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强化微生物修复石油污染海洋的研究进展*

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摘 要 海洋石油污染使石油烃(Petroleum hydrocarbons,PHCs)成为海洋重要有机污染物.快速有效清除石油污染物是当前急需解决的海洋环境问题之一.利用石油烃降解菌(Petroleum hydrocarbon−degrading bacteria,PHDB)进行的原位微生物修复是一种对环境友好且较经济的清除石油污染场所和管理海洋表面溢油的有效技术.本文综述了石油烃降解菌的特性和对环境的适应性.重点分析探讨了引入外源微生物,投加生物表面活性剂、添加营养物质和增加溶解氧含量、采用微生物固定化技术等方法强化原位微生物修复石油污染海洋的机制和研究进展.展望了海洋石油污染微生物修复领域的研究前景.

关键词 石油烃降解菌,生物表面活性剂,生物降解,强化修复,生物可利用性.

Enhanced microbial remediation of petroleum-pollutioned environment marine

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Abstract: Petroleum hydrocarbons (PHCs) have become major organic pollutants in marine environments. Therefore, there is an urgent need to develop technology for the rapid and effective removal of marine PHCs. Microbial remediation is an environmentally friendly, economical, and effective technology for the remediation of petroleum-polluted sites and management of marine oil spills. This review summarizes various types of petroleum hydrocarbon-degrading bacteria(PHDB) used in marine petroleum pollution control, as well as the environmental adaptability of these bacteria. Subsequently, the mechanisms and research progress of enhanced in-situ microbial remediation of petroleum-pollutioned marine environment through methods such as the introduction of exogenous microbes, dosing biosurfactants, addition of nutrients, increasing dissolved oxygen concentration and microbe immobilization technology are analyzed and reviewed. Lastly, based on the benefits and limitations of the current methods, the research prospects in the field of microbial remediation of marine petroleum pollution are presented.

Keywords: petroleum hydrocarbon-degrading bacteria, biosurfactant, biodegradation, enhanced remediation, bioavailability.

石油在勘测、开采、加工、运输等过程中因意外泄漏进入海洋,造成烷烃、环烷烃、芳香烃等石油烃

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(Petroleum hydrocarbons,PHCs)含量增加,引起海洋石油污染.疏水性的石油烃在海面形成油膜,阻断大气与海洋溶解性气体的交换和循环平衡,减少太阳辐射入海洋的能量,破坏海洋植物光合作用与食物链传递平衡,直接或间接影响人类生存和可持续发展.因此,发展与完善有效的海洋石油污染修复技术,快速彻底清除石油烃污染物,对减少油污引发的危害和保护海洋生态环境具有深远影响.

修复石油污染海洋的基本原则是在不对环境产生二次污染的情况下,采用物理法、化学法和微生物法,尽量缩小石油污染区域,并迅速清除和回收海面上的浮油.由图 1 可知,物理和化学法在石油污染发生初期,能快速有效的清除大面积浮油,但费用昂贵,易造成二次污染,不适合长期使用.经物化法清除后残留的石油烃通过土著石油烃降解菌(Petroleum hydrocarbon-degrading bacteria,PHDB)自身代谢被降解清除.微生物修复海洋石油污染的技术,具有修复持续时间长、对环境友好、处理成本低等优点,具有广阔的应用前景.但是,单一的石油烃降解菌对石油烃的降解能力有限,且自然降解过程较慢,在实际应用中,需要利用其它技术进行强化提高石油烃的修复效率.

