Bioresource Technology 155 (2014) 71–76

Contents lists available at ScienceDirect

Bioresource Technology

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

A stable synergistic microbial consortium for simultaneous azo dye removal and bioelectricity generation

Victor Bochuan Wang [a,c,1], Song-Lin Chua [a,b,1], Zhao Cai [a,d], Krishnakumar Sivakumar [a,d], Qichun Zhang [c], Staffan Kjelleberg [a,g], Bin Cao [a,f,*], Say Chye Joachim Loo [a,c], Liang Yang [a,e,*]

[a] Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, Singapore 637551, Singapore
[b] Graduate School of Integrative Sciences and Engineering, National University of Singapore, Singapore 117543, Singapore
[c] School of Materials Science and Engineering, Nanyang Technological University, Singapore 639798, Singapore
[d] Interdisciplinary Graduate School, Nanyang Technological University, Singapore 637551, Singapore
[e] School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore
[f] School of Civil and Environmental Engineering, Nanyang Technological University, Singapore 639798, Singapore
[g] School of Biotechnology and Biomolecular Sciences and Centre for Marine Bio-Innovation, The University of New South Wales, Sydney, NSW 2052, Australia

HIGHLIGHTS

- Co-culture system for simultaneous Congo red degradation/bioelectricity generation.
- Biofilm formation and spatial coverage affect bioelectricity generation.
- Interspecies interactions affect functions of synergistic microbial consortium.

ARTICLE INFO

Article history:
Received 1 November 2013
Received in revised form 16 December 2013
Accepted 19 December 2013
Available online 27 December 2013

Keywords:
Shewanella oneidensis
Pseudomonas putida
Bioremediation
Bioelectrochemical systems

ABSTRACT

Microbial species coexist in natural or engineered settings, where they encounter extensive competition and cooperation. Interactions occurring through metabolite exchange or direct contact might be important in establishment of functional biodegradation consortium. Understanding these interactions can facilitate manipulation of selected communities and exploitation of their capacity for specific industrial applications. Here, a simple dual-species consortium (*Pseudomonas putida* and *Shewanella oneidensis*) was established for examining simultaneous Congo red bioremediation in planktonic culture and power generation in anode biofilms. Compared to mono-species cultures, co-cultures generated higher current densities and could concurrently degrade Congo red over 72 h. Disabling the large secreted adhesion protein, LapA, of *P. putida* greatly enhanced *S. oneidensis* biofilm formation on the anode, which increased power generation in co-cultures. This demonstrates simultaneous control of specific planktonic and biofilm communities could be effective in manipulating microbial communities for targeted applications.

1. Introduction

Azo dyes are commonly employed in commercial textile industries and contain the aromatic functionality with one or more N=N groups. 70% (by weight) of all the dye used by society are composed of these compounds (Carliell et al., 1995). It was estimated that 15% of all used dye are eventually discharged into water bodies (Banat et al., 1996). The environmental release of dye-containing wastewater constitutes a major pollution hazard and public health concern as these synthetic compounds and their by-products are poisonous and mutagenic (Alves de Lima et al., 2007; De Aragão Umbuzeiro et al., 2005). However, conventional wastewater treatment methods are inefficient in degrading azo dyes (Nam and Renganathan, 2000; Oliveira et al., 2007). For example, only 3 of the 18 azo dyes were found to be degraded by activated sludge processes (Shaul et al., 1991). Better understanding of microbial interactions within a process setting can facilitate optimal manipulation of selected microbial communities for azo dye biodegradation. The bioremediation process would be more attractive when coupled with bioelectricity generation in a single platform.

* Corresponding authors. Address: School of Civil and Environmental Engineering, and Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore 639798, Singapore. Tel.: +65 6790 5277/6592 7895 (B. Cao). Tel.: +65 6592 3085 (L. Yang).
E-mail addresses: bincao@ntu.edu.sg (B. Cao), yangliang@ntu.edu.sg (L. Yang).
[1] These two authors contributed equally to this work.

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.12.078

Microorganisms live as complex multi-species communities in natural, industrial or engineered settings. Social traits such as competition and cooperation are frequently observed in microbial communities, which allow different microorganisms to coordinate their behaviors to determine the functions of the entire community. This form of interaction can be realized through endogenously secreted metabolites, which is demonstrated by secretion of antimicrobial compounds to inhibit the growth of neighbouring species. Consequently, certain microorganisms rely on these metabolites for survival (Hansen et al., 2007). The formation of surface-attached biofilm communities further complicates inter-species interactions. In biofilms, various microbial species are embedded in a self-generated extracellular polymeric substances (EPS) matrix (Flemming et al., 2007). Microbial cells interact extensively with each other within biofilms, which is critical for shaping structures and functions of microbial communities (Yang et al., 2011). In different environments, microorganisms can adopt a planktonic or biofilm mode of life.

Recent reports have demonstrated that microbial fuel cells (MFCs) can be used to couple bioremediation of azo dyes with bioelectricity generation (Fang et al., 2013; Solanki et al., 2013). MFCs employ electroconductive microorganisms as bio-catalysts to achieve oxidative breakdown of organic substrates which yields free electrons at the anode of the bioelectrochemical device (Logan et al., 2006; Lovley, 2006; Rabaey and Verstraete, 2005). In MFCs, the electrochemically active microorganism (EAM), such as *Shewanella oneidensis*, is able to transport electrons from its internal metabolic cycles to the anode via secretion of soluble redox mediators (Rabaey et al., 2005), physical appendages such as conductive pili (Reguera et al., 2005), and membrane associated cytochromes (Lovley et al., 2004). Liberated electrons move through an external circuit towards the cathode compartment which produces energy in the process. Generated protons diffuse across the selective proton exchange membrane (PEM) to the aerobic cathode chamber where a reduction process occurs with oxygen and electrons to produce water.

