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Review 综述

微生物菌群降解含石油烃污染废水的研究现状

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摘　要：石油烃废水的来源主要包括石油开采和石油产品加工使用过程中产生的废水。具体可分为石油开采过程中由于泄漏产生的污染、机械加工过程产生的废水、皮革印染过程中由于助剂使用产生的废水。石油烃废水具有有机物含量高、毒性大、可生化性差等特点。生物降解法因其无二次污染，已经成为石油烃废水处理的主要研究热点之一。本文结合最新文献分析及个人研究成果，详细介绍了石油烃废水的组成、生物降解菌群种类、生物降解机理、生物炭固定修复技术、降解基因和降解酶等方面内容，为后续进行微生物菌群降解石油烃废水的深入研究提供了参考。

关键词：微生物菌群；石油烃废水；降解机理；生物炭固定技术

Present situation of degradation of wastewater containing petroleum hydrocarbons by microbial flora

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Abstract: The wastewater containing petroleum hydrocarbons is mainly produced in the process of petroleum exploitation and petroleum products processing. It encompasses the water polluted by

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leakage in oil exploitation, the wastewater produced in machining, and the wastewater produced using auxiliaries in leather printing and dyeing. The wastewater containing petroleum hydrocarbons has high organic matter content, high toxicity, and poor biodegradability. Biodegradation has become one of the main research hotspots of the treatment of wastewater containing petroleum hydrocarbons because of no secondary pollution. Based on the latest literature and our research results, this paper details the composition of wastewater containing petroleum hydrocarbon, microbial species for biodegradation, biodegradation mechanism, biochar immobilization and remediation technology, and degradation genes and enzymes. This paper can provide reference for the further study of microbial flora degradation of wastewater containing petroleum hydrocarbon.

Keywords: microbial flora; wastewater containing petroleum hydrocarbons; degradation mechanism; biochar immobilization technology

石油和石油产品的勘探、生产、精炼、运输和储存过程会不可避免地产生环境污染[1]。石油的成分极其复杂，包括油性(主要成分)、胶质、沥青质、碳质。由 C 和 H 组成的烃类是石油的主要成分，如烷烃、环烷烃、多环芳烃[2]。石油烃污染物具有毒性、疏水性，且迁移能力较强，被许多国家列为优先控制污染物[3]。

石油烃废水的处理方法包括物理法[4-6]、化学法[7-8]和生物法。物理法降解效率低，化学法成本昂贵且有副作用，生物法因其无二次污染，已经成为石油烃降解的主要研究热点之一[9]。本文主要综述了利用微生物降解石油烃废水的现状，结合目前团队研究过程中出现的问题，如单一石油烃菌种对石油烃废水降解效果不理想、实验室菌种在实际石油烃废水处理时存活率低等，提出构建复合菌群、优化生物炭固定技术、研究功能基因等研究方向。

1 石油烃废水

1.1 石油烃废水特点

石油烃废水的来源主要包括石油开采和石油产品加工使用过程中产生的废水。具体可分为石油开采过程中由于泄漏产生的污染、机械加工过程产生的废水(例如研磨、切削、淬火、清洗过程产生的含油废水)、皮革印染过程中由于助剂使用产生的废水。石油烃废水的特点主要有 4 个方面。(1) 有机物含量高：烃类废水中含有大量的烃类化合物，化学需氧量(chemical oxygen demand, COD)和生化需氧量(biochemical oxygen demand, BOD)通常较高。例如，一些石油化工废水的 COD 可能高达每升数千甚至上万毫克[10]。(2) 乳化油含量高：石油加工产品由于表面活性剂的使用，使得废水中乳化油含量升高，难以破乳。(3) 可生化性差：由于烃类化合物的化学结构相对稳定，难以被微生物直接降解，因此烃类废水的可生化性较差。传统的生物处理方法对烃类废水的处理效果有限。(4) 毒性较大：一些烃类化合物对人体和环境具有一定的毒性[11]。例如，芳香烃类化合物(如苯、甲苯等)具有致癌性；某些烃类化合物可能会对水生生物造成毒害，影响水生生态系统的平衡。

