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Simultaneous electricity production and Direct Red 80 degradation using a dual chamber microbial fuel cell

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ABSTRACT

Microbial fuel cells (MFCs) are gaining tremendous interests for achieving simultaneous power production and recalcitrant wastewaters treatment. In this study, simultaneous electricity generation and tetra-azo dye (Direct Red 80) decolorization was examined in a dual chamber MFC. In addition, glucose and various volatile fatty acids were separately examined as co-substrates for anaerobic dye degradation and bioelectricity generation. Maximum power of 477.8 and 455.7 mW/m^2 were attained with glucose (1,000 mg/L) as a sole carbon source and glucose (1,000 mg/L) coupled with dye (200 mg/L), respectively. At this glucose and dye initial concentration, 85.8% color and 74.9% COD removal were resulted in 48 h batch studies. Color removal without any co-substrate's addition was 23%, indicating dye was degraded mainly in the presence of carbon sources. There was no substantial negative effect in electricity generation was observed with the dye degradation. Dye removal was decreased with the increase in dye initial concentration (25–800 mg/L) and increases notably with the increase in initial glucose concentration between 0 and 1,000 mg/L, while afterward insignificant effect up to 2,000 mg/L was observed. Glucose was determined as better co-substrate followed by acetic, propionic, and lactic acid in terms of dye removal and maximum power production. Gas chromatography-mass spectrometry showed sodium 4-aminoazobenzene-4′-sulfonate to be the subsequent metabolites formed during the decolorization of dye. This work demonstrated that MFC could be applied to achieve electricity generation and simultaneous azo dye degradation using glucose as the preferred co-substrate.

Keywords: Microbial fuel cell; Direct Red 80; Dye degradation; Anaerobic; Power density

1. Introduction

Global climate change and environmental pollution due to the abuse and potential shortfall of fossil fuels make it important to search for alternative energy sources that are cost-effective, environmentally friendly, and renewable [1–3]. In recent years, microbial fuel cells (MFCs) have attracted considerable attention as a suitable technology for simultaneous renewable energy production and wastewater

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treatment [4,5]. MFCs can efficiently convert variety of substrates (comprising organic compounds and even toxic substances) to electricity with the aid of biocatalytic reactions carried out by micro-organisms [6–9]. Mild conditions of ambient temperature, near to neutral pH and effectively unlimited range of potential fuels are obvious advantages of MFCs [10–12].

Azo dyes, which are aromatic compounds with one or more –N=N– group(s), comprise the largest and the most diverse band of synthetic dyes [13], and are widely used in a number of industries such as textile, food, cosmetics, and paper printing [14]. Azo dyes are quite toxic to living organisms due to their xenobiotic nature, and have been reported to contribute to the mutagenic activity of ground and surface water polluted by textile effluents [15]. Physicochemical methods, such as adsorption, coagulation–flocculation, filtration, and advanced oxidation have been used to treat wastewater containing azo dyes [16]. But these methods are not efficient in removing the recalcitrant dyestuff from wastewater due to their commercial insignificance and potential generation of secondary pollution [17]. Biological anaerobic processes provide a low-cost and efficient alternative for simultaneous color and organic matter removal [18,19]. Anaerobic environment of MFC anode can degrade azo dyes in the anaerobic chamber of the MFCs with readily degradable organic carbon sources. Simultaneous electricity generation and azo dye decolorization have been successfully achieved in MFCs [20–22]. Anaerobic azo dyes degradation processes are strongly influenced by operational conditions such as temperature, pH, alkalinity, and co-substrates [23,24]. Few researchers have studied the azo dyes decolorization in MFCs using various organic compounds and reported their substantial effects on dye decolorization [25,26]. However, to date, still little is known about the potential effect of metabolites produced after substrate (glucose) degradation (i.e. mainly into acetic, propionic, and lactic acids) on dye biodegradation and dye addition on these organics removal in MFCs. Azo bonds present in the dye effect the bacterial decolorization and degradation of azo dyes. It has been reported that dyes with simple structures (or dyes with lower number of azo bonds) and low molecular weights exhibit higher rates of color removal, whereas the removal rate is lower in the case of complex (or polyazo) and high molecular weight dyes during bacterial decolorization studies [27]. However, previous studies for azo dye removal in MFCs were carried out mainly in the presence of simple azo dyes (mono or diazo).

