Supporting information for the *ES&T* article

Towards energy neutrality: novel wastewater treatment incorporating acidophilic ammonia oxidation

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Text S1. Chemical analyses and calculation

COD concentration was measured using the standard test kits (range 25–1500 mg/L, Merck). NH_4^+-N, NO_2^--N, NO_3^--N, and PO_4^{3-}-P concentrations were analysed using a Flow Injection Analyser (Lachat Instrument, Milwaukee, Wisconsin). Analyses of total and dissolved iron, TP, and total kjeldahl nitrogen (TKN) concentrations were conducted by using Inductive Column Plasma Optical Emission Spectroscopy (ICP-OES, Perkin Elmer Optima 7300DV, Waltham, USA). The pH was measured using a portable pH monitor and probe (pH 5+, Oakton). Organic micropollutants were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). The dissolved oxygen (DO) concentration was measured using a portable DO monitor and probe (Optical DO sensor inPro 6960i, Mettler Toledo). Organic nitrogen was calculated as the difference between TKN and NH_4^+-N. Alkalinity was determined by titration with an ending pH value of 4.3 according to the standard method.[1] FNA concentration was calculated according to the equation $\mathrm{FNA\,(mg\,HNO_2-N/L)} = \frac{\mathrm{NO_2^-\ (mg\,N/L)}}{\mathrm{e}^{-2300/273\,+\,\mathrm{Temp}(°\mathrm{C})} \times 10^{\mathrm{pH}}}$. The apparent activation energy (Ea, kJ/mol) was estimated by the slope of linear regression of Arrhenius plot: $\ln r = -\frac{E_a}{R}\cdot\frac{1}{T} + \ln A$, where r is the biomass activity, R is the gas constant (8.32 J/mol/K), T is the temperature in kelvin, and A is the reaction frequency factor.

Text S2. Measurement of maximal activity for AOB, NOB and anammox bacteria

The maximal activities of AOB and NOB were measured in the aerobic MBBR, and the maximal anammox activity was assessed in the anoxic MBBR. Each test lasted for 3 h, during which all the controllers including two feeding pumps and two pH control systems were temporarily turned off. A NH_4HCO_3 stock solution (10 g N/L) of 10 mL and a Na_2NO_2 stock solution (10 g N/L) of 8 mL were added into the aerobic MBBR to increase NH_4^+-N and NO_2^--N concentrations to about 60–80 mg N/L. The DO concentration was maintained above 7.0 mg/L by constantly supplying compressed air to the reactor at a flow rate of 1.0 L/min via an air pump (whisper 100, China). The pH of aerobic MBBR was controlled between 7.0 and 7.5 by adding 0.1 M HCL and 0.1 M NaOH manually. Liquid samples were collected every 0.5 h to the end of test, and then filtered by 0.22 μm disposable sterile Millipore filters (Merck) for the analysis of NH_4^+-N, NO_2^--N and NO_3^--N concentrations. The maximal AOB and NOB activities were represented by the volumetric NH_4^+-N oxidation and NO_3^--N production rates, which were determined through linear regression of corresponding profiles obtained from the batch test. In the anoxic MBBR, 6 mL NH_4HCO_3 stock solution (10 g N/L) and 6 mL Na_2NO_2 stock solution (10 g N/L) were added to increase NH_4^+-N and NO_2^--N concentrations to 60–70 mg N/L. To remove oxygen in anoxic MBBR, compressed pure dinitrogen (N_2) gas was continually flushed in the reactor at a flow rate of 1.0 L/min during the test. The pH control and sampling strategy of anoxic MBBR were similar to the test performed in aerobic MBBR. The volumetric NH_4^+-N oxidation rate, which was determined through linear regression of the ammonium profile obtained from the batch test, represented the maximal anammox activity.

