Supplementary Information (SI) for

Insight into the removal of enoxacin in an anaerobic sulfur-mediated wastewater treatment system: performance, kinetics and mechanisms

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Chemical and regents

Enoxacin (ENO, 99% purity) and enoxacin-d8 (99% purity) were purchased from Dr. Ehrenstorfer Gmbh (Augsburg, Germany). The important physico-chemical properties of ENO were shown in **Table S1** in Supplementary Information (SI). HPLC-grade methanol, acetonitrile and formic acid were purchased from Labscience, Inc. (MA, USA). Oasis HLB (200 mg/6 mL) was purchased from Waters (MA, USA), 0.22-μm filter membrane was purchased from Jinteng (Tianjin, China). Ultra-pure water from Millipore System (Millipore, MA, USA) was used for preparing all the standards. The stock solutions of ENO were prepared at a concentration of 100 mg/L using methanol and stored in the dark at 4°C until further use.

Sulfate-reducing sludge used in batch experiments

The sulfate-reducing sludge used in batch experiments was taken from the SRUSB bioreactor after 366 days of continuous operation, and then washed using synthetic wastewater (see **Table S2** in SI for composition) for three times with centrifugation (1793×g) for separation of sludge pellets between two times of washing, until no residual ENO detected in the sulfate-reducing sludge.

ENO removal efficiency and specific removal rate

The removal efficiency and specific removal rate (q) of ENO were calculated using the following equations:

$$Removal\ efficiency\ (\%) = \frac{Q_{In} - Q_{En}}{Q_{In}} \times 100\% \qquad (S1)$$

$$q = \frac{Q_{In} - Q_{En}}{m} \qquad (S2)$$

Where, q is specific removal rate (μg/g/d); Q_{In} is the mass of ENO flowing into the SRUSB bioreactor daily (μg/d); Q_{En} is the mass of ENO flowing out from the SRUSB reactor daily (μg/d); m is the total mass of sludge (suspended solids) in reactor (g-SS).

The adsorption removal efficiency and the specific adsorption rate (q_s) of ENO were calculated using the Eq. (S3) and Eq. (S4):

$$Removal\ efficiency\ (\%) = \frac{\Delta Q_S}{Q_{In}} \times 100\% \qquad (S3)$$

$$q_S = \frac{\Delta Q_S}{m} \qquad (S4)$$

where, $\Delta Q_S = Q_{S(t+1)} - Q_{S(t)}$

where, q_S is specific sorption rate (μg/g/d); $Q_{S(t+1)}$ the mass of ENO in sulfate-reducing sludge detected on Day t+1 (μg/d); $Q_{S(t)}$ is the mass of ENO in sulfate-reducing sludge detected on Day t (μg/d); Q_{In} is the mass of ENO flowing into the SRUSB bioreactor daily (μg/d); m is the total mass of sludge (suspended solids) in reactor (g-SS).

The biodegradation removal efficiency and the specific biodegradation rate (q_b) of ENO were calculated using Eq. (S5) and Eq. (S6):

$$Removal\ efficiency\ (\%) = \frac{(Q_{In} - Q_{En} - \Delta Q_S)}{Q_{In}} \times 100\% \qquad (S5)$$

$$q_b = \frac{(Q_{In} - Q_{En} - \Delta Q_S)}{m} \qquad (S6)$$

where, $\Delta Q_S = Q_{S(t+1)} - Q_{S(t)}$; q_b is specific biodegradation rate (μg/g/d); Q_{In} is the mass of ENO flowing into the SRUSB bioreactor daily (μg/d); Q_{En} is the mass of ENO flowing out from the SRUSB reactor daily (μg/d); $Q_{S(t+1)}$ the mass of ENO in sulfate-reducing sludge detected on Day t+1 (μg/d); $Q_{S(t)}$ is the mass of ENO in sulfate-reducing sludge detected on Day t (μg/d); m is the total mass of sludge (suspended solids) in reactor (g-SS).

