Supporting information

Nano-Fe_3O_4 particles accelerating electromethanogenesis on an hour-long timescale in wetland soil

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1 Materials and methods

1.1 Determination of sulphur species

Solid-phase sulphur species (including AVS [acid-volatile sulfide] and CRS [chromium-reducible sulfide]) were determined by using the sequential extraction method developed by Burton *et al.*[1]. Further details can be found in a previous published study[2]. The concentration of SO_4^- was determined by ion chromatography (Dionex ICS-2000).

1.2 Analysis of bacterial and archaeal community composition

Polymerase chain reaction (PCR) amplification of 16S cDNA was undertaken using general bacterial primers (515F 5'-GTGCCAGCMGCCGCGGTAA-3' and 926R 5'-CCGTCAATTCMTTTGAGTTT-3') and archaeal primers (Arch519F 5'-CAGCCGCCGCGGTAA-3' and Arch915R 5'-GTGCTCCCCCGCCAATTCCT-3'). The primers with Illumina 5' overhang adapter sequences were used for two-step amplicon library building. The initial PCR reactions were carried out in a 25 μL reaction volume with 1–2 μL cDNA template, 250 mM dNTPs, 0.25 mM of each primer, 1X reaction buffer, and 0.5 U Phusion DNA Polymerase (New England Biolabs, USA).

After initial denaturation at 94 °C for 2 min, 25 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s were conducted with a final extension of 72 °C for 5 min. The second step PCR with dual 8-base barcodes was applied for multiplexing. The incorporation of two unique barcodes to either end of the 16S amplicons was via 8 cycle PCR reactions. The cycling conditions consisted of one cycle of 94 °C for 3 min, followed by 8 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s with a final extension cycle of 72 °C for 5 min. Prior to library pooling, a DNA gel extraction kit (Axygen, China) was used to purify the barcoded PCR products, which were quantified using a FTC-3000 TM real-time PCR system. The libraries were sequenced by 2×300 bp paired-end sequencing on the MiSeq platform.

To analyse the bioinformation, the raw reads, low quality sequences, clustering operational taxonomic units (OTUs), and taxonomic classifications were processed. The raw fastq files for all samples were demultiplexed based on the barcode. Trimmomatic (ver. 0.35) was adopted to remove low quality base pairs from the paired-end reads (parameters used: SLIDINGWINDOW –

50:20; MINLEN – 50). The trimmed reads were then further merged using the FLASH program (ver. 1.2.11) with default parameters. The low quality contigs were removed using the 'screen.seqs' command (filtering parameters: maxambig = 0, minlength = 200, maxlength = 580, and maxhomop = 8). A combination of various software: mothur (ver. 1.33.3), UPARSE (usearch ver. 8.1.1756, http://drive5.com/uparse/), and R (ver. 3.2.3) were applied to analyse the 16S sequences. The demultiplexed reads were clustered at 97% sequence identity into OTUs using the UPARSE pipeline (http://drive5.com/usearch/manual/uparse_cmds.html). The OTU representative sequences were used for taxonomy assignment against the Silva 119 database (confidence score ≥ 0.8) using the 'classify.seqs' command in mothur. The OTU taxonomies (from phylum to species) were determined based on the NCBI database. The sequence data have been submitted to the GenBank database under accession numbers SRP096926 (bacteria) and SRP097245 (archaea).

2 Results and discussion

2.1 The selection of an appropriate incubation time

The decomposition of some plant residues in anoxic soils may take three weeks or longer to reach a steady state. The incubation time in some cases has been over 30 days [3, 4]. For this study, the pre-set experimental period was relatively short (19 h). Before starting the experiments, changes in acetate and CO_2 concentrations were measured from the 12th day to the 13th day during the incubation period. Concentrations of acetate and CO_2 did not show any obvious change (Fig. S6), *i.e.*, the primary fermentation did not seem to produce much acetate and CO_2 in such a short time. Furthermore, in the case of inhibition of acetate consumption, we found that acetate did not exhibit significant accumulation in the vials (Fig. S1). It was an additional piece of evidence that the degradation of *Phragmites australis* straw did not produce acetate to any significant extent during the experiment (19 h). Actually, another potentially significance aspect of using soil incubated for 12 days is that it revealed more about the direction of the early acetate produced by fermentation of plant residues. If the soil is incubated for more than three weeks (hypothesis: the incubation was under steady-state conditions), some potential methanogenic pathways may be undetectable.

