A two-stage microbial fuel cell and anaerobic fluidized bed membrane bioreactor (MFC-AFMBR) system for effective domestic wastewater treatment

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Figure S1. Schematic diagrams of the SEA and SPA MFCs, showing the anode configurations (a) and the cathode assemblies (b).

Figure S2. Schematic diagram of the two-stage MFC-AFMBR system, showing the sampling points for liquid quality analyses. The green triangles indicated the sampling points of COD removals in the comparisons of SEA and SPA MFCs. The red circles indicated the sampling points for the treatment performance with the second stage AFMBR.
Figure S3. Part of the voltage and power data that was produced by the MFCs with steady operation during the 50 days continuous operation of the MFC-AFMBR system.
Table S1. COD removals of the SEA and SPA MFCs after 1 month.

| Characteristic | n   | Inf. (mg/L) | Eff. (mg/L) | Rem. (%) | Eff. (mg/L) | Rem. (%) | Rem. (%) |
|----------------|-----|-------------|-------------|----------|-------------|----------|----------|
|                |     |             | SEA-U       | SEA-D    | Overall     |          |          |
| tCOD           | 5   | 224 ± 17    | 161 ± 15    | 28.0     | 134 ± 8     | 16.8     | 40.0     |
| sCOD           | 5   | 140 ± 32    | 102 ± 14    | 27.3     | 82 ± 10     | 18.8     | 41.0     |
|                |     |             | SPA-U       | SPA-D    | Overall     |          |          |
| tCOD           | 5   | 224 ± 17    | 147 ± 6     | 34.3     | 119 ± 7     | 19.3     | 47.0     |
| sCOD           | 5   | 140 ± 32    | 96 ± 7      | 31.7     | 71 ± 7      | 26.1     | 49.5     |

The samples for COD analysis were taken daily from the sampling points (green triangles) as shown in Figure S2.

Table S2. Wastewater treatment efficiencies for the MFC-AFMBR system.

| Characteristic | n   | MFCs | AFMBR | Over all |
|----------------|-----|------|-------|----------|
|                |     | Inf. (mg/L) | Eff. (mg/L) | Rem. (%) | Eff. (mg/L) | Rem. (%) | Rem. (%) |
| tCOD           | 8   | 210 ± 11   | 107 ± 10   | 49.1     | 16 ± 3      | 85.2     | 92.5     |
| sCOD           | 8   | 114 ± 8    | 57 ± 14    | 50.3     | 16 ± 3      | 72.2     | 86.2     |
| TSS            | 5   | 45 ± 10    | 22 ± 4     | 51.0     | 0.7 ± 0.2   | 96.9     | 99.6     |
| pH             | 5   | 7.6 ± 0.1  | 7.1 ± 0.1  | 7.5 ± 0.2| 7.5 ± 0.2   |          |          |
| Conductivity   | 5   | 1473 ± 33  | 1457 ± 15  | 1420 ± 19|            |          |          |

The samples for COD analysis were taken daily during the polarization tests of MFCs for 5 days, and after that every 10 days. The samples for TSS, pH and conductivity analyses were taken every 10 days during the 50 days operation. The sampling points (red circles) are shown in Figure S2.
ESTIMATION OF BIOMASS SOLIDS PRODUCTION

Based on the reported values in literature, we can estimate the biomass solids productions in the AFMBR and MFCs, separately. For anaerobic fermentation to methane, the biomass solids yield is theoretically $0.077 \text{ g VSS/g COD}$ removed. The biomass solids production was estimated as $0.025 \text{ g VSS/g COD}$ removed in an AFMBR treating domestic wastewater. Thus, the averaged value was used for the estimation of the biomass solids production in the AFMBR as

$\frac{(0.077 \text{ g VSS/g COD} + 0.025 \text{ g VSS/g COD}) \times 0.5}{0.5} = 0.051 \text{ g VSS/g COD}$ \hspace{1cm} (1)