Adsorption isotherms and thermodynamics

Three adsorption isotherms (Henry, Freundlich and Langmuir) (Eq. (S7), Eq. (S8) and Eq. (S9)) were applied for ENO adsorption at a set of reaction temperatures (i.e., 5, 10, 15, 20, 25, 30 and 35ºC) in batch experiments.

$$q_e = k_H C_e \qquad (S7)$$

$$q_e = k_f {C_e}^{1/n} \qquad (S8)$$

$$q_e = \frac{k_L q_m C_e}{1+k_L C_e} \qquad (S9)$$

Where, k_d is the Henry adsorption coefficient (L/g), k_f is the binding energy constant reflecting the affinity of adsorbents to ENO; n is the Freundlich nonlinearity index; q_e is the mass of ENO adsorbed onto sulfate-reducing sludge at equilibrium (μg/g); q_m is the theoretical maximum adsorption capacity of ENO by sulfate-reducing sludge (μg/g); k_L is the Langmuir constant related to adsorption energy (L/μg).

The thermodynamic process, including parameters such as Gibbs free energy (ΔGº), enthalpy change (ΔHº), and entropy changes (ΔSº), were used to evaluate the feasibility and nature of the adsorption process, and calculated with the following Eq. (S10), Eq. (S11) and Eq. (S12):

$$\Delta G^o = \Delta H^o - T\Delta S^o \qquad (S10)$$

$$\ln k_H = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \qquad (S11)$$

$$\Delta G^o = -\mathrm{RT}\ln k_H \qquad (S12)$$

where, k_f is the Freundlich constant; R is the ideal gas constant (8.314 J/mol/K); T is the absolute temperature (K); ΔG^o is Gibbs free energy (kJ/mol); ΔH^o is enthalpy change (kJ/mol); ΔS^o is standard entropy changes (J/K/mol).

ENO analyses

The ENO in aqueous phase (influent and effluent) and sludge samples was determined regularly by an ultra-performance liquid chromatography (UPLC) with a DAD detector (Dionex, UltiMate 3000, CA, USA) using a Acclaim[120] C18 column (2.1×150 mm, 3 μm, Dionex, CA, USA) under mobile phase A (75% of ultra-pure water with 0.1% formic acid) and phase B (25% of acetonitrile). The flow rate, column oven temperature and injection volume were 0.3 mL/min, 30℃ and 25 μL, respectively. The monitoring was carried out at wavelength of 285 nm.

In the case of the solid phase (sulfate-reducing sludge), the samples were frozen and lyophilized. The dried sludge was extracted successively at room temperature in an ultrasonic bath three times with extraction solvent (methanol, sodium citrate buffer solution, EDTA buffer solution at a ratio of 3:1:1, v/v), evaporated and re-dissolved the samples with water and pre-concentrated by solid-phase extraction (SPE). An isotopically labelled internal standard (i.e., Enoxacin-d8) was added into the samples and used to correct for matrix effects during SPE. HLB cartridges (6 mL/200 mg, Waters, Sunnyvale, MA, USA) were used after preconditioning with 3×3 mL of menthol, 3 mL of menthol containing 0.1% formic acid, and 2×3 mL of ultra-pure water. Then, re-dissolved liquor percolated through the cartridges at 5 mL/min. The cartridges were subsequently air-dried by vacuum. Thereafter, the cartridges were eluted with 3×3 mL of menthol, and the eluent was dried by evaporation under a flow of nitrogen, and finally re-dissolved with 1 mL of acetonitrile/ultra-pure water with 0.1% formic acid (25:75, v/v) for ENO analysis using UPLC-DAD.

Table S1 Physical and chemical properties of ENO

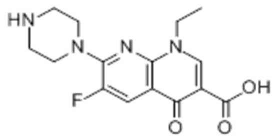
Name	Chemical formula	Molecular weight	pKa	LogKow	Molecular structure
ENO	$C_{15}H_{17}FN_4O_3$	320.32	6.3 8.7	-0.2	

Table S2 Composition of synthetic wastewater

Constituents	Concentration (mg/L)
Sodium acetate	640.6
NH_4Cl	95.5
$CaCl_2$	26.0
$MgCl_2$	38.9
K_2HPO_4	7.2
K_2HPO^3	19.2
Na_2SO_4	1213.0

Table S3 Batch experimental program for ENO adsorption and biodegradation by sulfate-reducing sludge

