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Treatment and electricity harvesting from sulfate/sulfide-containing wastewaters using microbial fuel cell with enriched sulfate-reducing mixed culture

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HIGHLIGHTS

- ▶ We started up microbial fuel cell (MFC) using enriched sulfate-reducing mixed culture.
- ► Sulfate-reducing bacteria and anode-respiring bacteria were enriched in anodic biofilms.
- ► The MFC effectively remove sulfate to elementary sulfur in the presence of lactate.
- ► The present device can treat sulfate laden wastewaters with electricity harvesting.

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ABSTRACT

Anaerobic treatment of sulfate-laden wastewaters can produce excess sulfide, which is corrosive to pipelines and is toxic to incorporated microorganisms. This work started up microbial fuel cell (MFC) using enriched sulfate-reducing mixed culture as anodic biofilms and applied the so yielded MFC for treating sulfate or sulfide-laden wastewaters. The sulfate-reducing bacteria in anodic biofilm effectively reduced sulfate to sulfide, which was then used by neighboring anode respiring bacteria (ARB) as electron donor for electricity production. The presence of organic carbons enhanced MFC performance since the biofilm ARB were mixotrophs that need organic carbon to grow. The present device introduces a route for treating sulfate laden wastewaters with electricity harvesting.

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1. Introduction

Sulfate-laden wastewaters are generated in production of sugar, alcohol, pharmaceutical products and monosodium glutamate. Anaerobic treatment of sulfate-laden wastewaters would produce H_2S , which is corrosive to metals and is toxic to living species [1]. Conventional biological process to treat sulfate-laden wastewater consists of two processes, sulfate reduction to sulfide by sulfate-reducing bacterial (SRB) and sulfide-oxidation to sulfur (S^0) by sulfide-oxidizing bacteria [2].

Microbial fuel cells (MFC) deliver the potential to directly generate electricity from degradation of organic and inorganic substrates in wastewater and in sludges [3,4]. Sulfide removal in MFC was

studied [5–12]. Rabaey et al. [13] cultivated mixed sulfide-oxidizing bacteria in MFC system for simultaneous sulfide removal and electricity generation. Sun et al. [14] demonstrated that bacterial catalysis played an important role in the electricity generation from a sulfide-fed MFC with anaerobic sludge. Sun et al. [15] investigated the microbial communities in MFC for sulfide conversion and electricity generation. The exoelectrogenic, sulfide-oxidizing and sulfate-reducing bacteria can harvest electricity during sulfide oxidation [16,17].

Zhang et al. [18] proposed the possibility to utilize the sulfate-reducing bacteria to convert sulfate to sulfide, then the MFC to convert the formed sulfide to elementary sulfur. Zhao et al. [19] studied the electricity generation potential from MFC with sulfate removal. Zhao et al. [20] demonstrated the anodic potential was controlled by the sulfide concentrations in the chamber compartment when treating sulfate-laden wastewaters. Ghangrekar et al. [21] noted that the performance of MFC declined as COD/SO₄²⁻ ratio was reduced. Efficient treatment of sulfate-laden wastewater

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Table 1Compositions adopted in the present tests.

	Stage 1 (10 d)	Stage 2 (30 d)	Stage 3 (12 d)
MFC-A MFC-B	3.0 g/L sodium lactate + 2.0 g/L MgSO ₄ ·7H ₂ O 3.0 g/L sodium lactate+2.0 g/L MgSO ₄ ·7H ₂ O	0.53 g/L Na ₂ S·9H ₂ O 3.0 g/L sodium lactate	3.0 g/L sodium lactate +0.53 g/L Na ₂ S·9H ₂ O
MFC-C	3.0 g/L sodium lactate+2.0 g/L MgSO ₄ ·7H ₂ O	2.0 g/L MgSO ₄ ·7H ₂ O	3.0 g/L sodium lactate+2.0 g/L MgSO ₄ ·7H ₂ O

needs multiple steps for its decontamination. Sulfate is an electron acceptor in redox reaction, which can compete with anodic surface in MFC for released electrons. This study cultivated sulfate-reducing mixed culture to start up MFC using sulfate or sulfide-containing wastewaters. The lactate was applied in some tests in excess so the organic compounds are not the limiting substrates.