In MFCs, it was reported that the observed growth yields varied between $0.07 – 0.22 \text{ g biomass/g COD}$ fed with glucose. Little information on biomass solids production in MFCs is available with domestic wastewater as the substrate. However, it is reasonable to assume that the biomass solids yield in MFCs will be between those of the aerobic treatment and the anaerobic fermentation, owing to the nature of the MFC process whereby some COD is removed using oxygen leaking in through the cathode, and some is used for biomass and energy production. A typical biomass solids production with aerobic treatment is $\sim 0.3 \text{ g VSS/g COD}$ removed. The biomass solids production in MFCs was estimated as

$\frac{(0.07 \text{ g VSS/g COD} + 0.22 \text{ g VSS/g COD}) \times 0.5}{0.5} = 0.145 \text{ g VSS/g COD}$ \hspace{1cm} (2)

The COD removal in MFCs was $(210 \text{ mg/L} – 107 \text{ mg/L}) = 103 \text{ mg/L}$, in AFMBR was $(107 \text{ mg/L} – 16 \text{ mg/L}) = 91 \text{ mg/L}$, and the overall removal was $(210 \text{ mg/L} – 16 \text{ mg/L}) = 194 \text{ mg/L}$ (Table S2).

Therefore, the biomass solids production of the MFC-AFMBR system is

$\frac{[(0.145 \text{ g VSS/g COD} \times 103 \text{ mg/L}) + (0.051 \text{ g VSS/g COD} \times 91 \text{ mg/L})]}{194 \text{ mg/L}}$

$= 0.10 \text{ g VSS/g COD}$ removed \hspace{1cm} (3)

This is about one-third of the typical biomass solids production in aerobic treatment.
ESTIMATION OF METHANE PRODUCTION IN THE AFMBR

1. Dissolved methane

As described in the Measurements and Chemical Analyses section, the serum bottles with the liquid sample from AFMBR (1.5 mL headspace and 5.0 mL liquid) were shaken for six hours at room temperature (25 °C), to establish gas-liquid equilibrium. After that, biogas (200 µL samples) in the headspace of the serum bottle was sampled using gastight syringes (250 µL; Hamilton Samplelock Syringe), and analyzed using gas chromatographs (SRI Instruments) for H₂, N₂, and CH₄. The pressure was 1 atm during the whole test period.

The methane composition in the headspace was y = 0.45 ± 0.10% at equilibrium. Using Henry’s constant $k_H = 0.0014 \text{ mol/kg-bar}$, the methane concentration in the liquid phase at equilibrium is

$$C_{\text{methane}} = k_H \times y \times P_T = 0.0014 \text{ mol/kg-bar} \times 0.45\% \times 1 \text{ atm}$$

$$= 0.0014 \text{ mol/kg-bar} \times 0.45\% \times 1.01325 \text{ bar} = 6.4 \times 10^{-6} \text{ mol/kg} \quad (4)$$

The molar volume of an ideal gas at 1 atmosphere of pressure is 24.465 L/mol at 25 °C, thus

$$V_{\text{methane}}^1 = 6.4 \times 10^{-6} \text{ mol/kg} \times 24.465 \text{ L/mol} = 0.156 \text{ mL/kg} = 0.156 \text{ mL/L liquid} \quad (5)$$

Moreover, all the methane in the headspace at equilibrium was coming from the dissolved methane in the liquid sample, and it equals to

$$V_{\text{methane}}^2 = 0.45\% \times 1.5 \text{ mL gas/ 5 mL liquid} = 1.35 \times 10^{-3} \text{ mL / mL liquid} = 1.35 \text{ mL/L liquid} \quad (6)$$

Therefore, the dissolved methane concentration in the liquid of AFMBR is

$$V_{\text{methane}}^L = V_{\text{methane}}^1 + V_{\text{methane}}^2 = 0.156 \text{ mL/L liquid} + 1.35 \text{ mL/L liquid} = 1.50 \text{ mL/L liquid}$$

$$= 6.15 \times 10^{-5} \text{ mol/ L liquid} \quad (7)$$

2. Gaseous methane

The methane composition in the headspace of AFMBR was measured as 3.5 ± 0.5%, and the gas production rate was 7.5 mL/day. Given the liquid flow rate of 1.56 L/day, the gaseous methane production is

