



Detoxification of simulated textile wastewater using a membraneless electrochemical reactor with immobilized peroxidase

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ABSTRACT

Simulated textile wastewater was degraded using a membraneless electrochemical reactor with immobilized peroxidase on the porous Celite. The optimal current density was 10 A m^{-2} , at which the highest amount of hydrogen (H_2O_2) could be generated. The decolorization efficiencies of the simulated wastewater using the electrochemical and electroenzymatic methods were 35% and 92%, respectively. Biodegradability, the ratio of 5-day biochemical oxygen demand to chemical oxygen demand (BOD_5/COD), was enhanced about 1.88 times when using the electroenzymatic treatment rather than raw wastewater, which could not be achieved by the electrochemical treatment. The toxic unit (TU), calculated using the lethal concentration (LC_{50}) of *Daphnia magna* (*D. Magna*), of effluent treated by electroenzymatic method was below 1, whereas those of simulated textile wastewater and effluent treated by electrochemical method were 11.4 and 3.9, respectively.

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1. Introduction

Synthetic dyes are currently being extensively used in the textile, paper, and printing industries as well as in dye houses. Annually, about one million tons of dyes are produced in the world, with about one tenth being released directly into the environment [1]. As such, serious environmental problems can emerge since these dyes contain chemicals that are toxic, carcinogenic, mutagenic, and/or teratogenic [2] when released into the ecosystem.

Due to their seriousness, a considerable amount of attention has been focused on effective treatments of dye wastewater. Currently, biological technologies have been conveniently employed to treat the dye wastewater by both aerobic and anaerobic cultures [3–5]. These technologies, however, have inherent drawbacks including poor biodegradability and slow degradation rates by microorganisms [6].

Physicochemical treatments such as filtration, adsorption/coagulation, and Fenton's process have been studied as alternative methods to biological processes in the decolorization of textile dyestuffs [7–9]. However, in spite of their high removal efficiency the practical applications of these treatments has been limited due to their high costs, small-scale operation and the

formation of secondary pollutants such as more toxic products and iron sludge.

The electrochemical treatment of dye wastewater has drawn attention due to its versatility and environmental compatibility through electron reactions [10]. However, it has been shown not to be economically viable for large-scale wastewater treatment because of its requirement of high electrical potential and/or current.

An electroenzymatic process, an advanced electrochemical process that combines enzymatic catalysis and the electrode reaction, was proposed as a novel treatment strategy for dyestuffs [11,12]. This method has attractive features such as lower energy consumption than, for example, electrochemical treatment, and a faster reaction rate since enzymatic catalysis is maintained in an electric field. However, its application in a large-scale facility has not been fully successfully as the scale-up of such a system is expensive and complex due to the usage of proton exchange membranes.

Ando et al. proposed a membraneless water electrolyzer to produce and recover acidic and/or alkaline water, and subsequently showed that the membraneless-type electrolyzer is simpler and more efficient at antifouling than a membrane-type electrolyzer [13].

The primary aim of this study is to degrade simulated textile wastewater for detoxification using a membraneless electrochemical reactor with immobilized peroxidase on the porous Celite. In addition, we aim to evaluate the degradation characteristics of the simulated textile wastewater using electrochemical process and

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Table 1
Composition of simulated textile wastewater and its function

Compound	Concentration (mg L ⁻¹)	Function
Orange II	25	Dyeing
Eriochrome® Black T	25	Dyeing
Allura Red AC	25	Dyeing
Sunset Yellow FPF	25	Dyeing
NaCl	1000	Promotes exhaustion of the dye onto cotton
Na ₂ CO ₃	100	Adjustment of the standard alkaline pH (8.8–9.3)
NaOH	100	Adjustment of the final dyeing pH (10.5–11.0)
Softener # 100	100	Lubricant for fiber
Snobil NH	50	Bleaching agent
M/C Cleaner	50	Machine cleaner
CH ₃ COOH	200	Reduces the alkalinity of effluent

electroenzymatic process in terms of UV/vis spectrometry, FT-IR spectra, biodegradability, and biotoxicity assessment.

