

共基质对10株细菌降解芘的作用*

苏丹^{1,2} 李培军^{1,*} 王鑫³

(¹ 中国科学院沈阳应用生态研究所, 沈阳 110016; ² 中国科学院研究生院, 北京 100039; ³ 沈阳大学沈阳环境工程重点实验室, 沈阳 110044)

摘要 从石油污染的污泥中分离出10株细菌(SB01—SB10),研究了有(或无)共基质(葡萄糖Glu,或菲PHE)对细菌降解芘(PYR)的影响。结果表明:当以PYR为唯一碳源和能源时(MS_1),SB01的PYR降解率最高,5d可降解30.4%;以Glu为共代谢基质时(MS_2),SB09的PYR降解率最高,可达37.7%;以PHE为共代谢基质时(MS_3),SB10的PYR降解率为50.2%。Glu抑制SB01、SB03对PYR的降解,对SB01抑制作用最明显,使SB01的PYR降解率降低7.9%;Glu对SB02、SB07、SB08、SB10降解率无明显促进或抑制作用。PHE对细菌降解PYR均有促进作用,对SB10的促进作用最明显,使其降解率提高29.8%。Glu与PHE对SB04和SB09降解PYR的促进作用无显著差异,而对其它各菌株而言,PHE对PYR降解的促进作用大于Glu。

关键词 菌株 生物降解 芘 葡萄糖 菲 共基质

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Effects of co-substrates on biodegradation of pyrene by ten bacterial strains. SU Dan^{1,2}, LI Pei-jun¹, WANG Xin³ (¹Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China; ²Graduate University of Chinese Academy of Sciences, Beijing 100039, China; ³Shenyang Key Laboratory of Environmental Engineering, Shenyang University, Shenyang 110044, China). -Chin. J. Appl. Ecol., 2007, 18(3): 636–640.

Abstract: A total of 10 bacterial strains represented as from SB01 to SB10 were isolated from a petroleum-contaminated sludge, and their potential of degrading pyrene (PYR) was investigated on the substrates pyrene (MS_1), pyrene plus glucose (MS_2), and pyrene plus phenanthrene (MS_3). The results showed that on MS_1 , the degradation rate of PYR by SB01 was the highest, with 30.4% of PYR degraded after 5 days. On MS_2 , the degradation rate of PYR by SB09 was the highest, being 37.7% after 5 days, while on MS_3 , 50.2% of PYR was removed by SB01. The degradation of PYR by SB01 and SB03 was inhibited by glucose, which was more obvious for SB01, but no significant difference was observed among SB02, SB07, SB08 and SB10. The biodegradation rate of PYR by all the ten bacterial strains was enhanced on MS_3 , and that by SB10 was increased by 29.8%. For SB04 and SB09, the biodegradation rate of PYR had no significant difference between MS_1 and MS_2 , but for other strains, the stimulation effect of phenanthrene on PYR degradation was higher than that of glucose.

Key words: bacterial strain; biodegradation; pyrene; glucose; phenanthrene; co-substrate.

1 引言

采用微生物固定化技术修复环境中多环芳烃污染时,高效降解菌的筛选是关键。国内外有关多环芳

烃降解菌筛选方面的研究很多^[12,18,30],但这些研究多是在无机盐培养基中以目标底物为唯一碳源和能源进行的^[11,13],而多环芳烃(polycyclic aromatic hydrocarbons, PAHs)是难降解的有机污染物,微生物很难直接降解^[4],这就给筛选高效降解高分子量PAHs菌株带来了挑战。

微生物降解高分子量PAHs的主要途径是共代谢^[8,22],相对毒性较低、价格便宜、容易获得、能维持

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** 通讯作者。E-mail: lipejun@iae.ac.cn

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PAHs 降解菌生长的物质,可用作 PAHs 的共代谢底物;和目标底物相似,是底物的代谢中间产物,能明显提高降解率的物质,也可作为 PAHs 的共代谢底物^[14]。文献中经常提到的共代谢底物有水杨酸^[15]、邻苯二甲酸^[21]和联苯等^[23,25]。

本文研究了10株细菌对4环PAHs的降解,探讨了有(或无)共代谢基质(葡萄糖Glu,或菲PHE)时细菌对芘(PYR)的降解能力,并初步探讨了细菌降解PAHs的代谢方式及生态因子的调控机理,最后筛选出2株高效降解菌株。本研究为固定化菌种选择以及微生物修复有机污染介质时生态因子调控方式提供科学依据。

