

Supporting information

For

Enhanced 4-chlorophenol biodegradation by integrating Fe₂O₃ nanoparticles into an anaerobic reactor: long-term performance and underlying mechanism

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Text S1. Synthesis and characterization of Fe₂O₃ nanoparticles

Hydrothermal method engaged with slight modification was used to synthesize Fe₂O₃ nanoparticles (Zhu et al., 2012). Briefly, FeCl₃·6H₂O (1.08 g), acetate (2.05 g) and polyvinyl pyrrolidone (0.75 g) were dissolved in 30 mL deionized water under stirring condition at 40°C to form a homogeneous solution. Then the obtained mixture was transferred into a Teflon-lined stainless-steel autoclave and sealed for heating at 200 °C for 5 h to get red muddy precipitate. The red solid products were collected by centrifugation, and then washed with deionized water and ethanol three times, respectively. Finally, the red powders were dried under vacuum at 75 °C for 8h.

For the characterization of Fe₂O₃ nanoparticles prepared, XRD analysis was carried out on a Bruker-AXSD8 system (Bruker AXS Inc., Germany). FTIR spectroscopy (Nicolet iS10, Thermo Fisher Scientific Inc., USA) was used for identifying the functional groups and the chemical bonding within wavenumber range of 4000-400 cm⁻¹. X-ray photoelectron spectra (XPS) were obtained using a PHI Quantera II ESCA System with Al Kα radiation at 1486.8 V.

The hydrothermal method is one of the most common procedures for fabrication of various metal oxide nanostructures for its easy conduction and high quality of the products. As shown in Fig. S1, TEM was performed to characterize morphology and crystal structure of as-synthesized Fe₂O₃ nanoparticles. The synthesized Fe₂O₃ nanoparticles showed polyhedral morphology (Fig. S1a), the single particle has a size in the range of 35-45 nm, which was similar with the hematite nanoparticles prepared

by Zhu et al. (2012). The XRD patterns (Fig. S1b) of the obtained Fe₂O₃ nanoparticles corresponded well with iron-oxide nanoparticles reported. The Fe₂O₃ sample exhibited three peaks centered at 2θ of 32°, 35° and 54° (JCPDS card No.89-0599). Therefore, polyhedral Fe₂O₃ nanoparticles within similar size was synthesized successfully in this study.

Text S2. Microbial community structure measurement

Three sludge samples, i.e., the initial seed sludge, the sludge collected from the control UASB and the coupled UASB at the end of long-term operation period, were taken for the analysis of microbial community structure. After being washed with phosphate buffer (7 mmol/L, pH = 7.0), the genomic DNA of each sample was extracted using the FastDNA SPIN for soil kit (MP Bio-medicals, USA) according to manufacturer instructions. For high-throughput sequencing, PCR amplification of the V3-V4 region of bacterial 16S rRNA was performed using primers (338F 5'-ACCTCCTACGGGAGGCAGCAG-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3'). Another primer combination of ArBa515F (5'-GTGCCAGCMGCCGCGGTAA-3') and Arch806R (5'-GGACTACVSGGGTATCTAAT-3') was used to selectively amplify the V4 region of archaea 16S rRNA sequences. The sequences were analyzed on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). DNA library building and data analysis were performed in accordance to Wu et al. (2019).

Text S3. Characterization of sludge morphology

To investigate the morphology of sludge at the long-term operation from the control UASB and coupled UASB, SEM images of sludges are given in Fig. S7. Compared to the sludge with a loose and rough surface in the control UASB, the sludge presented a rigid, dense and granular appearance in the coupled UASB. A multi-layer structure of the sludge was formed in the coupled UASB, where the cocci-shaped and rod-shaped were dominated on the surface of sludge and the filamentous ones were majorly located in the inner layer of sludge. A filamentous structure was observed on the surface and interior of the control UASB sludge. Zhang et al. (2011) indicated that the cocci-shaped species observed on the surface and filamentous species in the inner layer of sludge were likely related to acidogens and Methanothrix, respectively. This phenomenon can be attributed to the interactions of the nanoparticle materials, microbial species, and their induced EPS. The granule structure, microorganisms excrete EPS substances that attach themselves to the cells, could provide a formidable protective defense against any interference from the external environment such as low pH, oxygen and toxic substances (Sheng et al., 2010).

