白腐菌对制浆黑液中硫酸盐木素的降解*

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摘要 研究了白腐菌对硫酸盐木素的降解作用和影响因素. 结果发现, 2 种白腐菌对硫酸盐木素的降解能力不同, 培养 10d 后, 木云芝 Lu-11 的降解率达到 74.5%, 而黄孢原毛平革菌的降解率为 65.6%; 分子量在 1500- 3000kD 之间的硫酸盐木素部分被降解最为明显, 木云芝的降解产物只有一种主要组分, 而黄孢原毛平革菌的降解产物有 2 种主要组分; 培养条件如碳源、氮源、pH 值和温度对白腐菌降解硫酸盐木素的作用有明显的影响.

关键词 白腐菌, 硫酸盐蒸煮黑液, 硫酸盐木素, 生物降解.

白腐菌是一类担子菌(*Basidiomycetes*). 在自然界, 天然木素分子的降解主要是靠白腐菌完成, 木素的生物降解是碳循环的限速步骤[1]. 近几年来, 人们发现白腐菌对纸浆漂白废水有着明显的脱色和消除毒性的作用, 其原因是白腐菌能进一步降解漂白废水的氯化木素分子, 而氯化木素及其氧化产物是漂白废水颜色和毒性的主要来源[2]. 本文中笔者研究了白腐菌对硫酸盐木素的降解作用, 以探讨黑液污染的生物治理途径.

1 材料与方法

1.1 菌种及培养基

菌种为本室从自然界筛选分离的白腐菌木云芝(*Coriolus versicolor*)菌株 Lu-11 和美国 North Carolina State University 张厚民教授赠送的黄孢原毛平革菌(*Phanerochaete chrysosporium*)菌株 BKM/1767. 培养基制备按 Kirk 等的方法[3], 以葡萄糖作为碳源和酒石酸铵作为氮源分别加入到培养基中, 以 20mmol/L 的 2, 2-二甲基琥珀酸盐作为缓冲液(pH5.0).

1.2 硫酸盐木素制备和含量测定

按 Lindquist 的方法[4], 马尾松硫酸盐蒸煮黑液经过 70% 硫酸酸化至 pH 值 3.3, 加热到 60- 70℃, 使木素凝聚沉淀, 倾去上层清液得泥浆状粗木素, 离心分离除去大部分水并用蒸馏水洗涤沉淀数次, 粗木素沉淀于 70℃烘箱中干燥, 烘干后的木素经粉碎后, 过 60 目筛. 将此粗硫酸盐木素溶于 250ml 吡啶∶醋酸∶水为 9∶1∶1 的混合浴液中, 待木素完全溶解后加入 250ml 三氯甲烷萃取, 用力摇动使之充分混合后, 静置分层分离出有机溶剂层; 水层再用 250ml 三氯甲烷萃取, 合并 2 次分离的有机溶剂层. 所得的有机层液体于 50℃下真空蒸发, 浓缩至体积约为 100ml, 浓缩液在搅拌下慢慢滴入 2000ml 乙醚以沉淀木素, 离心分离除去乙醚层, 沉淀用 200ml 乙醚洗涤 3 次, 最后沉淀得到的木素于常温下真空干燥, 所得干燥的精制木素研细于 P_2O_5 中贮存备用. 经干燥后的硫酸盐木素配成 10% 浓度的培养基. 测定硫酸盐木素的浓度时, 将硫酸盐木素溶于 0.1mol/L 的 NaOH 水溶液中, 测定在吸收波长为 280nm 处的 OD 值, 根据标准曲线计算硫酸盐木素的含量.

1.3 硫酸盐木素分子量的测定

按 Kantelinen 等的方法[5], 经过精制的马尾松硫酸盐木素经 0.5mol/L 的 NaOH 溶解后, 过 Sephadex G-50 凝胶柱(Pharmacia 公司), 再用 0.5mol/L NaOH 溶液作洗脱液, 分子量大小用相应的蛋白质分子作为对照.

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2 结果与讨论

2.1 不同菌种对硫酸盐木素的降解作用

2 种白腐菌对硫酸盐木素的降解情况有所不同(图 1). 木云芝 Lu-11 对硫酸盐木素的降解能力更强一些, 8d 内已达到 74.5%, 而黄孢原毛平革菌 BKM/1767 在 10d 内达到的最大降解率为 65.6%; Lu-11 在培养第 4d 后, 降解硫酸盐木素的速度迅速上升, 第 8d 达到最大值; 而黄孢原毛平革菌 BKM/1767 在第 6d 后的降解速度才开始迅速增加, 到第 10d 后降解量基本不再变化.

