# Supplementary Materials

## Novel Dual-Particle Sulfur-Driven Partial Denitrification Coupled with Anammox for Robust Nitrogen Removal at Ambient Temperature

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**Table S1.** Operational parameters of the SADN system.

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**Fig S1.** The long-term performance of the SADN system; (a) influent and effluent nitrogen concentrations ($NO_3^-$-N and $NO_2^-$-N), NRE and NTR; (b) influent and effluent TIN concentrations and NLR.

**Fig S2.** Kinetic analysis of $NO_x^-$ competition and $NH_4^+$ degradation in the $S^0$PDA system, with nitrogen composition: batch 4: only $NO_3^-$ and $NO_2^-$.

**Note S1.** Calculations of nitrogen removal efficiency, nitrogen removal contribution ratios.

Nitrogen removal efficiency was calculated by Eq. S1-Eq. S3：

$$\mathrm{ARE} = (\mathrm{NH_4^+}_{\mathrm{Inf}} - \mathrm{NH_4^+}_{\mathrm{Eff}})/\mathrm{NH_4^+}_{\mathrm{Inf}} \times 100\% \quad \text{(Eq. S1)}$$

$$\mathrm{NRE} = (\mathrm{NO_3^-}_{\mathrm{Inf}} - \mathrm{NO_3^-}_{\mathrm{Eff}})/\mathrm{NO_3^-}_{\mathrm{Inf}} \times 100\% \quad \text{(Eq. S2)}$$

$$\mathrm{TIN_{RE}} = (\mathrm{TIN_{Inf}} - \mathrm{TIN_{Eff}})/\mathrm{TIN_{Inf}} - \mathrm{TIN_{Eff}} \times 100\% \quad \text{(Eq. S3)}$$

Where, ARE, NRE and $TIN_{RE}$ refer to the $NH_4^+$-N, $NO_3^-$-N and the total inorganic nitrogen (TIN) removal efficiency, respectively, where Inf and Eff are the concentrations of the influent and effluent in the long-term performance. Notably, the TIN concentration was defined as the sum of $NH_4^+$-N, $NO_2^-$-N and $NO_3^-$-N concentrations.

**Note S2.** Calculations of contribution ratio to the total inorganic nitrogen (TIN) removal. The contribution ratios of anammox, $S^0$ADN, and other potential nitrogen removal pathways were calculated following the relative method (Wang et al., 2024), as shown in Eq. S4 and Eq. S5:

$$\mathrm{Anammox}(\%) = \frac{\left[(1 + 1.32 + 0.26) \times (\mathrm{NH_4^+}_{\mathrm{Inf(0)}} - \mathrm{NH_4^+}_{\mathrm{Eff(t)}})\right]}{\mathrm{TIN_{Inf(0)}} - \mathrm{TIN_{Eff(t)}}} \times 100\% \quad \text{(Eq. S4)}$$

$$S^0\mathrm{ADN\ and\ others}(\%) = 100 - \mathrm{Anammox}\ (\%) \quad \text{(Eq. S5)}$$

Where, inf (0) and eff (t) are the concentrations (mg/L) of the influent and effluent in the long-term performance and in the initial and time t (h) in the ex-situ batch experiments, respectively.

**Note S3.** Calculations of NLR, NRR and $NO_3^-$ to $NO_2^-$ transformation ratio (NTR).

The total inorganic nitrogen loading rate (NLR) and the total inorganic nitrogen

removal rate (NLR) were calculated by Eq. S6 and Eq. S7; The $NO_3^-$ to $NO_2^-$ transformation ratio (NTR), while the $NO_3^-$ reduction rate and $NO_2^-$ reduction rate was calculated by Eq. S8:

$$NLR = (TIN_{Inf} \times Q)/V \quad \text{(Eq. S6)}$$

$$NRR = [(TIN_{Inf} - TIN_{Eff}) \times Q]/V \quad \text{(Eq. S7)}$$

$$NTR = \left[(NO_{2\,Eff}^- - NO_{2\,Inf}^-)/(NO_{3\,Inf}^- - NO_{3\,Eff}^-)\right] \times 100\% \quad \text{(Eq. S8)}$$

Where, Q was the influent flow rates of the $S^0$PDA system, and the V refers to the effective volume of the UASB bioreactor. Inf and Eff represented the influent and effluent nitrite concentrations in the SADN system.

**Note S4.** Calculations of the $NO_3^-$ reduction rate and $NO_2^-$ reduction rate.

The $NO_3^-$ reduction rate and $NO_2^-$ reduction rate was calculated by Eq. S9 and Eq. S10:

$$rNO_3\ (mg\ N/(L \cdot h)) = \Delta NO_3^-/(V \times \Delta t) \quad \text{(Eq. S9)}$$

$$rNO_2\ (mg\ N/(L \cdot h)) = \Delta NO_2^-/(V \times \Delta t) \quad \text{(Eq. S10)}$$

Where, $\Delta NO_x^-$ referred to the change in $NO_x^-$-N concentration from the initial reaction time to time t, and V was the volume of serum bottles in the batch experiments.

