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Coke-oven wastewater treatment in a dual-chamber microbial fuel cell with thiocyanate-degrading biofilm enriched at the air cathode

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ABSTRACT

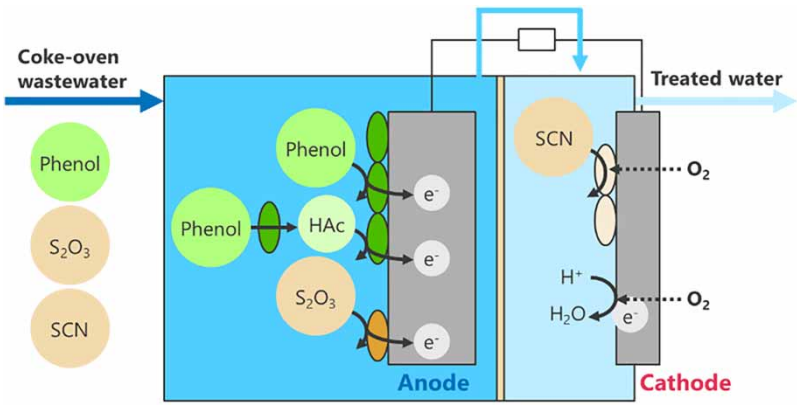
Coke-oven wastewater is usually treated with the activated sludge process, which requires large amounts of electrical energy for aeration and sludge disposal. A more sustainable treatment is strongly required. Recently, microbial fuel cells (MFCs) are focused as a technology for the production of electricity from wastewaters with simultaneous removal of organic matter. However, no MFC has been reported that can remove phenol, thiosulfate and thiocyanate simultaneously without aeration. Phenol can generally be removed well, whereas thiocyanate is relatively difficult to degrade. In this study, a dual-chamber MFC (D-MFC) was designed and equipped with a thiocyanate-degrading biofilm enriched on an air cathode. The D-MFC degraded phenol and thiosulfate in the anode chamber at the rate of 104 and 331 mg/L/day, respectively and subsequently degraded thiocyanate in the cathode chamber at the rate of 250 mg/L/day. The D-MFC showed high thiocyanate degradation rate. This suggests that pre-enrichment could accelerate thiocyanate degradation in MFC. In addition, thiocyanate degradation was not inhibited by phenol as thiocyanate was removed in the cathode chamber after phenol was removed in the anode chamber. This study demonstrated the feasibility of treating coke-oven wastewater by a D-MFC with a thiocyanate-degrading biofilm enriched at the air cathode.

Key words: coke-oven wastewater, microbial fuel cell, phenol degradation, thiocyanate degradation

HIGHLIGHTS

- A dual-chamber microbial fuel cell achieved simultaneous phenol, thiosulfate and thiocyanate degradation without active aeration.
- Pre-enrichment on an air cathode could accelerate thiocyanate degradation in MFC.
- The membrane is essential to avoid inhibition of thiocyanate degradation by phenol.

GRAPHICAL ABSTRACT



INTRODUCTION

Coke-oven wastewater is produced by the condensation and absorption of discharged gas from the coking process and contains various pollutants such as phenol, ammonia, cyanide, and sulfur compounds (Ashmore *et al.* 1967). These pollutants are generally measured as chemical oxygen demand (COD). Therefore, wastewater treatment is required to satisfy the COD discharge requirement. The main COD components in coke-oven wastewater are phenol, thiocyanate, and thiosulfate (Ashmore *et al.* 1967). The activated sludge process has been often applied to coke-oven wastewater treatment (Mishra *et al.* 2021).

The activated sludge process requires a large amount of electrical energy for aeration and sludge disposal (Rabaey & Verstraete 2005). Therefore, wastewater treatment technology with less energy consumption is strongly required. Recently, microbial fuel cells (MFCs) are focused as a next generation wastewater treatment technology. MFCs use electroactive bacteria which can convert biodegradable components in wastewater into electrical energy (Liu & Logan 2004). In MFCs, first, the biodegradable component is oxidized and generated electrons are transferred to the anodes by electroactive bacteria. Next, the electrons are transferred to the cathode via external circuit. Finally, the electrons reduce electron acceptors such as oxygen (Cheng *et al.* 2006). MFCs do not need electric power for aeration when using air cathodes, which have hydrophobic diffusion layers and can use passively diffusing oxygen (Cheng *et al.* 2006; Sim *et al.* 2015). In addition, the cell yield of MFCs is lower than for aerobic processes. This is because the energy available for biomass growth is reduced since a large part of the substrate energy is converted to electric energy (Logan *et al.* 2006). It was reported that MFC net yields range from 0.07 to 0.22 g biomass formed per g COD consumed, while typical aerobic yield for wastewater treatment is generally approximately 0.4 (Rabaey & Verstraete 2005). The low biomass production is very attractive since sludge disposal by dehydration and combustion has a high cost. Thus, MFC can reduce the electrical energy for aeration and sludge disposal. Therefore, MFC is expected as an attractive wastewater treatment technology with less energy consumption. In addition, coke-oven wastewater contains high concentrations of thiocyanate (3–9 mM), phenol (330–1,040 mg/L), and ammonia (24–364 mM) (Toh & Ashbolt 2002). Therefore, coke-oven wastewater is often diluted before biological treatments in order to decrease the concentration and toxicity of pollutants (Lim *et al.* 2002; Toh & Ashbolt 2002; Sueoka *et al.* 2009). Seawater is often used for dilution due to its availability and low cost (Oshiki *et al.* 2019). When seawater is used for dilution, the ionic strength will be higher than when freshwater is used, thus reducing Ohmic resistance. The performance of MFCs is high when the solution ionic strength is high (Liu *et al.* 2005). Therefore, MFCs may be highly effective when using coke-oven wastewater diluted by seawater and bacteria in a coke-oven wastewater treatment process.

