

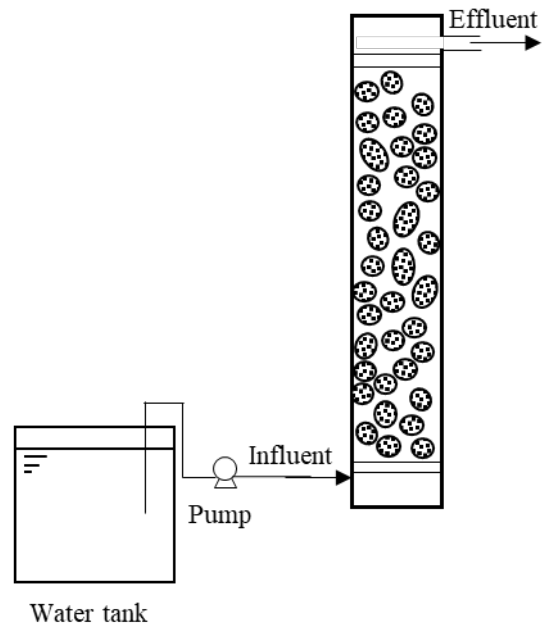
## **Materials and Methods**

### **DNA extraction and high-throughput sequencing**

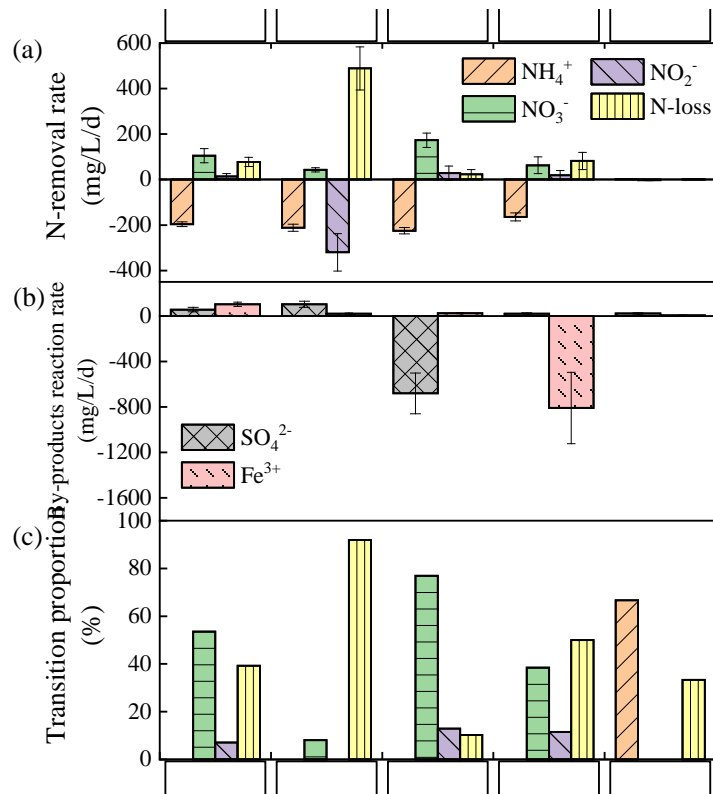
DNA extraction was conducted by using a rapid reagent extraction kit (Illumina, USA). The V4 gene of 16S rRNA was amplified by PCR using primers (515F and 806R), and high-throughput sequencing was performed on the Illumina NovaSeq platform. Then, the reads of samples were spliced and filtered by the FLASH software, and the operational taxonomic units (OTUs) were generated by clustering (similarity  $\geq$  97%). The representative sequence of OTU was selected and compared with the SILVA database (<https://www.arb-ilva.de/>) for species annotation.

