

固定化酶催化氧化去除水溶液中酚的研究

胡龙兴

(上海大学化学化工系, 上海 200072)

摘要 用海藻酸钙凝胶包埋辣根过氧化物酶制成固定化酶, 以过氧化氢为氧化剂, 进行固定化酶催化氧化去除水溶液中酚的实验研究. 对于 100 ml 酚含量为 100 mg/L 的单一苯酚或邻氨基酚水溶液, 控制条件为: 3% 海藻酸钠液 30 ml, 凝胶粒径 1 mm, 固定化时间 1 h, 酶包埋量 50 u, 反应时间 1 h, pH6 及过氧化氢加入量 2 mmol, 单一苯酚和单一邻氨基酚水溶液的酚去除率可分别超过 80% 和 70%. 对混合酚水溶液的处理, 存在明显的协同效应. 制备的固定化酶具有一定的重复使用性能.

关键词 固定化酶, 酶催化氧化, 辣根过氧化物酶, 酚.

造纸、石油化工、木材加工和煤气与炼焦等工业常排放出含酚废水. 含酚废水是一种危害严重的废水. 迄今, 对含酚废水的处理已有多种方法^[1-4], 但这些方法各有其局限性. 本文在用水溶性酶催化氧化含酚废水的研究工作基础上^[5], 探讨用固定化酶催化氧化去除水溶液中酚的可行性.

1 实验部分

1.1 试剂

辣根过氧化物酶(horseradish peroxidase, EC 1.11.1.7) 100 u/mg, 中科院上海生化研究所产品. 过氧化氢溶液、苯酚、邻氨基酚、海藻酸钠、无水氯化钙, 均为化学纯. 常用缓冲试剂, 酚分析试剂.

1.2 仪器

81-Z 型恒温磁力搅拌器, pH5-GD-A 型数字式酸度计, 721 型分光光度计, 全玻璃蒸馏器, 注射器等.

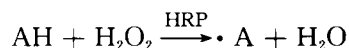
1.3 实验方法

固定化酶制备用海藻酸钙外胶凝法. 每次催化反应实验取 100 ml 已知酚浓度的溶液于烧杯中, 并置于磁力搅拌器上, 加入过氧化氢及固定化酶, 控制一定的反应条件, 当反应结束后, 将固定化酶、沉淀物及溶液相分离, 取水样

分析酚浓度, 计算出酚的去除率. 酚含量用 4-氨基安替比林比色法分析, 过氧化氢浓度用碘量法分析. 所有实验在 20—25℃ 的室温下进行.

1.4 处理方法的特点和基本原理

海藻酸钠易与蛋白质等共溶, 因此它能与酶溶合成均匀的悬浮液, 使固定化海藻酸钙凝胶具有酶分布的均匀性. 海藻酸钠完全无毒, 它不能被绝大多数微生物分解或作为基质, 所以是一种理想的生物载体. 将辣根过氧化物酶包埋在海藻酸钙凝胶的微细格子中, 使酶分子不从凝胶的网格中漏出, 而小分子的底物和产物则可以自由通过凝胶网格, 到达或离开酶分子. 这种固定化酶, 酶分子仅仅是包埋起来而未起化学反应, 酶活力较高, 机械强度较高, 并具有一定的反复利用率. 固定化酶催化反应是非均相反应, 反应发生在固液界面上, 固定化酶周围存在着扩散层, 一定程度上影响底物或产物的扩散. 本处理法的基本原理是: 通过固定化酶催化氧化反应, 生成水溶性差的聚合物, 使酚从水相中去除. 其基本反应为:



式中, AH 是还原性底物酚, $\cdot \text{A}$ 是自由基. 产生的高活性苯氧自由基等扩散到溶液各处, 与

酚分子或苯氧自由基本身发生反应,生成一系列高分子量和水溶性差的聚合物,从溶液中沉淀析出。

2 结果与讨论

2.1 固定化条件

(1) 海藻酸钙凝胶珠粒径的影响 取 3% 海藻酸钠液 30 ml, 加入含 50 u 的酶溶液, 充分混匀后, 用注射器滴入 2% 氯化钙溶液中, 搅拌 1 h, 将凝胶取出待用。控制注射方式, 制得粒径为 4、6 和 8 mm 的凝胶珠。考察了不同粒径凝胶珠催化氧化除酚率(图 1)及使用性能。反应条件: 酚含量 100 mg/L, 过氧化氢加入量 2 mmol, 溶液 pH6(为避免 PO_4^{3-} 对海藻酸钙凝胶的破坏, 用柠檬酸-柠檬酸钠缓冲液), 反应时间 1 h。

