生物工程学报
journals.im.ac.cn
cjb@im.ac.cn

Chin J Biotech 2009, December 25; 25(12): 1871-1881
Chinese Journal of Biotechnology ISSN 1000-3061

综 述

微生物代谢环境难降解性有机物的酶学研究进展

吴克，潘仁瑞，蔡敬民，刘斌

合肥学院生物与环境工程系 酶学与工程重点实验室，合肥 230022

摘 要：随着人类社会的快速发展，工业化水平不断提高，产生大量的污染物并排放到环境中，给人类的生活和身体健康造成了严重的影响。这些污染物中包含种类繁多的难降解有机物，如多芳香烃(PAHs)、环硝胺类物质(RDX、HMX 和 CL-20)、多氯联苯(PCBs)及烷烃类化合物等，对自然界的污染危害大。微生物可以消除它们对污染的影响，研究结果表明微生物的代谢或共代谢活动是降解这些物质的有效途径，降解起始阶段需要一些关键酶的参与活动，以氧化还原酶为主。这些氧化还原酶一般与细胞膜上其他的活性组分在一起，形成一个氧化还原系统氧化底物。被氧化的中间物质再通过一系列酶催化继续氧化成三羧酸中间代谢产物被微生物所利用。以下综述了与这些物质降解相关的代谢途径和关键的酶，展望今后在开展这类研究工作时要加强降解微生物的筛选和相关酶学的研究，进一步研究这些污染物的代谢或共代谢途径和机理，为工程化治理环境污染提供依据。

关键词：难降解有机物，芳香烃，环硝胺类物质，多氯联苯，微生物酶

Progress in study on microbial enzymes for the metabolism of environmental refractory organic compounds

Ke Wu, Renrui Pan, Jingmin Cai, and Bin Liu

Key Laboratory of Applied Enzymology & Engineering, *Department of Biological and Environmental Engineering*, *Hefei University*, *Hefei* 230022, *China*

Abstract: With the rapid development of socialization and industrialization, more and more pollutes were produced and discharged into natural environment. It is harmful to human health and life. These pollutes included refractory degradation organic compounds like PAHs, RDX, HMX, CL-20, PCBs and alkanes and their relative substances. Various compounds exist in nature with long life span. They are the most hazardous than other organics. The impact of pollutes can be treated by microorganisms. Result showed that it is an effective way for bioremediation of these pollutes with microbial metabolism or cometabolism. A few key enzymes, mainly oxidative and reductive enzymes, connected with the first step of initial degradation. Normally, enzymes grouped with other active fraction on the cell membrane are composed of one oxidative and reductive system for substrates oxidation. The metabolic intermediates can be used with TCA by microorganisms. The pathways of metabolism and the key enzymes were summarized. The further research topics should be focused on microorganism screen and its relative enzyme, pathway and mechanism of metabolism or cometabolism for such compounds degradation, and the result was hoped for the environmental protection.

Keywords: degradation of refractory organic compound, PAHs, cyclic nitramine, PCBs, microbial enzyme,

---

Received: October 9, 2009; **Accepted:** November 12, 2009

Corresponding author: Ke Wu. Tel: +86-551-2158448; E-mail: wuke@hfuu.edu.cn

全球人口增长迅速，工业和城市化进程加快，人类自身的发展使得人类对环境和资源的开发利用强度达到空前规模，造成的环境污染已经成为制约人类社会发展的重要因素。大量的合成有机化合物在人类活动的过程中进入环境，据估计每年约一千种新型的有机化合物投入市场，其中它们大多数是难生物降解性有机物。难降解性有机物具有的基本特性为：1) 长期残留性。污染物一旦排放到环境中，难于被分解，因此可以在水体、土壤和底泥等环境介质中存留数年或更长的时间。2) 生物蓄积性。难降解性有机物一般具有低水溶性、高脂溶性的特征，能够在多数生物脂肪中发生生物蓄积。3) 半挥发性和高毒性。有的难降解性有机物具有半挥发性，可以在大气环境中远距离迁移，它们对人和动物一般具有毒性作用，有的可以导致生物体内分泌紊乱、生殖及免疫机能失调，有的甚至引起癌症等严重疾病。

进入环境的难降解性的污染物，主要包括卤代有机溶剂、卤代苯环类化合物、稠环芳烃、杂环化合物以及农药等。传统的化学方法和物理方法，已经很难达到完全清除污染物的目的。微生物对环境的净化越来越引起人们的关注，其中以共代谢方法处理这些有机物效果最好[1]。共代谢作用就是微生物在厌氧或好氧的条件下，利用生长基质如碳源和能源时，释放酶对难降解性污染物进行降解作用，在污染物降解转化的过程中，微生物得不到维持生长的碳源或能源。共培养过程中也要注意基质浓度的影响，浓度高可与目标污染物之间发生关键酶的竞争作用，抑制难降解性物质的转化。

共代谢是普遍存在于自然界的一种代谢方式，影响着很多自然化合物和大量人工合成化合物的降解过程。其核心问题就是关键酶的诱导及其活性的维持、生长基质与目标污染物之间的竞争抑制、目标污染物及其中间降解产物对微生物毒性作用等，它们是影响共代谢过程的关键性因素。在影响共代谢过程的因素中，关键酶的诱导和作用机理研究、代谢的动力学等研究[2-3]，已引起广泛关注，以下以包括共代谢处理难降解有机物种类为对象，概括介绍相关降解过程中关键酶的研究情况，以期为今后的研究创造条件。

1 多环芳香烃(PAHs)的共代谢生物修复关键酶

PAHs 是一类对自然界和人类都具有毒害作用的化合物，产生于未完全燃烧的有机物、石油、汽油产品和各类工业活动[4-7]，达数百种之多，根据所含环的数量分为低分子量和高分子量两类物质。PAHs 在自然界的稳定性，取决于其物理和化学性质，一般情况下分子量越大，稳定性就越强，毒性也越大。这类物质对人类的危害主要来自大气、水体和食物等[8]。近年来研究结果显示，PAHs 的安全和有效的去除方法是生物修复，自然界中有很多能够利用 PAHs 的微生物如细菌、真菌和藻类等都已有报道[9]。这些微生物利用好氧或厌氧的代谢或共代谢途径来降解 PAHs，尤以共代谢途径为要。

PAHs 的好氧处理途径的第一步重要反应是由 dioxygenase/monoxygenase(双加氧酶/单加氧酶)参与氧化芳香环，通过代谢作用，最终将底物降解成三羧酸循环过程中的中间关键代谢产物，进入循环氧化；在厌氧处理过程中，电子受体为硝酸盐、铁离子和硫酸盐离子等，近年来的研究结果显示这一过程伴随着脱氮和硫的还原反应[10-11]。降解途径以萘为例(图 1)[12]。