In this study, the performance of a dual chamber MFC was investigated for simultaneous organic compounds removal and poly azo dye decolorization coupled with electricity production. Glucose and short chain fatty acids such as lactic, propionic, and acetic acids, which are mainly the fermentation products of anaerobic glucose degradation were employed to study their impacts on decolorization and electric power generation. In addition, the effects of initial co-substrate and dye concentrations on MFCs performance in terms of color removal and current production were evaluated. Biotransformation after the reductive cleavage of azo dyes was studied and their by-products were identified using gas chromatography-mass spectrometry (GC–MS).

2. Materials and methods

2.1. Dye

The Direct Red 80, a water soluble dye, was purchased from Sigma Aldrich, South Korea and was used as received without further processing.

2.2. MFC assembly

A dual chamber MFC reactor made up of plexiglass (each compartment: 5 cm × 5 cm × 8 cm) as previously reported [28,29]. Each anode compartment and cathode compartment was parted by a proton exchange membrane (Nafion® 117, DuPont Co.). Graphite felt (9.18 mm think, 5 cm × 5 cm) was used as the anode and platinum-coated graphite cloth facing the air side (20 wt% Pt., 5 cm × 5 cm) was used as the cathode. The MFC chambers were autoclaved for 20 min at 121˚C before use. The proton exchange membrane was pretreated with boiling distilled water, H_2O_2 (3%) and 0.5 M H_2SO_4 for 1 h each. The cathode and anode electrodes were dipped in distilled water for 1 d before using in chamber.

2.3. Inoculum and operation of MFC

Anaerobic digestion sludge collected from a domestic sewage treatment plant of Daegu, South Korea, was inoculated into the anaerobic compartment, i.e. the anode part of the MFC reactor after screening and washing. Main characteristics of the sludge analyzed were: pH 7.24, total suspended solids (TSS) = 14.91 g/L, volatile suspended solids (VSS) = 11.46 g/L, and filtered COD = 154 mg/L. To stabilize the MFC reactor, a defined medium containing glucose as a sole carbon source was supplied in a fed-batch mode over three months. The medium used comprise 560 mg/L $(NH_4)_2SO_4$, 420 mg/L $NaHCO_3$, 200 mg/L $MgSO_4 \cdot 7H_2O$, 20 mg $MnSO_4 \cdot H_2O$, 15 mg/L

$CaCl_2$, and other trace minerals. Magnetic stirring was used for proper mixing of the medium in the anode part. Nitrogen gas was initially purged into the anode part to attain strictly anaerobic conditions, whereas air was blown continuously into the cathode part to supply oxygen, i.e., an electron acceptor. All experiments were performed in batch mode at room temperature (20–25°C). Liquid samples were periodically taken from the anode part and filtered using 0.45 μm cellulose nitrate membrane for analyses of main components.

2.4. Analytical methods

Decolorization of Direct Red 80 was monitored by UV–vis (Hitachi U-2800) spectroscopy at the maximum visible absorbance wavelength of the dye (542 nm). Decolorization was quantified by correlating the absorbance at this wavelength.

Decolorization was determined by measuring the decrease in absorbance at the maximum wavelength of 542 nm. UV–vis (Hitachi U-2800) spectroscopy with a spectrometric quartz cell (1 cm path length) was used for this purpose. Color removal percentage was calculated as follows:

$$\text{Color removal } (\%) = \frac{C_\text{i} - C_t}{C_\text{i}} \times 100 \quad (1)$$

where C_i is the initial absorbance and C_t is the observed absorbance at time t. COD was measured using a Hach spectrophotometer. COD removal percentage was determined as follows:

$$\text{COD removal } (\%) = \frac{COD_\text{i} - COD_t}{COD_\text{i}} \times 100 \quad (2)$$

where COD_i is the initial COD concentration and COD_t is the COD concentration at time t. The TSS and VSS were analyzed using the standard methods [30].