1.2 石油烃废水组成

石油烃废水中的石油烃可分为饱和烃类、芳香烃类、沥青质类(酚类、脂肪酸类、酮类、酯类和卟啉类)和树脂类(吡啶类、喹啉类、咔唑类、亚砜类和酰胺类) 4 类[12]。石油烃废水中含有各种碳氢化合物，石油烃的碳氢化合物根据碳链的长度分为短链烃(C1−C10)、中长链烃(C10−C16)和长链烃(C>16)。短链烃由于其碳链短、分子量小，在自然条件下容易挥发和降解[13]。不同来源的石油烃废水成分差异较大，增加了处理的难度[14]。以机械加工过程的切削

液废水为例，废切削液中含有矿物油、表面活性剂、极压剂、防锈剂等各种助剂，有机物含量高，COD 值高，其 COD 可达每升数万至数十万毫克；成分复杂，含多种有害物质，生化性差，化学性质相对稳定[15]。切削液废水中碳氢化合物主要可分为链烷烃、环烷烃、芳香烃(表 1)。

1.3 生物法降解石油烃废水

Teli 等[16]使用浸没式厌氧膜生物反应器来处理废金属加工液，反应器的 COD 去除率为 64%，但甲烷产量很低，即该过程主要通过物化作用去除污染物，微生物的作用只是辅助。Zhang 等[17]研究了膜生物反应器与 Fenton 反应联用处理废切削液，在最佳条件下，COD 去除率可达到 97%。Ravi 等[11]从受石油污染的土壤中筛选出一株地衣芽孢杆菌，48 h 内总体石油产品降解效率可以达到 88%。Kodama 等[18]从油藏中分离得到反硝化氧化硫单胞菌(*Sulfurimonas denitrificans*) YK-1，该菌株能以硫酸盐、硝酸盐和氢为电子受体，不以原油为碳源，而是以原油中的含硫化合物为碳源。Qu 等[19]从受油田污染的土壤中筛选得到一株不动杆菌菌株(*Acinetobacter* sp.)，可以降解烷烃和芳香烃类污染物，短链烷烃(C14−C25)的平均降解效率为 78.8%，高于长链烷烃(C26−C35)的平均降解率 54.5%。不动杆菌可以通过脂肪酸 β 氧化途径降解烷烃[20]。Mishra 等[21]对铜绿假单胞菌(*Pseudomonas aeruginosa*)和红球菌(*Rhodococcus* sp.)进行了研究，发现这些细菌可以通过末端氧化途径降解石油污泥中的正十六烷。Zhang 等[22]发现铜绿假单胞菌在 12 d 内通过单线态和二氧途径降解了 34.5% 的芘。Yu 等[23]从不同油田分离驯化了 2 株高效油脂降解菌株枯草芽孢杆菌(*Bacillus subtilis*)和食异源物鞘氨醇菌(*Sphingobium xenophagum*)，并将其制备成高效微生物制剂，投入胜利油田污染场地。

目前，生物降解方法主要可分为两类：(1) 生物增强，将外源性活微生物添加到受污染的场地，将有害的石油污染物分解成二氧化碳和水等无毒成分[24]；(2) 生物刺激，通过添加营养物质来增强原生微生物的生物修复能力[25]。Wu 等[26]使用不动杆菌菌株进行生物强化，添加营养物质氮和磷进行生物刺激，对比生物强化和生物刺激过程中石油烃降解效率和微生物群落动态的变化，结果表明孵育 6 周后生物刺激、生物强化分别对总石油烃污染物降解了 60% 和 34%，第 7 周出现退化平台期。

2 石油烃降解菌

2.1 石油烃降解菌种类

α 变形菌(*Alphaprote obacteria*)、嗜盐菌(*Halophiles*)、β 变形菌(*Betaproteobacteria*)、γ 变

表1 石油烃废水中的碳氢化合物组成举例(以机械加工液废水为例)

Table 1 Example of hydrocarbon composition in petroleum hydrocarbon wastewater (e. g. machining fluid wastewater)

Type	Classification	Name
Hydrocarbon	Straight chain hydrocarbon	Tetratriacontane
	Branched hydrocarbon	11-methyltricosane
	Cyclic hydrocarbon	15-isobutyl-(13.alpha.H)-isocopalane
	Aromatic hydrocarbon	4-(2-fluorobenzoylamino)piperidine-1-carboxylic acid
Alcohols	Decanol	1-decanol, 2-octyl
	Heptanol	2-isopropyl-5-methyl-1-heptanol
Ethers/Esters	Esters	l-norvaline, N-(2-methoxyethoxycarbonyl)-, hexadecyl ester
	Ethers	Heptaethylene glycol monododecyl ether

