**Quorum sensing alters the microbial community of electrode-respiring bacteria
and hydrogen scavengers toward improving hydrogen yield in microbial
electrolysis cells**

Weiwei Cai^a, Zhaojing Zhang^{b,c}, Ge Ren^{b,d}, Qiuxuan Shen^{b,d}, Yanan Hou^a, Anzhou Ma^b, Ye Deng^b,

Aijie Wang^{a,b*}, and Wenzong Liu^{b*}

a State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), Harbin 150090, P.R. China

b Key Laboratory of Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

c State Key Laboratory of Fine Chemicals, Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China

d University of Chinese Academy of Sciences, Beijing 100049, China

***Corresponding authors:**

Dr. Aijie Wang

1 State Key Laboratory of Urban Water Resource and Environment
Harbin Institute of Technology
Harbin 150090, China

2 Key Laboratory of Environmental Biotechnology
Research Center for Eco-Environmental Sciences
Chinese Academy of Sciences
Beijing 100085, China
E-mail: waj0578@hit.edu.cn

Dr. Wenzong Liu

1 Key Laboratory of Environmental Biotechnology
Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences
Beijing 100085, China
E-mail address: wzliu@rcees.ac.cn

Abstract

Quorum sensing has been widely applied to enhance the energy recovery of bioelectrochemical system as a sustainable pathway to enhance communication between cells and electrodes. However, how signalling molecules (acyl-homoserine lactones, AHLs) regulate the microbial community to improve hydrogen generation in microbial electrolysis cells (MECs) is not well understood, especially the subsequent influence on interspecies relationships among not only electrode-respiring bacteria but also hydrogen scavengers. Understanding AHL regulation in a complicated and actual biofilm system will be valuable for future applications of microbial electrochemical technology. Herein, we added short-chain AHLs (3OC6) to regulate the biofilm community on bio-electrodes in MECs. As a result, hydrogen yields were enhanced with AHL addition, increasing by 5.57%, 38.68%, and 81.82% with varied external voltages (0.8 V, 0.6 V, and 0.4 V, respectively). Accordingly, overall reactor performance was enhanced, including coulombic efficiency, electron recovery efficiency, and energy efficiency. Based on an electrochemical impedance spectra analysis, the structured biofilm under simple nutrient conditions (acetate) showed a lower internal resistance with AHL addition, indicating that the microbial communities were altered to enhance electron transfer between the biofilm and electrode. The change in the cathodic microbial structure with more electrochemically active bacteria and fewer hydrogen scavengers could contribute to a higher electron recovery and hydrogen yield with AHL addition. The regulation of the microbial community structure by AHLs represents a potential strategy to enhance electron transfer and hydrogen generation in bioelectrochemical systems.

Keywords: quorum sensing, microbial electrolysis cell, microbial community, electron

transfer

1. Introduction

Quorum sensing is becoming a well-established strategy to regulate the cell-to-cell communication that naturally occurs in biofilms [1]. The mechanism was reported to involve secreted signalling molecules that regulate a diverse array of physiological activities, such as symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation [2]. Acylated homoserine lactones (AHLs) were shown to be the key factors that function as the universal language for intraspecific communication [3-5], and recently, they have been reported to promote respiration activity involving electron transfer from cells to electrodes [6]. The promotion of respiration activity has been reported to occur through two main pathways: one that involves the modification of bacterial physiology, including membrane permeability [7] and gene expression [8], and a second that involves the regulation of networks responsible for electron shuttle biosynthesis, such as phenazine [9-11]. Hence, strategies that use AHLs to regulate electron transfer have attracted increased attention [12-14]. By utilizing the versatile ability of quorum sensing, many studies have focused on regulating electron transfer or the respiration process to enhance electricity output by bioreactors in bioelectrochemical systems (BESs). However, AHLs are commonly tested in pure culture, especially *Pseudomonas sp.*, which can transfer electrons from organics to electrodes as an electron donor [10, 11], and mixed cultures are more complex in terms of the electron transfer pathway, leading to competition and syntrophism in different communities that subsequently determine the performance of the BES [15, 16]. In general, interspecies interactions involve communication, typically via quorum sensing, and metabolic cooperation or competition [17], which may induce some species to override others based on electron flow adjustment.

Although functional microorganisms that are enriched under the high pressure selectivity in BESs are more likely to improve electron transfer, the relationship between electrode-respiring bacteria and methanogens remains an important issue in BES development because methanogens are an inevitable factor that reduce electron flow from bioelectrochemical reactions to intracellular metabolism [18]. In microbial electrolysis cells (MECs) in particular, some hydrogen scavengers are always present, such as homo-acetogens and methanogens, whose growth depends on proton and electron recovery, which reduces hydrogen yield [19-21]. Conventional regulation or domestication of functional biofilms is generally based on competition for nutrients or growth inhibition [22, 23], whereas AHLs can regulate antagonistic interactions among species within a biofilm and be targeted to enrich functional microorganisms [24]. AHLs are intraspecific signalling molecules that affect biofilm formation and cell density. An autoinducer diffuses passively through the bacterial membrane and accumulates both intra- and extra-cellularly in proportion to cell density [25] It has been demonstrated that the community composition and structure of a bio-anode is mostly determined during the stochastic assembly process in a BES [26]. Thus, AHLs could regulate the community to establish a dominant community group with a better electron transfer ability, which could primarily occupy the electrode surface during the inoculation and start-up of a bioreactor when AHLs are added. This possibly leads to the advantageous competition between electrode-respiring bacteria and other microorganisms (hydrogen scavengers, such as methanogens). Thus, this approach represents a new strategy to domesticate application-required functional communities in a BES via adding AHLs that could trigger interspecies competition, leading to reduced growth of non-target species and enhanced