The voltage difference between the two electrodes was recorded across a fixed load of 22 0 Ω by a multicenter voltage collection and data acquisition system (Model 2700, Keithley Instruments Co., USA) connected to a personal computer. Current *(I)* was calculated from resistance *(R)* and voltage (V) by $I = V/R$. Power density (mW/m^2) was calculated according to relation, $P = VI/A$, where I is the current, V is the voltage, and A is the projected cross-sectional area of the anode. Power density curves were used to obtain the maximum power density by varying the external resistance from 0.5 to 1,000 Ω. Coulombic efficiency was calculated as follows:

$$\text{Coulombic efficiency } (\%) = \frac{8 \times I \times \Delta t}{\Delta\text{COD} \times V \times 96,485} \times 100 \quad (3)$$

where I is the average current, Δt is the batch time duration, ΔCOD is the difference of COD value between influent and effluent of anode chamber, V is the volume of wastewater treated, eight is constant based on 32 as the molecular weight of O_2, and four number of electrons for 1 mol of O_2. Dye metabolites were identified by gas chromatography-mass spectrometry (GC–MS). Supernatant taken was used to extract the metabolites with an equal volume of dichloromethane. The extracts were desiccated over anhydrous sodium sulfate at room temperature. The resulted crystals were dissolved in a small volume of HPLC grade methanol and used for GC–MS analysis. GC–MS analysis of the dye subsequent metabolites was carried out using a Finnegan/MAT (GCQ) at the ionization voltage of 70 eV in temperature programming mode on a DB-1 column. The initial column temperature was 80°C for 2 min, increased linearly to 280°C at 10°C/min, and retained at that temperature for 7 min. The temperature of the injection port was 280°C and the GC–MS interface was maintained at 290°C. The helium carrier gas flow rate was 1.0 ml/min. The degradation products were identified by the fragmentation pattern in the mass spectra. The concentrations of glucose and VFAs (butyric acid and lactic acid) were analyzed by high-performance liquid chromatography (Model 1200, Agilent Inc.) using a refractive index detector and an Aminex HPX-87H column (300 mm × 7.8 mm) with 8 mN H_2SO_4 as the mobile phase.

3. Results and discussion

3.1. Power production

To augment exoelectrogens and to stabilize their activity in the MFC reactor, easily fermentable glucose was supplied as a sole carbon source over three months in fed-batch mode. Fig. 1 shows the electricity production with glucose alone and glucose with the dye. Maximum current produced from glucose alone was 2.19 mA. Glucose was completely degraded within 4 h and acetate was formed as the main metabolite of its degradation. It is noteworthy that the current could be stably produced until the metabolite, acetate, was completely degraded in the anode part of the MFC reactor. After a stable voltage was obtained using glucose, the medium in the anode chamber was replaced with a defined medium containing glucose

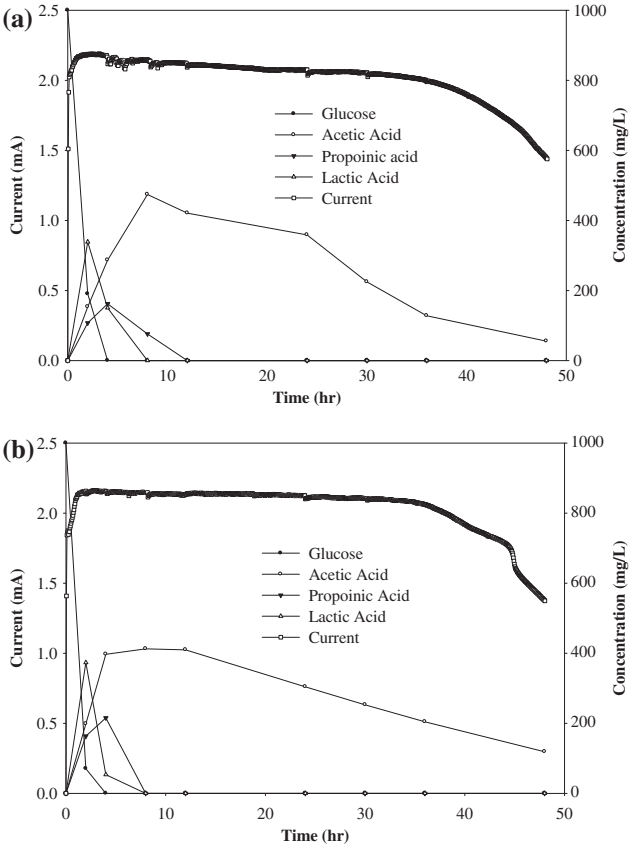

Fig. 1. Current generation and glucose degradation with (a) glucose (1,000 mg/L), (b) glucose (1,000 mg/L), and Direct Red 80 (200 mg/L).