References

- Sheng G P, Yu H Q, Li X Y (2010). Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnology Advance*, 28 (6): 882-894.
- Wu H, Sun Q, Sun Y, Zhou Y, Wang J, Hou C, Jiang X, Liu X, Shen J (2019). Co-metabolic enhancement of 1H-1,2,4 triazole biodegradation through nitrification. *Bioresource Technology*, 271: 236-243.
- Zhang Y, An X, Quan X (2011). Enhancement of sludge granulation in a zero valence iron packed anaerobic reactor with a hydraulic circulation. *Process Biochemistry*, 46 (2): 471-476.
- Zhu M, Wang Y, Meng D, Qin X, Diao G (2012). Hydrothermal synthesis of hematite nanoparticles and their electrochemical properties. *Journal of Physical Chemistry C*, 116 (30): 16276-16285.

Table S1 XPS results of the sludge sample from coupled UASB

Element	Energy	FWHM	Assignment
Fe 2p	711.4	2.61	Fe ³⁺
	716.3	4.89	Fe ³⁺
	724.6	3.72	Fe ₂ O ₃
O1s	530.8	2.05	Hydroxide
	531.8	1.50	Oxide
	532.8	1.69	Carbonates
C1s	284.5	1.23	C-C/C-H
	286.1	1.34	C-O
	288.2	0.80	CO ₃ ²⁻