Much research has been conducted to achieve coke-oven wastewater treatment systems by MFCs, where the anode is an anaerobic environment. The phenol degradation rate from 60 to 1,200 mg/L/day has been reported (Feng *et al.* 2015; Liu *et al.* 2017; Wu *et al.* 2018; Wang *et al.* 2019; Yang *et al.* 2020). However, no MFC has been reported that can remove phenol, thiosulfate and thiocyanate simultaneously without aeration. Especially, phenol can be removed well, while thiocyanate is relatively difficult to degrade. A thiocyanate degrading MFC has been reported (Ni *et al.* 2018). However, the degradation rate (7.1 mg/L/day) is not sufficiently high to replace activated sludge for coke-oven wastewater treatment. Therefore, to establish coke-oven wastewater treatment MFCs, it is necessary to consider how to treat thiocyanate in MFC.

To our knowledge, much research has been reported on aerobic thiocyanate degradation, while very limited information is available about the possibility of anaerobic thiocyanate degradation. For example, anaerobic thiocyanate-dependent denitrification has been reported (Sorokin *et al.* 2004). However, nitrate and nitrite are not included in coke-oven wastewater, and nitrification requires oxygen. Therefore, anaerobic degradation of thiocyanate in coke-oven wastewater may not be a feasible process. Some research has been conducted on the utilization of aerobic cathode biofilms using oxygen provided by air cathodes (Yan *et al.* 2012; Park *et al.* 2017). Thus, aerobic cathode biofilms are expected to degrade thiocyanate aerobically without aeration. To enrich thiocyanate-degrading biofilm on a cathode, there are two problems. Firstly, since it is considered that the growth rate of thiocyanate-degrading bacteria is lower than that of organo-heterotrophs, it can be difficult for them to thrive in a mixed culture system with abundant electron donor. Secondly, thiocyanate degradation is inhibited by phenol (Oshiki *et al.* 2019). Thus, we designed a dual-chamber MFC (D-MFC) equipped with a thiocyanate-degrading biofilm enriched on an air cathode. This MFC first degrades phenol in the anode chamber and subsequently degrades thiocyanate in the cathode chamber. In this study, we tested the feasibility and performance of coke-oven wastewater treatment using a D-MFC. A single-chamber MFC (S-MFC) without cathode biofilm was also run for comparison. Both batch-fed and continuous-flow experiments were conducted to examine the feasibility of simultaneous phenol, thiosulfate and thiocyanate removal from coke-oven wastewater using D-MFC. The degradation rates of phenol, thiosulfate and thiocyanate were assessed. The

structure and composition of the microbial community were analyzed to confirm which bacteria were enriched to enable the degradation of thiocyanate. Furthermore, the shifts in the relative abundance of the bacteria in response to different experimental conditions were investigated.

MATERIALS AND METHODS

MFC design

A dual-chamber air cathode MFC and a single-chamber air cathode MFC (referred to as D-MFC and S-MFC) were constructed as shown in Figure 1 (and Figure S1). The reactor bodies of D-MFC and S-MFC were made of polyvinyl chloride. The volumes of anode and cathode chambers of D-MFC were 108 (6 cm × 6 cm × 3 cm) and 72 mL (6 cm × 6 cm × 3 cm), respectively. The two chambers were separated by cation exchange membrane (Ultrex CMI-7000, Membranes International, United States). The volume of S-MFC was 108 mL (6 cm × 6 cm × 3 cm). Anodes were carbon felt (5 cm × 5 cm × 1 cm, LFP-210, Osaka gas chemicals, Japan), while cathodes were constructed as gas diffusion electrodes using carbon plates (2.5 cm × 2.5 cm, TGP-H-120, Toray, Japan) with a carbon base layer and four polytetrafluoroethylene (PTFE) layers on the air-facing side and a 0.5 mg-Pt/cm^2 catalyst layer on the solution-facing side (Cheng *et al.* 2006). D-MFC was equipped with air cathode with pre-enriched thiocyanate-degrading biofilm. A Ti wire (1.2 mm in diameter) was inserted inside the graphite felt and a Ti mesh (1 cm × 5 cm) was attached to the solution-facing side of cathode to connect the electrodes with the external circuit. A copper wire was used to close the circuit onto a resistor of 1,000 Ω. Voltages across the resistance were measured using a multi-meter, and data were automatically recorded by a data acquisition system.

Thiocyanate-degrading bacteria enrichment on the air cathode

An enrichment reactor consisting of two gas-diffusion electrodes (subsequently used as air cathodes in D-MFC) placed on the opposite ends (Figure S2) was used in the thiocyanate-degrading biofilm enrichment stage. The volume of the single chamber was 72 mL (6 cm × 6 cm × 2 cm). At the beginning of thiocyanate-degrading bacteria enrichment, a reactor was inoculated with 50 mL of return activated sludge taken from a full-scale coke-oven wastewater treatment plant (Kimitsu, Chiba, Japan) and 50 mL of phenol-free medium as shown in Table 1(a). Twice per week, half of media was replaced with fresh medium to avoid sedimentation. The enrichment period lasted 95 days.