product yield. However, no study has focused on mixed culture microbial community assembly at both the anode and cathode biofilms under quorum sensing regulation, especially the influence on interspecies communication and competition on the bio-cathode with the aim of enhancing energy recovery in MECs. According to our previous study, a higher hydrogen yield and better bioelectrochemical performance of the anode were obtained by using short-chain AHLs in MECs [27], but the associated mechanism requires further investigation regarding the relationship between bio-electrode community regulation and bioelectrochemical properties, as well as hydrogen yield.

Herein, we operated MEC reactors with a typical short-chain AHL (3OC6) to investigate the shift in the microbial communities in the anode and cathode biofilms in response to the addition of AHL. In addition, the consequent improvement in bioreactor performance was assessed to reveal the impact of AHL addition. To our knowledge, this is the first study to demonstrate hydrogen evolution enhancement regulated by quorum sensing in a mixed culture MEC. Hydrogen yields were measured in the presence of AHL at various external voltages. Electrochemical property and microbial community structure of bio-cathode were mainly disclosed to hydrogen yield change.

2. Material and methods

2.1 MEC construction and operation.

MEC reactors were constructed according to our previous study [28]. A single cube with a cylindrical chamber (4 cm long x 3 cm in diameter) was made of polycarbonate. The total volume was 28 mL, and a 10-mL injection syringe was attached to the cube and connected to a gas bag (0.2-L capacity, Dalian Delin CO. Ltd) to collect hydrogen. A carbon brush (25 mm

in diameter, 25 mm length; 0.22 m² surface area; fibre type: PANEX 33 160 K, ZOLTEK) was soaked in acetone for 24 h and heated in Muffin furnace at 450 °C for 60 min before being used as an anode [29]. A carbon cloth coated with a Pt catalyst layer (10% Pt/C mixed with Nafion solution to obtain 0.5 mg Pt/cm²) on one side was assembled as the cathode, and the total surface area was 7 cm². Six reactors were assembled to operate at room temperature (25 °C) in batch mode (24 h). Waste activated sludge used for inoculation was obtained from the secondary sedimentation tank at the Beijing Gaobeidian municipal wastewater treatment plant. All reactors were operated at an external voltage (supplied with a Switching Power Supply, FDPS-150, Fudantianxin Inc. China) adjusted from 0.8 V to 0.6 V to 0.4 V gradually. The MECs were fed with 26 mL of a 50:50 mixture of sodium acetate mixed with 100 mM PBS (Na₂HPO₄, 4.58 g/L; and NaH₂PO₄·H₂O, 2.45 g/L, pH=7.0 and nutrient solution, NH₄Cl, 0.31 g/L; KCl, 0.13 g/L; trace nutrient medium) at every cycle. AHL (3OC6, 10 mM) was mixed with the solution at the first inoculation, and the final concentration of AHL was 10 mM. The signal molecule was added at the end of each of the first three cycles at the same concentration of 10 mM.

2.2 Electrochemical analysis.

All electrochemical tests were performed with a saturated calomel reference electrode (SCE, model-217, Shanghai Precise. Sci. 91 Instru. Co., Ltd., China; 0.247 V vs. standard hydrogen electrode). A CHI 660e electrochemical station was employed for the cyclic voltammetry (CV) test that was performed after changing the substrate. The scan rate was 2 mV/s, ranging from -0.6 V to 0 V. Electrochemical impedance spectra (EIS) were obtained to investigate the resistance of individual electrodes in the MEC. The test was operated with an open circuit in

situ. A sine wave ranging from 1000 kHz to 10 mHz was applied to the cells at the open-circuit potential.

2.3 Analysis and calculation methods.

Scanning electron microscopy (SEM) images were collected with a field-emission scanning electron microscope (Hitachi 8020). AHL was detected by liquid chromatography-mass spectrometry (LC-MS). COD was determined by a standard method [30]. The current was automatically monitored with a Keithley instrument (2700, US). Gas production (hydrogen and methane) was detected by gas chromatography (GC) (EWAI, 4000A). All calculation methods in this study were based on our previous study [27]. The coulomb recovery ($Q=It$) from acetate at the anode rated with theoretical coulombs supplied from acetate was the coulombic efficiency (CE), which was applied to evaluate anode performance, including microbial activity and electron transfer. Electron recovery efficiency (ERE), which was determined by the ratio of coulombs in all products to the coulombs in the circuit, was used to reflect the performance of the cathode. Energy efficiency was used to evaluate the overall performance of the MEC in terms of thermodynamics feasibility. The specific equation was as follows:

$$\eta = W_{H_2} / W_E = (n \cdot \Delta H_{hydrogen}) / (E_{app} \cdot I \cdot t)$$

which only considered the input electric energy and heat energy in hydrogen (-237.1 kJ/mol).