2.2 Potential methanogens outside Euryarchaeota and Bathyarchaeota

As the microorganisms actively involved in methanogenesis have a restricted phylogenetic distribution, it was hypothesised that archaeal CH_4 metabolism originated within the phylum Euryarchaeota.[5] Kelly et al.[6] mentioned that archaeal phylogenomics provides evidence for a methanogenic origin of the phylum Thaumarchaea. To the best of our knowledge, there is still no experimental evidence to prove that Thaumarchaeota archaea can produce CH_4. Our findings show that some active archaea (denoted as 'Uncultured methanogen,' the yellow column in Fig. 5B), do belong to the phylum Thaumarchaeota. A recent genome-centric metagenomics study highlighted that CH_4 metabolism occurs in the archaeal phylum Bathyarchaeota (which lies outside the phylum Euryarchaeota).[7] Accidental discoveries in this study suggest the possibility of

discovering methanogens outside Euryarchaeota and Bathyarchaeota.

References

1. E.D. Burton, L.A. Sullivan, R.T. Bush, S.G. Johnston, A.F. Keene, *Appl Geochem*, 2008,23:2759-2766.

2. M. Luo, C.S. Zeng, C. Tong, J.F. Huang, Q. Yu, Y.B. Guo, S.H.Wang, *Appl Geochem*, 2014, 45:1-13.

3. F. H. Liu and R. Conrad, *Isme Journal*, 2011, 5, 1526-1539.

4. F. H. Liu and R. Conrad, *Environ. Microbiol.*, 2010, 12, 2341-2354.

5. S. Gribaldo and C. Brochier-Armanet, *Philos. T. R. Soc. B.*, 2006, 361, 1007-1022.

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Figures

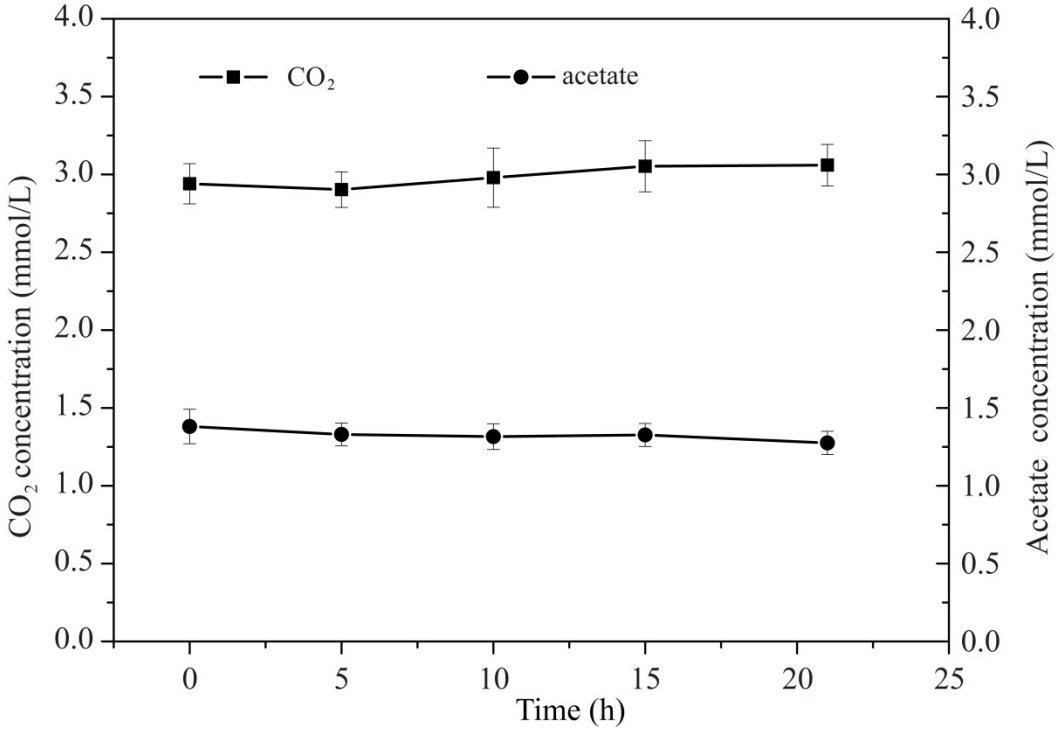

Fig. S1 Dynamics of CO_2 and acetate concentration from the 12^{th} to 13^{th} day during the incubation period.