2 材料与方法

2.1 供试材料

菲(phenanthren, PHE)、芘(pyrene, PYR)的纯度>97%,均为德国Fluka公司产品。供试微生物从石油污染土壤中分离、驯化、选育得到(10株细菌)。经权威鉴定^[1,24],SB01、SB02、SB03、SB04、SB08为芽孢杆菌(*Bacillus* sp.),SB07为微杆菌(*Microbacterium* sp.),SB06为球菌(*Micrococcus* sp.),SB05、SB10为黄杆菌(*Flavobacterium* sp.),SB09为动胶杆菌(*Zoogloea* sp.)。

2.2 降解试验

降解试验用3种降解培养基为:1)无机盐培养基(MS₁),每升水中含MgSO₄·7H₂O 0.2 g,CaCl₂ 0.02 g,KH₂PO₄ 1.0 g,K₂HPO₄ 1.0 g,NH₄NO₃ 1.0 g,FeCl₃ 0.05 g,pH=7.0~7.2;2)MS₁+0.1%Glu培养基(MS₂);3)MS₁+100 mg·L⁻¹PHE培养基(MS₃)。在150 ml三角瓶中加入30 ml降解培养基,1.01325×10⁵ Pa,灭菌30 min。冷却后加入PYR 50 mg·L⁻¹。在无菌操作条件下,将1 ml菌液接种于三角瓶中,悬浮细菌细胞数为1.0×10⁸ ml⁻¹,25℃,摇床震荡培养5 d。以不接菌为对照,每个处理3次重复。

2.3 水样中PAHs的测定

水样中PAHs的测定见参考文献^[20]。将三角瓶中水样称量后全部放入250 ml的分液漏斗中,向漏斗加入10 ml二氯甲烷,震荡萃取5 min,并不断放气,静止分层3 min,将下层的有机相移入容量瓶中,再用5 ml的二氯甲烷以相同的操作步骤重复萃取2次,3次萃取液合并,定容,然后取0.1 ml定容液转移至装有1.0 g硅胶的预处理柱中,再用正己烷/二氯甲烷(1:1)混合液洗脱。弃去第1组份1 ml洗脱

液,收集第2组份2 ml洗脱液于KD瓶中,再以氮气吹脱,甲醇定容后移入色谱进样瓶,用高效液相色谱(HPLC)测定PAHs。色谱条件:惠普1090-II高效液相色谱仪,配有二级管阵列检测器(diode array detector,DAD),色谱柱为C18烷基硅胶柱,柱温(39.9±1)℃;流动相:甲醇,流速0.8 μl·min⁻¹,进样量10 μl。检测波长:PYR 240 nm。

3 结果与分析

3.1 在MS₁中细菌对PYR的降解

在不同共代谢基质中菌株的PYR降解率见图1。由图1可见,当PYR初始浓度为50 mg·L⁻¹且以PYR为唯一碳源和能源时(MS₁),10株细菌对PYR均有一定的降解能力。ANOVA分析表明,不同菌株间降解率有显著差异($P < 0.05$)。多重比较(Tukey multiple comparison)表明,细菌对PYR的降解能力可分为5个等级。SB01对PYR的降解能力最强,5 d降解率为30.4%;SB02和SB03差异不显著($P > 0.05$),5 d降解率为23.6%~25.8%;SB07、SB08、SB09和SB10间差异不显著($P > 0.05$),5 d降解率均为20.0%左右;SB04与SB05差异不显著($P > 0.05$);SB06降解率最低,5 d降解率为11.1%(表1)。Kruskal-Wallis检验也证实了上述结论。由此可见,在有无共代谢基质时,细菌对PYR的降解效果都不明显。PYR含有4个苯环,属高分子量PAHs,在环境中占有一定比例,有明显的毒性,在环境中很难降解^[28~29]。

3.2 在MS₂中细菌对PYR的降解

由图1可见,在MS₂中不同菌株的PYR降解率间存在显著差异($P < 0.05$)。统计分析表明,Glu对PYR的去除有促进或抑制作用,而对某些细菌去除PYR没有影响;Glu对SB01和SB03降解PYR均有