**Reference:**

Wang L, Zhao Q, Zhang L, Wu D, Zhou J, Peng Y (2024). $S^0$-driven partial denitrification coupled with anammox ($S^0$PDA) enables highly efficient autotrophic nitrogen removal from wastewater. Water Research, 255: 121418

**Table S1.** Operational parameters of the SADN system.

| Phase | Time (day) | Electron donor | S/N ratio | Inf. $NO_3^-$-N (mg/L) | Inf. $NO_2^-$-N (mg/L) |
|---|---|---|---|---|---|
| I | I-1：1-27 | $Na_2S_2O_3·5H_2O$ (enrich SOB) | 2:1→3:1 | 34.2±1.2 | 0.2±0.1 |
| | I-2：28-37 | | | 43.0±1.9 | 0.2±0.1 |
| | I-3：38-50 | | | 65.4±2.7 | 0.5±0.8 |
| II | 50-91 | $S^0$ particles | - | 65.0±2.8 | 0.1±0.1 |

**Table S2.** Operational parameters and nitrogen-removal performance of the stable laboratory-scale anammox bioreactor.

| Phase | Inf. $NH_4^+$-N (mg/L) | Inf. $NO_2^-$-N （mg/L） | $\Delta NO_2^-$-N /$\Delta NH_4^+$-N | ARE（%） | $TIN_{RE}$（%） |
|---|---|---|---|---|---|
| I | 61.4±4.1 | 83.4±5.5 | 1.1±0.2 | 69.7±12.5 | 51.9±10.7 |
| II | 83.0±4.2 | 105.1±6.6 | 1.3±0.1 | 94.1±7.1 | 80.5±9.2 |

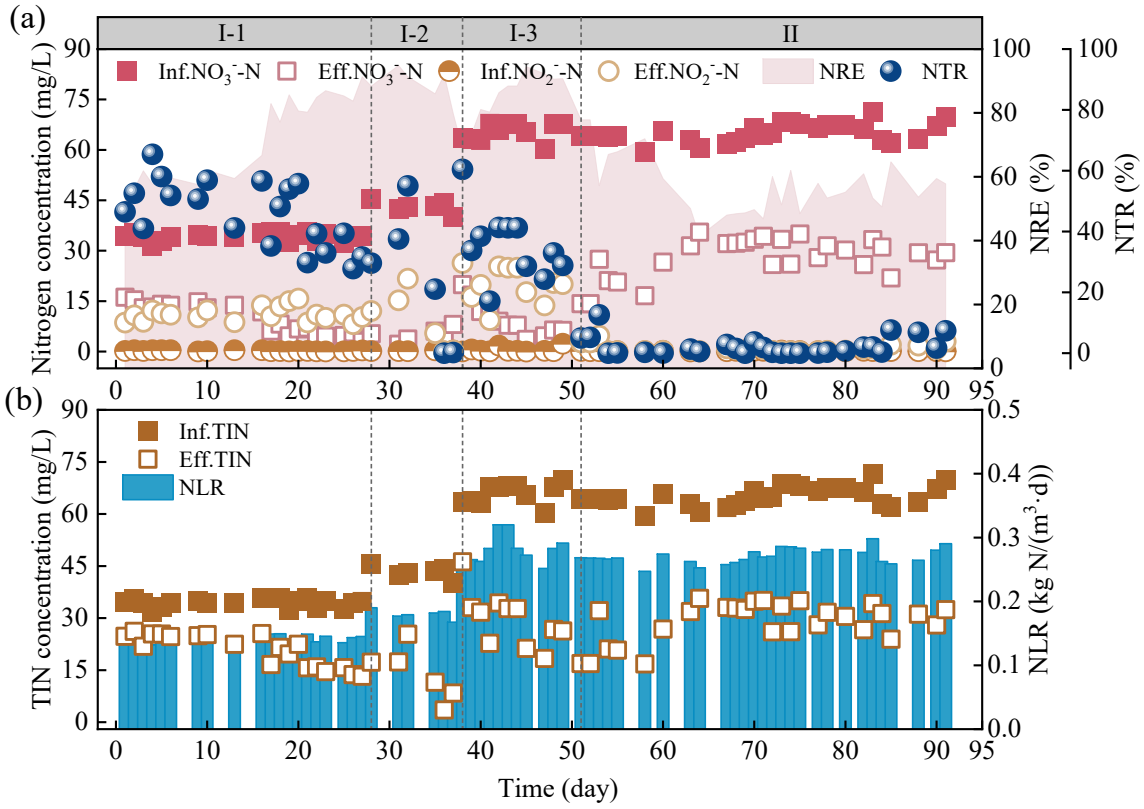


**Fig S1.** The long-term performance of the SADN system; (a) Nitrogen concentrations, NRE and NTR; (b) TIN concentrations and NLR.

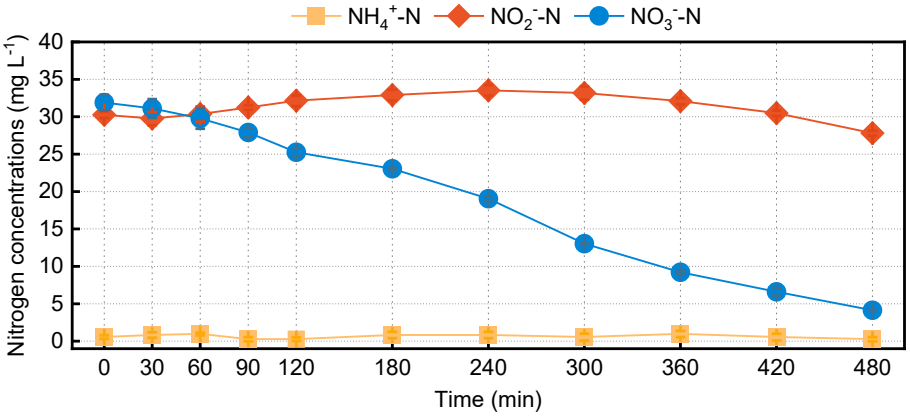


**Fig S2.** Kinetic analysis of $NO_x^-$ competition and $NH_4^+$ degradation in the $S^0$PDA system, with nitrogen composition: batch 4: only $NO_3^-$ and $NO_2^-$.