Text S3. DNA extraction, 16S rRNA gene amplicon sequencing, and data analyses

Two aerobic and two anoxic biofilm samples were collected at the end of Phase Ⅰ (i.e., operating temperature of 23°C) and Ⅳ (i.e., operating temperature of 12°C), respectively. After collection, all samples were stored at -80°C before being assessed by the Australian Centre for Ecogenomics at The University of Queensland (https://ecogenomic.org/). DNA was extracted from 50-200 mg of raw sample using Qiagen DNeasy Powersoil Pro Kit (cat #7016) following the manufacturer's protocol and checked with gel electrophoresis. The 16S rRNA gene encompassing the V6 to V8 regions was targeted using the 926F (5'- AAA CTY AAA KGA ATT GRC GG -3') and 1392wR (5'- ACG GGC GGT GWG TRC -3') primers modified to contain Illumina specific adapter sequence (926F: 5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG AAA CTY AAA KGA ATT GRC GG -3' and 1392wR: 5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GAC GGG CGG TGW GTR C -3'). The universal primer pair 926F-1392wR amplifies the small submit (SSU) ribosomal RNA of eukaryotes (18S) and prokaryotes (16S) specifically the V6, V7 and V8 regions. Raw sequencing data was processed by Quantitative Insights Microbial Ecology Ⅱ (QIIME Ⅱ) in multiple steps, including poor-sequences removal. After that, the sequences were clustered into operational taxonomic units (OTUs) at 97% identify threshold.

Text S4. Mass and energy balance assessments

The mass and energy balance assessments were carried out to further evaluate the feasibility of applying this novel system for domestic wastewater treatment. The evaluation of mass balance was performed based on the measured data of the laboratory-scale treatment system. The energy balance assessment was performed in a hypothetical WWTP with a treatment capacity of 10,000 m^3/d, according to Wu et al.[2] The main parameters used for the calculation are summarized in Table S5.

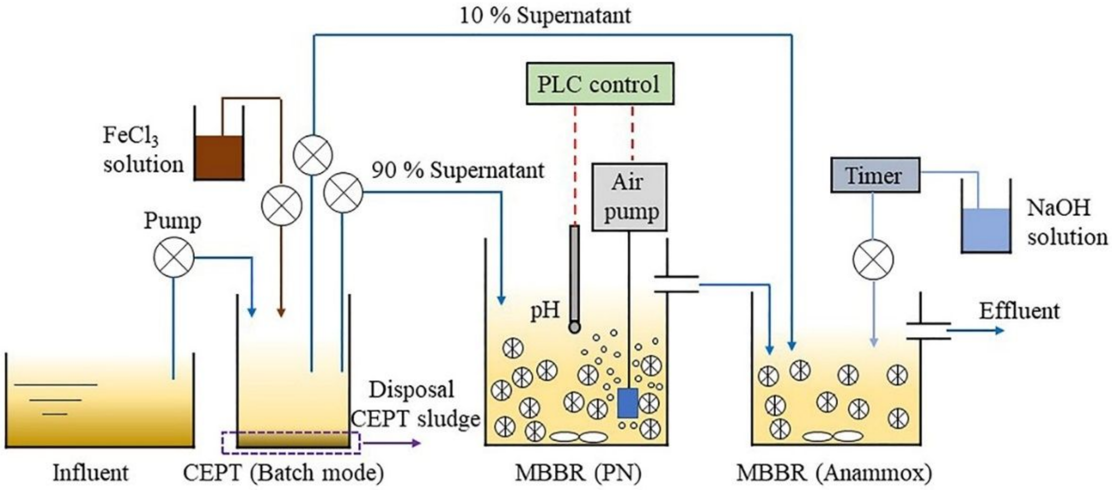

Figure S1. The schematic diagram of the laboratory-scale wastewater treatment system.