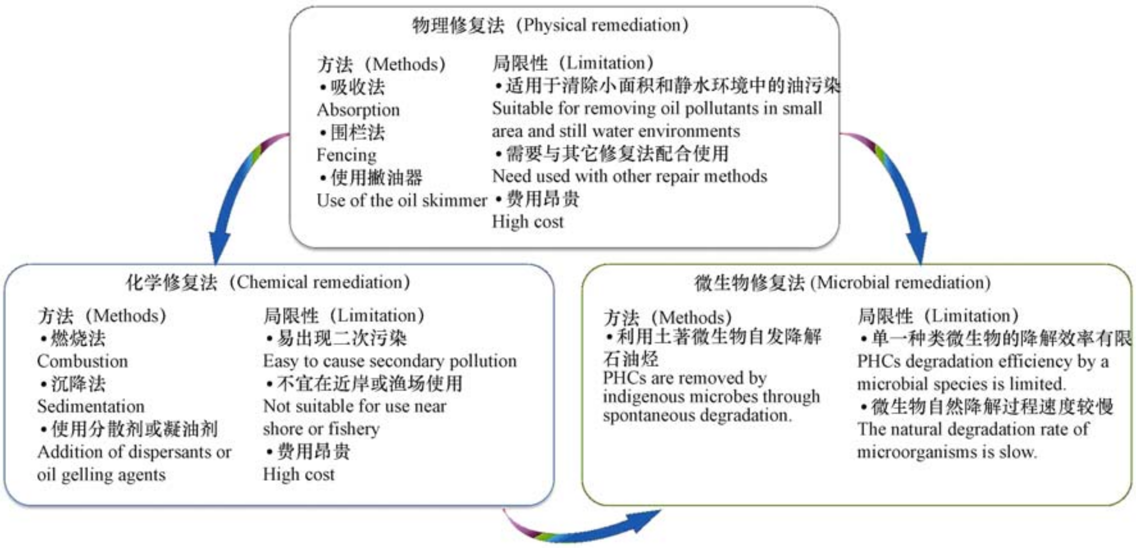

图 1　海洋石油污染修复技术的应用

Fig.1　Application and limitation of marine petroleum pollution remediation technology

本综述分析了石油烃降解菌的特性,探讨了引入外源微生物,投加生物表面活性剂,施加营养物质和增加溶解氧,以及采用微生物固定化技术等多种强化手段的修复机理和相关研究进展.本研究可为海洋或其他水体石油污染的强化微生物修复研究提供参考和借鉴.

1　石油烃降解菌(Petroleum hydrocarbon-degrading bacteria)

石油烃降解菌广泛存在于海洋和土壤等自然环境中,在修复治理受石油污染环境中起重要作用[1].通常一种石油烃降解菌只对一种或几种特定成分的石油烃有较强的降解活性.例如脱硫弧菌属(*Desulfovibrio*)、食烷菌属(*Alcanivorax*)、迪茨氏菌属(*Dietzia*)等对烷烃具有良好的降解效果[2-4];短杆菌属(*Brevibacterium*)、棒杆菌属(*Corynebacterium*)、解环菌属(*Cycloclasticus*)、假交替单胞菌属(*Pseudoalteromonas*)等对多环芳烃的降解能力较强[5-8].石油烃降解菌和用于海洋修复的石油烃降解菌专利菌分别列于表 1 和表 2.

石油烃降解菌能快速适应受石油污染的环境并对其有较强的修复能力.每次发生石油污染后,环境中的石油污染物诱导石油烃降解菌的生长,使其大量繁殖,快速聚集形成降解效率较高的微生物菌群[41-42].1989 年美国阿拉斯加威廉王子湾的 Exxon Valdez 邮轮泄漏事件导致 35500 t 原油泄入海洋后,Braddock 等[43]连续 3 年考察发现油膜覆盖区域的石油烃降解菌数量远高于非油膜覆盖区域.El-Sheshtawy 等[44]报道,从埃及红海被石油污染的吉萨湾分离出 15 种石油烃降解菌.