形菌(*Gammaproteobacteria*)、放线菌(*Actinomycetes*)和厚壁菌(*Bacillota*)被认为是降解碳氢化合物的关键菌群[27]。Govarthanan 等[28]和 Parthipan 等[29]研究表明，无色杆菌属(*Achromobacter* sp.)、假单胞菌属(*Pseudomonas* sp.)、芽孢杆菌属(*Bacillus* sp.)等生物群落可以消耗碳氢化合物，并具备降解芳烃以及产生酶和生物表面活性剂化合物的能力。目前，已报道的可使用受原油污染的土壤、受石油污染的水样、含油污泥等(表 2)，分离得到烃类降解菌株[30-36]。假单胞菌属(*Pseudomonas* sp.)、棒杆菌属(*Corynebacterium* sp.)、芽孢杆菌属(*Bacillus* sp.)、葡萄球菌属(*Staphylococcus* sp.)、不动杆菌属(*Acinetobacter* sp.)、肠杆菌属(*Enterobacter* sp.)、节杆菌属(*Arthrobacter* sp.)、红球菌属(*Rhodococcus* sp.)、青霉菌属(*Penicillium* sp.)等，均具有降解石油的潜力[37-42]。

2.2 石油烃降解菌的降解机理

微生物可以通过溶解、乳化或直接从油水界面黏附和吸收油来提高脂肪烃的生物利用度，从而促进脂肪烃的生物降解。微生物产生的细胞外生物表面活性剂或生物乳化剂，在通过假溶作用增强细胞摄取和通过降低表面张力来增加传质的界面面积方面发挥着重要作用[43]。Naaz 等[44]指出，许多细菌具有合成生物表面活性剂的能力，包括脂肪酸、磷脂、脂蛋白、糖脂、脂肽和中性脂质，这些生物表面活性剂会降低表面或界面张力，增加碳氢化合物在水中的溶解度或水在碳氢化合物中的溶解度，使微生物更容易接触到污染物。

根据石油烃类污染物的链长和类型，需要不同的酶将氧或其他电子受体引入底物以开始生物降解(图 1)[13,45]。(1) 微生物降解烷烃的途径不同，包括单端、二端和亚端氧化。烷烃进行有氧降解的第一步是由加氧酶催化的，加氧酶是一类能够将氧原子从分子氧引入烷烃底物的酶[46]。线性、中链和长链烷烃均可被烷烃单加氧酶或细胞色素 P450s 氧化，同时烷烃降解细菌拥有多个烷烃羟化酶基因[41]。正构烷烃比支链烷烃更易被微生物降解，因为支链甲基的裂变需要更大的能量消耗[47]。(2) 氧合酶、水解酶、脱氢酶和氧化酶都参与代谢多环芳烃，催化脱氢、芳香环羟基化和芳香环裂解等关键反应[48-49]。当微生物降解芳烃时，它们首先将芳

表2 部分已报道的可降解石油烃的细菌和真菌种类

Table 2 Some reported bacterial and fungal capable of degrading petroleum hydrocarbons

Category	Source	Name	Degradation object	Maximum degradation rate (%)	References
Bacteria	Surface sediments of oil-contaminated coastlines	*Pseudomonas aeruginosa*	Crude oil	55	[30]
	Oil contaminated rhizosphere soil of plants	*Rhodococcus hoagii*	Petroleum hydrocarbon	87	[31]
	Car workshop	*Staphylococcus hominis*, *Bacillus pumilus*	Petroleum	50	[32]
	Oil refinery sludge	*Ochrobactrum cicerir*	Total petroleum hydrocarbon	91	[33]
	Oil contaminated site	*Alcaligenes faecalis*, *Pseudomonas guariconensis*, *Pseudomonas monteilii*	polycyclic aromatic hydrocarbons	85	[34]
	Water samples from streams contaminated with oil	*Corynebacterium* sp.	Crude oil	60	[35]
Fungi	Oilfield contaminated soil	*Candida tropicalis*	Grease	61	[36]