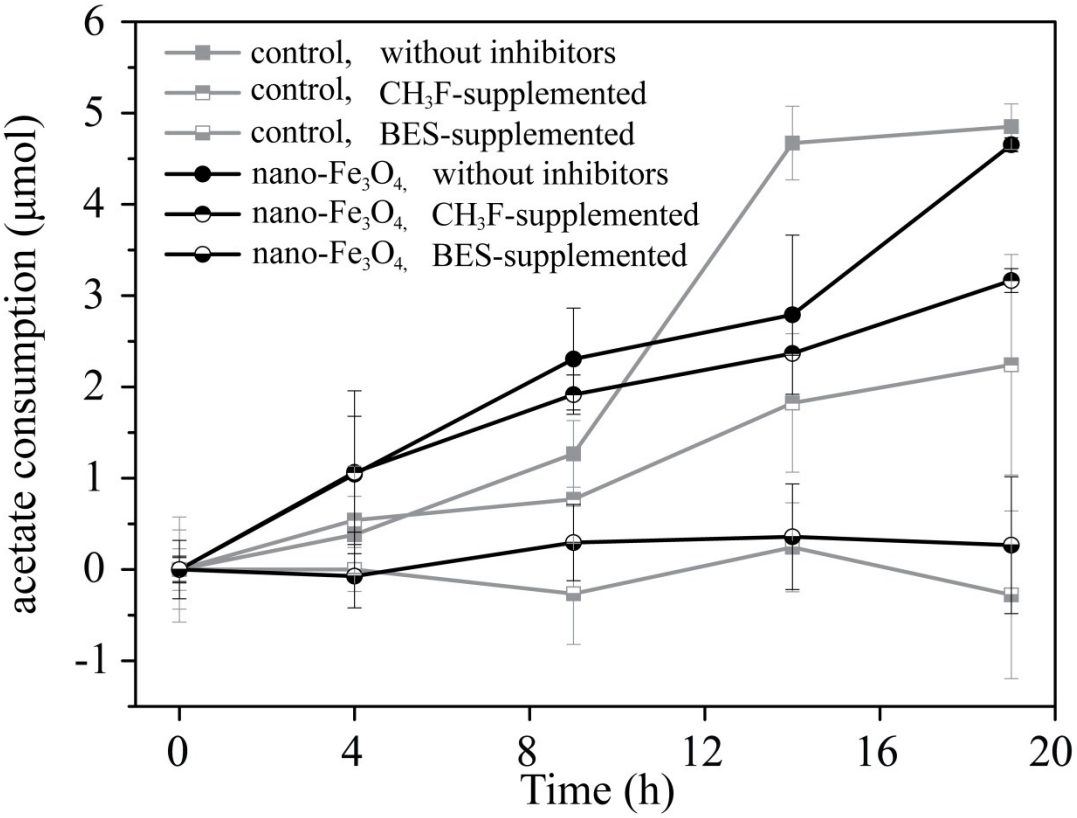

Fig. S2 The effect of nano-Fe_3O_4 particles on concentration of acetate.

