# 行政院國家科學委員會專題研究計畫 成果報告

## 結合臭氧與固定式酵素床法處理水中酚類污染物之研究
## 研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 99-2628-E-029-009-
執 行 期 間 ：99 年 08 月 01 日至 100 年 07 月 31 日
執 行 單 位 ：東海大學環境科學與工程學系

計 畫 主 持 人：宋孟浩

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報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 100 年 10 月 29 日

# 行政院國家科學委員會補助專題研究計畫 ■成果報告 □期中進度報告

# 結合臭氧與固定式酵素床法處理水中酚類污染物之研究

計畫類別：■個別型計畫　□整合型計畫
計畫編號：NSC　99-2628-E-029-009-
執行期間：2010 年 8 月 1 日至 2011 年 7 月 31 日

執行機構及系所：東海大學 環境科學與工程學系

計畫主持人：宋孟浩
計畫參與人員：許啟詮、呂晧瑜、蔡穎彰、楊宗憲、林沂侯

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

本計畫除繳交成果報告外，另須繳交以下出國心得報告：
□赴國外出差或研習心得報告
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■出席國際學術會議心得報告
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中　華　民　國　100 年　10　月　28　日

一、前言

環境中殘留許多化學物質皆會對環境生態造成影響，而影響程度則依化學物質之毒性強弱、暴露濃度劑量、暴露途徑、及時間長短等因素有關。「新興污染物(emerging contaminants)」為以汙水再生為終極目的之第三代汙水處理廠所面臨最具挑戰性的化學物質，其特質為"法規尚未規範或規範不全"及傳統生物處理程序無法有效分解去除，其易穿透汙水處理廠之整治防線而排放至水體環境中。Ibuprofen 與 Naproxen 為一類非固醇類的抗發炎藥物，由於大量使用而在水體環境中有較高之檢出率，這類藥品具有持久不易分解之特性，並易透過食物鏈關係，造成環境荷爾蒙效應。Laccase 是一種酵素，為含銅氧化酶，有四個銅離子，可催化基值之氧化反應(Riva, 2006)，其反應機制是利用電子轉移，在 Laccase 催化後產生活性自由基，透過自由基進一步造成基質分解或聚合反應，最後氧分子則被氧化成水。由於 Laccase 對於催化的基質比較沒有專一性(Majeau *et al*., 2010)，因此已被廣泛的應用在環境中的 PAHs、含氯有機物質、農藥、氟化芳香族化合物等，可以去除土壤與水中的毒性物質、處理工業廢水、紙漿和造紙業等應用(Zille *et al*., 2005)。固定化是將原本溶解態的(free)酵素改變成固定之非水溶液型態(immobilized)。將酵素固定於載體上，將使其環境適應力增加，且可有效的提升酵素活性、使用次數與回收。重要的是，由酵素固定床法所生成之催化氧化產物(product)和基質(substrate)能容易的被分離開來(Siddique *et al*., 1993)。Laccase 用在有機污染物分解上之優勢在於：1)容易分解不溶或者難溶解性的大分子有機化合物；2)由於酵素本身的分解基質範圍較廣，加上對於基質之專一性不高，所以分解之效率受到基質本身影響性不大。

二、研究目的

本研究之目的在於：(i)瞭解在不同水質條件下 Laccase 酵素分解 Ibuprofen 與 Naproxen 等新興汙染物質之成效，以及(ii)開發以褐藻酸(alginate)為載體來包覆 Laccase 作為環境永續之固定化方法。

三、實驗方法

1. 酵素活性分析

(i) Soluble Laccase：為了解 Laccase 於各種實驗條件下之活性，因此在降解實驗前由 ABTS 氧化之情形來決定 Laccase 之活性。此方法首先混合 0.2 mM ABTS 在 50 mM 酒石酸鈉緩衝液中(pH 4.0)，之後將 Laccase 原液稀釋 10 倍後，接著於 420 nm 測定吸光值($\varepsilon_{420}$ = 36,000 $M^{-1}$ $cm^{-1}$)，得到吸光值後代入 Beer's Law 公式(A=εbc)算其濃度。酵素表示單位設為 1U = 1μmol 氧化 ABTS/min。

(ii) Immobilized Laccase：將固定化之 Laccase beads 取出 20 顆並加入 ABTS 與之反應 10s、20s、30s、40、50s、60s，得其吸收值，由此可計算總製造顆數活性。

2. Degradation of Ibuprofen, Naproxen by soluble and immobilized Laccase

(i) Soluble Laccase：將反應使用的 Laccase 取 500 μL (pH 4.0)放置於透析膜中，並將兩端以透析夾夾住，置於反應槽中開始反應，其實驗設置如圖 1 所示。反應槽中之 Ibuprofen 或 Naproxen 溶液初始濃度為 20 ppm；在反應的過程中連續通入空氣，以提供反應所需要的氧氣，同時並放置於攪拌器上。在不同反應時間，將 Sample 經由 Millipore 0.22 μM filter 過濾後分析。於每組反應開始後，每天批次採樣之樣本以高效能液相層析儀(HPLC, Zobrax SB-C-18 column)去分析各種物質之殘留濃度。

(ii) Immobilized Laccase：褐藻酸包括法為本研究所使用之固定化酵素方法，褐藻酸鈉之黏稠水溶液，在含鈣的水溶液中，會與鈣離子形成鍵結，褐藻酸聚合物連接成網狀而凝固或膠狀。首先將 1 mL 酵素液加至 9 mL 去離子水稀釋，之後再加入 0.3 g 褐藻酸鈉，攪拌均勻後，若氣泡產生則使用超音波洗淨器進行去除氣泡。接著如圖 2 所示之三個步驟來進行固定化與測試：(i) Step 1 以針筒或滴管將混合