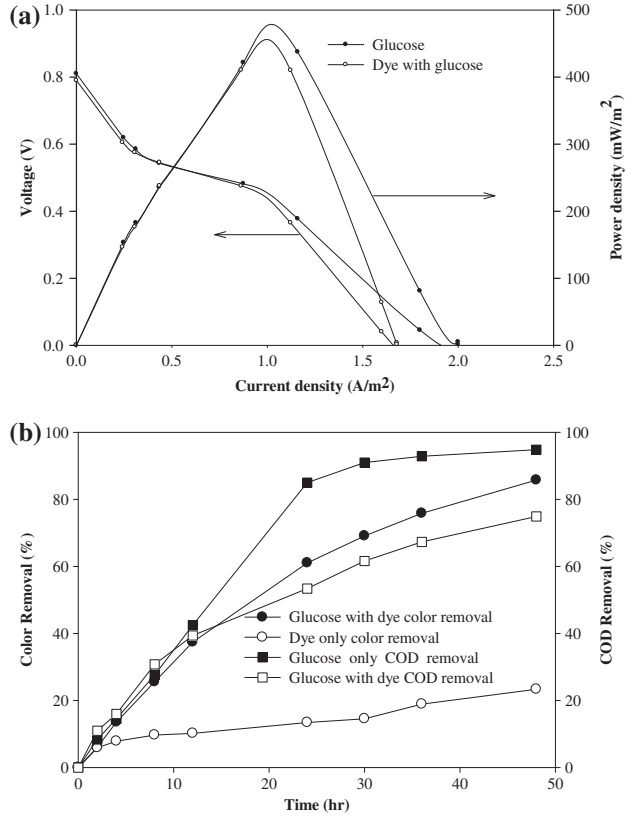

Fig. 2. (a) Power density and polarization curves with glucose only and glucose with dye and (b) color and COD removal performance.

(1,000 mg/L) and Direct Red 80 (200 mg/L) and operated further until the system was acclimated well with the dye. Degradation rate of glucose in the presence of the dye was still fast as electrons generated from glucose were used for azo dye reduction. Therefore, the addition of azo dye to the MFC did not affect maximum current generation at large. Fig. 2(a) shows polarization and power density curves. The maximum power density was 477.8 mW/m^2 at current density of 1.08 A/m^2 for glucose, compared with 455.7 mW/m^2 at current density of 1.00 A/m^2 for glucose with the dye. Hence, a decrease in maximum power density by 4.62% was observed, indicating the presence of the dye slightly influenced the MFC power density. The difference might be due to the competition between electrons for azo dye reduction and electrons transfer to anode. Coulombic efficiency achieved in this study for glucose as carbon source with and without dye was 13.7 ± 1.4%. Power production in this study was higher than previous similar studies of simultaneous power production and dye degradation. Power production reported by Sun et al. [22] was 234 mW/m^2 with glucose as carbon source and active red X-3B (300 mg/L) as dye in a single chamber MFC. While power production reported by Li et al. [18] were 364.5 mW/m^2 with glucose as carbon source and Congo Red (100 mg/L) as dye. These results revealed that the activity of electricity generating bacteria in MFC was not inhibited to greater extent and presented tolerance to dye with glucose as co-substrate. In practical applications, the possibility of MFC utilizations on the industrial wastewater treatment containing dyes and carbon sources can be established.

3.2. Dye degradation and COD removal

Due to lack of carbon in dyes, biodegradation is very difficult without an extra carbon source [31]. In several cases, the microbial discoloration of azo dyes or textile effluents was increased in the presence of glucose [23]. Glucose serves as the source of carbon and energy for the growth and survival of the microorganisms and as an effective electron donor, which is necessary for the breakage of the azo bond [32,33].

Fig. 2(b) shows dye and COD removal in the MFC. Dye was removed in MFC using glucose as co-substrate. About 85.8% color and 74.9% COD removal were obtained in the batch experiments at the dye concentration of 200 mg/L and glucose concentration of 1,000 mg/L, whereas COD removal with glucose (1,000 mg/L) only was 94.5%. This indicates that intermediates formed by dye degradation may be toxic in nature and slow down COD removal processes. The toxic intermediates with aromatic amines could be removed by coupling the process with aerobic treatment [34]. In this study, color removal without carbon source was only 23%, indicating Direct Red 80 was mainly removed in the presence of glucose which was in agreement with previous studies [35].

