Enhanced methane production from sludge anaerobic digestion with the addition of potassium permanganate

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Analytical Methods

Text S1. EPS extraction method

Firstly, the sludge samples were centrifuged at 4000 rpm for 10 min and the supernatant was collected as soluble EPS. The sludge pellet in the tube was then re-suspended to their original volume using 0.05% NaCl solution. After vigorously vibrated using a vortex mixer, the mixture was centrifuged at 4000 rpm for 10 min and the supernatant was collected as lightly bound EPS (LB-EPS). The collected sludge pellet was re-suspended to the original volume with 0.05% NaCl solution, then heated at 60 °C for 30 min, and finally centrifuged at 4000 rpm for 10 min and the supernatant was collected as tightly bound EPS (TB-EPS).

Text S2. The method for LIVE/DEAD cells analysis

Live cells and dead cells were discriminated using the Calcein-AM/PI Double Staining Kit (Dojindo Laboratories, Japan). Firstly, interfering components from bacterial suspension was removed through cell wash step using phosphate buffer solution. Then, 100 μL dye mixture which contained 2 μmol/L Calcein-AM and 4.5 μmol/L Propidium Iodide (PI) was added in 200 μL re-suspended bacterial suspension. After hatched in the dark at 37 °C for 15 min, slides with stained sludge samples (50 μl on each slide) were observed and photographed by a fluorescence microscope (Nikon Eclipse 80i, Japan) with No. 1 (for observing green fluorescence) and No. 2 (for observing red fluorescence) fluorescence microscope filters. Photos with red and green fluorescence were respectively taken by an annex digital camera. For each sample, 20 sets of stained photos were taken. Based on these photos, the average red and green areas were analyzed with the installed AxioVision software. The ratio of green/red fluorescence to total fluorescence was thus determined, which are equivalent to ratio of
live/dead cells to total cells.

**Text S3. Analysis of 3D-EEM fluorescence spectroscopy**

3D-EEM fluorescence spectra were measured using a luminescence spectrometry (Hitachi F-7000, Japan). The EEM spectra of EPS were gathered with corresponding scanning emission (Em) spectra from 200 nm to 500 nm at 5 nm increments by varying the excitation (Ex) wavelength from 200 nm to 450 nm at 5 nm increments. The excitation and emission slits were both maintained at 5 nm, and the scanning speed was set at 1200 nm/min for all the measurements. Under the same conditions, fluorescence spectra for Milli-Q water was subtracted from all the spectra to eliminate water Raman scattering and to reduce other background noise. The software Origin 8.0 (Origin Lab Inc., USA) was employed to handle the EEM data.

The calculation method for percent fluorescence response ($P_{i,n}$) allows all the fluorescence information with each region to be taken into account and could compare the differences among samples by analyzing the fluorescence variations. Volume of Fluorescence ($\phi_i$) beneath region of the EEM were calculated by the Equation 1:

$$\phi_i = \sum_{\lambda_{ex}} \sum_{\lambda_{em}} I(\lambda_{ex}\lambda_{em}) \cdot \Delta\lambda_{ex} \cdot \Delta\lambda_{em}$$

(1)

$\Delta\lambda_{ex}$ is the excitation wavelength interval (taken as 5 nm), $\Delta\lambda_{em}$ is the emission wavelength interval (taken as 1 nm) and the $I(\lambda_{ex}\lambda_{em})$ is the fluorescence intensity at each excitation-emission wavelength pair. $\phi_T$ is representative for the cumulative volume beneath the EEM which calculated as $\phi_T = \sum \phi_i$. It was reported that the values of $\phi_T$ and $\phi_i$ are closely related to the concentration of organic matters in different regions of EEMs. Moreover, the percent florescence response in a specific region ($P_{i}$) was calculated with the Equation 2:
\[ P_i = \frac{\varphi_i}{\varphi_T} \cdot 100 \quad (2) \]

According the equation 1 and 2, the percent fluorescence response of one region was calculated as Equation 3:

\[ P_{i,n} = \frac{\varphi_{i,n}}{\varphi_{T,n}} \cdot 100 \quad (3) \]

**Text S4. Model-based analysis.**

The first-order kinetic model (Eq. (4)) was adopted to assess the effect of different pretreatment conditions on methane production potential and hydrolysis rate of sludge.

\[ B(t) = B_0(1 - e^{-kt}) \quad (4) \]

where \( B(t) \) is the cumulative methane production at time \( t \) (mL/g VSS), \( t \) is digestion time (d), the two kinetic parameters \( B_0 \) and \( k \) represents methane production potential (mL/g VSS) and hydrolysis rate \((d^{-1})\), respectively. By inputting the methane yield data from batch anaerobic methane production experiment as \( B(t) \) and \( t \) into Eq. (4), the parameters \( B_0 \) and \( k \) were evaluated using Origin 8.0 software.