Groups	Synthetic wastewater	Sulfate-reducing sludge (1.5 g-SS/L)	ENO	0.1% NaN_3	Operation time	Removal mechanisms
I	+	-	+	-	5 days	hydrolysis
II	+	+	+	+	24 hrs	adsorption
II	+	+	+	-	5 days	hydrolysis adsorption biodegradation

Table S4 Performance of SRUSB bioreactor at different initial ENO concentrations

Parameters	Influent*	Effluent*						
		Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
COD (mg/L)	481.2±9.1	71.5±4.7	70.2±2.7	70.8±2.9	69.1±5.3	68.6±2.6	68.7±4.3	67.3±4.8
SO_4^{2-} (mg S/L)	268.1±4.9	107.1±8.5	107.2±6.7	107.3±7.6	105.9±3.4	106.1±2.1	104.9±3.8	104.3±2.7
SO_3^{2-} (mg S/L)	ND	ND	ND	ND	ND	ND	ND	ND
$S_2O_3^{2-}$ (mg S/L)	ND	ND	ND	ND	ND	ND	ND	ND
Total dissolved sulfide (H_2S+HS^-+S^{2-}) (mg S/L)	ND	144.5±8.0	144.7±8.9	145.6±4.3	146.1±7.0	146.8±11.2	147.0±8.7	147.9±10.5
pH	7.0	7.6±0.2	7.5±0.4	7.6±0.5	7.5±0.2	7.6±0.4	7.6±0.8	7.6±0.7
ORP (mv)	-	-463±5.0	-461±3.1	-473±4.7	-461±7.1	-469±5.8	-459±4.1	-465±4.5

*Mean value ± standard deviation

ND: Not detectable

Table S5 Adsorption kinetic (pseudo-first-order and pseudo-second-order) parameters for ENO adsorption onto sulfate-reducing sludge

Concentration (μg/L)	k_d (L/g)	Experimental data	Pseudo-first-order			Pseudo-second-order		
		qe (μg/g SS)	k_1 (1/hr)	q_e (μg/g SS)	r^2	k_2 (g/μg/hr)	q_e (μg/g SS)	r^2
100	22.68±2.1	53.8	0.51±0.037	2.49±0.25	0.711	1.73±0.11	53.8±4.11	0.999
300	23.47±1.7	157.4	0.31±0.015	0.59±0.13	0.381	1.02±0.021	156.25±3.93	0.999
500	22.79±0.9	269.9	0.30±0.003	0.96±0.07	0.382	0.68±0.045	270.27±6.07	0.999
1000	23.88±1.3	605.2	0.49±0.025	1.34±0.15	0.554	0.72±0.012	588.23±9.12	0.998
3000	28.27±0.7	1546.8	0.48±0.032	1.75±0.03	0.483	0.51±0.042	1666.7±7.06	0.964
5000	28.94±1.4	2752.8	0.49±0.024	1.81±0.09	0.496	0.8±0.091	2528.01±10.22	0.973

Table S6 Adsorption isotherm (Henry, Freundlich, and Langmuir) parameters of ENO by sulfate-reducing sludge under different temperature

Temperature	Henry		Freundlich			Langmuir		
(°C)	k_H (L/g)	r^2	k_F	1/n	r^2	$k_L \times 10^{-2}$ (L/g)	q_m (μg/g)	r^2
5	125.36±3.61	0.996	35.3±1.35	0.695	0.990	7.93±0.89	434.78±4.75	0.975
10	81.94±1.94	0.995	25.04±2.54	0.732	0990	5.35±0.53	500.00±8.09	0.971
15	52.17±2.31	0.997	20.80±0.82	0.804	0.995	3.11±0.13	714.28±8.25	0.972
20	36.84±2.48	0.995	16.00±3.51	0.825	0.992	2.52±0.05	666.67±7.69	0.964
25	27.99±0.99	0.996	12.69±2.63	0.848	0.987	1.78±0.08	769.23±6.29	0.937
30	19.56±1.65	0.998	7.41±1.34	0.817	0.990	1.37±0.03	666.68±9.18	0.978
35	13.51±1.50	0.999	3.89±0.78	0.800	0.990	1.02±0.15	526.32±5.56	0.952