The overall energy efficiency (including the chemical energy of the substrate, -872.9 kJ/mol for acetic acid) was as follows [31]:

$$\eta = W_{H_2} / W_E = (n \cdot \Delta H_{hydrogen}) / (E_{app} \cdot I \cdot t)(n \cdot \Delta H_{Substrate})$$

Hydrogen yield represented the conversion from acetate to hydrogen, and it was calculated by

the molar ratio of hydrogen to acetate.

2.4 DNA extraction and microbial community analysis.

Carbon brushes (bio-anode) and cloths (bio-cathode) were soaked in sterile water for several minutes to eliminate the effect of sludge at the anode. Subsequently, all bio-samples were collected to extract DNA for high-throughput sequencing. DNA was extracted with a FastDNA SPIN Kit for soil (MP). Based on the V4 hypervariable region of bacterial 16S rRNA genes, the following PCR primers were selected: 515F (GTGCCAGCMGCCGCGG) and 806R (GGACTACHVGGGTWTCTAAT), as they have been frequently used in previous related studies [32]. The raw sequencing data were generated with a MiSeq sequencing machine in fastq format. All sequencings were clustered at a 97% similarity level, and taxonomic assignments were made online by analysing the MiSeq data on the Galaxy pipeline: <http://172.16.60.161:8080/root>. The α -diversity of the microbial communities in the anode and cathode biofilms was described using the Shannon index. Principal coordinate analysis (PCoA) and heatmap analysis were used to assess the differences in microbial communities.

3. Results and discussion

3.1 Reactor performance.

Different voltages were applied to MEC reactors, and an improved hydrogen yield reflecting bioelectrochemical efficiency was observed following AHL addition. At 0.8 V for 24 h, there was a slight enhancement in hydrogen yield, increasing from 3.23 ± 0.18 mol H₂/mol acetate without AHL to 3.41 ± 0.17 mol H₂/mol acetate with AHL (fig. 1a). A significant improvement was observed when decreasing the external voltage to 0.6 V: only 2.12 ± 0.56 mol H₂/mol acetate was obtained from the control group without the addition of AHL, but a higher

hydrogen yield of 2.94 ± 0.29 mol H_2 /mol acetate indicated the positive effect of AHLs, representing a 38.7% increase. When the applied voltage was reduced to 0.4 V, there was an increase from 0.22 ± 0.04 mol H_2 /mol acetate to 0.40 ± 0.23 mol H_2 /mol acetate, showing an 81.8% increase over 24 h, although this was a very low hydrogen production rate at such a low voltage.

In addition to hydrogen yield, CE and ERE at both the anode and cathode indicated that the addition of AHLs influenced the cathode significantly (fig. 1 b and c). There was no obvious difference in CE, suggesting that AHLs maintained good electron transfer between microorganisms in the anode biofilm and from microbial cells to the electrode. It was interesting that similar CE values were obtained, around 82-85%, at 0.8 V and 0.6 V, where current value were similar (fig. S1). There was a clear decrease in CE at 0.4 V, as CE was lower than 50% with or without AHLs (42.93% and 43.91%, respectively). Importantly, the enhancement of ERE reflected the positive effect of AHLs on electron transfer at the cathode. As shown in fig 1c, the highest value was 97.73% for the AHL reactor under 0.8 V, whereas the value for the control reactor was 89.73%. At 0.6 V, a significant increase was achieved in accordance with the enhancement in hydrogen yield in the presence of AHLs; the ERE was 87.12%, which was higher than that of the control reactor (73.98%). The ERE with AHLs was also obviously higher than that of the control reactor at 0.4 V (52.70% vs 26.57%). Additionally, as shown in fig. 1d, there was a significant difference in energy efficiency at 0.6 V (214.64% vs 182.78%), whereas similar values were obtained at 0.8 V (both were close to 180%). Although AHLs greatly contributed to electron transfer and electron recovery at low voltages, the energy efficiency failed to improve further with high applied voltages because of

the low reaction rate in MECs at low voltages.

According to the results for overall reactor performance, hydrogen yield was enhanced in the presence of AHLs, which promoted electron transfer between the electrodes and microbial cells. Similar CE values suggested that there was no significant positive or negative effect on electron transfer at the bio-anode. However, the final increase in ERE directly indicated the positive influence on the hydrogen evolution reaction at the bio-cathode by the addition of AHLs. Considering the input electric energy, energy efficiency represents the energy revenue from hydrogen evolution [20]. Higher energy efficiencies that exceed 100% were achieved with or without AHLs, whereas the addition of AHLs showed an improvement over the control reactor at both 0.8 V and 0.6 V. Moreover, considering the contribution of the substrate, the overall energy efficiency that consisted of the input electricity and chemical energy of the substrate also showed the same trend. In sum, AHL addition improved hydrogen yield in MECs by enhancing electron transfer at the cathode, which facilitated the hydrogen evolution reaction.

