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Microbial fuel cell-based biosensor for toxic carbon monoxide monitoring

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ABSTRACT

This study presents an innovative microbial fuel cell-based biosensor for carbon monoxide (CO) monitoring. The hypothesis for the function of the biosensor is that CO inhibits bacterial activity in the anode and thereby reduces electricity production. A mature electrochemically active biofilm on the anode was exposed to CO gas at varied concentrations. A proportional linear relationship (R^2 = 0.987) between CO concentration and voltage drop (0.8 to 24 mV) in the range of 10% and 70% of CO concentration was observed. Notably, no further decrease of voltage output was observed by with further increasing CO concentration over 70%. Besides, the response time of the biosensor was 1 h. The compact design and simple operation of the biosensor makes it easy to be integrated in existing CO-based industrial facilities either as a forewarning sensor for CO toxicity or even as an individual on-line monitoring device.

1. Introduction

Carbon monoxide (CO) is considered as one of the most hazardous gasses for human health. Moreover, CO imposes negative environmental impact Unfortunately, significant emissions of CO are generated as a byproduct of power plants and other human chemical activities [15,4]. Thus, timely gas leakage detection or forewarning is important for protecting human beings and work places from CO inroad. Over the last decades, a great deal of research has been dedicated in development of optical, semiconductor or electrochemical CO sensors. These sensors present high sensitivity and could detect CO at very low concentrations ranging from 1 ppm to hundreds ppm [13,18,22]. However, CO liberated from incomplete combustion or steel mill industry is usually at much higher levels [8]. So far, there is not any compact form of sensors focusing on the detection of high concentrations of CO. Besides, most of conventional sensors need noble metal fabrications which make them relatively expensive. Moreover, many conventional CO sensors operate at high temperatures (above 100 °C), and thus, are energy intensive. Thus, a simple, efficient and cost-effective sensor capable of CO monitoring at high concentrations, is needed.

Recently, various microbial fuel cell (MFC)-based biosensors have been presented. MFC are attractive as biosensors due to the sustainability, and also the easily detected signal. MFC biosensors have been extensively studied for various monitoring applications such as microbial activity, chemical/biochemical oxygen demand [20], volatile fatty acids [10,11], nitric oxide reduction [23] and heavy metals (e.g. Cu^{2+}) [16,9]. MFC-based biosensors have also been employed to detect toxicity of specific pollutants (e.g. formaldehyde, Cu^{2+}) based on potential toxicity on exoelectrogens and reduction of electricity production [19].

Although CO could be utilized by Clostridium species as substrate [7], it is toxic to most of microorganisms. Thus, CO could also inhibit activity of bioanode in MFC and the drop of voltage could be an indicator of CO concentration. In this study, an MFC with mature anode biofilm was developed for the first time, to monitor CO concentration (termed as bioelectrical CO sensor). The objectives of this proof-of-concept study were (i) to demonstrate the feasibility of MFC for monitoring at high CO concentrations, (ii) to elucidate the correlation between voltage and CO concentration.

2. Materials and methods

2.1. Reactor configuration and startup

The MFC reactor used in this study consisted of two rectangular chambers made of nonconductive polycarbonate plates. The volume of anodic and cathodic chamber was of 70 and 35 mL, respectively. Two chambers were separated by a cation exchange membrane (CEM) (CMI 7000, Membrane international, NJ). The anode electrode was carbon brush (5.9 cm in diameter, 6.9 cm in length, Mill-Rose, USA) which was thermally pretreated at 450 °C for 10 min prior to use. Exoelectrogenic

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biofilm were enriched in the anode by inoculating with domestic wastewater which was taken from primary clarifier (Lyngby Wastewater Treatment Plant, Copenhagen, Denmark) and amended with 1 g/L sodium acetate (NaAc). Cathode electrode was made of Titanium mesh (4 cm × 3.5 cm) coated with Pt/C (0.5 mg (Pt)/cm^2). The cathode chamber was filled by 50 mM phosphate buffer (PBS, pH 7.0) and constantly aerated by a pump through a sterile needle connected with 45 μm filter. Electric circuit between anode and cathode electrodes was connected via a 1000 Ω external resistance for voltage recording. After approximately 40 days operation, stable electrical energy output (620 ± 20 mV, set as baseline of sensing) was achieved in several successive batch assays, which indicated that electro-active biofilm was well established on the anode.

