Partial nitritation under zero-pressure aeration in a membrane-aerated biofilm reactor: Nitrite accumulation, EPS molecular structure, and microbial community

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Text S1. Synthetic wastewater.

The reactor was fed with synthetic ammonium wastewater. The main components were as follows: NH_4HCO_3 at 0.282 $g \cdot L^{-1}$, equivalent to an NH_4^+-N concentration of 50 $mg \cdot L^{-1}$; $NaHCO_3$ at 0.3~2 $g \cdot L^{-1}$ for pH adjustment; KH_2PO_4 at 0.06 $g \cdot L^{-1}$; $MgSO_4 \cdot 7H_2O$ at 0.2 $g \cdot L^{-1}$; $CaCl_2$ at 0.05 $g \cdot L^{-1}$; and trace element solution SL-4 at 1 $mL \cdot L^{-1}$. The composition of SL-4 was as follows: EDTA at 0.5 $g \cdot L^{-1}$; $FeSO_4 \cdot 7H_2O$ at 0.2 $g \cdot L^{-1}$; and SL-6 trace element solution at 100 $mL \cdot L^{-1}$. The composition of SL-6 was as follows: $ZnSO_4 \cdot 7H_2O$ at 0.1 $g \cdot L^{-1}$; $MnCl_2 \cdot 4H_2O$ at 0.03 $g \cdot L^{-1}$; H_3BO_3 at 0.3 $g \cdot L^{-1}$; $CoCl_2 \cdot 6H_2O$ at 0.2 $g \cdot L^{-1}$; $CuCl_2 \cdot 2H_2O$ at 0.01 $g \cdot L^{-1}$; $NiCl_2 \cdot 6H_2O$ at 0.02 $g \cdot L^{-1}$; and $Na_2MoO_4 \cdot 2H_2O$ at 0.03 $g \cdot L^{-1}$. The synthetic wastewater was sterilized at 120℃ for 40 minutes before use and then cooled to room temperature.

Text S2. Analysis method.

After the completion of operation under constant-pressure aeration, intermittent aeration, and zero-pressure aeration, appropriate amounts of biofilm samples were collected for high-throughput sequencing. DNA extraction was performed using a Fast DNA spin kit (MP Biomedicals, USA), and DNA concentration and purity were measured using a Nanodrop ND-1000 spectrophotometer. The V3-V4 region of the 16S rRNA gene was amplified using the forward primer "CCTACGGRRBGCASCAGKVRVGAAT" and the reverse primer "GGACTACNVGGGTWTCTAATCC". Sequencing was conducted by GENEWIZ Inc. (Suzhou, China) on an Illumina MiSeq platform. The sequencing data were quality-filtered using Cutadapt, Vsearch, and QIIME and then clustered into operational taxonomic units (OTUs) at 97% similarity. Taxonomic assignment was performed against the Silva_132 database. Alpha diversity indices and Beta diversity were analyzed based on the OTU level, and statistical comparisons of microbial community composition at the genus level were performed.

The activities of ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), and nitrite oxidoreductase (NXR) were determined using commercial ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China) according to the manufacturer's instructions. Biofilm samples were collected from the reactor at the end of each operational phase, immediately frozen in liquid nitrogen, and stored at -80 °C until analysis. For each sample, three independent replicates were measured, and enzyme activities are expressed as $U \cdot L^{-1}$.

Table S1. The properties of the PDMS membrane used in this study**.**

Parameter	Value	Unit
Membrane outer diameter	1	mm
Membrane inner diameter	0.5	mm
Membrane thickness	0.25	mm
Effective length of membrane fiber	35	cm
Number of membrane fibers	850	/
Effective contact area	0.93	m^2
Specific surface area	465	$m^2 \cdot m^{-3}$
Porosity	4.5	%
Contact angle	100.21	°

Table S2. The operating parameters of each stage during the start-up of the partial nitritation reactor.

Stage	Operating days (d)	NH_4^+-N ($mg \cdot L^{-1}$)	Aeration pressure (kPa)	HRT (h)	pH
I	1-5	50	10	48	7.0
	6-13	50	10	24	7.0
	14-17	50	10	12	7.0
	18-21	50	10	18	7.0
	22-25	50	10	24	7.0
II	26-30	50	10	24	7.5
	31-34	50	10	24	8.0
III	35-40	50	5	24	8.0
	41-48	50	0	24	8.0
IV	49-71	70	0	24	8.0
	57-71	70	0	12	8.0