Microbial inoculum and feeding regime

First, D-MFC and S-MFC were inoculated and operated under batch condition (Figure S3). Next, D-MFC was operated under continuous-flow condition (Figure S4). The operating condition of D-MFC is shown in Table S1. For quick start-up of the MFCs, the anode chambers of D-MFC and S-MFC were inoculated using pre-acclimated bacteria from a single-chamber MFC (500 mL) with air cathode that had been running in fed-batch mode with synthetic coke-oven wastewater for over 4 years. The composition of synthetic coke-oven wastewater was determined based on the literature (Oshiki *et al.* 2019). The anode chambers of D-MFC and S-MFC were fed with the synthetic coke-oven wastewater as shown in Table 1(b), and the cathode chamber of D-MFC was fed with the same medium with the exception of phenol as shown in Table 1(c).

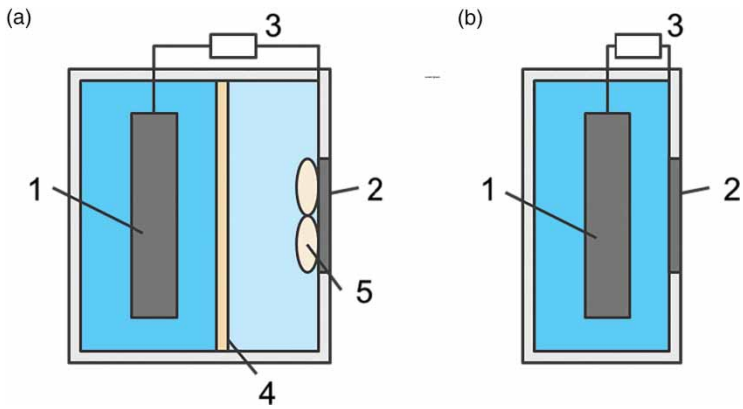


Figure 1 | Schematics of (a) dual-chamber air cathode MFC (D-MFC) and (b) single-chamber air cathode MFC (S-MFC). 1: carbon felt anodes; 2: air cathodes with PTFE; 3: external resistance; 4: cation exchange membrane; 5: pre-enriched thiocyanate-degrading bacteria.

Table 1 | Composition of media used for (A) pre-enrichment of the air cathodes, (B) start-up of the anode chamber of D-MFC and S-MFC and (C) start-up of the cathode chamber of D-MFC. (D) operation under continuous-flow condition

Component	A	B	C	D
Real sea water (Tokyo bay)	600 mL	600 mL	600 mL	600 mL
Industrial water	400 mL	400 mL	400 mL	400 mL
Phenol	–	400 mg/L	–	100 mg/L
Sodium thiocyanate	320 mg/L	320 mg/L	320 mg/L	320 mg/L
Sodium thiosulfate pentahydrate	–	557 mg/L	557 mg/L	557 mg/L
Ammonium chloride	1,650 mg/L	1,650 mg/L	1,650 mg/L	1,650 mg/L
Dipotassium hydrogen phosphate	29.2 mg/L	29.2 mg/L	29.2 mg/L	29.2 mg/L
Sodium hydrogen carbonate	650 mg/L	650 mg/L	650 mg/L	650 mg/L

After 9 days of start-up, D-MFC and S-MFC were operated under batch condition. The cathode solution was removed and the anode solution was transferred into the cathode, and newly-prepared synthetic coke-oven wastewater (Table 1(b)) was added to the anode chamber once a week. The concentrations of phenol, thiocyanate and thiosulfate in the anode chamber and the cathode chamber of D-MFC and S-MFC were determined at the start and end of each weekly batch. These MFC reactors were operated in a temperature-controlled lab at 25 $\pm$ 1 °C and remained covered to prevent any adverse effect of light.

From day 83, D-MFC was operated under continuous-flow condition. The synthetic coke-oven wastewater (Table 1(d)) was continuously supplied at a rate of approximately 10 mL/hour using a peristaltic pump. The wastewater in anode was continuously supplied to the cathode chamber using a peristaltic pump. The hydraulic retention time (HRT) of anode chamber and cathode chamber was 10.8 hours and 7.2 hours, respectively. Once a week, the raw wastewater tank (2 L) was replaced with a newly-prepared one. Two days after the raw water tank was replaced, the concentrations of phenol, thiosulfate and thiocyanate in raw water, anode chamber and cathode chamber were analyzed. To improve the degradation rates by the anode, the anode chamber was re-inoculated at day 114 using the same pre-acclimated bacteria used for start-up, and D-MFC was operated under batch condition from day 114 to day 140 (for 27 days). After day 140, D-MFC was operated under continuous-flow condition again.

In order to evaluate whether phenol inhibits thiocyanate degradation, the weekly changes of thiocyanate concentration of D-MFC before and after the removal of membrane (Figure S5) were compared. During the test, D-MFC was operated under batch condition. The anode chamber of D-MFC was fed with the synthetic coke-oven wastewater as shown in Table 1(b) and the anode solution was transferred into cathode solution once a week. The concentrations of thiocyanate in the cathode chamber of D-MFC were determined four times a week. The weekly batch experiments were repeated five and three times before and after removing the membrane, respectively.

Analysis

Samples were taken from the anode and cathode solutions once per week for measurements of phenol, thiosulfate and thiocyanate concentrations. The samples were filtered using 0.22 μm-diameter membrane filters and kept in the refrigerator at 4 °C prior to analysis. Phenol concentration was determined using the 4-aminoantipyrine spectrophotometric method. Thiosulfate concentration was determined via ion chromatography (ICS-2000, Thermo Fisher Scientific, Japan). Thiocyanate concentration was determined by the spectrophotometric method, using $Fe(NO_3)_3$ as the color developing agent under a wavelength of 460 nm.

