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Endocrine disruptive estrogens role in electron transfer: Bio-electrochemical remediation with microbial mediated electrogenesis

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ABSTRACT

Bioremediation of selected endocrine disrupting compounds (EDCs)/estrogens viz. estriol (E3) and ethynylestradiol (EE2) was evaluated in bio-electrochemical treatment (BET) system with simultaneous power generation. Estrogens supplementation along with wastewater documented enhanced electrogenic activity indicating their function in electron transfer between biocatalyst and anode as electron shuttler. EE2 addition showed more positive impact on the electrogenic activity compared to E3 supplementation. Higher estrogen concentration showed inhibitory effect on the BET performance. Poising potential during start up phase showed a marginal influence on the power output. The electrons generated during substrate degradation might have been utilized for the EDCs break down. Fuel cell behavior and anodic oxidation potential supported the observed electrogenic activity with the function of estrogens removal. Voltammetric profiles, dehydrogenase and phosphatase enzyme activities were also found to be in agreement with the power generation, electron discharge and estrogens removal.

1. Introduction

Endocrine disrupting compounds (EDCs)/estrogens are exogenous substances that interferes and alters the functions of endocrine system and consequently causes adverse health effects in an intact organism, or its progeny or (sub) populations (Damstra et al., 2002). Estrogens indirectly interact with the endocrine system that regulates the body's function resulting in excessive secretion or suppression of hormones by the phenomenon called endocrine disruption, which may involve the appearance of infertility, sexual under development, altered or reduced sexual behavior, attention deficit or hyperactivity, altered thyroid or adrenal cortical function, increased incidents of certain cancers, birth defects, etc. (Vogel, 2005). Even at nanogram levels these compounds increase the risk of cancer and decrease egg and sperm production, reduce gamete quality and tend to complete feminization of male fish (Notch et al., 2007). Occurrence of EDCs in the aquatic water bodies including fresh water environment and their possible disruptive effects on indigenous fauna was reported in many of the countries (Damstra et al., 2002). From the recent past, concern about the environmental fate and behavior of natural and synthetic organic chemicals detected in water has been increased. Natural or synthetic estrogens exhibit two or three orders of magnitude higher estrogenic activity than the chemical compounds and are considered to be dominant contributors to estrogenic activity in the treated wastewater (Barnes et al., 2008). Estrogens are considered as pseudo persistent pollutants due to their continuous entry into the environment through sewage/domestic wastewater. Removal of estrogens is extremely important to reduce the potential risk caused by them in treated wastewater. Various treatment methodologies have been reported for the removal of estrogens, viz. enzyme mediated, ultrasound destruction, ozonation, adsorption, potassium ferrate treatment, membrane bioreactors, and reverse osmosis, etc. in the literature (Huang et al., 2005; Auriol et al., 2006, 2008; Sei et al., 2008; Mao et al., 2009; Kiran Kumar et al., 2009, 2011). Estrogens degradation efficiency with biocatalyst was reported but with lower concentrations (Jobling et al., 1998). However, the removal efficiencies strongly dependent on the type

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Abbreviations: AO, activation over-potentials; BET, bio-electrochemical treatment; C, control operation; C1, concentration 1 (500 μg/L); C2, concentration 2 (1000 μg/L); C3, concentration 3 (2000 μg/L); COD, chemical oxygen demand; CP, concentration polarization; CV, cyclic voltammetry; DH, dehydrogenase; DSW, designed synthetic wastewater; DSWE, DSW and E3; e^-, electrons; E^0_{anodic}, initial anodic potential; E_{anodic}, anodic potential at resistance; EDCs, endocrine disruptive estrogens compounds; E3, estriol; EE2, ethynylestradiol; EIA, enzymatic immunoassay; ELISA, enzyme-linked immunosorbent assay; FAD^+, flavin adenine dinucleotide; $FADH_2$, flavin adenine dinucleotide (reduced); H^+, protons; *I*, current; LSV, linear sweep voltammetry; MFC, microbial fuel cells; NAD^+, nicotinamide adenine dinucleotide; $NADH + H^+$, nicotinamide adenine dinucleotide (reduced); OCV, potential difference/open circuit voltage; OL, ohmic losses; OLR, organic loading rate; PEM, proton exchange membrane; PT, phosphatase; RDAP, relative decrease in the anode potential; SDR, substrate degradation rate; TF, triphenyl formazan; TTC, 2,3,5-triphenyltetrazolium chloride; UASB, upflow anaerobic sludge blanket; VFA, volatile fatty acids; % *I*, percentage inhibition.

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of treatment applied and the physicochemical properties of the estrogens (Kiran Kumar et al., 2009, 2011).

Microbial fuel cell (MFC) is a hybrid bio-electrochemical system which directly transforms chemical energy stored in chemical bonds of substrate into electrical energy via electrochemical reactions involving biochemical pathways where microorganisms serve as biocatalyst (Lefebvre et al., 2011; More and Ghangrekar, 2010; Oh et al., 2010; Venkata Mohan et al., 2008b,c, 2009; Hamelers et al., 2010). The bio-potential developed between the bacterial metabolic activity [redox reactions generating electrons (e^-) and protons (H^+)] and electron acceptor conditions (separated by a membrane) leads to generate bioelectricity in MFC. Exploiting wastewater as substrate to harness electricity is considered as a sustainable approach and is the present age of energy research. More recently, MFC function as bioelectrochemical treatment system (BET) was established where several pollutants viz. azo dyes, phenol, nitrates, sulphates, chloroaniline, petroleum sludge, etc. have been reported through the anodic oxidation or cathodic reduction reactions (Luo et al., 2009; Mu et al., 2009a,b; Butler et al., 2010; Hamelers et al., 2010; Mohanakrishna et al., 2010; Venkata Mohan et al., 2010b; Venkata Mohan and Chandrasekhar, 2011). Anode chamber of MFC/BET resembles anaerobic treatment unit where substrates gets metabolized into its products through redox reactions and enzyme activities. Enzyme catalyzed transfer of electrons from an intracellular electron carrier molecule (redox mediator) to proton (H^+) will occur as a part of metabolic activity during fermentation process (Venkata Mohan et al., 2010a; Venkateswar Reddy et al., 2010). Oxidation of substrate in order to obtain energy and synthesis of cellular components from carbon source through energy coupling mechanisms is a general process during bacterial metabolic activities. The pollutants present in wastewater can also act as mediators between the fuel cell components, especially electrodes and biocatalyst, and get reduced.