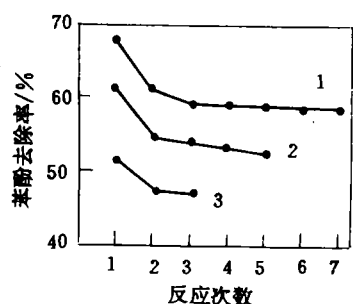


图 1 不同粒径凝胶珠对酚去除率的影响

1. 粒径 4 mm 2. 粒径 6 mm 3. 粒径 8 mm

由图 1 可见, 在间歇式反应方式下, 酚去除率第 2 次明显低于第 1 次, 以后则下降很少或几乎不变。小粒径的凝胶除酚率高于大粒径凝胶。在实验中发现, 对粒径为 8 及 6 mm 的凝胶珠, 分别在第 3 次和第 5 次使用后出现破裂、解体现象。而粒径为 4 mm 的凝胶珠在第 7 次使用后, 球形、机械强度及催化氧化效率基本不变。因此, 凝胶珠粒径宜控制在 4 mm 以下。

(2) 固定化时间的影响 固定化时间指将海藻酸钠及酶的混合液滴落到氯化钙溶液中的反应时间。取凝胶粒径为 4 mm, 固定化时间分别为 0.5、1、2、12 h, 其他条件同上, 结果如图 2。

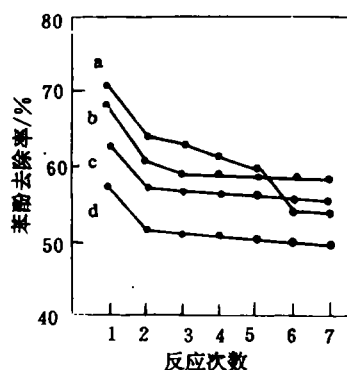


图 2 固定化时间对酚去除率的影响

a. 0.5 h b. 1 h c. 2 h d. 12 h

催化氧化效率下降。实验中发现: 固定化时间在 1 h 以上的凝胶具有同样的机械强度, 而固定化时间为 0.5 h 的凝胶珠的机械强度较低。因此, 固定化时间选 1 h 为宜。

2.2 不含酶的凝胶颗粒对酚的吸附

取凝胶珠粒径为 4 mm, 其他条件同上。观察不含酶的凝胶珠在间歇式反应中对酚的吸附, 结果如表 1 所示。

表 1 不含酶的凝胶珠对酚的吸附

使用次数	1	2	3	4
苯酚去除率/%	15	5	3	3

在凝胶珠重复使用前进行例行的清洗。由表 1 可见, 新凝胶珠对酚有一定的吸附能力, 而用过的凝胶珠吸附能力很弱。当用新凝胶珠催化氧化酚时, 由于催化氧化与吸附的共同作用, 使酚去除率高于使用过的凝胶珠。在第 1 次以后的使用中, 是催化氧化去除酚, 酚去除率较稳定。在以后实验中, 酚去除率取第 2 次处理时的数据。

2.3 处理单一含酚水溶液的正交试验

对固定化酶催化氧化酚的影响因素包括对固定化酶制备和对催化反应 2 方面的影响因素。在确定后者水平时, 参照水溶性酶试验结果。该结果表明: 当 pH 接近中性时, 酚的去除率最高。当用固定化酶催化反应时, 最适 pH 可能会向碱侧或酸侧偏移, 也可能不变。在本实验中

发现：固定化酶受 pH 影响较大，在 pH7.0 以上时，漏酶现象明显且稳定性变差。因此，本实验控制反应 pH 为 6。为了考察其他影响因素及寻求各因素水平的最佳组合，采用了正交试验设计法，选用 $L_9(3^4)$ 正交表，考察的因素，水平及结果如表 2 所示。其他条件同前。