For metagenomic sequencing, the Raw Data obtained from the Illumina HiSeq sequencing platform was preprocessed using Readfq to acquire the Clean Data. Then the Clean Data was assembled using MEGAHIT, while the assembled Scaffolds ( $\geq$  500bp) were used for the open reading frames (ORFs) prediction by the MetaGeneMark software, and the CD-HIT software was adopted to reduce sequence redundancy and obtain the unique gene catalogue (Unigenes). The Unigenes sequences were compared with the NCBI-NR database for species annotation using the DIAMOND software (e-value threshold of  $10^{-5}$ ). Blasted results for Unigenes were based on the KEGG database by the DIAMOND software to obtain annotation information of gene function and metabolic pathways (e-value threshold of  $10^{-5}$ ).

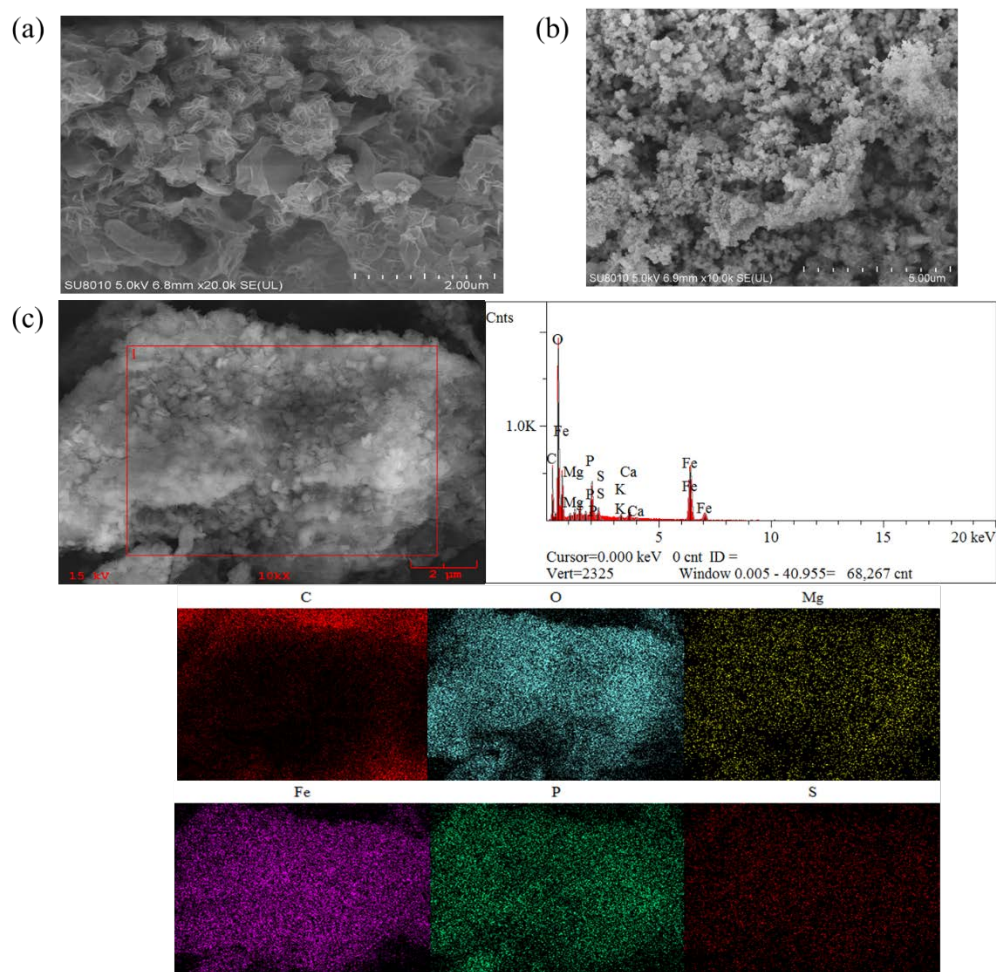
## Figures



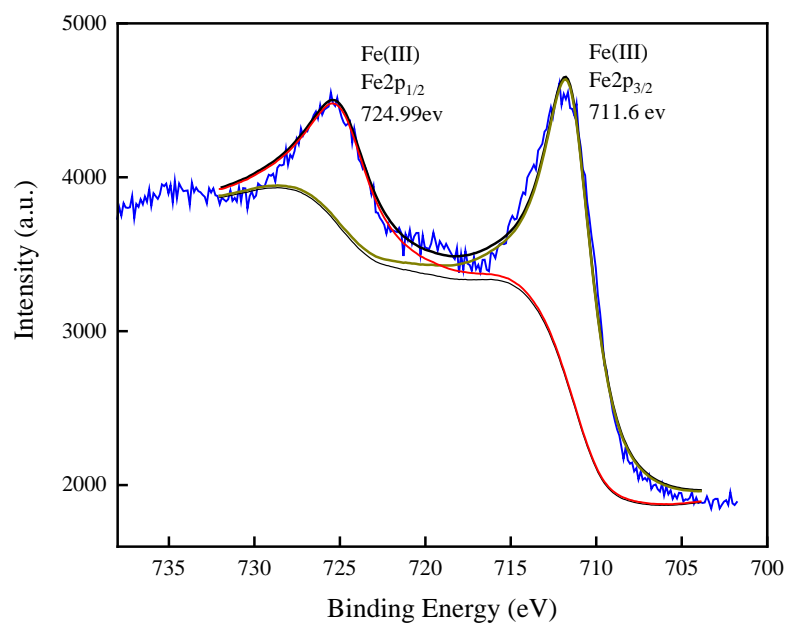
**Fig. S1** Diagram of FeS-driven autotrophic denitrification fixed-bed bioreactor.



**Fig. S2** Microbial activities of functional microorganisms for Sulfamox (Sulfam), Feamox (Feam), nitrification (Nitrifi), Anammox (Anam), denitrification (Denitri) processes at the bottom (0-10 cm) of the reactor (a, nitrogen removal rate; b, reaction rates of  $\text{SO}_4^{2-}$  and  $\text{Fe}^{3+}$ ; c, nitrogen removal of different process transformation proportion).



