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Volatile Fatty Acid Production through Arresting Methanogenesis by Electro-Synthesized Hydrogen Peroxide in Anaerobic Digestion and Subsequent Recovery by Electrodialysis

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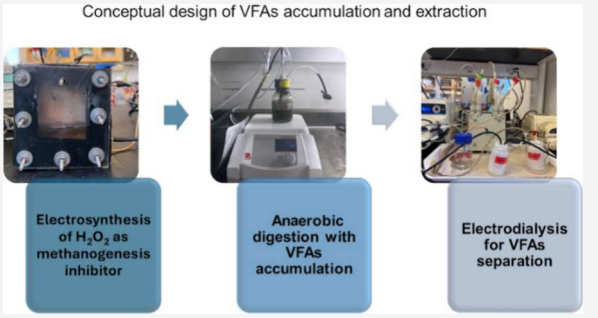
ABSTRACT: Producing volatile fatty acids (VFAs) in anaerobic digestion (AD) is of strong interest because of VFAs' potential values in biomanufacturing. Despite some success of VFA production via pretreatment, in situ inhibition of methanogens for VFA accumulation has yet to be explored. Herein, a system consisting of hydrogen peroxide (H_2O_2) production, application of H_2O_2 for inhibiting methanogens in AD, and VFA separation was investigated. A polytetrafluoroethylene-based electrospinning electrode was synthesized and capable of generating ∼4.2 g L^{-1} H_2O_2. When the generated H_2O_2 was applied to the AD, methanogens were inhibited, and VFA accumulation occurred. With the addition of 80 mg L^{-1} H_2O_2, an average VFA concentration of 10.6 g COD L^{-1} was obtained. The long-term H_2O_2 inhibition effect on methanogenesis was examined for nearly 100 days. A 2.3- to 3.3-fold increase in malondialdehyde levels, which indicated increased cell damage, along with a significant decrease in methane production and an increase in VFA concentration, might suggest that H_2O_2 could potentially inhibit methanogens while allowing acidogenic bacteria to remain functional. The accumulated VFAs were separated and then recovered using an electrodialysis unit, with a maximum VFA concentration of 26.7 g COD L^{-1}. The results of this study will encourage further exploration of the proposed system for VFA production by addressing several challenges, including a better understanding of the inhibition mechanism and a further increase in VFA yields.



KEYWORDS: *H_2O_2 electrochemical synthesis, wastewater sludge, volatile fatty acid, anaerobic digestion, electrodialysis*

1. INTRODUCTION

Anaerobic digestion (AD) is used to process around 50 million dry tons of organic wet wastes annually, including wastewater sludge, food waste, and manure, with products of valuable fuels and chemicals.[1] Particularly, AD is widely employed in wastewater treatment plants (WWTPs), where sludge management accounts for a significant portion of operational costs, and serves as a prevalent method for sludge treatment, facilitating solid dissolution, sludge volume reduction, and bioenergy recovery.[2] Although methane (CH_4) is a valuable product derived from organic wastes in AD that can be used as a source of renewable energy,[3] improper management of CH_4 presents new operational challenges, given its global warming potency 86 times that of carbon dioxide (CO_2) per kg on a 20-year scale.[4] It was reported that CH_4 emission from AD reactors and leaks from both treatment and transportation processes have been significantly underestimated.[5,6] Fewer than 10% of WWTPs in the United States harness biogas for beneficial purposes, leading to a waste of energy.[7] Additionally, less than 30% of organic carbon from AD feedstock is converted to CH_4, with about 8% emitted as CO_2.[8] Therefore, the recovery of more valuable products than CH_4 from the AD processes can be of great interest.

Volatile fatty acids (VFAs), the key intermediate products of AD, hold promise as precursors for high-value biofuels and biochemicals, such as polymers, food additives, and pharmaceuticals.[1] Traditionally, VFAs are derived from petrochemical processes, but the rising costs of petroleum and dwindling nonrenewable resources drive the exploration of utilizing wet wastes for VFA production.[9] Enhancing VFA production involves strategies such as improving hydrolysis rates and suppressing methanogenesis.[10] Various pretreatment methods

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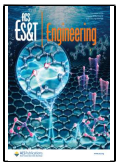

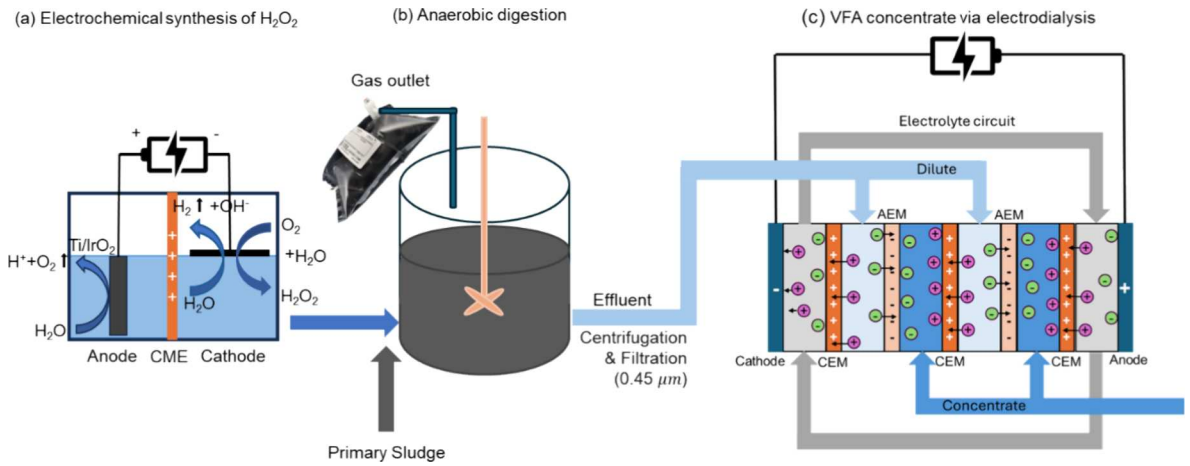

Figure 1. Schematic of the proposed system consisting of (a) electrosynthesis of H_2O_2, (b) AD reactor, and (c) ED reactor for VFA recovery.

