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High-rate stabilization of wastewater primary sludge in a single-chamber microbial H_2O_2 producing cell (sMPPC)

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Water impact statement

Wastewater sludge management is an important cost of wastewater treatment, yet new technologies are seldom developed due to the sludge complexity and high solids content. A single-chamber microbial peroxide producing cell (sMPPC) was demonstrated to treat sludge at high rates. In the sMPPC, the H_2O_2 produced enhances sludge stabilization, meet two important requirements for Class B biosolids.

High-rate stabilization of primary sludge in a single-chamber microbial hydrogen peroxide producing cell

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ABSTRACT: This study investigates the effect of sludge stabilization at high rates in a single-chamber microbial hydrogen peroxide (H_2O_2) producing cell (sMPPC). Unlike a typical microbial fuel cell operation, the sMPPC focuses on sludge treatment instead of power generation. Two different porous separators between the anode and the cathode, glass fiber (GF) and stitchbond polyester fabric (SPF), as well as two circuit modes, Closed and Open, were explored. The sMPPC in the Open-circuit mode (no current generation) had a COD removal rate of 0.89 g COD/L/day (removal flux of 22g COD/m^2-d) due to only passive oxygen diffused through air-cathode. The sMPPC in the Closed-circuit mode equipped with SPF increased the removal rate up to 2.4 g COD/L/day (5 g COD/L/day of loading rate). The high removal rate resulted from current production, oxygen diffused through air-cathode, and H_2O_2 produced, and was higher than a conventional anaerobic digester. This arrangement achieved a 52% VSS removal and 1.2 x 10^5 most probable number per gram solids of fecal coliforms, and the values met two important requirements (pathogen indicators and vector attraction reduction) for Class B biosolids production. The microbial community in the sMPPC showed a stratification of microorganisms at the anode, supporting crucial roles for aerobic metabolism as well as anaerobic hydrolysis, fermentation, and anode respiration. We demonstrate for the first time how sMPPC allows direct sludge stabilization at higher organic loads than traditional anaerobic digesters.

Keywords: organic loading rate; sludge treatment; microbial electrochemical cell; Class B biosolids, hydrogen peroxide

1. Introduction

The aim of wastewater treatment plants (WWTPs) is to treat liquid and solid waste and generate dischargeable water. At WWTPs, most waste organics are accumulated in primary and secondary settlement tanks as primary sludge (PS) and waste activated sludge, respectively. Anaerobic digestion (AD) is currently the most reliable method for treating sludges because of its economical, passive solids destruction and chemical oxygen demand (COD) removal as methane gas.[1-3] AD is typically operated at long retention times due to the slow growth and activity of methanogens, leading to loading rates in the order of 0.5-1.6 g VSS/L/d.[1-2] While new technologies have increased these rates by retaining methanogens,[4-5] the high solids content present in AD makes challenging to perform a biomass/liquid separation.

The treated sludge (digested sludge or biosolids) after AD can be used for land application as fertilizers if treated to regulatory standards. The United States EPA provides guidelines for treated sewage sludge under 40 CFR part 503.[6] The guidelines include concentration limits for heavy metals and other contaminants as well as treatment approaches. Among the provisions, Class B biosolids pertaining to solids digestion should meet pathogen indicators' levels equal or be less than 2 x 10^6 colony-forming units (CFU) or most probable number (MPN) per gram of biosoilds on a dry-weight basis. Additionally, vector attraction reduction must achieve a 38% or greater volatile solids removal. Achieving these regulatory levels is often challenging and requires a stable AD operation with solid retention times (SRTs) from 15 days at 35-55 °C to 60 days at 20 °C.[6]

Microbial electrochemical technologies (MET) is an alternative set of technologies that can treat sludge and other high-strength organic wastes.[7-8] Both MET and AD take advantage of the anaerobic food web for the removal of reduced equivalents in the waste. MET, however,

uses anode respiring bacteria for the ultimate removal of electrons while AD relies on methanogens to remove COD as methane gas.[9] In microbial peroxide producing cells (MPPCs), the current generated from anode respiration is used to produce hydrogen peroxide (H_2O_2) at the cathode which can be utilized as an oxidant to further treat the wastewater, removing additional COD.[10] Previous studies showed large amounts of H_2O_2 production are possible; ~3.1 g/L in MPPCs fed with acetate-containing synthetic wastewater in continuous catholyte flow.[11-13] H_2O_2 production using municipal wastewater has also been tested in MPPCs: domestic wastewater[14-16] and primary sludge.[17]

Stoichiometry of anodic and cathodic reactions for a generic organic compound in sludge (CH_2O) is followed:

$CH_2O + H_2O \rightarrow CO_2 + 4\ H^+ + 4\ e^-$ (anode)

$4H^+ + 4e^- + 2O_2 \rightleftharpoons 2H_2O_2$ (cathode)

If the efficiencies are 100%, 2.1 g H_2O_2 would be produced per 1 g COD removed by anode respiration. Dual-chamber MPPCs (dMPPCs) studied to collect H_2O_2 have achieved high efficiencies of up to 80% in our group.[11,12,17] The collected H_2O_2 as a significant oxidant can be utilized for wastewater treatment, thus increasing COD removal rates.

In this study, we focused on PS stabilization with the H_2O_2 produced in a single-chamber MPPC (sMPPC). The H_2O_2 serves as a water-soluble oxidant that could diffuse into the chamber from the cathode and oxidize 1 g COD per 2.1 g H_2O_2 generated. Then, the sMPPC can degrade twice the PS-COD by anode respiration and H_2O_2 oxidation than the typical microbial fuel cell at the same current density generated. As a strong oxidant, H_2O_2 can also assist in fecal coliform destruction to meet Class B biosolids regulations even in the reduced retention time (6-day hydraulic retention time, HRT) and low temperature (30 °C), as compared to standard conditions

for mesophilic AD. Different anode/cathode separators and circuit conditions (Open/Closed) were tested. Open-circuit experiments (no current), in which H_2O_2 is not produced, were used to estimate the rates of COD removal by passive O_2 diffusion from the air cathode. Lastly, we analyzed the microbial community from different locations in sMPPCs to further analyze the results of microbial roles for each condition of the sMPPC.

