Electrochimica Acta 199 (2016) 45–50

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

A laminar-flow based microfluidic microbial three-electrode cell for biosensing

Feifang Li[a], Zhanwang Zheng[b], Bin Yang[a], Xingwang Zhang[a], Zhongjian Li[a,*], Lecheng Lei[a]

[a] Key Laboratory of Biomass Chemical Engineering of Ministry of Education, College of Chemical and Biological Engineering, Zhejiang University, Hangzhou, 310027, China
[b] School of Environmental and Resource Science, Zhejiang A & F University, Hangzhou, 311300, China

ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form 21 March 2016
Accepted 23 March 2016
Available online 25 March 2016

Keywords:
microbial three-electrode cell
microfluidic chip
laminar flow
bioelectrochemical system
biosensor

ABSTRACT

Microbial three-electrode cells (M3Cs) have been widely used as a promising platform for developing biosensors and studying electrochemically active bacteria (EAB). Compared to conventional microbial two-electrode cells (e.g. microbial fuel cells and microbial electrolysis cells), M3Cs can offer more stable and better defined electrochemical environments for various research purposes. This work focused on developing a microliter scale microfluidic M3C, which had unique advantages over bench scale M3Cs. In this microfluidic M3C with a built-in three electrode system, laminar flow was exploited to separate the reference electrode from the working and the counter electrodes. Using laminar flow made it possible to integrate the M3C with a microliter scale microfluidic chip and maintain a stable Ag/AgCl electrode potential. With the help of the integrated three-electrode setup, the working electrode potential of the microfluidic M3C was able to be accurately controlled and thus a well-defined electrochemical environment was provided to *Geobacter sulfurreducens* to respire on the electrode. During 30 days operation, the reference electrode potential was stable, which guaranteed the accurate control of the working electrode potential. By taking advantage of the microliter scale and a short hydraulic retention time (HRT), fast responses to ferric citrate and formaldehyde with good reproducibility were achieved. Furthermore, a linear relationship between the output signals (peak area) and chemicals concentrations was obtained. The microfluidic M3C developed in this work will provide researchers in related areas a versatile platform for biosensor and EAB study.

1. Introduction

Bioelectrochemical systems (BESs), in which electrochemically active bacteria (EAB) catalyzes reactions by interacting with electrodes [1], have been widely used in biosensing, such as heavy mental detection [2], BOD measurement [3] and pesticide detection [4]. In a BES based biosensor, the input signals, biochemical molecules, can be converted into electric current (or voltage) as output signals via bacterial metabolism. Reproducibility, response time and recovery time are important parameters to evaluate the performance of biosensors. Good reproducibility is usually related to stable and well-defined electrochemical environment for EAB, since the current production is closely related to the bacterial metabolism [5]. On the other hand, to shorten the response and recovery time, fast mass transfer and short HRT are two key factors. Along this line, different solutions for improving BES based biosensors have been proposed. Developing miniaturized microbial three-electrode cell (M3C) based biosensors on microfluidic chips is a promising one.

A M3C is a typical BES, which can maintain stable and explicit electrochemical environment for EAB by accurately poising its working electrode at a constant potential level with the help of a three-electrode set-up. Similarly, a microbial fuel cell (MFC) is another typical BES and uses EAB as biocatalysts to transform chemical energy into electricity [6]. Therefore, MFCs can be used as an electric power source. MFCs are two-electrode systems. Compared to M3Cs, more factors can influence the produced electric current signal of MFCs, including the anode potential, ionic conductivity of medium and cathodic reactions [7]. Although MFCs and M3Cs have similar signal processing and transformation, the M3Cs have higher reproducibility and stability of the output signals.

Besides good stability and reproducibility, the fast response and recovery are also important for an ideal biosensor. In milliliter to liter scale BESs, mass transfer limitations [8] and long HRT result in the long response time and recovery time. Miniaturizing the BESs

* Corresponding author. Tel.: +86 571 87952525; fax: +86 571 87952525.
E-mail address: zdlizj@zju.edu.cn (Z. Li).

http://dx.doi.org/10.1016/j.electacta.2016.03.138
0013-4686/

can effectively improve the response and recovery speed and thus overcome the above mentioned disadvantages [9,10]. Several miniaturized MFC based biosensors (1.5 μL to 144 μL) have been developed [10,11], but miniaturizing a M3C is more challenging stemming from integrating a reference electrode into a micro or sub-micro liter scale device to build a three-electrode system. The main difficulty [12,13] is how to keep the inner electrolyte (e.g. KCl solutions for Ag/AgCl reference electrodes) of a reference electrode immiscible with the external medium but meanwhile allowing ion exchange between them. Usually, in a commercial reference electrode, porous ceramics are used as physical separators. However, in a microfluidic device, this is not feasible. Many researchers have made a lot of efforts. For example, two micro-fabricated M3C systems have been reported, in which the working electrode, the counter electrode and the Ag/AgCl electrode were integrated into the same chamber without separators [14,15]. The Ag/AgCl electrodes were immersed in bulk solution (i.e. bacterial growth medium). Although they were three-electrode systems, the reference electrolytes were not well-defined and the reference electrodes could be contaminated by bacteria during the experiment, which might result in the reference electrode potential shift. Another reported miniaturized M3C employed a dual-reference electrodes set-up, the reference electrode was placed far from the working electrode, and this set-up might result in unstable potential control [16]. In all these mentioned works, the unique flow pattern, laminar flow, in microfluidic channels was ignored. In a microliter scale channel, the laminar flow allows two different streams flow parallelly without convective mixture, but the ion exchange at the liquid-liquid interface is not hindered [17]. This feature provides a possibility of using laminar flow to replace porous ceramics. Microfluidic devices using laminar flow to separate the catholyte and anolyte in two-electrode systems have been reported [18,19]. Here, we tried to use laminar flow to develop a microfluidic M3C chip with fast response and recovery. E. Victoria Dydek et al. [20] used laminar flow to replace porous ceramics and successfully integrated the reference electrode into an abiotic microfluidic three-electrode system. The results indicated that using the laminar flow as a separator was feasible. But the design is for solely electrochemical applications, it needs modifications to be adapted to microbial biosensor applications.