现已分离出的许多革兰氏阳性菌，可以产生相关的氧化酶来降解 PAHs，这些酶分别由细菌的染色体和质粒编码。表 1 列出部分降解 PAHs 的关键酶类[13-15]。

上述这些酶都可以通过基因表达，用在 PAHs 的降解应用研究中。此外，还发现真菌的漆酶[16]、细胞色素 P450[17]等都可以降解 PAHs。

2 环硝胺类物质(RDX、HMX 和 CL-20)的生物修复关键酶

环硝胺类物质是众所周知的军事火药，它也广泛应用在化学工业和印染工业中，在日常的使用中对环境造成了严重的污染。这类物质的污染主要发生在军事基地和军事要塞及其周边地区，以及相关的工业区域。环硝胺类物质对环境造成的污染，除了其本身以外，还有在爆炸后产生的残留物。据美

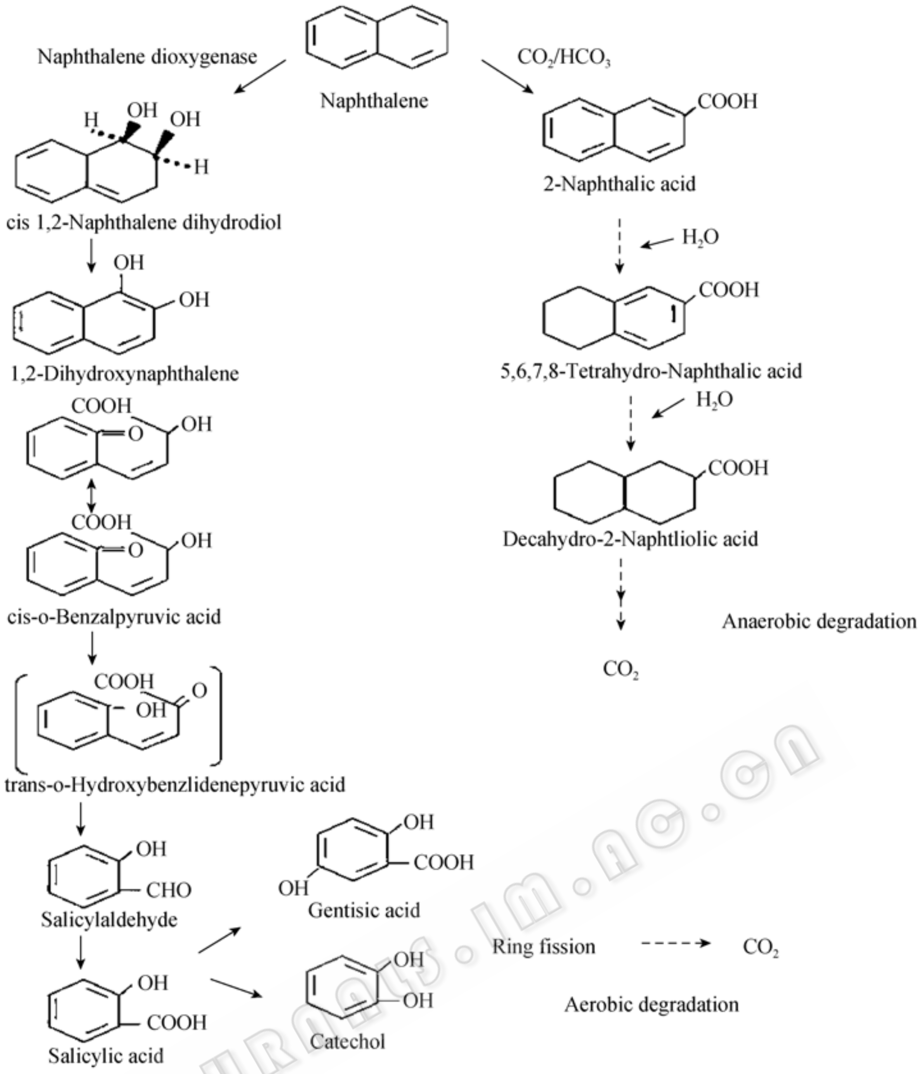

图 1 萘的好氧与厌氧降解途径[12]

Fig. 1 Pathways of naphthalene with aerobic and anaerobic bacterial degradation[12].

国和加拿大等国的调查, 上述的区域中, 这些物质在土壤中的含量较高, 已经污染了地表和地下水, 今后将严重影响周边人类的健康。环硝胺类物质对环境的污染已经得到各国政府的重视。目前已发现许多好氧细菌、厌氧细菌和真菌等都可以转化RDX、HMX和CL-20, 通过微生物的代谢来降低其对自然界的危害[18-19]。

对RDX、HMX和CL-20代谢途径的假设如图2所示[20]。在微生物代谢过程中一些关键的酶(表2)起到了重要作用[21-29]。为了进一步研究关键酶对这类物质的代谢效果, Hannik等、Rosser等[30-31]已经将细菌的PETNR(Pentaerythritol tetranitrate reductase)基因转入到烟草等植物体内, 获得成功表达, 植物在污染土地里生长良好。

3 烷烃降解过程中的关键酶类

对烷烃类物质污染的研究历史已久, 起初只是利用微生物的代谢作用处理水体中的浮油, 到了20世纪转变成油品的深加工和利用, 比如利用油品生产单细胞蛋白等。当面临处理油料污染的问题时, 人们不禁要了解微生物对烷烃类物质的氧化过程, 何种酶参与了烷烃类物质的羟基化作用等。研究结果表明许多微生物都具有较强的烷烃类物质的降解能力, 这类物质的氧化不能够在体外进行, 必须依赖一系列的酶的组成来完成羟化作用的过程, 代谢过程如图3所示[32]。