2. Materials and Methods

2.1. Primary Sludge

PS was obtained from the Greenfield Water Reclamation Plant (GWRP) located in Gilbert, AZ, USA, and stored at a 4 °C refrigerator. The raw PS was diluted for the MPPC influent with DI water to obtain a constant PS of 30,000 ± 2500 mg/L of total chemical oxygen demand (TCOD), 1400 ± 400 mg/L of soluble COD (SCOD), 19,000 ± 1500 mg/L of total suspended solid (TSS), and 16,000 ± 1900 mg/L of volatile suspended solid (VSS). There were a slight variations of PS influent concentrations applied in sMPPC per each experimental run, indicating a variation of organic loading rate even at the same HRT shown in Table 1 below. pH was 5.7 (± 0.2) and was adjusted to near neutral (~7). We measured the characteristics of the influent PS at the beginning and the end of each 6-day storage period.

2.2. sMPPC construction and operation

A flat-plate microbial electrochemical cell (0.5 L of reactor volume, anodes, and cathodes) used in our group was modified for the purpose of H_2O_2 production (sMPPC).[17] The projected area of each anode and cathode was ~100 cm^2 (10 cm x 10 cm), giving a specific surface area of 40 m^2/m^3. Two different types of porous separators between the anode and cathode were used and compared in sMPPC: 1) glass fiber (GF, 1 μm pore size, 330 μm thickness, Type A/E, PALL Corporation) and 2) stitchbond polyester fabric (SPF, ~30 μm pore size, Metacrylics, 360 μm thickness). As a single chamber (no cathode chamber), feed sludge filled in the reactor, soaked through the separators, and reached the cathode catalysts, where electrochemical reactions occurred. Silicon rubber gaskets were placed between each flat piece of anodes, cathodes, separators, and plexi-glass reactor parts. The distance between the anode

and cathode was ~0.25 cm. A reference electrode (Ag/AgCl, MF-2052, Bioanalytical Systems, INC., USA) was inserted in the middle of the chamber, at a ~2 cm distance from each of the anodes. We fixed the anode potential at -0.3 V (versus Ag/AgCl) using a multi-channel potentiostat (VMP3, BioLogic Science Instruments, Knoxville, TN) and recorded the current and anode and cathode potential every two minutes with EC-Lab software (v. 11.0). The details of reactor construction are available in the supplementary data (Figure S1).

Anode respiring bacteria (ARB) were firstly grown in the microbial electrolysis cell mode for more than two weeks with synthetic wastewater medium (50 mM acetate, 100 mM phosphate buffer, 14 mM ammonium chloride, and trace minerals) with a biofilm scraped from pre-acclimatized MEC that was also fed with an acetate medium.[9] Then, the reactor mode changed to sMPPC and operated for over 2 months with the same synthetic wastewater medium above. The sMPPC feed was then replaced with PS and the reactor operated semi-continuously at a 6- or 3-day HRT varying volumes at each HRT studied with one feeding cycle per day; for example, at 6-day HRT, we removed ~83 mL sludge from the chamber and fueled the same volume of PS to the chamber daily. Syringe with the volume of 120 mL and the tip of ~ 4 mm diameter was used for PS feeding into the sMPPC while avoiding filamentous solids (e.g., hair) and/or > 4 mm size of particles. During the sludge fed sMPPC experiments, the PS was mixed under the constant stirring at 220 rpm with a stirrer bar. An Open-circuit condition with SPF separator at a 6-day HRT was performed after the Closed-circuit operation.

The PS effluent was collected and characterized once the current had stabilized after each feeding. pH inside of the chamber was maintained at ~7 with the addition of 5 M sodium hydroxide during regular measurements of the PS effluent. Table 1 details the variations in sMPPC construction and operation: separators (GF-Closed vs SPF-Closed), and circuit modes

(SPF-Closed vs SPF-Open). We ran the sMPPC in a temperature-controlled room at 30 °C.

2.3. Analytical Methods

We followed *Standard Methods* for COD, suspended solids (TSS and VSS), total and fecal coliform enumeration.[18] For the quantification of volatile fatty acids (VFAs), the supernatant of the pelleted sludge was filtered through 0.2 μm membrane filters for high-performance liquid chromatography (HPLC). The liquid samples were injected into the HPLC (LC-20AT, Shimadzu) equipped with an Aminex HPX-87H (Bio-Rad) column according to the conditions described in Esquivel-Elizondo et al.[19]

2.4. Electrochemical Analysis

Once a stable 6-day HRT condition was reached, *j-V* curves for the potentials of anode, cathode, and cell voltages ($E_{cathode} - E_{anode}$) were developed using chronoamperometry following the protocol in Torres et al.[20] Each data point was collected at a 25-50 mV potential step. Ohmic resistances of the anode and cathode were measured by electrochemical impedance spectroscopy (EIS) at 100 kHz with an amplitude of 10 mV, and *iR* correction was performed for each potential.