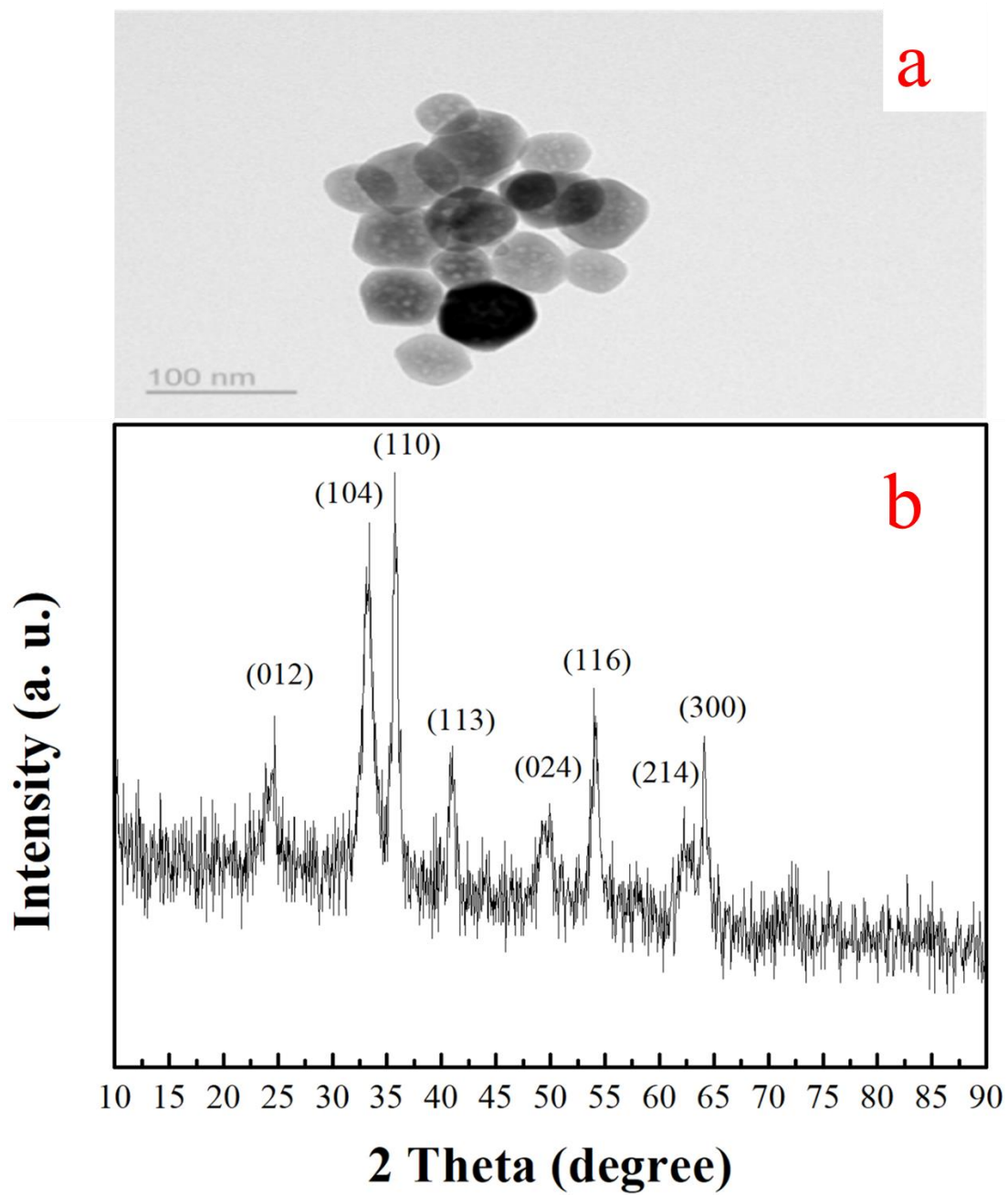


Fig. S1. TEM (a) and XRD (b) of Fe_2O_3 nanoparticles.