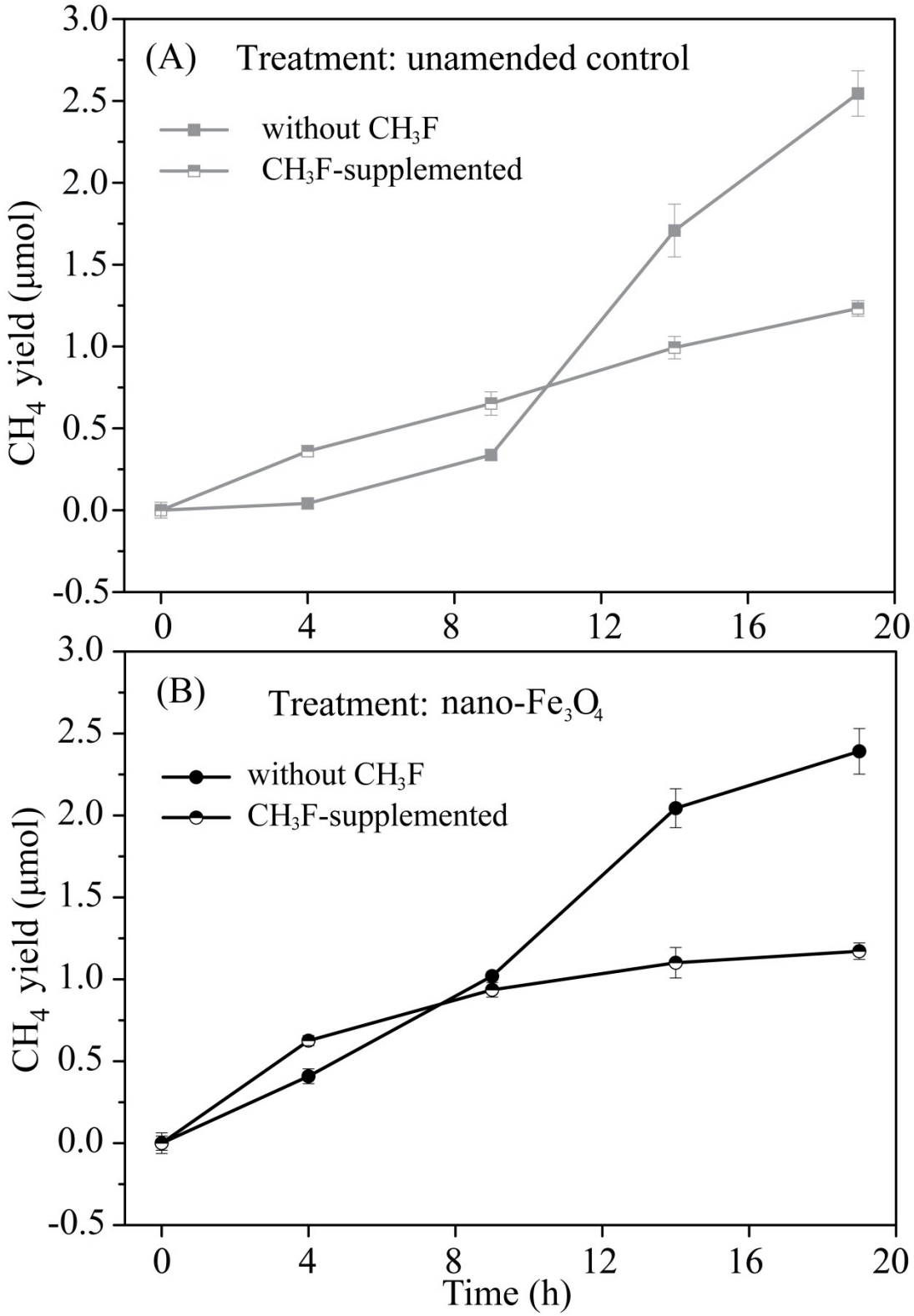

Fig. S3 Contributions from acetoclastic methanogenesis on methane production: CH_3F decreased the amount of methane in the second stage both in the absence (A) and presence (B) of nano-Fe_3O_4 particles.