Coulombic recovery (CR) and anodic Coulombic efficiency (CE) was calculated based on the COD variations of influent and effluent of PS and the cumulated Coulombs recovered as current, as described in our previous studies.[9]

2.5. Microbial Community Analysis

We collected 9 sludge samples of influent and effluent for microbial community analysis from three different locations of sMPPCs (suspension of the chamber, *SC*: anode biofilm facing the chamber side, *BfC*: and anode biofilm facing the separator/cathode side, *BfS*). Details for sampling locations are illustrated in Figure S2 of the supplementary data. Anode biofilm samples were obtained by scraping the anode surface using a sterilized spatula. Suspension samples from the chamber were centrifuged to produce pellets. From these wet biomass samples, we added ~0.25 g into the bead tubes as described in a Power Soil DNA extraction kit (MoBio Laboratories, Inc., Carlsbad, CA). The extracted DNA was sent to the Microbiome Analysis Laboratory at Arizona State University for amplicon sequencing (Illumina MiSeq). Then, the raw sequencing of the V4 region of the 16S rRNA genes was conducted using the QIIME community analysis platform.[21] This protocol is a modified from Ki et al.[17] For hierarchical clustering of the microbial community, we performed jackknifed beta diversity using QIIME.

3. Results and Discussion

3.1. Oxygen Diffusion with Air-cathode Equipped MPPC

The design of the air-cathode in any MFC technology, including the MPPC, allows delivery of oxygen (O_2) into the chamber. As the O_2 diffuses into the liquid chamber, it can serve as an electron acceptor for aerobic bacteria to consume sludge organics. Understanding O_2 diffusion into the sMPPC allows the determination of its contribution to PS destruction. Figure 1 schematizes the oxygen diffusion within the MPPCs, especially between the air-cathode and the anode chamber. Ambient air contains 21% of O_2 that diffuses through the gas-diffusion layer into the cathode catalyst layer. The O_2 concentration in liquid phase very near the cathode catalyst layer and in contact with ambient air is assumed to be saturated at 7.86 mg O_2/L (30 °C).

When we operated the sMPPC at a 6-day HRT in an Open-circuit configuration (no current flow), we observed significant PS destruction, with COD and VSS removals of 17 and 28% (Table 2). Since methane was not produced, we hypothesize that most of the decrease in COD is due to aerobic degradation. This corresponds to a rate of COD removal of 0.89 g/L/day, or 2.56 x 10^{-5} mg/cm^2/sec (22 g/m^2/day). This flux is similar to a previous MFC study (2.31 x 10^{-5} mg/cm^2/sec in the absence of membrane).[22] Open-circuit mode removed BOD at a rate faster than conventional aeration in an activated sludge process (~0.6 g BOD_5/L/d).[1] Interestingly, this high rate of removal does not match a calculated O_2 diffusion rate. Based on Fick's First Law,

$$J_L = \frac{D_{O_{2,liquid}}}{L}\Delta C$$

where J_L is the O_2 flux [mg/cm^2/sec], $D_{O2,\ liquid}$ is the diffusion coefficient 0.000025 cm^2/sec, L is the liquid diffusion layer, and ΔC is the change in O_2 concentration, 7.86 mg/L, we can estimate O_2 diffusion into the MPPC chamber. Since there is no mixing in the space between anode and

cathode, at a distance of > 1 mm, we can assume that L is at least 1mm. The calculated O_2 flux of is 2.0 x 10^{-6} mg/cm^2/sec, which is about an order of magnitude smaller than the observed consumption flux. The much larger O_2 flux suggests that PS-COD was consumed in the space between anodes and cathodes, where soluble COD diffused from the bulk anode was oxidized by aerobic bacteria in suspension or attached to the separators, anodes or cathodes surfaces. This consumption-assisted flux is similar to those obtained in membrane-aerated biofilm reactors (MABRs, 0.9-64 g COD/m^2/d of COD removal) that closely resemble the cathode design in MFCs.[23] When the sMPPC operated in Closed-circuit, allowing H_2O_2 production, the removal rate enhanced with anode respiration and H_2O_2 effect discussed in the next section.

3.2. Sludge Treatment in Closed-circuit Conditions (GF vs SPF)

Figure 2 shows the PS-COD balances from the sMPPC using GF or SPF separators at a 6-day HRT. Columbic recovery (CR) was approximately 20% when using either GF or SPF as a separator (Table 2). Also, as shown in Table 2, COD and VSS removals in the GF-equipped sMPPC were similar at ~50% and ~55%, respectively. CR (anode respiration by ARB) accounted for ~37% of total COD removal. The H_2O_2 produced is expected to consume 1 g COD per g COD circuited. Based on this, the MPPC is expected to have a CE < 50%. The O_2 diffusion into the anode further decreases the CE to the values observed (35 ± 3 and 38 ± 6% for GF and SPF, respectively). CR tracks this low CE value when combined with the total COD removal of 56 and 49%. A low CE and CR is advantageous in the treatment-focused operation of the MPPC, where COD removal is less dependent on achieving a high current generation. The PS effluent from both GF- and SPF-equipped sMPPC met the Class B biosolid requirement of > 38% of volatile solids removal. Even though the GF- and SPF-equipped sMPPC yielded

similar levels of total coliforms in PS effluent, the SPF had a ~30-fold reduction in fecal coliforms, compared to the GF. As the reduction of fecal coliforms over PS feed, SPF had 2.8 log-reduction while GF had 1.3 log-reduction. More importantly, only SPF met the pathogen indicators' levels of the Class B biosolids requirement (< 2 x 10^6 CFU or MPN per gram of biosolids); the measured fecal coliform in the PS effluent was an order of magnitude below the requirement. The Closed-circuit experiments with two separators show two important trends: 1) a more efficient H_2O_2 transport from cathode to anode with the large-pore SPF than GF, and 2) no effect on anode respiration with H_2O_2 production, as shown by CR, CE, and current density (Table 2 and Figure 3). Larger pores of SPF (~30 μm) may result in efficient H_2O_2 transport to the chamber and thus more chances of H_2O_2-to-pathogen contact, while smaller pores (1 μm) of GF may lead to slow diffusion of H_2O_2 and thus possibly increase of H_2O_2 degradation before reaching to PS pathogens.