液，逐滴加入攪拌中之 30 mL 0.2 M $CaCl_2$ 溶液中，加完後繼續緩慢攪拌 20 min，使其硬化。接著以蒸餾水洗滌後備用。(ii)將固定化酵素珠子放置於燒杯中進行 batch 實驗，實驗條件如圖 2 之 Step 2 所示，透過攪拌器攪拌經設定反應時間採樣後利用 Millipore 0.22 μM filter 過濾。(iii)使用高效能液相層析儀去分析，分析條件如圖 2 之 Step 3 所示。圖 3 為本實驗室所產製之 Laccase beads。

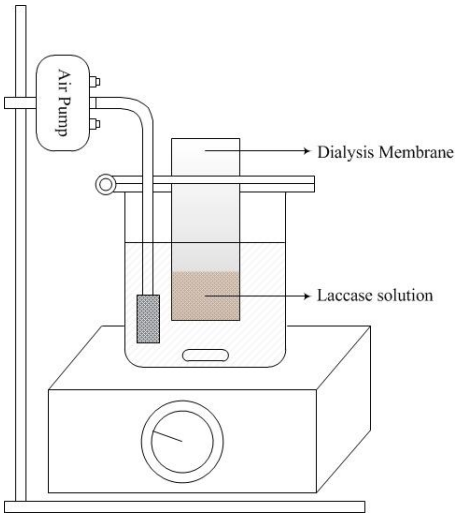


圖 1、 Free enzyme 於水相降解汙染物反應系統圖

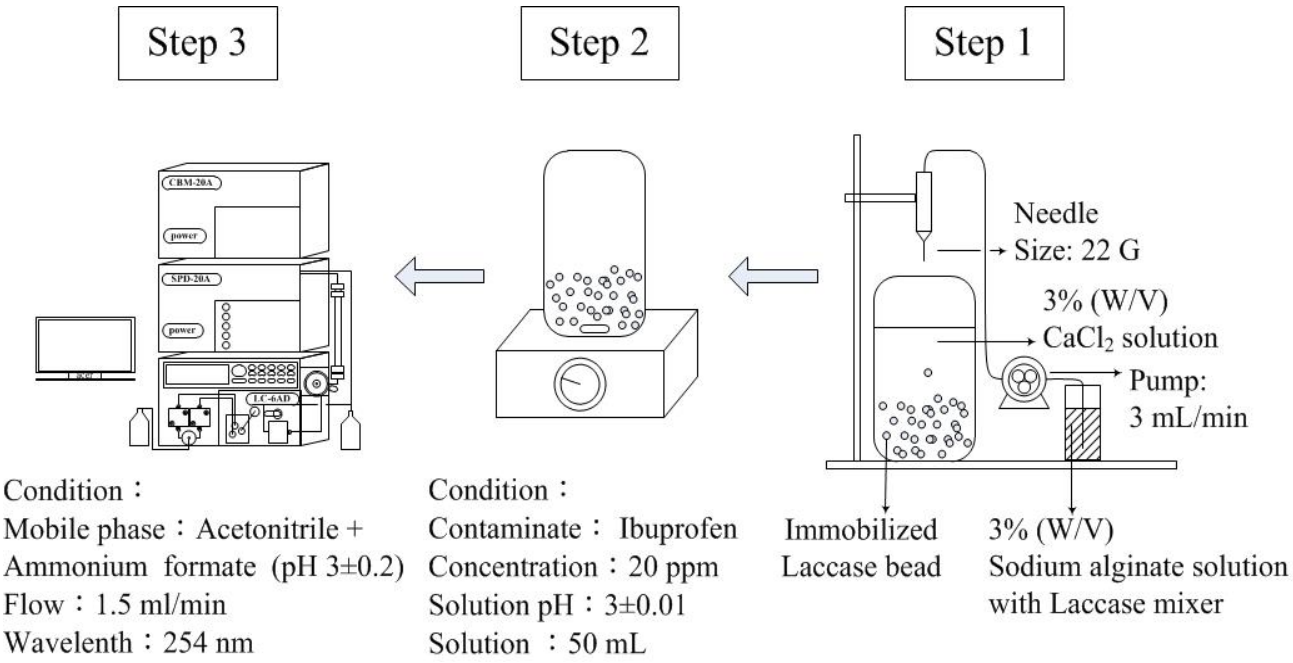


圖 2、固定化酵素材料、方法與實驗分析步驟圖

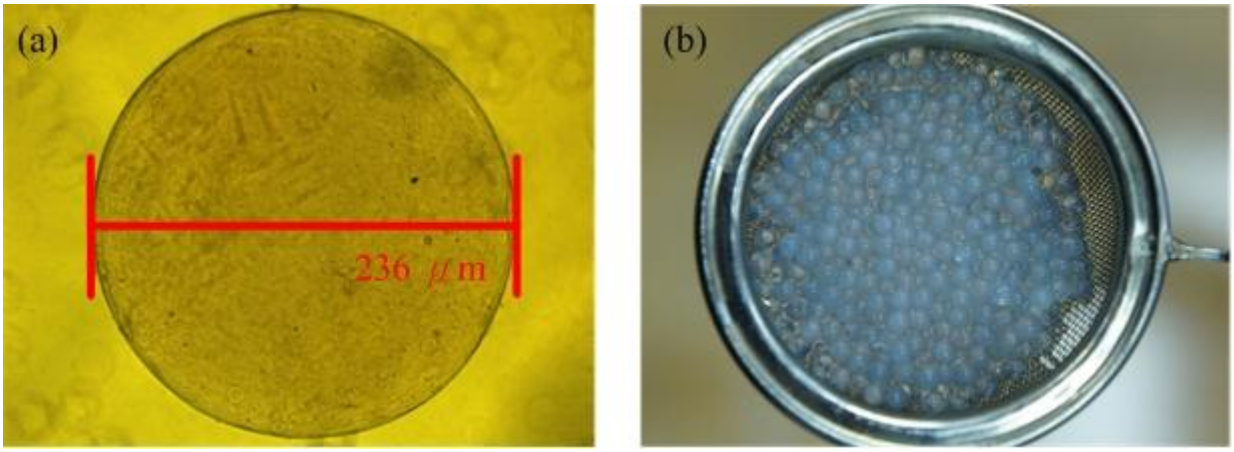


圖 3、(a)倒立式共軛雷射顯微鏡下固定化珠子 Size；(b)本實驗室所產製 Laccase beads 之圖片

三、結果與討論

1. Soluble Laccase

圖 4 為水相之 Laccase 在 30℃~60℃的活性測試結果。利用酵素活性分析法得知在 30℃，酵素活性為 1.62 U/mL，而 40℃為 1.12 U/mL，50℃為 0.80 U/mL，60℃為 0.24U/mL 等。此結果顯示當溫度越高，Laccase 的活性就越低，溫度與活性成反比的趨勢。因此接下來之降解反應將溫度選於室溫下進行。而圖 5 為水相之 Laccase 分別在 pH 3、pH 4 及 pH 5 的活性分析，其反應於室溫下進行。結果是 pH 3 時，酵素活性為 4.02 U/mL，而 pH 4 時為 3.82 U/mL，pH 5 時為 3.07 U/mL。因此水相的 Laccase 酵素特性是適合於室溫且 pH 3 的條件下有最佳的活性。接著將利用 Laccase 容易分解不溶或者難溶解性的大分子之特性，應用在傳統污水處理程序無法有效分解的 Ibuprofen(IBP)與 Naproxen(NPX)。