3.2 Electrochemical analysis.

In order to interpret the enhancement in electron transfer due to AHLs, CV and EIS were assessed to analyse the electrochemical property changes after adding AHLs to the MEC. The CV results for the anode were reported in our previous study, and the redox peak was around -300 mV. There was a slight increase in the current value at -300 mV; moreover, the onset potential showed no obvious change, indicating that there was no obvious change due to the presence of AHLs. The onset potential and current value were both similar in the CV test. The linear sweep voltammetry (LSV) test for the cathode showed a similar change (shown in fig.

S2). However, there were several differences associated with the current indicated by slightly different peak positions, which might be ascribed to some exogenous electron mediators being excreted from microbes in the presence of AHL [33]. The overall similarity was homologous with the change in the current, as the anodic current density was limited by the electron transfer resistance [34]. In order to further investigate the impedance for electron transfer at the anode and cathode, EIS, which indicate the electron transfer at the interface between the electrode and electrolyte, were obtained to assess the resistance of the individual electrodes in the MECs. As shown in fig. 2a, the first semicircle occurring in the high-frequency (HF) section was possibly induced by the ionic migration through the electrolyte film that formed on the interface between the electrode and electrolyte [35]. The addition of AHLs reduced the ionic migration resistance, as a smaller semi-diameter was obtained. The second semicircle mainly depended on the resistance of charge transfer. AHLs also caused a lower charge transfer resistance, according to the lower semi-diameter shown in fig. 2b. A similar improvement was achieved not only at the bio-anode but also at the bio-cathode, as it also showed a lower resistance in both ionic migration and charge transfer. The difference in the ohmic resistance of the cathode could have been caused by the cathode reassembly process. However, the lower resistance in the charge transfer contributed to the higher ERE at the cathode, suggesting that the electron transfer improved with the addition of AHLs.

3.3 Biofilms on the anode and cathode.

FE-SEM images revealed the visible micro-structures of the biofilms formed on the surfaces of the anode and cathode. Many microorganisms attached to the electrode, whereas there was

a visible difference in the biofilm structure between the reactor with AHLs and the control reactor (fig. 3). AHL addition led to a piled community cluster on the anode surface, but a flat community structure formed in the control reactor. This result indicated that efficiency was improved by AHL addition because more strains worked together and more electrons could be transferred to the anode by strains farther away. This phenomenon was also observed on the surface of the cathode. AHLs increased the colonising ability of cathodic communities. The electrode contained activated sites, favouring the transfer of electrons by exoelectrogens, which promoted biofilm formation by co-aggregation and metabolic cooperation in which similar species or synergetic species developed beneficial phenotypes. Thus, communities can grow in thick biofilms because energy can be transferred over longer distances through their neighbours [17]. However, some antagonistic species or those with different growth rates will lose some efficient “energy supply sites,” gradually leading to less growth, especially on the cathode.

As we reported in our preliminary results, short-chain AHLs have a positive effect on enhancing the bioelectrochemical properties of anodic communities [27]. It was proven that a significant amount of AHLs was produced by some bacteria such as *Pseudomonas* sp., and thus, QS regulation mechanisms were triggered to achieve the complete degradation of complex compounds [36]. In addition, the abilities of the bio-anode for substrate utilisation and electron production were increased [7]. Moreover, cell aggregates, as shown in the SEM images, are commonly observed in both industrial (such as wastewater treatment plants) and natural (such as marine snow) settings, which is important to the formation of biofilms [37]. The SEM images indicate the feasibility of controlling the biofilm formation structure in a

mixed culture with a simple substrate while also enhancing substrate utilisation and biofilm formation.

3.4 Microbial community structure analysis.

With AHL addition, the Shannon index decreased from 5.04 to 4.47, indicating that the α -diversity of the bio-cathode may have reduced by competition or occupation. However, the diversity of the bio-anode was not substantially changed according to the Shannon index with or without AHL (5.13 and 5.10, respectively). The distribution of the microbial community was subsequently identified at the phylum level (fig. S3). *Proteobacteria* were predominant in all biofilms, accounting for >70% at the bio-anodes and >60% at the bio-cathodes. *Firmicutes*, as a reportedly dominant anodic bacteria, accounted for the second highest relative abundance in all biofilms and presented a higher enrichment at the bio-cathode (16-20%) than the bio-anode (5-10%). AHL addition led to a noticeable increase in *Firmicutes* at the anode (10.11%) compared to that in the control anode (5.44%) without AHL addition, whereas AHL addition caused a change in *Firmicutes* from 16.09% (without AHLs) to 19.94% (AHLs) at the bio-cathodes. In general, AHLs acted on gram-negative bacteria; therefore, the change in *Firmicutes* attributed to gram-positive bacteria could be ascribed to the indirect influence caused by AHLs. There is an internal relationship in electrode biofilms among various microorganisms, and the community structure could shift in response to any change in individual species and affect reactor performance. In the recent report, some species of *Firmicutes* were shown to play an important role in carbon recycling, providing suitable by-products for anode-respiring bacteria [38], because *Firmicutes* were located close to anode-respiring bacteria. Moreover, *Euryarchaeota* (archaea) were enriched at the control

cathode (accounting for 3.18%).