3.3. Current Density

Each HRT studied in the MPPC was evaluated for at least 5 reactor volumes in order to achieve a pseudosteady state. Current densities at this pseudosteady state are shown in Figure 3 at 6- and 3-day HRTs with the separators. Current densities increased up to ~ 3.5 A/m^2. In previous work, we operated a dMPPC fed with PS from the same facility at a 6-day HRT, but with the diluted PS, 8 g COD/L and 4 gVSS/L (Ki et al., 2017b). Compared to the dMPPC, the sMPPC on this work was fed ~3.8 and 4.4 times higher strength of PS as COD and VSS, yet the current densities showed around 3.5 times higher and COD and VSS removal rates of PS increased ~2.8 and ~3.9 times. Thus, COD and VSS removal was proportional to the current production. The higher COD concentrations of the influent sludge as well as higher alkalinity (>

3000 mg/L as $CaCO_3$, Table S1) of the stabilized sludge in the chamber might result in the increase of current density. The current density of ~3.5 A/m^2 from this study is the highest reported in sludge-fed METs.[17,24-26]

Further decreasing the HRT to 3-day did not increase the current density, while CR was reduced to 8%, indicating that an organic loading rate higher than ~5 gCOD/L/d (or 2.5 gVSS/L/d) does not affect current density, which is not limited by substrate availability. Moreover, the higher organic loading, ~10.5 gCOD/L/d (or 5.3 gVSS/L/d) at 3-day HRT, stemmed a failure of fecal coliform destruction (8.2 x 10^7 MPN/GDW) to reach Class B biosolids requirements.

3.4. Open vs Closed Circuit Operations

The O_2 gas flux into the sMPPC cathode is theoretically 3-fold higher in the Closed-circuit experiment due to the additional oxygen demand by the cathode for H_2O_2 production (2 e^- reaction) along with the oxygen demand for the Open-circuit. However, since the cathode catalyst is adjacent to the gas-diffusion layer, it is assumed that there is no significant gas diffusion limitation into the cathode and thus the water adjacent to it is at saturation. Thus, oxygen diffusion to the anode in the Closed-circuit condition would be the similar to one in the Open-circuit. If we assume a similar O_2 liquid diffusion rate occurred in the Closed-circuit mode, then we can expect that at least 13% of the influent PS-COD was likely degraded solely by the H_2O_2 produced in the sMPPC since 19 ± 2% of the COD was removed by anode respiration (Table 2). This is equivalent to 27% of the total COD removal.

The theoretically estimated production is equivalent to adding 8.3 g H_2O_2/L into the 30 g COD/L sludge. Also, we explore more details in the possible H_2O_2 production rate based on

electrochemical analysis in the next section. Despite the high production, all anode effluent samples contained less than 5 mg H_2O_2/L, suggesting that the H_2O_2 was fully utilized at a fast rate within the sMPPC by radical generation,[17,27,28] auto-decay,[11] or consumption through peroxidases produced by microorganisms.[29] In addition to electron consumption by ARB, the degradation of PS-COD might have resulted from direct mineralization with H_2O_2 or reactive oxygen species (ROS or radicals) derived from H_2O_2 or from microbial oxidation by O_2 produced from H_2O_2 degradation.

We also found a ~2-fold soluble COD accumulation (1660 ± 240 mg COD/L) in the Open-circuit mode (Figure S3) with more propionate and long chain (recalcitrant) organics, indicating a limitation of an electron sink in the system (e.g. methanogenesis producing methane or anaerobic respiration transferring electrons to anode). Thus, a higher fraction of longer chain fatty acids, (e.g., propionate) directs a thermodynamic feedback inhibition of ß–oxidation enabling the conversion of longer fatty acids to acetate.[1,30,31]

3.5. sMPPC electrochemical analysis

As shown in *j-V* curves operated at 6-day HRT with the SPF in Figure 4, open-circuit potentials of 0.35 ± 0.03 V were lower than the theoretical potential of 0.56 V for H_2O_2 production. This discrepancy is likely to be a pH gradient between the anode and cathode.[32] Although the PS effluent pH were almost neutral (~6.9), the local anode and cathode pHs could drift from the bulk pH due to the proton-dependent reactions occurring at its surface.[20,33]

Since the sMPPC is constructed more compactly than the dMPPC, having shorter distance between the anode and cathode, we can therefore expect to decrease Ohmic overpotential.[11] The *j-V* curves with real wastewater (PS) also show that energy-neutral

operations at 0 V occurred at 1.63 ± 0.10 A/m^2, which is comparable or greater to previous research conducted by Rozendal et al.[33] at 1.6 A/m^2 and Modin and Fukushi[15] at 0.54 A/m^2. Assuming 100% conversion from recovered electrons to H_2O_2 at the energy-neutral condition, the theoretical H_2O_2 production rate would be 0.25 g/d (or 0.5 g/L/d).