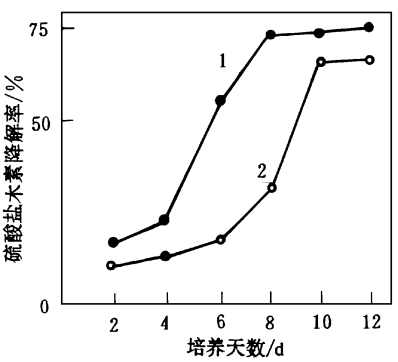

图 1 白腐菌降解硫酸盐木素的时间曲线

1. 木云芝 2. 黄孢原毛平革菌

2.2 分子量变化

黑液中的硫酸盐木素是低分子量木素, 即天然木素在化学药品和高温作用下的降解产物, 经过白腐菌作用后其分子量可进一步降解而显著降低, 图 2 是白腐菌对硫酸盐木素降解的分子量变化情况. 极限排阻层析法分析硫酸盐木素分子量在降解过程中的变化表明, 当硫酸盐木素降解时, 高分子量部分含量减少了, 特别是在 3000-1500kD 之间的减少量最为明显, 而分子量小于 1000kD 的部分却有所增加, 显然低分子量硫酸盐木素分子的增加部分是由于高分子量硫酸盐木素降解的低分子量产物的累积形成; 由于增加和减少的部分并不完全对称, 高分子量硫酸盐木素的降解产物也可能发生了部分的聚合反应. 2 种白腐菌对硫酸盐木素降解的产物组成情况有一定的差异, 硫酸盐木素经 Lu-11 培养 8d 后, 降解产物只有一种主要组分, 分子量在 500- 100kD 之间有一个峰值; 经 BKM/1767 培养 10d 后, 降解产物有 2 种主要的组分, 表现在 500- 100kD 之间有 2 个高峰值.

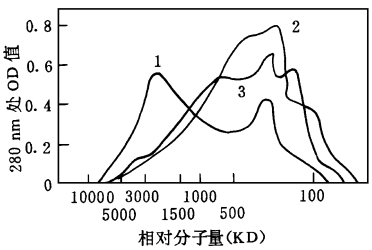

图 2 硫酸盐木素被白腐菌降解后的分子量变化

1. 对照 2. 木云芝 3. 黄孢原毛平革菌

硫酸盐木素分为水溶性和水不溶性, 后者占了 90% 以上的比重. 白腐菌对水溶性硫酸盐木素的降解作用相对小得多; 高分子量组分的降解主要是对水不溶性硫酸盐木素分子的降解. 水溶性组分在白腐菌培养过程中, 平均分子量也发生了变化, 以降解反应为主, 但也存在一定程度的缩合反应, 尽管降解量非常小.

2.3 影响微生物降解硫酸盐木素的因子

营养条件对白腐菌降解硫酸盐木素有一定的影响, 但对不同菌种的影响有所差异. 当加入 0.3% 的葡萄糖时, 硫酸盐木素的降解率最大, 加入 0.06% 的酒石酸铵时, 木云芝 Lu-11 有最大的降解硫酸盐木素的能力. 对于黄孢原毛平革菌 BKM/1767, 酒石酸铵浓度达到 0.12% 时, 硫酸盐木素的降解率最大(图 3). 白腐菌在偏酸性条件下生长最好, 在 pH5- 6 范围内, 对硫酸盐木素的降解量最多(图 4); 温度对白腐菌降解硫酸盐木素有明显的影响, 在 35℃左右降解率最高(图 5).

3 结论

(1) 在纯培养条件下, 白腐菌对于硫酸盐木素具有一定的降解能力, 不同菌种的降解能力有所差异.

(2) 白腐菌对硫酸盐木素的不同级分的降解也有差异, 降解最多的是 3000- 1500kD 之间的高分子量部分.