have been extensively studied to enhance hydrolysis, such as the use of ultrasound, alkaline pretreatment, and hydrothermal conversion of sludge.[11] Because VFAs are important substrates for methanation, arresting methanogenesis will prevent VFA consumption and help with their accumulation,[12] and this approach is still in its infancy (compared to the pretreatment approach).[13] Early attempts include thermal treatment to deactivate methane-producing microorganisms at the expense of energy consumption[14] and the use of chemical inhibitors like 2-bromoethanesulfonic sodium (BES) to inhibit methane producers with potential impacts on other coexisting microbes.[15] However, these inhibitors are costly, making up over 80% of VFA production costs, and pose contamination risks due to their toxicity, requiring specialized storage facilities.[8] Creating microaerobic conditions in which oxygen may inhibit methanogens due to their inadequate ability to remove reactive oxygen species (ROS) generated by oxygen seems promising, but it is challenging to precisely control the oxygen (O_2) level due to its low solubility in liquids.[16] An alternative to gaseous O_2 is hydrogen peroxide (H_2O_2), which is a key ROS species and also serves as a significant signaling agent involved in metabolic regulation and stress response, facilitating cellular adaptation to environmental changes and stress.[17] The use of H_2O_2 allows for easier implementation, precise control of dosage, and better mass transfer, thereby accelerating the inhibition of methanogenesis with microaerobic conditions and possible oxidants.[18]

The prior efforts of using H_2O_2 in AD mostly focused on the pretreatment of feedstock to promote CH_4 production from waste.[19] Direct addition of H_2O_2 to inhibit methanogens in AD was reported in an earlier study that used glucose as a substrate for CH_4 production and obtained a maximum VFA concentration of 1,233.1 ± 55.9 mg L^{-1} with a one-time addition of 666 mg L^{-1} H_2O_2 in a batch reactor.[15] However, a high level of H_2O_2 can induce oxidative stress in both methanogens and acidogenic bacteria. Therefore, optimizing the H_2O_2 dosage is crucial to inhibit methanogens in the long term while ensuring the survival and functionality of acidogenic bacteria. Those preliminary findings encouraged further exploration of direct H_2O_2 addition to the treatment of actual sewage sludge, which was more difficult to degrade, and evaluation of the long-term performance. To ensure the successful inhibition of methanogens by H_2O_2 for VFA production, two technological challenges must be properly addressed: sustainable supply of H_2O_2 and VFA separation/recovery. Currently, industrial production of H_2O_2 relies on expensive catalysts and complex organic solvent extraction, while the direct synthesis of H_2O_2 from H_2 and O_2 carries safety risks.[20] The extraction and subsequent concentration of VFAs from the fermentation broth can be energy-intensive and often require multistep technologies, particularly for concentrating and isolating them from low-concentration acid mixtures.[13,21]

In this study, a system consisting of electrochemical H_2O_2 synthesis, AD with arrested methanogenesis, and electrodialysis (ED) recovery was developed and investigated for VFA production. Direct electrosynthesis of H_2O_2 enables on-site production without the need for large-scale infrastructure and reduces environmental impact by coupling with renewable electricity.[22] Although ED is able to effectively recover VFAs,[23,24] previous research predominantly focused on extracting VFAs from low-concentration solutions due to the ineffective utilization of front-end fermentation processes to enhance VFA accumulation.[25] The specific objectives of this study were to (1) evaluate the on-site production of H_2O_2 through electrosynthesis using a polytetrafluoroethylene (PTFE)-based electrospun electrode, (2) examine the impact of H_2O_2 on the VFA production in AD, and (3) investigate the effectiveness of ED in recovering highly concentrated VFAs from the AD effluent.

2. MATERIALS AND METHODS

2.1. Reagents and Materials. PTFE (60 wt %), carbon black (XC72) powder, Nafion dispersion (D520, 5 wt %), and carbon paper (Sigracet 39 BB, 10 cm × 10 cm) were purchased from the Fuel Cell Store. Poly(ethylene oxide) (PEO, M_w = 100,000 g/mol), hydrogen peroxide (30 wt %), potassium titanium oxide oxalate dihydrate ($K_2[TiO(C_2O_4)_2 \cdot 2H_2O]$, 2-propanol, and thiobarbituric acid were obtained from Sigma-Aldrich. Solutions were prepared using Milli-Q water (18.2 MΩ cm, Millipore). The feedstock for the AD reactor was the primary sludge collected from the primary settling tanks at the Missouri River Wastewater Treatment Plant (St. Louis, MO, USA), which receives an average of 144,000 m^3 of municipal wastewater per day.

2.2. Preparation of the Gas Diffusion (Cathode) Electrode. An electrospun gas diffusion (cathode) electrode was prepared according to a previous study[26] and composed of three layers: a gas diffusion layer, a carbon black coating layer

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using Nafion as a binder, and a layer of electrospun PTFE nanofibers. To prepare the catalyst ink, CB powder (30 mg) was sonicated with 5 wt % Nafion (1.9 mL) and 2-propanol (1.1 mL) for 30 min. The resulting ink was then sprayed onto carbon paper (5 cm × 6 cm) using an airbrush. The coated carbon paper was sintered at 350 °C for 40 min. PTFE nanofibers were electrospun on the coated carbon paper by using an electrospinning and electrospraying unit (Taisiman Technology Co., Ltd., Dalian, China). The precursor solution was prepared by sonicating PEO (0.55 g) and PTFE (4.64 mL, 60 wt %) in 2.36 mL of DI water for 30 min and stirred for at least 1 h, before being transferred to a syringe pump. The coated carbon paper was fixed on a collator facing a syringe needle. A voltage of 20 kV was applied between the needle tip and the plate at 10 cm. The pump flow rate was set as 1 mL h^{-1} and operated for 20 min.