In this work, we fabricated a 3 μL microfluidic M3C, in which laminar flow was used to separate the reference electrode and the working/counter electrodes. Gold electrodes (the working and the counter electrodes) and an Ag/AgCl electrode were integrated on a microfluidic chip fabricated with the soft lithograph technology. *Geobacter sulfurreducens*, a model EAB, was grown in the device. The aim of this work is to develop a stable and microliter scale platform for biosensing and EAB study.

2. Materials and methods

2.1. Design of the microfluidic M3C

The microfluidic M3C consisted of a glass wafer with gold electrodes sputtering on it and a polydimethylsiloxane (PDMS) channel (Fig. 1). Our design was based on the work of E. Victoria Dydek et al. [20], but with enlarged electrodes. The design of E. Victoria Dydek et al. was a solely electrochemical three-electrode microfluidic system and was for short-term abiotic applications. Our device was developed for microbial biosensing, which usually took 10–30 days (including biofilm formation and biosensing process) and the AgCl of the integrated Ag/AgCl reference electrode would constantly be reduced into Ag in the long-term process. This could result in a potential shift of the Ag/AgCl reference electrode, especially a reference electrode with small area was used. To provide a stable reference potential, the reference electrode area was enlarged. To obtain stronger electric current signals, the working electrode area was also enlarged. In our design (Fig. 1b), both the working and the counter electrodes had an area of 8×1 mm, and the reference electrode had an area of 17×0.5 mm. All of them had the same thickness (Au/Ti, 200 nm/100 nm). As the stream flows from the working electrode to the counter electrode, the reactions occur at the counter electrode will not affect the working electrode. The gap between the working electrode and the reference electrode was 0.5 mm. The PDMS channel was designed to be a Y shape (Fig. 1a, b, c). The anolyte leg was 1 mm wide and the catholyte leg was 0.5 mm wide. Designing the two legs with different widths was to avoid reference electrode contamination by making the convection time across the y-junction to the end of the channel shorter than the diffusion time across the dividing line

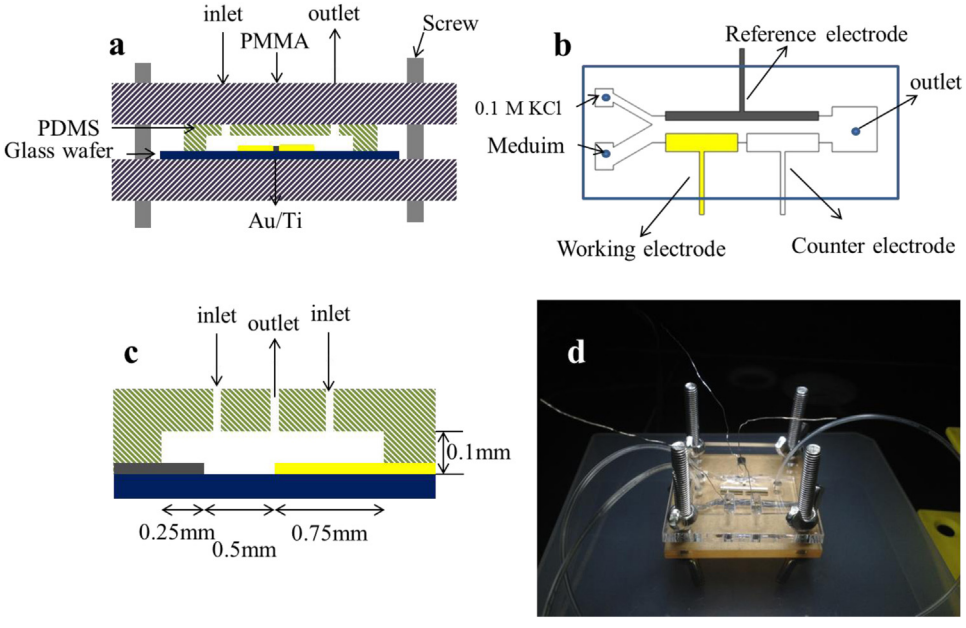

Fig. 1. Scheme of the microfluidic channel device. (a) Front view. (b) Top view. (c) Side view. (d) A photograph of the assembled device.