WATER RESEARCH 60 (2014) 182–196

Available online at www.sciencedirect.com
ScienceDirect
journal homepage: www.elsevier.com/locate/watres

Azo dye load-shock on relative behavior of biofilm and suspended growth configured periodic discontinuous batch mode operations: Critical evaluation with enzymatic and bio-electrocatalytic analysis

*A. Naresh Kumar, C. Nagendranatha Reddy, R. Hari Prasad, S. Venkata Mohan**

Bioengineering and Environmental Centre (BEEC), CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad 500007, India

ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form
4 April 2014
Accepted 11 April 2014
Available online 24 April 2014

Keywords:
Derivative cyclic voltammetry (DCV)
Extracellular electron transfer (EET)
Azo reductase
Dehydrogenase activity
Sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE)
Sequencing batch reactor (SBR)

ABSTRACT

Effect of dye (C.I.Acid Black 10B) load-shock was comparatively evaluated in biofilm (self-immobilized) and suspended growth systems operated in periodic discontinuous batch mode (PDBR, anoxic-aerobic-anoxic) was investigated. At higher dye load (1250 mg dye/l), biofilm system showed relatively higher dye (74.5%) and COD (46%) removal efficiencies than the corresponding suspended mode operation (dye/COD removal efficiency, 42%/65%). Increment in dye load showed increment in azo reductase and dehydrogenase enzyme activities. Voltammograms (cyclic) showed higher reduction currents (RC) with increment in dye load specifically in biofilm system. Derivative cyclic voltammograms analysis depicted the involvement of mediators (NAD^+, FAD^+, etc.) which presumably played a major role in electron transport chain and dye degradation. Disappearance of peak (1612 cm^{-1}) specific to azo group in FTIR spectrum, at higher loading rate in both the systems indicates the non-inhibitory and robust nature of PDBR operation.

1. Introduction

On an annual basis, it is estimated that 4500 kg/year of dyes are released as wastewater in which 70% of the dyes are insoluble (Frijters et al., 2006). Azo dyes are the largest and versatile group of dyes having a wide range of applications in the textile, paper, food, leather, cosmetics and pharmaceutical industries (Franciscon et al., 2012). These dyes are xenobiotic and toxic in nature and found to have high

* *Corresponding author*. Tel./fax: +91 40 27191664.
E-mail addresses: vmohan_s@yahoo.com, svmohan@iict.res.in (S. Venkata Mohan).

http://dx.doi.org/10.1016/j.watres.2014.04.027

Nomenclature

ΔG	Gibbs free energy
COD	chemical oxygen demand
CV	cyclic voltammetry
DCV	derivative cyclic voltammetry
DH	dehydrogenase
DO	dissolved oxygen
DSW	designed synthetic wastewater
e^-	electron
EA	electron acceptor
EET	extracellular electron transfer
Ef	formal potential
Ep	peak position
E_{pa}	potential at anodic current
E_{pc}	potential at cathodic current
FAD	Flavin Adenine dinucleuotide
FTIR	Fourier transforms infrared spectroscopy
H^+	proton
HRT	hydraulic retention time
i_{pa}	oxidation peak current
i_{pc}	reduction peak current
KBr	potassium bromide
KDa	Kilo Dalton
MW	molecular weight
NAD	nicotinamide adenine
OC	oxidation current
OLR	organic loading rate
PDBR	periodic discontinuous batch reactor
RC	reduction current
Rpm	rotations per minute
R_p	polarization resistance
SBR	sequencing batch reactor
SDR	substrate degradation rate
SDS-PAGE	Sodium dodecyl sulphate-poly acrylamide gel electrophoresis
SDW	synthetic dye wastewater
TF	triphenylformazan
TTC	2, 3, 5-triphenyltetrazolium chloride
UV–Vis	Ultra Violet–Visible
VFA	volatile fatty acids
βa	oxidative slope
βc	reductive slope

recalcitrance thus they present in environment creating aesthetic and environment problems (Rathod and Archana, 2013; Venkata Mohan et al., 2012, 2013b). Treatment of dye bearing wastewater through biological process is particularly challenging due to the recalcitrant, inhibitory nature and complex aromatic structures of these dyes (Shah et al., 2013; Malachova et al., 2013; Venkata Mohan et al., 2013a). Conventional aerobic wastewater treatment processes are seldom capable of efficiently decolorizing azo dye contaminated effluents due to the strong electron-withdrawing property of the azo groups that protects them against the attack by oxygenases (Hai et al, 2011; Venkata Mohan et al., 2007a,b). However, anaerobic metabolic function facilitates reductive breakdown of azo dye molecule by cleaving the azo bond to the corresponding colorless aromatic amines. These aromatic amines resist further anaerobic degradation, due to their mutagenic nature (Basibuyuk and Forster, 1997; Ong et al., 2005; Venkata Mohan et al., 2004, 2007c,d, 2012). Conversely, the aromatic amines could be mineralized in aerobic microenvironment by non-specific enzymes through hydroxylation and ring-fission of aromatic compounds (O'Neill et al., 2000; Khalid et al., 2010; McMullan et al., 2001; Venkata Mohan et al., 2005a,b). Thus although anaerobic process alone cannot handle the complete mineralization of the dye bearing wastewater, combining anaerobic and aerobic treatments can remove color efficiently with simultaneous degradation the organic matter (Fu et al., 2001; O'Neill et al., 2000; Venkata Mohan et al., 2007b,d). Sequential integration of anaerobic/micro-aerophilic/aerobic process facilitates initial reduction of the dye molecule (anaerobic) and followed by degradation of dye metabolites (aerobic) (Rao et al., 2005; Muda et al., 2011: Venkata Mohan et al., 2013a). Sequencing batch reactor (SBR) or periodic discontinuous batch reactor (PDBR) enforces controlled stable steady state conditions in the long run and also imposes selective pressures that can select a defined biocatalysts which are able to degrade complex wastewater (Wilderer et al., 2001; Venkata Mohan et al., 2004a, 2005a, 2009).

The biological process efficiency depends on the type of reactor configuration used and the associated operating conditions adopted along with the nature and characteristics of the wastewater being treated. Among the reactor configurations, biofilm (self-immobilized) configured systems are considered to be effective over the corresponding suspended growth systems due to the possibility of higher hydraulic loading rates and optimal utilization of the biomass (Venkata Mohan et al., 2013b). Biofilm operation also induces both aerobic and anoxic microenvironments along biofilm depth and competition for space, oxygen and organic carbon, thus results in a spatial distribution of microorganisms within the biofilm (Venkata Mohan et al., 2005). In the present communication, detailed experiments were performed to study the influence of azo dye shock-loads on biofilm and suspended growth configurations operated in PDBR/SBR mode on treatment efficiency by keeping organic load constant. The bioprocess during treatment was critically analyzed by enzyme activities viz. azo reductase and dehydrogenase (DH) along with bio-electrochemical analysis to comprehensively understand the process dynamics.

2. Materials and methods

2.1. *Synthetic dye wastewater*

C.I.Acid Black 10 B (4-amino-5-hydroxy-3-[(4-nitrophenyl) azo]-6-(phenylazo)-2, 7-naphthalene disulfonic acid disodium salt; $C_{22}H_{14}N_6O_9S_2Na_2$; MW, 616.49; CAS No. 1064-48-8), an azo dye (acid application class)was used as test dye. Simulated dye wastewater (SDW) was prepared by dissolving corresponding concentration 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