2.3. Electrosynthesis of H_2O_2. An electrochemical cell for H_2O_2 production consisted of two chambers separated by a piece of cation exchange membrane (CEM, Ultrex CMI7000, Membranes International, Inc., Glen Rock, NJ, USA) with an effective area of 10 × 10 cm (Figure 1a). The CEM was sandwiched between neoprene sheets (10 × 10 × 0.8 cm) and bolted to a polycarbonate outer plate to create anode and cathode chambers with a working volume of 30 cm^3 each. The anolyte was 0.1 M sodium sulfate (Na_2SO_4) and the catholyte was 0.1 M sodium perchlorate ($NaClO_4$). The anode electrode was a tantalum and iridium oxide-coated titanium plate measuring 2 × 5 cm (Xinfeng Technology, Hunan, China), which facilitates oxygen precipitation and proton generation. The cathode electrode was the gas diffusion electrode, as described in the previous section. A power supply (3644 A, Circuit Specialists, Inc., Mesa, AZ, USA) was connected in series with a 1 Ω resistor to provide a constant current density of 10/20 mA cm^{-2} for the electrochemical cell.

2.4. AD Reactor. The AD reactor was a glass bottle with a working volume of 200 mL (Figure 1b). Every 3 days, 40 mL of digestate was replaced by 40 mL of primary sludge (including H_2O_2 addition volume), resulting in a total solid retention time of 15 days. The detailed characteristics of primary sludge used for AD are shown in Table S1. After sludge replacement, nitrogen gas was used to flush the reactor for 5 min to remove oxygen. The AD was placed on a shaker bed with a shaking speed of 150 rpm and operated at ∼37 °C. A gas bag was used for collection of the generated biogas. The AD operation consisted of three stages. Stage I (days 0−30) was the control period without H_2O_2 addition (only 4 mL 0.1 M $NaClO_4$ was added). During stage II (days 30 to 51), H_2O_2 was added with each sludge replacement to achieve an initial H_2O_2 concentration of 40 mg L^{-1} in the reactor. In stage III (days 51 to 150), the amount of H_2O_2 added was increased to (initially) 80 mg L^{-1} in the AD reactor. To ensure a consistent addition of 4 mL of a H_2O_2 solution and avoid the difference introduced by adding different amounts of electrolytes and primary sludge in the reactor, the original 4.2 g L^{-1} H_2O_2 electrolyte was diluted with 0.1 M $NaClO_4$. A blank test was also conducted to assess the impact of $NaClO_4$ on AD performance.

2.5. ED Setup for VFA Recovery. A benchtop ED module (64002, PCCell GmbH, Heusweiler, Germany) consisted of three CEMs (with an effective area of 8 × 8 cm) and two anion exchange membranes (AEMs, with an effective area of 8 × 8 cm) that were alternately partitioned to create two concentrate chambers and two dilute chambers (Figure 1c). A platinum-/iridium-coated titanium anode electrode and a V4A steel cathode electrode (effective area of 7 × 7 cm) were placed in the bilateral anode and cathode chambers, respectively. These electrodes were then connected to an external power supply that provided a constant DC voltage of 15 V. The anode and cathode chambers contained a total of 120 mL of 0.05 M Na_2SO_4 electrolyte that was recirculated at a rate of 1 mL min^{-1}. The digestate from the AD reactor was centrifuged and filtered through a 0.45 μm filter, and then the liquid was directed to the dilution chamber. Concurrently, 60 mL of 0.05 M H_2SO_4 was supplied to the concentrate chamber as the initial electrolyte. A batch of ED operation period spanned 2.5 h. After each operation cycle, the dilute electrolyte was replenished with the freshly collected AD effluent, while the concentrate electrolyte remained unchanged to accumulate VFAs. The pH of the concentrate electrolyte was adjusted to below 4 after each cycle, which is below the pK_a of VFAs, to ensure the predominance of VFAs in the concentrate chamber in their molecular form rather than as ions, thereby facilitating the migration of more VFA ions from the dilute chamber to the concentrate chamber driven by the ionic concentration gradient and current flow, and to prevent phosphoric precipitation (the effluent of AD also contains PO_4^{3-}) that might create scaling on the membrane.[27]

2.6. Measurement and Analysis. The synthesized gas diffusion cathode was characterized by scanning electron microscopy (SEM) (Thermo Fisher Quattro S, USA) to elucidate its structure, and its contact angle with water was tested using optical contact angle measurements and contour analysis systems (DataPhysics Instruments GmbH, Germany). The concentration of H_2O_2 was determined using the potassium titanium oxalate spectrophotometric method: a 100 μL sample was mixed with 500 μL of 0.05 M $K_2[TiO(C_2O_4)_2]\cdot 2H_2O$ and 500 μL of 3 M H_2SO_4. Subsequently, the solution was diluted to a total volume of 3 mL with deionized water and quantified at a wavelength of 400 nm using a UV−vis spectrophotometer (Genesys 20, Thermo Scientific, MA, USA).[28] The solution pH was measured using a benchtop pH meter (Oakton Instruments, Vernon Hills, IL, USA). The VFA content was analyzed using a gas chromatograph (GC) fitted with a flame ionization detector (Thermo Fisher, St. Louis, MO, USA). The soluble chemical oxygen demand (SCOD) was measured using a DR/890 (HACH Co., Ltd., USA) colorimeter to aid in the estimation of the percentage of VFAs in the organic load. The nonenzymatic antioxidant response of microorganisms was evaluated by measuring the malondialdehyde (MDA) level.[29] MDA quantification was based on its chromogenic reaction with thiobarbituric acid.[30] The concentration of Ca^{2+} was measured by a cation chromatograph equipped with an IonPac CS12A (Dionex Easion, Madison, WI, USA). The contents of CH_4 and CO_2 in the collected biogas were analyzed using a GC with a thermal conductivity detector (Thermo Fisher, St. Louis, MO, USA), and the relevant CH_4 percentage in biogas was calculated using eq 1.

$$CH_4\ (\%) = \frac{CH_4\ \text{volume}}{CH_4\ \text{volume} + CO_2\ \text{volume}} \times 100 \quad (1)$$