**Fig. S3** SEM Morphology of pyrite solid particle surface in different stages (Days 1, 45) (a-b), and SEM-EDS of the used FeS (on Day 45) (c).



**Fig. S4** Fe(2p) orbital XPS spectra of the used FeS (background (black dash); Fe(III) 2p<sub>1/2</sub> (red dash), Fe(III) 2p<sub>3/2</sub> (gray dash) and the solid black line represents the sum of the component peaks).

**Table****Table S1** Effluent sulfate and Total-Fe concentrations of theoretical and measured values in the bioreactor.

Phase	Effluent		Removal percentage		SO <sub>4</sub> <sup>2-</sup>		T-Fe	
	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	measured	theoretical	measured	theoretical
	(mg/L)		(%)		(mg/L)			
I	33.9±18.2	39.4±13.7	68.0	60.0	70.4±85.2	269.6	2.4±0.9	31.3
II	30.5±22	34.1±28.2	69.8	64.8	136±82.6	289.3	3±1.1	33.5
III	24.5±19.5	58.1±18.2	75.8	41.0	180±75.8	184.7	4.1±2.6	21.4
IV	2.2±1.1	14.7±8.4	95.6	69.6	127.6±18.3	154.4	1.1±0.4	17.9
V	3.3±2.8	43.7±9.3	93.1	11.2	54.1±30.4	118.2	2.4±1.1	2.8
VI	8.6±4.4	47.7±7.4	82.2	3.5	19.8±4.2	106.3	1.9±0.2	1.0
VII	0.9±0.7	5.9±6.4	98.1	88.0	138.5±31.5	293.3	2.9±0.5	22.7
VIII	8.3±5.7	9.2±4.6	82.8	0	93.8±26.2	164.7	2.1±0.6	0.0