表 1 降解 PAHs 的关键酶类

Table 1 Key enzymes of PAHs degradation

Strain	Substrate	Enzyme	Reference
Pseudomonas putida OUS82	Naphthalene, phenanthrene	Ferredoxin reductase, cis-dihydrodiol dehydrogenase, dioxygenase, isomerase, hydrate-adolase, dehydrogenase	13-15
P. aeruginosa PaK1	Naphthalene	Ferredoxin reductase, cis-dihydrodiol dehydrogenase, dioxygenase, isomerase, hydrate-adolase, dehydrogenase	13-15
P. stutzeri AN10	Naphthalene, 2-metthylnaphthalene	Saliylate 1-hydroxylase	13-15
Rhodococcus sp. 124	Naphthalene, toluene, indene	Cis-dihydrodiol dehydrogenase, putative aldolase	13-15
Nocardidoes sp. KP7	Phenanthrene	Dioxygenase, ferredoxin reductase, 2-carboxybenzaldehyde dehydrogenase	13-15
Mycobacterium sp. PYR-1	Anthracene, phenanthrene, fluoranthene, pyrene	Aldehyde dehydrogenase	13-15
Pseudomonas putida strains	Naphthalene, salicylate	Reductase, *cis*-naphthalene dihydrodiol dehydrogenase, salicyaldehyde dehydrogenase, 1,2-dihydroxynaphthalene oxygenase, 2-hydroxybenzalpyruvate aldolase, 2-hydroxychromene-2-carboxylate isomerase, salicylate hydroxylase, catechol oxygenase, 2-hydroxymuconic semialdehyde dehydrogenase, 2-oxo-4-pentenoate hydratase, 4-hydroxyl-2-oxovalerate aldolase, acetaldehyde dehydrogenase, 4-oxalocrotonate decarboxylase, 2-hydroxymuconate tautomerase ferredoxin reductase, *cis*-dihydrodiol dehydrogenase, dioxygenase, isomerase, hydrate-adolase, dehydrogenase, saliylate 1-hydroxylase	13-15
P. putida NCIB9816	Naphthalene	Naphthalene-dioxygenase	13-15
P. sp. Strain C18	Naphthalene, dibenzothiophene, phenanthrene	Naphthalene-dioxygenase, *cis*-naphthalene dihydrodiol dehydrogenase, salicyaldehyde dehydrogenase, 1,2-dihydroxynaphthalene dioxygenase, isomerase, hydratase-adolase	13-15
P. sp. Strain C18	Naphthalene	Ferredoxin reductase, naphthalene cis-dihydrodiol dehydrogenase, salicyaldehyde dehydrogenase	13-15
Ralstonia sp. U2	Naphthalene	Ferredoxin reductase, *cis*-dihydrodiol dehydrogenase, aldehyde dehydrogrnase	13-15
Alcaligenes faecalis AFK2	Phenanthrene	Ferredoxin reductase, *cis*-dihydrodiol dehydrogenase, dihydroxyphenanthrene dioxygenase, isomer, hydratase-aldolase, 1-hydroxy-2-naphtholdehyde dehydrogenase, 1-hydroxy-2-naphthooate dehydrogenase, trans-2-carboxybenzaldehyde dehydrogenase, 2-carboxybenzaldehyde dehydrogenase, glutathione-s-transferase	13-15

烷烃类物质种类较多，对于不同的烃类物质，微生物产生的烷烃羟化酶的种类也不同。实验表明，根据烷烃强化水解酶的底物性质可将烃类物质分为 3 类，即 C_1-C_4、C_5-C_{16}和 C_{17}及其以上。近来的相关氧化烷烃类物质的关键酶见表 3[33-37]。

4 多氯联苯(PCBs)类物质降解过程中的关键酶类

PCBs 是工业化学品，属于卤代芳烃，已有 209 种同系物或异构体。具有特殊高温下一般不可燃、低电导率以及化学稳定性和生物难降解性，已被用作绝缘油、阻燃剂、导热剂、液压油、增塑剂等。PCBs 也被用于铁路变压器、矿井设备、无碳复写纸、颜料、电磁设备中，作为一种衬纸介质和浸没油、光学液体以及天然气管道液体。

PCBs 的化学性质极其稳定，在自然界中降解极其缓慢，联苯的氯化程度增加，持久性也随着增加，是一种长寿命的环境污染物。可通过各种途径迁移入大气、土壤、水体环境中，造成环境污染。PCBs 对生物肌体脂肪的亲和力，很容易在脂肪中溶解，具有很强的脂溶性。很容易进入食物链，影响人类和动物的生存能力，被列入世界八大公害。对这类

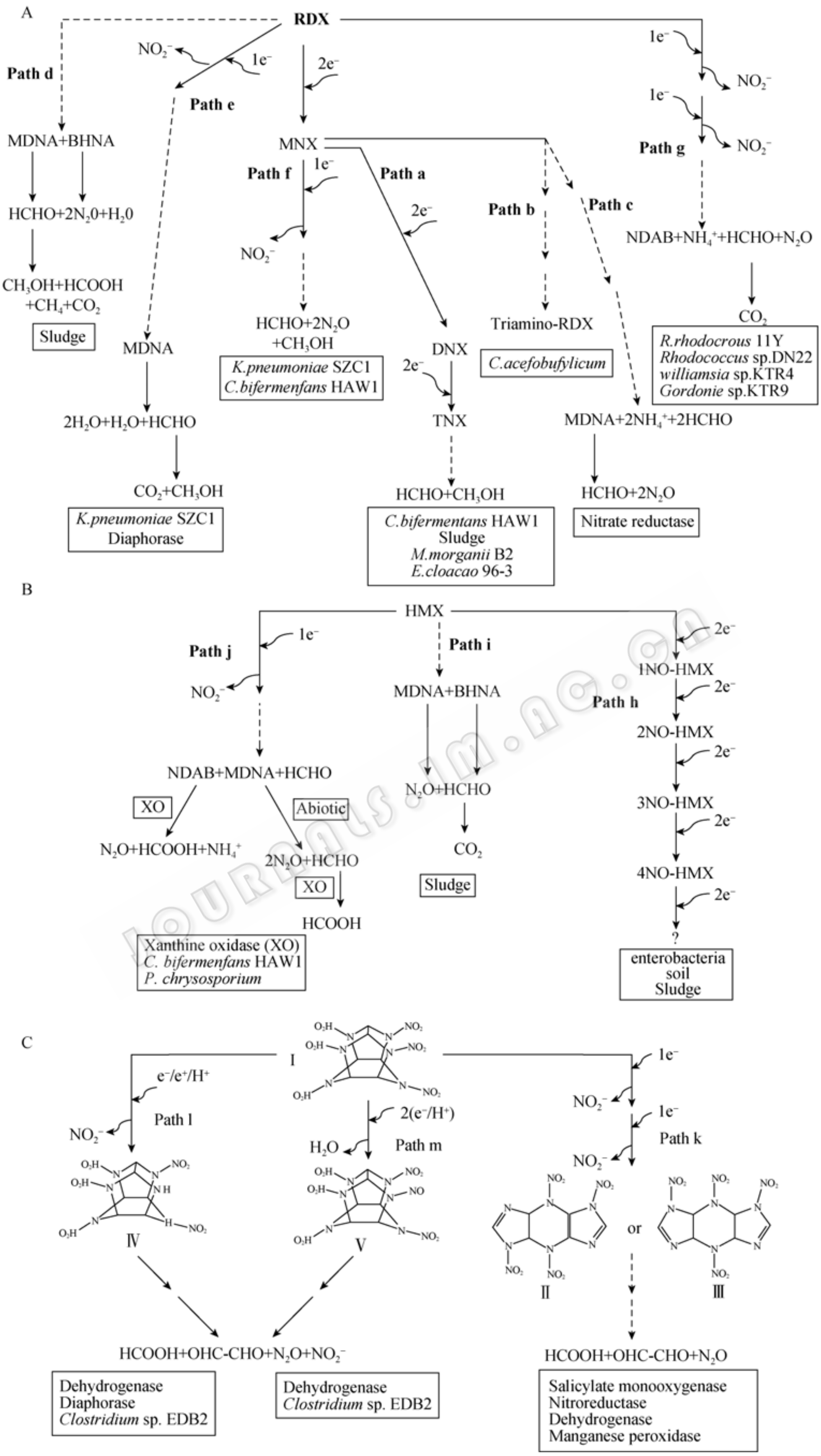

图 2　RDX(A)、HMX(B)和 CL-20(C)的代谢途径[20]

Fig. 2　Proposed biodegradation pathways of RDX(A), HMX(B), and CL-20(C)[20].