**Text S5. Evaluating the impacts of KMnO\(_4\) on all biochemical processes involved in sludge anaerobic digestion**

After disintegration, the largely produced soluble organics are utilized by anaerobes for methane production through the following hydrolysis, acidogenesis, acetogenesis and methanogenesis processes. This experiment was carried out to evaluate the impacts of KMnO\(_4\) on each biochemical process using several model substances. In brief, 15 serum bottles with a working volume of 500 mL each were adopted and equally separated into five groups, named as BSA-Group, Glucose-Group, Butyrate-Group, H\(_2\)-CO\(_2\)-Group, and Acetate-Group, respectively, with three bottles in each.
BSA-Group: Three bottles firstly received 270 mL pure water containing 0, 142.3 and 284.6 mg KMnO₄, respectively, with the contents be consistent with 0, 0.04 and 0.08 g/g VSS in batch methane producing experiment. After 30 min, each reactor received 30 mL the same inoculum and 945 mg bovine serum albumin (BSA). At last, all bottles were excluded the internal air and sealed for anaerobic digestion according to that operated in batch methane producing experiment. By detecting the degradation of BSA in different reactors, the effect of KMnO₄ on hydrolysis process was revealed.

Glucose-Group: All operations of this test were conducted the same as the BSA-Group, apart from that the BSA was substituted by 216 mg glucose.

Butyrate-Group: This test was carried out in accord with that operated in the BSA-Group, except that the BSA was replaced by 540 mg sodium butyrate.

H₂-CO₂-Group: Each bottle firstly received 270 mL pure water containing 0, 142.3 and 284.6 mg KMnO₄, respectively. After 30 min, each reactor received 30 mL the same inoculum. Afterwards, all bottles were flushed with a hybrid gas containing 50% N₂, 40% H₂ and 10% CO₂. At last, the bottles were sealed for anaerobic digestion, and the specific operations were in accordance with that illustrated in batch methane producing experiment.

Acetate-Group: The operations of this test were consistent with that performed in the BSA-Group, other than that the BSA was replaced by 270 mg sodium acetate.

Text S6. The measuring method of fecal coliform in digested sludge

Measurement of fecal coliforms was conducted with Colilert-18 Test kit from the IDEXX laboratories. The sludge samples were diluted to 100 mL in sterilized bottles. The Colilert-18 reagents were afterwards dissolved in the 100 mL diluted samples. The 100 mL mixed solution
was transferred into the Quanti-Tray 2000 and then sealed with a Quanti-Tray sealer. The sealed Quanti-Tray/2000 was next incubated at an incubator at 44.5 ± 0.5 °C for 18 h. Finally, the positive wells were counted and the Most Probable Number (MPN) of Fecal Coliform was obtained based on the IDEXX Quanti-Tray/2000 MPN Table.
Figure S1. The linear fitting of $B_0$ (a) and $k$ (b) values to KMnO$_4$ dosages at the range of 0 to 0.08 g/g VSS.
Figure S2. The fractions of live cells to total cells in sludge with different KMnO₄ dosages.

Error bars represent standard deviations of triplicate tests.
Figure S3. The Venn diagram of sludge microbial OTUs between the control and 0.08 g/g VSS KMnO₄ pretreated reactors.
Figure S4. The numbers of fecal coliform in the control and KMnO$_4$ pretreated reactors after 30 d anaerobic digestion.
### Table S1 The main characteristics of WAS and inoculum used in this study.

| Parameters                              | WAS            | Inoculum     |
|-----------------------------------------|----------------|--------------|
| pH                                      | 6.8 ± 0.1      | –            |
| TSS (total suspended solids) (mg/L)     | 20458 ± 262    | 23851 ± 295  |
| VSS (volatile suspended solids) (mg/L)  | 13174 ± 144    | 11453 ± 138  |
| TCOD (total chemical oxygen demand) (mg/L) | 17052 ± 334   | 18754 ± 307  |
| SCOD (soluble chemical oxygen demand)   | 84 ± 3         | 294 ± 17     |
| Total carbohydrates (mg/L)              | 1275 ± 131     | –            |
| Total proteins (mg/L)                   | 5689 ± 138     | –            |
| Humic acid (mg/L)                       | 1348 ± 102     | –            |
| Fulvic acid (mg/L)                      | 972 ± 114      | –            |
| Lignin (mg/L)                           | 1035 ± 67      | –            |
| Cellulose (mg/L)                        | 1277 ± 84      | –            |
| Hemicellulose (mg/L)                    | 1148 ± 75      | –            |
Table S2 The variations of several microbial ecological diversity indicators with the addition of 0.08 g/g VSS KMnO₄.

| Reactors          | Indicators | Chao          | Simpson       | Shannon       |
|-------------------|------------|---------------|---------------|---------------|
| Control           |            | 2754.4 ± 68.1 | 0.0055 ± 0.0007 | 6.18 ± 0.06   |
| 0.08 g/g VSS KMnO₄ |            | 2421.7 ± 63.4 | 0.0121 ± 0.0010 | 5.73 ± 0.05   |