细菌发光传感器在快速检测污染物急性毒性中的应用*

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摘要 本研究以明亮发光杆菌作为指示生物, 将细胞固定化技术、生物传感器技术与发光细菌毒性测试技术有机结合起来, 建立一种细菌发光传感器. 确定了固定化菌膜的发光强度及稳定时间. 以这一检测系统测定了 3 种金属离子及 3 种有机化合物的急性毒性(以抑制菌膜发光强度 50% 所需的受试物浓度 EC_{50}值表示), 并分析了毒性作用的动力学过程. 实验结果表明: 在 pH7.0, 温度 20℃, 3.0% NaCl 底液条件下, 固定化菌膜的发光强度达 250–300×10^{-7}mW, 稳定时间达 60–80min; 各受试物毒性强弱及 EC_{50}值(mg/L)为: Hg^{2+}(0.15) > Cu^{2+}(14) > Zn^{2+}(130), 苯酚(35) > 乙醛(210) > 醋酸乙酯(1200), 与哺乳动物毒性试验的LD_{50}值顺序一致, 有较高的灵敏度和稳定性; 不同毒物之间对细菌发光反应的抑制速率有差异.

关键词 发光细菌, 生物传感器, 急性毒性, 发光强度, 灵敏度, 稳定性.

近几十年来, 许多微生物学检测手段[1,2]被用于分析环境污染物的急性毒性, 其中发光细菌因其独特的生理特性[3]而被看作是一种较理想的指示生物, 发光细菌毒性试验(Microtox)[4]因其检测时间短(15min)、灵敏度高(细胞基本物质代谢受到影响前发光反应先被抑制)而被世界各国广泛采用. 我国于 1995 年也将这一方法列为环境毒性检测的标准方法(GB/T 15441–1995)[5]. 但该方法亦存在细胞发光强度本底差异较大, 检测期间发光自然变化幅度较宽的问题, 细胞固定化技术是解决这一问题的有效方法. 近年来生物传感器技术由于将生物敏感材料与现代光电检测手段完美结合, 实现连续在位检测等特点在环境监测中的运用愈来愈广泛, 如 BOD 传感器已进入标准化和商品化的前期阶段[6].

本研究将细胞固定化技术、生物传感器技术与发光细菌毒性测试技术有机结合起来, 以硅光片作为细胞光信号与电信号转换的敏感元件, 建立一种细菌发光传感器, 改善细菌发光的稳定性, 实现急性毒性的连续动态检测, 同时能对急性毒性的动力学过程进行分析.

1 实验部分

1.1 仪器

DJ–Ⅱ型微光光功率计(含硅光片, 机械电子工业部第 23 研究所), CS501 型超级恒温器(重庆试验设备厂), 蠕动泵(德国 Melsungen GA 公司), 78HW–Ⅰ型磁力搅拌器(杭州仪表电机厂), 自制暗盒.

1.2 菌种及培养基

明亮发光杆菌(Photobacterium phosphoreum)由中国科学院南京土壤所提供.

ASW 培养基(W/V): 胰蛋白胨(0.5%), 酵母浸出汁(0.5%), 氯化钠(3%), 磷酸氢二钠(0.5%), 磷酸二氢钾(0.1%), 甘油(0.3%), 琼脂粉(1.5%), pH7.0

1.3 受试物

HgCl, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, C_6H_6O, C_2H_4O, $C_4H_8O_2$. 所用试剂均为分析纯.

1.4 固定化菌膜的制备

从斜面接菌种于 ASW 培养液中, 20℃恒温, 200r/min 振摇培养 12h, 使菌数达到 10^8 个

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/ml, 吸取 5ml 菌液经蔡氏滤器过滤. 同时在直径 25mm, 孔径 0.65μm 的混合纤维素微孔滤膜上刷上一薄层热溶的 ASW 培养基, 待其自然冷却成膜, 将膜的培养基面粘附到蔡氏滤器的截菌面, 使培养基面均匀吸附一层明亮发光杆菌(直接将细胞固定于微孔滤膜易导致菌体流失和细胞活力不能持久), 将固定好的菌膜有菌面覆盖在硅光片上, 构成细菌发光传感器的敏感探头(图 1).