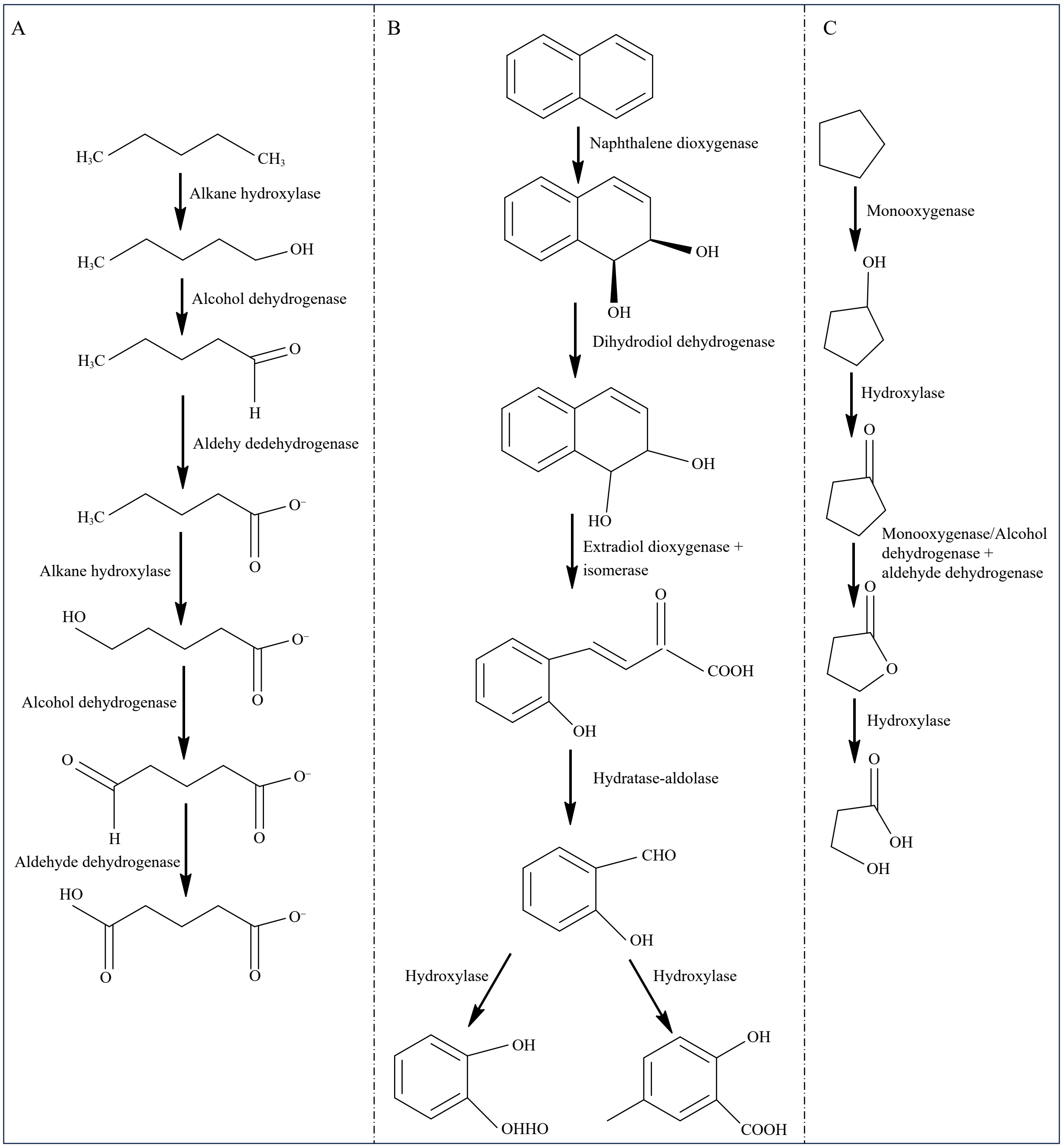

图1 石油烃生化阶段降解路径。A：正烷烃[45]；B：芳香烃[45]；C：环烷烃[13]。

Figure 1 Degradation pathway of petroleum hydrocarbon during biochemical stage. A: n-alkanes[45]; B: Aromatic hydrocarbon[45]; C: Naphthenes[13]。

烃降解为儿茶素，然后在双加氧酶的作用下通过原位开裂和偏心开裂的过程打破苯环[50]。(3) 负责激活脂肪烃和芳烃的单加氧酶和双加氧酶被认为是石油污染的特定标志物[51]。

