Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Assessment of start-up of Passive Aeration Simultaneous Nitrification and Denitrification (PASND) process using activated sludge as sole seed for low-energy C and N removal

Appendix A. Supplementary data

1. Formulation of synthetic wastewater

Table A.1: Synthetic wastewater component.

Synthetic wastewater component	Concentration (mg/ L)	Solution of trace element	Concentration (mg/ L)
CH_3COONa	660	Ethylene Diamine Tetraacetic Acid (EDTA)	15
NH_4Cl	60	$ZnSO_4 \cdot 7H_2O$	0.43
KH_2PO_4	44	$CoCl_2 \cdot 6H_2O$	0.24
$MgSO_4 \cdot 7H_2O$	25	$MnCl_2 \cdot 4H_2O$	0.99
$CaCl_2 \cdot 2H_2O$	300	$CuSO_4 \cdot 5H_2O$	0.25
$FeSO_4 \cdot 7H_2O$	6.25	$NaMoO_4 \cdot 2H_2O$	0.22
trace element solution	1 mL/ L	$NiCl_2 \cdot 6H_2O$	0.19
		$NaSeO_4 \cdot 10H_2O$	0.21
		H_3BO_4	0.014
		$NaWO_4.2H_2O$	0.050

2. Complete removal of acetate

Acetate sodium was measured after COD removal efficiency was stable at about 88.26%. Similar to the previous study by Hossain et al. (1), the outlet acetate also reached 0 mg/L, which shows complete removal of volatile fatty acids. Thus the outlet COD (54.57±19.35 mg/L) may be some metabolites (e.g., EPS, DNA, protein) that could not be used as carbon sources for PHAs synthesis (2).

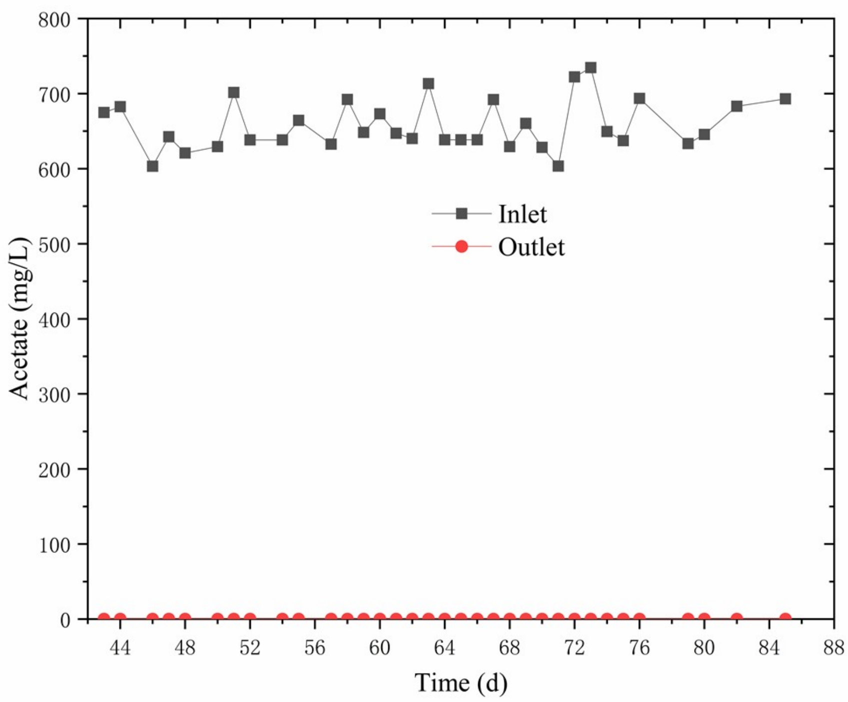

Figure A.1: Complete removal of acetate in long operation.

3. Measurement of methane production from biofilms in anaerobic phase

To verify that no methane was generated in the reactor during the anaerobic stage, the biocarrier was placed in an anaerobic bottle (the filling ratio of the biocarriers was 30%), and anaerobic sodium acetate solution (COD=500 mg/L, purged by nitrogen gas for 30 mins) was added to 90% of the volume of the bottle. A rubber plug was used to seal the bottle. Periodic gas sampling from the head space of the anaerobic bottle was carried out and the gas was sent to gas chromatography machine for methane detection. Test method parameters are as follows:

The chromatographic column: 30 m×0.32 mm×0.25 μm;

The column temperature: 40°C;

Residual time: 3 min;

Inlet temperature: 200°C;

FID Detector temperature: 250°C;

Split ratio: 50:1;

Sample quantity: 50 μL;

CO_2 gas injection: 50 μL;

As shown in **Fig. A.2**, no methane peak was observed during the anaerobic phase. It should also be noted that there was also no gas pressure build-up observed. Therefore, it can be concluded that no methane was produced during the anaerobic process.