UV–vis spectral scan of supernatants with increase in time showed that the maximum absorbance in visible spectra was decreasing at 542 nm (Fig. 3). There was also a decrease in absorbance around 298 nm and an increase around 254 nm in ultraviolet region. The peak in visible region corresponds to azo bond, whereas peaks in ultraviolet region correspond to naphthalene and benzene structures [36]. These changes occurred in UV and visible region interpreted that the molecular structure of sulfonated dye Direct Red 80 changed after decolorization. It could be presumed that the azo bonds cleaved during the biological reaction and primary chromophore was annihilated. The absorbance peaks did not disappear completely, indicating the formation of intermediates resulting from the biodegradation of the azo dye, which still contains aromatics.

The metabolites produced after dye degradation were analyzed by GC–MS. The structures of the detected metabolites were identified as sodium 4-aminoazobenzene-4′-sulfonate. In most studies of azo dye degradation, naphthalene derivatives were reported to be the end products. Biodegradation products proposed from Congo Red dye in anaerobic–aerobic MFC reactors by Li et al. [18] contains amine, naphthalene, and phenyl-based compounds. Rasool et al. [15] studied Direct Red 80 degradation in the presence of the mixed anaerobic sulfate-reducing bacteria culture and detected metabolites were identified as aniline and 1, 4 diamine benzene. Fig. 4 shows a probable pathway for dye degradation of Direct Red 80.

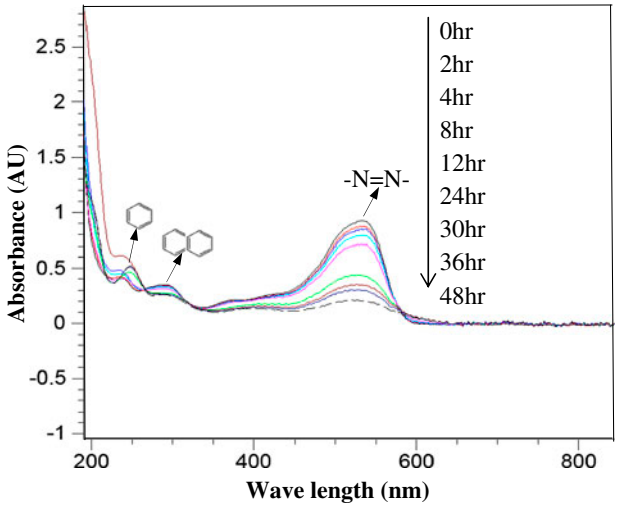

Fig. 3. UV–vis spectra of Direct Red 80 at different time intervals.

3.3. Effect of initial dye and glucose concentrations on color removal and current generation

The effects of initial dye and glucose concentrations on the MFC system performance were evaluated in terms of color removal and current generation. The initial dye concentrations in the range of 25–800 mg/L at the fixed glucose concentration of 1,000 mg/L were applied to the batch reactors. Fig. 5(a) shows that color removal decreases with increase in dye concentration. Color removal was 91.1% at the dye concentration of 25 mg/L and more than 85% of Direct Red 80 was decolorized up to the dye concentration of 200 mg/L. Dye decolorization rate was significantly deteriorated to 54.7% as the dye concentration further increased to 800 mg/L. The decolorization rate decrease might be due to the inhibition of high concentration of the dye as the sulfonic group attached to aromatic rings significantly hindered the micro-organisms growth [37]. However, the azo dye concentration in textile wastewater is typically in the range of 150–250 mg/L, and the MFC system is an excellent system for the rapid decolorization of dyes in practical terms. The glucose concentrations were varied from 0 to 2,000 mg/L with constant dye concentration of 200 mg/L. Fig. 5(b) shows the changes in the color removal percentages with time. Color removal percentage was below 70% at the glucose concentration in range of 0–500 mg/L. Decolorization rate increased as glucose concentration increased up to 1,000 mg/L. After which, variation in color removal percentage was insignificant up to 2,000 mg/L. More than 80% of dye color was removed at the glucose concentrations between 1,000 and 2,000 mg/L. Fig. 6 shows the effects of initial dye and glucose concentrations on current production. It remained mainly between 1.9 and 2.2 mA; only a slight decrease in current production at high initial glucose and dye concentrations was observed. This suggests that the MFC system was not significantly influenced by initial glucose and dye concentrations in terms of current production. Similar