2.2. CO sensing tests

During batch tests, wastewater was replaced by nutrient solution (pH 7.2 ± 0.5) containing 50 mM PBS, 0.13 g/L KCL, 0.31 g/L NH_4Cl, 1 g/L NaAc, vitamin solution and trace mineral solution as previously described [21]. This nutrient solution was added to the anode chamber prior to each test to maintain the same conductivity and pH. Previously study indicated that effect of CO on conductivity and pH was negligible due to its poor solubility.

Before initiation of each batch test, the anode was flashed with nitrogen to remove oxygen. Subsequently, different concentrations of CO (from 0% to 100% at 10% interval, mixed with nitrogen) were fed into the anode through a peristaltic pump at 4 mL/min (OLE DICH, Instrumentmakers Aps, Denmark) (Fig. 1). A gas-diffuser was set on the bottom of anode chamber to cut CO into micro-bubbles upwards. After each test, the anolyte was refilled with fore-mentioned fresh nutrient solution to acquire fully recovery of electricity production. All the tests were conducted in replicate in water bath at 25 ± 0.5 °C.

2.3. Chemicals and analyses

All chemicals were analytical pure without further purification. CO was purchased from AGA Industrial Gases (Denmark). Voltage across the resistance was recorded at 5 min intervals throughout the test using a potentiostat (CT-4008W, Neware Battery Testing System, China).

3. Results and discussion

The bioelectrical CO sensor was tested with different CO concentrations (as percentage). Fig. 2 illustrates the correlation of voltage drop which was determined as the voltage difference before and 1 h after adding CO in the anode. It can be seen that voltage drop was

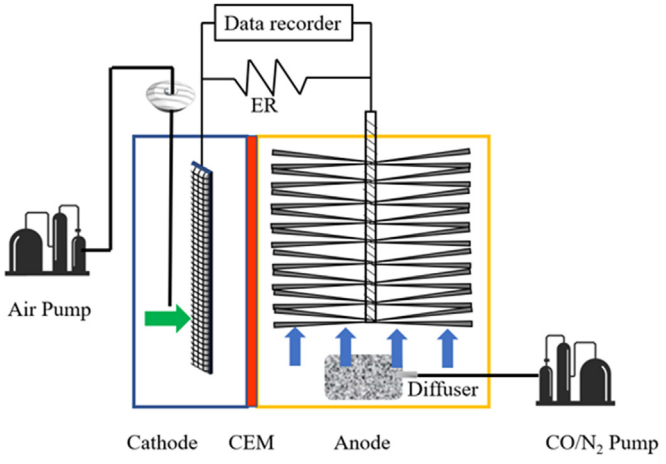

Fig. 1. Schematic illustration of bioelectrical CO sensor.

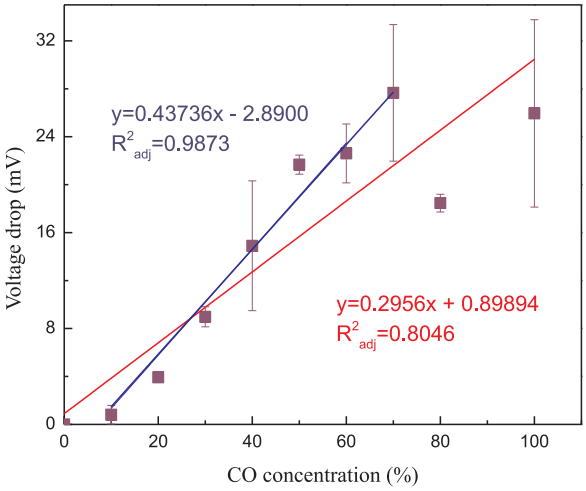

Fig. 2. The relationship between voltage drop (△mV) and CO concentrations after 1 h operation and linear correlation in the CO concentration ranges of 10–70% (Blue line) and 0–100% (Orange line), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