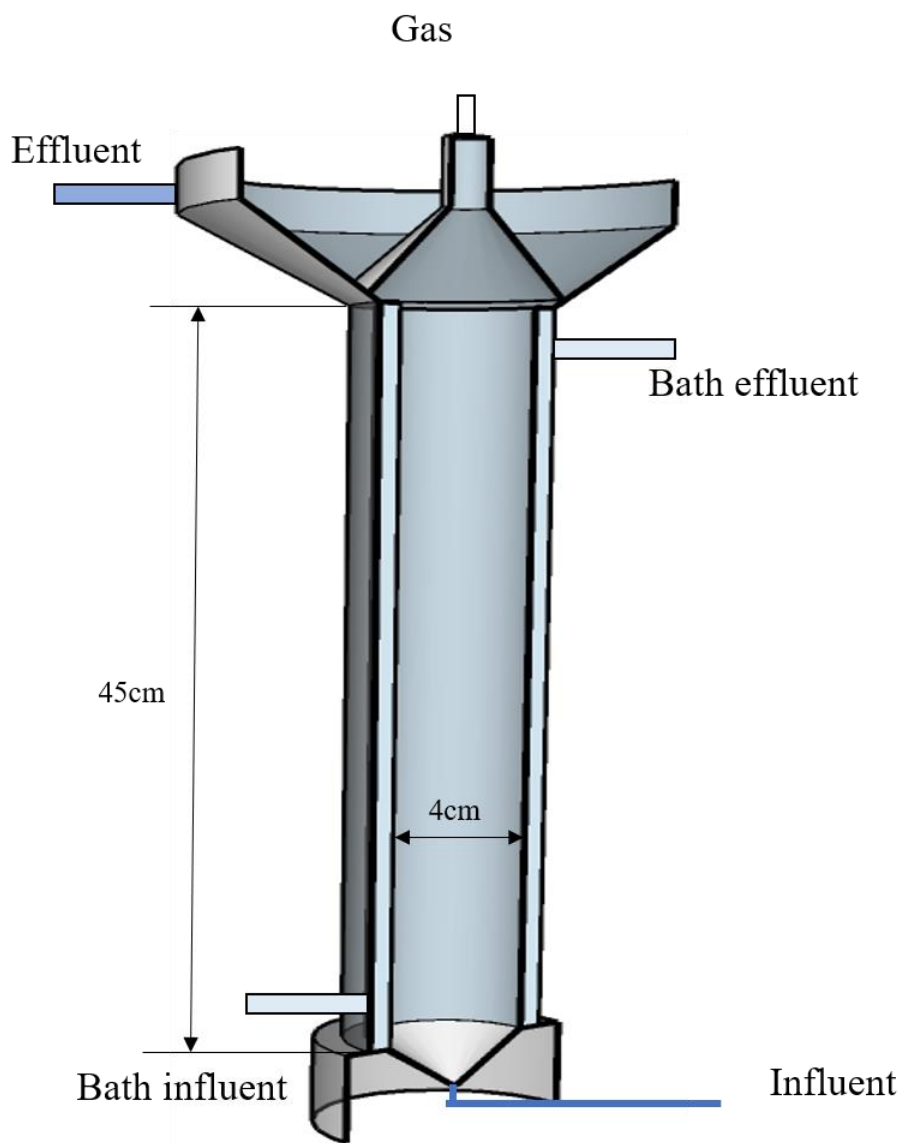


Fig. S2. Schematic diagram of Fe_2O_3 nanoparticles coupled UASB bioreactor.

