Supporting Information

High-rate stabilization of primary sludge in a single-chamber

microbial hydrogen peroxide producing cell

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SUPPORTING INFORMATION

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Figure S1. Details of single-chambered microbial H_2O_2 production cell (sMPPC): (a) carbon fiber anode woven with titanium plate, (b) two different filters used in the sMPPC, (c) cathode coated with Vulcan carbon with PTFE coating to air-side, and (d) assembled and potentiostat channel connected sMPPC.



Figure S2. Schematic view for the sampling locations (marked with yellow dotted circles) in the sMPPC for microbial community: suspension of chamber (*SC*), anode biofilm of chamber side (*BfC*), and anode biofilm of filter side (*BfF*).

Calculation of oxygen concentration in gas and liquid phase

Ambient air contains nearly 21% of O₂, partial pressure of O₂ is ~0.21 atm. Based on the Ideal gas law,

PV = nRTwhere, P is 0.21 atm (O_2), V is the volume (L), n is the number of moles of O_2 , R is the ideal gas constant (0.0825 L·atm/mole·K), and T is absolute temperature (here 303 K at 30 °C). Thus, O₂ concentration in the air at 30 °C becomes $\frac{n}{V} = \frac{mole}{L} = \frac{0.21 \text{ atm}}{0.0825 \text{ L} \cdot \text{ atm/mole} \cdot \text{K} \times 303\text{K}} = 0.0084 \text{ mole}/\text{L}, \text{ which is } 0.269 \text{ g O}_2/\text{L}.$

To determine saturation concentration of O₂ in water near the cathode catalyst layer coming from gas diffusion layer at 30 °C and 1 atm, we use Henry's law below since the solubility of O₂ gas in water is related to the partial pressure of the gas in the atmosphere,

 $\mathbf{P}_{\mathbf{g}} = \mathbf{K}_{\mathbf{h}} \cdot \mathbf{X}_{\mathbf{g}}$

where, P_g is the partial pressure of the O₂ gas (0.21 atm), K_h is the Henry's law constant (4.75 x 10⁴ atm/mole at 30 °C), and X_g is the equilibrium mole fraction of dissolved O₂ gas (mole of O₂ $[n_g] / \{ \text{mole of } O_2 [n_g] + \text{mole of water } [n_w] \}).$

Thus, $X_g = \frac{P_g}{K_h} = \frac{0.21 \ atm}{4.75 \ \times 10^4 \ atm/mole} = 4.421 \times 10^{-6} \ mole = \frac{n_g}{n_g + n_w}$

Since 1 mole of water is 18 g and water density is 1000 g/L, n_w is 55.6 mole/L. Thus, based on the above equation, calculated n_g is 2.458 x 10⁻⁴ mole /L, which is 0.00786 g O₂/L or 7.86 mg O_2/L .



Figure S3. Soluble organics of effluent PS such as COD and VFAs in Exp. 1-4. Total VFAs are sum of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate (C_2 - C_5). We defined cyclic organics (e.g., alcohols, sugars, esters, and amino acids) as the unaccounted fraction in SCOD (SCOD - total VFAs).

Predictive metagenomic analysis

We also conducted the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) pipeline to predict the metagenomics compositions of the samples from the 16S rRNA gene data and used the HMP (Human Microbiome Project) Unified Metabolic Analysis Network (HUMAnN) pipeline to determine the presence/absence and abundance of microbial pathways in a community accurately from metagenomics data (v0.99). The odds ratios (OR) was used and calculated to define whether genes encoding particular metagenomics functions were over or under presented in the anode biofilm toward chamber as compared to the separator (*SPF-BfC* vs *SPF-BfS*) as

 $OR = \frac{\varphi_{SPF-BfS}/(1-\varphi_{SPF-BfS})}{\varphi_{SPF-BfC}/(1-\varphi_{SPF-BfC})}$

where φ is the relative abundance of a certain metabolic category to a given gene in the metagenomes of each biofilm (Zhou et al., 2017).





Figure S4. Odds ratios of selective 16S-rRNA-based predictive metagenomics functions in *BfF* compared to those in *BfC* in sMPPC with (a) SPF and (b) GF, as assigned by KEGG category database through PICRUSt and HUMAnN analyses.

		Alkalinity	Ammonia
		mg/L as CaCO ₃	mg NH ₃ -N/L
Influent		310 (± 43)	18 (± 7.6)
Effluent	GF 6-day HRT	1900 (± 470)	26 (± 6.6)
	SPF 6-day HRT	3400 (± 890)	38 (± 6.9)
	SPF 3-day HRT	3500 (± 360)	85 (± 3.6)
	SPF 6-day HRT control	1400 (± 72)	99 (± 0.3)

Table S1. Alkalinity and ammonia concentrations on PS in sMPPC

References

 C. Zhou, Z. Wang, A. Ontiveros-Valencia, M. Long, C.-y. Lai, H.-p. Zhao, S. Xia, B. E. Rittmann, *Appl. Catal. B-Environ.*, 2017, 206, 461-470.