increased with the increasing of CO concentrations. Detectable inhibition (~ 1 mV drop) firstly appeared when 10% CO (mixed with 90% of N_2) was pumped into the anode chamber. Approximately 24 mV of voltage drop was observed when CO concentration was up to 70% and then leveled out in the range of 22–34 mV for higher CO concentrations. Overall, the regression coefficient (R^2_{adj}) was 0.987 which indicated that the biosensor could be used to monitor the CO concentrations in the range of 10–70%. As anolyte was amended with sufficient sodium acetate which provided sufficient substrate for continuous electricity generation, the voltage decrease could only be explained by the slight inhibition effect of CO on the exoelectrogens, and thereby reducing the amounts of electrons generated from microbial metabolism. When the CO concentration was below 10%, toxicity was hardly detected. However, when CO was higher than 70%, the voltage did not decrease further probably due to the limited solubility of CO in the anolyte. These results indicate that MFC can be an effective biosensor for CO monitoring. CO could affect the current generation of MFC biosensor in two different ways. Firstly, similar to human CO poisoning, carbon monoxide might chelate with enzymes, which are involved in extracellular electron transfer or cell growth [6]. Secondly, CO may influence the metabolic processes.

The response time (RT), defined as the minimum time needed to acquire a reliable voltage measurement correlating to CO concentration was assessed. The biosensor became more sensitive (shorter RT) at higher CO concentrations (Fig. 3a). Accordingly, circuit current needed more time to recover to the former level after CO exposure. To be more specific, no lag phase of recovery was needed when CO inlet concentration was lower than 10% while it would take more than an hour to fully recover when 70–100% CO was tested. Actually, the voltage output would not immediately stop decreasing after the exposure at high CO levels. Instead, the voltage continued decreasing for approximately 20 min after CO was replaced by nitrogen. It could be deduced that the toxicity of CO might have negatively affected the microbial metabolism rather than thermodynamic electron-transfer reactions. Fig. 3b presents the different regression coefficients (R^2) between current and CO concentrations at different operating-time. Apparently, the correlation became more linear when the reaction time increased. The results indicated that in order to acquire an accurate monitoring of CO concentration, at least one-hour response time (R^2 of 0.987) should be applied (Fig. 3b).

Benefit from better characteristics of chemical catalysts, chemical based CO sensors (Table 1) generally presented higher selectivity and

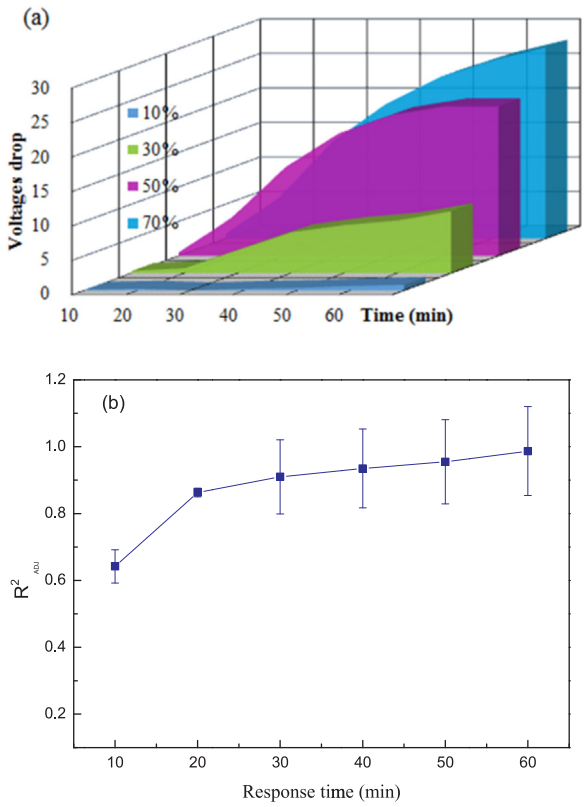

Fig. 3. Voltage of different CO concentrations against time (presented as mean values of multiple tests) (a) and R^2 against response time (b).