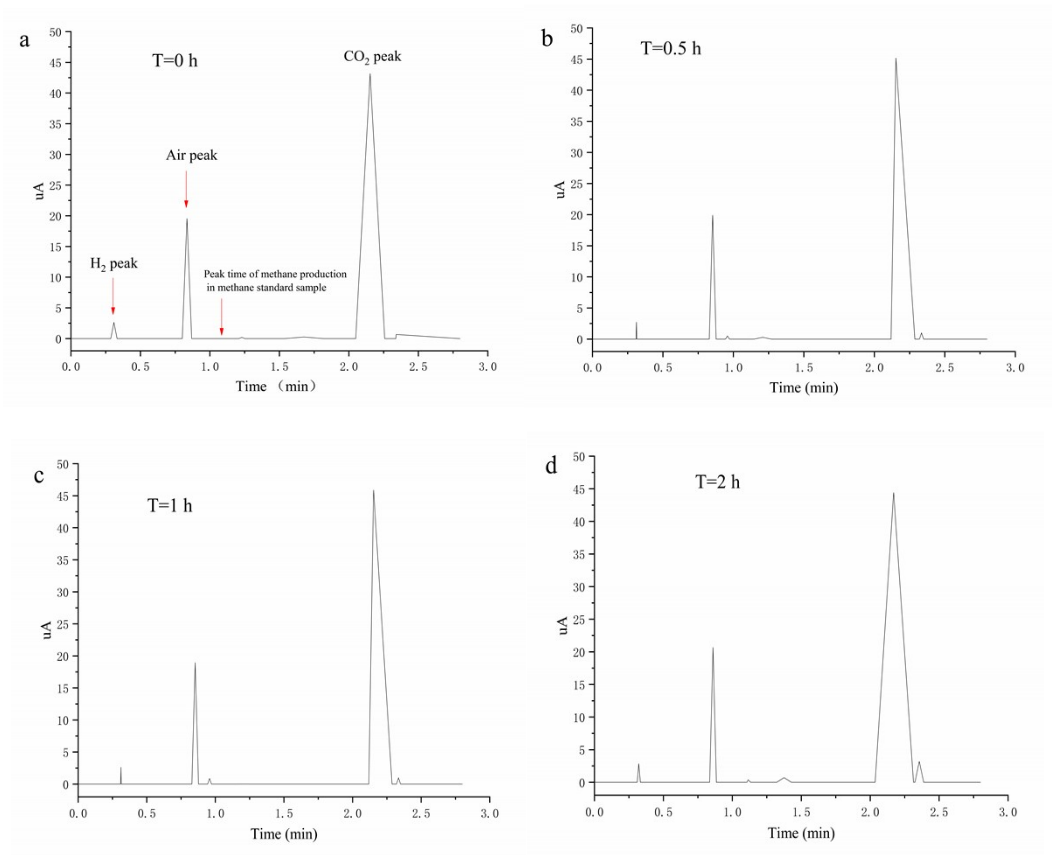

Figure A.2: Change of methane content in reactor headspace during anaerobic process.

4. Investigation of multi-stages PASND system efficiency

Table A.2: Pollutant concentration after multi-stage treatment.

HRT (h)	Pollutant (mg/L)	Influent wastewater	Single treatment	Repeated treatment
12((3+3)×2)	COD	550±18	87.5±12.5	39.5±13.5
	NH_4^+-N	40.45±0.68	15.45±0.86	3.86±0.67
	NO_2^--N	0	0	0.07±0.05
	NO_3^--N	0	0	0

	TN	40.45±0.68	15.45±0.86	3.93±0.72

5. Thick biofilm in biocarrier

Figure A.3: Thick biofilm in biocarrier (after 65 d long-term operation).

6. Denitrification via PHAs and sodium acetate as carbon source

To eliminate the biomass disturbance, the denitrification rate was investigated under batch tests. Batch tests were carried out under three stages named R1, R2, and R3. In the R1 stage, the biofilm on the carriers was contacted with sodium acetate solution (COD=500 mg/L) to synthesize PHAs. After that, the carriers were removed and the potassium nitrate/sodium nitrite solution (N=50 mg/L) was added, respectively (R2 stage). In R3 stage, the biofilm was directly in contact with a mixture of sodium acetate and potassium nitrate/sodium nitrite (N=50 mg/L), and the concentration of sodium acetate was the same as the amount of carbon conversion of PHAs in R2 stage. The working reaction volume for each reactor was 85 mL with a filling ratio of about

30%. Nitrite and nitrate in the reactor are determined periodically. COD concentration, PHAs content, and glycogen content in the biofilm were measured at the beginning and end of each stage. At the end of the reaction, the biofilms in the carriers were collected and dried, and weighed. The sum of its mass and the biofilm mass used to test PHAs and glycogen was the total biomass (m_0) involved in the reaction.