$$V_{\text{methane}}^G = (3.5\% \times 7.5 \text{ mL/day}) / (1.56 \text{ L/day}) = 0.17 \text{ mL/L liquid} = 6.9 \times 10^{-6} \text{ mol/ L liquid} \quad (8)$$
Using Henry’s constant $k_H = 0.0014 \text{ mol/kg-bar}$, the saturated methane concentration in the liquid phase should be

$$C_{\text{methane}}(\text{Saturation}) = k_H \times y \times P_T = 0.0014 \text{ mol/kg-bar} \times 3.5\% \times 1 \text{ atm}$$

$$= 0.0014 \text{ mol/kg-bar} \times 3.5\% \times 1.01325 \text{ bar} = 4.96 \times 10^{-5} \text{ mol/kg} \tag{9}$$

As the molar volume of an ideal gas at 1 atmosphere of pressure is 24.465 L/mol at 25 °C,

$$V_{\text{methane}}(\text{Saturation}) = 4.96 \times 10^{-5} \text{ mol/kg} \times 24.465 \text{ L/mol} = 1.21 \text{ mL/kg} = 1.21 \text{ mL/L liquid} \tag{10}$$

Therefore, the measured dissolved methane concentration of 1.50 mL/L liquid is 125% of the saturated value. This methane oversaturation was also observed in other studies of anaerobic membrane reactors.²

3. **Total methane**

The total methane production in AFMBR is

$$V_{\text{methane}} = V_{\text{methane}}^L + V_{\text{methane}}^G = 1.50 \text{ mL/L liquid} + 0.17 \text{ mL/L liquid} = 1.67 \text{ mL/L liquid}$$

$$= 6.83 \times 10^{-5} \text{ mol/L liquid} \tag{11}$$

As the COD removal in AFMBR was (107 mg/L – 16 mg/L) = 91 mg/L, the methane yield in AFMBR is

$$\gamma_{\text{methane}}(\text{AFMBR}) = (1.67 \text{ mL/L}) / (91 \text{ mg/L}) = 0.018 \text{ L/g COD} = 7.5 \times 10^{-4} \text{ mol/g COD} \tag{12}$$

Given that one mole of methane is equivalent to 64 g COD, the methane fraction of the removed COD is $(7.5 \times 10^{-4} \text{ mol/g COD}) \times 64 \text{ g COD/mol} = 0.048 = 4.8\%$.

This is much less than that of 50 – 60 % typically obtained in anaerobic methanogenic digestion.²,¹⁰
BATCH TEST RESULTS USED TO ESTIMATE METHANE PRODUCTION

Methane production of the domestic wastewater was measured in batch test using serum bottles (165 mL, Wheaton, Millville, NJ, USA). Primary clarifier effluent (100 mL) and anaerobic sludge (1%, 20 mL) collected from the Pennsylvania State University Wastewater Treatment Plant was first added into the serum bottle, and then sparged with N₂ gas for 20 min. Deionized water (100 mL) was used instead of the primary effluent as a control to measure the methane production by the anaerobic sludge alone. All tests were conducted and analyzed in duplicate at ambient temperature (25 °C). Biogas (200 µL samples) in the headspace of the serum bottle was sampled using gastight syringes (250 µL; Hamilton Samplelock Syringe), and analyzed using gas chromatographs (SRI Instruments) for H₂, N₂, and CH₄ every day until the methane content reached plateau after 7 days. Chemical oxygen demand (COD) was measured using a standard method (method 5220, HACH COD system, HACH Company, Loveland, CO).

The initial tCOD of the domestic wastewater was 247 ± 7 mg/L. At the end of the batch test, the methane measured in the head space of the test bottles was 7.6 ± 0.3 mL (corresponding to a gas composition of 19%), and that of the control bottles was 1.9 ± 0.2 mL (corresponding to a gas composition of 4.7%). As the serum bottles were incubated on a shaker during the tests, equilibrium between the gas and liquid phase was assumed. Thus, the dissolved methane concentrations in the tested and control bottles could be calculated as above.

