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Mixotrophic operation of photo-bioelectrocatalytic fuel cell under anoxygenic microenvironment enhances the light dependent bioelectrogenic activity

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ABSTRACT

Electrogenic activity of photo-bioelectrocatalytic /photo-biological fuel cell (PhFC) was evaluated in a mixotrophic mode under anoxygenic microenvironment using photosynthetic consortia as biocatalyst. An acetate rich wastewater was used as anolyte for harnessing energy along with additional treatment. Mixotrophic operation facilitated good electrogenic activity and wastewater treatment associated with biomass growth. PhFC operation documented feasible microenvironment for the growth of photosynthetic bacteria compared to algae which was supported by pigment (total chlorophyll and bacteriochlorophyll) and diversity analysis. Pigment data also illustrated the association between bacterial and algal species. The synergistic interaction between anoxygenic and oxygenic photosynthesis was found to be suitable for PhFC operation. Light dependent deposition of electrons at electrode was relatively higher compared to dark dependent electron deposition under anoxygenic condition. PhFC documented for good volatile fatty acids removal by utilizing them as electron donor. Bioelectrochemical behavior of PhFC was evaluated by voltammetric and chronoamperometry analysis.

1. Introduction

Life on earth ultimately depends on the energy derived from sun. Photosynthesis is the only process of biological importance that can harvest light energy (Taiz and Zeiger, 2010). In fact, a large fraction of the planet's energy resources (fossil fuels) results from photosynthetic activity. During the last decade, escalating use of fossil fuels associated with CO_2 emissions, and related environmental issues initiated the search for alternative technologies which generate energy from renewable resources (Tilche and Galatola, 2008). With increasing concern about sustainable energy supplies and waste minimization, solar energy gained much attention to tap enormous resource for powering future generations. Photo-bioelectrocatalytic/photo-biological fuel cell (PhFC) is one such application where the photosynthetic organisms act as biocatalyst to transform light energy to bioelectricity by utilizing CO_2 or organic sources as substrates. Microbial fuel cell (MFC) has gained a great deal of attention in recent years for its capacity to convert organics to bioelectricity through dark-fermentation (Venkata Mohan et al., 2007, 2008a; Chae et al., 2009; You et al., 2008). Unlike dark-fermentative condition, very few and specific reports are available on the usage of photosynthetic mechanism for fuel cell operation. Most of these studies relates to single strains of green algae, cyanobacteria and photosynthetic bacteria acting as anodic biocatalyst by adopting either photoautotrophic or photoheterotrophic mode of nutrition. The metabolic activity of the biocatalyst used and nutrition mode adopted generally governs the efficiency of PhFC. Photoautotrophic mode integrated with oxygenic photosynthesis condition was studied with *Chlamydomonas reinhardtii*, *Phormidium*, *Nostoc*, *Spirulina*, *Anabaena*, *Synechocystis PCC*-6803, etc. as anodic biocatalyst (Venkata Mohan et al., 2008d; Pisciotta et al., 2010; Rosenbaum et al., 2005; Zou et al., 2009). Photoheterotrophic mode of nutrition was mostly evaluated with anoxygenic photosynthesis condition using photosynthetic bacteria like *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Rhodobacter*, *Rhodopseudomonas*, etc. as biocatalyst (Xing et al., 2008; Cho et al., 2008; Rosenbaum et al., 2005; Scheuring et al., 2006; Yeliseev et al., 1996). Photoautotrophic mode of PhFC operation reported to yield lower power output compared to the photoheterotrophic mode of operation. The oxygenic photosynthetic microenvironment prevailing under photoautotrophic condition neutralizes the electron prior to reaching the electrode which result in lower power output.

The aim of this study is to explore the potential of mixotrophic operation, on photo-bioelectrocatalytic/PhFC operation using photosynthetic consortia as biocatalyst. The possibility of light dependent electrogenic activity (i.e., capability of depositing electrons to the extracellular environment in response to illumination) will be evaluated in mixotrophic operation, by harvesting light energy through utilization of organic carbon from wastewater and CO_2 in a PhFC operated with an open-air cathode and non-catalyzed

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graphite electrodes. Mixotrophic operation, facilitates the feasibility of syntrophic association between oxygenic and anoxygenic photosynthetic conditions in a single system. This condition enables the heterotrophic consumption of oxygen generated by autotrophs during microbial respiration, which will have positive influence on the fuel cell power output. Besides bioelectricity generation, mixotrophic operation can neutralize the atmospheric carbon dioxide (CO_2) as well as the CO_2 which is produced due to respiration during PhFC operation and also utilizes the organic carbon present in wastewater (anolyte) facilitating its treatment.

2. Methods

2.1. Photo-biological fuel cell (PhFc) configuration

PhFC designed for single chamber was fabricated using 'perspex' i.e., poly (methyl methacrylate) (PMMA); $C_5H_2O_8$; density, 1.19, MP, 130–140 °C) material which has good light penetrance (92%). The anodic compartment has 0.6 l of total volume with a working volume of 0.5 l. Non-catalyzed plain graphite plates (5 × 5 cm; 10 mm thick; surface area, 70 cm^2) were used as electrodes. Prior to use, the electrodes were soaked in deionized water for overnight. NAFION-117 (Sigma–Aldrich) was used as proton exchange membrane (PEM). Top portion of the cathode was exposed to open-air while bottom portion was fixed to PEM and exposed to wastewater. Prior to use the PEM was treated sequentially with 30% of H_2O_2 and 0.5 M of H_2SO_4. Anode was completely submerged in the anolyte along with PEM while half of the cathode remained submerged (Venkata Mohan et al., 2008b). Provisions were made in the PhFC design for sampling ports, wire input points (top), inlet and outlet ports, gas outlet, etc. Leak proof sealing was provided at the joints in the anode compartment. Copper wires were used for contact with electrodes after sealing with epoxy sealant. Parallaly a dimensionally similar system without providing electrode-membrane assembly was designed and designated as control system.

