, which will be discussed in the P species analysis section. The effects of each factor on methane

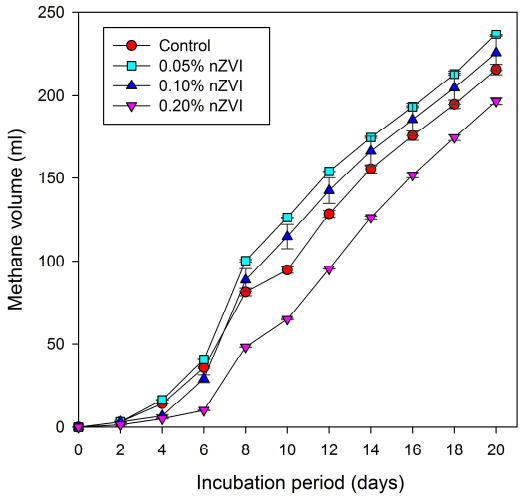

Fig. 5. The final cumulative volumes of methane produced during 20-d anaerobic digestion.

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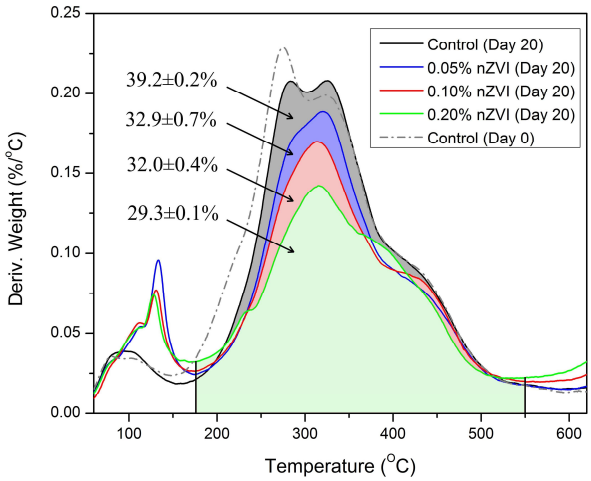

Fig. 6. TG-DSC curves of sludge at 20 d

yield were not quantified, and further studies are needed to distinguish between these potential mechanisms.

Fig. 6 shows the differential thermal gravimetric analysis (DTG) data of sludge samples after 20 d of anaerobic digestion. As suggested by a peak in the DTG curves, the weight loss occurred at approximately 100-150 °C in the NZVI dosed sludge samples, which could be attributed to the evaporation of bound water in the core-shell structure of the NZVI when situated in the sludge. The largest sludge sample peaks appeared at 175~550 °C, mainly as a result of organic matter pyrolysis. The introduction of NZVI and the biodegradation of organic matter could both result in decreased peak intensities at approximately 175~550°C in the NZVI dosed sludge samples.

Due to the formation of the core-shell structure of NZVI in the sludge, the difference of LOI_{550}-LOI_{105} cannot reflect the organic matter content in the sludge. In this study, the weight loss of the sludge samples at 175~550 °C by TG analysis was attributed to the organic matter content in the sludge samples. Based on this assumption, the VS contents (%, dry basis) decreased from 39.2% to 32.9, 32.0, and 29.3% in the treatments with 0.05%, 0.10%, and 0.20% NZVI after 20 d of anaerobic digestion. Because the amended Fe would change the composition of the solids, which would change the fraction of VS generated per g of total solids, the values of VS in g/L were calculated according to Eqn.(2). The VS contents (g/L, wet basis) of the sludge decreased from 3.38±0.02 g/L to 3.14±0.07 and 3.24±0.04 g/L in the presence of 0.05% and 0.10% NZVI, and were not different from those in the 0.20% NZVI-dosed sludge (3.39±0.01 g/L). The methane production and VS degradation implied that NZVI doses of 0.5-0.10% could accelerate sludge stabilization during anaerobic digestion. Methane production could be inhibited at elevated NZVI doses (e.g., 0.20%) due in part to limited soluble phosphorus availability.

$$\text{VS (g/L, wet basis)} = \text{VS (\%, dry basis)} \times \text{TS (g/L)} \quad (2)$$