表 1　石油烃降解菌

Table 1　Petroleum hydrocarbon-degrading bacteria

序号 No.	菌属名称及主要参考文献 Bacterial species and references	序号 No.	菌属名称及主要参考文献 Bacterial species and references
1	无色杆菌属(*Achromobacter*)[9-10]	13	迪茨氏菌属(*Dietzia*)[11]
2	不动杆菌属(*Acinetobacter*)[12]	14	黄杆菌属(*Flavobacterium*)[13]
3	气单胞菌属(*Aeromonas*)[14]	15	戈登氏菌属(*Gordonia*)[15]
4	产碱菌属(*Alcaligenes*)[16]	16	海杆菌属(*Marinobacter*)[8]
5	食烷菌属(*Alcanivorax*)[2-3]	17	小单胞菌属(*Micromonospora*)[4]
6	芽孢杆菌属(*Bacillus*)[17]	18	分支杆菌属(*Mycobacterium*)[18]
7	短杆菌属(*Brevibacterium*)[8]	19	诺卡氏菌属(*Nocardia*)[15]
8	伯克霍尔德氏菌属(*Burkholderia*)[19]	20	假单胞菌属(*Pseudomonas*)[20]
9	柠檬酸杆菌属(*Citrobacter*)[21]	21	假交替单胞菌属(*Pseudoalteromonas*)[5]
10	棒杆菌属(*Corynebacterium*)[17-22]	22	红球菌属(*Rhodococcus*)[23]
11	解环菌属(*Cycloclasticus*)[6-7]	23	鞘氨醇单胞菌属(*Sphingomonas*)[24]
12	脱硫弧菌属(*Desulfovibrio*)[4]	24	深海弯曲菌属(*Thalassolituus*)[25]

表 2　海洋石油烃降解菌专利菌(中国)

Table 2　Patent bacteria of marine petroleum hydrocarbon-degrading bacteria (China)

序号 No.	菌种名称(代号) Name (code) of bacterial species	菌种保藏号 Preservation No. of bacterial species
1	克什米尔小陌生菌(*Advenella kashmirensis*)6-10-1[26]	CGMCC# No. 13003
2	柴油食烷菌(*Alcanivorax dieselolei*)T6-6[27]	CGMCC No. 9033
3	侧孢芽孢杆菌(*Brevibacillus laterosporus*)G-40[28]	CCTCC## No. M2017400
4	耐碱海旋菌(*Thalassospira alkalitolerans*)[29]	CGMCC No. 13079
5	交替假单胞菌(*Pseudoalteromonas* sp.)QN-1[30]	CGMCC No. 11635
6	红球菌(*Rhodococcus* sp.)QY-2[31]	CGMCC No. 11634
7	交替假单胞菌(*Pseudoalteromonas* sp.)DC-2[32]	CGMCC No. 11633
8	菌 CN-3[33]	CCTCC No. M2015537
9	威尼斯不动杆菌(*Acinetobacter venetianus*)LCL-1[34]	CCTCC No. M2015538
10	极地杆菌(*Polaribacter* sp.)DL8-7[35]	CGMCC No. 9011
11	柠檬胞菌(*Citreicella* sp.)DLFJ3-3[36]	CGMCC No. 9014
12	硫膨大杆菌(*Thioclava* sp.)DLFJ1-1[37]	CGMCC No. 9013
13	克伯村鸟氨酸微菌(*Ornithinimicrobium kibberense*)[38]	CGMCC No. 6844
14	食烷烃戈登氏菌(*Gordonia alkanivorans*)[38]	CGMCC No. 6845
15	威尼斯不动杆菌(*Acinetobacter venetianus*)DW3[39]	CGMCC No. 5215
16	短小芽孢杆菌(*Bacillus pumilus*)[40]	CGMCC No. 3384
17	威尼斯不动杆菌(*Acinetobacter venetianus*)[40]	CGMCC No. 3385
18	门多萨假单胞菌(*Pseudomonas mendocina*)[40]	CGMCC No. 3386
19	皮氏罗尔斯顿菌(*Ralstonia pickettii*)[40]	CGMCC No. 3387

#菌种保藏在中国普通微生物菌种保藏管理中心; ## 菌种保藏在中国典型培养物保藏中心.

