

Supplementary material

Single-stage PN/A technology treating saline ammonia-rich wastewater: finding the balance between efficient performance and less N₂O and NO emissions

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Table S1 salinity in different wastewater effluents

| Type of wastewater | Salinity | Ammonia concentration | Ref. |
|--------------------|------------------------|--|----------------------|
| | g NaCl L ⁻¹ | mg NH ₄ ⁺ -N L ⁻¹ | |
| Fish canning | 8–10 | 700-1000 | 1 |
| Gas fields | 30 | 150-200 | 2 |
| Tanning leather | 1.65–8.24 | 10-100 | 3 No.206 Talbe 15.14 |
| Flue gases | 3–40 | 300 | 4 |
| desulphurization | | | |

Supplementary Methods

16S rRNA gene high-throughput sequencing. Biomass samples were collected on the last day at each elevated salinity Period (day 122, 184, 229, 268 and 300) and stored at -80°C. The Genomic DNA was extracted from samples for polymerase chain reaction of V3-V4 region of the 16S rRNA. Then the purified amplicons were quantified by QuantiFluor-ST Fluorometer (Promega, USA), and a composite sequencing library was constructed by combining equimolar ratios of amplicons from all samples. The resulting library was sent for paired-end sequencing (2×250 bp) on an Illumina Miseq platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The obtained 16S rRNA gene sequences were compared with sequences in the GenBank database using the NCBI Blast search program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Low quality reads (ambiguous nucleotides and quality value <20) were removed from the raw sequence data. Eventually, the numbers of high quality sequences were 45508 (Period A, day122), 69341 (Period B, day 184), 47831 (Period B, day 229), and 37380 (Period D day 268) and 34364 (Period E, day 300) with an average length of 433 bp. To facilitate the comparison between different samples, the numbers of sequences were normalized to the same sequencing depths of 29114 by MOTHUR program. Subsequently, number of operational taxonomic units (OTUs) (clone sequences with >97% similarity were grouped together and regarded as one OTU) was obtained by Usearch program (version 7.1) using furthest neighbor algorithm and established the phylogenetic tree with the relative abundances of OTUs (exhibited by color variation) by the maximum parsimony.

Calculation of ammonia oxidation rate (AOR). Ammonia oxidation rates (AOR) by aerobic ammonia oxidation bacteria (AOB) in the single-stage PN/A system was estimated as $\text{mg-N L}^{-1} \text{ min}^{-1}$ based on nitrogen balances and the stoichiometry of nitrification process and the anammox process

$$AOR = \frac{\Delta NH_4^+ - \frac{\Delta TN}{2.04}}{T_a} \quad \text{Eq. S1}$$

Where Δ is the nitrogen concentration difference between nitrogen concentrations in the beginning and at the end of aerobic stage, mg-N L⁻¹; 2.04 is the molar ratio converting the dinitrogen gas removed from CANON system (1.02 moles of dinitrogen gas produced per mole of ammonium reacted in anammox process) into nitrogen; T_a is the aeration time in each aerobic stage, 35 min.

References

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