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Reductive behaviour of acid azo dye based wastewater: Biocatalyst activity in conjunction with enzymatic and bio-electro catalytic evaluation

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HIGHLIGHTS

- Azo reductase and dehydrogenase activities correlated with dye removal.
- Cleavage of azo compounds to metabolic intermediates were detected.
- Redox shuttlers facilitated enhanced electron transfer.

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ABSTRACT

Present study illustrates the significance of biocatalyst's reductive behaviour in the degradation of dye molecules using glucose as co-substrate. An anaerobic system was operated at a dye concentration of 50 mg/l with an organic loading rate (OLR) of 1.36 kg COD/m^3-day. Decolourization and COD removal efficiencies were observed to be 42% and 48% respectively. Azo reductase (18.9 U) and dehydrogenase enzyme (1.4 μg/ml) activities showed increment with operation time. Anaerobic microenvironment showed dye reduction converting them into aromatic amines. The presence of mediators viz., cytochromes, quinines and Fe–S proteins depicted in the cyclic voltammetry profiles played a crucial role in transfer of electrons for the reduction of dye molecules. Bio-electro kinetic profiles obtained through Tafel analysis showed persistent reduction behaviour, which is in good correlation with dye degradation in the anaerobic microenvironment.

1. Introduction

Azo dyes are the group of synthetic colourants characterized by aromatic moieties, which are linked with one or more azo groups (R_1-N = N-R_2). Different substitutions on the aromatic nucleus give structurally diverse and versatile group of compounds owing to their recalcitrance and toxicity (Jain et al., 2012; Linda et al., 2009). Degradation of these recalcitrant compounds is most commonly carried out through various techniques viz., physical, chemical, biological etc. Azo dye often remains chemically unchanged in effluents of wastewater treatment plants due to the presence of sulfo and azo groups that render recalcitrance for oxidative biodegradation by protecting the dye molecule against the attack of oxygenases (Nigam et al., 1996; Keharia and Madamwar, 2003). Therefore, conventional activated sludge process (ASP) is not considered to be a viable option for the treatment of azo dye based wastewater (Kulla et al., 1983; Shaul et al., 1991). Decolourization of azo dyes requires an initial cleavage of azo bond in anaerobic microenvironment prior to aeration processes (O'Neill et al., 2000; Venkata Mohan et al., 2013a). Anaerobic biological processes are considered to be advantageous compared to the physico-chemical methods due to their eco-friendly and cost effective nature (Katuri et al., 2009; Pandey et al., 2007; Venkata Mohan et al., 2005, 2007, 2012; Balapure et al., 2015). Biological decolourization via anaerobic reductive processes usually require exogenous electron donors, which create conducive conditions that support reductive break down of the azo bond to the corresponding colourless aromatic amines (Vander Zee and Villaverde., 2005; Ong et al., 2005). The metabolic capabilities of individual microbial species are limited and do not support effective mineralization of azo dyes. However, mixed population consisting of diverse bacteria are efficient in degrading the azo compounds (Moosvi et al., 2007; Pearce et al., 2003) due to the synergistic interactions among the various species. In the light of these premises, the present study is designed and carried out in an

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anaerobic system using mixed culture as biocatalyst for the treatment of azo dye based wastewater under reductive microenvironment with a dye concentration of 50 mg/l with an organic loading rate of 1.36 kg COD/m^3-day. The reductive behaviour of biocatalyst in anaerobic microenvironment was studied with the function of enzymatic and bio-electrocatalytic activities along with the biochemical parameters.

2. Methods

2.1. Experimental details

C.I. Acid black 10B (4-amino-5-hydroxy-3-[(4-nitrophenyl) azo]-6-(phenylazo)-2,7-naphthalene disulfonic acid disodium salt ($C_{22}H_{14}N_6O_9S_2Na_2$), an azo dye belonging to acid applications class was used as test dye. Simulated dye wastewater (SDW) was prepared by dissolving 50 mg/l of dye in designed synthetic wastewater [DSW (g/l): glucose-3.0, NH_4Cl-0.5, KH_2PO_4-0.25, K_2HPO_4-0.25, $MgCl_2$-0.3, $CoCl_2$-0.025, $FeCl_3$-0.025, $ZnCl_2$-0.0115, $NiSO_4$-0.050, $CuCl_2$-0.0105, $CaCl_2$-0.005 and $MnCl_2$-0.015]. pH of the DSW was adjusted to 7.0 ± 0.2. Prior to startup, the bioreactor was inoculated with mixed anaerobic sludge (15%; collected from wastewater treatment plant, Hyderabad). Parent biomass was washed twice with phosphate buffer saline (PBS) and re-suspended in DSW (chemical oxygen demand (COD) of 3 g/l (without dye)) for overnight at 28 °C. The overnight grown culture was inoculated to the bioreactor by re-suspending through feed (VSS, 4870 mg/l). The bioreactor was operated in an air tight sealed condition to ensure the prevalence of anaerobic conditions with a total/working volume of 0.50/0.45 l in fed-batch mode at an hydraulic retention time (HRT) of 48 h. The reactor was kept for biomass settlement (15 minutes) and the supernatant was decanted.