目前，大多数研究者仅关注了一种或多种石油烃降解菌在特定反应步骤中的降解途径。然而，在多种石油烃降解菌共同存在的情况下，对石油烃污染场地的修复，以及对石油烃降解

菌降解途径的完整分析，包括所有反应步骤的报道尚未见文献[13]。根据本人前期研究结果，微生物对石油烃污染物中碳氢化合物降解的难易程度可排序如下：直链烷烃>支链烷烃>芳香烃>杂环化合物[15]。以厌氧+好氧联合处理石油烃废水为例，厌氧段含石油烃废水的变化包括4个方面。(1) 长链烷烃消失，如11-甲基二十三烷、四十三烷。(2) 醋酸、醇、醇胺、酰胺类物质消失。(3) 生成饱和脂肪酸，如乙酸、丙酸、丁酸等；生成醇类物质，如乙醇；生成环酮和内酯。(4) 苯环聚合物种类增多。好氧段含石油烃废水的变化包括硅氧烷消失、苯环聚合物种类减少。经生物降解后，石油烃废水中最难降解的物质为醇胺类杂环化合物。

3 石油烃降解菌研究方向

3.1 复合功能菌群构建

构建复合功能菌群是指通过组合不同优势菌株，以增强其生态功能和应用效果。单一菌株的能力有限，而通过将不同菌株组合起来，可以发挥它们各自的优势，产生协同作用，从而提高整体功能。在降解石油烃的过程中，微生物菌群之间可能产生协同效应，也可能存在竞争抑制作用，随着环境变化不同微生物种群优势菌的数量不同[52]。通过基因技术构建共现微生物网络，可以展示微生物之间的种间相互作用，并分析复配后菌落种群间的拮抗关系[14]。不同的微生物菌群之间存在强烈的代谢相互关系[53]，这种代谢相互关系可以通过宏基因组学技术进行分析。在生物降解石油烃的好氧和厌氧过程中，微生物之间的关系主要涉及代谢过程，其次是环境信息处理。微生物代谢主要涉及氨基酸代谢、碳水化合物代谢、能量代谢、辅因子和维生素代谢，而环境信息处理主要涉及膜运输和信号转导[15]。

获得高效功能菌株是构建复合菌群的前提。传统的筛菌方法需经过采样、预处理、增殖培养、选择培养、菌株分离纯化、菌落选取、初筛、纯化、复筛等过程，筛菌过程繁琐。由于培养基的成分与菌株原生环境及待处理污染物的环境差异较大，部分目标菌株可能无法存活[54]。下文介绍了目前几种热门的筛菌技术。(1) 生物合成工程菌：利用合成生物学技术，以将重组基因或突变基因导入感受态细胞中，从而获得具有高效降解功能的工程菌株。Zhang等[55]以枯草芽孢杆菌为感受态细胞，使用靶向全基因组的CRISPRi技术对关键基因进行高通量筛选，并将筛选出的关键基因组合导入感受态细胞中，筛选出具有增强蛋白表达能力的菌株。Luo等[56]通过适应性实验室进化(adaptive laboratory evolution, ALE)确定基因 *hcaE*、*hcaK* 的突变在高浓度香草酸耐受性的提高中起主要作用，随后将这2种突变组合引入亲本菌株中，获得了高香草酸耐受性菌株。(2) 高通量筛菌技术：例如基于荧光检测的高通量筛选技术、微液滴高通量筛选技术等。Luu等[57]采用微流控液滴平台培养和筛选出能够产生过量纤维素酶的 *Trichoderma reesei* 突变体，利用微流体平台可在几个小时内根据其分泌的纤维素酶培养和筛选一百万个突变体。液滴系统消除了生长较慢和生长较快的菌株变体之间的营养竞争，进而能够分离生长较慢的突变体，从而提高代谢效率[58]。(3) 原位筛菌技术：本团队前期研究将从不同来源(油田土壤、含油污泥等)得到的除油菌加入含高浓度切削液废液的生物反应器中，进行切削液废液的连续培养，当除油效果稳定后，COD去除率可达90%以上[15]。(4) 原位电离质谱筛菌技术：原位电离质谱筛菌技术也是高效筛菌技术之一，可在敞开的大气压环境中进行原位、表面和实时采样分析活菌落，并可现场提取DNA，该技术单个样品分析时间短，能可视化菌落细胞表型分子的空间分布[59]。