2. Materials and methods

2.1. Inoculum and MFC

The waste activated sludge sample was collected from a municipal wastewater treatment plant in Taipei City, Taiwan, which treats sewage at biological oxygen demand (BOD5) of $185 \,\mathrm{mg}\,\mathrm{L}^{-1}$ and suspended solids (SS) of $190 \,\mathrm{mg}\,\mathrm{L}^{-1}$. The collected sludge had the following characteristics: pH 7.3, total suspended solids of

 $16,700\,\mathrm{mg}\,\mathrm{L}^{-1}$ and volatile suspended solids of $12,800\,\mathrm{mg}\,\mathrm{L}^{-1}$. The sludge was filtered using coarse screen and was then incubated anaerobically to enrich sulfate-reducing consortium for one month in a medium of composition (per liter): $2.0\,\mathrm{g}\,\mathrm{MgSO_4}\cdot\mathrm{7H_2O}$; $5.0\,\mathrm{g}\,\mathrm{sodium}\,\mathrm{citrate}$; $1.0\,\mathrm{g}\,\mathrm{NH_4Cl}$; $0.5\,\mathrm{g}\,\mathrm{K_2HPO_4}$; $5\,\mathrm{g}\,\mathrm{sodium}\,\mathrm{lactate}$; $1.0\,\mathrm{g}\,\mathrm{Fe}(\mathrm{NH_4})_2(\mathrm{SO_4})_2$; $1.0\,\mathrm{g}\,\mathrm{yeast}\,\mathrm{extract}$; $1\,\mathrm{mL}\,\mathrm{Wolf}$'s vitamin solution and $1\,\mathrm{mL}\,\mathrm{Wolf}$'s mineral solution. The wastewater pH was adjusted to $7.5\pm0.1\,\mathrm{using}\,1\,\mathrm{N}\,\mathrm{HCl}$.

Two-chamber MFCs comprising anode and cathode cylindrical chambers (inside diameter 5 cm; length 4 cm each) were connected to a cation exchange membrane (CEM) (Ultrex CMI-7000; Membrane International, Inc., Glen Rock, NJ, USA). Anode was made of carbon felt (area, 6 cm²), cathode was made of carbon cloth (area, 9 cm²) (W0S1002; CeTech Co., Taichung, Taiwan). The cathode had a surface loading of 0.5 mg cm² Pt catalyst. The electrodes were placed at the centers of each chamber and were parallel to the CEM.

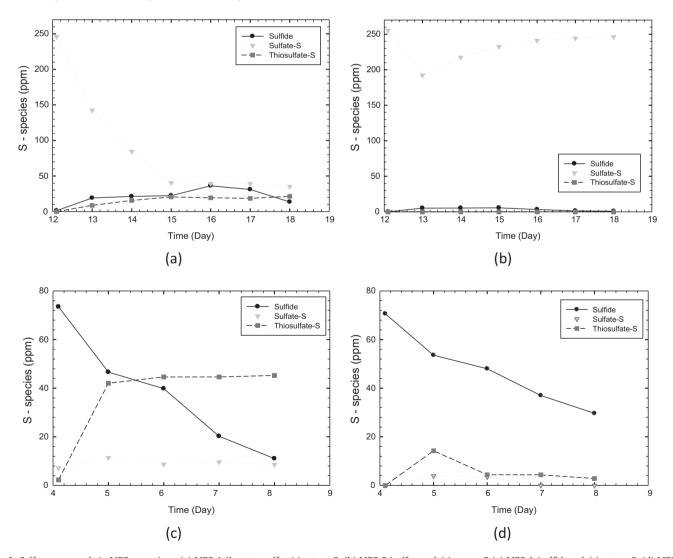


Fig. 1. Sulfur compounds in MFC operations. (a) MFC-A (lactate+sulfate) in stage 2. (b) MFC-C (sulfate only) in stage 2 (c) MFC-A (sulfide only) in stage 3. (d) MFC-C (lactate+sulfide) in stage 3.