2.2. Enzymes activity

2.2.1. Azo reductase activity

Azo reductase activity was monitored to understand the reductive cleavage of azo bond in the dye molecule. Colorimetric method was employed to estimate the extracellular azo reductase enzyme activity using NADH as co-substrate (Leelakriangsak and Borisut, 2012; Naresh Kumar et al., 2014). Reaction mixture constituting of 600 µl sample, 600 µl test dye, 1.2 ml of potassium phosphate buffer and 7 mg/ml of NADH was incubated for 12 h (37 °C). The absorbance of overnight incubated reaction mixture was monitored at λ_{max} of 618 nm. The linear decrease of absorption was used to calculate the azo reductase activity. One unit of azo reductase accounts for the amount of enzyme required to decolourize 1 µmol of dye per minute (Venkata Mohan et al., 2013a; Nagendranatha Reddy et al., 2014).

2.2.2. Dehydrogenase (DH) activity

Dehydrogenase enzyme (DH) activity (substrate linked) of the anaerobic biocatalyst was estimated using redox sensitive 2,3, 5-triphenyltetrazolium chloride (TTC) based on its reduction to insoluble formazan. TTC (colourless soluble dye) serves as the terminal electron acceptor in biochemical reactions (Zhenhua et al., 2008; Venkata Mohan et al., 2013b). 5 ml of TTC (5 g/l) and 2 ml of glucose solution (0.1 mol/l) were added to 5 ml of culture and stirred continuously for 20 minutes (200 rpm) followed by its incubation at 37 °C for 12 h. Thereafter, one millilitre of concentrated sulphuric acid was added to the reaction mixture (to stop the oxidization) followed by addition of 5 ml toluene to extract the triphenylformazan (TF) formed in the reaction mixture and the sample was agitated at 200 rpm (30 min). After keeping the reaction mixture idle for 3 min, it was then centrifuged at 4000 rpm (5 min) and the supernatant was collected to measure the absorbance at 492 nm using spectrophotometer (TF forms a coloured complex with toluene).

2.3. Analysis

Samples from the reactor were collected at selected time intervals and the resultant supernatant, after centrifugation (4000 rpm; 5 min; 28 °C) was analyzed for dye concentration, change in COD concentration and enzymes activities. The dye concentration was monitored colorimetrically at λ_{max} 618 nm using UV–vis spectrophotometer (Thermo Electron). The performance of the reactor was assessed by monitoring COD, pH and volatile fatty acids (VFA) as per the procedures outlined in the standard methods (APHA, 1998). Cyclic voltammetry (CV) technique was employed to observe redox variations in bioelectrochemical behaviour of anaerobically operated biocatalyst using potentiostat–glavanostat system (Autolab-PGSTAT12, Ecochemie). A potential ramp of +0.5 to −0.5 V was applied (30 mV/s scan rate) to record the voltammograms (Srikanth and Venkata Mohan, 2012). All the electrochemical assays were performed using platinum wire and glassy carbon rod as working electrode and counter electrodes respectively against reference electrode (Ag-AgCl(S)) in wastewater as anolyte (Velvizhi and Venkata Mohan, 2015). Derivative Cyclic Voltammogram (DCV), Tafel plots, slopes (β_a and β_c) and polarization resistance (R_p) were derived from the CV profiles to understand the extracellular electron transfer sites (EETs), redox reactions and potentials, resistance offered, respectively with respect to time intervals (Annie Modestra and Venkata Mohan, 2014).

3. Results and discussions

3.1. Colour removal

Initially, the anaerobic system was operated with designed synthetic wastewater (DSW; without dye) for 10 cycles with the retention time of 48 h to facilitate the biomass growth and acclimatization. After acclimatization with respect to constant COD removal (75.3%), the reactor was fed with synthetic azo dye bearing wastewater (SDW-50 mg/l) and operated continuously accounting for total operation time of 720 h with an OLR of 1.36 kg COD/m^3-day. Improvement in dye degradation pattern was observed with the operation time (15 cycles) representing the adaptation of the biocatalyst to the toxic dye microenvironment (Fig. 1a). During initial cycles of operation, dye removal efficiency of 30% (cycle 1; 15 mg dye removal) was observed and after 10 cycles of operation, the removal efficiency increased to 39% depicting the increment in dye removal efficiency due to adaptation of biocatalyst to the dye microenvironment. Significant variation in colour removal efficiency was observed during initial cycles from cycle-1 to 11, respectively. However, continuous operation of bioreactor with dye bearing wastewater improved the colour removal efficiency and resulted in marginal variations from cycle-12 to 15. Maximum colour removal efficiency of 42% was observed at 15th cycle operation. Colour removal efficiency was observed to vary at various time intervals with respect to the cycle length. Maximum colour removal was observed during the initial hours of operation (6 h), which increased gradually thereafter reaching maximum removal efficiency by the end of cycle operation. During 6 h, cycle-1 operation depicted dye removal efficiency of 16% followed by cycle 10 (24.7%) and cycle 15 (30.54%). The breakdown and removal of chromophore group of azo dye was assessed by UV–visible spectroscopy using multi-scan spectrum analysis (200–800 nm) for every 6 h time interval (Fig. 1b).