圖 6 為水相之 Laccase 在 pH 3~5 去除 IBP 之降解反應結果，此實驗之酵素活性為 3.56 U/mL，溫度在 27~28℃進行，總反應時間為 120 hr。結果顯示在 pH 3 的條件下，其去除 IBP 效率最佳，約 55%被去除，其次為 pH 4 時去除效率約 35%，最後在 pH 5 時去除效率約 10%。此去除效率與圖 5 之結果有相關，pH 3 為活性最高的時候，因此在 pH 3 時其降解率也具有最佳的效果。利用相同的實驗方法而將目標物質換成 Naproxen 後在酵素活性為 3.63 U/mL 而反應時間為 148 hr。其結果如圖 7 所示也是在 pH 3 之條件下具有最佳之去除效率(43%)，其次為 pH 4 之 16%以及 pH 5 之 22%。而在圖 6 之 pH 3 及 pH 5 之條件下有觀察到汙染物濃度有些微上升之現象，其原因是因為在反應中曝大量的氣體，以致於水分蒸發，造成物質濃度上升，此現象在圖 7 也有相同的情況出現。但利用 Soluble Laccase 來降解 Ibuprofen 與 Naproxen 為可行性之作法且降解效率非常好。但如何有效回收再利用，則必須利用固定化來達到此目標。

2. Immobilized Laccase

圖 8 為利用 Immobolized Laccase 於曝氣條件下降解 Ibuprofen 之效率，由結果可知再有包覆以及無包覆(Blank) Laccase 之條件下，Ibuprofen 均有一定之去除效果，這結果顯示初期的吸附作用扮演非常重要之角色，亦即水中之 Ibuprofen 將首先被吸附於載體上接著再進行擴散進入載體內與 Laccase 反應。此外，圖 9 為在較少 Immobilized Laccase (400 beads)之作用下之長期降解結果，如前所述因為吸附作用快速，擴散作用以及酵素化學反應作用為其速率決定步驟(rate limiting step)。在反應 300 小時之後，約有近 50%之 Ibuprofen 被去除，此結果與圖 6 之結果相較(約 100 小時可達 50%之去除率)，可知擴散作用之尺度(因為圖 6 為利用透析袋之實驗結果相對擴散作用較低)約與反應速率之尺度相當。