precision than other conventional sensors. However, chemical catalysts are mostly heavy metals and expensive and may cause environmental pollution upon use due to the utilization of potential toxic chemicals. Furthermore, most of other recently developed CO sensors require either high temperature or involve complex noble metals which make them uneconomical feasible. The bioelectrical CO sensor reported here is a simple and cheap alternative as no additional signal transducer and chemicals were needed. The electricity consumption of operation was calculated as only 0.2 kW h, which was mainly consumed by air pump. It is the first time that bioelectrochemical system has been proposed for CO sensing. The bioelectrical CO sensor developed in this study is suitable on relatively higher concentrations of CO than other sensors. Therefore the bioelectrical CO sensor may fulfil its potency in respect to various industrial processes related to high emissions of carbon monoxide [3].

It has been reported that some acetogenic bacteria like *Clostridia* sp. could use CO as substrate in MFC for electricity production [3,6]. However, electricity generated from CO was not observed in this study, which could be due to the different anodic conditions (e.g., inocula). It could be evidenced that even with 100% CO, voltage was stabilizing at background value (~20 mV) during operation time after fully depletion of acetate (Fig. 4). Besides, in this study, the voltage drop started to

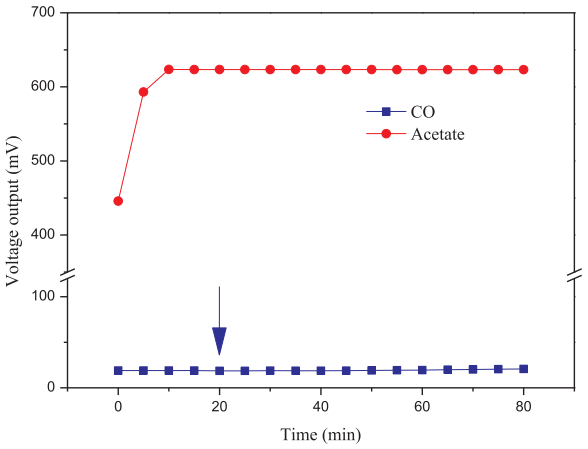

Fig. 4. Typical electricity generation from acetate (red line, without CO) and pure CO (blue line, no acetate). The arrow indicates the time of 100% CO input (4 mL/min). Before CO introduction, the MFC was starved for 18 h to make sure the depletion of organic matters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

decline at 10% CO even with adequate acetate addition (Fig. 2), which indicated that the pre-acclimated mature electro-activated biofilm was adapted to acetate rather than carbon monoxide. It has been reported that CO flux higher than 1.02 L/d (corresponding to 25% in this study) severely inhibited anodic activity [14]. To date, the most common intermediate/product for CO fermentation was acetic acid [1], while a large amount of which was added in anode chamber as electron donors. CO fermentation was consequently hindered due to the thermodynamics balance. It could also be evidenced that no ethanol was detected in the system (data not shown).

4. Implications

The bioelectrical sensor developed here shows promising perspectives. For instance, it can find applications in areas where high CO emissions can arise such as incineration facilities. Furthermore, the sensor has economic merits as it operates at room temperature, does not need expensive noble metals as sensing elements and can operate without electricity input if a single-chamber MFC with air cathode is employed.