表 2 降解 RDX、HMX 和 CL-20 的关键酶

Table 2 Key enzymes of RDX, HMX and CL-20 transformation

Strains	Substrates	Enzymes	References
Aspergillus niger	RDX	Nitrate oxidoreductase	21
C. kluyveri	HMX	Xanthine oxidase	22
Pseudomonas sp. strain FA1	CL-20	Nitroreductase	23
Pseudomonas sp. strain ATCC 29352	CL-20	Monooxygenase	24
Clostridium sp. EDB2	CL-20	Dehydrogenase	25
N	RDX	Manganese	26
Rhodococcus strain DN22	RDX	Cytochrome p-450	27
Morganella morganii B2	TNT	Nitroreductase	28
Enterobacter cloacae PB2	TNT	Penterythritol tetranitrate reductase	29

N: no determination

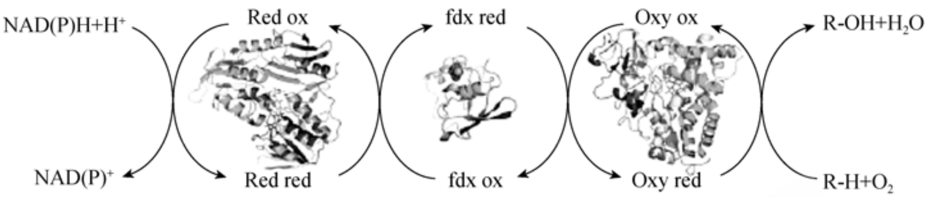

图 3 烷烃类物质羟化反应的电子传递链[32]

Fig. 3 Way of alkane hydroxylase system and its electron transfer system[32].

表 3 烷烃类物质的氧化关键酶类

Table 3 Key enzymes of the oxidation of alkanes

Strains	Substrates	Enzymes	References
Methylococcus, *Methylosinus*, *Methylocystis*, *Methylomonas*, *Methylocella*	C_1-C_8(halogenated)-alkanes, alkanes, cycloalkanes	Soluble methane monooxygenase	33
Methylococcus, *Methylosinus*, *Methylocystis*, *Methylomonas*, *Methylobacter*	C_1-C_5(halogenated)-alkanes, alkanes	Particlate methane monooxygenase	33
Acinetobacter, *Alcanivorax*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Rhodococcus*	C_5-C_{16} alkanes, fatty acids, alkylbenzenes, cycloalkanes	AlkB-related alkane hydroxylases	34
Candida maltosa, *Candida tropicalis*, *Yarrowia lipolytica*	C_{10}-C_{16} alkanes, fatty acids	Eukaryotic P450	35
Acinetobacter, *Alcaniverorax*, *Caulobacter*, *Mycobacterium*	C_5-C_{16} alkanes, cycloalkanes	Bacterial P450 oxygenase system	36
Rhodococcus, *Sphingomonas*, *Acinetobacter* sp. M1	C_{10}-C_{30} alkanes, alkylbenzenes	Dioxygenase	37

物质的处理，保护环境也引起了广泛重视。

尽管这类物质在自然界寿命长，微生物处理 PCBs 是一种有效的途径，研究表明以革兰氏阳性细菌和阴性细菌为主的微生物能够降解 PCBs，降解作用很复杂，这主要取决于 PCBs 的种类，还取决于氯的含量。有些微生物尽管含有编码的 PCBs 酶基因，但是只能降解含 3~4 个氯基团的 PCBs，但是也有些微生物表现出很强的降解能力。对这类物质降解基本途径以联苯及其类似物等[38]分解过程为例(图 4)。降解过程中还需要一些关键性的酶如 biphenyl-2,3-dioxygenase 等的参与。表 4 列出 PCBs 降解过程中的关键酶系，在染色体上以基因簇形式存在[39-63]。