In this context, detailed studies were performed to evaluate the function of BET system in remediation of selected estrogen compounds with simultaneous power generation. The functional role of estrogens in electron discharge during BET operation was also studied. Phosphatase (PT) and dehydrogenase (DH) are important enzymes involved in the carbon degradation and redox reactions for the inter-conversion of metabolic intermediates. PT and DH activities were also evaluated during estrogens bioremediation under microbial electrogenesis. Synergistic interaction of biochemical and electrochemical oxidation processes during estrogens removal was discussed in detail.

2. Experimental design

2.1. Endocrine disruptive compound (EDC)

Estriol [E3, $C_{18}H_{24}O_3$; 1,3,5 (10)-estratriene-3,16α,17β-triol; Sigma–Aldrich] and 17α-ethynylestradiol [EE2; $C_{20}H_{24}O_2$, 17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta [α] phenanthrene-3,17-diol; Sigma–Aldrich] were used as model estrogen compounds after diluting to the required concentrations in water.

2.2. Biocatalyst

Anaerobic consortia from operating full scale UASB reactor treating wastewater was used as biocatalyst (Venkata Mohan et al., 2008a). Parent culture was enriched under anaerobic microenvironment at pH 6.0 in designed synthetic wastewater (DSW) constituting glucose (3 g/L) as carbon source along with the other nutrients such as, 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), $CoCl_2$ (25 g/L), $ZnCl_2$ (11.5 mg/L), $CuCl_2$ (10.5 mg/L), $CaCl_2$ (5 mg/L), $MnCl_2$ (15 mg/L), $NiSO_4$ (16 mg/L) and $FeCl_3$ (25 mg/L), with a chemical oxygen demand (COD) of 3600 mg/L (80 rpm; 48 h).

2.3. System configuration

Single chambered MFC/BET was fabricated with open-air cathode using ‘perspex’ material (total/working volume: 0.5/0.42 L). Non-catalyzed graphite plates [5 × 5 cm; 10 mm thick; surface area 70 cm^2 (plain cathode) and 83.5 cm^2 (perforated anode; 0.1 cm diameter)] were used as electrodes. Prior to use, electrodes were soaked overnight in deionized water (∼18 h). Proton exchange membrane (Nafion 117; Sigma–Aldrich) was fixed between anode and cathode. Top portion of the cathode was exposed to air while bottom portion was fixed to PEM and was in contact with wastewater. Anode was completely submerged in the anolyte (Sfig. 1). Copper wires were used for contact with electrodes after sealing with epoxy sealant. Provisions were made in the design for sampling ports, wire input points (top), inlet and outlet ports and gas outlet. Leak proof sealing was provided at joints to maintain anaerobic microenvironment in the anode compartment.

2.4. Operation

Anodic chamber was inoculated with enriched anaerobic culture (0.04 L) by dissolving in DSW (0.38 L). Prior to feeding, pH of the wastewater was adjusted to 6 (acidophilic) using concentrated orthophosphoric acid (88%) or 1 N NaOH. Wastewater was fed to reactor from inlet provided at the bottom of anode chamber to facilitate the flow in upward direction passing through anode towards cathode (advective flow). Anolyte was continuously stirred at 80 rpm to eliminate concentration gradient during operation. BET was operated in fed batch mode at room temperature (29 ± 2 °C). Before every feeding event, inoculum was allowed to settle down (30 min; settling) and exhausted feed (0.38 L) was removed (decanted; 15 min) under anaerobic conditions. Settled inoculum (∼0.04 L by volume) was used for subsequent operations. Feeding, decanting and recirculation operations were performed using peristaltic pumps controlled by electronic timer. After every feeding event, anode chamber was sparged with oxygen free N_2 gas for 2 min to maintain anaerobic microenvironment. Initially, BET was operated at an OLR of 0.195 kg COD/m^3 day for stabilized performance with respect to power generation and substrate removal. After obtaining stable performance the anode chamber was fed with EDC compounds dissolved in DSW. OLR was maintained constantly (0.458 kg COD/m^3 day) throughout the operation and the concentration of EDCs was varied. Different concentrations of E3 was dissolved in DSW and fed to reactor [C1, 500 μg/L; C2, 1000 μg/L; C3, 2000 μg/L]. Remediation of E3 was also evaluated under 2000 mV poised potential (PP) with C2 concentration. 2000 mV was selected as external potential based on the observations made in previous study where increased treatment efficiencies were observed (Srikanth et al., 2010). Remediation of EE2 was also evaluated at C2 [1000 μg/L] concentration. Operation details along with the performance were as depicted in Table 1.

2.5. Analysis

Power output and substrate degradation were considered to assess the performance of BET during operation with varying nature and concentrations of estrogens. Potential difference/open circuit voltage (*OCV*) and current (*I*) (in series; 100 Ω) measurements were recorded by digital multi-meter. Power (mW) was calculated using $P = IV$. Power density (mW/m^2) and current density (mA/m^2) were calculated by relating the obtained power and current with