Here, a simple dual-species microbial consortium was defined, based on a model bioremediation organism *Pseudomonas putida* and electrochemically active *S. oneidensis*, for examining inter-species interactions. *P. putida* and *S. oneidensis* were cultivated in MFCs. The azo dye, Congo red, was added to the MFCs with both planktonic *P. putida* and *S. oneidensis* biofilm to examine the inter-species interactions within the co-culture system. *P. putida* is a well-known azo dye degrading microorganism (ADM) that can break down azo dyes by reductive cleavage of the molecular bonds (Nelson et al., 2002). In this contribution, the dynamics of power generation, Congo red bioremediation, as well as the anode biofilms were monitored in order to elucidate inter-species interactions to achieve simultaneous power generation and bioremediation.

2. Methods

2.1. Bacterial strains and growth media

The M1 salt solution consisted of 7.2 g/L of HEPES, 0.3 g/L of sodium hydroxide, 1.5 g/L of ammonium chloride, 0.1 g/L of potassium chloride, 0.52 g/L of monosodium phosphate, and trace amounts (1% v/v) of mineral stock solution, vitamin stock solution and amino acids stock solution (Cao et al., 2011). Before use, 20 mL of sodium lactate and 10 mL of calcium chloride were added to the salt solution to yield final concentrations of 20 mM sodium lactate and 0.68 mM calcium chloride. Monocultures of *S. oneidensis* MR-1 strain or wild type *P. putida* OUS82 and its mutant (*P. putida* OUS82 Δ*lapA*) for inoculation of MFCs were prepared aerobically in lysogeny broth (LB) medium at 37 °C.

2.2. Set up of MFCs

All materials were used as received, unless otherwise stated. Angled tubular glass compartments (17 mm O.D. × 1.8 mm wall thickness) forming the anode and cathode chambers of the MFCs, carbon felt (3.18 mm thickness) and stainless steel pinch clamps (#28) were purchased from VWR Singapore Pte. Ltd. (Singapore). Titanium wire (0.25 mm diameter) and serrated silicone septa (18 mm O.D.) were purchased from Sigma–Aldrich, Singapore (Singapore). Nylon screws and nuts were purchased from Spectra-Teknik (Singapore). Nafion® N117 proton exchange membrane (PEM) was purchased from Ion Power (United States of America).

Dual chamber *U*-tube MFCs were constructed as reported previously (Sund et al., 2007, 2009; Wang et al., 2013a,b). Two 90° 28/15 ball-to-plain-end and socket-to-plain-end glass tubes were separated by a piece of Nafion® N117 PEM. Grease was applied to the joints of the glass tubes and sealed against a circular piece of PEM (diameter of 2 cm). This was to ensure proper sealing of the joints to prevent cross contamination of inoculants to the cathode chamber. The device was secured in place and tightened with a stainless steel pinch clamp. Carbon felt electrodes cut to 2 cm × 5 cm dimensions (width × length) were fastened to the titanium wire with the nylon screw and nut. The assembled electrodes were then seated inside the glass tubes. Before MFC operation, the devices were filled with ultrapure water and autoclaved to sterilize the devices and internal components. After sterilization and decanting off the ultrapure water, the anode and cathode chambers were each filled with the growth medium. As reported in literature, 2 mL of Congo red solution (5 mM stock solution) was introduced only to the anode chamber to a final concentration of 0.5 mM (Hou et al., 2011a,b; Sun et al., 2013; Xu et al., 2013). 1 mL of culture ($OD_{600} \sim 1.0$) for each bacterial strain was then inoculated into the anode chamber only. The final total volume of solution in each of the anode and cathode chambers was 20 mL. A serrated silicone septum was used to seal the anode chamber, while the cathode chamber was loosely capped with an inverted glass scintillation vial to provide an aerobic environment. The titanium wire was threaded through the septum. The cathode electrodes were partly submerged in the catholyte to allow for an 'air-wicking' aerobic configuration. The electrodes were then connected to a 1 kΩ resistor and voltage measurements across the resistors were recorded at a rate of 1 point per 5 min using an eDAQ e-corder® data acquisition system (Bronjo Medi, Singapore), equipped with Chart® software. Data collection started immediately after inoculation of the devices. MFCs were kept inside an incubator set to 30 °C for up to approximately 3 days.

2.3. Characterization of azo dye

Congo red was purchased from Sigma–Aldrich, Singapore and used as received. 5 mM stock solution was prepared with ultrapure water and filter sterilized subsequently. Samples were collected from the anode chambers at different time intervals. These aliquots were centrifuged at 10,000 rpm for 10 min to remove suspended biomass from the liquid media. Decolorization of Congo red was monitored through optical absorbance spectroscopy at $\lambda = 496$ nm using a Shimadzu UV–visible spectrophotometer (UVmini-1240). Actual concentration was acquired against a standard calibration curve (absorbance vs. concentration).

2.4. Biofilm imaging

Brightfield, red fluorescent protein (RFP) and green fluorescent protein (GFP) images of biofilms attached to carbon felt electrode fibers were taken by Zeiss Observer.Z1 inverted epifluorescence microscope at 20× magnification.