Though promising, the bioelectrical sensor still has some challenges in terms of operation, sensitivity and selectivity. Toxic gases are unlikely emerged as pure CO, thus further research is in progress to focus on the selectivity and stability, identifying the potential affecting factors and underlying sensing mechanisms with respect to microbiology. Last but not least, as microbes in anode are easily transformed, which means they are vulnerable if being exposed for the long-term in toxic conditions, the microbial flora should be examined. Thus the system need to be further optimized for ensuring correct monitoring in field application.

Table 1
Comparisons of various forms of CO sensors developed recently.

Type	Sensing element	Temperature (°C)	Detection range	Correlation	References
Chemical translation sensor	Human olfactory system	N/A	500 ppm – 1600 ppm[a]	N/A	[2]
Organometallic chemistry sensor	CuO Nanoparticles	210	100 ppm[a]	N/A	[12]
Optical gas sensor	Au/ZnO sensing film	Room temperature	0.5 – 100 ppm	Linear	[15]
Impedancemetric sensor	layered perovskite-type oxides	400	50 – 600 ppm	Linear	[17]
Time division multiplexing technique	Single-reflection spherical optical mirror	N/A	2.96 – 2500 ppm	Exponential	[5]
Bioelectrical CO Sensor	Bioanode	25	(1– 7) $\times 10^5$ ppm	Linear	This study

[a] The minimum detection test is not provided by authors.

5. Conclusion

This study for the first time investigated the feasibility of MFC-typed sensor for CO detection, employing anode exoelectrogens as key element. Reproducible voltage drops caused by CO toxicity as indicating signals were obtained to designate CO concentrations. A linear relationship (R^2 = 0.987) between the electric signals and CO concentrations was discovered over the range of 10–70% and 50 min to one-hour response time was recommended. This novel biosensor could be in situ employed in remote places where electricity is in absence as the monitoring system operates without electricity addition. Though the bioelectrical CO sensor has a potential, further improvements of anti-interference and selectivity, as well as reactor optimization for more compact configuration, is of significance to fit applicable demands.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