3. RESULTS AND DISCUSSION

3.1. H_2O_2 Synthesis by the Gas Diffusion Electrode. The gas diffusion electrode was successfully synthesized, and

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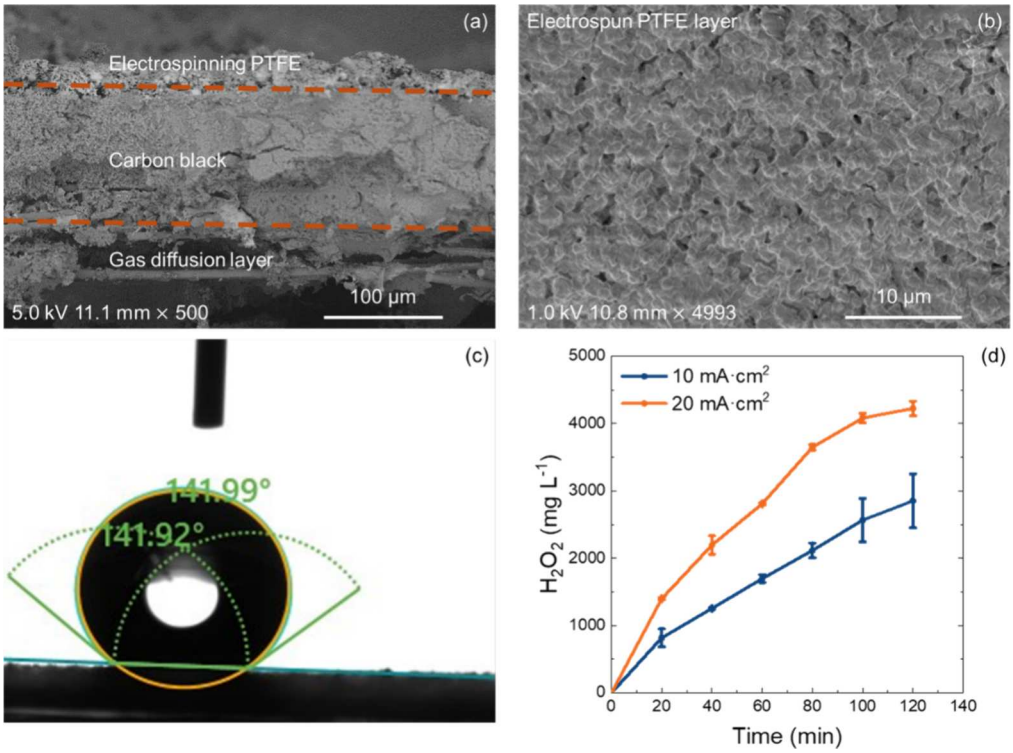

Figure 2. Electrochemical H_2O_2 synthesis: (a) SEM side view of the composite gas diffusion cathode; (b) SEM top view of the composite gas diffusion cathode; (c) contact angle of the composite gas diffusion cathode; and (d) H_2O_2 production under 10 and 20 mA cm^{-2}.

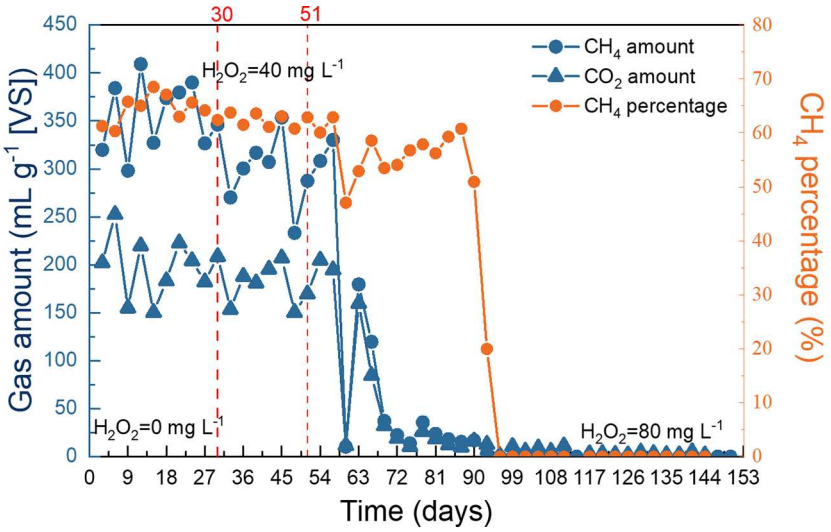

Figure 3. Changes of CH_4 and CO_2 at different stages of H_2O_2 addition.

its three-layer structure was viewed in the SEM image: a gas diffusion layer, a carbon black catalytic layer, and an electrospinning layer (Figure 2a). The porous structure composed of fibers that can be seen in the top view allowed the carbon black catalyst to access a large amount of electrolyte through the cracks in the PTFE coating (Figure 2b).[26] The contact angle of this electrode was around 141° (Figure 2c), suggesting the excellent hydrophobicity of the surface. This hydrophobic structure is essential for the formation of H_2O_2 while inhibiting the formation of H_2 through water reduction. Structuring a three-phase interface would facilitate the continuous diffusion of oxygen from the air through the electrode into the water, where it is then reduced to H_2O_2.[31]

The synthesized gas diffusion cathode was tested for H_2O_2 formation under current densities of 10 and 20 mA cm^{-2} (Figure 2d). Both current densities led to successful H_2O_2 production, and, as expected, the higher current density of 20 mA cm^{-2} achieved a maximum of 4,224 ± 107 mg L^{-1} after 120 min, higher than 2,851 ± 400 mg L^{-1} at 10 mA cm^{-2}, because more electrons were available to reduce more O_2 in the electrochemical reaction, thereby promoting the rate of H_2O_2 generation. The H_2O_2 produced under 20 mA cm^{-2} was then used for the subsequent inhibition of methanogens in AD. It was reported that the energy consumption for electrosynthesis of H_2O_2 could range from 6.0 to 22.1 kWh/kg.[26] In the present study, approximately 0.48−1.77 kWh of energy would