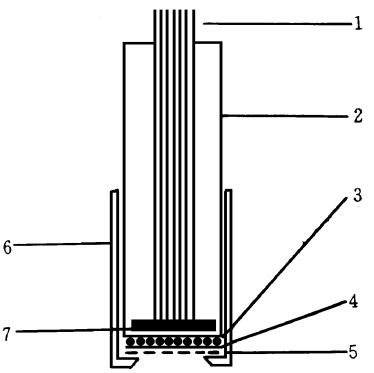

图 1　细菌发光传感器敏感探头的结构简图

1. 电信号输出　2. 玻璃保护套　3. 明亮发光杆菌　4. 薄层 ASW 培养基　5. 微孔滤膜　6. 探头夹套　7. 硅光片

1.5　菌膜发光强度及受试物毒性测定

将敏感探头、微光光功率计、蠕动泵、暗盒等组成一套连续测定系统(图 2). 向带有夹层的流通反应池中以 1ml/min 流速通入 3% NaCl 底液或待测样品液(待测样品按 GB/T 15441-1995 方法处理), 用磁力搅拌器搅拌, 夹层内通入 20℃恒温水, 敏感探头插入暗盒反应池中, 在避光条件下通过 DJ-Ⅱ型微光光功率计测定菌膜发光强度及其变化值, 毒性试验结果以固定化菌膜与受试物作用 15min 后发光强度被抑制 50% 所需的受试物浓度(mg/L), 即 EC_{50} 值表示.

2　结果与讨论

2.1　菌膜发光强度及稳定时间

图 3 显示在 pH 7.0, 温度 20℃, 3.0% NaCl 底液条件下, 固定化菌膜发光强度可达 250-300×10^{-7}mW, 即达纳瓦级, 满足 DJ-Ⅱ型微光光功率计的灵敏度要求. 稳定发光(发光强

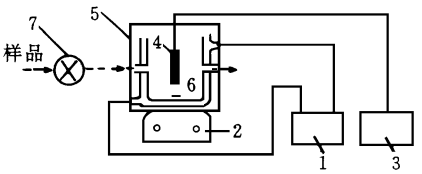

图 2　细菌发光传感器的组成示意图

1. 恒温水浴　2. 磁力搅拌器　3. 微光光功率计　4. 敏感探头　5. 暗盒　6. 反应池　7. 蠕动泵

度平值±5% 范围) 时间可持续 60-80min. 毒性检测时间为 15min, 在此期间固定化菌膜发光强度变化不超过±2%, 而 Microtox 方法中无论是采用菌悬液或是冻干粉测定, 细菌发光强度 15min 的自然变化值可达±10%, 因此其稳定性不及细菌发光传感器.

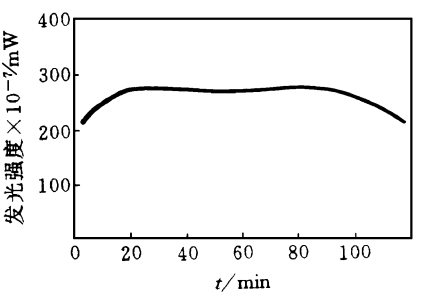

图 3　固定化菌膜的发光强度及稳定时间

2.2　污染物急性毒性的测定

在本实验条件下, 各受试物急性毒性测定的 EC_{50} 值见表 1, 同时将 Microtox 测定的 IC_{50} 值, 哺乳动物毒性试验(大鼠, 经口) 的 LD_{50} 值及各污染物在地面水中的最高容许浓度(国标 TJ36-79[11]) 列入表 1 中进行比较.

由表 1 可知, 细菌发光传感器的毒性测试结果灵敏度略低于 Microtox 方法, 可能有以下原因: ①由于 Microtox 方法中菌悬液与受试物作用充分, 而细菌发光传感器方法中受试物是经过扩散通过固定化膜与菌细胞接触, 在相同检测时间内作用程度不如 Microtox 方法充分, 这可以通过进一步优化固定化条件加以改进. ②Microtox 方法中光电信号的转换器件是光电倍增管, 与本方法所用的硅光片相比对光信号的灵敏度更高(内有多级放大结构), 这也是 Microtox 方法灵敏度更高的一个因素. 但光电

倍增管的结构较硅光片复杂得多,从经济和技术角度看目前尚难于直接与固定化菌膜结合构成生物传感器的敏感部件. 细菌发光传感器的优点是发光稳定,在满足菌膜一定的检测灵敏度条件下,稳定性是应着重考虑的因素之一. 发光被抑制后,在底液中通入培养液可使发光强度得以恢复而重复使用. 从表 1 中还可以看出,细菌发光传感器的检测结果与哺动物毒性试验的结果吻合,但灵敏度高出约 1 个数量级,各受试物的毒性强度顺序均为: Hg^{2+} > Cu^{2+} > Zn^{2+}, 苯酚> 乙醛> 醋酸乙酯,说明 2 种方法有可比性,而且测试结果也与国家标准对各污染物的最高容许浓度要求一致,这些都说明了细菌发光传感器这一方法在污染物急性毒性测定方面的可行性.