In test bottles,

\[
C_{\text{methane}}^T = k_H \times y \times P_T = 0.0014 \text{ mol/kg-bar} \times 19\% \times 1 \text{ atm} \\
= 0.0014 \text{ mol/kg-bar} \times 19\% \times 1.01325 \text{ bar} = 2.7 \times 10^{-4} \text{ mol/kg} \\
\]

\[
m_{\text{methane}}^T = 2.7 \times 10^{-4} \text{ mol/kg} \times 0.12 \text{ L} \times 1.0 \text{ kg/L} = 3.2 \times 10^{-5} \text{ mol} \\
\]

\[
V_{\text{methane}}^T = 3.2 \times 10^{-5} \text{ mol} \times 24.465 \text{ L/mol} = 0.8 \text{ mL} \\
\]

In control bottles,

\[
C_{\text{methane}}^C = k_H \times y \times P_T = 0.0014 \text{ mol/kg-bar} \times 4.7\% \times 1 \text{ atm} \\
= 0.0014 \text{ mol/kg-bar} \times 4.7\% \times 1.01325 \text{ bar} = 6.7 \times 10^{-5} \text{ mol/kg} \\
\]
\[
m_{\text{methane}}^C = 6.7 \times 10^{-5} \text{ mol/kg} \times 0.12 \text{ L} \times 1.0 \text{ kg/L} = 8.0 \times 10^{-6} \text{ mol} \quad (17)
\]

\[
V_{\text{methane}}^C = 8.0 \times 10^{-6} \text{ mol} \times 24.465 \text{ L/mol} = 0.2 \text{ mL} \quad (18)
\]

Then, the total methane production in the test bottles is

\[
m_{\text{methane}}^T = 3.2 \times 10^{-5} \text{ mol} + \frac{7.6 \text{ mL}}{24.465 \text{ L/mol}} = 3.2 \times 10^{-5} \text{ mol} + 3.1 \times 10^{-4} \text{ mol}
\]

\[
= 3.4 \times 10^{-4} \text{ mol} \quad (19)
\]

\[
V_{\text{methane}}^T = 0.8 \text{ mL} + 7.6 \text{ mL} = 8.4 \text{ mL} \quad (20)
\]

And, the total methane production in the control bottles is

\[
m_{\text{methane}}^C = 8.0 \times 10^{-6} \text{ mol} + \frac{1.9 \text{ mL}}{24.465 \text{ L/mol}} = 8.0 \times 10^{-6} \text{ mol} + 7.76 \times 10^{-5} \text{ mol}
\]

\[
= 8.6 \times 10^{-5} \text{ mol} \quad (21)
\]

\[
V_{\text{methane}}^C = 0.2 \text{ mL} + 1.9 \text{ mL} = 2.1 \text{ mL} \quad (22)
\]

Therefore, the methane production from the domestic wastewater is

\[
m_{\text{methane}}^D = 3.4 \times 10^{-4} \text{ mol} - 8.6 \times 10^{-5} \text{ mol} = 2.54 \times 10^{-4} \text{ mol} \quad (23)
\]

\[
V_{\text{methane}}^D = 8.4 \text{ mL} - 2.1 \text{ mL} = 6.3 \text{ mL} \quad (24)
\]

Assuming that the COD removal efficiency was 75%, \(^{11}\) the amount of COD removed from the domestic wastewater is 247 mg/L \times 75% = 185 mg/L.

However, not all these removed COD could be converted into methane, due to other COD consuming processes such as sulfate reduction and biosolids production.

A typical sulfate concentration in domestic wastewater was \(~30\) mg/L, \(^5\) and thus sulfate reduction would represent a COD equivalent of 20 mg/L [One mole of sulfate (96 g) is equivalent to 64 g COD].

For anaerobic fermentation to methane, the biomass yield is theoretically 0.077 g VSS/g COD removed.\(^1\) Thus, the biomass production is 0.077 g VSS/g COD \times 185 mg/L = 14.2 mg VSS/L, and this represents a COD equivalent of 21.3 mg/L (COD/VSS ratio of 1.5 \(^{12}\)).