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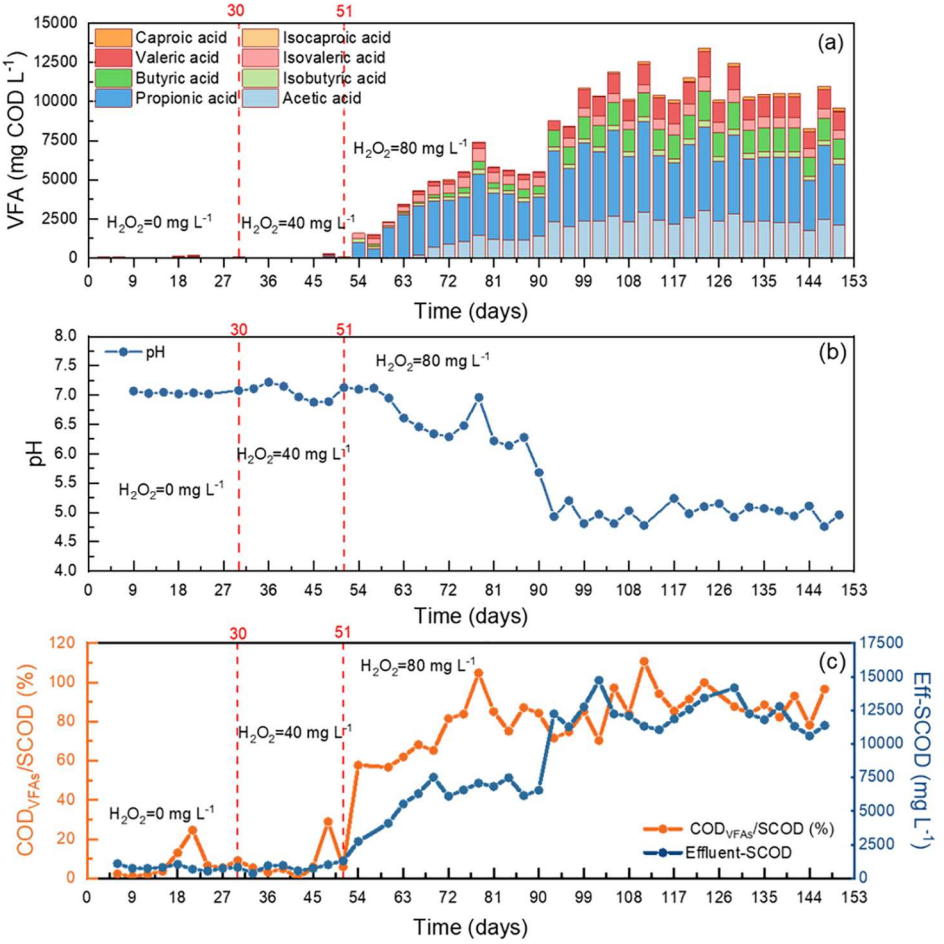

Figure 4. Production of VFAs: (a) individual VFA species; (b) pH change; and (c) effluent SCOD and VFAs/SCOD.

be required to process each cubic meter of sludge for complete methanogen inhibition. For comparison, BES, an effective inhibitor for methanogens,[32] needs to be added at 1.055 g per cubic meter of sludge ($1.12 g^{-1} ~ $1.62 g^{-1}, Sigma-Aldrich),[33] a higher cost than that of electrosynthesis of H_2O_2. Another inhibition method, heat pretreatment, typically requires a temperature exceeding 100 °C for 10−30 min,[14] resulting in more than 87 kWh for heating 1 m^3 of sludge. Please note that the above-mentioned analyses were preliminary and a more comprehensive comparison would need to be conducted at pilot or full-scale applications.

3.2. Biogas Production under the Influence of H_2O_2 Addition. The presence of the electrolyte ($NaClO_4$) in the H_2O_2-containing solution did not significantly impact AD performance, supported by the insignificant difference between the $NaClO_4$ addition group and the non-$NaClO_4$ addition group (p-values of 0.6 and 0.4 for CH_4 and VFA production, respectively) (Figure S1). In contrast, the production of CH4 was notably affected by the addition of H_2O_2 (Figure 3). During stage I, which had no H_2O_2 addition, the average CH_4 production measured at three-day intervals was 355.5 ± 36.7 mL g^{-1} [VS], accompanied by a relative CH_4 percentage of 64.3 ± 2.6%. Upon adding 40 mg L^{-1} H_2O_2 in stage II, the average CH_4 production slightly decreased to 295.6 ± 37.8 mL g^{-1} [VS], with a relative percentage of 62.4 ± 1.2%. A two-tailed two-sample unequal variance *t* test on the sample data yielded a *p*-value of 0.00637, indicating a statistically significant difference between the means of the two samples ($p < 0.05$). Compared to stage I, the CH_4 production decreased by 20.3%, and the relative percentage decreased by 3.1%, indicating the occurrence of a suppressive effect by H_2O_2 on methanogens; however, this inhibition was insufficient to completely halt the methanogenesis process.

In stage III, the initial amount of H_2O_2 was increased to 80 mg L^{-1}, but this change did not immediately generate an inhibitory effect. In fact, a lag phase occurred from day 54 to day 57, during which there was a slight increase in CH_4 production. The lag phase is a natural delay in microbial growth kinetics, when microbial populations take time to adjust to environmental changes.[34] A sharp decrease in the CH_4 production occurred on day 60, and after day 96, the CH_4 production was completely inhibited. The CO_2 production also dropped from 198.2 ± 31.3 mL g^{-1} [VS] (stage I) to 4.7 ± 3.2 mL g^{-1} [VS] (stage III from days 93 to 150). The relative CH_4 percentage remained stable during days 60−90, because the amounts of both CH_4 and CO_2 decreased significantly and the decreasing trends in CO_2 and CH_4 yields were similar. The CH_4 yield gradually decreased from 179.6 mL g^{-1} [VS] to 17.0 mL g^{-1} [VS], while the CO_2 yield also followed the same trend, reduced from 159.9 mL g^{-1} [VS] to 16.3 mL g^{-1} [VS]. Those results indicated that both the acetogenesis and methanogenesis phases were inhibited because CO_2 is a major product of the acetogenesis phase, while CH_4 is the primary product of the methanogenesis phase. The decreased gaseous products also suggested that most of the organic matter remained in the fermentation broth.

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Therefore, the addition of H_2O_2 at 80 mg L^{-1} could effectively arrest the methanogenesis process.