3.2 生物炭固定化菌群技术

实验室筛选获得的石油烃降解菌群，由于无法适应高含油浓度、性质多变、毒性强的石

油烃废水，需要利用固定化生物技术将石油烃降解菌固定化制备成一种适用性更强、活性更高、耐受性更高的固定化复合材料。目前，关于固定化微生物技术的研究多聚焦于受污染土壤，对含石油烃废水的研究较少[60]。生物炭材料例如秸秆、稻壳、玉米芯、果壳、林业固废(如木屑、树枝)等，由于具有成本低、比表面积大、可固废资源化利用等优点，已成为固定化技术的主要载体(表 3)[61-68]。Kumagai 等[69]选用 1.0 g 稻壳经精炼后，在 600−700 ℃的温度下进行热解，可吸附 0.5−6.0 g 的重油。

生物炭固定技术中，生物炭的使用可以为生物降解提供更多的固定支撑和间隙，从而引发生物降解[70]。生物炭材料富含有机碳，其含有的氮、磷、钾等元素可作为营养物质，促进微生物的生长和分裂[71]。生物炭表面大量的活性羟基、羧基、磺酸基团、氨基、亚氨基、酰氨基等官能团对微生物细胞的黏附和增殖具有重要作用。利用生物炭作为载体时，接种微生物的成活率显著提高[72]。Yin 等[73]以稻壳生物炭和海藻酸钠作为复合真菌的固定化基质，构建了吸附体系和包埋体系，在 60 d 的修复期内表现出最高的柴油去除效率(64.10%)。Han 等[74]研究发现，生物炭载体固定微生物可以降低污染环境对微生物的毒性，改性后的生物炭表现出更高的比表面积、更强的吸附能力和更高的比活性。生物炭在酸性条件下发生质子化，能够产生大量正电荷，从而促进微生物的固定和吸附[75]。Liu 等[76]利用壳聚糖-生物炭复合材料作为固定化微生物制剂的载体，固定铜绿假单胞菌和地衣芽孢杆菌。壳聚糖上的氨基(−NH_2)和生物炭上的羧基(−COOH)通过共价键结合，形成新的化学键(−NH−CO−)。对油污土壤进行 60 d 修复后，原油去除率可达 45.82%[76]。尽管生物炭固定化技术是目前研究的重点，但仍存在一些知识空白，例如生物炭固定的力学性能、

表3 生物炭制备方法总结

Table 3 Summary of biochar preparation methods

Preparation material	Biochar preparation method	Application	References
Camellia oleifera shell powder	The camellia oleifera shell powder was pretreated with 0.5 mol/L $FeCl_3$ and pyrolyzed at 300 ℃	Soil conditioner	[61]
Rice straw	The dried rice straw was crushed and sieved. The powder was put into a ceramic crucible and pyrolyzed at 500 ℃	Remediation of heavy metal pollution	[62]
Sewage sludge and coconut shell	Sewage sludge and coconut shell were heated at 500 ℃. And then activating *via* potassium hydroxide solution. Subsequently, the solid pyrolyzed at 800 ℃, and then washed with HCl solution	Remediation of polyacrylamide contamination	[63]
Masson pine wood	The granulated waste wood sawdust was sieved to a size between 0.063−2.000 mm. The pyrolysis was performed at 500 ℃ and 700 ℃	Cement additive	[64]
Hickory wood	The hickory powder was pyrolyzed at 600 ℃. After washing and drying, soaked in NaOH solution for 4 h	Adsorption of heavy metals	[65]
Rice straw	The straw was dried at 80 ℃, ground and sieved to produce <2.0 mm particles. Pyrolysis was performed at 700 ℃	Adsorption of Cd	[66]
Peanut shell	The peanut shell was pyrolyzed at 500 ℃, and processed by 60-mesh sieve	Remove electroplating mixed-wastewater	[67]
Pomelo peel	Pomelo peel was pyrolyzed at 500 ℃, then activating with KOH at a mass ratio of 1:4. Biochar obtained was washed with 35% HNO_3 and water until the pH reaches 7.0±0.2. Finally, the produced biochar was oven-dried at 80 ℃ for 24 h	Remove tetracycline antibiotics	[68]

