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

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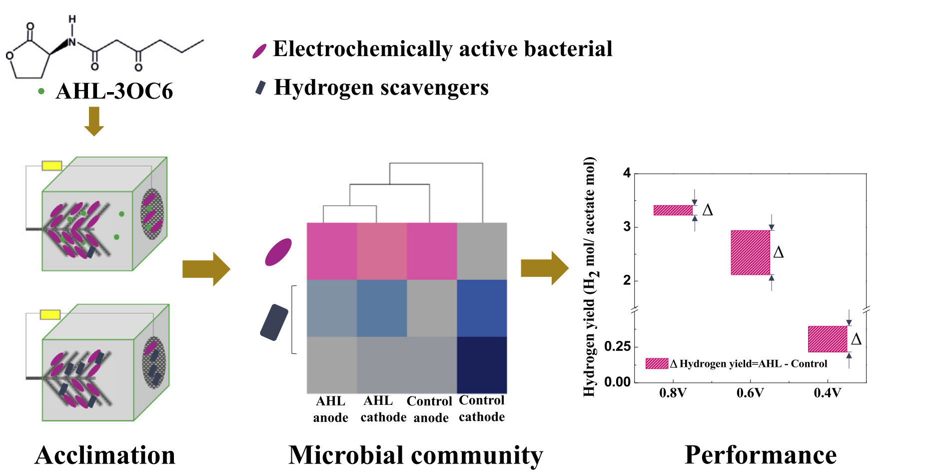
HIGHLIGHTS

- Enhanced hydrogen yield has been achieved with addition of AHL.
- AHL regulated exoelectrogens resulting in electrochemical activity enhancement.
- Microbial community shift in cathodic biofilm inhibited hydrogen loss.

GRAPHICAL ABSTRACT



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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

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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.

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 utilising 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 bioelectrode 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 × 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 m^2 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/cm^2) on one side was assembled as the cathode, and the total surface area was 7 cm^2. 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