3.3. VFA Production. The addition of H_2O_2 led to an increased accumulation of VFAs (Figure 4a). In the absence of H_2O_2 (stage I), the maximum VFA concentration was 166.8 mg COD L^{-1}, while for most of the time, the VFA concentration was below 50 mg COD L^{-1}. Adding 40 mg L^{-1} H_2O_2 (stage II) increased the total VFA content to a maximum of 295.0 mg COD L^{-1}. When the initial H_2O_2 concentration increased to 80 mg L^{-1} (stage III), the total VFAs dramatically increased to 1,582.2 mg COD L^{-1} on day 54, more than five times the maximum in the second stage. From day 54 to day 90, the total VFA content further increased to 5,530.2 mg COD L^{-1}, which then reached 8,763.1 mg COD L^{-1} on day 93. The highest total VFA concentration was obtained on day 123 at 13,423.8 mg COD L^{-1}. The average VFA concentration in the AD effluent reached 10,585.5 mg COD L^{-1} between days 93 and 150. Generally, VFA production through sludge fermentation is challenging because most of the organic matter in the sludge is encapsulated by microbial secreted extracellular polymeric substances (EPS) and microbial cell membranes, making hydrolysis more restrictive and thus slowing down VFA production.[35] This challenge drives many studies to focus on using pretreatment to release the SCOD from sewage sludge and then employ methanogenesis inhibition to achieve a high VFA yield. The present study has demonstrated that even without energy-intensive or costly pretreatment, a relatively high VFA yield rate could be obtained with the proposed in situ inhibition. The comparison of VFA production between the present study and the prior literature using pretreatment can be found in Table S2. The VFA accumulation decreased the solution pH from 7 to 5 (Figure 4b). Without external pH adjustment, the solution pH stabilized at around 5, which is a key indicator of continuous VFA production in this system. Although the VFA accumulation led to a pH drop with potential effects on microbial activity, we did not intend to mitigate organic acid accumulation other than regularly replacing the fermentation substrate and removing some of the accumulated VFAs. This would allow us to evaluate the behavior of the anaerobic fermentation system under H_2O_2 stress without other interference. In the later stage of the experiment, methane production dropped to zero, while VFA production became stabilized at approximately 3,529 mg COD L^{-1} day $^{-1}$, implying that methanogens were inhibited but acidogenic bacteria continued to function effectively. Therefore, this system proved to be effective in maintaining consistent VFA production at pH 5, aligning with previous reports that acidogenic bacteria are most active within the pH range of 4–7, whereas extremely acidic conditions (pH < 3) could inhibit the acidogenesis process.[36]

The composition of the VFAs was analyzed, focusing on eight specific VFAs (Figure 4a). It was observed that propionic acid exhibited a marked increase and became prominent from day 54 onward. The accumulation of acetic acid was not evident until day 93, at which point the composition of VFAs stabilized. Propionic acid and acetic acid were identified as the main VFA products, accounting for an average of 41.6 and 22.7% of the total VFA mass, respectively, followed by butyric acid and valeric acid, both of which together accounted for approximately 11.7–13.7%. The accumulation of propionic acid in the present system could be attributed to thermodynamic conditions unfavorable for propionic acid degradation, as well as the hindered growth of syntrophic propionate oxidizers under uncontrolled VFA accumulation conditions.[37] Prior studies have shown that different microbial populations have different optimal pH ranges for their growth and VFA production, with propionic acid production being predominant at a pH of 5–5.5,[36] a range where the solution pH of the present study fell into. For comparison, many studies reported the VFA production under controlled pH conditions and found that acetic acid was the dominant VFA species. In particular, an alkaline condition can be more favorable for acetic acid due to the promotion of sludge hydrolysis.[12] For example, it was observed that when the pH increased to 8.9, the acetate concentration could reach 2,563 mg COD L^{-1}, while propionate was less than 897 mg COD L^{-1}.[38] Likewise, an alkaline pH led to an acetic acid concentration exceeding 6,000 mg COD L^{-1}, accounting for more than 70% of total VFAs.[47] Given that propionic acid is sold at a higher price per ton compared to acetic acid,[39] producing propionic acids may yield a higher economic value.

In addition to VFAs, the AD process can also generate byproducts, such as lactic acid and alcohol, which would decrease the enrichment of the target products and create challenges for the subsequent isolation and purification.[40] To examine the products in the aqueous phase, the COD_{VFAs}/SCOD ratio was analyzed (Figure 4c).[41] During stage I and stage II, the effluent SCOD and the COD_{VFAs}/SCOD ratios remained relatively stable. The average SCOD was 801 mg L^{-1} in stage I and 840 mg L^{-1} in stage II. The average COD_{VFAs}/SCOD ratios in stage I and stage II were 8.66% and 7.18%, respectively. Both SCOD and COD_{VFAs}/SCOD ratios started to increase in stage III. The effluent SCOD reached 5,540 mg L^{-1} on day 63, surpassing that of the influent (4780 mg L^{-1}), suggesting that the AD process was primarily in the hydrolysis and acidification stages, which would limit the conversion of organic matter to CH_4. From day 93 to 150, the SCOD ratio was 12,217.8 ± 1,083.1 mg L^{-1} and the COD_{VFAs}/SCOD ratio was 87.5 ± 10.3%, suggesting that a large amount of insoluble organic compounds was decomposed into VFAs. The COD_{VFAs}/SCOD ratio obtained in this study is higher than 75%, as reported in the prior literature.[42]

3.4. Preliminary Exploration of the H_2O_2 Inhabitation Mechanism. Although this study focused on the demonstration of a complete system for VFA recovery (including H_2O_2 production, VFA production, and VFA separation), a preliminary investigation of the H_2O_2 inhibition mechanism was conducted. H_2O_2 is a ROS species and can cause the destruction of cell membranes, proteins, and DNA.[43] Because bacteria encode a greater variety of antioxidant metabolism genes compared to archaea, methanogens are less resistant to stressful environments, and thus ROS may particularly affect methanogens, resulting in the failure of the methanation process.[44] The elevated levels of intracellular ROS will lead to lipid peroxidation, which is typically determined by the MDA level. Thus, MDA is commonly used as a valuable indicator for analyzing cellular damage.[45] The average MDA level in stage II was 0.57 ± 0.06 μmol L^{-1}, increased by 2.3 times compared to 0.24 ± 0.09 μmol L^{-1} in stage I, implying the occurrence of cell damage in the presence of H_2O_2 (Figure 5). From day 60 to 84, the average MDA level exhibited a continued upward trend, reaching 0.79 ± 0.13 μmol L^{-1}, 3.3 times that observed during stage I. Between day 33 and 84, the MDA content increased, while CH_4 production decreased, and VFAs accumulated with the addition of H_2O_2. The shift of the