2. Experimental

2.1. Chemicals

Peroxidase (EC 1.11.1.7) from *Aspergillus oryzae* and Celite beads (Celite®R-646, 8/14 mesh) were purchased from Novozyme Co. (Novozyme Nordisk, Bagsvaerd, Denmark) and Celite Korea Ltd. (Celite Korea, Seoul, Korea), respectively. Simulated textile wastewater was prepared using various dyestuffs, inorganic salts and acetic acid, with respective compositions and functions given in Table 1. To realize actual textile wastewater, a fabric softener and detergent from Dae Young Chemical Co. Ltd. (Korea) were added. Other chemicals used in this study were obtained from the Sigma–Aldrich Chemical Company (USA).

2.2. Immobilization of peroxidase and determination of its characteristics

For the immobilization of peroxidase on the porous Celite, aminopropylation with 3-aminopropyltriethoxysilane (3-APTES, Sigma–Aldrich, USA) and linkage with glutaraldehyde (GA,

Sigma–Aldrich, USA) were performed, as previously described in Ref. [12]. The enzymatic activity of the immobilized peroxidase was spectrometrically measured using H₂O₂ and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) as the oxidizing and reducing substrate, respectively [14]. The protein yield of the immobilized peroxidase was then determined using a bicinchoninic acid (BCA) assay [15].

2.3. Membraneless electrochemical reactor

The schematic of the membraneless electrochemical reactor is illustrated in Fig. 1. The reactor is composed of three cell pairs and arranged alternatively with platinum coated on titanium plate and stainless steel as anode and cathode, respectively. The effective area of each electrode is 50 cm², and simulated textile wastewater was continuously supplied into the membraneless system by a peristaltic pump (Masterflex®, USA).

2.4. Analysis

The concentration of electrogenerated H₂O₂ was measured using the DMP method, where copper(II) ions and 2,9-dimethyl-1,10-phenanthroline were used in the absorbance measurement at 453 nm, recorded using a UV/vis spectrophotometer (Shimadzu, Japan) [16]. The concentration of the simulated textile wastewater was also determined by a UV/vis spectrophotometer at 489 nm and the decolorization efficiency was calculated based on change of the peak height. The chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in the wastewater were measured by a closed reflux colorimetric method and 5-day BOD test, respectively according to Standard Methods [17]. To compare the degradation characteristics of the simulated textile wastewater, a Fourier transform infrared spectrometer (FT-IR, 460 Plus, Jasco, Japan) equipped with an attenuated total-reflection (ATR) was used.

2.5. Acute biotoxicity assessment

The *Daphnia magna* used as the test species in this study were standardized to assess aquatic toxicity [18]; the effluents acquired through each treatment were exposed to the neonates of *D. magna* aged <24 h. Here, five neonates were used in four replicates of each

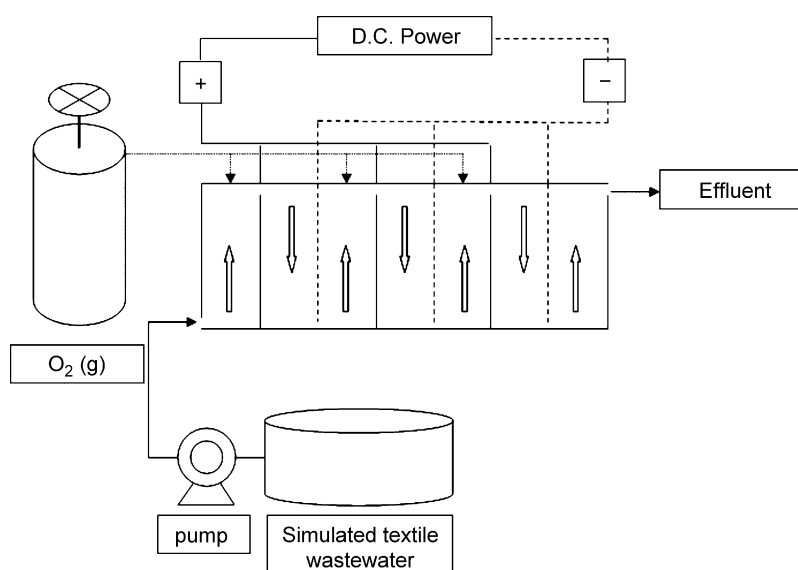


Fig. 1. Schematic diagram of a membraneless electrochemical reactor with three cell pairs (anode: Pt/Ti, cathode: stainless steel, effective area of each electrode: 50 cm²).

test effluent. The test solutions, 6.25%, 12.5%, 25%, 50% and 100%, were diluted from 100% effluent using a dilution factor of 2, using synthetic moderately hard water. Prior to toxicity tests, the hardness was adjusted by adding an appropriate content of CaSO_4 and MgSO_4 to the treated effluent samples. *D. magna* mortality was then measured after a 48 h exposure.