Calculations

The average degradation rate of the anode of S-MFC under batch condition r_{anode} was calculated as:

$$r_{anode} = \frac{C_0 - C}{\Delta t}$$

where, C_0 and C are the concentrations of pollutants of start and end of weekly batch and Δt is the batch period.

The degradation rate of the anode and cathode of D-MFC under batch condition r_{anode}, $r_{cathode}$, r_{total} was calculated as:

$$r_{anode} = \frac{C_0 - C_{anode}}{\Delta t_{anode}}$$

$$r_{cathode} = \frac{C_{anode} - C_{cathode}}{\Delta t_{cathode}}$$

$$r_{total} = \frac{C_0 - C_{cathode}}{\Delta t_{anode} + \Delta t_{cathode}} = \frac{r_{anode} \cdot \Delta t_{anode} + r_{cathode} \cdot \Delta t_{cathode}}{\Delta t_{anode} + \Delta t_{cathode}}$$

where, C_0, C_{anode}, $C_{cathode}$ are the raw wastewater, the anode chamber and the cathode chamber concentrations of pollutants of weekly batch, and Δt_{anode} and $\Delta t_{cathode}$ are the batch periods of anode and cathode chamber, respectively.

The degradation rate of the anode and cathode of D-MFC under continuous-flow condition r_{anode}, $r_{cathode}$, r_{total} (mg/L/day) was calculated as:

$$r_{anode} = \frac{(C_{in} - C_{anode}) \times q}{V_{anode}}$$

$$r_{cathode} = \frac{(C_{anode} - C_{cathode}) \times q}{V_{cathode}}$$

$$r_{total} = \frac{(C_{in} - C_{cathode}) \times q}{V_{anode} + V_{cathode}}$$

where C_{in}, C_{anode} and $C_{cathode}$ are the influent, effluent from the anode chamber and effluent from the cathode chamber concentrations of pollutants, q is the flow rate of influent (10 mL/hour), V_{anode} and $V_{cathode}$ are the volume of the anode chamber and the cathode chamber, respectively.

The Coulombic efficiency ϵ is the ratio of electron equivalents recorded as electrical current to those of phenol and thiosulfate degraded. The Coulombic efficiency for D-MFC under continuous-flow mode is calculated as

$$\epsilon = \frac{I}{Fq\left\{\frac{(C_{in}^{Phenol} - C_{anode}^{Phenol})b^{Phenol}}{M^{Phenol}} + \frac{(C_{in}^{Thiosulfate} - C_{anode}^{Thiosulfate})b^{Thiosulfate}}{M^{Thiosulfate}}\right\}}$$

where $M^{Phenol} = 94$ is the molecular weight of phenol, $M^{Thiosulfate} = 112$ is the molecular weight of thiosulfate, I is the current, F is Faraday's constant, $b^{Phenol} = 28$ is the number of electrons exchanged per mole of phenol and $b^{Thiosulfate} = 8$ is the number of electrons exchanged per mole of thiosulfate, respectively.

Microbial community

Biomass samples at anode electrode (AE), cathode electrode (CE), anode solution (AS) and cathode solution (CS) of D-MFC were collected four times for microbial community analysis. The first sample was collected from the initial biofilm on the CE (Figure S5A). Second samples were collected from AE, CE, AS and CS before removing membrane on day 441 (Figure S5B). Third samples were collected from AE, CE and AS before adding membrane on day 477 (Figure S5C). Final samples were collected from AE, CE, AS and CS at the end of operation (Figure S5D). Microbial community analysis was conducted at the Nippon Steel Eco-tech Corp., Chiba, Japan. The detailed experimental steps were described elsewhere (Yu *et al.* 2018). Briefly, DNA was extracted by using an Extrap Soil DNA Kit Plus ver.2 (Nippon Steel Eco-tech Corp.). The V4-V5 region of the 16S RNA gene was amplified. Next generation sequencing was conducted by using an Illumina MiSeq. The sequence data were analyzed using Quantitative Insights into Microbial Ecology (QIIME) (Caporaso *et al.* 2010). Sequence similarities of 97% were clustered in operational taxonomic units (OTUs). The phylogenetic analysis of OTU representatives was performed by using the Greengenes database (DeSantis *et al.* 2006).

RESULTS AND DISCUSSION

Comparisons of phenol, thiocyanate and thiosulfate removal between D-MFC and S-MFC

The concentrations of phenol, thiocyanate and thiosulfate in the anode chamber and the cathode chamber of D-MFC and S-MFC under batch condition were determined at the beginning and at the end of the batch cycles (Figure S6), and average degradation rates were calculated. The degradation rates of phenol, thiosulfate and thiocyanate in the anode and cathode chambers of D-MFC and S-MFC are shown in Figure 2. In D-MFC, phenol was removed completely by both the anode and cathode chambers within 7 days. Thiosulfate was removed completely within 7 days by the anode chamber. Thiocyanate was removed completely within 7 days in the cathode chamber. It seems that, in D-MFC, phenol, thiosulfate and thiocyanate degradations occurred in both anode and cathode chamber, anode chamber only, and cathode chamber only, respectively. S-MFC also removed phenol and thiosulfate completely within 7 days. However, thiocyanate could not be removed. It was hypothesized that it is unfeasible to treat thiocyanate in coke-oven wastewater by electroactive or anaerobic bacteria. Although thiocyanate was reported to be removed by an MFC (Ni *et al.* 2018), thiocyanate in coke-oven wastewater could not be removed by MFCs in that study. This suggests that phenol inhibits thiocyanate-degrading bacteria and that is possibly the main reason for the inability of the cathode biofilm to remove thiocyanate in S-MFC. In addition, our data showed that thiosulfate and thiocyanate were degraded by different microbial species, since thiocyanate could not be removed, while thiosulfate could be removed in S-MFC. The current of D-MFC depended on phenol and thiosulfate concentrations (Figure S7). This indicated that the substrates are used in the bioelectrochemical process.