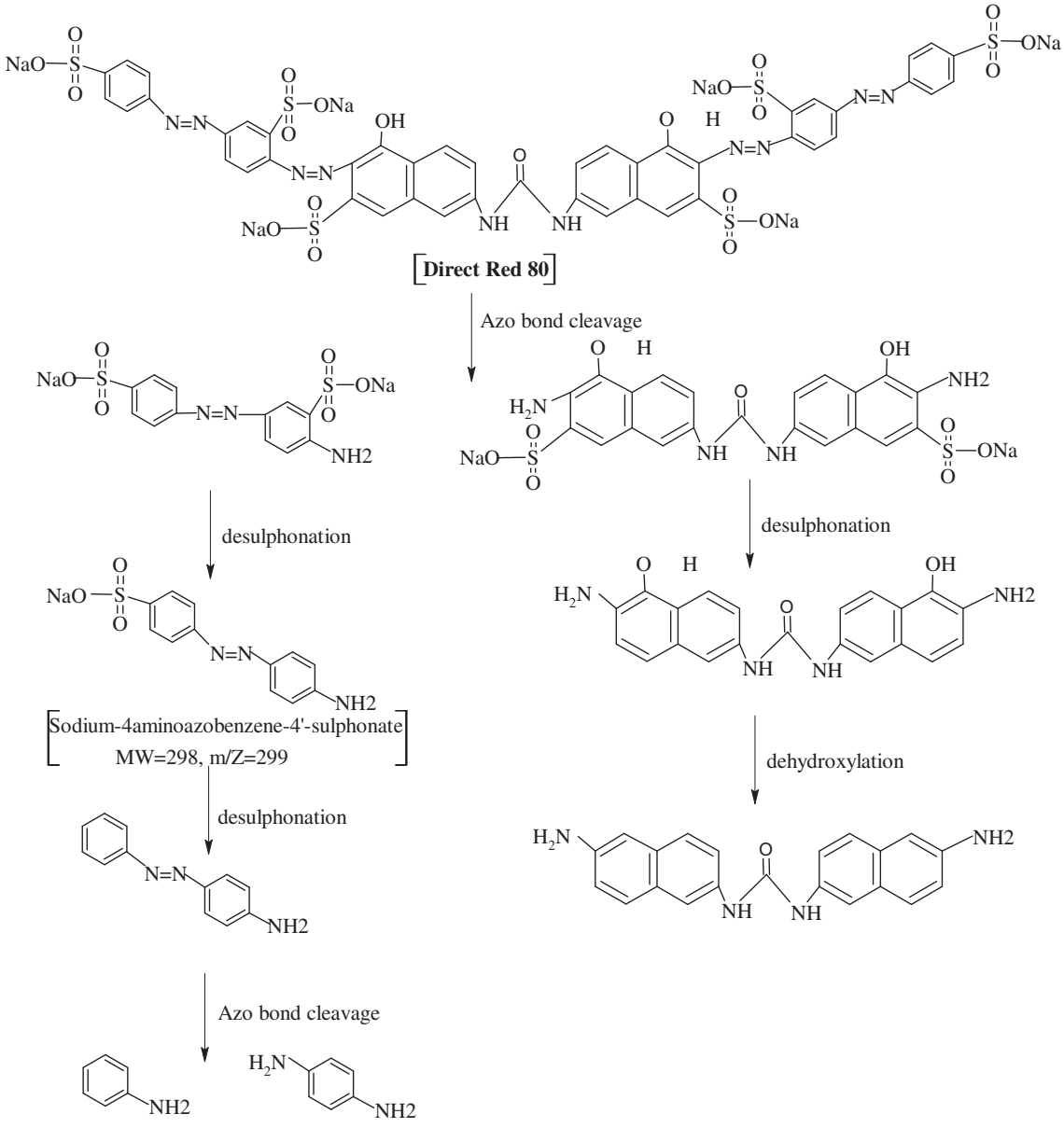

Fig. 4. A proposed mechanism for degradation of Direct Red 80.

inhibition for current generation at high co-substrate concentration was reported by Sharma and Li [38].