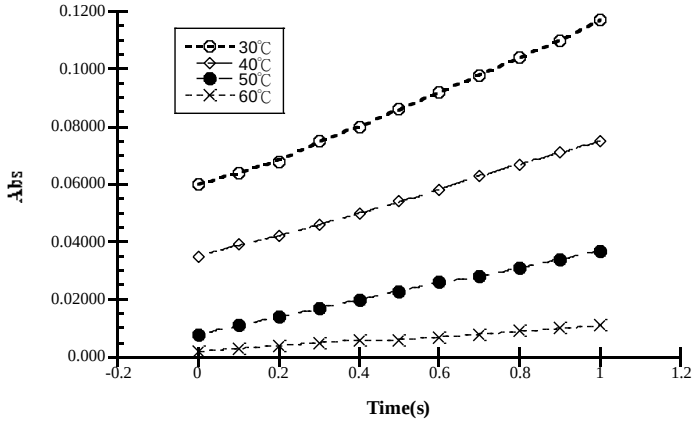


圖 4、Soluble Laccase 在不同溫度下之活性圖

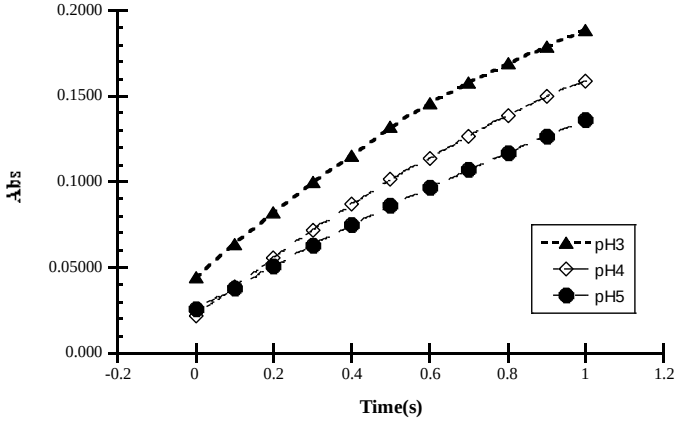


圖 5、Soluble Laccase 在不同 pH 下之活性圖

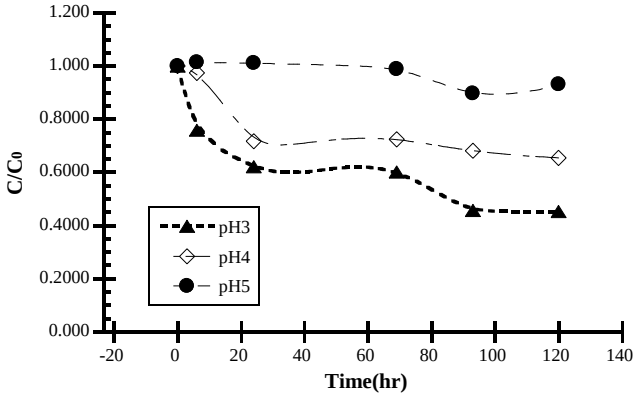


圖 6、Soluble Laccase 在不同 pH 條件下去除 Ibuprofen 之降解圖
(實驗條件:酵素活性=3.56U/mL，IBU 濃度=20ppm)

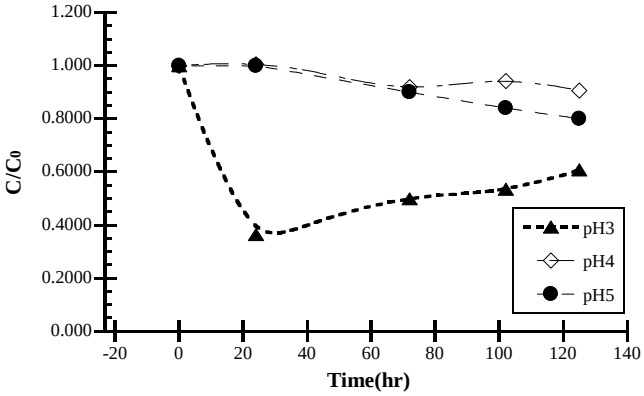


圖 7、Soluble Laccase 在不同 pH 條件下去除 Naproxen 之降解圖
(實驗條件:酵素活性=3.63U/mL，NPX 濃度=20ppm)

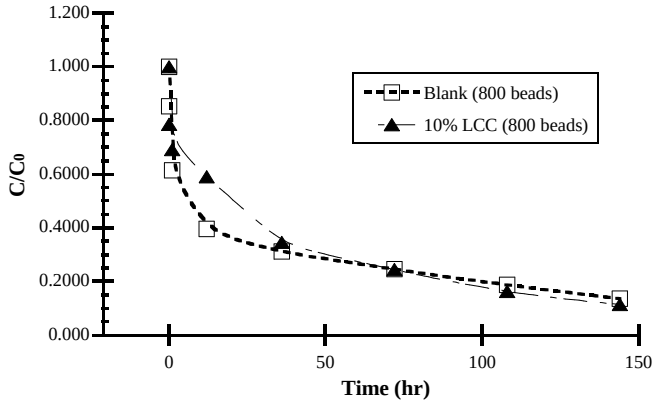


圖 8、利用曝氣盤輔助 Immobilized Laccase beads 降解 Ibuprofen (Laccase 濃度= 3.1498U/mL；Immobilized LCC bead loading = 50% (W/V))

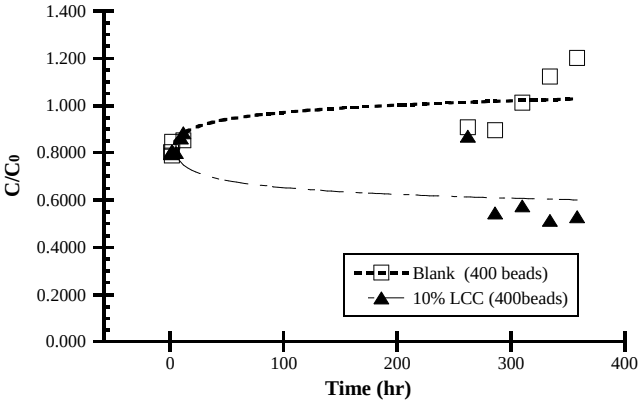


圖 9、於燒杯中攪拌 Immobilized Laccase beads 降解 Ibuprofen (Laccase 濃度= 3.1498U/mL; Immobilized LCC bead loading = 50% (W/V))

四、結論

本研究顯示用酵素來降解代表性之藥物類新興汙染物 Ibuprofen 以及 Naproxen 為可行之方法，目前利用生物科技已可將 Laccase 酵素大量生產，故此降解方法在新興汙染物之處理上將具有很大之競爭力。而本實驗也利用海藻膠為載體材料，成功的將 Laccase 酵素包覆其中並測試其降解新興汙染物 Ibuprofen 之成效。這種以環境友善材料(海藻膠)作為包附載體之作法，未來在實用上將有很大之發展潛力。

五、參考文獻


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4. Siddique M.H., Pierre C.C. St., Biswas N., Bewtra J.K., Taylor K.E. (1993). Immobilized enzyme catalyzed removal of 4-chlorophenol from aqueous solution, *Water Research*, 27, 883-890.