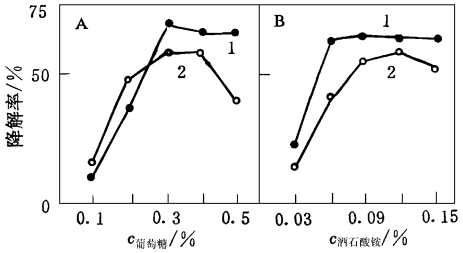

图 3　外源营养对白腐菌降解硫酸盐木素的影响

A. 葡萄糖　　B. 酒石酸铵

1. 木云芝　2. 黄孢原毛平革菌

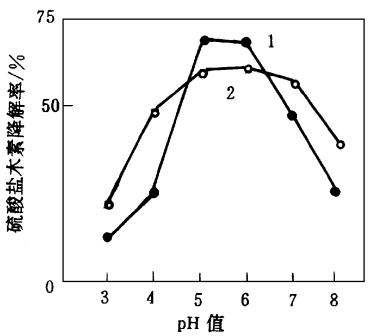

图 4　pH 值对白腐菌降解硫酸盐木素的影响

1. 木云芝　2. 黄孢原毛平革菌

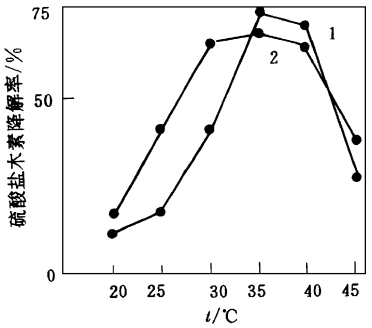

图 5　温度对白腐菌降解硫酸盐木素的影响

1. 木云芝　2. 黄孢原毛平革菌

(3) 培养条件对白腐菌降解硫酸盐木素有着明显的影响, 添加一定量的碳源与氮源、酸性 pH 培养条件和 35℃左右的温度能促进硫酸盐木素的降解作用.

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一种甲硫醇试剂储藏法

甲硫醇(Methanethiol Methylmercaptan)是无色液体或气体, 有不愉快的气味。不溶于水, 溶于乙醇和乙醚。熔点- 123.1℃, 沸点 7.6℃, 闪点- 17.8℃, 常用于喷气机汽油添加剂、杀虫剂、催化剂及甲硫氨基酸的合成。由于低沸点、低嗅觉阈值(空气中 1.1ppb, 水中 0.0011mg/L)的特点, 导致了甲硫醇的分析在试剂储存和采样 2 方面的诸多困难, 使得甲硫醇的分析在实际工作中难以开展。甲硫醇原试剂的保存温度一般要求低于- 20℃, 否则易挥发出来成为不断的难闻的臭味污染源。CH_3SH 分子将吸附于室内物体表面, 即使将原试剂抛却, 室内浓重的气味仍将延续很长时间, 严重干扰和影响了工作的正常进行。根据笔者的经验, 甲硫醇原试剂瓶被打开后再封闭, 一般的措施是很难阻止 CH_3SH 分子的逸出的, 即使密封后埋于冰块中, 恶臭味仍将穿过厚厚的冰层源源不断地渗透出来。

一种简便的甲硫醇储藏办法为: 把配制完标准使用液后的甲硫醇原试剂瓶尽量封闭紧密, 用锡箔纸包住瓶口和瓶颈, 埋于装满细颗粒活性炭的容器中, 放于普通冰箱的冷柜中储藏。配制的甲硫醇标准使用液标定后于冰箱中放置备用, 超过一个月使用需要重新标定。

采用上述储藏法, 可以有效地免除 CH_3SH 逸出时的臭味, 便于普通实验室的运行操作。

同济大学环境工程学院汪立忠和陆雍森供稿

dexes controlled in producing.

Key words: dye intermediates, J-acid, wasteliquor, extraction, resource recovery.

Study On Full-scale Test of Biological Contact Oxidation Pretreatment in Drinking Water Treatment from Huaihe River Source Water (Bengbu Reach). Liu Wenjiun, He Beiping et al. (Dept. of Environ. Eng., Tsinghua University, Beijing 100084), Lu jianhong et al. (Bengbu Water Company, Bengbu, 233000): *Chin. J. Environ. Sci.*, **18**(1), 1997, pp. 20- 22

In this study, the test of full-scale biological contact oxidation pretreatment in drinking water treatment was discussed, which is first in domestic. The results demonstrated that biological pretreat process can remove organic compounds and ammonia of source water by 13. 6%- 20. 5% and 70%- 90% respectively when the ratio of water to air is 1 ∶ 1; the key factor to affect the biological pretreat process performance is dissolved oxygen and temperature.