As shown in fig. S4 at the class level, *Delta-proteobacteria* were mainly enriched at the anode, reaching 59.37% at the AHL anode and 62.04% at the control anode. A slight difference in the relative abundance of *Gamma-proteobacteria* at the anode was detected, as AHLs resulted in a higher relative abundance compared with the control anode (5.42% vs 1.03%). *Gamma-proteobacteria* were obviously enriched at the cathode and accounted for 25.42% and 24.45% of the total at the AHL and control anode, respectively. According to the phylogenetic tree based on 16S rRNA reported in a previous study [39], many groups of *Gamma-proteobacteria* are strongly affected by AHLs, especially *Pseudomonadales*. A further analysis at the genus level supported this assumption. In addition to the enrichment of *Geobacter* in the biofilms on the anodes, *Acinetobacter* that belonged to *Pseudomonadales* were enriched at the AHL anodes (3.84%) compared with the controls (0.84%). However, they were enriched at the cathode substantially, accounting for 10.88% at the AHL cathode compared to only 0.38% at the control cathode. However, some hydrogen scavengers, such as methanogens and homo-acetogens that contributed to the loss of electrons, showed different changes in the cathode biofilms [21]. As shown in fig. 5, electrochemically active bacteria (EAB), classified according to a previous study [40], accounted for around 60% of the population at the anode with or without AHLs. A significant difference in EAB was visible at the cathode, as it clearly increased after the addition of AHLs. The value for the cathode with AHLs was 46.72%, which was higher than the value of 32.01% for the control. The sum of methanogens accounted for 0.25% at the anode and 0.49% at the cathode with AHLs, whereas the corresponding values were 0.46% and 3.16% without AHLs. Specifically,

Methanobrevibacter, which is a hydrogenotrophic methanogen, accounted for 2.46% of the total in the biofilm on the electrode in the absence of AHL, and the value decreased to 0.28% in the presence of AHLs. Homo-acetogens of *Acetoanaerobium* and *Acetobacterium* were also decreased to 4.03% compared with 8.15% for the control cathode. Apparently, AHLs favoured EAB on the cathode rather than hydrogen scavengers, which could result in electrons flowing to methane or acetate.

The heatmap analysis provided a visible method to compare the differences in microbial community structure. It was obvious that *Acetoanaerobium* and *Methanobrevibacter* were enriched much more at the control cathode than at the AHL cathode and anode (fig 6a). AHL addition changed the biofilm communities on the anode and cathode (fig. 6b). For anode biofilms, there were few changes in the dominant communities by AHL addition. However, the AHL cathode was markedly changed compared to the control due to increases in EAB and decreases in hydrogen-utilising bacteria. As a result, the anode performance remained stable under varied external voltages due to the stable relative abundance of EAB, because the dominant population was primarily composed of synergetic communities. The microbial community structure was noticeably changed at the cathode by AHL addition, resulting in the enrichment of *Acinetobacter* and lower relative abundance of hydrogen scavengers, which caused a significant enhancement in ERE and hydrogen yield. Quorum sensing, regulated interspecies cooperation, and competition could favour EAB growth while overwhelming other bacteria trying to obtain energy from the cathode [24]. The shift in the cathodic community contributed to the improvement in the performance of MEC with AHL addition. Additionally, it has been shown that *Acinetobacter* could be regulated by AHLs [41, 42]. In

previous studies, *Acinetobacter* was detected at the anode or cathode in bioelectrochemical systems [43, 44], proving that *Acinetobacter* could secrete a redox compound similar to pyrroloquinoline quinone (PQQ) [45], which could further boost extracellular electron transfer via using outer-membrane-bound redox compounds [46]. In some way, electron mediators would help to extend the pathway for electron transfer from microbes to the electrode over a long distance, which would also lead to lower electron transfer resistance at both the anode and cathode, according to the EIS results. Moreover, the increased peak current in the CV results for the anode in our previous study and the CV results for the cathode in this study (fig. S2) also suggest that a lower resistance impedes electron transfer between bacteria and electrodes.

Considering the regulation mechanism of quorum sensing for gram-negative bacteria relies on the release of AHLs, the surplus from the decomposition of AHLs was important in this AHL-dependent regulation strategy. In order to confirm if AHLs truly had a regulatory effect in MECs, the residual AHLs in the effluent were detected with LC-MS (fig. S5). Although it was possible that AHLs were energy and nitrogen sources for the growth of microbes [47], the final AHL detection results proved that they were not completely degraded by chemical or biological decomposition. Importantly, the presence of AHLs in the effluent was evidence that they play a role in the MEC rather than being rapidly degraded.