# 國科會補助專題研究計畫項下出席國際學術會議心得報告

日期：100 年 8 月 10 日

| | | | |
|---|---|---|---|
| 計畫編號 | NSC－99-2628-E-029-009 | | |
| 計畫名稱 | 結合臭氧與固定式酵素床法處理水中酚類污染物之研究 | | |
| 出國人員<br>姓名 | 宋孟浩 | 服務機構<br>及職稱 | 東海大學環工系 助理教授 |
| 會議時間 | 100 年 7 月 4 日至<br>100 年 7 月 7 日 | 會議地點 | 新加坡 |
| 會議名稱 | （中文）變遷環境中永續水處理技術研討會<br>（英文）Sustainable Water Solutions for a Changing Environment | | |
| 發表論文<br>題目 | （中文）利用溶解及固定之 Laccase 酵素處理水中布洛酚與奈普生之研究<br>（英文）Removal of Ibuprofen and Naproxen in Water by Soluble and Immobilized Laccase | | |

一、參加會議經過

第一天(7 月 4 日)：抵達新加坡住進旅館並隨即前往 Suntec Convention Center 議場報到領取資料並確定行程。傍晚參加開幕儀式與開幕演講。

第二天(7 月 5 日)：參加研討會以及前往展場參觀各國廠商展出之先進水處理設備。下午 poster session，與各國學者交流研究成果。

第三天(7 月 6 日)：參加研討會。

第四天(7 月 7 日)：上午參加研討會，下午搭機返台。

二、與會心得

1. 本次會議參與人數眾多，除學術界外水處理工業界之各國廠商亦參與積極；因此

除學術研討外亦學習到許多業界相關之最新資訊。

2. 與國際間其他學者交流建立人脈。

3. 低耗能汙水處理廠將是未來之趨勢。

三、考察參觀活動(無是項活動者略)

四、建議

無

五、攜回資料名稱及內容

會議集(proceedings)

六、其他

論文接受函與研討會全文如後附件

东海大學 GMail

宋孟浩 **<mhsung@go.thu.edu.tw>**

# Your Conference Submission

1 封郵件

**Water Convention 2011 Programme Committee <waterconvention@siww.com.sg>**
收件者: Menghau Sung <mhsung@thu.edu.tw>

**2011**年**1**月**18**日下午**2:22**

Article title: Application of immobilized laccase enzyme in removal of ibuprofen in water
Reference No: IWA-6340
Water Convention Conference (4 - 8 July 2011 Singapore)

Dear Dr. Sung,

Following the review of all the conference outline papers, I am pleased to inform you that your submission for the Water Convention Conference (4 - 8 July 2011 Singapore) is being accepted onto the conference programme for poster presentation. A Poster Presentation is a visual representation of the reviewed material at the confernece.

If you accept this invitation, you should now prepare a poster in the correct format - please see http://www.iwahq.com/uploads/Conference_Graphics/Leading-Edge%20Sustainability/Sydney%202004/Authors/Poster-template.ppt to obtain a poster template. You might also find the publication Communicating Science Effectively
- http://www.iwapublishing.com/template.cfm?name=isbn1843391252 - helpful when creating your poster.

In order to create the preliminary programme for the Water Convention Conference (4 - 8 July 2011 Singapore) I will really appreciate if you could reply to Ms Brenda Lai, brenda.lai@iwahq.org, indicating whether you accept or decline this invitation by 7 February 2011. Please indicate the manuscript number in your reply.

If you decline this invitation, I will appreciate if you could log in at the IWA conference website and in your authors website you accessed "Submissions Needing Revision". Once there please click on "Decline to Revise" in the Action Link of the relevant submission.

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Yours sincerely

Water Convention 2011 Programme Committee

Reviewers' comments:

# Removal of Ibuprofen and Naproxen in Water by Soluble and Immobilized Laccase

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**Abstract**
The efficient removal of emerging pollutants from water by a chemical-free approach is important for a sustainable water reclamation system. Laccase is an enzyme that is capable of degrading a wide variety of phenolic contaminants in water. Its stability and lack of inhibitors have made it an attractive agent in enzymatic degradation. In this study, soluble laccase and laccase immobilized on resins are prepared to test its efficiency on the removal of Ibuprofen and Naproxen, which are both emerging pharmaceutical pollutants. Batch systems were adopted for the degradation tests. Various mediators, including 2,2 A-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO), and violuric acid (VA), are used to test the performance of the enzyme-mediator system. Results indicate that VA is the only mediator that can increase the overall removal efficiency of Ibuprofen and Naproxen. The immobilized laccase can remove approximately 50% of Ibuprofen in water, but cannot remove noticeable amount of Naproxen.

**Keywords**
Laccase; immobilization; emerging pollutants; water reclamation

## INTRODUCTION

The continuing presence of emerging water pollutants such as pharmaceuticals, personal care products, surfactants, and various industrial additives has made water reclamation more expensive than before due to the use of membrane filtration process, which is highly energy-intensive. Other alternatives to remove these pollutants are unconventional chemical and biological treatment processes. The Advanced Oxidation Processes (AOPs) have been proved to be an effective chemical treatment method in degrading these pollutants; however, their costs could be high if large quantities of water are to be treated. Consequently, various enzymatic treatments that are used alone (Garcia et al., 2011) or in combination with the filtration method (Kim et al., 2011) have been developed recently so that the goal of cost-effective treatments of these pollutants can be achieved.