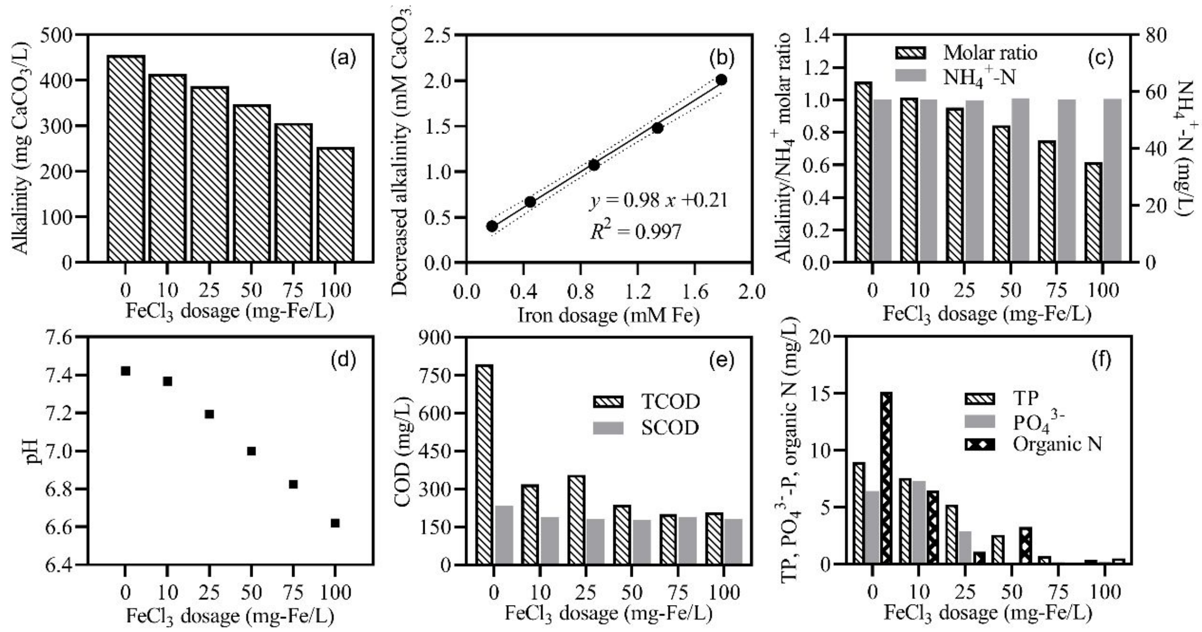

Figure S2. Changes of wastewater composition with the addition of $FeCl_3$ at different concentrations. (a) The change of wastewater $CaCO_3$-alkalinity with the increased Fe dosage. (b) The linear relationship between decreased $CaCO_3$-alkalinity and dosed Fe. (c) The changes of $CaCO_3$-alkalinity/NH_4^+-N molar ratio and NH_4^+-N concentration with the increased Fe dosage. (d) The profile of wastewater pH with the increased Fe dosage. (e) the shifts of TCOD and SCOD concentrations with the increased Fe dosage. (f) The changes of TP, PO_4^{3-}-P, and organic nitrogen concentrations with the increased Fe dosage.

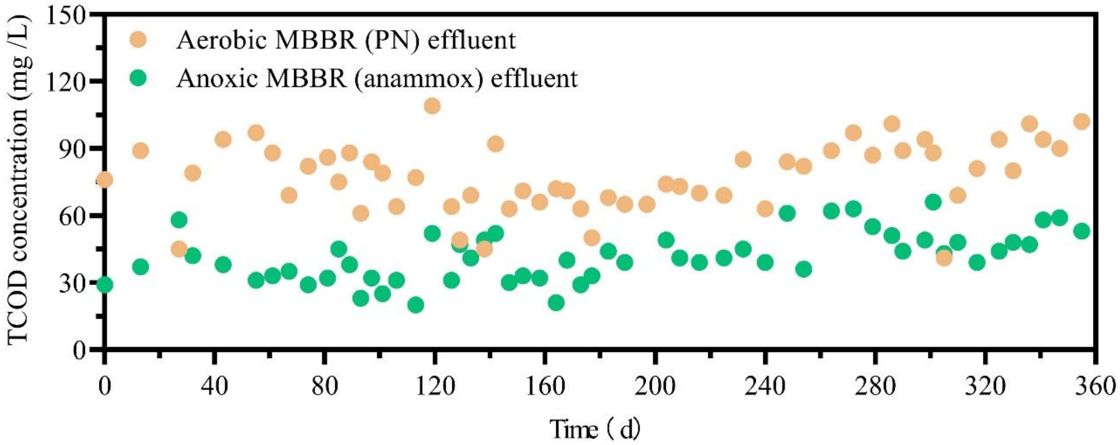

Figure S3. COD concentration in the effluent of aerobic and anoxic MBBRs.