Key words: drinking water, biological pretreatment, pilot scale test, Huaihe River.

Degradation of Black Liquor Lignin Produced from Kraft Pulping Process of Pine by White-Rot Fungi. Lin Lu, Yang Gao et al. (State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, 510641): *Chin. J. Environ. Sci.*, **18**(1), 1997, pp. 23- 25

Black liquor lignin is the main pollutant in the black liquor produced from kraft pulping process of paper-making raw materials. In this paper, effect of white-rot fungi on degradation of black liquor lignin produced from pine kraft cook was studied. Results showed that white-rot fungus could degrade more than 74. 5% of black liquor lignin in the medium after 10 days of culture, the main part of black liquor lignin degraded was in the range of 1500- 3000kD of molecular weight. Culture factors such as cabon and nitrogen source, pH value in the medium and temperature exerted during the culture had an important role respectively on the effect of degrading black liquor lignin by white-rot fungus.

Key words: white-rot fungus, black liquor from kraft pulping process, sulfonate lignin, biodegradation.

Pilot Scale Petrochemical Wastewater Treatment Using Inner Loop Fluidized Bed Bioreactor. Zou Ping, Wang Chengwen and Qie Yi (Dept. of Environ. Eng., Tsinghua University, Beijing 100084): *Chin. J. Environ. Sci.*, **18**(1), 1997, pp. 26- 29

A Pilot scale experiment on petrochemical wastewater treatment using inner loop fluidised bed bioreactor and floatation process was conducted. The effluent COD from the process is about 200 and 100 mg/L when influent COD is 800 and 500mg/L, respectively. The loading rate of the bioreactor can be achieved above 15kgCOD/(m^3.d).

Key words: inner loop fluidised bed bioreactor, petrochemical wastewater, floatation process.

Investigation of the Landfill Gas Composition and Its Yield in South China. L. Y. Chan and S. C. Lee (Dept. of Civil and Structural Engineering, The Hong Kong Polytechnic University, Hong Kong), Y. Qin (Institute of Environmental Science, Zhongshan University, Guangzhou, 510275): *Chin. J. Environ. Sci.*, **18**(1), 1997, pp. 30- 34

Five landfill gas monitoring wells were installed and the composition of landfill gases were monitored in Wufengshan landfill in Foshan, south China. For the wells located in the late landfilled region, CH_4 and CO_2concentrations of landfill gases are high and stable. For the wells located in the early landfilled region, CH_4 and CO_2 concentrations of landfill gases are low and variable. In the last field measurement, the gases in the well located in early landfilled region has lost the characters or landfill gas. It's implication is that the biological decomposition process of the refuse underground has completed or the anaerobic environment has been destroyed. It just lasted for about 4 years and is much shorter than the expected time of 10- 20 years. The differences of landfill gas between Foshan Wufengshan landfill and Hong Kong Shuen Wan landfill were compared and discussed. The yield of landfill gas in Wufengshan landfill was estimated according to the original carbon component of the refuse.

Key words: landfill, waste gas, biological decomposition, monitoring well, CH_4, CO_2, gas yield, Foshan.

Photolysis of α-Naphthaleneacetic Acid in Aqueous Solution. Zufei Zhou, Weichuan Jiang and Weiping Liu (Dept. of Chemistry, Zhejiang University, Hangzhou 310027): *Chin. J. Environ. Sci.*, **18**(1), 1997, pp. 35- 37

Photolysis of α-naphthaleneacetic acid(NAA) has been investigated at 25℃ in aqueous solutions by irradiation at different wavelengths. The shorter wavelength of 254nm is considerably more effective in promoting degradation than wavelength of 365nm. The primary degradation of NAA follows a pseudo-first-order kinetics. The photolysis half-life and rate constant were determined to be 60min and $1.15\times10^{-2}min^{-1}$ respectively. The optimum photolysis rate has been observed using TiO_2 powder as photocatalyst. Several reaction intermediates were identified using GC/MS technique. The photolysis of NAA involves decarboxylation and oxidation on aromatic ring. On the basis of the analytical data, a mechanism of the process has been proposed.

Key words: photolysis, α-naphthaleneacetic acid, ultraviolet light.

Mn^{2+}-Oxidizing Bacteria and the Mn^{2+}-Removing Activity of the Filter Sand Used in Water Plants. Bao Zhirong et al (Dept. Molecular Biology, Jilin Univ,