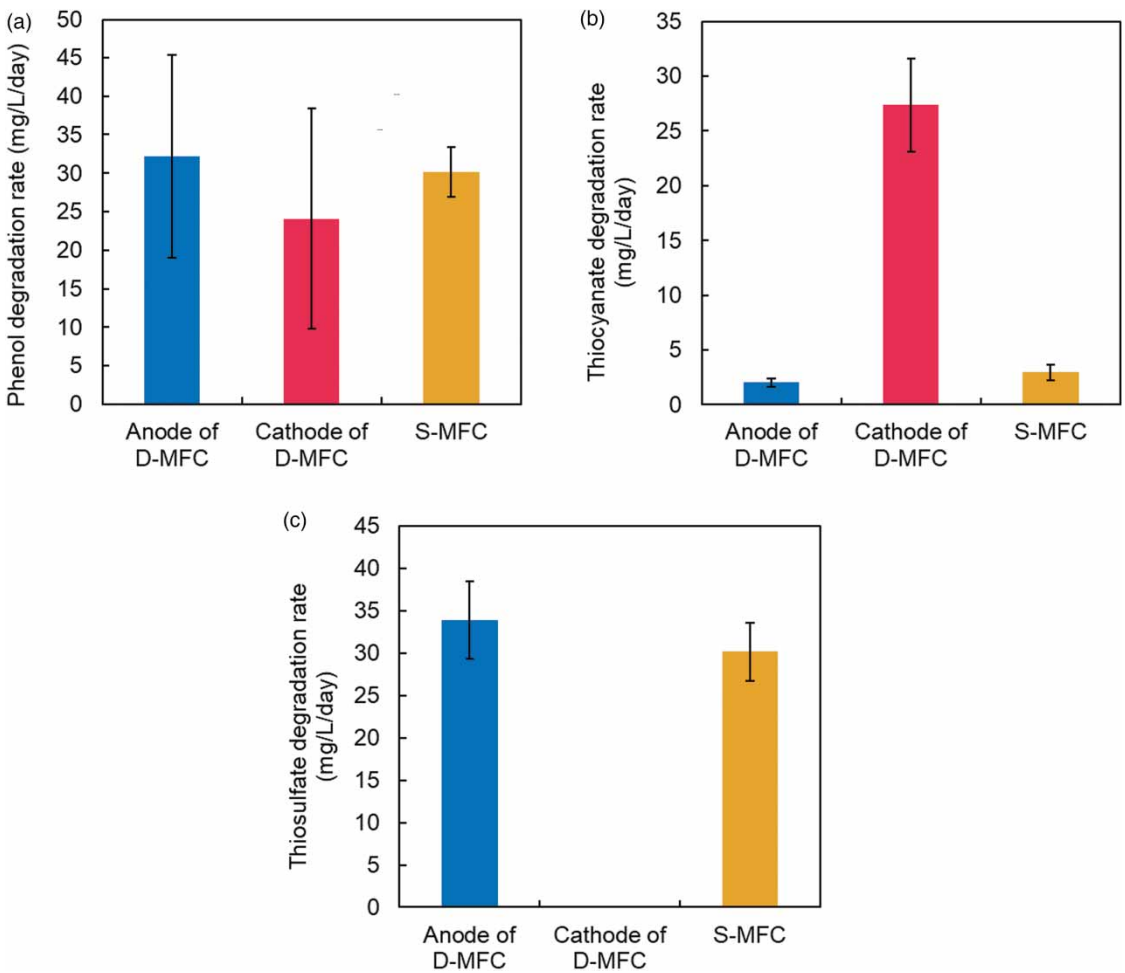


Figure 2 | Degradation rates of (a) phenol, (b) thiosulfate and (c) thiocyanate in D-MFC and S-MFC operated in batch mode. Error bars are standard deviations ($n = 5$).

Degradation rate and Coulombic efficiency under continuous-flow condition

The concentrations of phenol, thiocyanate and thiosulfate in the anode chamber and the cathode chamber of D-MFC under continuous-flow condition were determined once a week, and average degradation rates were calculated. The concentrations and degradation rates of phenol, thiosulfate and thiocyanate in the anode and cathode chamber of D-MFC are shown in Figures 3 and 4, respectively. From day 83 to day 114, the phenol and thiosulfate were removed completely and the total degradation rate of D-MFC under continuous-flow condition showed much higher degradation rates than under batch condition. However, most phenol and thiosulfate were removed in the cathode chamber and thiocyanate degradation rate was slow. To improve the degradation rates of the anode chamber, it was re-inoculated using the same pre-acclimated bacteria used for start-up and was operated under batch condition from day 114 to day 140. After that, D-MFC was operated under continuous-flow condition again. As a result, the phenol and thiosulfate degradation rates of anode chamber gradually increased and the thiocyanate degradation rate of the cathode chamber increased simultaneously. This suggests that it is important for improvement of thiocyanate degradation to decrease phenol inhibition and competition with phenol-degrading bacteria and thiosulfate-degrading bacteria in the cathode chamber. The Coulombic efficiency and the current are shown in Figure 5. The current was increasing and became almost stable from 0.3 to 0.6 mA when the phenol degradation rate by anode was stable (after day 250). The current was comparable to that of other MFCs (Luo *et al.* 2009; Liu *et al.* 2017; Wang *et al.* 2019) which have same external resistance (1,000 Ω) and substrate (phenol). This suggested that D-MFC showed sufficient power generation performance despite having a membrane. Although the phenol degradation rate of D-MFC gradually increased, the Coulombic efficiency decreased. This implies that anaerobic phenol-degrading bacteria other than electroactive phenol-degrading bacteria may have been prevalent, although it is not clear what electron acceptors they utilized (possibly