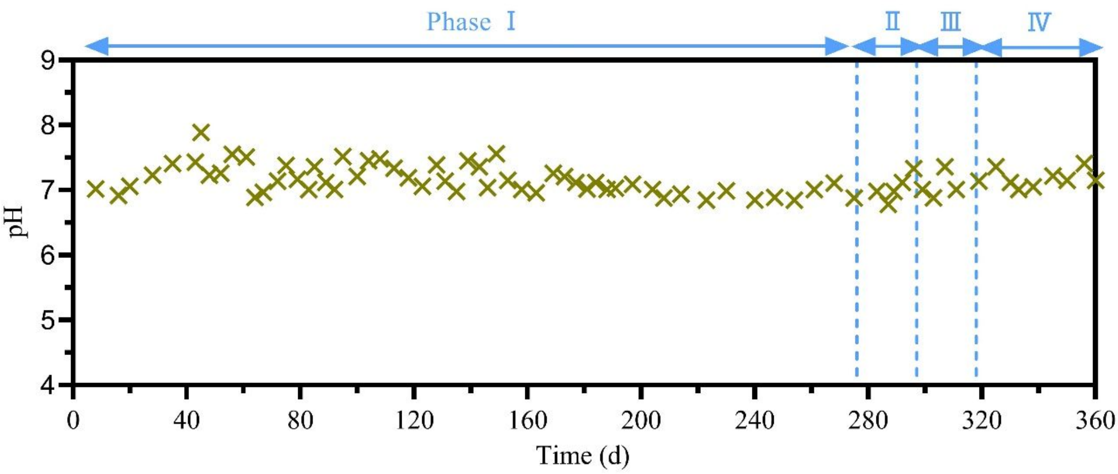

Figure S4. The pH of anoxic MBBR during the study period.

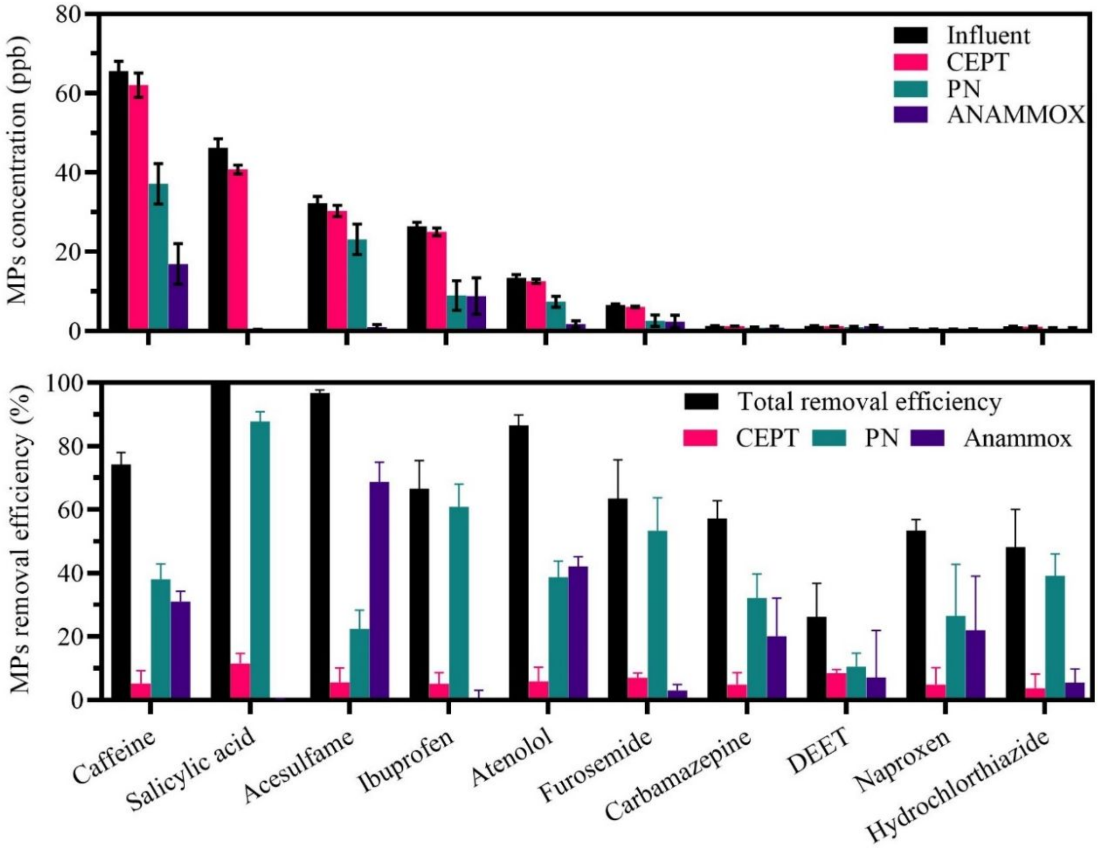

Figure S5. Micropollutants concentration (upper) and removal efficiency (down) in different units of the wastewater treatment system.

