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# Wastewater treatment and microbial communities in an integrated photo-bioelectrochemical system affected by different wastewater algal inocula

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ABSTRACT

Integrated photo-bioelectrochemical (IPB) systems are a newly emerging technology for sustainable wastewater treatment through synergistic cooperation between microbial fuel cells (MFCs) and algal bioreactors. This study aimed to advance our understanding of the IPB performance in contaminant removal and bioenergy production and how IPB function is affected by different algal–bacterial inocula. Fed with a synthetic solution, the IPB system could achieve more than 90% removal of solution organic compounds and nearly 100% of ammonium nitrogen. Production of algal biomass was significantly different among three inocula, varying from 5.9 to 53.3 mg $L^{-1}$. The highest energy production was 0.089 kWh $m^{-3}$, including direct electrical energy of 0.055 kWh $m^{-3}$ and indirect electrical energy of 0.034 kWh $m^{-3}$ from biomass conversion, and a positive energy balance could be achieved. The natural algal inocula resulted in the cyanobacteria *Leptolyngbya* and green alga *Acutodesmus* as dominant photoautotrophs in cathode suspension and biofilms, providing oxygen for MFC function. Differences in IPB efficiency could be related to microbial composition; one inoculum resulted in absence of Xanthomonadaceae bacteria, while another had more γ-proteobacteria. Specific taxa identified could be important for optimizing electricity generation and algal biomass for biofuel production.

## 1. Introduction

Recovering bioenergy from contaminants will promote and facilitate sustainable wastewater treatment, and to achieve this, new technologies such as microbial fuel cells (MFCs) and algal bioreactors are being developed. MFCs are a bio-electrochemical reactor in which exoelectrogenic bacteria oxidize organic compounds and produce bioelectricity [1]. The direct energy recovery from wastewater makes MFCs a promising approach for simultaneous wastewater treatment and bioenergy production. The electricity-generating processes in MFCs promote the oxidation of organic compounds [2]; however, nutrients such as nitrogen and phosphorus are not effectively removed, unless special processes are linked to MFCs [3]. Treatment of wastewater with algae has a long history, especially in removing nutrients [4,5] and wastewater can also be regarded as a resource for growing algal biomass for production of biofuels [6]. Algal bioreactors are typically based on algae using photosynthesis to fix carbon dioxide into organic compounds, resulting in biomass growth and oxygen production. During algal growth, concentrations of dissolved nutrients, e.g., nitrogen (N) and phosphorus (P), are drawn down by algal uptake [7,8]. Combining MFCs and algal bioreactors may complement the functions of these two components to improve the removal of organics (in MFCs) and nutrients (algal uptake), reduce the need of aeration (via algal oxygen production), and harvest bioenergy (bioelectricity in MFCs and biomass in algal bioreactors). While MFC energy production and algal nutrient bioremediation have been studied separately, we lack information about the how different algae and bacteria might affect functions of integrated algal bio-electrochemical reactors.

Algae linked to MFCs have been examined through several approaches [9]. First, algae can provide a substrate for the exoelectrogenic bacteria in the anode of MFCs. Electricity can be successfully produced from the MFC fed with living or dry algal biomass cultivated in a separate photobioreactor [10–13]. However, the efficiency of this approach is low, as it still uses two separated reactors and it is hard to transfer chemical energy from algae (via the degradation of the macromolecules in algae) to exoelectrogenic bacteria in the anode of an MFC. Second, algae may be able to catalyze the anode reaction. A study observed electrogenic activity associated with high biomass algal growth and chlorophyll content in a photo-MFC that used light as the input energy, but the dissolved oxygen (DO) produced by algae via oxygenic photosynthesis inhibited the anode process [14]. Third, algae have been cultivated in

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the cathode to provide DO as an electron acceptor to the cathode reaction, providing DO levels comparable to or better than the mechanic aeration typically required, thereby greatly decreasing energy consumption through eliminating mechanical aeration [15–19]. In addition to providing DO, algae in the cathode compartment could also help improve wastewater treatment through nutrient removal. Therefore, combining algae in the cathode of MFCs provides a promising approach for wastewater treatment.

We have previously developed an integrated photo-bioelectrochemical (IPB) system to synergistically link an MFC with an algal bioreactor for wastewater treatment and bioenergy production [20]. With algae in the cathode, the IPB system effectively removed both organic and nutrient compounds during the one-year operation: more than 92% of organics, 98% of ammonium nitrogen and 82% of phosphate were removed from synthetic wastewater [20]. The analysis of energy balance indicated that the IPB could theoretically produce more energy than it consumed (related to energy required for mixing of the anolyte and the catholyte), thereby making the treatment process energy neutral or positive. The cathode of the IPB was initially inoculated with green algae laboratory organism *Pseudokirchneriella subcapitata*, and over the operation the cathode microbial community became mixture of both bacteria and algae, both of which contributed critical metabolic activities to the IPB performance. In this previous IPB, we used laboratory cultures of algae to inoculate the IPB. However there is growing interest in using natural algal–bacterial assemblages both for wastewater treatment, but also for production of biofuels [21]. Wastewater contains a range of algal and cyanobacterial taxa which could work in wastewater treatment [22]. It is believed that the source of algal inocula plays a key role in shaping microbial communities in the cathode. However, how different algal–bacterial communities might function together for synergistic MFC function has not been explored.

To address these outstanding issues, the aim of this current study is to examine the IPB system with different sources of algal inoculum, sampled from several wastewater treatment facilities. The present IPB system is different from our previous one mainly in two aspects: first, instead of cation exchange membrane employed previously, an anion exchange membrane is used here to facilitate nitrate movement from the anode to the cathode, and second, algal inocula used are from natural source, rather than laboratory cultures used in the previous study. The specific objectives are: (1) to evaluate the performance of the IPB with algal sources from different wastewaters; (2) to analyze the energy balance and production of algal biomass; and (3) to characterize the microbial community in the cathode related to these different algal sources.