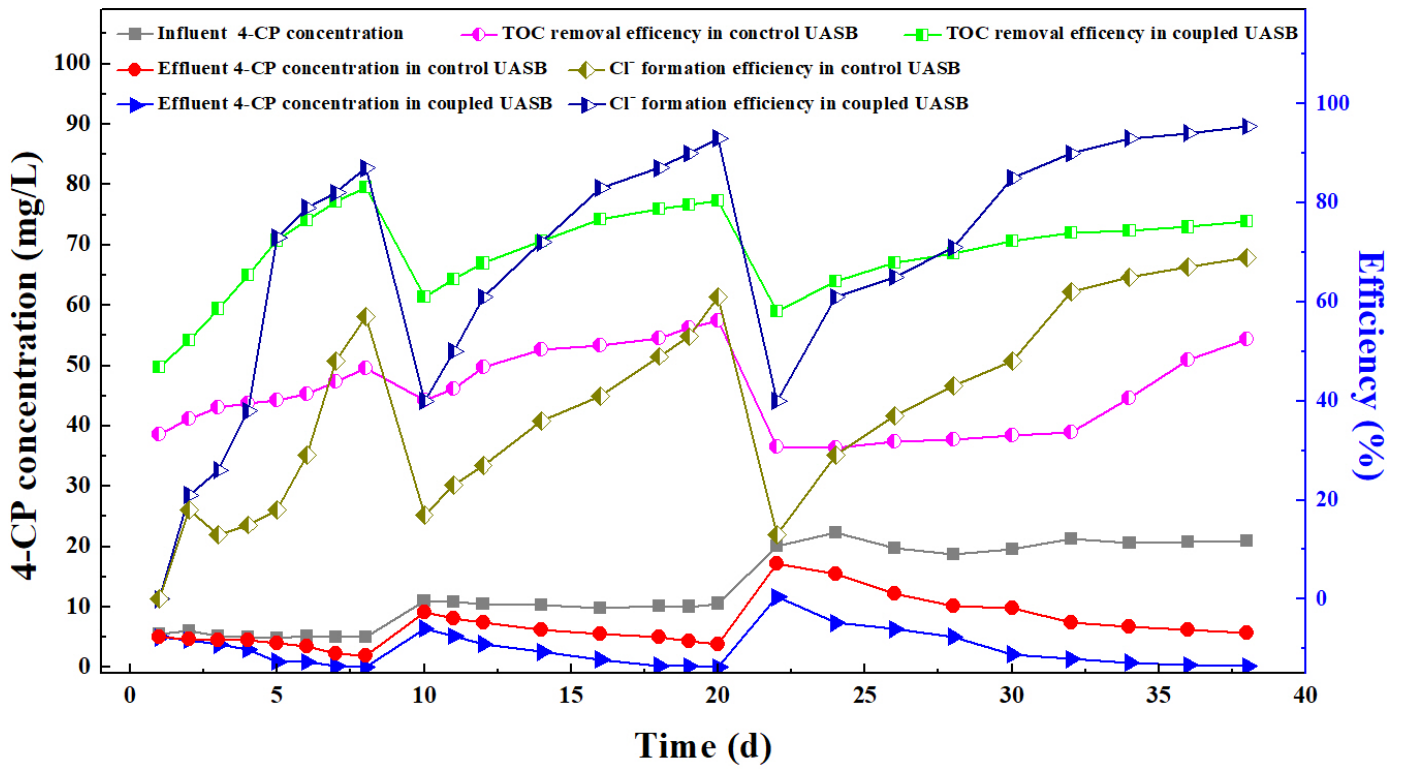


Fig. S3. 4-CP biodegradation, TOC removal and Cl⁻ formation during the start-up period of the both control UASB and coupled UASB.