5 氯乙烯类降解的关键酶

氯乙烯类化物中常见的是三氯乙烯(TCE)。在受

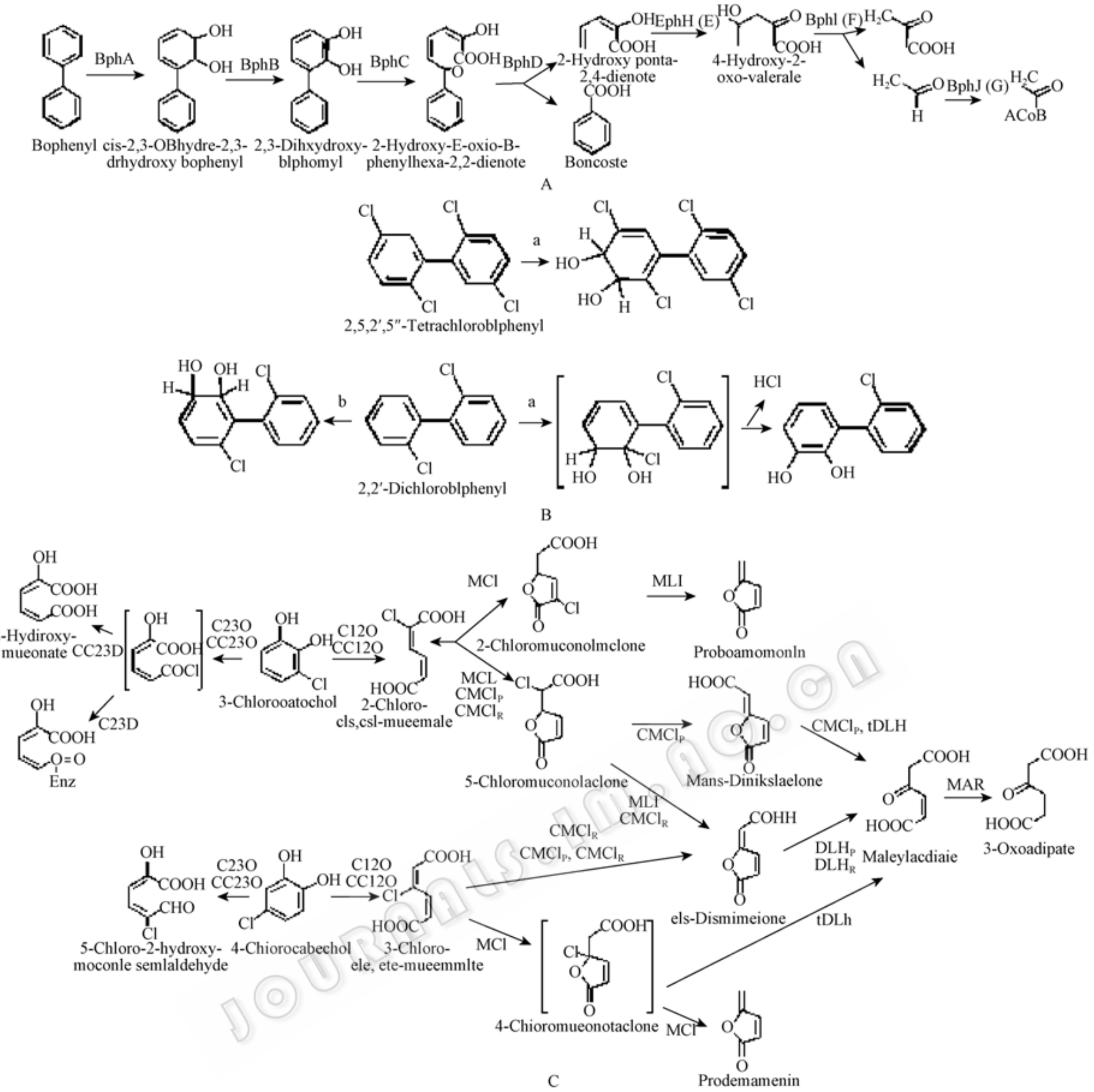

图 4　联苯(A)、多氯联苯(B)和氯儿茶酚(C)的微生物降解代谢途径[38]

Fig. 4　Selected pathways for biphenyl(A), PCB (B) and chlorocatechols (C) degradation[38].

污染的地下水和土壤中, 三氯乙烯是最主要的有害污染物之一。TCE 是致癌物质, 人类直接饮用含 TCE 的地下水或食用受污染土壤中生长出来的粮食对健康危害极大。由于它不能够作为微生物生长的碳源和能源, 单独存在时难以被好氧生物降解。高氯化乙烯可通过厌氧生物通风处理。近年来大量研究发现氯化物可通过共代谢好氧降解, 是解决这类物质污染的好途径。在生长基质和其他可转化化合物存在下, 微生物才能够对非生长基质进行转化。生长基质是电子供体, 为细胞的生长和维持提供碳源和能源, 甲烷、苯酚、甲苯和丙烷等已被证明是有效的电子供体, 另外好氧共代谢还需要加入氧作为电子受体, 从而达到这类物质的生物降解。不同生长基质上, TCE 降解微生物酶见表 5[64-73]。

6　展望

大量的污染物质如多芳香烃(PAHs)、环硝胺

类物质(RDX、HMX 和 CL-20)、多氯联苯(PCBs)及氯烯化物等被生产出来，随着人类的应用，逐渐被排放到环境中，带来了严重的环境影响。利用微生物的代谢或共代谢活动可有效降解这些物质，在降解过程需要一些关键的氧化还原酶类、以及相关联的代谢物酶类的参与活动。以上概述了国外相关的研究成果包括代谢途径和关键的酶，很多酶学的研究工作已进入分子水平，并有工程应用的范例。

对这些物质的污染现象也引起了我国学者的高度重视，先后开展了相关的研究工作，并取得了相关的研究成果。但是，目前研究的内容大多还是以微生物的细胞代谢为主，来评价污染物的降解程度。也有作者提出了氧化酶在环境保护中的应用[74]，也建立了相关三氯乙烯的代谢过程中关键酶的模型[75]，但是很少开展代谢过程中酶学的研究工作。建议今后要加强开展对这类有机物降解的微生物的筛选和相关酶学的研究，进一步研究这些污染物的代谢或共代谢途径和机理，为工程化治理环境污染提供依据。

表4　降解PCBs过程的关键酶类
Table 4　Key enzymes of degradation of PCBs

Strain	Substrate	Enzyme	Reference
Burkholderia sp. LB 400,	Bip5hynel	Oxygenase, oxidoreductase, biphenyldihydrodiol dehydrogenase, 2,3-dihydroxybiphenyl 1,2-dioxygenase, glutathione transferase, 2-hydroxypenta-2,4-dienoate hydratase, acetaldehyde dehydrogenase, 4-hydroxy-2-oxovalerate aldolase	39-40
Pseudomonas putida KF715	Biphynel	Oxygenase, oxidoreductase, biphenyldihydrodiol dehydrogenase, 2,3-dihydroxybiphenyl 1,2-dioxygenase	41-42
Rhodococcus sp. strain M5	Biphynel	Oxygenase, oxidoreductase, biphenyldihydrodiol dehydrogenase, 2,3-dihydroxybiphenyl 1,2-dioxygenase	43-44
Rhodococcus sp. strain RHA1	Biphynel	Oxygenase, oxidoreductase, biphenyldihydrodiol dehydrogenase, 2,3-dihydroxybiphenyl 1,2-dioxygenase	45-46
Achromobacter georgiopolitanum KKS102	Biphynel	Oxygenase, oxidoreductase, biphenyldihydrodiol dehydrogenase, 2,3-dihydroxybiphenyl 1,2-dioxygenase, glutathione transferase, 2-hydroxypenta-2,4-dienoate hydratase, acetaldehyde dehydrogenase, 4-hydroxy-2-oxovalerate aldolase	47-49
Sphingobium yanoikyae B1, *Novosphingobium aromaticvorans* F1999	Biphynel	Oxygenase, oxidoreductase, didrodiol dehydrogenase, glutathione transferase, 2-hydroxypenta-2,4-dienoate hydratase, acetaldehyde dehydrogenase, extrediol dioxygenase, 4-hydroxy-2-oxovalerate aldolase, HOPODA/hydroxymuconic somialdehyde hydrolase, xylene monooxygenase	50-53
Pseudomonas putida AC858, *Pseudomonas aeruginosa* JB2, *Pseudomonas* sp. P51, *P. chlororaphis* RW71, *Wautersia eutropha* NH9	Chlorrocatechol	Dienelactone hydrolase, phenol hydroxylase, chlorolcatechol 1,2 dioxygenase, maleylacetate reductase, chloromuconate cycloisomerase	54-58
Burkholderia sp. NK8	Chlorrocatechol	Dienelactone hydrolase, chlorolcatechol 1,2 dioxygenase, maleylacetate reductase, chloromuconate cycloisomerase	59
Wautersia eutropha JMP134	Chlorrocatechol	Dienelactone hydrolase, phenol hydroxylase, chlorolcatechol 1,2 dioxygenase, maleylacetate reductase, 2,4-D α–ketoglutarate dioxygenase, chloromuconate cycloisomerase	60
Delftia acidovorans P4a	Chlorrocatechol	Dienelactone hydrolase, phenol hydroxylase, chlorolcatechol 1,2 dioxygenase, maleylacetate reductase, 2,4-D α–ketoglutarate dioxygenase, chloromuconate cycloisomerase	61-62
Rhodococcus opacus 1CP	Chlorrocatechol	Dienelactone hydrolase, chlorolcatechol 1,2 dioxygenase, chloromuconate cycloisomerase, chloromuconolactone *de*halogenase	63