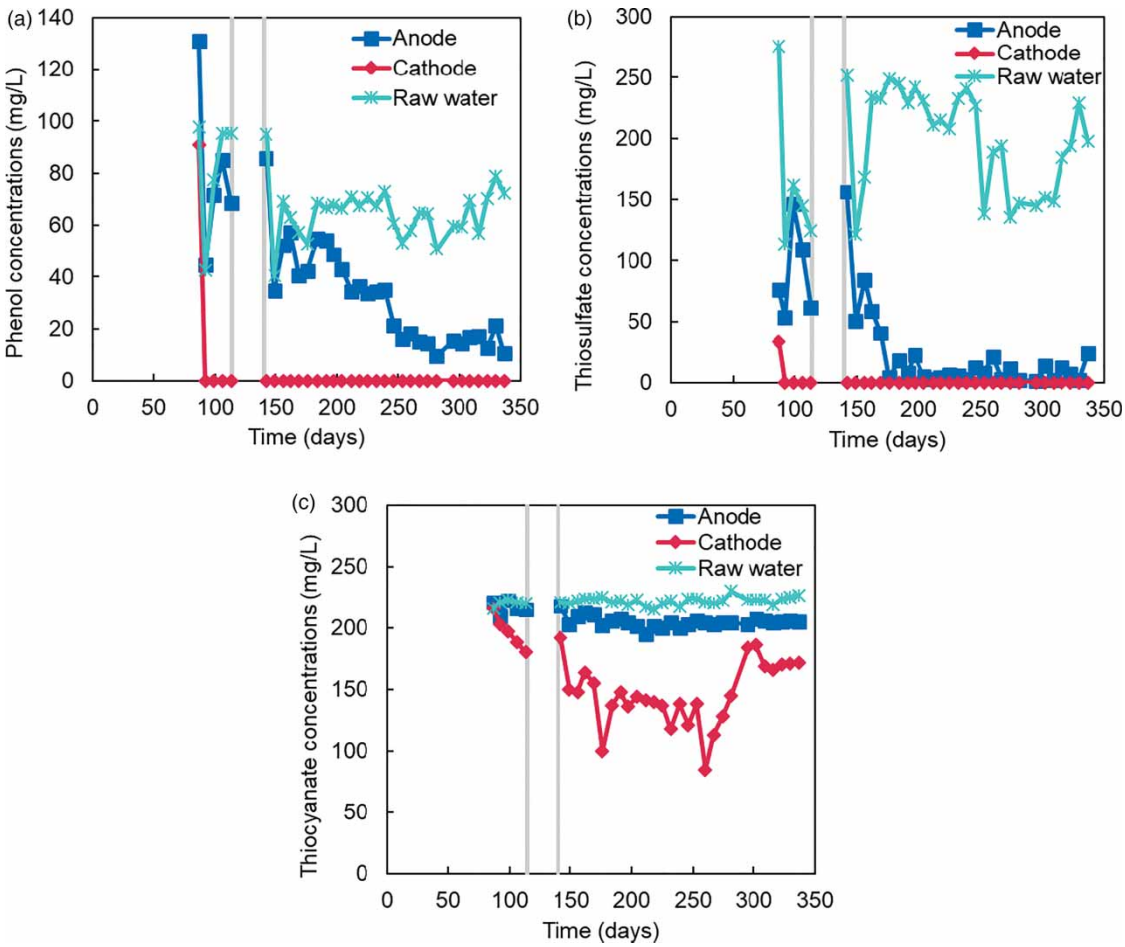


Figure 3 | Concentrations of (a) phenol, (b) thiosulfate and (c) thiocyanate in anode chamber, cathode chamber and raw water of D-MFC under continuous-flow condition. The data under batch condition was excluded.