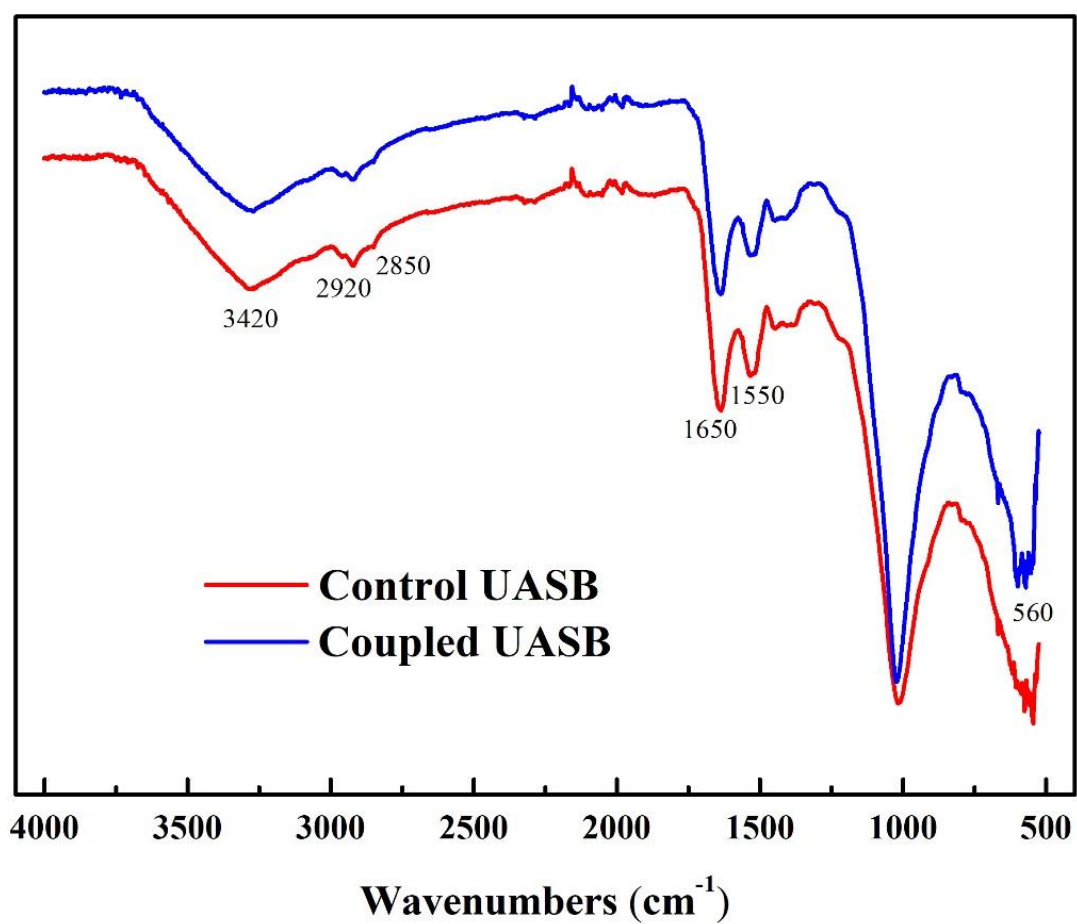


Fig. S4. FTIR spectra of anaerobic sludge taken from the coupled UASB.

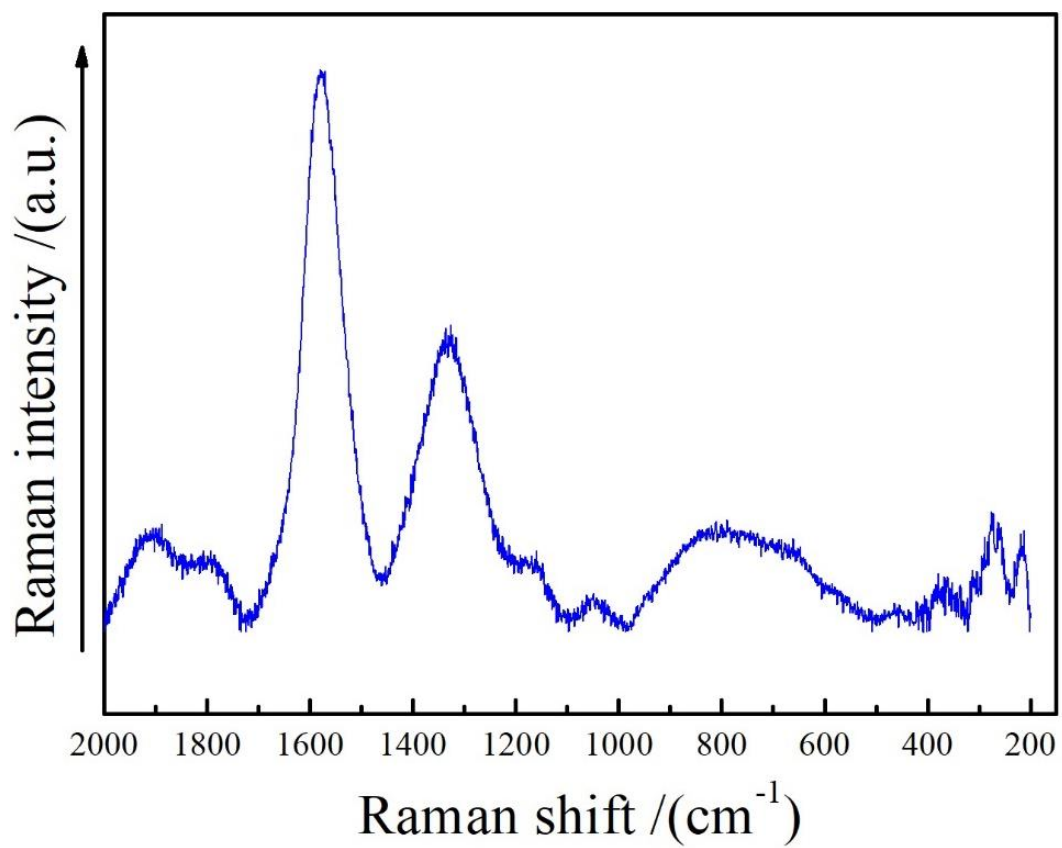


Fig. S5. Raman spectra of anaerobic sludge taken from Fe₂O₃ nanoparticles coupled UASB.