表 5 不同生长基质中降解 **TCE** 过程的关键酶类

Table 5 Key enzymes of degradation of TCE with different growth substrates

Strain	Growth substrate	Enzyme	Reference
Rhodococcus corallinus B-276	Propylene	Alkane monooxygenase	64
Xanthobacter Py2	Propylene	Alkane monooxygenase	65
Nitrosomonas europaca	Ammonia	Ammonia monooxygenase	66
Ralstonia eutropha JMP 134	Phenol + 2,4-dichlorophenoxyacetate	Phenol hydroxylase	67
Mycobacterium vaccae JOB5	Propane	Propene monooxygenase	68
Pseudomonas butanavora	Butane	Butane monooxygenase	73
Methylosinus trichosporium OB3b	Methane	Particulate methane monooxygenase, soluble methane monooxygenase	70
Methylomonas methanica 68-1	Methane	Soluble methane monooxygenase	71
Pseudomonas putida F1	Toluene	Toluene dioxygenase	68
Burkholderia cepacia G4	Toluene	Toluene 2-monooxygenase	72
Ralstonia pickettii PKO1	Toluene	Toluene 3-monooxygenase	69
Pseudomonas mendocina KR1	Toluene	Toluene 4-monooxygenase	69

REFERENCES

[1] Janssen DB, de Koning W. Development and application of bacterial culture fort he removal of chlorinated aliphatics. *Water Sci Technol*, 1995, **31**(1): 237–247.

[2] Alvarez-Cohen L, Speitel GE Jr. Kinetics of aerobic cometabolism of chlorinated solvens. *Bidegradation*, 2001, **12**: 105–126.

[3] Sun WJ, Liu YD. Models of the kinetics of biological co-metabolism. *J Zhengzhou Univ* (*Eng Sci*), 2003, **24**(2): 108–112.
孙文杰, 刘勇弟. 生物共代谢动力学模型. 郑州大学学报(工学版), 2003, **24**(2): 108–112.

[4] Blumer M. Polycyclic aromatic compounds in nature. *Sci American*, 1976, **23**(4): 35–45.

[5] Saraswathy A, Hallberg R. Degradation of pyrene by indigenous fungi from a former gasworks site. *FEMS Microbiol Lett*, 2002, **210**: 227–232.

[6] Brandt HCA, Watson WP. Monitoring human occupational and environmental exposures to polycyclic aromatic compounds. *Ann Occup Hyg*, 2003, **47**: 349–378.

[7] Hammond EC, Selikof IJ, Lawther PL, *et al*. Inhalation of benz[a]pyrene and cancer in man. *Ann NY Acad Sci*, 1976, **271**: 116–124.

[8] Mastrangela G. Polycyclic aromatic hydrocarbons and cancer in man. *Environ Health Perspect*, 1997, **104**: 1166–1170.

[9] Pritchard PH. Strategy using bioreactors and specially selected micro-organisms for bioremediation of groundwater contaminated with creosote and pentachlorophenol. *Environ Sci Technol*, 1993, **27**: 691–698.

[10] Ambrosoli R, Petruzzelli L, Minati JL, *et al*. Anaerobic PAH degradation in soil by a mixed bacterial consortium under denitrifying conditions. *Chemosphere*, 2005, **60**: 1231–1236.

[11] Zhang X, Sullivan ER, Young LY. Evidence for aromatic ring reduction in the biodegradation pathway of carboxylated naphthalene by a sulphate-reducing consortium. *Biodegradation*, 2000, **11**: 117–124.

[12] Bamforth SM, Singleton I. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *J Chemical Technol Biotechnol*, 2005, **80**: 723–736.

[13] Habe H, Omori T. Genetics of polycyclic aromatic hydrocarbon degradation by diverse aerobic bcaeria. *Biosci Biotechnol Biochem*, 2003, **67**: 225–243.

[14] Van Hamme JD, Singh A, Ward OP. Recent advances in petroleum microbiology. *Microbiol Mol Biol Rev*, 2003, **67**: 503–549.

[15] Chauhan A, Fazlurrahman, Oakeshott JG, *et al*. Bacterial metabolism of polycyclic aromatic hydrocarbons: strategies for bioremediation. *India J Microbiol*, 2008, **48**: 95–113.

[16] Steffen KT, Schubert S, Tuomela M, *et al*. Enhancement of bioconversion of high-molecular mass polycyclic aromatic hydrocarbons in contaminated non-sterile soil by litter-decomposing fungi. *Biodegradation*, 2007, **18**: 359–369.

[17] Hammel KE, Kalyanaraman B, Kirk TK. Oxidation of polycyclic aromatic-hydrocarbons and dibenzo Pdioxins by phanerochaete-chrysosporium ligninase. *J Biol Chem*, 1986, **261**: 6948–6952.

[18] Hawari J, Beaudet S, Halasz A, *et al*. Microbial degradation of explosives: biotransformation versus mineralization. *Appl Microbiol Biotechnol*, 2000, **54**: 605–618.

[19] Strigul N, Braida W, Christodoulatos C, *et al*. The assessment of the energetic compound 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (CL-20) degradability in soil. *Environ Pollut*, 2006, **139**: 353–361

[20] Croker FC, Indest KJ, Fredrickson HL. Biodegradation of the cyclic nitramine explosive RDX, HMX, and CL-20. *Appl Microbiol Biotechnol*, 2006, **73**: 274–290.

[21] Bhushan B, Halasz A, Spain JC, *et al*. Diaphorase catalyzed biotransformation of RDX via N-denitration mechanism. *Biochem Biophys Res Commun*, 2002, **296**: 779–784

[22] Bhushan B, Halasz A, Spain JC, *et al*. Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine catalyzed by a NAD(P)H: nitrate oxidoreductase from *Aspergillus niger*. *Environ Sci Technol*, 2002, **36**: 3104–3108

[23] Bhushan B, Paquet L, Halasz A, *et al*. Mechanism of xanthine oxidase catalyzed biotransformation of HMX under anaerobic

conditions. *Biochem Biophys Res Commun*, 2003, **306**: 509–515.

[24] Bhushan B, Halasz A, Hawari J. Nitroreductase catalyzed biotransformation of CL-20. *Biochem Biophys Res Comm*, 2004, **322**: 271–276.