3.4. Effects of NZVI on the distributions of phosphorus, iron and RIS species

Table 2 demonstrates the fractionation profile of P for WAS after anaerobic digestion. The addition of NZVI reduced the proportion of H_2O-P and acetate-P and elevated the proportion of NaOH-P. A chi-square test revealed significant differences in the P species distributions between the treatments and the control (P<0.001). The percentages of the bioavailable fractions of P (H_2O-P+ acetate-P) relative to the total concentrations were significantly decreased (P<0.001) from 58.1% to 5.32, 0.78 and 0.38% in the treatments with 0.05, 0.10 and 0.20% (wt) NZVI, respectively. Among the fractions accessible by the microorganisms, the fraction of H_2O-P significantly decreased from 79.4 mg/L in the control to 0.4, 0.2 and 0.2 mg/L for the three NZVI dosages. Meanwhile, the P in Fe(III)-hydroxy-P and iron phosphate (mainly extracted by NaOH)[20, 38] increased from 73.2 mg/L to 200, 209 and 206 mg/L for the NZVI treatments of 0.05%, 0.10%, and 0.20%.

Table 2 The effects of NZVI-dosing on the concentration and distributions of phosphorus in the sludge after anaerobic digestion.

Treatments	H_2O-P		Acetate-P		NaOH	Residual	Bioavailable fractions[b]		Total concentration
	Soluble	UPW[a]	Acetate 1	Acetate 2					
Undosed	66.05±0.04	13.38±0.05	39.91±0.08	11.54±0.29	73.17±0.21	21.16±0.61	130.88±0.31	58.12%	225.21±0.71
0.05% nZVI	0.10±0.01	0.30±0.04	3.36±0.01	8.57±0.39	200.10±0.30	19.55±0.76	12.33±0.39	5.32%	231.98±0.91
0.10% nZVI	0.08±0.00	0.14±0.00	0.40±0.00	1.17±0.01	208.66±6.62	18.33±0.03	1.79±0.01	0.78%	228.78±6.62
0.20% nZVI	0.11±0.01	0.12±0.00	0.36±0.01	0.27±0.02	205.70±8.54	19.95±0.32	0.86±0.02	0.38%	226.51±8.85
P species[c]	Soluble P species	P weakly bound to sludge particles	Struvite; P adsorbed to $CaCO_3$; P from amorphous Ca-P precipitates	Soluble reactive P from Al, or Fe. Organic P	Iron phosphate				

[a] ultra-pure water (18 MΩ•cm)
[b] made up of the first four fractions for P (soluble, UPW, acetate 1, acetate 2) and considered to be accessible by the microorganisms.
[c] interpretation of phosphorus species[38]

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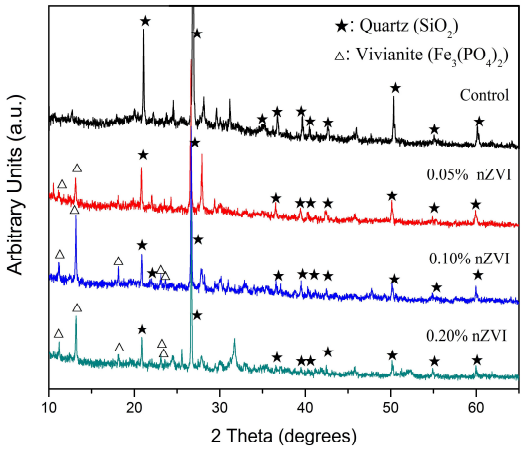

Fig. 7. XRD spectra of the crystalline phases in sludge after anaerobic digestion.

The P species distribution results indicated that soluble and loosely bound phosphorus were immobilized by the addition of NZVI due to formation of Fe (III)-hydroxy-P or iron phosphates. Further XRD analysis (Fig. 7) confirmed a clear increase in the intensity of vivianite [$Fe_3(PO_4)_2$] in WAS treated with NZVI. The lower concentrations of bioavailable P (especially soluble P) in the digest reactors could impair digestibility by limiting soluble P, which would further decrease biogas production and decelerate sludge stabilization. Dentel and Gossett[39] proposed that the organics (e.g., fatty acids) could be physical enmeshed by Fe–hydroxy–phosphate 'chains', which would cause them to become less available or unavailable to microorganisms during anaerobic digestion. Another interesting point is that, the immobilization of P by the addition of NZVI could help recover P in phosphate–containing wastewater, especially in WAS when using alkali extraction technology[40].