References

[1] H.N. Abubackar, F.R. Bengelsdorf, P. Duerre, M.C. Veiga, C. Kennes, Improved operating strategy for continuous fermentation of carbon monoxide to fuel-ethanol by clostridia, Appl. Energy 169 (2016) 210–217.
[2] M. Bergmann, M. Egert, H. Plenio, Malodorogenic sensing of carbon monoxide, Chem.-A Eur. J. 23 (2017) 13328–13331.
[3] M. Devarapalli, H.K. Atiyeh, J.R. Phillips, R.S. Lewis, R.L. Huhnke, Ethanol production during semi-continuous syngas fermentation in a trickle bed reactor using Clostridium ragsdalei, Bioresour. Technol. 209 (2016) 56–65.
[4] M. Diender, A.J. Stams, D.Z. Sousa, Pathways and bioenergetics of anaerobic carbon monoxide fermentation, Front Microbiol 6 (2015) 1275.
[5] M. Dong, C. Zheng, S. Miao, Y. Zhang, Q. Du, Y. Wang, et al., Development and measurements of a Mid-infrared multi-gas sensor system for CO, CO_2 and CH_4 detection, Sensors (Basel, Switzerland) (2017) 17.
[6] A. Hussain, G. Bruant, P. Mehta, V. Raghavan, B. Tartakovsky, S.R. Guiot, Population analysis of mesophilic microbial fuel cells fed with carbon monoxide, Appl. Biochem. Biotechnol. 172 (2014) 713–726.
[7] A. Hussain, S.R. Guiot, P. Mehta, V. Raghavan, B. Tartakovsky, Electricity generation from carbon monoxide and syngas in a microbial fuel cell, Appl. Microbiol. Biotechnol. 90 (2011) 827–836.
[8] C.H. Im, C. Kim, Y.E. Song, S.-E. Oh, B.-H. Jeon, J.R. Kim, Electrochemically enhanced microbial CO conversion to volatile fatty acids using neutral red as an electron mediator, Chemosphere 191 (2017) 166–173.
[9] Y. Jiang, P. Liang, C. Zhang, Y. Bian, X. Yang, X. Huang, et al., Enhancing the response of microbial fuel cell based toxicity sensors to Cu(II) with the applying of flow-through electrodes and controlled anode potentials, Bioresour. Technol. 190 (2015) 367–372.
[10] X. Jin, I. Angelidaki, Y. Zhang, Microbial electrochemical monitoring of volatile fatty acids during anaerobic digestion, Environ. Sci. Technol. 50 (2016) 4422–4429.
[11] X. Jin, X. Li, N. Zhao, I. Angelidaki, Y. Zhang, Bio-electrolytic sensor for rapid monitoring of volatile fatty acids in anaerobic digestion process, Water Res 111 (2017) 74–80.
[12] J. Jonca, A. Ryzhikov, S. Palussiere, J. Esvan, K. Fajerwerg, P. Menini, et al., Organometallic synthesis of CuO nanoparticles: application in low-temperature CO detection, Chemphyschem 18 (2017) 2658–2665.
[13] S. Kundu, R. Sudharson, M. Narjinary, Pd impregnated gallia: tin oxide nanocomposite an excellent high temperature carbon monoxide sensor, Sens. Actuators B-Chem. 254 (2018) 437–447.
[14] P. Mehta, A. Hussain, B. Tartakovsky, V. Neburchilov, V. Raghavan, H. Wang, et al., Electricity generation from carbon monoxide in a single chamber microbial fuel cell, Enzym. Microb. Technol. 46 (2010) 450–455.
[15] A. Paliwal, A. Sharma, M. Tomar, V. Gupta, Carbon monoxide (CO) optical gas sensor based on ZnO thin films, Sens. Actuators B-Chem. 250 (2017) 679–685.
[16] Y. Shen, M. Wang, I.S. Chang, H.Y. Ng, Effect of shear rate on the response of microbial fuel cell toxicity sensor to Cu(II), Bioresour. Technol. 136 (2013) 707–710.
[17] Y. Shimizu, S. Yamamoto, S. Takase, A thick-film impedancemetric carbon monoxide sensor using layered perovskite-type cuprate, Sens. Actuators B-Chem. 249 (2017) 667–672.
[18] M. Shojaee, S. Nasresfahani, M.H. Sheikhi, Hydrothermally synthesized Pd-loaded SnO2/partially reduced graphene oxide nanocomposite for effective detection of carbon monoxide at room temperature, Sens. Actuators B-Chem. 254 (2018) 457–467.
[19] J.Z. Sun, G.P. Kingori, R.W. Si, D.D. Zhai, Z.H. Liao, D.Z. Sun, et al., Microbial fuel cell-based biosensors for environmental monitoring: a review, Water Sci. Technol. 71 (2015) 801–809.
[20] Y. Zhang, I. Angelidaki, Submersible microbial fuel cell sensor for monitoring microbial activity and BOD in groundwater: focusing on impact of anodic biofilm on sensor applicability, Biotechnol. Bioeng. 108 (2011) 2339–2347.
[21] Y.F. Zhang, I. Angelidaki, Submersible microbial desalination cell for simultaneous ammonia recovery and electricity production from anaerobic reactors containing high levels of ammonia, Bioresour. Technol. 177 (2015) 233–239.
[22] Q. Zhou, L.N. Xu, A. Umar, W.G. Chen, R. Kumar, Pt nanoparticles decorated SnO2 nanoneedles for efficient CO gas sensing applications, Sens. Actuators B-Chem. 256 (2018) 656–664.
[23] S.F. Zhou, S.B. Huang, J.X. He, H. Li, Y.Q. Zhang, Electron transfer of Pseudomonas aeruginosa CP1 in electrochemical reduction of nitric oxide, Bioresour. Technol. 218 (2016) 1271–1274.