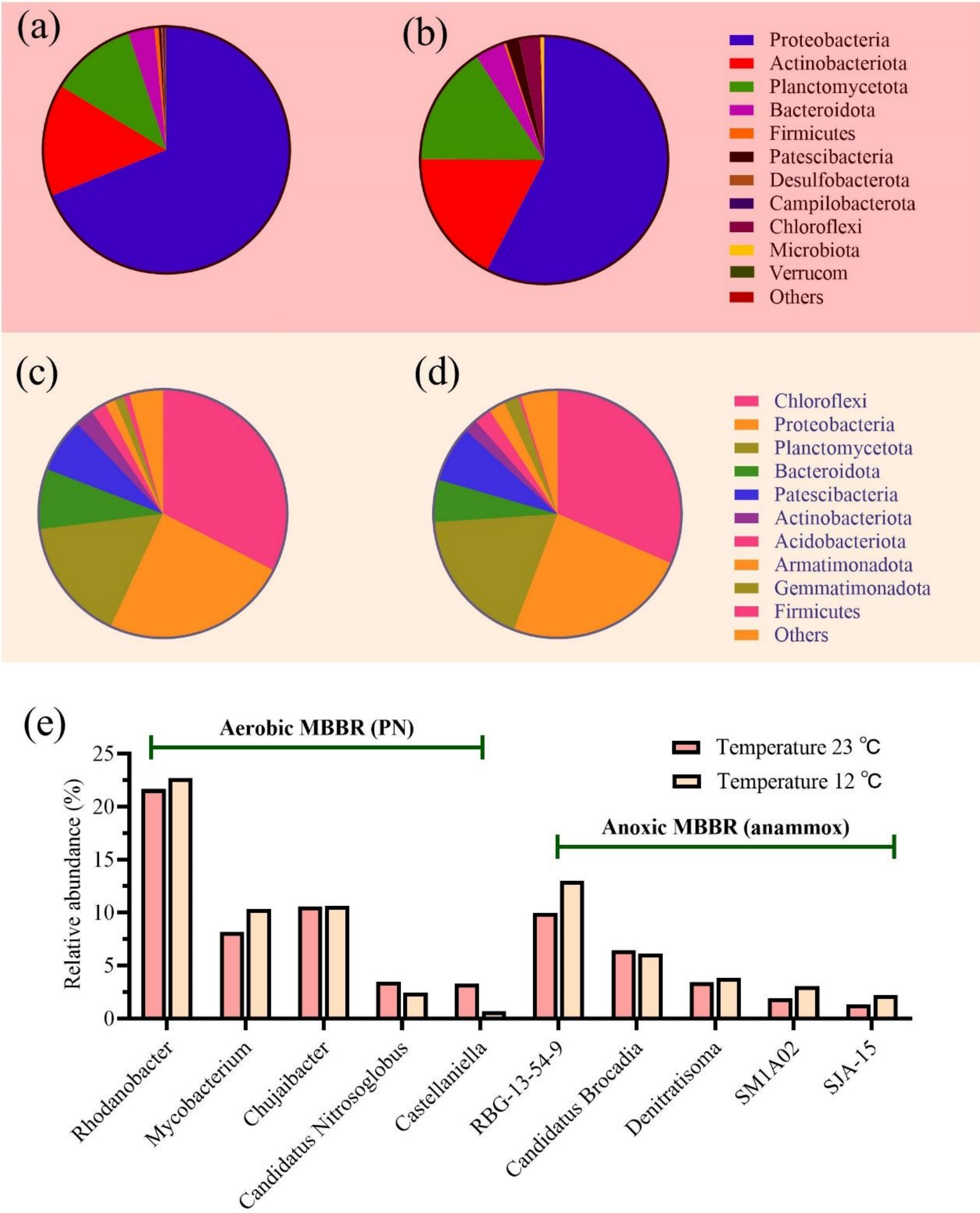

Figure S6. The microbial composition of the two-stage PN/A system. (a) The top ten phyla of aerobic MBBR at 23°C. (b) The top ten phyla of aerobic MBBR at 12°C. (c) The top ten phyla of anoxic MBBR at 23°C. (d) The top ten phyla of anoxic MBBR at 12°C. (e) The top five genera in both aerobic and anoxic MBBRs at 23°C and 12°C.