3.6. Community Analysis

In Figure 5, all samples of chamber suspension and anode biofilms facing the chamber side (-*SC*, -*BfC*) were largely dominated (> 60%) by *Bacteroidia* and *Clostridia*, which are hydrolysis and fermentative bacteria that degrade complex organics.[17,35] While the both *Bacteroidia* and *Clostridia* classes were present as the dominant groups in the anode biofilms (*GF-BfC* and *SPF-BfC*), *Deltaproteobacteria,* which include known anode-respiring bacteria,[17,36,37] was a significant fraction of the *BfC* sample, 8% in *GF-BfC* and 27% in *SPF-BfC*. This was a different trend from a previous study which showed a large fraction of *Deltaproteobacteria* (~70%) in anode biofilm of PS-fed dMPPC with 0.89 gCOD/L/d and 0.44 gVSS/L/d of an organic loading rate.[17] This suggests that the 5-fold higher organic load in this study enriched for anode biofilm microbial communities with solid degradation and fermentation functions. Anode biofilms toward the cathode and next to the porous separators (*GF-BfS* and *SPF-BfS*) were dominated with *Gammaproteobacteria* (> 42 ± 2%). Among *Gammproteobacteria*, aerobes such as *Alteromonadales* and *Oceanospirillales*, were the largest fractions (> 35%). Similar trends were also seen in the dMPPC biofilm on the membrane side, where the community was affected by oxygen diffusing in through the air-cathodes.[17]

3.7. Outlook: High-strength Wastewater Treatment in MPPCs

The sludge loading in our sMPPC was 2.5-2.8 g VSS/L/d at 6-day HRT (Table 1), a value that fall higher than the "standard rate" of anaerobic digestion of 0.5-1.6 g VSS/L/d.[1,2] Sludge stabilization or removal rates were 1.5 and 0.8 g VSS/L/d (or 2.4 and 0.9 g COD/L/d) in Closed- and Open-circuit, respectively.

This study demonstrated a new approach for high-strength wastewater (HSW, e.g., PS) treatment with H_2O_2 produced in a sMPPC. PS-COD and other HSW can be oxidized by various electron acceptors: (1) the anode, (2) the H_2O_2 produced, and (3) O_2 that either diffuses directly from the cathode or is derived from the H_2O_2 degradation. This allows large solid destruction of PS and lowers the pathogenic level below the requirements for Class B biosolids. Produced H_2O_2 or O_2 derived from decomposed H_2O_2 also inhibits methanogens, which are competitors (electron sinks) for ARB in anaerobic conditions,[9] thus directing more PS-electrons to the anode. Based on the results of microbial community, a syntrophic interaction with aerobic bacteria is suggested as shown in Figure 5, where aerobes consume the diffused O_2 in the space between the anode and cathode, creating an anaerobic environment for ARB to grow at the inner portions of the anode. Thus, the sMPPC is expected not only to reduce pathogenic levels in PS, but also to degrade hazardous compounds that exist in the PS with aerobic bacteria on the separator side of the anode biofilm. Predictive metagenomic functions are available in the Supplementary data. The stratification of ecosystems within the sMPPC creates a compound environment that effectively treats PS using biological and electrochemical processes.

In a previous study, Class B pathogen indicators and vector attraction reduction criteria were achieved in AD with the operating conditions of 24-38 °C mesophilic temperatures, 24-90 days HRTs, and 0.3-2.3 gVSS/L/d organic loading rates.[38] We demonstrated achievements of some Class B biosolids metrics (pathogen indicators and vector attraction reduction) in PS-fed

sMPPC with a lower HRT (6-day) and temperature (30 °C) and at a high organic loading rate (~2.7 gVSS/L/d) by anode respiration, produced H_2O_2, and passively diffused O_2. Thus, our results suggest that an sMPPC can become a compact alternative to traditional sludge treatment system.

4. Conclusion

This is the first demonstration of high-rate sludge stabilization in an sMPPC fed with PS. The sMPPC in the Open-circuit mode, which is no electron flow through the circuit, had a COD removal rate of 0.89 g COD/L/day (removal flux of 22g COD/m^2-d) due to only passive oxygen diffused through air-cathode. The sMPPC in the Closed-circuit mode and equipped with SPF increased the removal rate up to 2.4 g COD/L/d (5 g COD/L/d of loading rate). In the SPF-equipped sMPPC, the produced H2O2 diffused into the chamber, resulting in a faster stabilization of the PS and larger reduction of pathogen indicators and vector attraction that met two important requirements for Class B biosolids. At least 27% of the COD removal (equivalent to the removal rate of 0.95 g COD/L/d) was attributed to the H_2O_2 produced in the system. The synergistic work in diverse microbial consortium successfully occurred in hybrid aerobic/anaerobic sMPPC system via aerobic degradation of hazardous compounds, and anaerobic hydrolysis and fermentation of high-strength sludge, and anode respiration along with H_2O_2 produced at the cathodes to achieve better effluent sludge quality. The reactor configuration was essential in obtaining high rates of PS treatment. The flat electrode configuration avoided clogging or high accumulation of sludge that would impede current and H_2O_2 generation.[9] Yet, the specific surface area (40 m^2/m^3) was high enough to obtain a high flux removal based on processes occurring at the electrodes. Further optimization of the reactor configuration can lead to better performance of the sMPPC.

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Appendix A. Supplementary materials

Details of single-chambered microbial H_2O_2 producing cell (sMPPC), schematic view for sampling locations of microbial community, soluble organics of effluent PS, odd rations of selective 16S-rRNA-based predictive metagenomics functions in sMPPC, and PS alkalinity and ammonia concentrations are all available in the Supplementary Data.