#China Center for Type Culture Collection, CCTCC, ##China General Microbiological Culture Collection Center, CGMCC

利用石油烃降解菌快速响应石油污染的特性,通过特定石油烃基质富集培养、诱导等人工方式可以获取丰富的石油烃降解菌资源.这些微生物的活性和降解能力均优于非石油污染区域的微生物.Wang 等[45]以中国蓬莱 19-3 石油平台采集的沉积物与原油为基质共同培育出以假单胞菌和食烷菌为主要菌株的微生物群落,在 30 d 内迅速降解原油.夏瑛铭等[23]从天津市大沽排污河下游受多环芳烃(PAHs)污染的河道底泥中筛选、纯化得到一株红球菌属的荧蒽降解菌,分离培养 42 d 后该细菌对荧蒽的降解率达到 31.69%.

研究表明,在未经石油污染的环境中,石油烃降解菌占环境中细菌总数的比例很低;石油污染越严

重,石油烃降解菌的数量越多;当污染逐渐减轻时,石油烃降解菌的数量也随之减少[46-47].因此,石油烃降解菌的数量和菌群的变化也是石油污染物在海面或沉积物中随时间迁移和降解程度的良好指标[48].

2 强化微生物修复石油污染技术(Enhanced microbial remediation techniques of petroleum pollution)

2.1 引入外源石油烃降解菌

利用微生物进行石油污染海洋的原位修复过程中,单一土著石油烃降解菌对组分复杂的石油污染物存在降解效率较低与修复风险较大等缺点.将外源石油烃降解菌引入到修复场地与土著微生物形成混合菌群,利用不同石油烃降解菌对不同碳含量石油烃的降解互补作用,可以最大程度的减少或避免由于单一菌种对环境的不适应性或碳源选择性等引起的问题,有效提高石油污染物的去除率[49].由印度古吉拉特邦 ONGC 油田的原油污染地分离出的 6 种石油烃降解菌组成的混合菌群经过 75 d 的培育,对 C8—C35 的降解率达到 83.70%,显著高于每种石油烃降解菌的单独降解率[50].Alisi 等的研究发现,利用 10 种石油烃降解菌组成的 ENEA-LAM 微生物配方修复重金属和柴油污染土壤时,75%的柴油在 42 d 内被清除,其中 C12—C20 的正烷烃被完全降解[51].

通常,单一石油烃降解菌对性质稳定的环烷烃和多环芳烃等进行截止式转化[52].例如能将环已烷转化为环已酮的微生物不能进行内酯化和开环,而能将环已酮内酯化和开环的微生物却不能将环已烷转化为环已酮[53].因此,利用混合菌群清除石油烃时,一种微生物的代谢产物可能是另一种微生物生长所需的底物,避免了因转化产物积累造成的降解效率低下,有效促进和加快受石油污染环境的修复[46, 54].

但是,在引入外源石油烃降解菌时,需要充分考虑外源微生物与土著微生物之间的相容性.如果二者相容性较好,通过协作和代谢互补作用,可以实现污染物的完全降解.但是,如果外源微生物在营养物质和石油烃的吸收利用等方面与土著微生物处于竞争关系,外源石油烃降解菌的引入则会降低土著微生物的降解效果[55].