Laccases are multi-nuclear copper-containing oxidases that can catalyse the monoelectronic oxidation of substrates at the expense of oxygen (Riva, 2006). After the catalytic oxidation by laccases, the substrate becomes reactive radicals, which can further undergo laccases-catalyzed or nonenzymatic reactions such as polymerization, hydration, and hydrogen abstraction to produce less toxic derivatives (Majeau et al., 2010). With their low specificity on substrates, laccases have been applied to pulp delignification (Camarero et al., 2007) and to the oxidation of various environmental pollutants such as phenols, dyes (Zille et al., 2005), pesticides, endocrine disrupters, and polycyclic aromatic hydrocarbons (Collins et al., 1996). Very often laccases are employed together with the mediators, which are easily oxidizable substrates that can act as redox intermediates between the active sites of enzyme and a non-phenolic substrate. Due to the high redox potential of the mediator, laccases-mediator system can further oxidize contaminants that cannot be oxidized using laccases alone (Bourbonnais et al., 1997).

The immobilization of laccases is an important step in field applications because it can eliminate some undesirable constraints of enzymatic proteins such as their non-reusability and high sensitivity to denaturing agents (Durán et al., 2002). Both physical and chemical immobilization methods are available. In general, chemical immobilization has the advantage to provide strong stable enzyme

attachment, and, in some cases, to reduce enzyme deactivation rate. For the laccase enzyme to be workable in field operations, a simple and effective immobilization method must be developed. The goal of this study is to understand the performance of enzymatic degradation of emerging pollutants, exemplified by Ibuprofen and Naproxen, using soluble and immobilized laccase. A simple procedure to crosslink the laccase to the support resin was used. The effects of mediators were also examined.

## MATERIALS AND METHODS

### Preparation of soluble laccase

A buffer solution of pH 4.5 was first prepared using 68.2 mM of soduim tartrate dibasic dehydrate. Then, 1 mg of laccase (EC 1.10.3.2, from *Rhus vernificera*) was dissolved in 1 mL of the buffer solution to make the stock solution of soluble laccase. The laccase activity was determined from the degradation rate of the ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) substrate. One unit of activity represents 1 mM of ABTS*, oxidized ABTS, produced per minute. The concentration of ABTS* was measured via Beer's law under the wavelength of 420 nm.

### Preparation of immobilized laccase

The procedure used in the immobilization of tyrosinase (Wada et al., 1993) was employed as a reference method for the immobilization of laccase on resins in this study. A weakly acidic cation exchange resin (Daiaion WK10, Mitsubishi Chemical Co., Japan) was used as support. The crosslinking agent used was EDC (1-Ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride). To start the immobilization procedure, 500 mg of the resin was first added to a 2 mL solution of laccase in 0.1 M phosphate buffer controlled at pH 7. Then, 0.9 mL of the EDC solution, containing 57.6 mg of EDC, was added fractionally to the buffer solution, with 60-μL aliquots every 4 minutes under constant stirring at 4 ℃. After the addition of all EDC, the mixture was stirred 4 ℃ for another 17 hours. Then, the immobilized laccase was washed multiple times with buffer solution to remove residual soluble laccase. After the washing process, the immobilized laccase is now ready for use.

### Degradation experiments

The degradation of Ibuprofen by soluble laccase was conducted in an amber glass reactor. The reactor was operated under continuous air bubbling to provide oxygen. In the experiment without mediator addition, 22.75 mL of Ibuprofen solution (20 ppm) was first prepared in the glass reactor; then, 2.25 mL of water plus 0.25 mL of the soluble laccase enzyme was added to start a run. Samples at selected times were withdrawn, filtered with 0.22 μm Millipore filter, and analyzed by HPLC. In experiments with mediators, the experimental procedures were identical as above except 2.25 mL of mediators were added instead of water. Mediators tested in this study include ABTS, VA (Violuric acid), and TEMPO (2,2,6,6-Tetramethyl-1-piperidinyloxy), having concentrations of 0.056 mM, 0.56 mM, and 0.83 mM, respectively. In the degradation experiments by immobilized laccase, Degradation experiments were initiated by adding immobolized laccase (1.5 U/L) to a beaker containing Ibuprofen (20 mg/L). This system was gently mixed using a stir bar. At selected reaction times, aqueous samples were withdrawn, filtered, and analyzed for their residual Ibuprofen concentration by HPLC. An identical degradation experiment was also carried out for Naproxen.

## RESULTS AND DISCUSSION

### Degradation by soluble laccase

Figure 1 shows the results of Ibuprofen removal by soluble laccase under the influences of various mediators. It is evident that there is a very rapid decrease in Ibuprofen concentration after 1 minute

regardless of the presence of mediators. This proves that laccase is quite effective in removing Ibuprofen in water. After 1 minute, the degree of removal becomes insignificant. Such rapid initial removal implies that multiple mechanisms are involved during the removal process. In fact, it is suspected that the initial rapid removal of Ibuprofen is mainly due to adsorption onto laccase, which is always the first step in an enzymatic degradation process. Subsequent slow removal also implies that the step of oxidation is the rate limiting step in the entire process. Among three mediators tested, the VA is the only one that can enhance the removal. It has already known that the mediator can act as strong oxidants, carrying higher redox potentials than the central metal ions in the laccase, to degrade highly recalcitrant compounds. In addition, the 1-minute data for VA also implies that not only adsorption, but also a certain degree of oxidation has occurred within 1 minute. Because if the mechanism is pure adsorption, the 1-minute data for VA and for laccase alone would be the same. With the presence of VA, the removal efficiency is enhanced more than 20% at the 1-minute reaction time. Figure 2 shows the results for Naproxen. These results also have rapid removal at 1 minute, implying the existence of the initial adsorption behaviour as observed for Ibuprofen. The VA is also found to be the best mediator for Naproxen and can cause a 20% increase in degradation as well.