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Table and Figures

Table 1. Design of experiments with separators, circuit connection, and PS loading rate

Exp.	HRT	Separator	Circuit mode	PS loading rate
GF-Closed	6 day	GF	Closed	4.3 gCOD/L/d (2.5 gVSS/L/d)
SPF-Closed	6 day	SPF	Closed	5.0 gCOD/L/d (2.7 gVSS/L/d)
SPF-Closed2	3 day	SPF	Closed	10.5 gCOD/L/d (5.3 gVSS/L/d)
SPF-Open	6 day	SPF	Open	5.2 gCOD/L/d (2.8 gVSS/L/d)

GF: glass fiber, SPF: stitchbond polyester fabric

Table 2. Summary of performances in single- and dual-chambered MPPCs

MPPCs		sMPPC* (this study)			dMPPC**
Exp.		GF-Closed	SPF-Closed	SPF-Open	AEM
CR	%	20 (±1)	19 (±2)	0	31 (±1)
CE	%	35 (±3)	38 (±6)	0	64 (±3)
COD removal	%	56 (±4)	49 (±8)	17 (±7)	49 (±1)
VSS removal	%	61 (±5)	52 (±5)	28 (±8)	43 (±4)
COD removal rate	gCOD/L/d	2.42 (±0.05)	2.46 (±0.16)	0.89 (±0.04)	0.42 (±0.00)
VSS removal rate	gVSS/L/d	1.52 (±0.03)	1.36 (±0.03)	0.81 (±0.09)	0.19 (±0.01)
Total coliform	MPN /GDW	6.3×10^7 ($\pm 4.5 \times 10^6$)	5.6×10^7 ($\pm 4.0 \times 10^7$)	2.9×10^8 ($\pm 3.5 \times 10^7$)	-
Fecal coliform	MPN /GDW	3.91×10^6	1.2×10^5 ($\pm 1.2 \times 10^4$)	1.4×10^7 ($\pm 4.0 \times 10^6$)	-

* 6-day HRT

** Operating conditions: 9-day HRT, 8 gCOD/L and 4 gVSS/L as influent PS, 0.89 gCOD/L/d and 0.44 gVSS/L/d as organic loading rate when used anion exchange membrane (AEM) as a separator (Ki et al., 2017b)