3.5 Potential application.

The influence of quorum sensing in BESs was investigated by adding exogenous signalling molecules in this study. Hydrogen yield was improved in MECs with varied external voltages by AHL addition, which changed the community structure and aggregation on both the anode

and cathode. The results of this study reveal the possibility of improving hydrogen production in MECs via regulating the microbial community structure to strengthen energy recovery with exogenous AHLs. The results are valuable, as they can aid in preventing hydrogen loss in individual MECs, in terms of hydrogen recycling by homo-acetogens and methanogenesis by methanogens [21, 48]. The results indicate that AHLs could help enhance electrode-respiring bacteria that compete with other by-product-utilising bacteria in electrochemical biofilms. As a biological strategy to prevent hydrogen from being consumed by undesired microorganisms, the addition of AHLs is a straightforward approach to adjust the microbial community structure toward hydrogen generation. The pH control strategy, air exposure, and temperature adjustments had a limited impact on the shift in the microbial community [49, 50]. Although optimising the applied voltage and hydrogen collection procedures have been shown to have positive effects on the anodic microbial community, there are the disadvantages of requiring indirect regulation and an external energy input [51, 52]. Importantly, all of the above strategies are limited to a non-selective role in the fundamental process of bio-methanogenesis, whereas quorum sensing can specifically act on microbial interactions to inhibit methanogens. Moreover, compared to the use of chemical inhibitors that specifically impede methanogenesis in MECs [53], AHLs can be synthesised by bacteria and degraded via chemical or biological pathways. As mentioned above, AHLs would not persist in natural systems, hence, their environmental friendly characteristic is an advantage over chemical inhibitors. In addition to triggering quorum sensing, AHLs can supply energy or carbon sources after the mature microbial community is successfully formed. In sum, the introduction of quorum sensing to inhibit or stimulate the growth of specific bacteria opens a

new approach to prevent hydrogen loss by regulating the community structure in bioelectrochemical systems [54].

4. Conclusion

The influence of quorum sensing in BESs was investigated by adding exogenous signalling molecules. Hydrogen yield was enhanced in MECs with varied external voltage conditions. In addition, a higher ERE was obtained at the cathode with AHL addition, which resulted in a lower charge transfer resistance at the cathode. AHLs changed the community aggregation on both the anode and cathode. The higher electron recovery and hydrogen yield were related to the change in the cathodic microbial structure, with an increased proportion of *Gamma-proteobacter* and fewer hydrogen scavengers with AHL addition. Prospectively, this study for the first time provides insights into improving hydrogen production performance in MECs via regulating the microbial community structure to improve energy recovery by adding exogenous AHLs.

Acknowledgements

This research was supported by the National Science Foundation for Distinguished Young Scholars (Grant No. 51225802), National Natural Science Foundation of China (No. 51578534), “Hundred Talents Program” of the Chinese Academy of Sciences, and Project 135 of the Chinese Academy of Sciences (No. YSW2013B06).

References

- [1] McLean RJ, Whiteley M, Stickler DJ, Fuqua WC. Evidence of autoinducer activity in naturally occurring biofilms. *FEMS Microbiol Lett.* 1997;154:259-63.
- [2] Miller MB, Bassler BL. Quorum sensing in bacteria. *Annu Rev Microbiol.* 2001;55:165-99.
- [3] Perez-Rodriguez I, Bolognini M, Ricci J, Bini E, Vetriani C. From deep-sea volcanoes to human pathogens: a conserved quorum-sensing signal in Epsilonproteobacteria. *ISME J.* 2015;9:1222-34.
- [4] Rader BA, Campagna SR, Semmelhack MF, Bassler BL, Guillemin K. The quorum-sensing molecule autoinducer 2 regulates motility and flagellar morphogenesis in *Helicobacter pylori*. *J Bacteriol.* 2007;189:6109-17.
- [5] Sperandio V, Mellies JL, Nguyen W, Shin S, Kaper JB. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA.* 1999;96:15196-201.
- [6] Toyofuku M, Nomura N, Fujii T, Takaya N, Masada H, Sawada I, et al. Quorum Sensing Regulates Denitrification in *Pseudomonas aeruginosa* PAO1. *J Bacteriol.* 2007;189:4969-72.
- [7] Yong YC, Yu YY, Yang Y, Liu J, Wang JY, Song H. Enhancement of extracellular electron transfer and bioelectricity output by synthetic porin. *Biotech bioeng.* 2013;110:408-16.
- [8] Hu Y, Yang Y, Katz E, Song H. Programming the quorum sensing-based AND gate in *Shewanella oneidensis* for logic gated-microbial fuel cells. *Chem. Commun.* 2015;51:4184-7.
- [9] Rabaey K, Boon N, Höfte M, Verstraete W. Microbial phenazine production enhances electron transfer in biofuel cells. *Environ Sci Technol.* 2005;39:3401-8.
- [10] Venkataraman A, Rosenbaum MA, Perkins SD, Werner JJ, Angenent LT. Metabolite-based mutualism between *Pseudomonas aeruginosa* PA14 and *Enterobacter aerogenes* enhances current generation in bioelectrochemical systems. *Energy Environ Sci.* 2011;4:4550.
- [11] Wang Y, Kern SE, Newman DK. Endogenous phenazine antibiotics promote anaerobic survival of *Pseudomonas aeruginosa* via extracellular electron transfer. *J Bacteriol.* 2010;192:365-9.
- [12] Venkataraman A, Rosenbaum M, Arends JBA, Halitschke R, Angenent LT. Quorum sensing regulates electric current generation of *Pseudomonas aeruginosa* PA14 in bioelectrochemical systems. *Electrochem Commun.* 2010;12:459-62.
- [13] Yong YC, Yu YY, Li CM, Zhong JJ, Song H. Bioelectricity enhancement via overexpression of quorum sensing system in *Pseudomonas aeruginosa*-inoculated microbial fuel cells. *Biosens Bioelectron.* 2011;30:87-92.
- [14] Yong YC, Wu XY, Sun JZ, Cao YX, Song H. Engineering quorum sensing signaling of *Pseudomonas* for enhanced wastewater treatment and electricity harvest: A review. *Chemosphere.* 2015;140:18-25.
- [15] Alatraktchi FAa, Zhang Y, Angelidaki I. Nanomodification of the electrodes in microbial fuel cell: Impact of nanoparticle density on electricity production and microbial community. *Appl Energy.* 2014;116:216-22.
- [16] Lobato J, González del Campo A, Fernández FJ, Cañizares P, Rodrigo MA. Lagooning microbial fuel cells: A first approach by coupling electricity-producing microorganisms and algae. *Appl Energy.* 2013;110:220-6.
- [17] Elias S, Banin E. Multi-species biofilms: living with friendly neighbors. *FEMS Microbiol Rev.* 2012;36:990-1004.
- [18] Chae K-J, Choi M-J, Kim K-Y, Ajayi FF, Park W, Kim C-W, et al. Methanogenesis control by