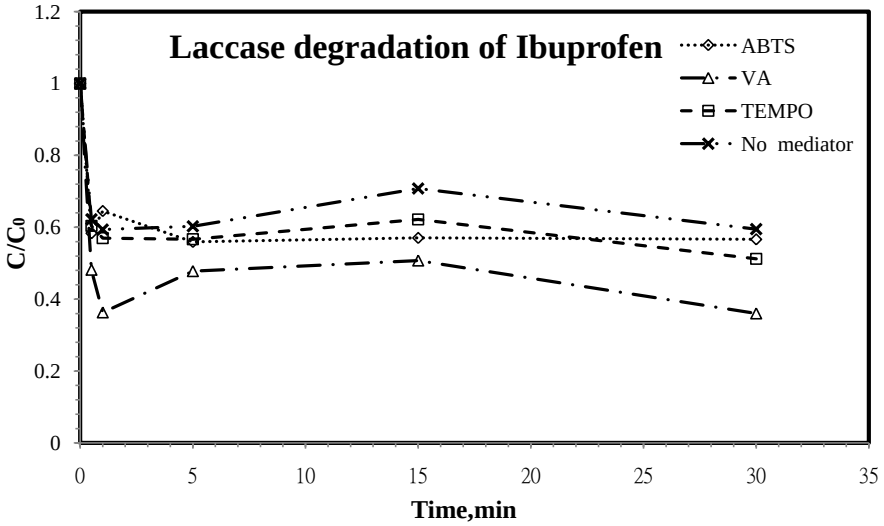


**Figure 1.** Degradation of Ibuprofen by soluble laccase (activity = 30 U/L) alone and in the presence of three different mediators.

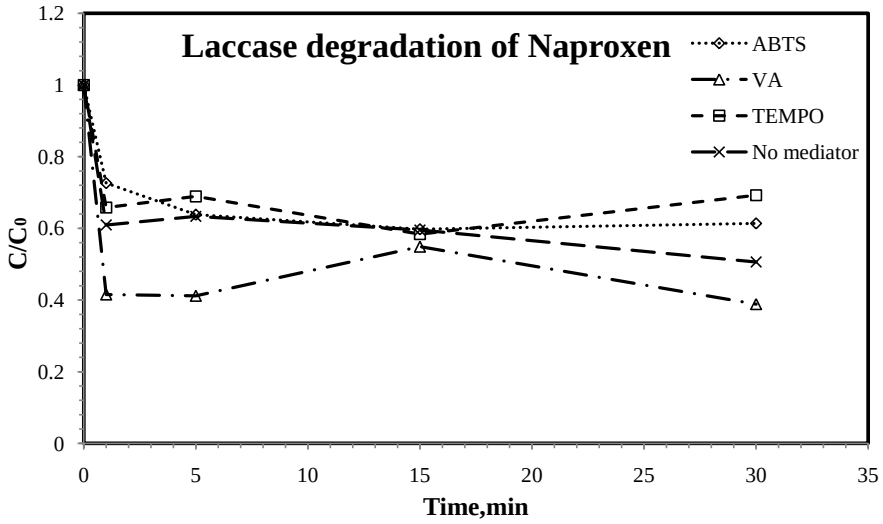


**Figure 2.** Degradation of Naproxen by soluble laccase (activity = 30 U/L) alone and in the presence of three different mediators.

**Effects of mediator concentrations**

Figure 3 shows the results of Ibuprofen removal by soluble laccase under three different ABTS concentrations of 0.5, 5, and 50 μM. The Ibuprofen concentration employed here is higher but the laccase activity is lower than conditions employed in Figure 1. Such design is to create an experimental condition so that effects of mediator concentration can be observed appropriately. From Figure 3, it is evident that the best mediator effect occurs at the concentration of 5 μM. At a higher ABTS concentration of 50 μM, the Ibuprofen removal efficiency becomes lower. This is mainly due to the relatively rigorous oxidation and destruction of the laccase protein by the mediator. This causes the number of active sites on laccase to decrease.

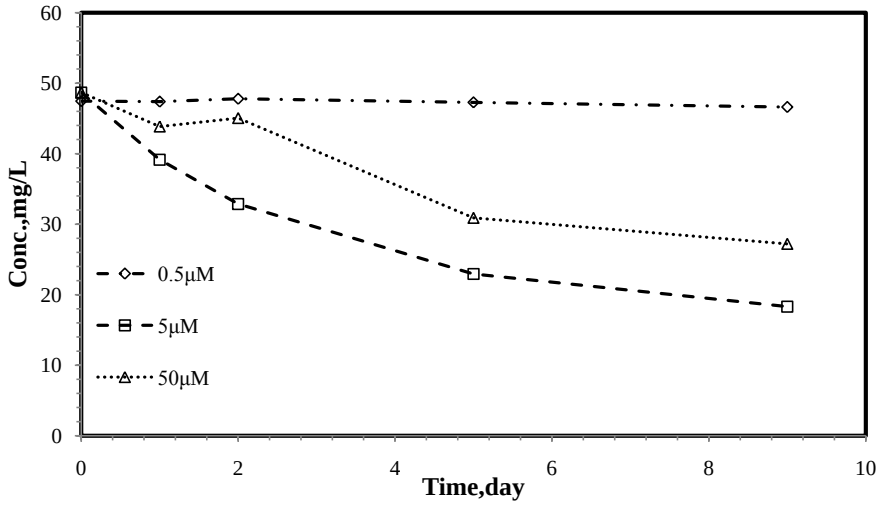


**Figure 3.** Degradation of Ibuprofen by soluble laccase under 3 different ABTS concentrations.

**Degradation by immobilized laccase**

Figure 4 shows the degradation results of Ibuprofen and Naproxen by immobilized laccase. It is evident that the degradation of Naproxen is low, but is relatively high for Ibuprofen. This differs from previous results in soluble laccase, where both Naproxen and Ibuprofen have close efficiencies of removal. It is hypothesized that the size of the substrate molecule (i.e., Naproxen or Ibuprofen) plays dominant roles in contributing to these discrepancies. Since there is no mediator in the immobilized laccase system, molecules in the system can only be oxidized when they bind to the central metals of the enzyme. Thus, if a molecule is too large it may have difficulty accessing the active sites. Consequently, the presence of mediator could be important in immobilized laccase systems.