Table S7 Thermodynamic parameters of ENO adsorption onto sulfate-reducing sludge

Temperature (°C)	ΔG°(KJ/mol)	ΔH°(KJ/mol)	ΔS°(J/(mol/K))
5	-51.354		
10	-51.364		
15	-51.373		
20	-51.381	-51.882	-146.738
25	-51.390		
30	-51.398		
35	-51.406		

Table S8 Biodegradation kinetic (zero, first and second-order) parameters for ENO in sulfate-reducing sludge system

Concentrations (μg/L)	Zero-order kinetic			First-order kinetic			Second-order kinetic		
	K_0' (μg/L/d)	$t_{1/2}$ (d)	r^2	*K_1' (d^{-1})*	$t_{1/2}$ (d)	r^2	*K_2' ×10^{-2}* (L/μg/d)	$t_{1/2}$ (d)	r^2
100	3.30±0.10	15.88	0.984	0.034±0.0009	20.26	0.988	0.04±0.02	23.85	0.980
300	17.14±4.81	8.75	0.997	0.067±0.0010	10.35	0.997	0.03±0.01	11.14	0.991
500	23.91±8.73	10.60	0.981	0.053±0.0002	13.05	0.989	0.01±0.003	19.75	0.986
1000	55.10±5.69	9.51	0.995	0.061±0.0001	11.45	0.994	0.007±0.002	13.64	0.987
3000	154.66±11.53	9.78	0.982	0.058±0.0010	12.00	0.982	0.002±0.001	16.52	0.975
5000	263.95±16.27	9.51	0.985	0.060±0.0002	11.61	0.985	0.001±0.001	19.91	0.981