The lethal concentration (LC_{50}) was calculated using the Probit program, which was downloaded from the US EPA website. The term toxic unit (TU) is introduced to compare the relative toxicity as follows:

$$\text{TU} = \frac{100\%}{\text{LC}_{50}} \quad (1)$$

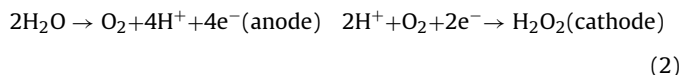
where LC_{50} corresponds to 1 TU; thus, 1 TU refers to 50% mortality at 50% effluent.

3. Results and discussion

3.1. Electrogeneration of hydrogen peroxide

Peroxidase is known to be effective for the degradation of aromatic amine and azo dye in dye compounds through enzymatic catalysis in the presence of H_2O_2 [19]. The electroenzymatic process utilizes electrogenerated H_2O_2 in the activation of peroxidase. To form H_2O_2 in the reactor, a proton is produced on the anode surface through water dissociation and the produced proton moves to the cathode surface by electromigration and convection in an electric field. Then, hydrogen peroxide is generated by combining the proton with the supplied dissolved oxygen.

Fig. 2 presents the concentration of continuously electrogenerated H_2O_2 within the membraneless electrochemical system as a function of current density in the range of 3– 15 A m^{-2} . As shown in Fig. 2(a), the energy consumption increased linearly with current density since the Ohmic relation was governed in a membraneless electrochemical system. Moreover, the production rate of hydrogen peroxide increased with a corresponding current increase from 3 to 10 A m^{-2} and the highest production of H_2O_2 was at 10 A m^{-2} where protons were generated vigorously at the anode surface by water dissociation. The generated protons were reduced to hydrogen peroxide at the cathode surface by combination with oxygen in the supplied air through a 2-electron reaction as follows [20]:



Beyond 13 A m^{-2} , however, the production rate of H_2O_2 decreased with respect to current density (Fig. 2(a)), and the amount of electrogenerated H_2O_2 decreased according to time in a different way below 10 A m^{-2} (Fig. 2(b)) since the reduction of electrogenerated H_2O_2 to H_2O occurred ($\text{H}_2\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow 2\text{H}_2\text{O}$) on the cathode surface. Thus, the optimal condition of current density for electrogeneration of H_2O_2 was determined to be 10 A m^{-2} , where water dissociation occurred predominantly with the 2-electron reaction on the cathode surface.

3.2. Decolorization of simulated textile wastewater using membraneless system

The decolorization efficiency of the simulated textile wastewater using electrochemical method was 35% since the oxidant produced at the applied current of 10 A m^{-2} was not sufficient for complete wastewater oxidation. Conversely, 92% of the simulated textile wastewater was decolorized using electroenzymatic degradation since the immobilized peroxidase was activated by the