3.4. Effect of different co-substrates on color removal and power production

Percentage color removal of Direct Red 80 and current production in the presence of glucose and its subsequent metabolites such as acetic, propionic, and lactic acid as co-substrates were examined. Initial dye concentration and each co-substrate used was identical i.e. 200 and 1,000 mg/L COD, respectively. As shown in Fig. 7(a) maximum color removal was achieved by glucose (84.7%) and followed by acetic (77.7%), propionic (68.6%), and lactic acids (60.2%), respectively. This corresponded to 3.60 mg dye/L h in case of glucose and 3.34, 3.00 and 2.66 mg dye/L h for acetic, propionic, and lactic acids, respectively. These results agree with others in the literature in that glucose was the preferred substrate for anaerobic dye decolorization under anaerobic conditions, whereas volatile fatty acids were poor electron donors [25,26,39]. The differences among volatile fatty acids could attribute to the microbial activity and bacteria specific [40,41]. Fig. 7(b) shows power and current density curves with dye and different co-substrates. The maximum power

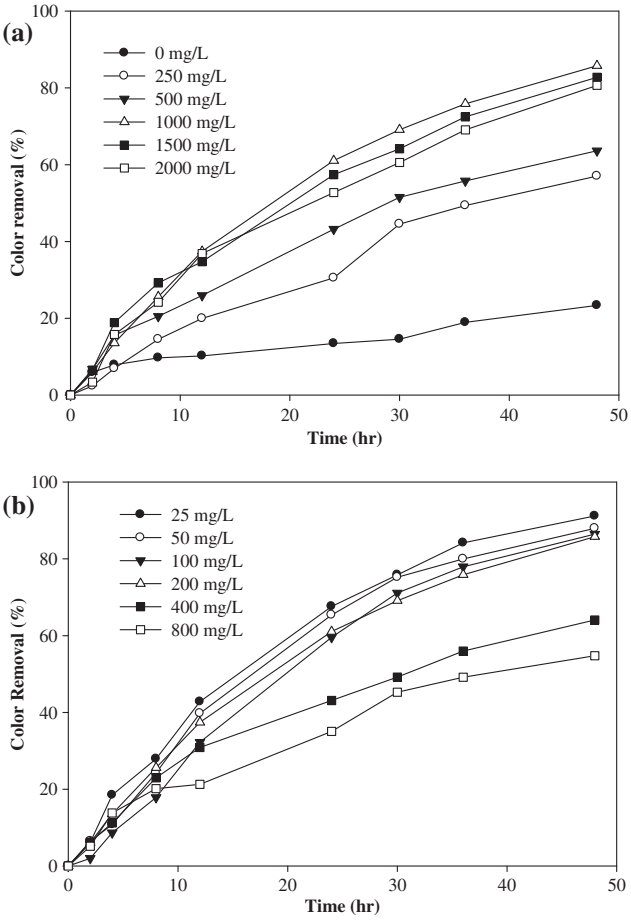

Fig. 5. Effect of (a) glucose and (b) initial dye concentrations on color removal.

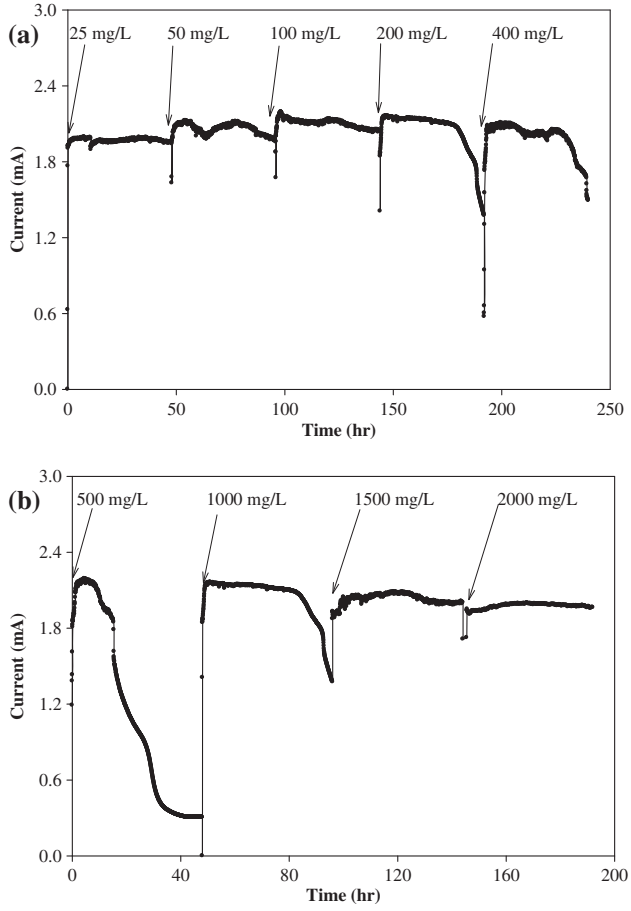

Fig. 6. Effect of initial (a) dye and (b) glucose concentrations on current production.