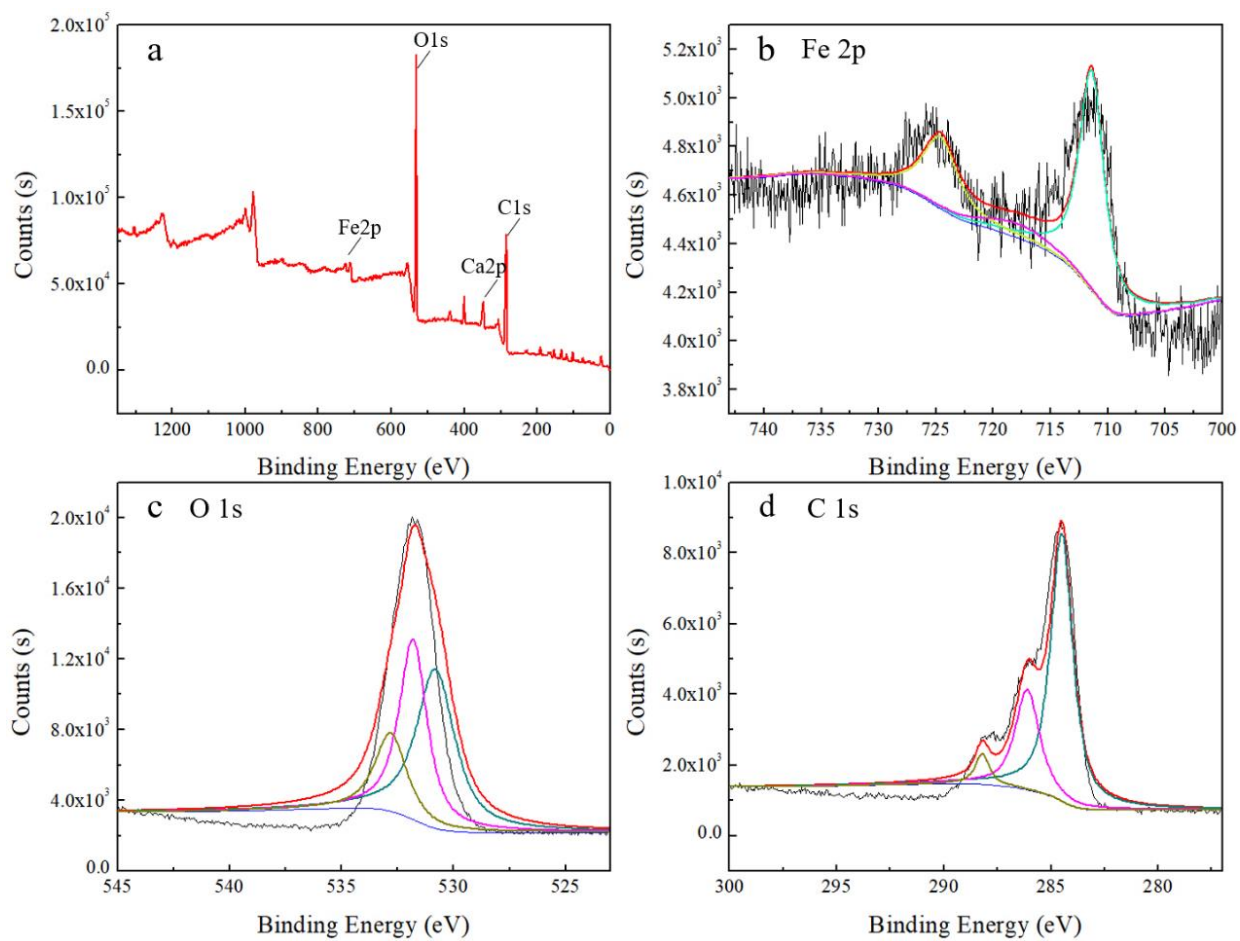


Fig. S6. XPS spectra of anaerobic sludge taken from the coupled UASB.