employing various environmental stress conditions in two-chambered microbial fuel cells. *Bioresour Technol.* 2010;101:5350-7.

[19] Liu W, Cai W, Guo Z, Wang L, Yang C, Varrone C, et al. Microbial electrolysis contribution to anaerobic digestion of waste activated sludge, leading to accelerated methane production. *Renew Energ.* 2016;91:334-9.

[20] Cai W, Han T, Guo Z, Varrone C, Wang A, Liu W. Methane production enhancement by an independent cathode in integrated anaerobic reactor with microbial electrolysis. *Bioresour Technol.* 2016;208:13-8.

[21] Parameswaran P, Zhang H, Torres CI, Rittmann BE, Krajmalnik-Brown R. Microbial community structure in a biofilm anode fed with a fermentable substrate: the significance of hydrogen scavengers. *Biotechnol Bioeng.* 2010;105:69-78.

[22] Yan H, Yates MD, Regan JM. Effects of constant or dynamic low anode potentials on microbial community development in bioelectrochemical systems. *Appl Microbiol Biotechnol.* 2015;99:9319-29.

[23] Ullery ML, Logan BE. Anode acclimation methods and their impact on microbial electrolysis cells treating fermentation effluent. *Int J Hydrogen Energy.* 2015;40:6782-91.

[24] Chandler JR, Heilmann S, Mittler JE, Greenberg EP. Acyl-homoserine lactone-dependent eavesdropping promotes competition in a laboratory co-culture model. *ISME J.* 2012;6:2219-28.

[25] Bassler BL. Small talk: cell-to-cell communication in bacteria. *Cell.* 2002;109:421-4.

[26] Zhou J, Liu W, Deng Y, Jiang Y-H, Xue K, He Z, et al. Stochastic assembly leads to alternative communities with distinct functions in a bioreactor microbial community. *mBio.* 2013;4:e00584-12.

[27] Liu W, Cai W, Ma A, Ren G, Li Z, Zhuang G, et al. Improvement of bioelectrochemical property and energy recovery by acylhomoserine lactones (AHLs) in microbial electrolysis cells (MECs). *J Power Sources.* 2015;284:56-9.

[28] Liu W, Huang S, Zhou A, Zhou G, Ren N, Wang A, et al. Hydrogen generation in microbial electrolysis cell feeding with fermentation liquid of waste activated sludge. *Int J Hydrogen Energy.* 2012;37:13859-64.

[29] Wang X, Cheng S, Feng Y, Merrill MD, Saito T, Logan BE. Use of carbon mesh anodes and the effect of different pretreatment methods on power production in microbial fuel cells. *Environ Sci Technol.* 2009;43:6870-4.

[30] Zhou A, Zhang J, Cai W, Sun R, Wang G, Liu W, et al. Comparison of chemosynthetic and biological surfactants on accelerating hydrogen production from waste activated sludge in a short-cut fermentation-bioelectrochemical system. *Int J Hydrogen Energy.* 2016.

[31] Rader GK, Logan BE. Multi-electrode continuous flow microbial electrolysis cell for biogas production from acetate. *Int J Hydrogen Energy.* 2010;35:8848-54.

[32] Zhang Y, Cong J, Lu H, Li G, Xue Y, Deng Y, et al. Soil bacterial diversity patterns and drivers along an elevational gradient on Shennongjia Mountain, China. *Microb Biotechnol.* 2015;8:739-46.

[33] Marsili E, Baron DB, Shikhare ID, Coursolle D, Gralnick JA, Bond DR. *Shewanella* secretes flavins that mediate extracellular electron transfer. *Proc Natl Acad Sci USA.* 2008;105:3968-73.

[34] Fan Y, Sharbrough E, Liu H. Quantification of the internal resistance distribution of microbial fuel cells. *Environ Sci Technol.* 2008;42:8101-7.

[35] Wang Q, Dong H, Yu H, Yu H, Liu M. Enhanced electrochemical reduction of carbon dioxide to formic acid using a two-layer gas diffusion electrode in a microbial electrolysis cell. *RSC Adv.* 2015;5:10346-51.