(461 mW/m^2) was obtained with glucose at current density of 0.98 A/m^2, compared with 342, 322, and 296 mW/m^2 for acetic, propionic, and lactic acids at current densities of 0.80, 0.77, and 0.75 A/m^2, respectively. These differences in power generations were associated with different electron donors in MFCs as the influence of substrates was quite significant on composition of bacterial community as well as power densities [42]. Especially, inoculum sources applied to MFCs can result in different power densities for different substrates. As MFCs were inoculated with acetate-fed MFCs, the power density obtained by acetate as the sole substrate was much higher (360 mW/m^2) than glucose (9.8 mW/m^2) [43]. However, the power density produced from glucose was the highest at 401 mW/m^2 followed by acetate and ethanol at 368 and 302 mW/m^2, respectively, when MFCs were inoculated with domestic wastewater [38]. Similarly, in another study, the power density was highest in case of glucose (156 mW/m^2), followed by acetate (64.3 mW/m^2), propionate (58 mW/m^2), and butyrate (51.4 mW/m^2), respectively [42]. In this study, the MFCs were inoculated with anaerobic sludge from a domestic wastewater treatment plant and the highest power density was obtained by glucose followed by acetic, propionic, and lactic acids. In comparison to glucose with azo dye (12.7%), CE was higher in case of acetic acid (19.2%), followed by propionic (16.6%) and lactic acid (14.9%). These results were well in agreement with earlier studies [38,42], where glucose produced higher power density than other substrates, but gave the lowest CE. The low CE of organic substrates in MFCs was mainly due to the utilization of substrates for the growth of bacterial communities or the consumption of generated electrons by other electron acceptors (such as oxygen from cathode to anode, nitrate, and sulfate.) and azo bonds reduction.

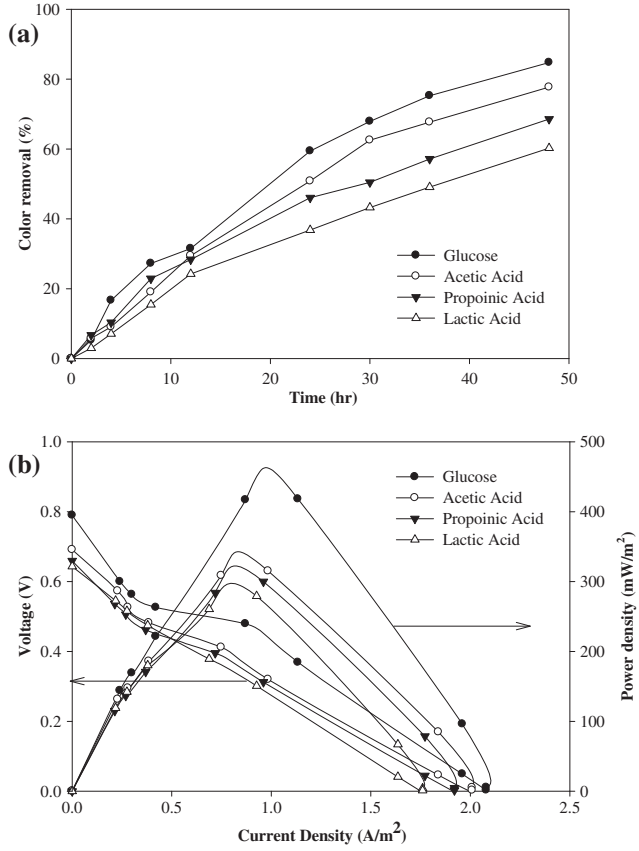

Fig. 7. (a) Effect of co-substrates on color removal and (b) power density and polarization curve with different co-substrates.

4. Conclusion

In this study, a stable current along with color and COD removal was successfully attained using the dual chamber MFC. There was no inhibition in current production during co-substrate degradation after addition of dye; however, slight decrease in COD removal was observed due to dye intermediates formation. Glucose was determined as a better co-substrate than acetic, propionic, and lactic acid for dye removal and current production. Increase in initial dye and glucose concentrations affected the color removal, but less changes were observed in current generation. Sodium 4-aminoazobenzene-4′-sulfonate was identified as the main intermediate dye product by GC–MS.

Supplemental material

The supplemental material for this paper is available at http://dx.doi.org/10.1080/19443994.2015.1049410.

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