Table S1. Main characteristics of raw domestic wastewater used in this study.

Parameter	Unit	Average value ± standard error	Number of samples
TCOD	mg/L	461.7 ± 46.1	27
SCOD	mg/L	218.3 ± 13.2	27
NH_4^+-N	mg/L	46.1 ± 5.7	76
NO_2^--N	mg/L	ND[a]	76
NO_3^--N	mg/L	ND[a]	76
PO_4^{3-}-P	mg/L	6.8 ± 1.3	76
Alkalinity	mg $CaCO_3$/L	382.4 ± 35.6	35
pH	-	7.1 ± 0.1	38

[a] ND: Not detected as the nitrite and nitrate was always below 0.5 mg/L and could be neglected during the experiments.

Table S2. Removals of organic carbon and nutrients by the integrated wastewater treatment system (CEPT, acidic PN and anammox) at different temperatures.

Parameter	Unit	Temperature	Raw wastewater	CEPT effluent	Acidic PN effluent	Anammox Effluent
COD concentration	mg COD/L	23°C	468.3 ± 50.7	176.3 ± 19.2	73.9 ± 13.9	38.8 ± 10.8
		20°C	432.5 ± 4.9	168.3 ± 3.8	92.3 ± 7.6	50.0 ± 5.6
		15°C	439.5 ± 33.2	167.7 ± 21.0	66.0 ± 23.6	52.3 ± 12.1
		12°C	449.7 ± 20.6	178.0 ± 8.5	93.5 ± 7.7	49.8 ± 5.3
PO_4^{3-}-P concentration	mg P/L	23°C	6.6 ± 1.5	0.6 ± 0.4	0.7 ± 0.3	0.3 ± 0.2
		20°C	7.1 ± 1.2	0.7 ± 0.3	0.5 ± 0.2	0.4 ± 0.1
		15°C	6.5 ± 0.8	0.5 ± 0.3	0.5 ± 0.1	0.5 ± 0.2
		12°C	6.8 ± 0.7	0.5 ± 0.3	0.5 ± 0.2	0.4 ± 0.1
NH_4^+-N concentration	mg N/L	23°C	49.2 ± 4.9	48.4 ± 5.3	16.9 ± 4.2	2.7 ± 1.4
		20°C	42.9 ± 4.7	43.4 ± 4.5	17.4 ± 3.4	2.4 ± 0.7
		15°C	44.1 ± 0.9	42.9 ± 0.6	17.3 ± 1.6	1.8 ± 0.7
		12°C	46.8 ± 1.2	45.4 ± 1.8	20.1 ± 1.5	2.1 ± 0.6
NO_2^--N concentration	mg N/L	23°C	ND[a]	ND[a]	28.8 ± 4.2	1.5 ± 1.1
		20°C			24.3 ± 0.6	1.8 ± 0.3
		15°C			25.9 ± 2.1	2.4 ± 0.4
		12°C			25.5 ± 1.9	1.9 ± 0.6
NO_3^--N concentration	mg N/L	23°C	ND[a]	ND[a]	0.6 ± 0.8	1.5 ± 1.1
		20°C			0.6 ± 0.6	1.8 ± 0.3
		15°C			0.2 ± 0.2	2.4 ± 0.4
		12°C			0.2 ± 0.1	1.9 ± 0.6
TN concentration	mg N/L	23°C	49.5 ± 4.9	48.6 ± 5.3	47.2 ± 6.2	5.1 ± 1.8
		20°C	43.0 ± 4.7	43.7 ± 4.5	42.1 ± 3.3	5.5 ± 1.1
		15°C	44.3 ± 0.9	43.0 ± 0.6	43.3 ± 1.4	5.1 ± 1.1
		12°C	47.1 ± 1.2	45.7 ± 1.8	45.6 ± 2.2	5.1 ± 1.2

[a] ND: Not detected.

Table S3. The iron concentration in each part of system.

Parameter	Total iron	Dissolved iron
Unit	(mg Fe/L)	
Iron dosage	50	
CEPT effluent	2.8 ± 1.2	0.3 ± 0.2
Acidic MBBR effluent	2.4 ± 1.2	0.2 ± 0.1
Anoxic MBBR effluent	2.3 ± 0.8	0.2 ± 0.1

Table S4. The performance of mainstream PN/A process in treating low-strength wastewater at low temperature.