MPN: most probable number

GDW: gram of solids, dry-weight basis

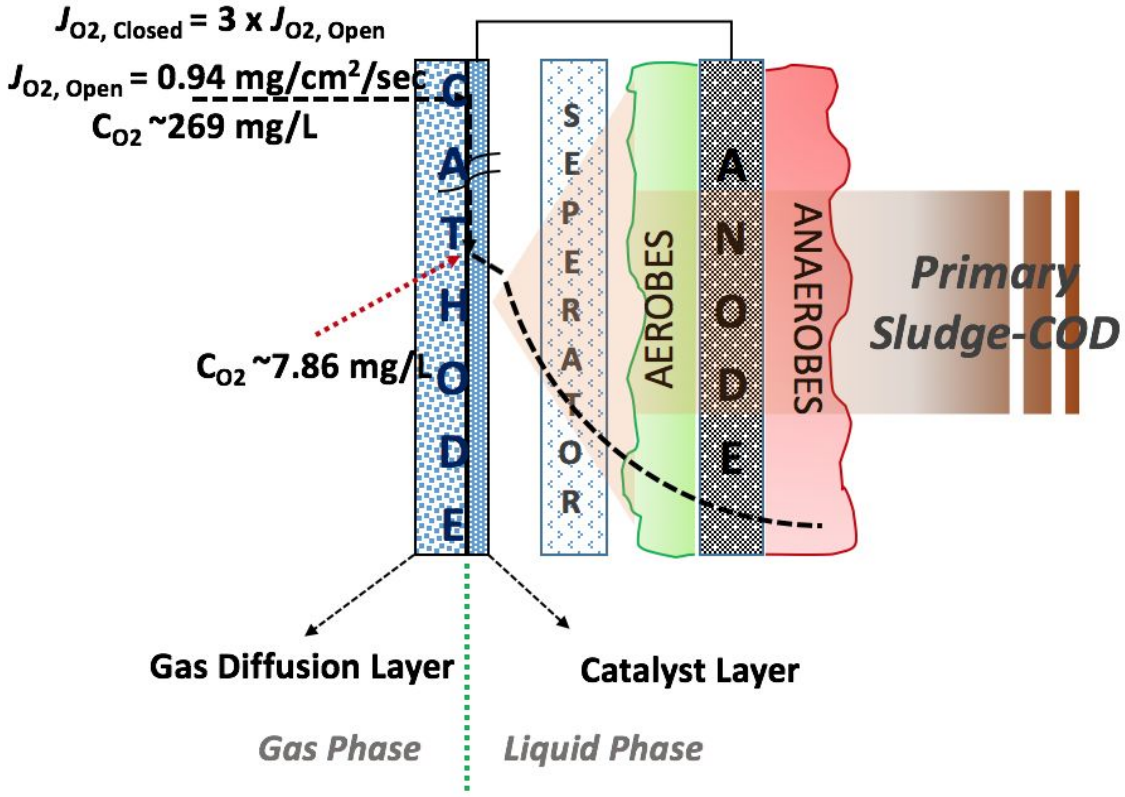

Figure 1. Oxygen diffusion to sMPPC and consumption phenomenon

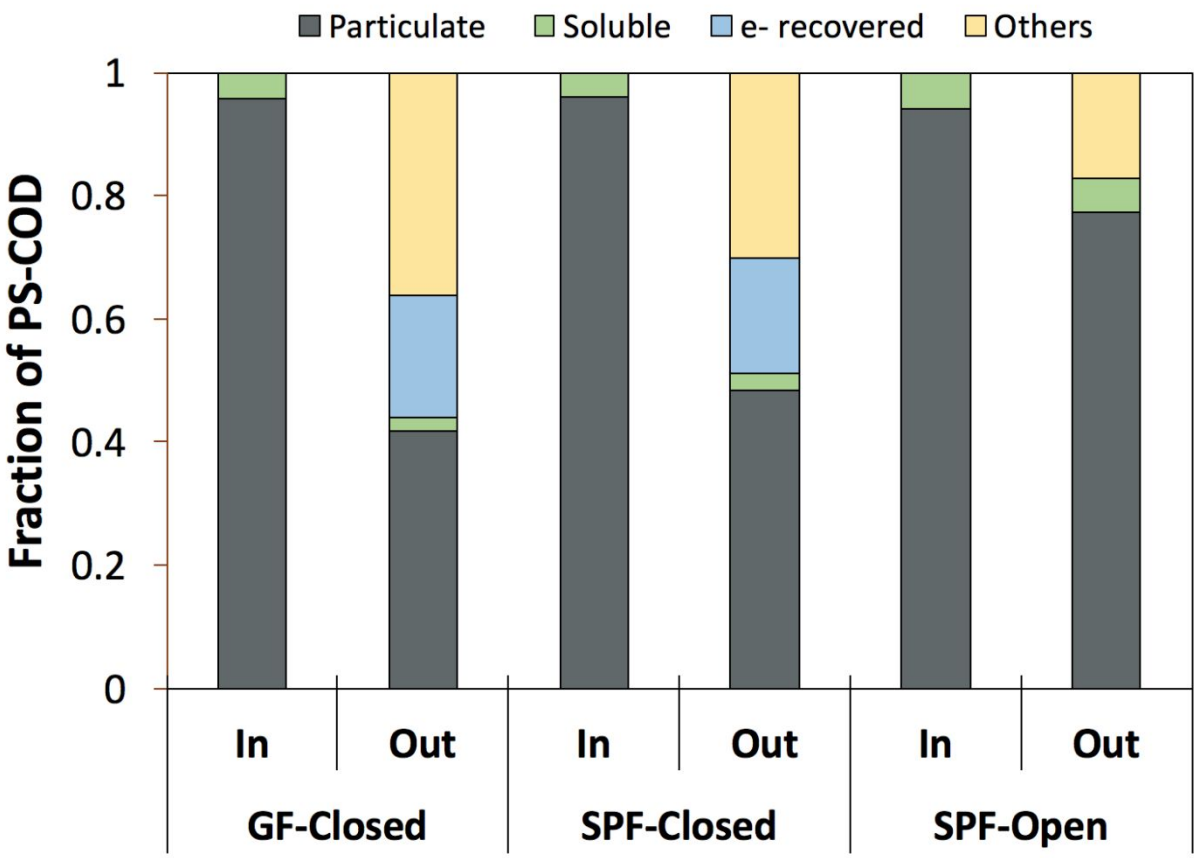

Figure 2. COD-based mass balances of PS_{in} and PS_{out} in three experiments at 6-day HRT. Particulate is the solids fraction of PS; soluble is the soluble fraction of PS; e^- recovered is accumulated Coulombs; Other is the unaccounted fraction of PS removed, including removal by H_2O_2 or O_2 diffusion.

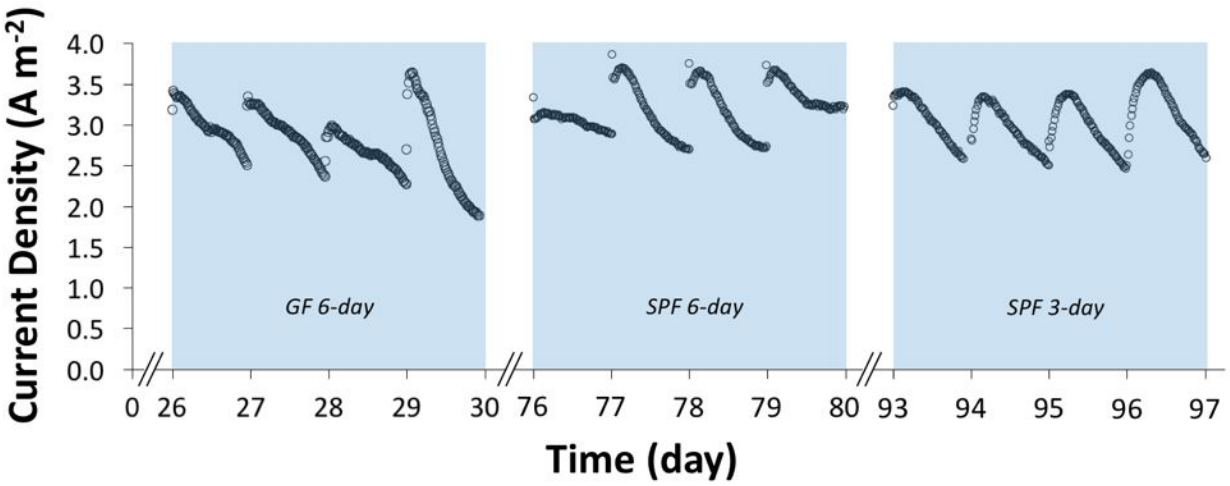

Figure 3. Current densities under stabilized conditions at 6- and 3-day HRTs in the sMPPC equipped with GF and SPF separators.