For denitrification process driven by both PHAs and sodium acetate, about 20 mg/L nitrate was removed within 1 hour without any nitrite produced. A similar nitrite removal rate was also obtained.

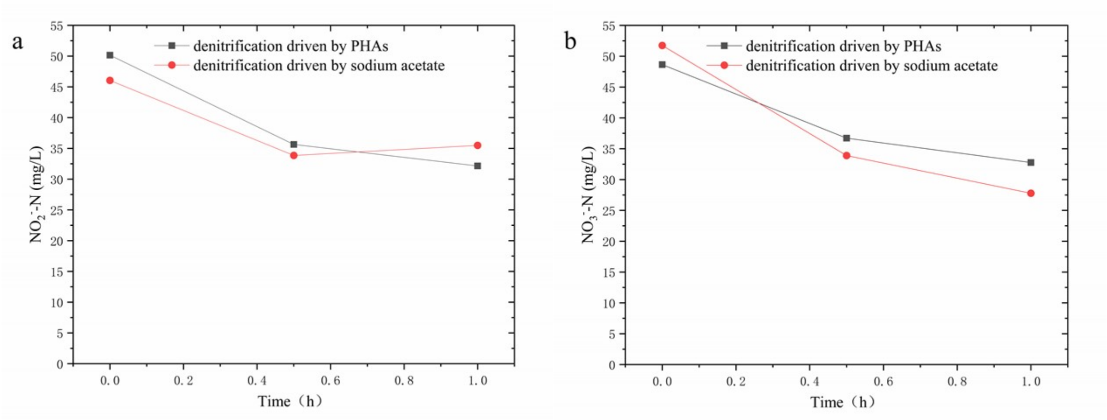

Figure A.4: Denitrification via PHAs and sodium acetate (a: using nitrate as electric acceptor; b: using nitrite as electric acceptor).

7. Performance on COD and nitrogen removal when C/N ratio was 2.5

As shown in **Fig. A.5**, the COD removal efficiency was about 66% when C/N ratio was 2.5, and the TN removal efficiency is about 19.3%. When the sewage is pumped into the reactor, 21.2mg/L nitrate is observed immediately, which is more than the decrease of ammonia, indicating that nitrate generated in the aerobic stage is not completely consumed and the nitrogen left over from the previous cycle affects the total nitrogen removal in the cycle.