表 2 单一含酚水溶液处理的试验方案及结果分析¹⁾

试验号	A 海藻酸钠浓度/%	B 凝胶粒径/mm	C 酶包埋量/u	苯酚去除率/%
1	1	1	40	54(48)
2	1	2	50	68(61)
3	1	3	60	46(41)
4	2	1	50	77(69)
5	2	2	60	56(50)
6	2	3	40	40(36)
7	3	1	60	65(58)
8	3	2	40	53(47)
9	3	3	50	71(63)
K_1	168(150)	196(175)	147(131)	
K_2	173(155)	177(158)	216(193)	
K_3	189(168)	157(140)	167(149)	
k_1	56(50)	65(58)	49(44)	
k_2	58(52)	59(53)	72(64)	
k_3	63(56)	52(47)	56(50)	
R(极差)	7(6)	13(11)	23(20)	

1) 括号内为邻氨基酚数值

由表 3 可知，对于苯酚和邻氨基酚 2 种水溶液的处理结果， $F_{0.1} > F_A > F_{0.2}$ ， $F_{0.01} > F_B > F_{0.05}$ ， $F_{0.01} > F_C > F_{0.05}$ ，故酶包埋量和凝胶珠粒径 2 因素对除酚率影响显著，海藻酸钠浓度对除酚率也有一定影响。由均方或极差大小可知，影响酚去除率的因素依次是：酶包埋量、凝胶珠粒径、海藻酸钠浓度。用 A、B、C 3 列的 k_1 、 k_2 、 k_3 作图 3。

由图 3 可见，(1) 海藻酸钠浓度越高，酚去除率越高，但浓度为 4% 时固定化较困难，以

3% 为最好。(2) 凝胶珠粒径越小，酚去除率越高，以凝胶珠粒径 1 mm 为最好。(3) 酶包埋量以 50 u 为最好，酶包埋量再增加，酚去除率反而有所下降。

综上所述， $A_3B_1C_2$ 可能是最好的工艺条件，对该条件进行了验证性实验，制备的固定化酶重复使用 7 次，单一苯酚水溶液中酚的去除率在 81%—83%，单一邻氨基酚水溶液中酚的去除率在 71%—74%。这表明海藻酸钠浓度 3%，凝胶粒径 1 mm 及酶包埋量 50 u 确是最佳的工艺条件。

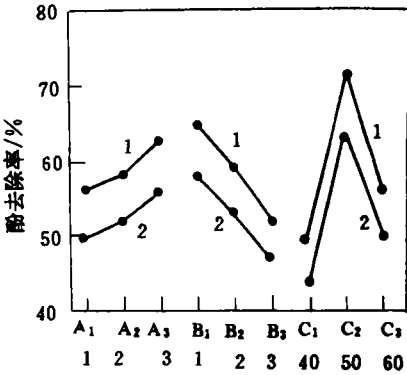


图 3 3 因素与酚去除率关系

1. 苯酚溶液 2. 邻氨基酚溶液

2.4 苯酚含量对催化氧化效率的影响

考察了苯酚含量变化对酚去除率的影响。酚浓度分别为 1, 10, 100, 300, 500, 700 mg/L，其他条件同正交试验所得最佳条件，结果如图 4 所示。

由图 4 可见，酚含量较低时，酚的去除率可达 90% 以上。随着水溶液中酚含量增加，酚去除率逐渐下降。因此，如要保持高去除率，就要适当增加过氧化氢和固定化酶的加入量。

表 3 单一含酚水溶液处理试验的方差分析¹⁾

方差来源	平方和	自由度	均方	F	临界值
A	80(57)	2(2)	40(28.5)	7.27(8.14)	$F_{0.05}=4$ $F_{0.01}=9$
B	254(204)	2(2)	127(102.0)	23.09(29.14)	$F_{0.05}=19$ $F_{0.01}=99$
C	840(678)	2(2)	420(339.0)	76.36(96.86)	$F_{0.05}=19$ $F_{0.01}=99$
误差	11(7)	2(2)	5.5(3.5)		
总和	1185(946)	8(8)			