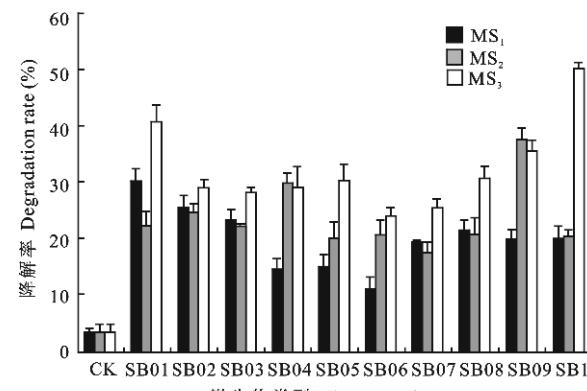


图1 在不同基质中菌株对PYR的降解

Fig. 1 Biodegradation of PYR by bacteria strains on different substrates.

表1 在MS₁中细菌PYR降解率的Tukey分析Tab. 1 Tukey multiple comparisons performed on the degradation rate of PYR in MS₁

细菌 Bacteria	SB01	SB02	SB03	SB04	SB05	SB06	SB07	SB08	SB09	SB10
SB01	-	4.60	6.80	15.70	15.10	19.33	11.13	8.70	10.13	10.00
	*	*	*	*	*	*	*	*	*	*
SB02	-	2.20	11.10	10.47	14.73	6.53	4.10	5.53	5.40	
	0.065	*	*	*	*	*	*	*	*	
SB03	-	8.90	8.30	12.53	4.33	1.90	3.30	3.20		
	*	*	*	*	*	0.096	*	*		
SB04	-	-0.60	3.60	-4.57	-7.00	-5.63	-5.76			
	0.058	*	*	*	*	*	*			
SB05	-	4.27	-3.97	-6.40	-5.00	-5.17				
	*	*	*	*	*	*				
SB06	-	-8.17	-10.60	-9.20	-9.41					
	*	*	*	*	*					
SB07	-	-2.43	-1.03	-1.13						
	0.065	0.759	0.661							
SB08	-	1.40	-1.30							
	0.695	-1.249								
SB09	-	-0.11								
	*									
SB10	-									

* $P < 0.05$. 下同 The same below.

抑制作用,但对SB01降解PYR的抑制作用更明显,使其PYR降解率降低7.9%;在MS₁和MS₂中,SB02、SB07、SB08和SB10间PYR降解率差异不显著($P > 0.05$),在降解PYR时它们对环境营养状况没有依赖作用;Glu促进SB09对PYR的降解,使SB09在富营养条件下发挥高效的降解作用,5 d降解率可达37.7%,较在MS₁中提高17.4%;Glu对SB04、SB05和SB06降解PYR也有促进作用。由此可见,添加营养物质或添加一些共代谢底物等调控措施,可提高某些细菌的PAHs去除率,但这种促进作用不具有普遍性。因此,在实际应用中应根据所选微生物的种类选择不同的调控方式,这与一些学者的研究是一致的。张杰等^[29]研究表明,外加的Glu浓度小于100 mg·L⁻¹时,可提高PYR的降解率;当Glu超过一定量时,PYR的降解率反而下降。郭楚玲等^[9]认为,外加酵母浸出液和Glu,可促进微生物生长,提高PYR的降解率。聂麦茜等^[17]研究表明,Glu对两株不同的黄杆菌(*Flavobacterium* sp. FCN1, FCN2)降解PHE有抑制作用。

Glu促进PYR降解的机理可能是:Glu改变了细菌的碳源和能源的底物结构,从而增大了细菌对碳源和能源的选择范围,使难降解的PYR最终被细菌降解^[10]。另外,Glu在氧化过程中产生NADH,为降解酶提供辅助因子^[7]。Wang等^[26]研究表明,Glu可代替苯酚作为降解4-氯苯酚(4-CP)的共代谢底

物,可为微生物的生长提供碳源和能源;Glu由4-CP完成对共代谢酶的诱导。

综上所述,高效降解菌的选择要因地制宜。在富营养条件下,一些微生物的降解能力可能受到限制,使降解效果不佳,而一些微生物的降解能力被促进。例如,SB09在富营养条件下对PYR的降解效果更好,为高效降解菌株。