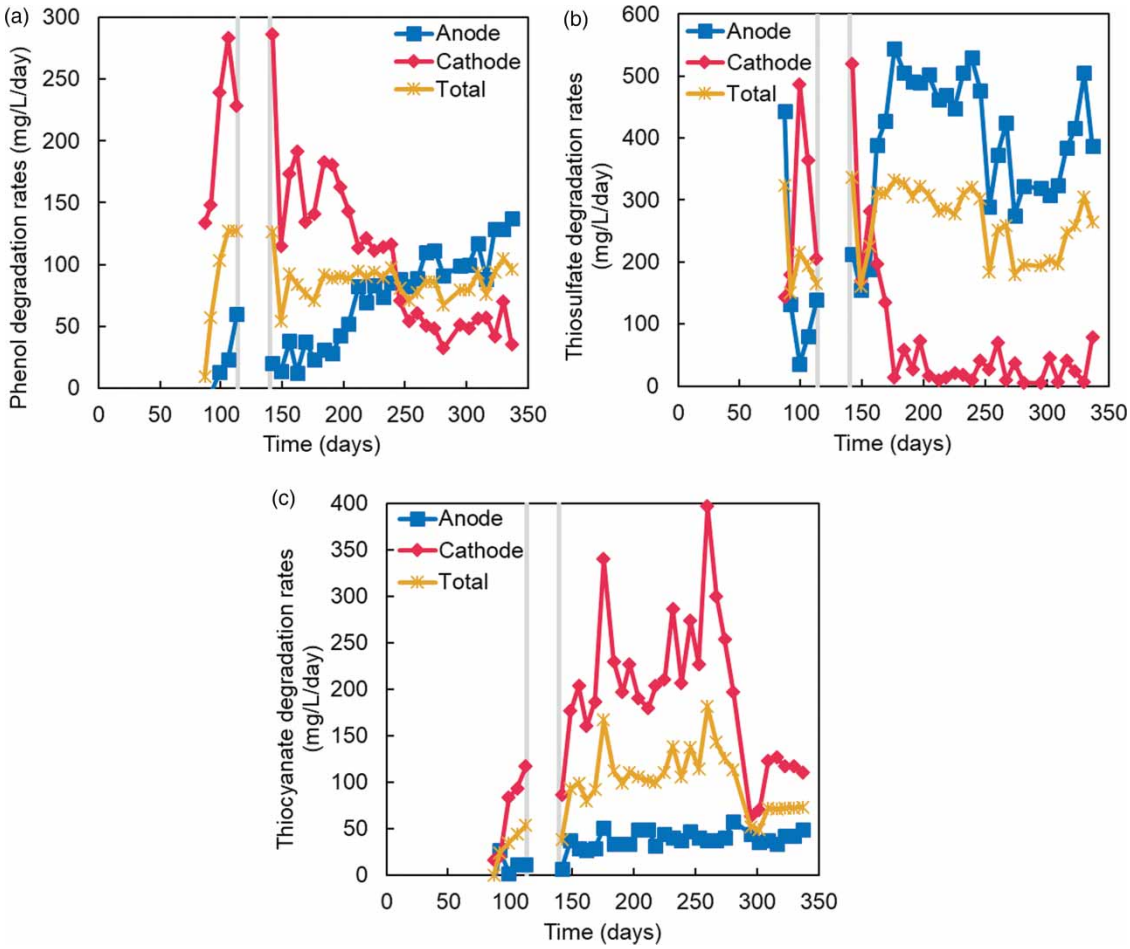


Figure 4 | Degradation rates (a) phenol, (b) thiosulfate and (c) thiocyanate by anode, cathode and total of D-MFC under continuous-flow condition. The data under batch condition was excluded.

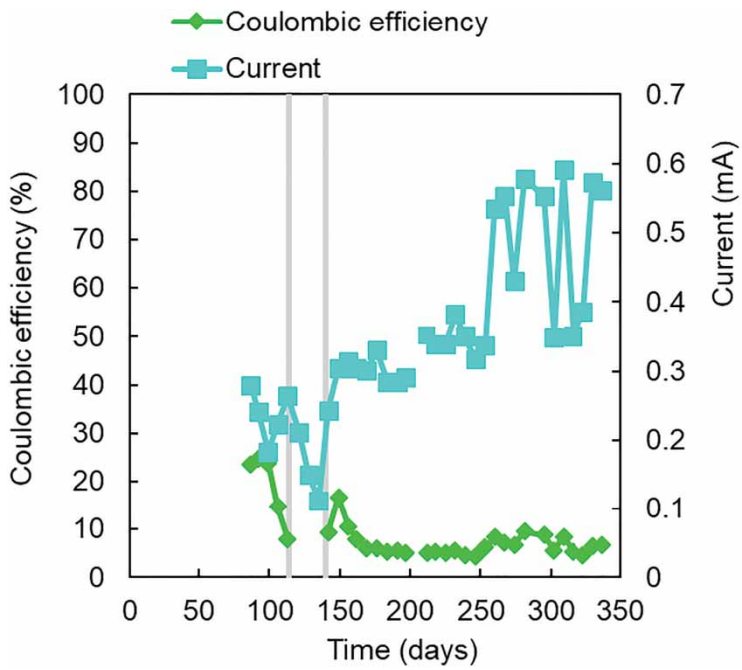


Figure 5 | Coulombic efficiency and current of D-MFC under continuous-flow condition. The data under batch condition was excluded.

Table 2 | Comparison of removal rates of phenol, thiocyanate and thiosulfate

Substrate type	MFC type	Degradation rate (mg/L/day)			Reference
		Phenol	Thiocyanate	Thiosulfate	
Synthetic coke-oven wastewater	Dual	1,200 (Cathode, with aeration)			Feng *et al.* (2015)
Real coke-oven wastewater	Dual	34.3 (Cathode)	16.5 (Cathode)		Wu *et al.* (2018)
Real coke-oven wastewater	Single	216			Liu *et al.* (2017)
Synthetic coke-oven wastewater	Dual	60 (Anode)			Wang *et al.* (2019)
Thiocyanate	Dual		7.1 (Anode)		Ni *et al.* (2018)
Phenol	Dual	500 (Anode)			Luo *et al.* (2009)
Phenol	Single	90			Mousavi *et al.* (2016)
Phenol	Dual	105.6 (Anode)			Yang *et al.* (2018)
This study (batch condition)	Dual	26.2 (Anode) 30.4 (Cathode)	1.7 (Anode) 24.1 (Cathode)	35.7 (Anode)	
This study (continuous-flow condition)	Dual	104 (Anode) 43.6 (Cathode)	45.2 (Anode) 250 (Cathode)	331 (Anode) 17.3 (Cathode)	