Table 2Reactor performances of testing conditions.

Condition	OCV (mV)	$P_{max}(\mathrm{mWm^{-2}})$	Sulfate removal %	Sulfide removal%	COD removal%	Coulombic efficiency(CE%)
Lactate + Sulfate	720	255	84.2	_	24.3	17.5
Lactate only	571	12.7	_	_	21.8	10.5
Sulfate only	644	2.0	3.5	_	-	-
Sulfide only	742	21.9	_	85.3	_	6.7
Lactate + Sulfide	656	276	-	57.7	25.0	9.6

2.2. Startup and operation

Three identical MFCs (MFC-A, MFC-B, MFC-C) were tested. The MFCs were operated in fed-batch mode. All electrochemical experiments and MFC operations reported in this study were carried out in duplicate at 30 $^{\circ}$ C and only the mean values were reported. These three MFCs were operated in the feed compositions as listed in Table 1.

The enriched mixed culture was fed into the MFC anodic chamber. The cathodic medium was a mixture of 50 mM phosphatebuffered saline (PBS) buffer (pH 6.9) and 50 mM K₃Fe(CN)₆. The MFC was inoculated with 10 mL of enriched mixed culture at 30 °C. The synthetic wastewater was fed to the anodic chamber containing (per liter) 3.0 g sodium lactate, 2.0 g MgSO₄·7H₂O; 2.0 g NaHCO₃; 1.0 g NH₄Cl; 1.8 g KH₂PO₄; 1.2 g K₂HPO₄ and 10 mL of trace element (stage 1). After 10-d testing, the three MFCs were successfully started up and having similar performance. Then the feed to MFC-B was changed to lactate only mode (the sulfate salt removed), the feed to MFC-C was changed to sulfate only mode (the lactate sale removed), while the feed to MFC-A was kept unchanged. This period lasted for 30 d. Afterward, the feed to MFC-A were changed to sulfide only mode, that to MFC-B to lactate+sulfide mode, and MFC-C back to lactate + sulfate mode. The wastewater pH was adjusted to 8.0 ± 0.1 using 1 N NaOH or HCl for preventing loss of hydrogen sulfide in the beginning of the tests.

2.3. Electrochemical analysis

Voltage cross the $1\,k\Omega$ resistor was recorded at an interval of 2 min using a digital acquisition system (611D, CH Instruments Inc., USA). The current density was normalized by the projected surface areas of anodic sides. Polarization curves were measured by applying a linear potential decrease of $1\,mV\,s^{-1}$ from the open circuit voltage to $0\,mV$ [22,23]. The Coulombic efficiency was calculated by dividing coulomb output (integrating current and time) by total coulomb input (based on total lactate or sulfide in feeds) (see Table 2).

The sulfide concentration was determined by potential titration using Sure-FlowTM Combination Silver/Sulfide Electrodes. Concentrations of sulfate, thiosulfate and sulfite were measured using an ion chromatography (Dionex ICS-3000). Element sulfur in the MFC discharge was measured following the sulfite method [24]. All measurements were repeated at least three times for data assurance.