3.3 在MS₃中细菌对PYR的降解

在MS₃中不同菌株PYR降解率间存在显著差异($P < 0.05$) (图1)。由表2可见,PHE对细菌降解PYR均有促进作用,使SB10的PYR降解率达50.2%,较无PHE时提高29.8%;SB02、SB07、SB08和SB10的PYR降解率在MS₁和MS₂间差异不显著($P > 0.05$),即Glu对它们的降解率提高没有贡献;SB04、SB09的PYR降解率在MS₂和MS₃间差异不显著($P > 0.05$);SB04、SB09的PYR降解率在MS₁和MS₂间有显著差异($P < 0.05$);Glu和PHE都能促进SB04、SB09对PYR的降解,即Glu使SB04和SB09的PYR降解率分别提高15.2%和17.4%,PHE使SB04和SB09的PYR降解率分别提高14.6%和15.5%。

有的共代谢底物起微生物生长基质的作用,有的共代谢底物虽然不能作为微生物生长的基质,但能使微生物产生可降解PAHs的酶类。PHE是3个苯环的PAHs。向底物中添加低分子量的PAHs,可诱

表2 有无共代谢基质(Glu, PHE)时细菌对 PYR 的降解率差值

Tab. 2 Differences in degradation rate for ten bacteria strains between MS₁ and MS₂ or MS₃

处理 Treatment	降解率差值 Differences in degradation rate (%)									
	SB01	SB02	SB03	SB04	SB05	SB06	SB07	SB08	SB09	SB10
MS ₂ - MS ₁	-7.9*	-0.7	-1.2*	15.2*	4.9*	10.0*	-1.7	-0.7	17.4*	0.2
MS ₃ - MS ₁	10.4*	3.3*	4.7*	14.6*	15.0*	13.0*	6.4*	9.0*	15.5*	29.8*
MS ₃ - MS ₂	18.3*	4.0*	5.9*	-0.6	10.1*	3.1*	8.1*	9.7*	-1.9	29.6*

导产生降解高分子量 PAHs 的酶类。Chen 等^[3]也发现, PHE 在细菌 *Pseudomonas saccharophila* P15 降解 BaP 中起重要作用。巩宗强等^[5-6]研究表明:用 PHE 对土壤进行预处理, 消除 BaP 的降解滞后期, 可提高 BaP 的降解率; PYR 与低分子量 PAHs 之间有共代谢关系; PHE 可促进 PYR 的降解, 这与本文研究结论一致。环境中 PAHs 不是单一存在的, 因此 SB10 适用于降解环境中的总 PAHs(包括低分子量和高分子量)。

PAHs 的种类复杂, 高分子量 PAHs 占有一定比例^[18], 且有较明显的毒性特征^[2,16,27]。因此, 采用微生物修复 PAHs 污染时, 应着重于对高分子量 PAHs 的控制, 通过添加营养物质或一些共代谢底物等调控措施, 来提高微生物的修复作用。

采用微生物法修复环境中多环芳烃污染时, 除筛选高效降解菌之外, 还要掌握降解菌的降解特性及降解条件; 通过对生态因子进行合理调控来启动降解菌对高分子量 PAHs 的降解能力, 或者通过诱导环境本身所具有的自净功能来提高微生物的修复能力, 减少生态修复成本。

4 小 结

1) 当 PYR 初始浓度为 50 mg · L⁻¹、以 PYR 为唯一碳源和能源时, SB01 的 PYR 降解率最高, 5 d 可降解 30.4%; 以 Glu 为共代谢基质时, SB09 的 PYR 降解率最高, 5 d 可达 37.7%; 以 PHE 为共代谢基质时, SB10 的 PYR 降解率为 50.2%。

2) Glu 抑制 SB01 和 SB03 对 PYR 的降解; Glu 对 SB02、SB07、SB08 和 SB10 对 PYR 的降解无明显促进或抑制作用; Glu 促进 SB09 对 PYR 的降解, 比在 MS₁ 中高 17.4%; Glu 对 SB04、SB05 和 SB06 的 PYR 降解也有促进作用。

3) PHE 对 PYR 降解具有促进作用, 对 SB10 降解 PYR 的促进作用最强, 使其降解率提高 29.8%。

4) 在 MS₂ 和 MS₃ 中, SB04 的 PYR 降解率差异不显著($P > 0.05$), SB09 的 PYR 降解率差异也不显著($P > 0.05$); 在 MS₂ 和 MS₃ 中, SB04 的 PYR 降解率差异不显著($P > 0.05$), SB09 的 PYR 降解率差异