[25] Bhushan B, Halasz A, Spain JC, *et al*. Initial reaction(s) in biotransformation of CL-20 is catalyzed by salicylate 1-monooxygenase from Pseudomonas sp. strain ATCC 29352. *Appl Environ Microbiol*, 2004, **70**: 4040–4047.

[26] Bhushan B, Halasz A, Hawari J. Biotransformation of CL-20 by a dehydrogenase enzyme from *Clostridium* sp. EDB2. *Appl Microbiol Biotechnol*, 2005, **69**: 448–455.

[27] Bradley PM, Dinicola RS. RDX (hexahydro-1,3, 5-trinitro-1,3,5-triazine) biodegradation in aquifer sediments under manganese-reducing conditions. *Biorem J*, 2005, **9**: 1–8.

[28] Coleman NV, Spain JC, Duxbury T. Evidence that RDX biodegradation by *Rhodococcus* strain DN22 is plasmid-borne and involves a cytochrome p-450. *J Appl Microbiol*, 2002, **93**: 463–472.

[29] Kitts CL, Green CE, Otley RA, *et al*. Type I nitroreductases in soil enterobacteria reduce TNT (2,4,6-trinitrotoluene) and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine). *Can J Microbiol*, 2000, **46**: 278–282.

[30] Hannink NK, Rosser SJ, French CE, *et al*. Uptake and metabolism of TNT and GTN by plants expressing bacterial pentaerythritol tetranitrate reductase. *Water Air and Soil Pollution*, 2003, 3: 251–258.

[31] Rosser SJ, French CE, Bruce NC. Special symposium: phytoremediation engineering plants for the phytodetoxification of explosives. *In Vitro Cell Dev Biol-Plant*, 2001, **37**: 330–333.

[32] van Berlen JB, Funhoff EC. Alkane hydroxylases involved in microbial alkane degradation. *Appl Microbiol Biotechnol*, 2007, **74**: 13–21.

[33] McDonald IR, Miguez CB, Rogge G, *et al*. Diversity of soluble methane monooxygenase-containing methanotrophs isolated from polluted environments. *FEMS Microbiol Lett*, 2006, **255**: 225–232.

[34] van Beilen JB, Li Z, Duetz WA, *et al*. Diversity of alkane hydroxylase systems in the environment. *Oil Gas Sci Technol*, 2003, **58**: 427–440.

[35] Iida T, Sumita T, Ohta A, *et al*. The cytochrome P450ALK multigene family of an n-alkane-assimilating yeast, *Yarrowia lipolytica*: cloning and characterization of genes coding for new CYP52 family members. *Yeast*, 2000, **16**: 1077–1087.

[36] van Beilen JB, Funhoff E G, van Loon A, *et al*. Cytochrome P450 alkane hydroxylases of the CYP153 family are common in alkane-degrading eubacteria lacking integral membrane alkane hydroxylases. *Appl Environ Microbiol*, 2006, **72**: 59–65.

[37] Maeng JH, Sakai Y, Tani Y, *et al*. Isolation and characterization of a novel oxygenase that catalyzes the first step of nalkane oxidation in *Acinetobacter* sp. strain M-1. *J Bacteriol*, 1996, **178**: 3695–3700.

[38] Pieper DH. Aerobic degradation of polychlorinated biphenyls. *Appl Microbiol Biotechnol*, 2005, **67**: 170–191.

[39] Erickson BD, Mondello FJ. Nucleotide sequencing and transcriptional mapping of the genes encoding biphenyl dioxygenase, a multicomponent polychlorinated-biphenyl-degrading enzyme in *Pseudomonas* strain LB400. *J Bacteriol*, 1992, **174**: 2903–2912.

[40] Hofer B, Backhaus S, Timmis KN. The biphenyl/polychlorinated biphenyl-degradation locus (bph) of *Pseudomonas* sp. LB400 encodes four additional metabolic enzymes. *Gene*, 1994, **144**: 9–16.

[41] Hayase N, Taira K, Furukawa K. *Pseudomonas putida* KF715 bphABCD operon encoding biphenyl and polychlorinated biphenyl degradation: cloning analysis, and expression in soil bacteria. *J Bacteriol*, 1990, **172**: 1160–1164.

[42] Nishi A, Tominaga K, Furukawa K. A 90-kilobase conjugative chromosomal element coding for biphenyl and salicylate catabolism in *Pseudomonas putida* KF715. *J Bacteriol*, 2000, **182**: 1949–1955.

[43] Peloquin L, Greer CW. Cloning and expression of the polychlorinated biphenyl-degradation gene cluster from *Arthrobacter* M5 and comparison to analogous genes from gramnegative bacteria. *Gene*, 1993, **125**: 35–40.

[44] Masai E, Yamada A, Healy JM, *et al*. Characterization of biphenyls catabolic genes of gram-positive polychlorinated biphenyls degrader *Rhodococcus* sp. strain RHA1. *Appl Environ Microbiol*, 1995, **61**: 2079–2085.

[45] Labbe D, Garnon J, Lau PCK. Characterization of the genes encoding a receptor-like histidine kinase and a cognate response regulator from a biphenyl/polychlorobiphenyl-degrading bacterium, *Rhodococcus* sp. strain M5. *J Bacteriol*, 1997, **179**: 2772–2776.

[46] Takeda H, Yamada A, Miyauchi K, *et al*. Characterization of transcriptional regulatory genes for biphenyl degradation in *Rhodococcus* sp. strain RHA1. *J Bacteriol*, 2004, **186**: 2134–2146.

[47] Kimbara K, Hashimoto T, Fukuda M, *et al*. Cloning and sequencing of two tandem genes involved in degradation of 2,3-dihydroxybiphenyl to benzoic acid in the polychlorinated biphenyl-degrading soil bacterium *Pseudomonas* sp. strain KKS102. *J Bacteriol*, 1989, **171**: 2740–2747.

[48] Ohtsubo Y, Delawary M, Kimbara K, *et al*. BphS, a key transcriptional regulator of bph genes involved in polychlorinated biphenyl/biphenyl degradation in *Pseudomonas* sp. KKS102. *J Biol Chem*, 2001, **276**: 36146–36154.

[49] Kikuchi Y, Yasukochi Y, Nagata Y, *et al*. Nucleotide sequence and functional analysis of the metacleavage pathway involved in biphenyl and polychlorinated biphenyl degradation in *Pseudomonas* sp. strain KKS102. *J Bacteriol*, 1994, **176**: 4269–4276.

[50] Zylstra GJ, Kim E. Aromatic hydrocarbon degradation by *Sphingomonas yanoikuyae* B1. *J Ind Microbiol Biotechnol*, 1997, **19**: 408–414.

[51] Pinyakong O, Habe H, Omori T. The unique aromatic catabolic genes in sphingomonads degrading polycyclic aromatic hydrocarbons (PAHs). *J Gen Appl Microbiol*, 2003, **49**: 1–19.