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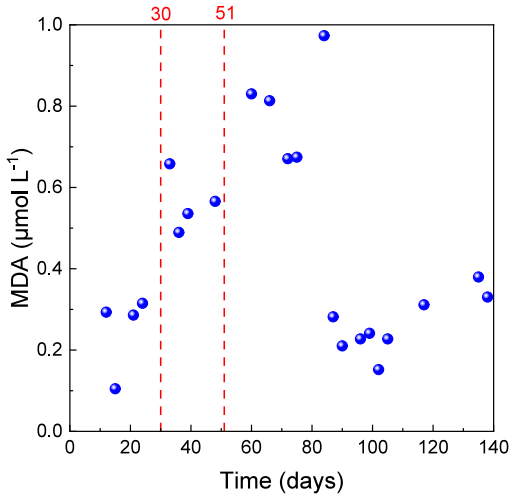

Figure 5. Changes in the MDA level during the three stages of H_2O_2 addition.

main product from methane to VFAs indicated that the activity of methanogens was inhibited, while acidogenic bacteria became dominant in the microorganism communities, with the varied MDA level over time and the different tolerance levels to ROS between methanogens and acidogenic bacteria. From day 96 to 144, the MDA level decreased to 0.26 ± 0.07 μmol L^{-1}, similar to that of stage I, indicating that the level of cell damage returned to a normal metabolic state. This suggests that acidogenic bacteria were able to manage the level of H_2O_2-induced stress through their own antioxidant mechanism. The MDA levels revealed that cell damage increased with a higher H_2O_2 dosage and returned to normal once VFA production stabilized, and methane production was completely inhibited. Those results imply selective inhibition of methanogens under the optimal H_2O_2 stress level.

3.5. ED Performance Evaluation. Separation of the produced VFAs is an important step in VFA recovery, and to do so, ED was employed to separate VFAs from the AD effluent and then concentrate them for recovery. The average separation efficiency of VFAs from the dilute solution to the concentrated solution was 62.7% over the first six cycles, peaking at 81.6% during the second cycle (Figure 6a). This increased VFA recovery in the second cycle may be related to the ion exchange mechanism, in which ions in the target solution initially exchange with the charged groups on the ion exchange membrane (first cycle), causing some ions to remain on the membrane and thus a relatively lower recovery efficiency. Once the membrane is saturated with the target ions, further migration of VFA anions can proceed without the need to displace other ions, resulting in a higher separation efficiency.

As the operation cycles progressed (beyond the second cycle), the separation efficiency declined, likely due to the increased ion concentration in the concentrate chamber relative to that in the dilution chamber, which created a reverse concentration gradient. This made further transferring VFAs more difficult. Despite the decreased separation efficiency, the VFA concentration in the concentrate solution increased from 4,431 to 26,721 COD mg L^{-1} after six cycles at a rate of 4,341 COD mg L^{-1} per cycle. There was less than 500 COD mg L^{-1} increase in cycle 7, indicating that further ED operation would not be able to recover more VFAs and the

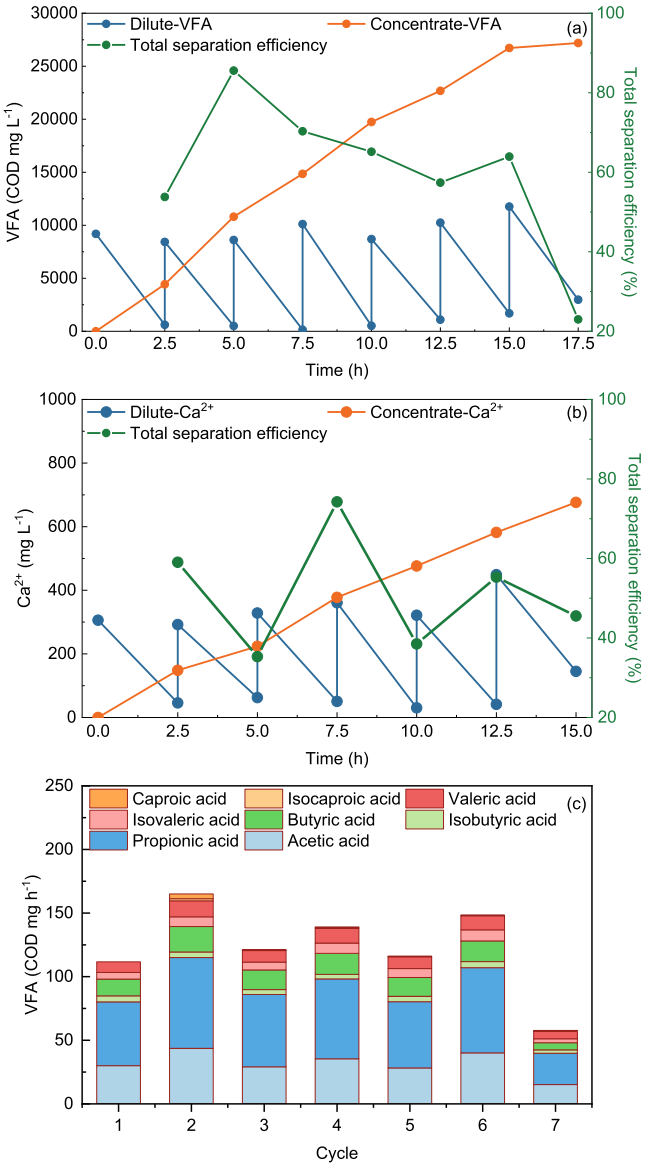

Figure 6. VFA separation by ED: (a) changes in VFA concentration in both the dilute and concentrated solutions and total separation efficiency per cycle; (b) Ca^{2+} concentration in both the dilute and concentrated solutions and total separation efficiency; and (c) VFA composition per cycle.

concentrate solution should be replaced with a fresh solution after cycle 6.