[36] Yong Y-C, Zhong J-J. N-Acylated homoserine lactone production and involvement in the

biodegradation of aromatics by an environmental isolate of *Pseudomonas aeruginosa*. *Process Biochem.* 2010;45:1944-8.

[37] Parsek MR, Greenberg EP. Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends Microbiol.* 2005;13:27-33.

[38] Liu W, He Z, Yang C, Zhou A, Guo Z, Liang B, et al. Microbial network for waste activated sludge cascade utilization in an integrated system of microbial electrolysis and anaerobic fermentation. *Biotechnol Biofuels.* 2016;9:1.

[39] Huang Y, Zeng Y, Yu Z, Zhang J, Feng H, Lin X. In silico and experimental methods revealed highly diverse bacteria with quorum sensing and aromatics biodegradation systems--a potential broad application on bioremediation. *Bioresour Technol.* 2013;148:311-6.

[40] Zhang Y-C, Jiang Z-H, Liu Y. Application of Electrochemically Active Bacteria as Anodic Biocatalyst in Microbial Fuel Cells. *Chinese J Anal Chem.* 2015;43:155-63.

[41] González RH, Nusblat A, Nudel BC. Detection and characterization of quorum sensing signal molecules in *Acinetobacter* strains. *Microbiol Res.* 2001;155:271-7.

[42] Sarkar S, Chakraborty R. Quorum sensing in metal tolerance of *Acinetobacter junii* BB1A is associated with biofilm production. *FEMS Microbiol Lett.* 2008;282:160-5.

[43] Liu X-W, Li W-W, Yu H-Q. Cathodic catalysts in bioelectrochemical systems for energy recovery from wastewater. *Chem. Soc. Rev.* 2014;43:7718-45.

[44] Heidrich ES, Edwards SR, Dolfing J, Cotterill SE, Curtis TP. Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12 month period. *Bioresour Technol.* 2014;173:87-95.

[45] Freguía S, Tsujimura S, Kano K. Electron transfer pathways in microbial oxygen biocathodes. *Electrochim Acta.* 2010;55:813-8.

[46] Laurinavicius V, Razumiene J, Kurtinaitiene B, Lapenaite I, Bachmatova I, Marcinkeviciene L, et al. Bioelectrochemical application of some PQQ-dependent enzymes. *Bioelectrochemistry.* 2002;55:29-32.

[47] Flagan S, Ching WK, Leadbetter JR. *Arthrobacter* Strain VAI-A Utilizes Acyl-Homoserine Lactone Inactivation Products and Stimulates Quorum Signal Biodegradation by *Variovorax paradoxus*. *Appl Environ Microbiol.* 2003;69:909-16.

[48] Lee H-S, Rittmann BE. Significance of biological hydrogen oxidation in a continuous single-chamber microbial electrolysis cell. *Environ Sci Technol.* 2009;44:948-54.

[49] Ruiz Y, Baeza JA, Guisasola A. Enhanced performance of bioelectrochemical hydrogen production using a pH control strategy. *ChemSusChem.* 2015;8:389-97.

[50] Chae K-J, Choi M-J, Kim K-Y, Ajayi FF, Chang I-S, Kim IS. Selective inhibition of methanogens for the improvement of biohydrogen production in microbial electrolysis cells. *Int J Hydrogen Energy.* 2010;35:13379-86.

[51] Lu L, Hou D, Wang X, Jassby D, Ren ZJ. Active H₂ Harvesting Prevents Methanogenesis in Microbial Electrolysis Cells. *Environ. Sci. Technol. Lett.* 2016;3:286-90.

[52] Wang A, Liu W, Cheng S, Xing D, Zhou J, Logan BE. Source of methane and methods to control its formation in single chamber microbial electrolysis cells. *Int J Hydrogen Energy.* 2009;34:3653-8.

[53] Catal T, Lesnik KL, Liu H. Suppression of methanogenesis for hydrogen production in single-chamber microbial electrolysis cells using various antibiotics. *Bioresour Technol.* 2015;187:77-83.

[54] Rabaey K, Rodriguez J, Blackall LL, Keller J, Gross P, Batstone D, et al. Microbial ecology meets

electrochemistry: electricity-driven and driving communities. ISME J. 2007;1:9-18.

Figure captions:

Figure 1. MEC performance: (a) hydrogen yield; (b) coulombic efficiency; (c) electron recovery efficiency; (d) energy efficiency.

Figure 2. Electrochemical tests for the anode and cathode (a): EIS for the anode; (b): EIS for the cathode.

Figure 3. FE-SEM images of biofilms at the anode and cathode for both groups (a,b): anodic biofilm with AHL; (c,d): cathodic biofilm with AHL; (e,f): cathodic biofilm without AHL; (g,h): cathodic biofilm without AHL.

Figure 4. Genus distribution for both the anode and cathode in the presence and absence of AHL.

Figure 5. Specific functional genera classification for electrodes in all reactors, EAB: electrochemically active bacteria.

Figure 6. Heatmap and PCoA analyses of the microbial communities.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

● Electrochemically active bacterial

■ Hydrogen scavengers

Acclimation

Microbial community

Performance