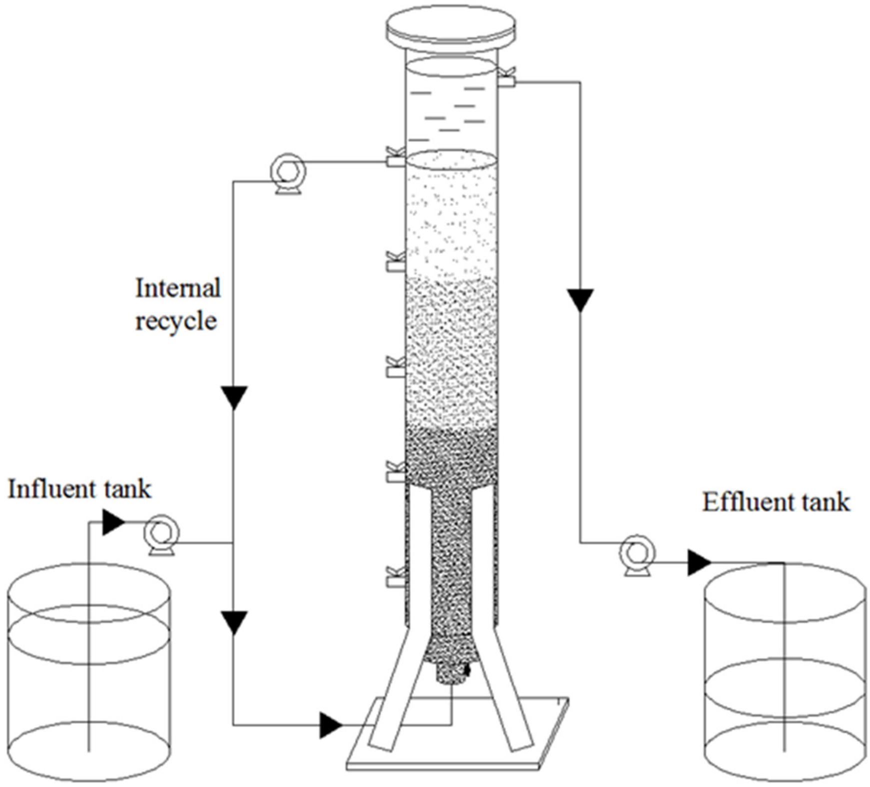


Figure S1 Schematic diagram of the lab-scale SRUSB bioreactor

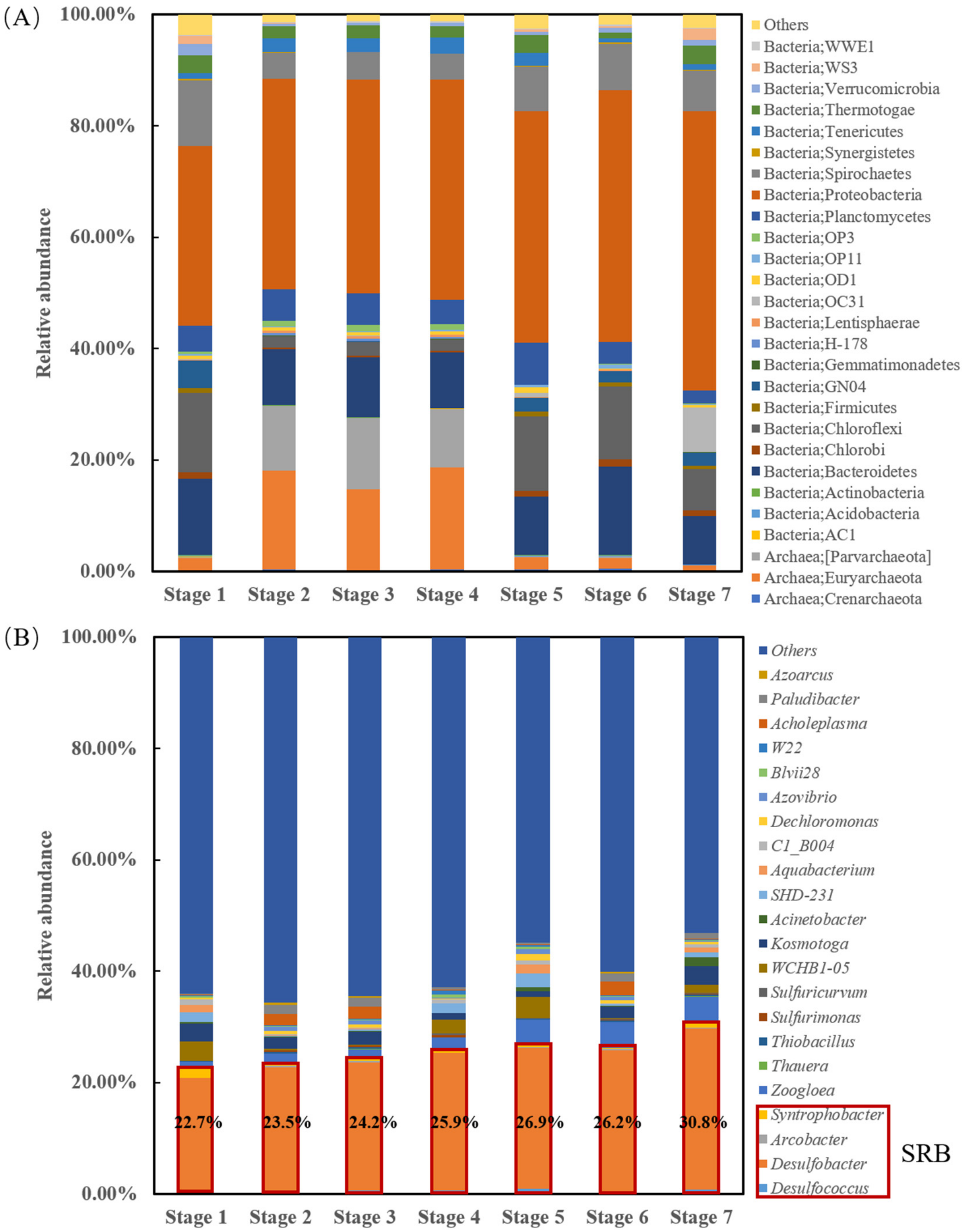


Figure S2 Microbial community characterization of sulfate-reducing sludge samples from SRUSB bioreactor at each stage at phylum (A) (relative abundance＞0.1%); and genus (relative abundance ＞0.1%) (B) levels (Others include that microorganism of relative abundance less than 0.1% and unclassified).