## 2. Materials and methods

### 2.1. IPB system setup

The IPB system consisted of a tubular MFC installed in a glass beaker (Fig. 1), which functioned as both the cathode compartment and the algal bioreactor. The MFC was constructed in an anion exchange membrane tube (AEM, Ultrex CMI7000, Membranes International, Inc., Glen Rock, NJ, USA) containing an interior supporter of a polyvinyl chloride (PVC) tube with a diameter of 2.54 cm and a height of 24 cm, resulting in an anode liquid volume of about 180 mL. The AEM was used to improve nitrate removal by promoting nitrate migration from the cathode into the anode for heterotrophic denitrification. A 20 cm-long carbon brush (Gordon Brush Mfg. Co., Inc., Commerce, CA, USA) was used as the anode electrode. The carbon brush electrode was pretreated by immersion in acetone overnight and heated to 450 °C for 30 min [23]. The cathode electrode was a layer of carbon cloth with Pt/C as a catalyst that wrapped the AEM tube. To coat the catalyst to the cathode electrode, the Pt/C powder was mixed with Nafion solution, and then applied to the carbon cloth surface with a brush to a final loading rate of ~0.5 mg Pt cm$^{-2}$. The anode and cathode electrodes were connected by copper wires to an external circuit across a 100-Ω resistor. The glass beaker that held the MFC had a diameter of 10 cm and height of 29 cm, with a liquid volume of 1800 mL. A compact fluorescent bulb (32 W, 120 V, Energy Wiser, color temperature 4000 K, Bulbrute Industries, Inc., China) was installed near the cathode to provide the illumination on a 16-h on/8-h off cycle.

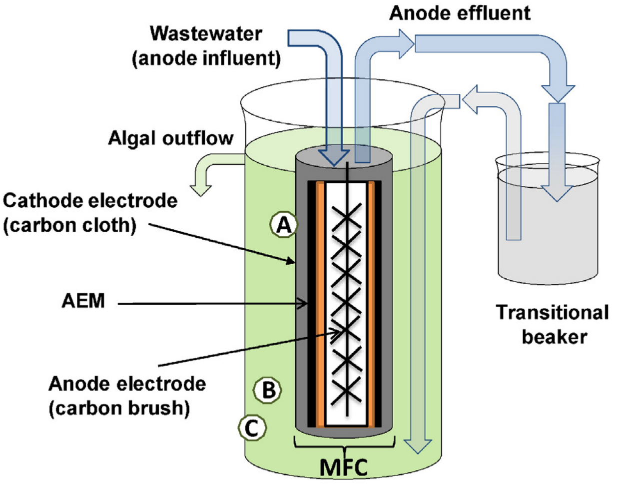

**Fig. 1.** Schematics of the integrated photo-bioelectrochemical (IPB) system consisting of an MFC with anion exchange membrane (AEM) and an algal bioreactor. Synthetic wastewater (anode influent) is fed into the anode compartment, where organic compounds are biologically degraded for generating electrons. Electrons move from the anode electrode (carbon brush) to the cathode electrode (carbon cloth), where oxygen reduction occurs to complete the electrical circuit. The treated wastewater (anode effluent) is discharged into in a transitional beaker, and this solution then is supplied to the cathode compartment (algal bioreactor), where algae grow and produce dissolved oxygen for supporting the cathode reaction. The final effluent from the cathode compartment is discharged containing suspended algal cells. Samples for microbial community analysis were collected from the cathode membrane (A), the suspension (B) and the algal bioreactor wall (C). The algal bioreactor was mixed with a magnetic stirrer and provided with a light source.

### 2.2. Operating conditions

The IPB system was continuously operated at room temperature of ~20 °C. The anode compartment was inoculated with the anaerobic sludge from a municipal wastewater treatment plant (South Shore, Milwaukee, WI, USA). The cathode compartment was inoculated with algal solutions from one of three different wastewater sources: the algal inocula “Sherwood” and “Francisville” were collected from domestic treatment ponds for small towns in the states of Ohio (Sherwood: 41.2897°N, 84.5511°W) and Indiana (Francisville: 40.9858°N, 86.8839° W), respectively; the algal source “WWExp” was a mixed sample taken from several oxidation ponds of wastewater treatment facilities in Indiana and Ohio, USA. The IPB systems tested with these algal sources were denoted as the IPB-S (Sherwood), IPB-F (Francisville) or IPB-W (WWExp). To reduce the effect of (different) reactors, the same IPB was used for testing all three inocula in a sequential order: after testing one inoculum, the cathode electrode of the IPB was cleaned using bleach before inoculating next algal sample, and data was only collected after the system had been operating with the new inocula for at least one month, which ensured that each IPB system had sufficient time to re-establish microbial communities. The anode compartment was fed with a synthetic solution containing sodium acetate as a carbon source and the following compounds: $NH_4Cl$, NaCl, $MgSO_4$, $CaCl_2$, $NaHCO_3$, $KH_2PO_4$, and trace element solution according to a previous study [24] and as detailed in Table S1. This solution was fed into the MFC anode at a flow rate of 0.4 mL min$^{-1}$ (with an analyte recirculation rate of 30 mL min$^{-1}$), resulting in a hydraulic retention time (HRT) of 7.5 h and an organic loading rate of 0.87 kg COD m$^{-3}$ d$^{-1}$ in the anode compartment. The effluent of the anode flowed into a 200-mL transitional beaker, which acted as a stabilization unit, and then the liquid in the