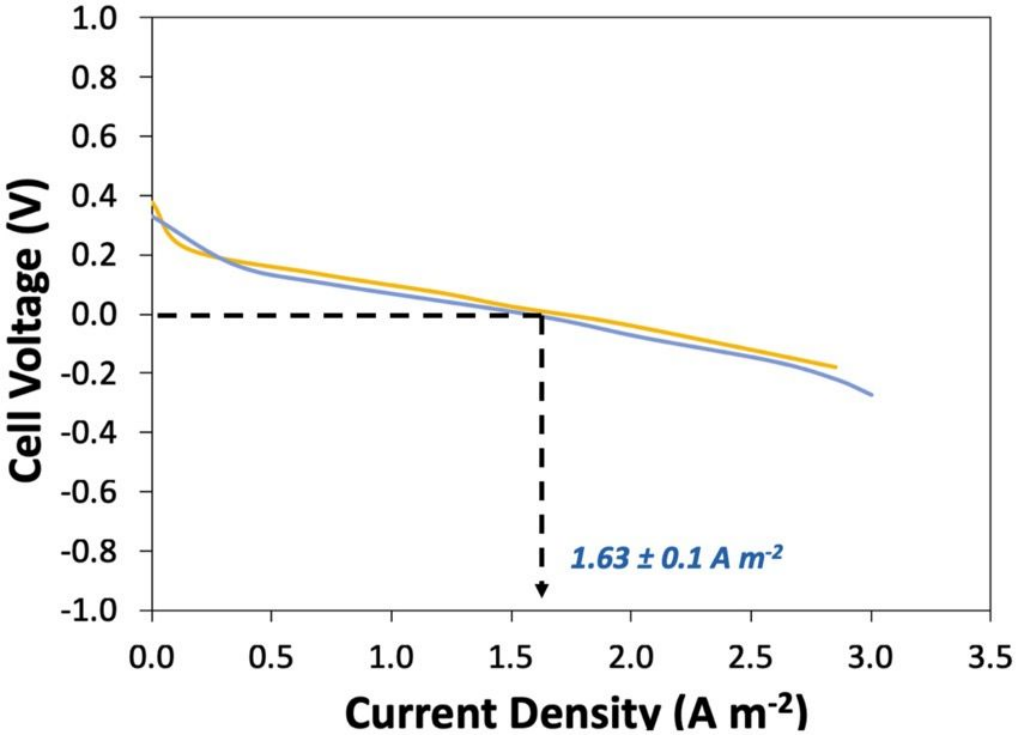

Figure 4. Relationship between cell voltages (whole cell) and current density through *j-V* curves (replicates) in SPF-equipped sMPPC at 6-day HRTs. 0 V cell voltage indicates energy neutral.

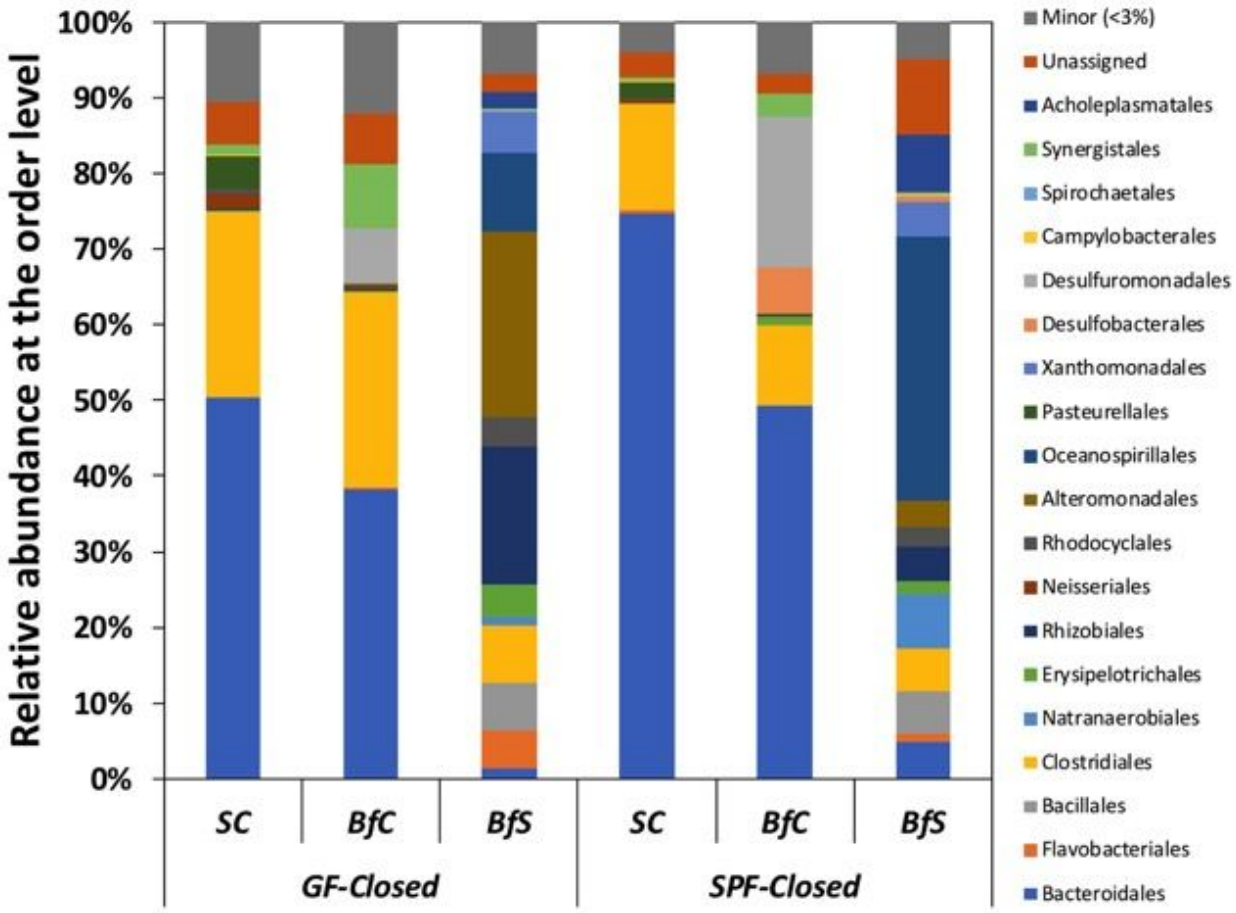

Figure 5. Comparing microbial community profiles. Unknown and minor (<3 %) phylotypes in samples are not shown. 6 samples for the relative abundance at the order level of bacterial community include three different sampling locations: suspension of chamber (*SC*), anode biofilm of chamber side (*BfC*), anode biofilm of separator side (*BfS*) of each experiment.

Table of Contents Entry

High-rate sludge stabilization of wastewater primary sludge was achieved in single-chamber microbial peroxide producing cells meeting pathogen and vector attraction reductions for Class B biosolids.