In terms of total mass balance, 62.7% VFAs from the dilute chamber entered the concentrate chamber, 7.4% remained in the dilute chamber, and the remaining 29.9% VFAs likely evaporated as gas or precipitated during the process. The recovery efficiency in this study is lower than that of a synthetic VFA stream, which can reach over 90% recovery efficiency,[46] likely related to the presence of competing cations and anions in the real fermentation broth that interfere with the migration process of VFAs. The presence of metabolite components and other salts has been reported to negatively impact the ED recovery performance. Due to their larger molecular sizes, VFAs are less preferentially transferred compared to inorganic ions like Na^+, K^+, and Cl^-, resulting in a reduced recovery of 70−35%.[47] In the present study, a relatively high initial VFA concentration of 9,214 mg COD L^{-1} was used in the dilute

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chamber, increasing the conductivity and reducing the solution's resistance. Compared to an initial concentration of 3,210 mg COD L^{-1} acetic acid in that previous study, which achieved only a 43.2% recovery rate,[48] the higher concentration in this study not only lowered the solution resistance but also made the VFAs more competitive against other coexisting ions.

The precipitates were observed on the membrane surface and analyzed to reveal VFAs upon dissolution. VFAs can precipitate with calcium salt and then be released through acid-dissolving calcium salts.[49] The precipitates observed on the membrane might be because both Ca^{2+} and VFAs were concentrated in the concentration chamber and then precipitated when a certain concentration was reached. In a previous study, the precipitation of long-chain fatty acids occurred with the addition of 500 mg L^{-1} Ca^{2+} to the AD reactor.[50] In the present study, the Ca^{2+} concentration in the concentrate chamber reached 582 mg L^{-1} by cycle 5 with an average separation efficiency of 51.3% counting only Ca^{2+} accumulation in the concentrate (Figure 6b). After six cycles, only 76.2% of Ca^{2+} lost from the dilute chambers was accounted for in the concentrated chambers and electrolyte, suggesting that the remaining 23.8% might have precipitated. However, the specific mechanism of deposition and the methods to mitigate it for improving recovery efficiency warrant future investigation.

Different VFA species exhibited different recovery rates (Figure 6c). Propionic acid had the highest recovery rate of 60.0 mg COD h^{-1}, succeeded by acetic acid (34.4 mg COD h^{-1}), butyric acid (16.0 mg COD h^{-1}), valeric acid (10.3 mg COD h^{-1}), isovaleric acid (7.1 mg COD h^{-1}), isobutyric acid (4.3 mg COD h^{-1}), caproic acid (0.9 mg COD h^{-1}), and isocaproic acid (0.4 mg COD h^{-1}). The recovery rate is mostly affected by both the initial concentration of these VFAs in the dilute solution and their molecular weights. A higher initial concentration results in a greater concentration gradient between the dilute and concentrate solutions, thereby favoring VFA migration.[51] The VFAs with smaller molecular weights find it easier to move through the ion exchange membrane and are consequently more readily recovered.[52]

3.6. Perspectives. Despite the success of VFA production through arresting methanogens with the electro-synthesized H_2O_2, several challenges need to be addressed toward further development of this system. First, while the present study achieved a relatively high VFA concentration without any pretreatment for sludge, the complex EPS and biologically recalcitrant materials in sludge can reduce organic matter degradation rates and anaerobic fermentation efficiency.[53] Disrupting the EPS structure will release adsorbed particulate and macromolecular organic matter and extracellular enzymes, thereby increasing organic matter concentration and enhancing dissolution, hydrolysis, and fermentation rates. The application of H_2O_2 inhibition after the existing pretreatment such as thermal hydrolysis may potentially enhance the conversion of organic matters to VFAs. Second, to better understand how H_2O_2 inhibits methanogenesis, it is essential to investigate both the evolution of microbial communities in its presence and the fate of H_2O_2 in the AD reactor, for example, whether it yields oxygen, generates hydroxyl radicals, or uses other mechanisms. Such an understanding is crucial for optimizing the use of H_2O_2 while maintaining a robust community for VFA production. Third, a relatively high VFA loss was observed during the ED recovery process, likely attributed to the high concentration of VFAs in the concentration chamber and the accumulation of Ca^{2+}, which would make VFAs susceptible to evaporation or precipitation.[49] It should be noted that the previously reported high recovery efficiency (>90%) was mostly from the synthetic VFA solvent.[54] The actual fermentation broths are more complex, with other coexisting components potentially hindering VFA recovery. This typically results in VFA recovery efficiency below 70%, which could further decrease as the length of the VFA chain increases. How to ensure a high recovery efficiency with a real VFA-rich fermentation broth requires further investigation. For example, using electrochemical pretreatment techniques to recover nitrogen and phosphorus and remove alkali metal ions, followed by an ED process, may efficiently manage the complexity and coexisting components in the actual fermentation broth. Fourth, one of the key tasks for AD treatment is to reduce the sludge contents. Thus, the sludge after VFA production needs to be characterized for a better understanding of sludge properties, for example, dewaterability, VS reduction, and organics in the solid phase.

4. CONCLUSIONS

In this study, the feasibility of incorporating on-site H_2O_2 electrochemical synthesis and its subsequent application to halt methanogenesis, along with VFA recovery, has been demonstrated. The proposed system proved to be efficient and potentially cost-effective, offering a controllable approach to inhibiting methanogenesis for more valuable products from AD. Significant accumulation of VFAs, particularly propionic and acetic acids, was achieved. Both the VFA production efficiency from available VS and the VFA concentration were competitive with those using sludge pretreatment or other in situ inhabitation methods. The analysis of MDA concentration and CH_4 production under different H_2O_2 additions indicated that methanogens had a lower tolerance to ROS compared to other anaerobic microorganisms, making them more susceptible to inhibition by H_2O_2.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestengg.4c00384.

Discussion on the significance of VFA production through anaerobic digestion, including a hypothesis on using H_2O_2 to inhibit methanogenesis and a comparison of various methods for enhancing VFA production (PDF)

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CRediT: **Jiasi Sun** conceptualization, data curation, formal analysis, investigation, methodology, writing - original draft, writing - review & editing; **Xi Zhang** formal analysis, investigation, writing - original draft.

Notes

The authors declare no competing financial interest.

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