表 **1** 各受试物的急性毒性测试结果/ mg · L^{-1}

受试物	EC_{50}	IC_{50}	LD_{50}[10]	TJ36-79
Hg^{2+}	0.15	0.1[5]	1	0.001
Cu^{2+}	14	9.9[7]	300	0.1
Zn^{2+}	130	55.5[8]	1890	1.0
苯酚	35	17.6[9]	414	0.01
乙醛	210		1930	0.05
醋酸乙酯	1200		11300	

2.3 污染物对细菌发光作用的动力学分析

由于细菌发光传感器的发光稳定性好,而且也是一个动态连续的检测过程,所以可用于分析污染物对细菌发光作用的动力学过程.

图 4 显示在 EC_{50}值剂量条件下,苯酚和 Zn^{2+} 使固定化菌膜的发光强度随时间变化的曲线. 由图 4 可知,苯酚使菌细胞的发光强度在初始阶段迅速下降,而后进入一个缓慢减弱的过程,15min 时发光强度降至初始值的一半. 可能是苯酚在初始阶段迅速抑制了菌细胞的发光反应(使细菌荧光素酶失活),但在此剂量下还未产生致死效应,此后由于细胞的‘耐受性’而使发光下降速率减缓,而 Zn^{2+} 的动力学曲线与前者相比初始阶段并无一个明显的急速下降过程,可能是苯酚与 Zn^{2+} 使细菌荧光素酶失活的速率不同所致. 用该检测系统可比较不同污染物对菌细胞发光反应抑制的速率,得到相应的毒性反应动力学曲线,使对不同污染物的毒性作用有更深的认识.

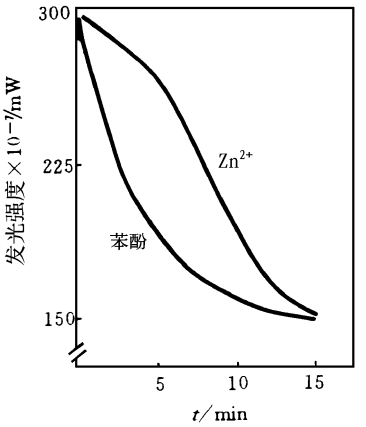

图 4 苯酚、Zn^{2+} 对细胞发光作用的动力学曲线

3 小结

本研究在细胞固定化技术、生物传感器技术和发光细菌毒性测试技术的基础上,发展了一种细菌发光传感器,对急性毒性测定有较高的灵敏度和稳定性,与哺乳动物毒性测试结果有良好的相关性,并能进行连续动态检测和动力学分析. 菌膜的固定化条件及保存手段尚需进一步深入研究.

致谢 在仪器分析方面本研究所周宜开教授和任恕教授给予了大力支持,谨致谢意.

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Kinetics of Photocatalytic Oxidation of Phenol on TiO_2. Hu Chun, Wang Yizhong, Tang Hongxiao (State Key Laboratory of Environmental Aquatic Chemistry, Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085): *Chin. J. Environ. Sci.*, **18**(4), 1997, pp. 1—4

The kinetics of the photodegradation of phenol were studied in TiO_2 aqueous suspensions under mercury lamp irradiation. The effects of initial concentration (c_0) of phenol, oxygen patial pressure (P_{O_2}), light intensity (I), the amount of catalyst and calcination temperature of catalyst on photodegradation of phenol have been investigated. The characteristics of photocatalytic oxidation of phenol were verified, the reaction apparently proceeded according to the first-order kinetics with the apparent rate constant dependencing on c_0, P_{O_2}, I and $[TiO_2]$. Photocatalytic activity can be improved by suitable calcination. The experimental results indicates that the oxygen patial pressure, light intensity and dose of catalysts were important parameters in the degradation process. A chemical kinetic equation was proposed, which could be in agreement with experimental results.

Key words: photocatalytic, kinetics, phenol, TiO_2, photocatalytic oxidation.

Research on Basic Theory of Determining Critical Loads for Acid Deposition with Steady-state Mass Balance Method. Xie Shaodong, Hao Jiming, Zhou Zhongping (Dept. of Environ. Eng., Tsinghua Univ., Beijing 100084): *Chin. J. Environ. Sci.*, **18**(4), 1997, pp. 5—9

Some basic conceptions were clearly stated in the theory of determining critical loads for acid deposition. Basic formula to calculate critical loads for acid deposition was obtained by establishing steady-state mass balance equation for alkalinity production in leaching solution. From this basic formula, mathematical expressions to calculate critical loads for acidity, potential acidity, sulfur deposition and nitrogen deposition were derived. The methods of calculating critical alkalinity leaching by means of critical chemical values that will not cause harmful effects on a selected indicator organism were also studied. Therefore, a complete and systematic theory for steady-state mass balance method was presented in the paper.

Key words: acid deposition, critical load, ecosystem, soil, steady-state mass balance method.