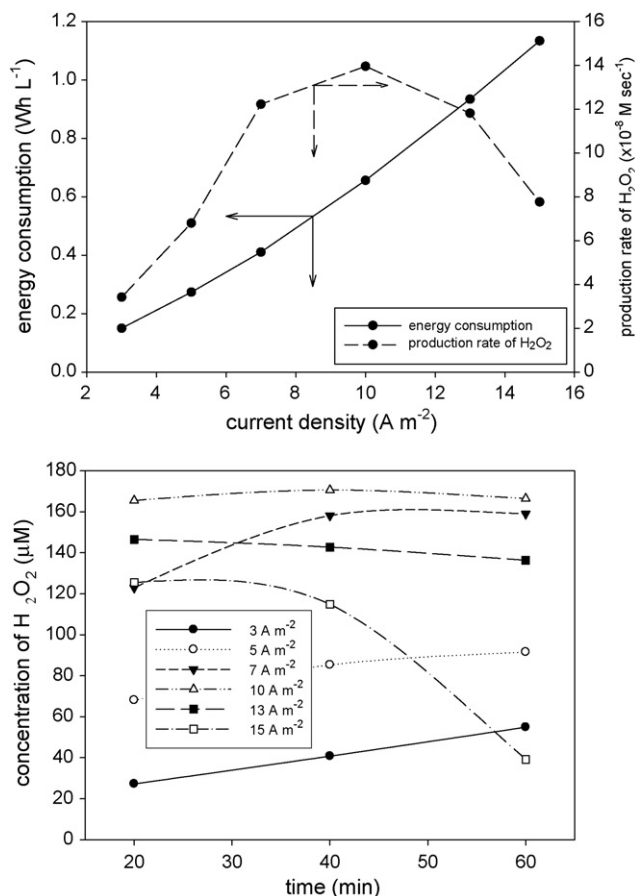


Fig. 2. Characteristics of electrogenerated H_2O_2 within a membraneless electrochemical reactor: (a) the energy consumption and amount of electrogenerated H_2O_2 for the initial 20 min, and (b) the amount of electrogenerated H_2O_2 as a function of current density.

electrogenerated H_2O_2 , thereby following the activated peroxidase to catalyze the degradation of simulated textile wastewater.

Fig. 3 presents the UV/vis spectra of the simulated wastewater and effluents using electrochemical and electroenzymatic treatments. Note that the peaks at 310 and 489 nm did not disappear during electrochemical treatment, implying that aromatic $\pi-\pi^*$

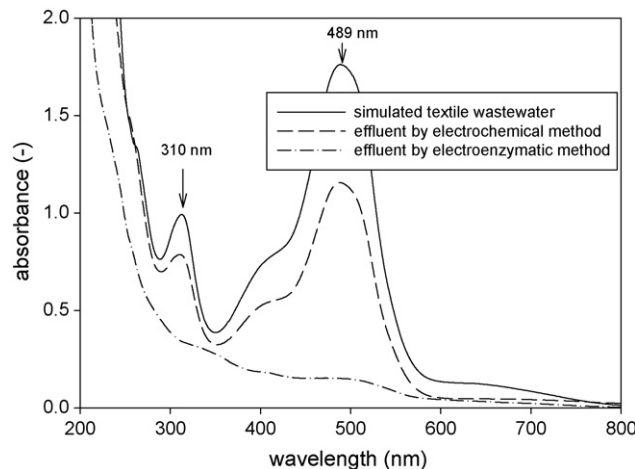


Fig. 3. Scanning spectra of the raw simulated textile wastewater and effluent after treatment by electrochemical and electroenzymatic methods. Current density = 10 A m^{-2} ; retention time = 45 min; and temperature = 25°C .

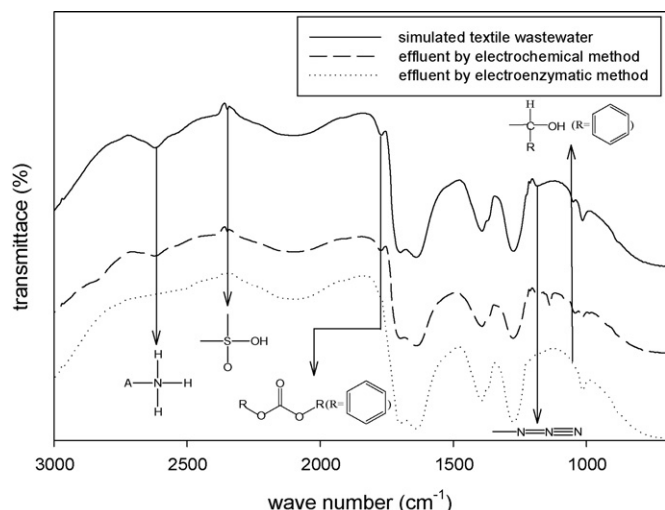


Fig. 4. FT-IR spectra with ATR of simulated textile wastewater and effluents treated by electrochemical and electroenzymatic processes. Current density = 10 A m^{-2} , retention time = 45 min and temperature = 25°C .

compounds and azo bonds remained in the effluent [21]. On the other hand, the peaks at 310 and 489 nm disappeared when the electroenzymatic method was employed since the aromatic compounds and azo dyes were effectively degraded by electroenzymatic process.

3.3. Degradation characteristics of the simulated textile wastewater

Biodegradability, the ratio of BOD_5 to COD (BOD_5/COD), defines something as biodegradable if all of its organic compounds are subject to decomposition through biological activity. The BOD_5/COD of the simulated textile wastewater was as low as 0.32 since the compounds in the simulated textile wastewater were biorefractory compounds such as aromatic amines and azo dyes. After electrochemical treatment, the BOD_5/COD did not change remarkably (to 0.35) since biorefractory compounds remained in the effluent. On the other hand, the BOD_5/COD ratio from the electroenzymatic method was enhanced by a factor of 1.88 (0.60) as compared to the raw wastewater, implying that the refractory compounds in textile wastewater were converted into more easily biodegradable compounds such as lower molecular organic acids (data not shown).