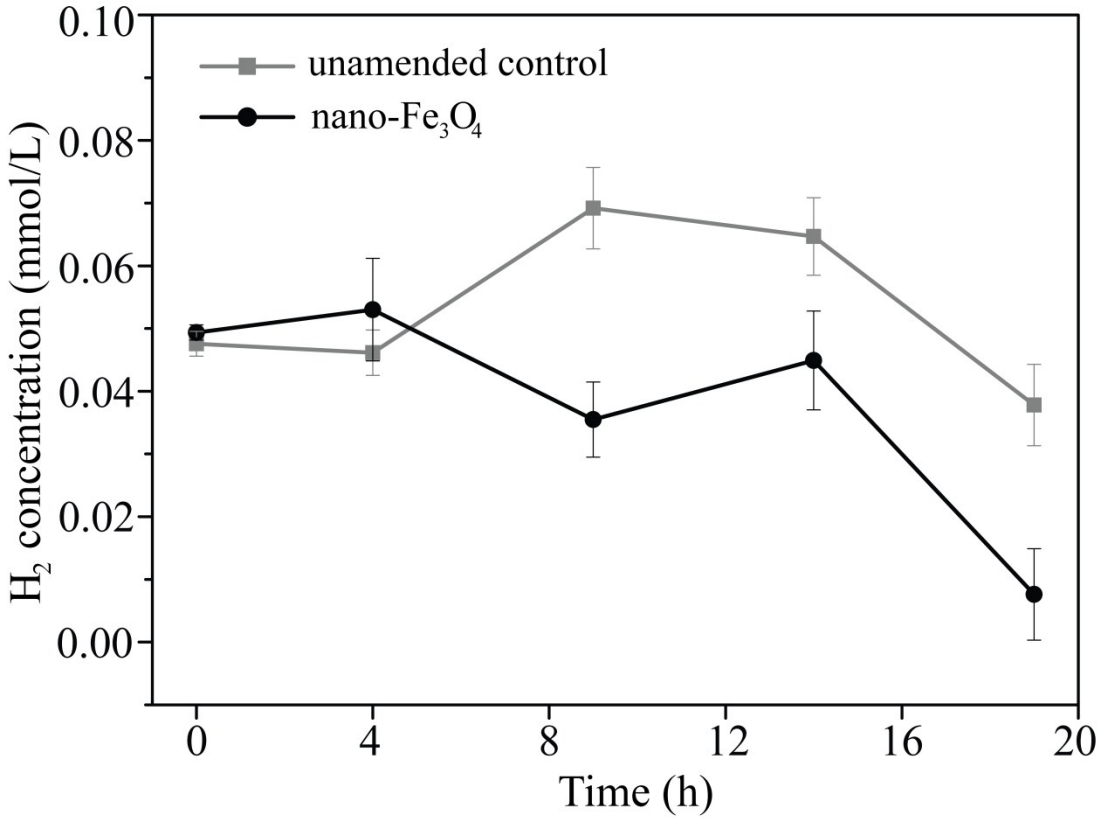

Fig. S4 The effect of nano-Fe_3O_4 particles on H_2 concentration.