The characteristics of Fe in the sludge are shown in Fig. 8. Understandably, the addition of NZVI markedly increased the total Fe content in the sludge from 148.6 mg/L for the control to 617.8, 1155 and 1905 mg/L when NZVI was added at rates of 0.05%, 0.10% and 0.20%, respectively. The concentrations of bioavailable Fe extracted in the first three fractions (soluble+UPW+KNO_3+KF) were not vastly different in the presence of NZVI. Our results indicated that adding NZVI resulted in the conversion of most of the Fe to the fraction of Fe hydroxides or phosphates during anaerobic digestion after 20 days of anaerobic digestion. The formation of Fe hydroxides and Fe phosphate most likely resulted from the H_2O-NZVI reaction and resulted in immobilization of the bioavailable P fraction. Moreover, the determination of Fe-ASC indicated the

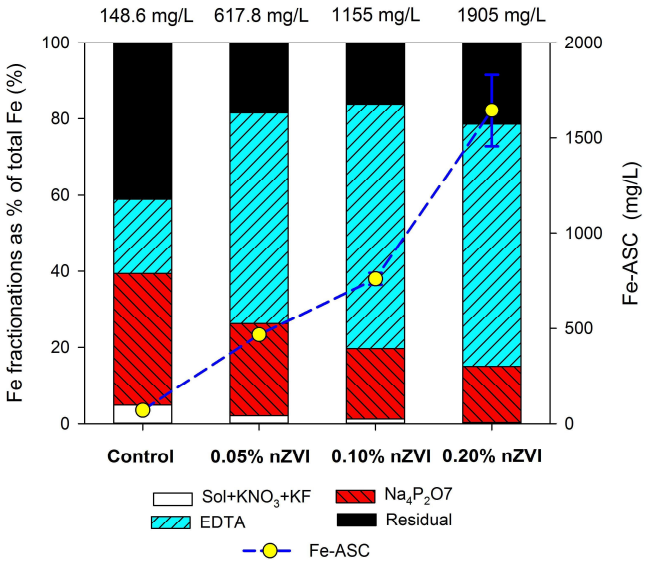

Fig. 8 The effects of NZVI-dosing on the distribution of iron in sludge after anaerobic digestion. Interpretation of iron species[38], $Na_4P_2O_7$-Fe: Organically-bound Fe; EDTA-Fe: iron hydroxides and Fe-hydroxy-phosphates; and Residual Fe: tailings from Fe–P, Fe phosphate and FeS

presence of a boundary of reactive iron oxides in the NZVI-dosed sludge after anaerobic digestion. The amorphous Fe concentration increased from 69 mg/L to 466, 760 and 1644 mg/L, while the percentages of the remaining amorphous Fe relative to the total concentrations was increased from 46.51% to 75.51%, 65.84%, 86.30%, when NZVI was added at 0.05, 0.10 and 0.20%, respectively, which implied that reusing NZVI–dosed sludge to eliminate hydrogen sulphide could be conducted to reduce operation costs.

The distribution of reduced inorganic sulphur (RIS) species was determined to elucidate the effects of NZVI in sulphide reaction, as showed in Fig. 9. After 20 days of incubation, the presence of NZVI at 0.05, 0.10 and 0.20% enhanced the acid-volatile sulphide (AVS) concentrations by 3.85, 4.42 and 7.49 mg/L, the Cr(II)-reducible sulphide (CRS) concentrations by 0.12, 0.15 and 0.14 mg/L and the elemental sulphur (ES) concentrations by 0.08, 0.11 and 0.11 mg/L (Fig. 9), respectively. Repeated ANOVA tests revealed significant differences in AVS (P<0.05) and CRS (P<0.05) between the treatments and the control. According to Rickard and Morse[41], potential sources of AVS include dissolved S(-II) species (H_2S, HS^-, $FeHS^+$), FeS clusters, iron sulphide nanoparticles, mackinawite (FeS), pyrite, and greigite (Fe_3S_4). Hsieh et al. [23]indicated that pyrite could not be recovered using the AVS

