# Regulating secretion of extracellular polymeric substances through dosing magnetite and zero-valent iron nanoparticles to affect anaerobic digestion mode

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Numbers of pages: 3

Numbers of figures: 1

## Materials and Methods

### DNA extraction, PCR amplification and high-throughput 16S rRNA pyrosequencing.

The sludge samples were transferred into 2 mL sterile centrifuge tube with 1 mL 70% ethanol, then centrifuged in 10000 g at room temperature. After discarding the supernate, the samples were rinsed by phosphate-buffered saline (0.13 M NaCl and 10 mM $Na_2HPO_4$ at pH 7.2) and then harvested by centrifugation (10000 g for 3 min at room temperature), discarding the supernate and dried in oven under 55$^{o}$C for 10 min to volatilize any residual ethanol. The E.Z.N.ATM Mag-Bind Soil DNA Kit (Omega Bio-Tek, UAS) was used to extract DNA from the sludge samples following the manufacturer's protocol. Integrity and purity of the extracted DNA were determined by agarose gel electrophoresis and then stored at -20$^{o}$C for the subsequent analyses.

The DNA samples were qualified by Qubit 2.0 DNA detection kit (Sango, China) and then analyzed by Sango Company (Sango, China). The pyrosequecing gene libraries were constructed using V3-V4 universe primers 341F/805R (341F:5'-CCTACGGGNGGCWGCAG-3', 805R: 5'-GACTACHVGGGTATCTAATCC-3'). The first step of amplification cycling scheme was measured as follows: 94$^{o}$C for 3 min, followed by 5 cycles of 94$^{o}$C for 30 s, 45$^{o}$C for 20 s and 65$^{o}$C for 30 s, then with 20 cycles of 94$^{o}$C for 20 s, 55$^{o}$C for 20 s and 72$^{o}$C for 30 s, after that, the final elongation step at 72$^{o}$C for 5 min was performed. The second step of amplification cycling scheme was measured as follows: 95$^{o}$C for 30 s, followed by 5 cycles of 95$^{o}$C for 15 s, 55$^{o}$C for 15 s and 72$^{o}$C for 30 s and finally 72$^{o}$C for 5 min.

After amplification, the PCR products were checked by agarose gel electrophoresis to determine the quality of the amplification and the molecular weight and DNA concentrations of the samples. After that, samples were purified using Agencourt AMPure XP magnetic beads (Backman Coulter, USA) according to the manufacture's protocol. Then the purified PCR product was precisely quantified using Qubit 2.0 DNA detection kit (Sangon, China) to prepare DNA library

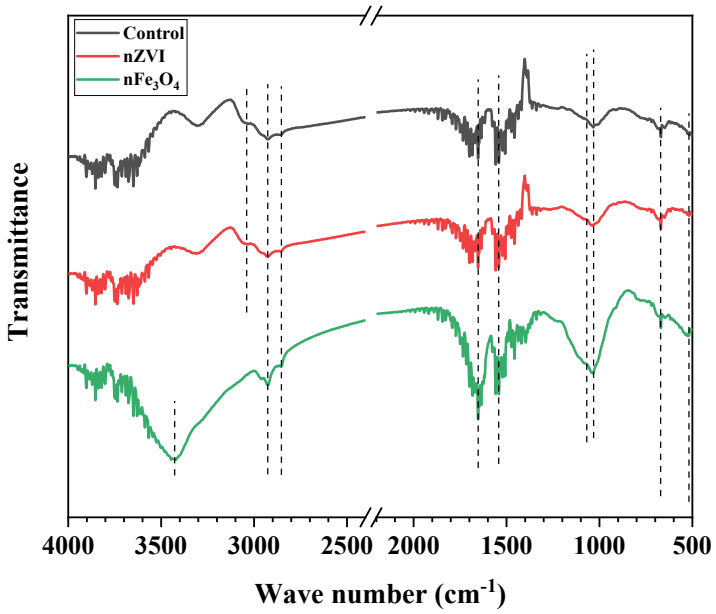


Figure S1: FTIR spectra of EPS in the three anaerobic reactors.

The peaks at around 3400 $cm^{-1}$ were assigned to O−H stretching vibrations; 1658,1568, and 1448 $cm^{-1}$ were assigned to the C−O stretching of amide; The peaks at 1163 and 1076 $cm^{-1}$ correspond to the C−O stretching and C−O−C vibrations of polysaccharides