1) 括号内为邻氨基酚数值

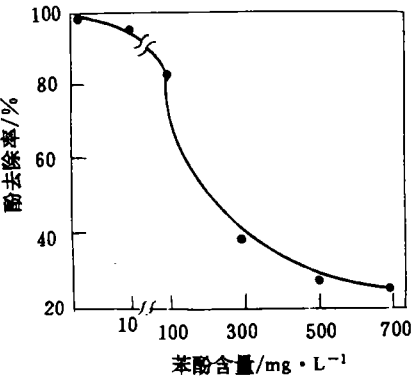


图 4 苯酚含量对去除率的影响

2.5 处理混合酚水溶液的试验

控制混合液总酚含量为 100 mg/L，总体积

为 200 ml，由等体积单一含酚液混合而成，改变苯酚和邻氨基酚比例，其他条件同上，考察了混合酚和对应的单一酚水溶液的酶催化反应效果，结果如表 4 所示。

由表 4 可见，混合酚水溶液的酚去除率高于单一酚溶液的去除率，存在明显的协同效应。

3 结论

控制适当的条件，单一苯酚和单一邻氨基酚水溶液的酚去除率分别达到 80%和 70%以上。在混合酚水溶液的处理中，存在明显的协同效应，酚去除率高达 85%以上。固定化酶具有一定的重复使用性能，与水溶性酶处理相比，

表 4 混合酚溶液的催化氧化去除率

水溶液组号	混合液酚含量/mg · L ⁻¹		酚去除率/%	含单一苯酚液		含单一邻氨基酚液	
	苯酚	邻氨基酚		酚含量/mg · L ⁻¹	去除率/%	酚含量/mg · L ⁻¹	去除率/%
1	30	70	85	60	84	140	51
2	50	50	86	100	82	100	71
3	70	30	88	140	60	60	76

本法能减少酶消耗，降低处理成本。

致谢 本工作得到张仲燕教授指导，姚海宏、徐萍同志参加了部分实验，在此一并表示感谢。

参 考 文 献

1 张芳西，金承基等. 含酚废水的处理与利用. 北京：化学工

业出版社，1983：77—378

2 Bettmann H et al. Appl. Microbiol. Biotechnol. , 1984, 20 (5): 285

3 Yen chenyu et al. J. Environ. Eng. , 1984, 110(5): 976

4 Goto Motonobu et al. Environ. Sci. Technol. , 1986, 20 (5): 463

5 胡龙兴，张仲燕. 环境科学，1992, 13(4): 40

欢迎购阅《中国小造纸、小印染和高浓度有机废水治理技术交流会论文汇编》

《中国小造纸、小印染和高浓度有机废水治理技术交流会论文汇编》(《环境科学》增刊)精选了由国家环保局科技标准司科技处主持的“小造纸厂污染防治技术交流会”和“小印染、高浓度有机废水污染防治技术交流会”交流的优秀科技论文 39 篇，介绍了适用性强、投资少、成本低和能耗低的新型治理工艺、新设备、新药剂等，包括小造纸厂黑液碱回收，木素综合利用产品开发，石灰法草浆厌氧处理技术，处理高浓度有机废水的厌氧生物技术和处理印染废水的兼

氧-好氧-生物炭技术，高压脉冲电解法新设备等。

《汇编》约 20 万字，定价 8 元/册(含邮资)。编辑部还有刊，欲购者请书款汇到北京 2871 信箱(邮编 100085)《环境科学》编辑部，并用正楷在汇款单上写清双方地址、邮政编码、姓名、款数、所购书名及数量，切勿多寄或少寄书款及在信中夹邮书款，也可携款到编辑部购买，联系电话：62545511—2138。

their composition and concentration. The experimental results showed that the concentration of volatile organic acids increased after the thermophilic digestion of the mixed substrates and decreased greatly after their further digestion in the second mesophilic digester, which were averagely 3346—5529 mg/L and 43—433 mg/L, respectively. Acetic acid, propionic acid, butyric acid and valeric acid are the main acids in the mixed substrates and in the effluent of thermophilic digester.