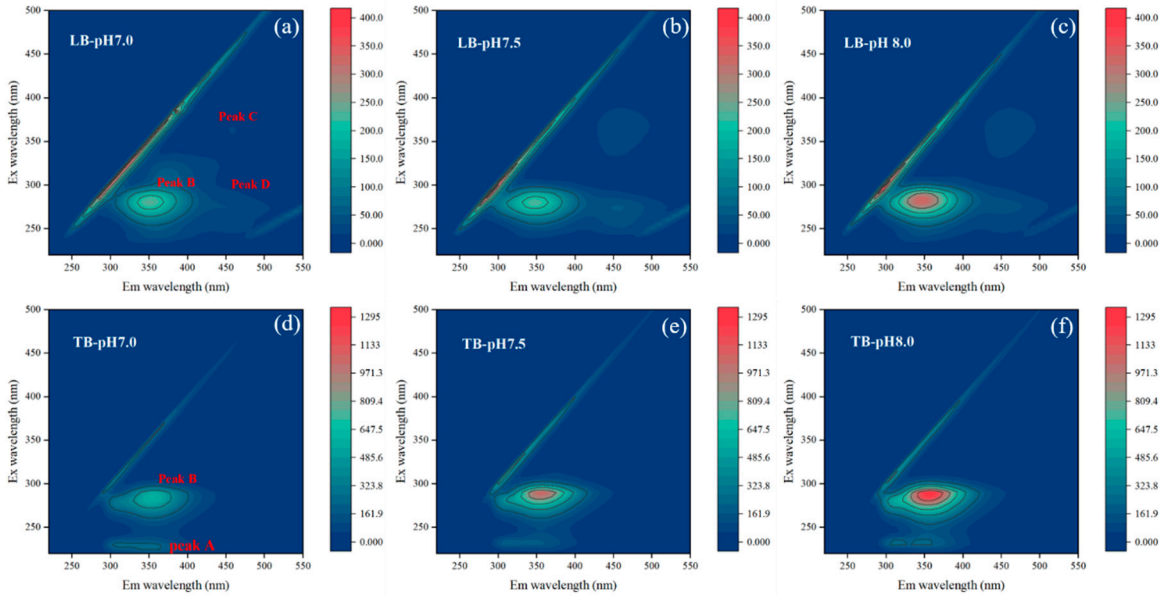


Figure S1. 3D-EEM fluorescence spectra of EPS in reactor: LB-EPS (a-c) and TB-EPS (d-f) at influent pH values of 7.0, 7.5, and 8.0.

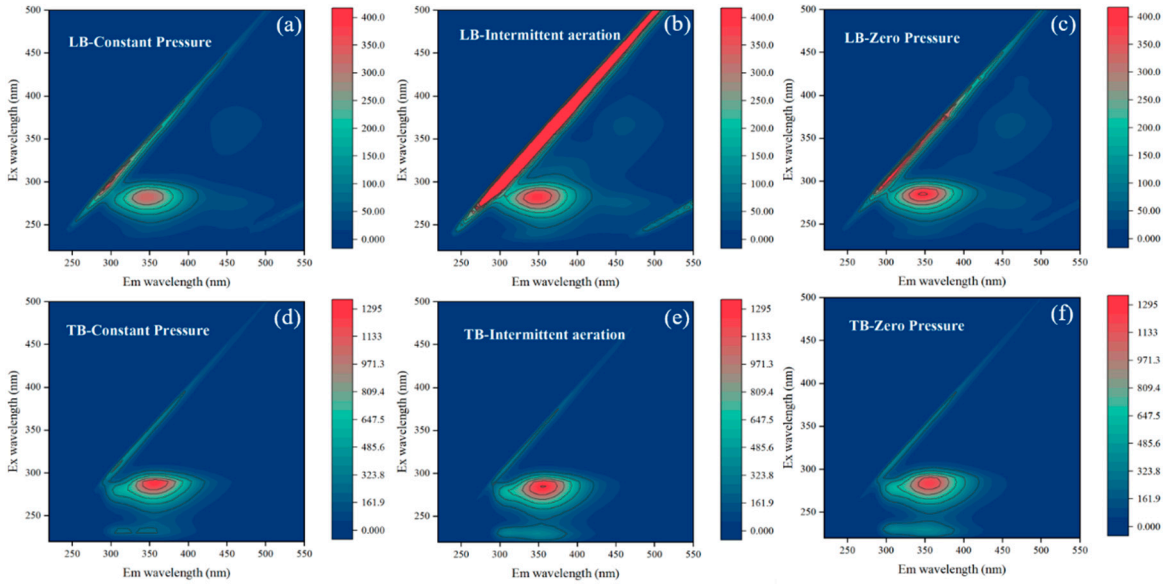


Figure S2. 3D-EEM fluorescence spectra of EPS in biofilm: LB-EPS (a-c) and TB-EPS (d-f) under constant-, intermittent, and zero-pressure aeration.