也不显著($P > 0.05$); Glu 和 PHE 都能提高 SB04 和 SB09 的 PYR 降解率。对其他各菌株而言, PHE 对细菌降解 PYR 的促进作用大于 Glu。

参考文献

- [1] Buchanan RE, Gibbons NE. 1974. Trans. The Translation and Editing Group of Bergey's Manual of Determinative Bacteriology of Microbiological Institute of Chinese Academy of Science (中国科学院微生物研究所伯杰细菌鉴定手册编译组). 1984. Bergey's Manual of Determinative Bacteriology. 8th. Ed. Beijing: Science Press. (in Chinese)
- [2] Calder JA, Lader JH. 1976. Effect of dissolved aromatic hydrocarbons on the growth of marine bacteria in batch culture. *Applied and Environmental Microbiology*, 32(1): 95 - 101
- [3] Chen SH, Aitken MD. 1999. Salicylate stimulates the degradation of high molecular weight polycyclic aromatic hydrocarbons by *Pseudomonas saccharophila* P15. *Environmental Science and Technology*, 33(3): 435 - 439
- [4] Gao X-S (高学晟), Jiang X (姜霞), Ou Z-Q (区自清). 2002. Behaviors of polycyclic aromatic hydrocarbons (PAHs) in the soil. *Chinese Journal of Applied Ecology* (应用生态学报), 13(4): 501 - 504 (in Chinese)
- [5] Gong Z-Q (巩宗强), Li P-J (李培军), Wang X (王新), et al. 2001. Co-metabolic degradation of pyrene in soil. *Chinese Journal of Applied Ecology* (应用生态学报), 12(3): 447 - 450 (in Chinese)
- [6] Gong Z-Q (巩宗强), Li P-J (李培军), Wang X (王新), et al. 2002. Effects of aromatics on the degradation of benzo(a)pyrene in slurry reactors. *Environmental Science* (环境科学), 23(6): 69 - 73 (in Chinese)
- [7] Gottschalk G. 1979. Bacterial Metabolism. New York: Springer-Verlag.
- [8] Günther T, Dornberger U, Fritzsche W. 1996. Effects of ryegrass on biodegradation of hydrocarbons in soil. *Chemosphere*, 33: 203 - 215
- [9] Guo C-L (郭楚玲), Maskaoui K (哈里德), Zheng T-L (郑天凌), et al. 2001. Degradation on polycyclic aromatic hydrocarbon (PAHs) by mixed microorganism isolated from coastal sediments. *Journal of Oceanography in Taiwan Strait* (台湾海峡), 20(1): 43 - 47 (in Chinese)
- [10] Kanaly RA, Bartha R. 1999. Cometary mineralization of benz[a]pyrene caused by hydrocarbon additions to soil. *Environmental Toxicology and Chemistry*, 18