[52] Kim E, Zylstra GJ. Functional analysis of genes involved in biphenyl, naphthalene, phenanthrene, and m-xylene degradation by *Sphingomonas yanoikuyae* B1. *J Ind Microbiol Biotechnol*, 1999, **23**: 294–302.

[53] Romine MF, Stillwell LC, Wong KK, *et al*. Complete sequence of a 184-kilobase catabolic plasmid from

Sphingomonas aromaticivorans F199. *J Bacteriol*, 1999, **181**: 1585–1602.

[54] Chatterjee DK, Kellogg ST, Watkins DR, *et al*. Plasmids in the biodegradation of chlorinated aromatic compounds//Levy SB, Clowes RC, Koenig EL, eds. Molecular Biology, Pathogenicity, and Ecology of Bacterial Plasmids. New York: Plenum Pub Corp Plenum, 1981: 519–528.

[55] Hickey WJ, Sabat G, Yuroff AS, *et al*. Cloning, nucleotide sequencing, and functional analysis of a novel, mobile cluster of biodegradation genes from *Pseudomonas aeruginosa* strain JB2. *Appl Environ Microbiol*, 2001, **67**: 4603–4609

[56] van der Meer JR, Ravatn R, Sentchilo V. The clc element of *Pseudomonas* sp. strain B13 and other mobile degradative elements employing phage-like integrases. *Arch Microbiol*, 2001, **175**: 79–85.

[57] Potrawfke T, Armengaud J, Wittich RM. Chlorocatechols at positions 4 and 5 are substrates of the broad-spectrum chlorocatechol 1,2-dioxygenase *Pseudomonas chlororaphis* RW71. *J Bacteriol*, 2001, **183**: 997–1011.

[58] Ogawa N, Miyashita K. The chlorocatechol-catabolic transposon Tn5707 of Alcaligenes eutrophus NH9, carrying a gene cluster highly homologous to that in the 1,2,4-trichlorobenzenedegrading bacterium *Pseudomonas* sp. strain P51, confers the ability to grow on 3-chlorobenzoate. *Appl Environ Microbiol*, 1999, **65**: 724–731.

[59] Liu S, Ogawa N, Miyashita K. The chlorocatechol degradative genes, tfdT-CDEF, of *Burkholderia* sp. strain NK8 are involved in chlorobenzoate degradation and induced by chlorobenzoates and chlorocatechols. *Gene*, 2001, **268**: 207–214.

[60] Poh RPC, Smith ARW, Bruce IJ. Complete characterization of Tn5530 from Burkholderia cepacia strain 2a (pIJB1) and studies of 2,4-dichlorophenoxyacetate uptake by the organism. *Plasmid*, 2002, **48**: 1–12.

[61] Vedler E, Koiv V, Heinaru A. Analysis of the 2,4-dichlorophenoxyacetic acid-degradative plasmid pEST4011 of *Achromobacter xylosooxidans* subsp. denitrificans strain EST4002. *Gene*, 2000, **255**: 281–288.

[62] Hoffmann D, Kleinsteuber S, Müller RH, *et al*. A transposon encoding the complete 2,4-dichlorophenoxyacetic acid degradation pathway in the alkalitolerant strain *Delftia acidovorans* P4a. *Microbiology*, 2003, **149**: 2545–2556.

[63] Moiseeva OV, Solyanikova IP, Kaschabek SR, *et al*. A new modified ortho cleavage pathway of 3-chlorocatechol degradation by *Rhodococcus opacus* 1CP: genetic and biochemical evidence. *J Bacteriol*, 2002, **184**: 5282–5292.

[64] Saeki H, Akira M, Furuhashi K, *et al*. Degradation of trichloroethylene by a linear plasmid-encoded alkene monooxygenase in *Rhodococcus corallinus* (*Nocardia corallina*) B-276. *Microbiol*, 1999, **145**: 1721–1730.

[65] Ensign SA, Hyman MR, Arp DJ. Cometabolic degradation of chlorinated alkenes by alkene monooxygenase in a propylenegrown *Xanthobacter* strain. *Appl Environ Microbiol*, 1992, **58**: 3038–3046.

[66] Hyman MR, Russell SA, Ely RL, *et al*. Inhibition, inactivation, and recovery of ammonia-oxidizing activity in cometabolism of trichloroethylene by *Nitrosomonas europaea*. *Appl Environ Microbiol*, 1995, **61**: 1480–1487.

[67] Harker AR, Kim Y. Trichloroethylene degradation by two independent aromatic degrading pathways in *Alcaligenes eutrophus* JMP134. *Appl Environ Microbiol*, 1990, **56**: 1179–1181.

[68] W 1881 ckett LP, Householder SR. Toxicity of trichloroethylene to *Pseudomonas putida* F1 is mediated by toluene dioxygenase. *Appl Environ Microbiol*, 1989, **55**: 2723–2725.

[69] Leahy JG, Byrne AM, Olsen RH. Comparison of factors influencing trichloroethylene degradation by toluene-oxidizing bacteria. *Appl Environ Microbiol*, 1996, **62**: 825–833.

[70] DiSpirito AA, Gulledge J, Shiemke AK, *et al*. Trichloroethylene oxidation by the membraneassociated methane monooxygenase in Type I, Type II and Type X methanotrophs. *Biodegradation*, 1992, **2**: 151–164.

[71] Oldenhuis R, Oedzes JY, van der Waarde JJ, *et al*. Kinetics of chlorinated hydrocarbon degradation by *Methylosinus trichosporium* OB3b and toxicity of trichloroethylene. *Appl Environ Microbiol*, 1991, **55**: 2819–2826.

[72] Folsom BR, Chapman PJ, Pritchard PH. Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: kinetics and interactions between substrates. *Appl Environ Microbiol*, 1990, **56**: 1279–1285.

[73] Hamamura N, Storfa RT, Semprini, *et al*. Diversity in butane monooxygenases among butane-grown bacteria. *Appl Environ Microbiol*, 1997, **65**(10): 4586–4593.

[74] Chen J, Du Y, Song YT, *et al*. Research progress of catalysis of reduction of nitro compounds by oxidoreductases. *J Chem Ind Eng*, 2008, **29**(3): 8–12.
陈洁, 杜赟, 宋雨婷, 等. 氧化还原酶催化还原硝基化合物的研究进展. 化学工业与工程技术, 2008, **29**(3): 8–12.

[75] Zhang XH, Bajpai R. Development of a key-enzyme based model for cometabolism-a case study on cometabolism of trichloroethylene by methanotrohpic bacteria. *Acta Sci Circum*, 2000, **20**(5): 558–562.
张锡辉, Bajpai R. 以关键酶为基础共代谢模型的建立—以甲烷细菌共代谢三氯乙烯为例. 环境科学学报, 2000, **20**(5): 558–562.