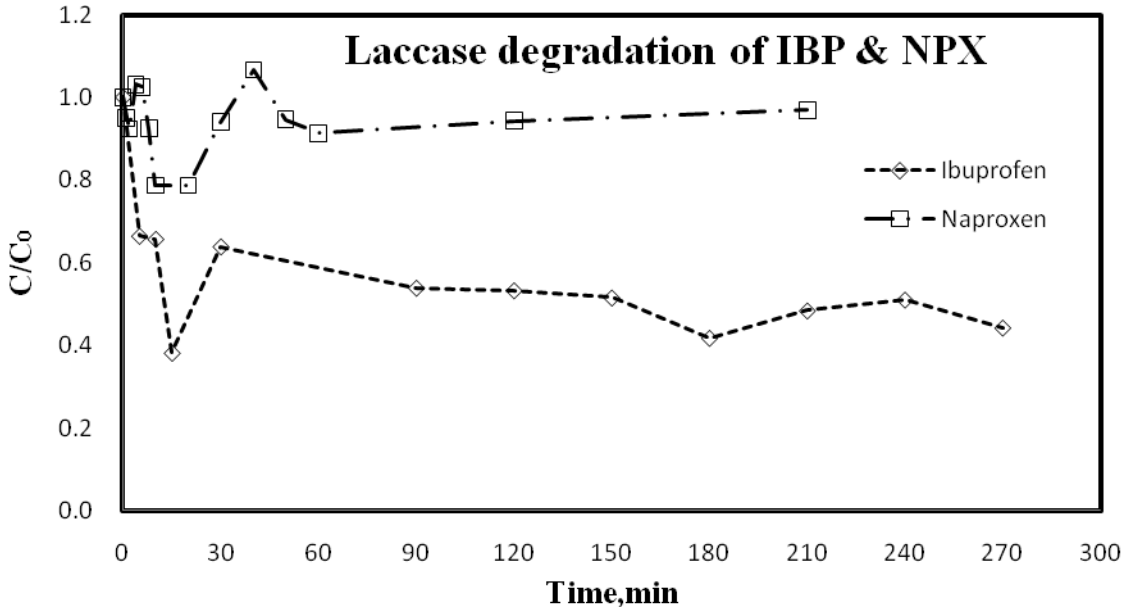


**Figure 4.** Degradation of Ibuprofen and Naproxen by immobilized laccase

## CONCLUSIONS

From this study, it is found that in the soluble laccase system, both Ibuprofen and Naproxen have very close removal efficiencies under all conditions. The removal efficiency of up to 50% can be achieved. The only mediator effective for these two pollutants is violuric acid. In addition, it is observed that an optimum mediator concentration exists for their degradation. In the case of ABTS, the optimum concentration is 5 μM. Finally, in experiments using immobilized laccase it is found that Ibuprofen can be removed effectively, but not Naproxen, which is too large to access active sites on laccase.

## ACKNOWLEDGEMENT

This study is funded by the National Science Council of Taiwan (Grant No. NSC 99-2628-E-029-009).

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# 國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/28

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|---|---|
| 國科會補助計畫 | 計畫名稱: 結合臭氧與固定式酵素床法處理水中酚類污染物之研究 |
| | 計畫主持人: 宋孟浩 |
| | 計畫編號: 99-2628-E-029-009- 學門領域: 環境工程 |

無研發成果推廣資料

# 99 年度專題研究計畫研究成果彙整表

| 計畫主持人：宋孟浩 | 計畫編號：99-2628-E-029-009- |
|---|---|
| 計畫名稱：結合臭氧與固定式酵素床法處理水中酚類污染物之研究 | |

| 成果項目 | | | 量化 | | | 單位 | 備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等） |
|---|---|---|---|---|---|---|---|
| | | | 實際已達成數（被接受或已發表） | 預期總達成數(含實際已達成數) | 本計畫實際貢獻百分比 | | |
| 國內 | 論文著作 | 期刊論文 | 0 | 0 | 0% | 篇 | |
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| | 技術移轉 | 件數 | 0 | 0 | 0% | 件 | |
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| | 課程/模組 | 0 | |
| | 電腦及網路系統或工具 | 0 | |
| | 教材 | 0 | |
| | 舉辦之活動/競賽 | 0 | |
| | 研討會/工作坊 | 0 | |
| | 電子報、網站 | 0 | |
| | 計畫成果推廣之參與（閱聽）人數 | 0 | |

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本研究開發新穎之酵素利用，對永續水處理提供一個新的方法，尤其是在新興汙染物之處理上。本研究所利用之海藻膠是非常廉價且完全安全且與人體組織可相容的材料，而酵素在生物科技之幫助下，目前已可以低成本大量生產。因此整體而言，本研究所開發之固定化酵素顆粒，除有具體處理效果外，在價格上亦將具有很大之競爭力，可與其他水處理技術競爭。