Water-Particulate Distribution Coefficient of Heavy Metal and Application in Sediment Quality Criteria in China River. Huo Wenyi and Chen Jingsheng. (Dept. of Urban and Environmental Sciences, Peking University, Beijing 100871): *Chin. J. Environ. Sci.*, **18**(4), 1997, pp. 10—13

Water-particulate distribution coefficient of heavy metal was calculated in natural condition (both physical and chemical) in China rivers. Due to difference of grain size composition, distribution coefficient of heavy metal in the Changjiang (Yangtze River) is greater than it in the Huanghe (Yellow River). For the Changjiang, that distribution coefficient estuary is greater than it in Wuhan station mainly reflects effect of ion intensity. Based on preceding data, sediment quality criteria was proposed in the study.

Key words: water-particulate distribution coefficient, heavy matel, sediment quality criteria, Yangtze River, Yellow River.

The Application of Bacterial Luminescent Biosensor in Rapid Determination of Acute Toxicity of Pollutants. Huang Zheng et al. (Institute of Environ. Medicine, Tongji Medical Univ. Wuhan 430030): *Chin. J. Environ. Sci.*, **18**(4), 1997, pp. 14—16

Photobacterium phosphoreum was used as the indicator bacteria. The techniques of cell immobilization, luminous bacteria toxicity test and biosensor were combined to develope a bacterial luminescent biosensor. The luminescent intensity of immobilized bacteria film and its stable time were determined. The acute toxicity of 3 metallic ions and 3 organic compounds was detected by this system (based on the EC_{50} value 50% inhibition rate of luminescent intensity of immobilized bacteria film). The kinetic process of toxicants on bacterial luminescence was analyzed. The results showed that the luminescent intensity could reach 250– 300×10^{-7} mW when the immobilized bacteria film was measured in 3.0% NaCl of pH 7.0 at 20℃. The stable time could reach 60– 80min. The toxicity and EC_{50} (mg/L) sequence of toxicants were: Hg^{2+} (0.15) > Cu^{2+} (14) > Zn^{2+} (130), phenol (35) > acetaldehyde (210) > ethyl ester (1200). The EC_{50} sequence was coincident with the LD_{50} of mammal toxicity test and this system had good sensitivity and stability. There was difference among toxicants in the inhibition rate of bacterial luminescence.

Key words: bacterial luminescence, biosensor, acute toxicity, luminescent intensity, stability, sensitivity.

Biodegradation of Poly-β-Hydroxyalkanoates Membrane in Aerobic and Anaerobic Sludge. Gao Haijun, Chen jian et al. (Environ. Biotechnol. Lab., Sch. Biotechnol., Wuxi Univ. Light Industry, Wuxi 214036): *Chin. J. Environ. Sci.*, **18**(4), 1997, pp. 17—20

Biodegradation process and mechnism of poly-β-hydroxybutyrate (PHB) and poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (PHBV) were studied in aerobic and anaerobic sludge. Microorganisms in sludge can grow using PHB (V) as sole carbon source. Biodegradation rate of PHB is faster than that of PHBV. Different conditions, such as pH and temperatures, have different influence on microbial degradation abilities of PHB(V) in sludge. Product configuration, especially specific surface area, has close correlation with the rate, and the larger specific surface area is, the faster biodegradation rate is.

Key words poly-β-phydroxybutyrate (PHB), poly (β-hydroxybutyrate-co-β-hydroxyvalerate) (PHBV), sludge, biodegradation.

Comparative Study on the Biodegradability of Chlorobenzenes by Chlorobenzene Acclimated Sludge. Qu Fuping, Zhang Xiaojian, He Miao, Gu Xiasheng (Dept. of Environ. Eng., Tsinghua University, Beijing 100084): *Chin. J. Environ. Sci.*, **18**(4), 1997, pp. 21—24

A study on the biodegradability of five priority pollutants, which include chlorobenzene, o-, m-, p-dichlorobenzene and 1, 2, 4-trichlorobenzene, was conducted by measuring the respiratory consumption. Seed sludge and chlorobenzene acclimated sludge were used in the test. The experimental observations indicated the respiratory of seed sludge was completely inhibited by the five organic compounds, the degree of inhibitory is linked with the degree of chlorination, the site of chlorine atom substitution and the substrate concentration. The chlorobenzene acclimated sludge not only shows the biodegradable ability for the chlorobenzene, but the cometabolic ability for the o-, and m-dichlorobenzene, while the p-dichlorobenzene and 1, 2, 4-trichlorobenzene presents the strong inhibition, this shows the characteristic of the enzymes induced by chlorobenzene, i. e. they require the substrate must have at least one "continuous three vacant site structure" in the benzene ring. The kinetic biodegradable/inhibitory constants are also presented in this paper.

Key words: chlorobenzenes, priority pollutant, aerobic biodegradability, active sludge, acclimation.

The Study on the Relationship between the Activation of Al in Soil and Decline of Fir Forest