Electrochemical monitoring of biodegradation of phenolic pollutants using nanoporous gold

Badal Shah and Aicheng Chen

Department of Chemistry, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario P7B 5E1, Canada

Phenolic compounds are widely present in the wastewater that is discharged as a result of many industrial processes. Chlorophenol is one of the most toxic phenolic pollutants, which causes skin and eye irritation and has carcinogenic activity too. Conventional methods for 4-chlorophenol removal from wastewater include extraction, adsorption on activated carbon, steam distillation, chemical oxidation and photochemical degradation. However, they are costly and the removal efficiency is marginal; indeed some are prone to form even more toxic intermediate. In the recent years, researchers have explored the biodegradation of phenolic compounds employing enzyme horseradish peroxidase (HRP). Although HRP is effective, it requires hydrogen peroxide for its activity and forms a number of toxic intermediates.

Tyrosinase is a peroxidase enzyme, which is widely distributed in fruits, plants and animals. It catalytically oxidizes phenol into quinone through a two-step process: (i) hydroxylation of the phenol at the ortho position to form o-diphenol; and (ii) dehydrogenation of o-diphenols to produce o-quinone. These quinones may react spontaneously with each other to form oligomers.

Recently, noteworthy efforts have been made in the removal of phenolic pollutants using tyrosinase by exploiting its advantages over HRP (1). In these studies, either high performance liquid chromatography (HPLC) or UV-Visible spectroscopy (UV) is required to monitor the biodegradation process. Although these methods are selective and sensitive, high cost of instrumentation and tedious and lengthy analytical process make them less attractive. Here, we propose, for the first time, a facile electrochemical approach for monitoring the biodegradation of phenolic compounds using a nanoporous Au electrode, where tyrosinase was selected as a model enzyme. Our study has revealed that the proposed approach is effective not only for the monitoring of the biodegradation process but also for the rapid evaluation of enzymatic activity under different circumstances (2).

The nanoporous gold supported on Ti substrate was fabricated via the chemical reduction of a gold precursor ($HAuCl_4$) using a hydrothermal method (3,4). Morphology and composition of the fabricated nanoporous Au materials were characterized by field-emission scanning electron microscopy (FE-SEM) and energy dispersive X-ray spectrometry (EDS).

Cyclic voltammetry and differential pulse voltammetry with a pulse width of 0.2s, pulse period of 0.5s and potential increment of 4mV, were performed using CHI 660B electrochemical workstation (CH Instrument Inc., USA). A Pt coil and a 3M KCl saturated Ag/AgCl electrode was used as counter electrode and reference electrode respectively. The fabricated nanoporous gold, as well as a gold disc for comparison was used as the working electrode. A phosphate buffer solution containing various phenolic compounds and 50µL of tyrosinase enzyme (2mg/ml) were used as electrolytes, which were blended using a magnetic stir bar.

We have demonstrated a novel electrochemical approach based on differential pulse voltammetry for the effective monitoring of biodegradation processes and for the rapid determination of enzymatic activity. Through the use of a nanoporous Au electrode we have, for the first time, successfully monitored the biodegradation process of 4-chlorophenol using a facile electrochemical method, to replace expensive, tedious and lengthy UV and/or HPLC techniques (2). The effects of pH and temperature on tyrosinase activity in the biodegradation of 4-chlorophenol were determined, which demonstrate the applicability of this approach in determination of enzyme activity. The facile and effective electrochemical approach described in this study opens the door for the rapid assessment and optimization of enzyme activity and high throughput screening for specific enzymes and/or receptors in demanding medical and environmental applications encompassing biosensing, enzyme engineering, drug design and biodegradation.

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References

1. A. Dincer, S. Becerik, T. Aydemir, *Int. J. Biol. Macromol.* **50,** 815 (2012).
2. B. Shah, A. Chen, *Electrochem. Commun.* **25**, 79 (2012).
3. J. Wang, D.F. Thomas, A.Chen, *Chem. Commun.* **40,** 5010 (2008).
4. A.K.M. Kafi, A. Ahmadalinezhad, J. Wang, D.F. Thomas, A. Chen, *Biosens. Bioelectron.* **25,** 2458 (2010).