3. Results and discussion

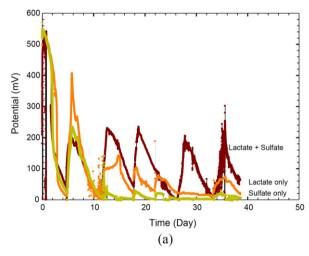
3.1. Wastewater characteristics

Take the 2nd cycle in stage 2 for demonstration purpose. With lactate + sulfate feed, the sulfate concentration was reduced from its initial value ($248 \, \text{mg} \, \text{L}^{-1}$ as S) to $39.3 \, \text{mg} \, \text{L}^{-1}$ as S in 3-d testing (Fig. 1a). The concentrations of thiosulfate and sulfide were only slightly increased. No sulfite was detected in the suspension. Hence the principal product from lactate + sulfate feed was S⁰ or polysulfides (S_X^{2-}) at 84.1% conversion (the use of protocols by Ref. [24]

revealed the presence of S^0 in the MFC liquid. We did not measure the overall S^0 concentration for not deteriorating the mature biofilm on anodic surface for further tests). In the present test we use S_x to present the sum of S^0 and S_x^{2-} for brevity sake. Meanwhile, the solution COD was reduced by 24.3% (data not shown).

With sulfate only feed, the concentration of sulfate was reduced in one day from 262 mg L^{-1} to 192 mg L^{-1} as S (Fig. 1b). No accumulation of thiosulfate, sulfite or sulfide was noted. Hence, most sulfate was converted to S_x . In Fig. 1b the formed S_x was utilized by SRB to sulfide, probably using the lysed biomass as the carbon source. The use of formed sulfur as substrate for SRB to regenerate sulfide was previously reported [25].

When the feed to MFC-A was shifted from lactate + sulfate to sulfide only, the sulfide dropped in concentration from 78 mg L^{-1} to 11 mg L^{-1} as S in 4-d testing (Fig. 1c). Neither sulfate nor sulfite was



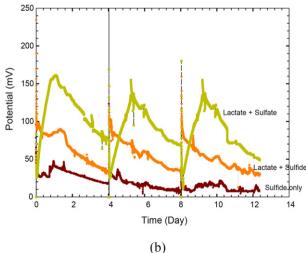


Fig. 2. Voltage drops of MFC at different feeds. (a) Stage 1 (d 1-12) and stage 2 (d 13-d 40). (b) Stage 3.

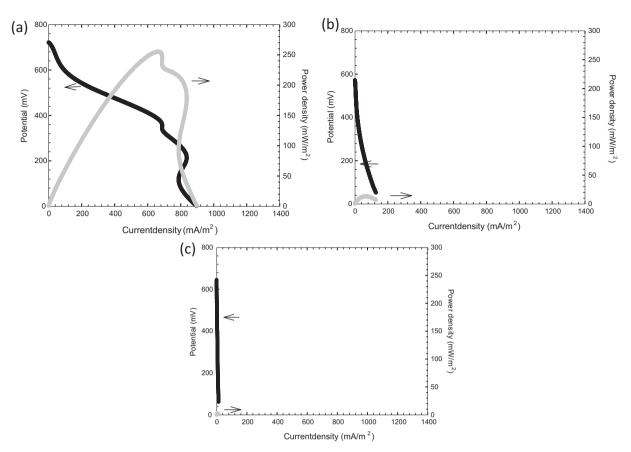


Fig. 3. Polarization curves and power density curves at the end of stage 2. (a) MFC-A (lactate + sulfate). (b) MFC-B (lactate only). (c) MFC-C (sulfate only).

formed. Instead, thiosulfate was accumulated to a level of $40 \, \text{mg L}^{-1}$ as S on day 1. During day 2–4, most sulfide consumed was converted to S_x .

In lactate+sulfide tests, the sulfide was reduced in concentration from $70\,\mathrm{mg}\,L^{-1}$ to $29.6\,\mathrm{mg}\,L^{-1}$ as S in 4-d testing (Fig. 1d). Only thiosulfate concentration was increased to $14.2\,\mathrm{mg}\,L^{-1}$ as S on day 1, then it was reduced over testing time. Hence, most sulfide consumed by lactate+sulfide feed was converted to S_x (95.9% conversion).