The degradation rate of D-MFC under continuous-flow condition was the average from day 267 to day 281 ($n = 3$).

oxygen being transferred through the cation exchange membrane (CEM) from the cathode side). These degradation rates were compared with other literature values as presented in Table 2. The thiocyanate degradation rate of D-MFC (250 mg/L/day) was higher than for other studies. This suggests that D-MFC has remarkable ability of thiocyanate degradation. On the other hand, some reported higher phenol degradation rates than this study (104 mg/L/day). This suggests that the improvement of Coulombic efficiency is key to enhancing phenol degradation rates of D-MFC. These results showed that the degradation rate was expected to be further improved; however, D-MFC had enough potential for practical use based on the concentrations of coke-oven wastewater.

Effect of phenol on thiocyanate removal

As previous work indicated that thiocyanate degradation was inhibited by phenol (Oshiki *et al.* 2019), the membrane of D-MFC was removed and thiocyanate degradation before and after the removal of membrane was compared in order to evaluate whether phenol was an inhibitor. Thiocyanate concentrations during the treatment in the cathode chamber of D-MFC before and after removal of the membrane are shown in Figure 6. It seems that the thiocyanate degradation rate decreased immediately after removal of the membrane. This suggests that phenol may have an inhibitory role on thiocyanate degradation, due to its toxicity to thiocyanate-degrading bacteria. Therefore, the membrane is essential for an MFC to treat phenol, thiosulfate and thiocyanate simultaneously. S-MFC did not have a membrane. Therefore, even if S-MFC was equipped with air cathode with pre-enriched thiocyanate-degrading biofilm, S-MFC could not degrade thiocyanate due to inhibition by phenol.

Microbial community

The microbial communities sampled at AE, CE, AS and CS of D-MFC were analyzed. The relative abundance of OTUs closely related to order *Chromatiales*, which is known as thiocyanate-degrading bacteria (Oshiki *et al.* 2019) was shown in Table 3. Some species within the order were reported as thiocyanate-degrading bacteria (Sorokin *et al.* 2014; Watts *et al.* 2017; Oshiki *et al.* 2019). The relative abundance of *Chromatiales*-related bacteria of CE was much larger (>24%) than that of AE, AS and CS (<3%). The results suggested that *Chromatiales*-related bacteria were enriched and maintained on the cathode during D-MFC operation and thiocyanate was mainly degraded by cathode biofilm. The microbial communities other than *Chromatiales* were different on the electrodes and in the solutions of anode and cathode (Figure S8). Halophilic phenol-degrading electroactive bacteria could not be characterized since this bioprocess has never been reported in literature. Further studies are necessary to characterize them.

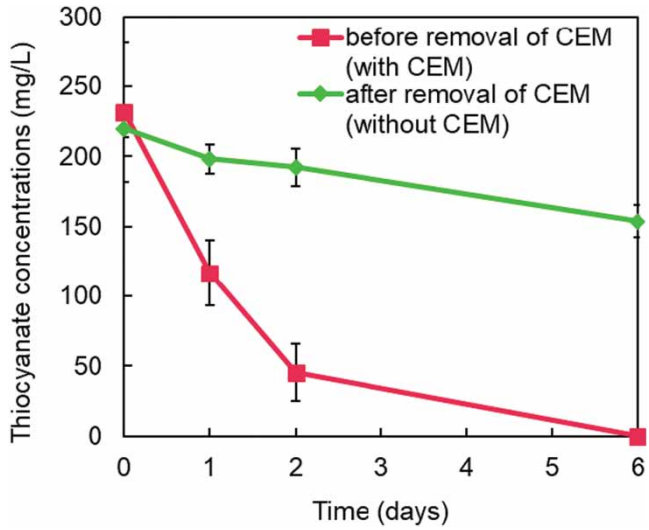


Figure 6 | Changes of thiocyanate concentration in the cathode chamber of D-MFC before and after removal of membrane (red: before removal of the membrane, green: after removal of the membrane). Error bars are standard deviations (red: $n = 3$, green: $n = 5$).

Table 3 | Relative abundance of *Chromatiales*

	AE	CE	AS	CS
(A) Start-up	–	26.2%	–	–
(B) Dual-chamber	1.6%	25.4%	1.9%	0.4%
(C) Single-chamber	1.0%	27.0%	1.6%	–
(D) Dual-chamber	2.8%	24.2%	1.9%	0.9%

Chromatiales was enriched and maintained on the cathode during D-MFC operation (AE: anode electrode, CE: cathode electrode, AS: anode solution, CS: cathode solution).

CONCLUSIONS

A dual-chamber microbial fuel cell (D-MFC) with a thiocyanate-degrading biofilm enriched at its air cathode achieved simultaneous phenol, thiosulfate and thiocyanate degradation without active aeration. The D-MFC showed much higher thiocyanate degradation rate than the control single-chamber MFC. This suggested the pre-enrichment could accelerate thiocyanate degradation in MFC. In addition, after removing membrane, the thiocyanate degradation rate decreased immediately. This suggests that phenol may have an inhibitory role on thiocyanate degradation, due to its toxicity to thiocyanate-degrading bacteria. Therefore, the membrane is essential for an MFC to treat phenol, thiosulfate and thiocyanate simultaneously. This study demonstrated the feasibility of treating coke-oven wastewater by a D-MFC with a thiocyanate-degrading biofilm enriched at the air cathode.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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