2.2. Anodic biocatalyst

Mixed photosynthetic consortia acquired from an ecological water body near Nacharam, Hyderabad was used as parent culture for inoculation into the anodic chamber. Parent culture was washed thrice in saline buffer (1000 rpm, 20 °C) and enriched with designed synthetic wastewater (DSW; glucose-3 g/l; NH_4Cl-0.5 g/l, KH_2PO_4-0.25 g/l, K_2HPO_4-0.25 g/l, $MgCl_2$-0.3 g/l, $FeCl_3$-0.025, $NiSO_4$-0.016, $CoCl_2$-25 mg/l, $ZnCl_2$-11.5 mg/l, $CuCl_2$-10.5 mg/l, $CaCl_2$-5 mg/l, $MnCl_2$-15 mg/l, chemical oxygen demand (COD)-3.2 g/l) (Venkata Mohan et al., 2008a, b, c, 2011) under microaerophilic microenvironment at pH 7.0 (100 rpm; 48 h).

2.3. Operation

DSW was modified by replacing glucose with sodium acetate (CH_3COONa, 2 g/l). (Srikanth et al., 2009) Modified DSW was used as substrate throughout the experiments after adjusting the required pH and organic load. Prior to feeding, pH of the wastewater was adjusted to 7 ± 0.1 using orthophosphoric acid (88%)/ 0.1 N NaOH. Both the systems after inoculation with mixed photosynthetic culture (Total solids (TS) 0.5 g/l) were operated with acetate rich wastewater in suspended growth mode employing fed-batch (up-flow) operation at ambient room temperature (27 ± 2 °C). Sequential operations were followed for every feeding event in a stipulated time interval (settling-30 min, decanting-10 min, feeding-10 min). Mixing was done using magnetic stirrer to maintain biomass in suspension and to prevent the formation of substrate gradient during operation. Voltage drop was considered as an indicator for changing the feed. Before changing the feed, inoculum was allowed to settle down (30 min) and exhausted wastewater (0.5 l) was replaced with fresh wastewater under anaerobic condition. The settled biomass (0.1 l) was used for subsequent operations. Wastewater was fed through inlet port provided at the top of anode chamber. Anode chamber was sparged with oxygen free N_2 gas for 30 s after every feeding and sampling event to remove oxygen microenvironment. Constant voltage outputs and substrate (COD) removal efficiency were considered as indicators to assess the stabilized performance of the PhFC. The performance of both the systems was monitored under photo-mixotrophic conditions with 12 h of illumination (altering between 12 h light (3000 ± 200 Lux) and 12 h dark condition) at constant organic load rate (OLR) of 0.182 kg COD/m^3-day.

2.4. Assessment of electrogenic activity

The electrogenic activity of fuel cell was assessed in terms of voltage, current and electron discharges (Logan and Heilmann, 2006). Potential difference/open circuit voltage (OCV) and current (I) (in series; 100 Ω) measurements were recorded using auto range digital multi-meter. Polarization curve was plotted with the function of current density against potential and power density measured at different resistances (30–0.05 kΩ). Anodic oxidation potential and electron motive force (emf) were also measured across various resistances (30–0.05 kΩ). Sustainable power calculations were also made after the fuel cells reached a stable cell potential at respective experimental cycles. Relative change in anodic oxidation potential (RDAP) was calculated to evaluate the sustainable power generation. Volatile fatty acids (VFA), oxidation reduction potential (ORP), pH and COD (soluble, dichromate closed refluxing method) were determined as per the procedures outlined in the standard methods (APHA, 1998). Dissolved oxygen (DO) and pH were measured in anode chamber during operation. Light intensity was measured by Lux meter (LT 300, Extech Corp.).

2.4.1. Bio-electrochemical analysis

Bio-electrochemical behavior of the photo systems was studied *in situ* by cyclic voltammetry (CV), linear sweep voltammetry (LSV) and chronoampeometry (CA) using potentiostat–galvanostat system (Autolab-PGSTAT12, Ecochemie, Netherlands). Voltammograms (cyclic) were recorded by applying a potential ramp at a scan rate of 30 mV/s over the range from +0.6 to −0.6 V. A scan rate of 30 mV/s was optimized for the system, which showed significant interfacial electron-transfer kinetics (Srikanth et al., 2010). LSV (polarizing between −0.6 and 0.6 V) was studied to evaluate oxidation and reduction potentials during the operation. LSV was extended to negative potential to exploit the redox phenomenon of photosynthetic bacteria with the function of bio-anode. CA analysis was performed for 900 s at a poised potential of 0.5 V at the end of the experiment to evaluate the sustainable current generation. All the electrochemical assays in PhFC were performed considering anode (graphite) as working electrode (WE) and cathode (graphite) as counter electrode (CE) against Ag/AgCl (s) reference electrode (RE). In the case of control, two graphite electrodes were inserted at the time of measurement and used as WE and CE against RE.

2.4.2. Biomass and pigments estimation

Total biomass was calculated by measuring the OD of the anolyte at 600 nm (Blankenship et al., 1995; Li et al., 2008). Cell density (D) of algae was estimated by taking optical density (OD) at 650 nm using Eq. (1) (Xin et al. (2010).

$$D\ (\text{cells/ml}) = 9.52 \times 10^6 OD_{650} + 70957 \quad (1)$$

Total chlorophyll (chl *a* and *b*) bacteriochlorophyll *a* (Bchl *a*) were detected calorimetrically in *in vivo* conditions. Anolyte