Therefore, the amount of COD that could be converted into methane is

\[
185 \text{ mg/L} - 20 \text{ mg/L} - 21.3 \text{ mg/L} = 143.7 \text{ mg/L} \quad (25)
\]
This represents 58% of the initial COD, which was comparable to that 50% of the COD could be converted into methane in a previous study.\textsuperscript{10}

The mass of COD that could be converted into methane is

\[143.7 \text{ mg/L} \times 0.1 \text{ L} = 14.4 \text{ mg COD}\] (26)

The methane yield is

\[y_{\text{methane}} = \frac{2.54 \times 10^{-4} \text{ mol}}{14.4 \text{ mg COD}} = 0.017 \text{ mol CH}_4/\text{g COD}\] (27)

In general, this methane yield is in accordance with the theoretical value that one mole of methane is equivalent to 64 g COD.
COD BALANCE FOR THE AFMBR

The COD balance was made to better evaluate the methane production ability from AFMBR. There were many processes that were related to the COD balance in AFMBR, because of the complex composition of the domestic wastewaters and the processes occurring in the primary MFCs. Thus, a crude estimation was made, which was believed important to demonstrate possible methane production.

The COD removal in AFMBR was \((107 \text{ mg/L} - 16 \text{ mg/L}) = 91 \text{ mg/L}\).

Sulfate is a competing electron acceptor and its reduction is considered in the COD balance. A typical sulfate concentration in domestic wastewater was \(\sim 30 \text{ mg/L}\), and \(\sim 50\%\) of the sulfate could be reduced in MFCs. Thus, the sulfate concentration entering AFMBR was \(\sim 15 \text{ mg/L}\), and its reduction would represent a COD equivalent of 10 mg/L (One mole of sulfate (96 g) is equivalent to 64 g COD).

The biomass solids production in the AFMBR was estimated as \(0.051 \text{ g VSS/g COD removed}\). Thus, the biomass production is \(0.051 \text{ g VSS/g COD} \times 91 \text{ mg/L} = 4.64 \text{ mg VSS/L}\), and this would represent a COD equivalent of 7.0 mg/L (COD/VSS ratio of 1.5).

Usually, nitrites and nitrates are not considered as competing electron acceptors in anaerobic systems treating domestic wastewater, as the nitrogen present in domestic wastewater are in the form of organics and ammonia. However, the organic nitrogen and ammonia could be oxidized during treatment in air-cathode MFCs, due to the oxygen diffusion through cathode. Total nitrogen decreased from the influent concentration of 48 mg/L to effluent of 13 mg/L, with no ammonia detected in the effluent based on the measurements. Thus, we could assume that all the nitrogen in the effluent was in the form of nitrate. A typical total nitrogen concentration in domestic wastewater is \(\sim 40 \text{ mg/L}\), and thus a nitrate-nitrogen concentration of 11 mg/L is estimated in the effluent of MFCs here. The nitrate and its reduction should also be considered in the COD balance in AFMBR. With a concentration of \(\sim 11 \text{ mg/L}\) (nitrate-nitrogen), nitrate reduction would represent a COD equivalent of 32 mg/L (One mole of nitrate (14 g as N) is equivalent to 40 g COD).

Therefore, the amount of COD that could be converted into methane is

\[91 \text{ mg/L} - 10 \text{ mg/L} - 7 \text{ mg/L} - 32 \text{ mg/L} = 42 \text{ mg/L},\] which represents 46\% of the initial COD.
METHANE PRODUCTION OF THE AFMBR

Using the methane yield \( y_{methane} = 0.018 \) mol CH\(_4\)/g COD measured in the batch test, the methane production ability in AFMBR is

\[
V_{methane} \text{ (Ability)} = 0.017 \text{ mol CH}_4/\text{g COD} \times 42 \text{ mg/L} = 7.1 \times 10^{-4} \text{ mol/L liquid}
\]

\( = 17.4 \text{ mL/L liquid} \) \( \text{(29)} \)

The total methane produced in AFMBR of 1.67 mL/L liquid, was only about one-tenth of the theoretical production (17.4 mL/L liquid). This caveat might indicate methane losses from the system, or the existing of other COD sinks that require further study for a better understanding.

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