3.2. Cell performance

Fig. 2 shows the voltage drop data of the three MFCs. The current density patterns were similar to those of voltage drop and were not shown here for brevity sake.

When both lactate and sulfate were present, the maximum voltage drop in the stage 2 test for MFC-A ranged 240–280 mV, with maximum current density thus yield of 513 mA m $^{-2}$ (Fig. 2a). With lactate only feed, the MFC-B can produce maximum voltage drop of 65–145 mV. The MFC-C fed with sulfate only yielded negligible electricity owing to lacking of carbon source.

Comparing data in stage 1 and stage 2, the lactate + sulfate reactor (MFC-A) reached a stable MFC performance; the absence of sulfate led to decline in performance of MFC-B; and the shortage of lactate ceased electricity production in MFC-C. Although sulfate is an electron acceptor, a natural competitor with the anode surface, the presence of sulfate in lactate-fed MFC yielded more electricity than that with only lactate (Fig. 2a). Fig. 2b shows the voltage drop data of the three tested MFCs in stage 3. Comparing data in stage 2 and stage 3, when the feed to MFC-A was shifted from lactate+sulfate to sulfide only, its performance dropped over time. In MFC-B in which sulfide was added to the lactate only feed, the MFC

was revived to a stable performance with maximum voltage drop at 100–150 mV (ignoring the initial spikes at feeding). The presence of sulfide enhanced reactor performance when compared with the lactate only feeds. Adding lactate to the sulfate only feed in MFC-C increase electricity outputs, with maximum voltage drop at around 160 mV. The present tests revealed that the presence of sulfate and sulfide can enhance MFC performance.

3.3. Electrochemical characteristics

Fig. 3 shows the polarization curves and power density curves for the studied MFC at different feeds at the end of stage 2. The MFC-A with lactate + sulfate feed at the end of stage 2 had an open-circuit voltage (OCV) of 720 mV and the maximum power density (P_{max}) of 255 mW m⁻² (Fig. 3a). Additionally, the polarization curve for lactate + sulfate feed revealed the characteristics for power overshooting. This occurrence indicated that the resistances for the biofilms on anodic surface had reached its maximum capacity to electron transfer, so yielding insufficient biological reaction within the biofilm [23]. The corresponding Coulombic efficiency (CE%) was 17.5%. When the feed contained only lactate (MFC-B at the end of stage 2), the OCV was 571 mV and the P_{max} was 12.7 mW m⁻² (Fig. 3b). The corresponding CE% = 10.5%. With the sulfate only feed (MFC-C at the end of stage 2), OCV = 644 mV and P_{max} = 2.0 mW m⁻² only (Fig. 3c).

Fig. 4 shows the polarization curves and power density curves for the studied MFC at different feeds at the end of stage 3. The MFC-A with sulfide only feed had OCV of 742 mV and P_{max} = 21.9 mW m⁻² (Fig. 4a), with CE% = 6.7%. When the feed contained both lactate and sulfide, the OCV was 656 mV and P_{max} was 276 mW m⁻² (Fig. 4b), and CE% = 9.6%.

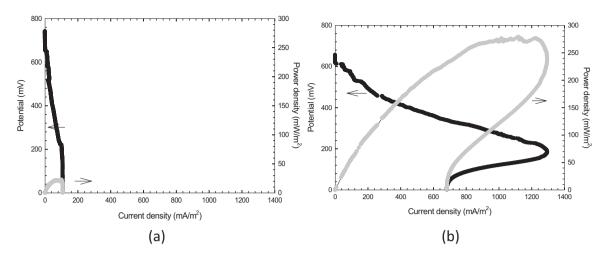


Fig. 4. Polarization curves and power density curves at the end of stage 3. (a) MFC-A (sulfide only). (b) MFC-C (lactate + sulfide).