- (10): 2186 – 2190
- [11] Kelly I, Cerniglia CE. 1995. Degradation of a mixture of high-molecular-weight polycyclic aromatic hydrocarbons by a *Mycobacterium* strain PYR-1. *Journal of Soil Contamination*, **4**(1): 77 – 91
- [12] Kiyohara H, Nagao K, Yana K. 1982. Rapid screen for bacteria degrading water-insoluble, solid hydrocarbons on agar plates. *Applied and Environmental Microbiology*, **43**(2): 454 – 457
- [13] Li P-J (李培军), Xu H-X (许华夏), Zhang C-G (张春桂). 2001. The degradation of B(a)P by microorganism in contaminated soil. *Techniques and Equipment for Environmental Pollution Control* (环境污染治理技术与设备), **2**(5): 37 – 40 (in Chinese)
- [14] Liu S-L (刘世亮), Luo Y-M (骆永明), Cao Z-H (曹志洪), et al. 2002. Prospects on combined remediation with microorganisms and plants for polycyclic aromatic hydrocarbons in the contaminated soils. *Soils* (土壤), **(5)**: 257 – 265 (in Chinese)
- [15] Mahaffey WR. 1988. Bacterial oxidation of chemical carcinogens: Formation of polycyclic aromatic acids from benz(a)anthracene. *Applied and Environmental Microbiology*, **54**(10): 2415 – 2423
- [16] Menzie CA, Potocki BB, Santodonato J. 1992. Exposure to carcinogenic PAHs in the environment. *Environmental Science and Technology*, **26**(7): 1278 – 1284
- [17] Nie M-Q (聂麦茜), Zhang Z-J (张杰), Zhao G-F (赵桂芳), et al. 2001. The study on effects of co-substrates on the biodegradation of polycyclic aromatic hydrocarbons by preponderant bacterium. *Research of Environmental Sciences* (环境科学研究), **14**(5): 30 – 32 (in Chinese)
- [18] Park KS, Sims RC, Dupont RR, et al. 1990. Fate of PAH compounds in two soil types: Influence of volatilization, abiotic loss and biological activity. *Environmental Toxicology and Chemistry*, **9**: 187 – 195
- [19] Saeed T, Al-bloushi A, Al-Matrouk K. 1995. Assessment of levels of polycyclic aromatic hydrocarbons in the oil from Kuwait lakes. *Achieves of Environmental Contamination and Toxicity*, **29**(1): 45 – 51
- [20] Song Y-F (宋玉芳), Ou Z-Q (区自清), Sun T-H (孙铁珩), et al. 1995. Analytical method of polycyclic aromatic hydrocarbons in soil and plants samples. *Chinese Journal of Applied Ecology* (应用生态学报), **6**(1): 92 – 96 (in Chinese)
- [21] Stringfellow WT. 1995. Induction of PAH degradation in a phenanthrene-degrading pseudomonad// Hinchee RE, Vogel CM, Brockman FJ, eds. *Microbial Processes for Bioremediation*. Columbus: Battelle Press: 83 – 89
- [22] Tao X-Q (陶雪琴), Dang Z (党志), Lu G-N (卢桂宁), et al. 2003. Biodegradation mechanism of polycyclic aromatic hydrocarbons (PAHs) in soil: A review. *Bulletin of Mineralogy, Petrology and Geochemistry* (矿物岩石地球化学通报), **22**(4): 356 – 360 (in Chinese)
- [23] Tao X-Q (陶雪琴), Lu G-N (卢桂宁), Yi X-Y (易筱筠), et al. 2006. Isolation of phenanthrene-degrading microorganisms and analysis of metabolites of phenanthrene. *Journal of Agro-Environment Science* (农业环境科学学报), **25**(1): 190 – 195 (in Chinese)
- [24] The Group of Systematic Bacteriology in the Institute of Microbiology, Chinese Academy of Sciences (中国科学院微生物研究所细菌分类组). 1978. *Method of Common Identification of Bacteria*. Beijing: Science Press. (in Chinese)
- [25] Title PC, Liu YT, Strand SE, et al. 1995. Use of alternative growth substrates to enhance PAH degradation// Hinchee RE, Anderson DB, Hoeppel RE, eds. *Bioremediation of Recalcitrant Organics*. Columbus: Battelle Press: 1 – 8
- [26] Wang SJ, Loh KC. 1999. Facilitation of cometabolic degradation of 4-chlorophenol using glucose as an added growth substrate. *Biodegradation*, **10**(4): 261 – 269
- [27] Wilson SC, Jones KC. 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): A review. *Environmental Pollution*, **81**: 229 – 249
- [28] Yu SH, Ke L, Wong YS, et al. 2005. Degradation of polycyclic aromatic hydrocarbons (PAHs) by a bacterial consortium enriched from mangrove sediments. *Environment International*, **31**(2): 149 – 154
- [29] Zhang J (张杰), Liu Y-S (刘永生), Meng L (孟玲), et al. 2003. Isolation and characteristics of PAHs-degrading strains. *Chinese Journal of Applied Ecology* (应用生态学报), **14**(10): 1783 – 1786 (in Chinese)
- [30] Zhou X-T (周贤涛), Wu J (吴娟), Lin L (林鹿). 2002. Biodegradation pathway of aromatic compounds by white rot fungus. *Techniques and Equipment for Environmental Pollution Control* (环境污染治理技术与设备), **3**(12): 1 – 8 (in Chinese)

作者简介 苏丹,女,1980年生,博士研究生。主要从事PAHs污染的微生物修复技术方面研究,已发表论文8篇。
E-mail: sudangirl@sohu.com

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