Influent ammonium concentration (mg N/L)	Type of wastewater	HRT (h)	NLR (kg N/(m^3·d))	Type of reactor	Temperature (°C)	TN removal efficiency (%)	Biomass growth type	Reference
53 ± 5	diluted sludge digester	12	0.11 ± 0.01	SBR	15	70.6 ± 19.5	granules	[3]
21.2 ± 5.2	aerobically pre-treated municipal wastewater	9-14	0.04-0.06	SBR	15	73.1	biofilm	[4]
		12-14	0.04			62.3	biofilm + suspended	
23-46	synthetic wastewater	1.57	0.18-0.35	RBC	15	36 ± 9	biofilm	[5]
		1.09	0.25-0.51		14	42 ± 4		
61	synthetic wastewater	5.4	0.27[a]	SBAR	10	39	granules	[6]
50	synthetic wastewater	64.8 - 139.2[a]	0.01-0.02	MBBR	10	71.8[a]	biofilm	[7]
50	synthetic wastewater	>240	<0.05	SBR	10	~30	suspended	[8]
		24-48	0.03-0.05	SBR		~40	granules	
		24-48	0.03-0.05	MBBR		~40	biofilm (2 mm)	
		24-48	0.03-0.05	MBBR		~77	biofilm (10 mm)	
~70	synthetic wastewater	60	~0.03	SBR	12	>90	suspended	[9]
60-80	synthetic wastewater	~8	~0.2	SBR	15	<50	granules	[10]
45.4 ± 1.8	CEPT pre-treated municipal wastewater	12.2	~0.09	MBBR	12	88.9 ± 2.5	biofilm	This study

RBC: rotating biological contactor; SBR: sequencing batch reactor; SBAR: sequencing batch air-lift reactor

Table S5. Main parameters used in the energy balance assessment.

Parameters	Unit	Value
Wastewater flow rate	m^3/d	10000
Fe dosage	mg/L	50
Influent COD concentration	mg/L	468
Effluent COD concentration	mg/L	40
Influent NH_4^+ concentration	mg N/L	46
Effluent NH_4^+ concentration	mg N/L	3
COD removal efficiency of CEPT	%	62.4
Ratio of NH_4^+ oxidized by AOB	%	57
Autotrophic bacteria yield[11]	g cell formed / g NH_3-N oxidized	0.24
Autotrophic bacteria decay[11]	/day	0.1
Heterotrophic bacteria yield[11]	g cell COD formed / g COD removed	0.64
Heterotrophic bacteria decay[11]	/day	0.2
Anammox bacteria yield[11]	g cell formed / g NH_3-N oxidized	0.13
Anammox bacteria decay[11]	/day	0.1
Energy recovery efficiency of AD	%	30
Energy density of CH_4	MJ/m^3	36

133 **Table S6**. Ammonium nitrogen and alkalinity concentrations in domestic wastewater in the literature.

Type of wastewater	Country	Ammonia nitrogen concentration (mg N/L)	Alkalinity concentration (mg $CaCO_3$/L)	Molar ratio of alkalinity to ammonia nitrogen	Reference
HRAS (high rate activated sludge) effluent	Australia	44.7 ± 4.5	380 ± 10	1.17 ± 0.03	[12]
Raw domestic wastewater	China	79.1	510.6	0.90	[13]
Raw domestic wastewater	Korea	35.2	195	0.78	[14]
Raw domestic wastewater	Greece	150	350	0.33	[15]
Raw domestic wastewater	Venezuela	21.3	115.2	0.76	[16]
Raw domestic wastewater	Spain	29 ± 5	250 ± 28	~1.21	[17]
		8.6 ± 0.5	73.5 ± 2.4	~1.20	
Raw domestic wastewater	India	8.0 ± 0.2	58.8 ± 3.3	~1.03	[18]
		7.2 ± 0.1	62.8 ± 2.8	~1.22	
Raw domestic wastewater	France	44.2	342	1.08	[19]
		12	50	0.58	
Raw domestic wastewater	United States	25	100	0.56	
		50	200	0.56	
Raw domestic wastewater	Brazil	17 ± 3	155 ± 17	~1.28	[20]
Raw domestic wastewater	India	30–45	230–300	0.72–1.40	[21]
Raw domestic wastewater	Australia	46.1 ± 5.7	382.4 ± 35.6	1.1 ± 0.1	This study

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