生物炭特性优化、微生物群落动力学、标准化和可扩展性、现场规模应用和监测、环境影响和风险评估[77]。

3.3 降解基因及降解酶的研究

Izmalkova 等[78]指出降解相关基因及其编码的酶在微生物代谢中发挥重要作用，降解酶的活性可作为判定生物降解能力的指标。Zheng 等[79]将脱氢酶、固体多酚氧化酶和固体脲酶作为评价活性土壤微生物群落降解有机物能力的指标。微生物对废水中石油烃类污染物的降解主要由相关功能基因编码的酶类驱动，降解过程中伴随着氧化、还原、脱氢、羧化等生化反应。Elumalai 等[80]研究发现，在原油降解过程中，包括烷烃羟化酶、醇脱氢酶和脂肪酶在内的许多酶在生物降解过程中起关键作用。以 2 级厌氧+2 级好氧法处理机械加工液废水为例，厌氧降解过程和好氧降解过程中发挥作用的酶类型(图 2)，主要包括糖基转移酶(glycosyl transferases)、糖苷水解酶(glycoside hydrolases)、碳水化合物结合酶(carbohydrate-binding modules)、碳水化合物酯酶(carbohydrate esterases)和多糖裂解酶(polysaccharidelyases)等。

探究降解菌的基因及酶对石油烃污染物降解的变化规律，可利用多组学分析技术[81-83]。基因组学分析，例如基因扩增子测序分析技术[84]和宏基因组学技术[85]，可以分析石油烃降解过程中菌群的组成和丰度变化，并注释功能性基因。转录组学可以探索差异表达的基因，分析降解的代谢类型[3]。蛋白质组学是对基因组学的补充，用于从感兴趣的生物样品中发现新的酶。代谢组学是一种检测内源性和外源性代谢产物的方法[86]，可以通过分析菌群产生的代谢产物变化，寻找烃类代谢途径。

4 总结与展望

石油烃废水的产生会对人类和环境造成严重危害，生物降解法因其无二次污染，已经成为石油烃降解的主要研究热点之一。然而，目前关于石油烃废水的研究在以下方面报道较少：针对高浓度石油烃废水的特种生物菌群组成及生物炭复合菌剂特性优化；多种石油烃降解菌共同存在的情况下对石油烃污染场地的修复；对石油烃降解菌降解途径的完整分析以及所有反应步骤。未来可在以下方面对石油烃废水的生物降解法进行进一步研究。

(1) 简化功能菌株筛选步骤，建立可针对高浓度含油废水的特种菌株库。构建复合功能菌群，研究复合菌群之间菌落种群间的拮抗关系及代谢关系。

(2) 优化生物炭固定降解菌剂技术：以农林固废为原料制备生物炭材料载体，通过表面改性、磁性改性等方式对生物炭材料进行改性，增加生物炭的比表面积和吸附位点数。未来可深入研究生物炭固定的力学性能、生物炭特性

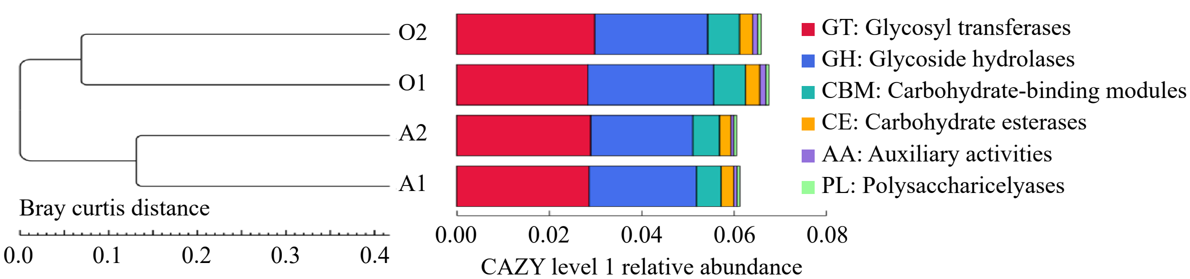

图2 2级厌氧+2级好氧法处理机械加工废水过程中主要作用酶

Figure 2 The main enzymes in the treatment of mechanical processing wastewater by 2-level anaerobic+2-level aerobic.

优化、微生物群落动力学、标准化和可扩展性、现场规模应用和监测、环境影响和风险评估。

(3) 通过多组学结合的方式，研究石油烃类污染物的微生物降解途径、降解过程中的关键酶及其基因。确定降解酶活性测定的方法，评估石油烃污染场地的生物降解能力。

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