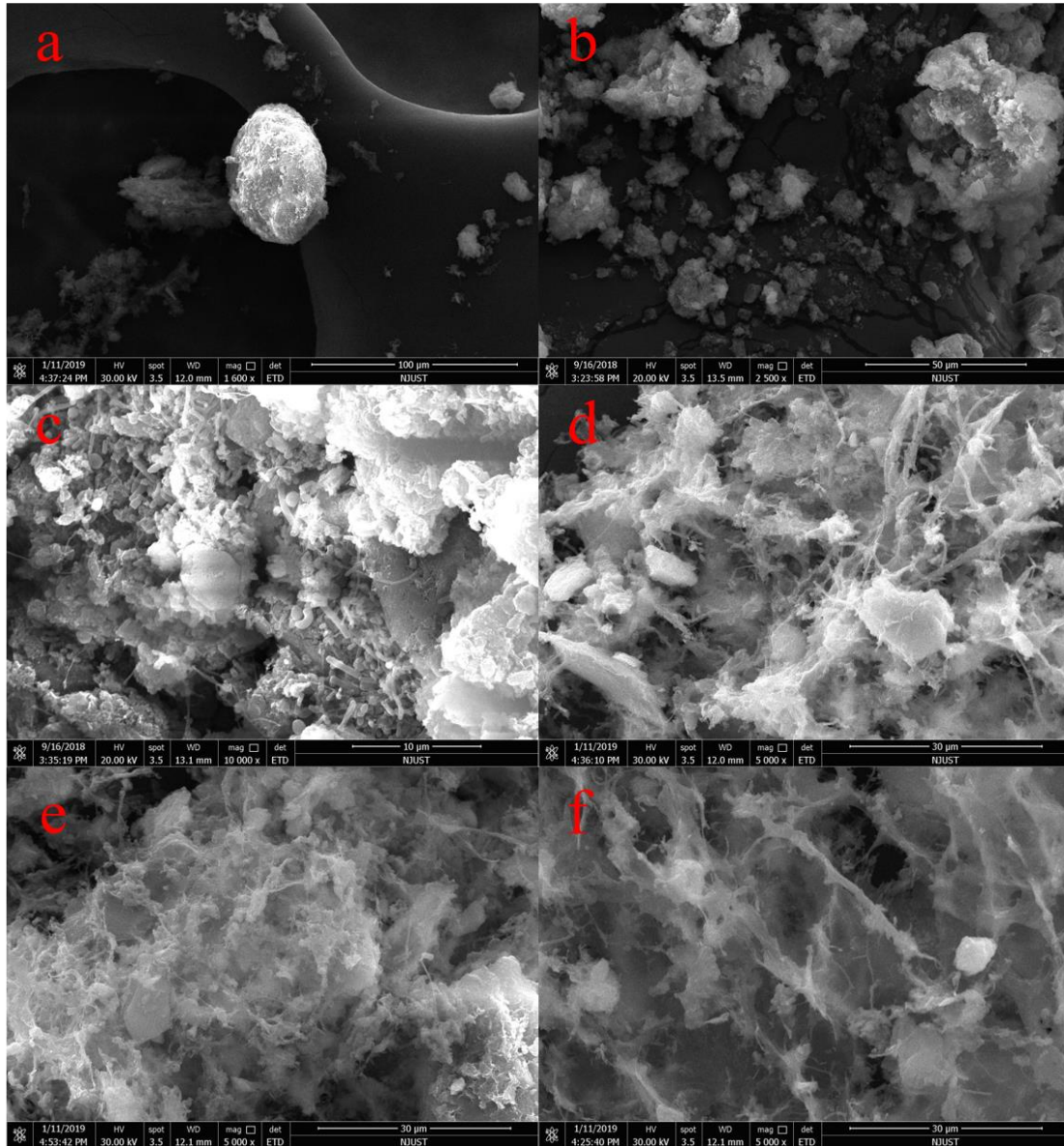


Fig. S7. SEM of the sludge from the coupled UASB (a, c and e) and the control UASB (b, d and f).