The OCV presents the potential produced by the chemical reactions on two electrodes. Tests with lactate, sulfate, sulfide and their mix had similar OCV, therefore, the very different P_{max} obtained in Fig. 4 should be attributable to the very different R_{int} yielded in the response to the different compositions in feeds. The internal resistance of the MFC (R_{int}) can be estimated using OCV and P_{max} data as follows $R_{int} = \text{OCV}^2/4P_{max}$ [26]. The internal resistance of MFC with lactate + sulfate, lactate only, sulfide only and lactate + sulfide were estimated 0.51, 6.4, 6.3 and 0.39 k Ω , respectively.

4. Discussion

The present MFC with enriched SRB effectively converted most sulfate to S^0 with COD consumed as an energy source. Experimental observations suggest that in both systems the sulfide is the principal electron donor for the present MFC on electricity production. Restated, the enriched biofilm cannot utilize lactate as substrate for electricity generation. However, when both lactate and sulfate are present, the sulfate reducing bacteria (SRB) can reduce sulfate to sulfide using lactate as carbon and energy source; then the biofilm converts the formed sulfide to elementary sulfur with electricity. The R_{int} values by lactate + sulfade and lactate + sulfide feeds were both low. When lactate only or sulfide only was fed the MFC was with very high internal resistance. Only when both lactate and sulfate or sulfide was present in feed the R_{int} would be low.

The R_{int} of the MFC is composed of anodic (R_{anode}) and cathodic ($R_{cathode}$) resistances [27], with the former being further divided into a sum of mass transfer (R_{mt}), substrate utilization (R_{su}), extracellular electron transfer (R_{et}) and charge transfer (R_{ct}) [28]:

$$R_{\text{int}} = (R_{mt} + R_{su} + R_{et} + R_{ct}) + R_{cathode}$$
(1)

When feed compositions in the MFC tests were "swapped" the MFC performance could be shifted in a "reversible" manner, so the R_{et} should not be changed much in different stages of testing. Nien et al. [23] noted that in the tested MFC R_{ct} is generally less than other resistance components. The present tests were worked on diluted suspensions with fixed MFC geometry, suggesting similar R_{mt} for all systems. The remaining resistance that could contribute to significant change in R_{int} with the presence of lactate is R_{Su} . In lactate + sulfate tests the SRB converted sulfate to sulfide to supply as electron donor for their neighboring anode respiring bacteria. In lactate + sulfide tests SRB were not working, while organic carbon was needed by ARB to grow. This observation suggests that the principal ARB are mixotrophs that have a similar growth behavior

as the strain, *Pseudomonas* sp. C27, isolated by [16], that can grow only with organic carbons.

The power generation level by the MFC was low (up to 200–300 mW m $^{-2}$), rendering the MFC unfit as a practical power source. Conversely, the ARB presented on anodic surface could work together with SRB in MFC biofilm for treating sulfate-laden wastewaters. This study clearly demonstrated the feasibility of using SRB-MFC for treating sulfate-laden wastewater in a single unit, by firstly converting sulfate to sulfide and from yielded sulfide to electricity. The yielded element sulfur can be a potential material for recycling.

5. Conclusions

In this study, the MFC system inoculated with enriched SRB consortium has been successfully applied to treat sulfate and sulfide-laden wastewaters. In the presence of lactate, the sulfate in water could be reduced from 248 mg L⁻¹ to 39.3 mg L⁻¹ as S in 3-d testing with 84.1% conversion to S⁰. With or without the addition of lactate, the present MFC effectively oxidized sulfide in waters to S⁰. The MFC produced electricity from sulfate- or sulfide-laden wastewaters if lactate was presented. The internal resistance of MFC was contributed by the substrate resistance, mainly being attributable to the activities of ARB which were mixotrophs that need lactate as carbon source. The results presented in this study revealed the potential of the present SRB-MFC to effectively treat sulfate-laden wastewaters with electricity harvesting.

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