Key words: thermophilic/mesophilic, anaerobic digestion, organic acids.

Study on the Effect of Tourism on the Ecological Environment of Mountain Emei and Protective Strategies.

Jiang Wenju et al. (Dept. of Environ. Sci. and Eng., Sichuan Union Univ., Chendu 610065); *Chin. J. Environ. Sci.*, **17** (3), 1996, pp. 48—51

With development of tourism and increase of visitors, the ecological environment of Mt. Emei has been seriously damaged. SO_2 concentrations in the atmosphere in some places have exceeded the national standard and NO_x concentrations are increasing gradually. The acidization of rain, fog and soil are getting more serious. *Abies fabri*, main scenic forest at high altitudes, have also been on the way to decline. The damaged extent at tourist spots is obviously higher than that in other places. In this paper, the policy of protection prior to development was proposed, with strategies of increasing capacity of tourism, rationally dispersing tourists, advocating electricity in replacement of coal, concentratedly disposing sewage and solid wastes, devoting major efforts to plant vegetation, controlling residential population and emphasizing consciousness of environmental protection.

Key words: Mountain Emei, tourism, ecological environment, protective strategy.

Catalytic Combustion of Pyridine Over Metal Oxides Catalysts.

Luo Mengfei et al. (Institute of Catalysis, Hangzhou University, Hangzhou 310028); *Chin. J. Environ. Sci.*, **17**(3), 1996, pp. 52—53

The Oxidation activity of pyridine and NO_x control in pyridine oxidation over metal oxides catalyst were studied. As the temperature increased, the content of nitrogen oxide generated by pyridine oxidation went through a maximum value.

Oxidation activity of catalysts was found to be in direct proportion to their NO_x control ability. Ag-Mn and Ag-Co bi-metal oxides catalysts exhibit higher activity and NO_x control ability than the individual composition catalyst. The oxidation mechanism of pyridine was discussed.

Key words: pyridine catalytic oxidation, metal oxide catalyst, NO_x control.

The Photolysis Character of HCFC-22 in Presence of Hydrogen Peroxide.

Zhong Jinxian et al. (Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085), Chen Dazhou (Chinese Center for Certified Reference Materials, Beijing 100013); *Chin. J. Environ. Sci.*, **17**(3), 1996, pp. 54—56

Under simulated atmospheric condition, the HCFC-22 + H_2O_2 in the presence or absence of oxygen was irradiated by low pressure mercury lamp and the products of photolysis were determined by a Fourier Transform Infrared Spectroscopy with a 20 m long path cell. The products are COF_2 , CO_2 , HF and HCl for HCFC-22 + H_2O_2 system, CO_2 , H_2O , HF and HCl for HCFC-22 + $\text{H}_2\text{O}_2 + \text{O}_2$ system. Based on these results, the mechanisms of photolysis were suggested.

Key words: photooxidation, HCFC-22, H_2O_2 .

Study on the Catalytic Removal of Phenols from Aqueous Solution by Immobilized Enzyme.

Hu Longxing (Dept. of Chem. & Chem. Eng., Shanghai University, Shanghai 200072); *Chin. J. Environ. Sci.*, **17**(3), 1996, pp. 57—60

The removal of phenols from aqueous solution by immobilized enzyme catalytic oxidation was studied. The immobilized enzyme was prepared by containing horseradish peroxidase with calcium alginate gel, and hydrogen peroxide was used as oxidizing agent. For 100 ml phenol or o-aminophenol aqueous solution containing 100 mg/L phenol, under the following appropriate conditions: 3% sodium alginate solution 30 ml, gel particle diameter 1 mm, immobilization time 1 h, containing amount of enzyme 50 u, reaction time 1h, pH 6 and hydrogen peroxide dose 2 mmol, the removal rates of phenol and o-aminophenol exceeded 80% and 70% respectively. There was synergistic effect between various phenols in the treatment of mixed phenol aqueous solution. The treatment result after mixing dif-

ferent phenol wastewaters is better than their separate treatment result. The immobilized enzyme prepared has fair repeating usage performance.

Key words: immobilized enzyme, enzyme catalyzed oxidation, horseradish peroxidase, phenol.