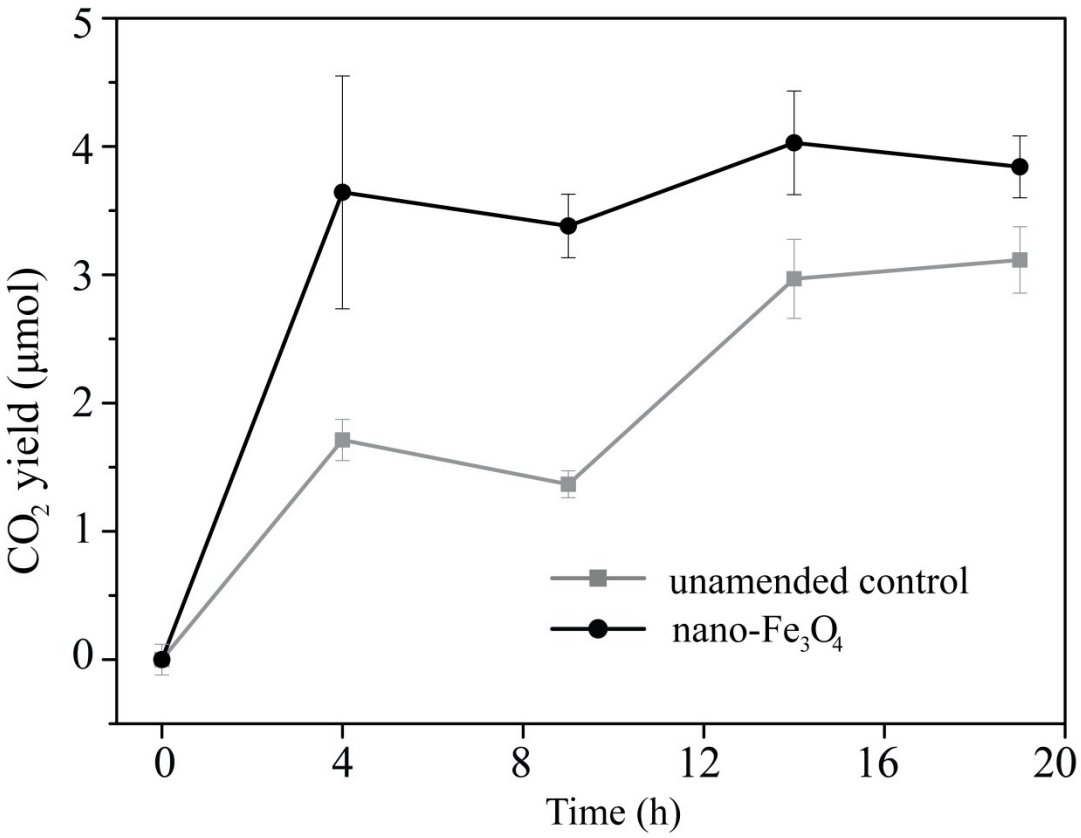

Fig. S5 The effect of nano-Fe_3O_4 particles on CO_2 concentration.

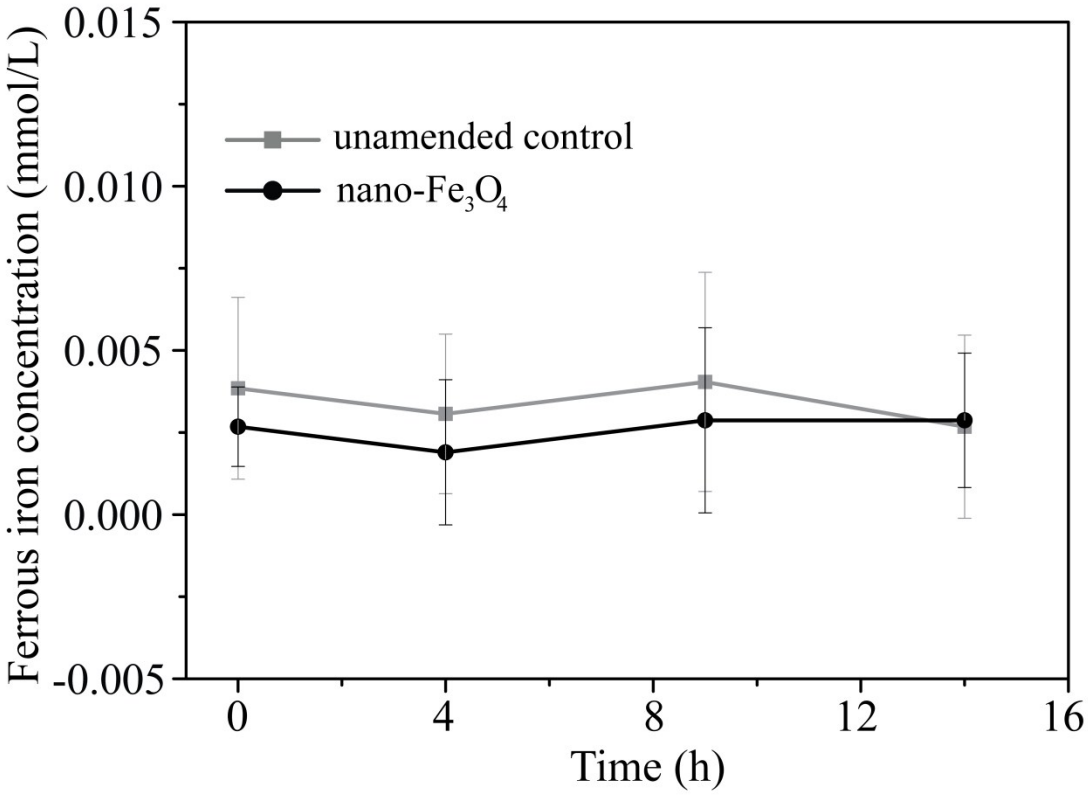

Fig. S6 Increased Fe (II) concentration in nano-Fe_3O_4-treated and unamended control vials.