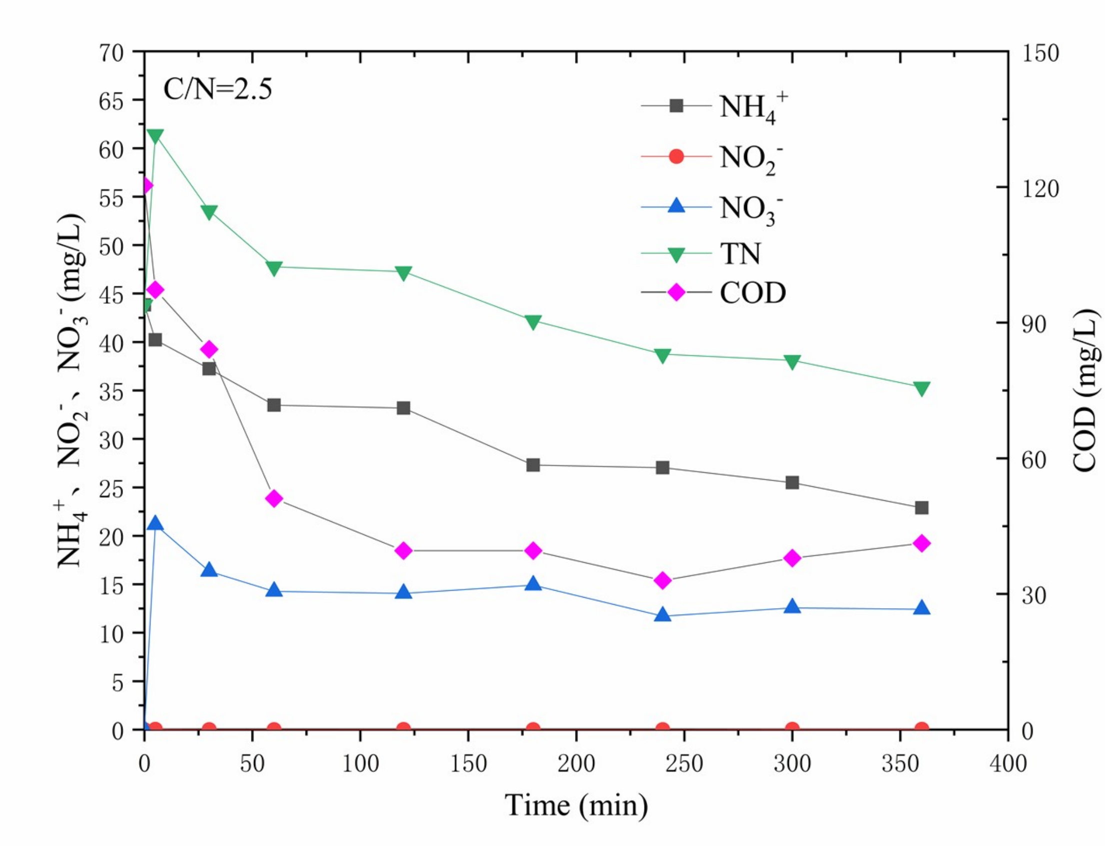

Figure A.5: Detailed profiles of pollutants removal on day 20.

8. Energy consumption calculation and comparison between energy consumption of inlet pump and conventional aggressive aeration.

The power consumption of inlet pump can be calculated through Eq A.1(3, 4):

$$E_{Inlet} = \frac{Q \times \rho \times g \times h}{102 \times \eta_1 \times \eta_2} \times t \tag{A.1}$$

While E_{Inlet} refers to energy consumption of water pump for inflow, kWh. Q is flow rate of water pump, m^3/s; ρ is density of wastewater water, 1000 kg/m^3; g is acceleration of gravity, 9.81m/s^2; h is actual lifting height of wastewater, m; η_1 is pump efficiency, 0.65; η_2 is engine efficiency, 0.7; t refers to the running time of pump, h.

The specific energy consumption (kWh/m^3) is the E_{Inlet} per m^3 wastewater treated.

$$The\ specific\ energy\ consumption = \frac{\rho \times g \times h}{102 \times \eta_1 \times \eta_2} \times 1m^3 \tag{A.2}$$

The actual lifting height (h) of water equals the height of gravity center of the bioreactor, when water is fed from the bottom. In this study, it was set as 1, 1.5 and 2-m for the bioreactors with heights of 2, 3, and 4-meter, respectively. The comparison between the energy consumption of the inlet pump at different hydraulic lifting heights and that of the conventional aggressive aeration process is shown in **Table A.3.**

Table A.3: The comparison between the energy consumption of the inlet pump at different hydraulic lifting heights and that of the conventional aggressive aeration process.

	PASND process energy for pumping (Reactor Height, m)			Conventional activated sludge process(5)	
	2	3	4	Aggressive aeration	The whole process
Energy consumption (kWh/m^3)	0.006	0.009	0.012	0.3	0.6

E_{COD} and E_{TN} was calculated through Eq A.3 and A.4:

$$E_{COD} = \frac{The\ specific\ energy\ consumption}{C_{COD-removed}} \times 1000 \tag{A.3}$$

$$E_{TN} = \frac{The\ specific\ energy\ consumption}{C_{TN-removed}} \times 1000 \tag{A.4}$$

While E_{COD} was the energy needed to remove 1 kg COD (kWh/kg COD); $C_{COD-removed}$ was the COD removal per unit volume of sewage treated (mg/L); E_{TN} was the energy needed to remove 1 kg TN (kWh/kg TN); $C_{TN-removed}$ was the TN removal per unit volume of sewage treated (mg/L).

References:

1. Hossain MI, Cheng L, Cord-Ruwisch R. Energy efficient COD and N-removal from high-strength wastewater by a passively aerated GAO dominated biofilm. Bioresource technology. 2019;283:148-58.
2. Cui Y-W, Shi Y-P, Gong X-Y. Effects of C/N in the substrate on the simultaneous production of polyhydroxyalkanoates and extracellular polymeric substances by Haloferax mediterranei via kinetic model analysis. RSC advances. 2017;7(31):18953-61.
3. Qi X, Xiong Y, Jin W, Liu Y, Cong B. Domestic urban sewage treatment energy consumption calculation method. Journal of University of Science and Technology Liaoning. 2015;38(02):155-60.
4. Zhang Z. Drainage engineering. Beijing: China Architecture & Building Press; 2015.
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