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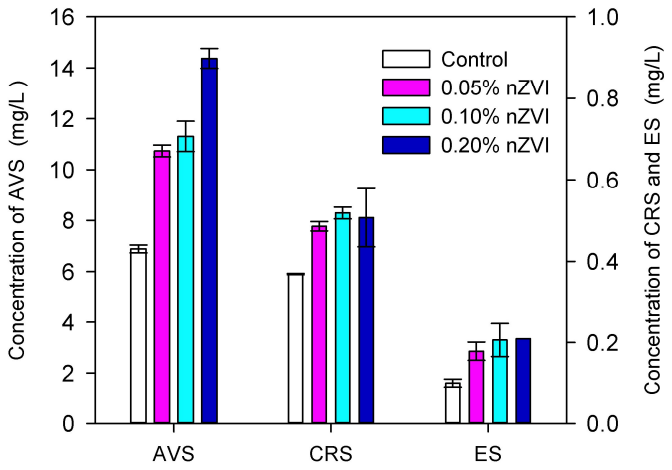

Fig. 9 The effects of NZVI-dosing on the distribution of reduced inorganic sulphur (RIS) species in sludge after anaerobic digestion.

analysis procedure adopted in this study. Therefore, the increase of AVS-S in sludge treated with NZVI suggested that the dissolved S(-II) produced during sulphate reduction was immediately immobilized in the form of insoluble FeS, potentially due to the direct reaction between sulphide accumulated in the sludge and zero-valent iron, as showed in Eq. (3)[42].

$$Fe^0+H_2S=FeS+H_2(g) \quad (3)$$

The variations in CRS-S (pyrite) and ES between the treatment and control suggested that FeS_2 and S^0 were by-products of the reactions between hydrogen sulphide and NZVI during sludge anaerobic digestion. Previous studies indicated that NZVI can form a surface layer of iron oxide when situated in the aqueous environment, as shown in Eq (4) for simplification[31, 43].

$$Fe^0+2H_2O=FeOOH+1.5H_2(g) \quad (4)$$

Apart from Eq.(3), the hydrogen sulphide produced from the reduction of sulphate by SRB can effectively react with the hydrous Fe(II)/Fe(III) oxide layers of the nanoparticles via Eqs. (5) ~ (6), in which S^{2-} was immobilized as FeS or FeS_2 or oxidized as S_8 [43]:

$$2FeOOH+3H_2S=2FeS+1/8S_8+4H_2O \quad (5)$$

$$2FeOOH+3H_2S=FeS_2+FeS+4H_2O \quad (6)$$

The distribution of RIS in this study indicated that most of the hydrogen sulphide produced was immobilized as iron (II) sulphide and that a minor portion of the RIS was oxidized to pyrite and elemental sulphur on the surface of NZVI.

According to the results of this study, the NZVI presented multiple physical-chemical properties during sludge anaerobic digestion that were closely correlated with the core-shell structure. The hydrogen and hydroxyl ions generated by the Fe^0-H_2O reaction under anaerobic conditions favoured an increase in pH. After the core-shell structure was formed when NZVI was added to the aqueous environment, the nanoscale surface oxide layer significantly decreased the hydrogen sulphide concentration in the biogas by forming iron sulphide (mostly), pyrite and elemental sulphur, which improved the quality of the biogas and was beneficial for energy use and odour abatement. The methane yield was enhanced at a low dose of NZVI (0.05-0.10%) but decreased as the dose was elevated (0.20%). Methane production and volatile solid degradation analyses implied that doses of 0.5-0.10% NZVI could accelerate sludge stabilization in anaerobic digestion. However, the immobilization of soluble or weakly bound P through the formation of vivianite with nanoscale oxide layers at the surface of NZVI reduced the bioavailable P fraction and could further inhibit methane production processes at elevated NZVI doses (e.g., 0.20%).

Conclusions

Supplying NZVI to anaerobic sludge digesters can be an efficient method for the long-term removal of hydrogen sulphide in biogas. The formation of FeS and a small portion of FeS_2 and S^0 via reactions between hydrogen sulphide and oxides shell on NZVI surfaces were mainly responsible for the elimination of hydrogen sulphide according to the RIS species analysis. The methane yield was enhanced at the low NZVI dose (0.05-0.10%) but decreased at the elevated dose (0.20%). Methane production and the volatile solid degradation analysis indicated that NZVI doses of 0.5-0.10% accelerate sludge stabilization in anaerobic digestion. The phosphorus fractionation profile suggests that methane production could be inhibited at the elevated dose of NZVI (e.g., 0.20%), partly due to limited soluble phosphorus availability as a result of the immobilization of bioavailable-P through the formation of vivianite.

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