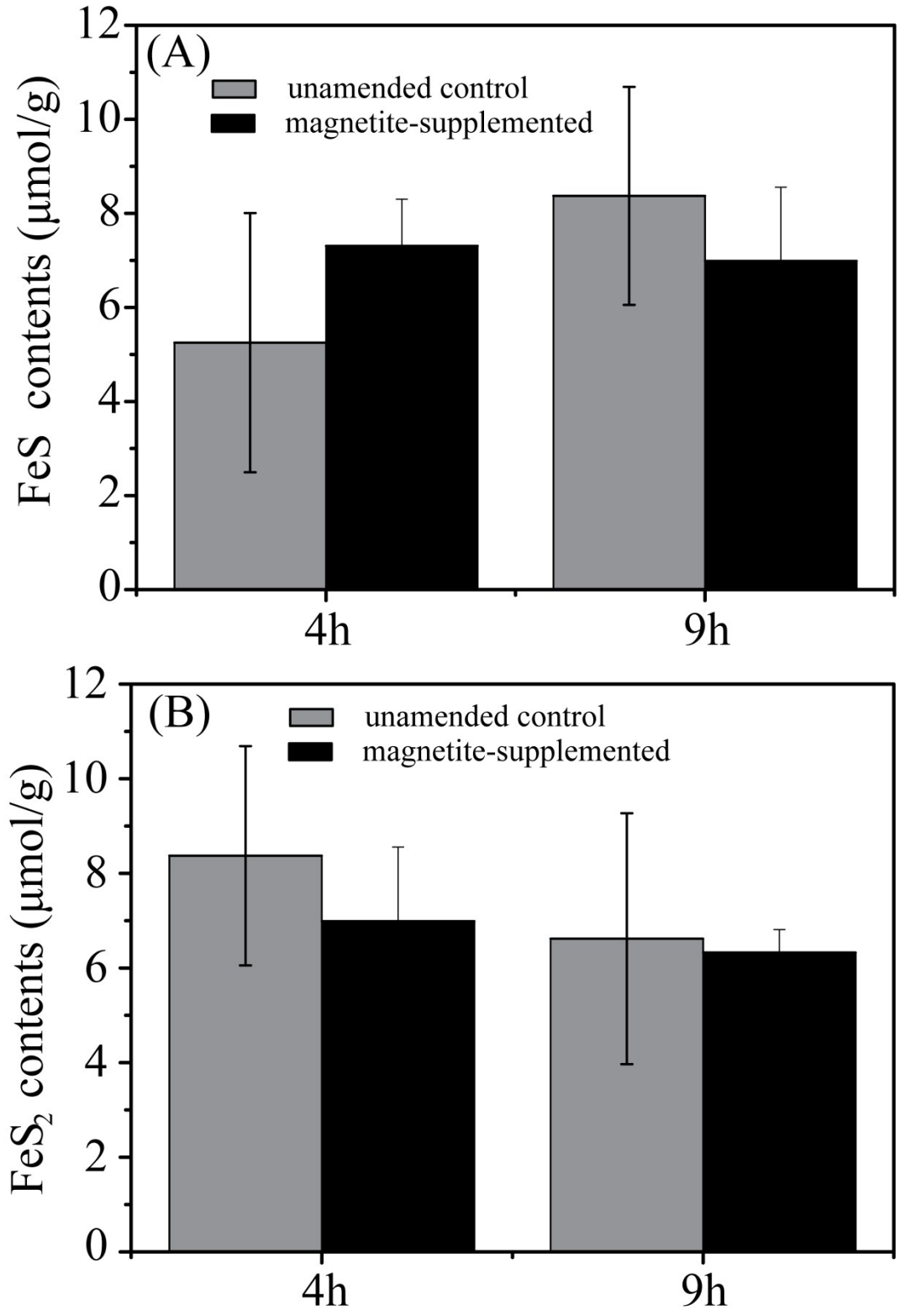

Fig. S7 Contents of sulphur species at 4th and 9th hours.