Determination of Trace Copper, Lead and Cadmium in Seawater by Graphite Furnace Atomic Absorption Spectrometry with Dithizone-n-Butyl Acetate Extraction. Jin Xuegen (Zhejiang Environmental monitoring Centre Station, Hangzhou 310012); *Chin. J. Environ. Sci.*, **17**(3), 1996, pp. 61—63

This paper studied the optimum conditions for the determination of trace copper, lead and cadmium in seawater by graphite furnace atomic absorption spectrometry with dithizone-n-butyl acetate extraction. The results showed that copper, lead and cadmium in the weakly alkaline medium (pH8—10) can be quantitatively extracted simultaneously. The relative standard deviation is less than 3% and the recoveries for the added standard are between 92%—102%. The detection limit ($\mu\text{g/L}$) for copper, lead and cadmium are 0.06, 0.14 and 0.002, respectively. This method is simple and rapid. The method has been successfully applied to the determination of trace copper, lead and cadmium in seawater with satisfactory results.

Key words: dithizone, extraction, graphite furnace, seawater, copper, lead, cadmium.

Determination of Lead from Automobile Exhaust Using Anodic Stripping Voltammetry at Carbon Fiber Beam Mercury Film Electrodes. Shao Mengxin et al. (Dept. of Environ. Eng., Tianjin Professional College, Tianjin 300402); *Chin. J. Environ. Sci.*, **17**(3), 1996, pp. 64—66

In this paper, the preparation for carbon fiber beam mercury film microelectrodes was described. The electrode voltammetric properties were investigated. Lead content from automobile exhaust was determined using anodic stripping voltammetry. The suitable experimental conditions were assigned. This technique is simple, quick, with high sensitivity and fine reproducibility. The detection limit is about $0.1 \text{ ng} \cdot \text{ml}^{-1}$, the linear range is $1.0 \text{ ng} \cdot \text{ml}^{-1}$ — $20 \text{ ng} \cdot \text{ml}^{-1}$. The relative standard deviation is 9.4%.

Key words: carbon fiber microelectrodes, anodic stripping voltammetry, automobile exhaust, lead.

Determination of Phenol in Wastewater by Alternating-current Oscilloscopic Polarography Titration. Qiu Zhiguo (Dept. of Chemistry Northeast Forestry University, Harbin 150040); *Chin. J. Environ. Sci.*, **17**(3), 1996, pp. 67—68

A method to use a mixed solution of hydrochloric acid and potassium bromide as base solution to titrate phenol in water sample directly was indicated. The method is simple and rapid. The measuring range is 11.07—2113.2 mg/L , variation coefficient is below 0.9%, recovery rate is 95%—102%.

Key words: phenol, alternating-current oscillographic titration, wastewater determination.

Analysis on the Typical Photochemical Processes of Pollution in Autumn. Zhu Yuxiu et al. (Dept. of Environ. Eng., Shanghai Institute of Urban Construction, Shanghai 200092); *Chin. J. Environ. Sci.*, **17**(3), 1996, pp. 69—71

Synchronous observations of O_3 - NO_x concentrations and relative meteorological material have been lasted for one month, in order to study the characteristics of photochemical pollution and their relations with meteorology. Photochemical pollution processes in the autumn have been found. It not only includes the mechanisms of producing and accumulating of local sources as in summer and winter, but also includes the feature of high ozone pollution from outer sources as in spring. The highest and mean maximum concentrations of O_3 reaches 168.7 $\mu\text{g/m}^3$ and 114.8 $\mu\text{g/m}^3$, respectively.

Key words: autumn, processes of ozone pollution, photochemical pollution.

Study on the Indirect Determination of the Soluble Sulfide in Water by Spectrophotometry. Wang Lihong et al. (Dept. of Environ. Sci., Hangzhou University, Hangzhou 310029); *Chin. J. Environ. Sci.*, **17**(3), 1996, pp. 72—74

In this paper, a new method for indirect determination of trace sulfide ion was developed. The optimum conditions of precipitate reaction of copper with sulfide and color reaction of copper with T(4-MOP) PS_4 were discussed. At about pH 8.5, CuS was formed, and at pH4.0 the surplus