Fig. 4 shows the FT-IR spectra of the simulated textile wastewater and the effluent treated by electrochemical and electroenzymatic processes, comparing their degradation characteristics. The effluent spectrum of the electrochemical method was similar to that of raw wastewater since the degradation of dye chemicals through electrochemical oxidation at 10 A m^{-2} in the membraneless electrochemical reactor was not efficient. After the electroenzymatic reaction, however, some chemical bonds such as N–H stretching (2629 cm^{-1}), sulfur (2351 cm^{-1}), carbonate (1777 cm^{-1}), azide (1218 cm^{-1}), and alcohol (1046 cm^{-1}) were decomposed since the simulated textile wastewater was effectively degraded using the immobilized peroxidase in the membraneless electrochemical reactor.

3.4. Biotoxic assessment of the simulated textile wastewater

In order to identify the toxicity of the simulated textile wastewater, a biotoxicity assessment using *D. magna* was performed as the simulated textile wastewater and effluents using the elec-

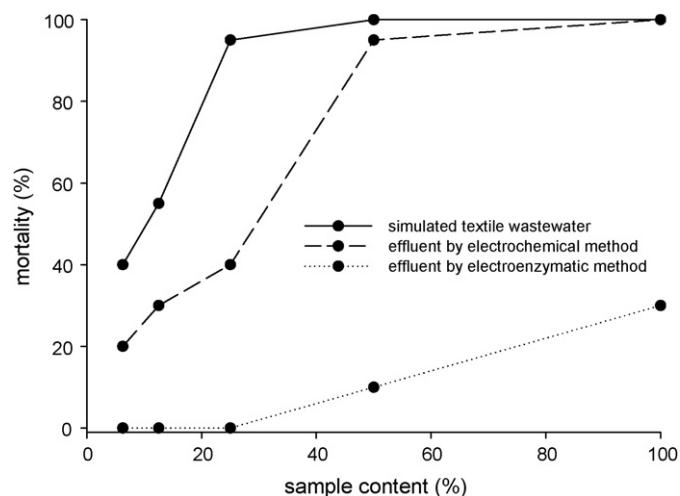


Fig. 5. Acute biotoxicity assessment using *D. magna* after 24 h of incubation in effluent concentrations of 6.25%, 12.5%, 25%, 50%, and 100%.

trochemical and electroenzymatic processes. As shown in Fig. 5, most *D. magna* died when they were exposed to effluent content of over 25% in the simulated textile wastewater. This suggests that the mortality of *D. magna* was induced by the dye chemicals in the simulated textile wastewater as there was no mortality in the control solution. The mortality in the effluent treated by the electroenzymatic process was 30%, even in 100% effluent, although that for the electrochemical process was more than 95% when *D. magna* was exposed to effluent content over 50%. It was thereby shown that the toxicity of the simulated textile wastewater was dramatically reduced when the electroenzymatic process was employed.

Finally, a toxic unit (TU) was employed to numerically compare the relative toxicity of the effluents from each process. The TU values of the simulated textile wastewater and the effluent treated by electrochemical method were 11.4 and 3.9, respectively; both samples affected the mortality of *D. magna* due to their toxicity. Conversely, the TU of effluent treated by the electroenzymatic method was below 1 since less than 50% mortality was observed, even at 100% concentration, implying that the toxic compounds in the simulated wastewater were detoxified by enzymatic catalysis using the electrogenerated H_2O_2 .

4. Conclusion

The detoxification of simulated textile wastewater was performed using a membraneless electrochemical reactor with immobilized peroxidase. The decolorization efficiencies of the simulated textile wastewater using the electrochemical and electroenzymatic methods were 35% and 92%, respectively, since the azo bonds in the wastewater were efficiently degraded by electroenzymatic reaction. Also, the biodegradability of the effluent by electroenzymatic method was enhanced 1.88 times and the biotoxicity of the effluent using the electroenzymatic method was decreased since toxic compounds in the wastewater such as aromatic compounds and azo dyes were effectively degraded by the electroenzymatic process.

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