2.2 投加生物表面活性剂

生物表面活性剂是微生物在其细胞表面产生的具有较强乳化活性和增溶作用的次生代谢产物,包括低分子表面活性剂(糖脂、脂肽、磷脂等)和高分子表面活性剂(脂蛋白等)[56-58].表 3 列出部分石油烃降解菌属及其产生的生物表面活性剂.投加生物表面活性剂,可将海面或海底沉积物中的石油乳化分散成微小液滴,增加石油的溶解度,降低油滴与微生物细胞壁以及油滴与水的界面张力,使石油烃通过一定的通道和途径进入到吸附在其表面的石油烃降解菌体内,而后被吸收和利用,有效提高石油烃的生物可利用性和修复效率[59-63].铜绿假单胞菌(*Pseudomonas aeruginosa*)S6 在中性及弱碱性条件下产生的糖脂类生物表面活性剂可使水中菲的溶解度增加约 23 倍[64].雷依丰[65]的研究结果指出,添加 100 mg L^{-1} 的海藻糖脂后,以萘和蒽为唯一碳源的红平红球菌 FF 的最大生长量分别提高了 145%和 51.86%,FF 菌对萘和蒽的去除率在 6 d 内分别提高了 23.86%和 22.69%.Wang 等[66]报道,用生物表面活性剂处理后的土著微生物对海洋沉积物中石油烃的总降解率为 51.29%,比未经生物表面活性剂处理提高了 17.8%.

相对于商业合成的化学表面活性剂,生物表面活性剂具有较低的临界胶束浓度和更高的表面活性,能有效提高疏水性难降解有机物(如多环芳烃)的溶解度,使石油烃降解菌容易利用和降解这些物质.Pi 等[67]在修复模拟近海水域石油漏油的研究中发现,鼠李糖脂对石油烃的生物修复系数是商用化学表面活性剂 GM-2 的 2.04 倍.生物表面活性剂对石油烃降解菌没有毒害作用,可降解性高,不易造成二次污染[68-69].

很多生物表面活性剂生产菌本身就是石油烃降解菌.这些微生物不仅能够分泌生物表面活性剂,同时还会消耗部分石油烃,对石油烃的降解有良好的正向辅助作用.因此,生物表面活性剂生产菌与土著微生物形成的混合菌群能有效提高石油污染海洋的修复效率,降低修复成本[70-71].苏增建等[59]的研究指出,添加以葡萄糖为基质培养的生物表面活性剂生产菌 BSM-301 后,土著弧菌属(*Vibrio*)细菌 SJDQ-112 和 SJDQ-1122 对石油烃的降解效率分别提高了 64.9%和 86.2%.多种生物表面活性剂生产菌混合后具有更好的稳定性和更强的增溶能力.Cerqueira 等[72]的研究结果显示,由巨大芽孢杆菌(*Bacillus megaterium*),铜绿假单胞菌(*Pseudomonas aeruginosa*)和蜡样芽孢杆菌(*Bacillus cereus*)等 5 种石油烃降

解菌组成的混合菌群的乳化活性高达 55.1%,对脂肪烃和芳香烃的去除率分别达到 90.7%和 51.8%.

表 3 生物表面活性剂及其生产菌

Table 3 Biosurfactants and biosurfactant-producing bacteria

生物表面活性剂 Biosurfactants	生物表面活性剂生产菌 Biosurfactant-producing bacteria
脂肽	芽孢杆菌属(*Bacillus*)[73]; 气单胞菌属(*Aeromonas*)[74]; 微球菌属(*Micrococcus*)[59]
鼠李糖脂	迪茨氏菌属(*Dietzia*)[75]; 假单胞菌属(*Pseudomonas*)[57]; 戈登氏菌属(*Gordonia*)[76]
海藻糖脂	红球菌属(*Rhodococcus*)[65]; 节杆菌属(*Arthrobacter*)[77]
脂蛋白	不动杆菌属(*Acinetobacter*)[58]

2.3 施加营养物质和增加溶解氧

石油烃类污染物在自然条件下可为石油烃降解菌提供足够的碳源,但是并不能为微生物生长提供充足的氮磷等营养元素[78].在石油烃降解过程中加入适量的尿素、磷酸盐和钾肥等可有效促进微生物的生长和活性,有利于生物表面活性剂的产生,有效提高石油烃的微生物降解速度和降解程度[79]. Nikolopoulou 和 Kalogerakis[80]将含尿酸的亲脂肥与鼠李糖脂生物表面活性剂共同作用清除海洋石油污染,C19—C34 在第一周和首 18 d 内的去除率分别达到 80%和 96%.但是,施加过量的含氮营养物质可能促进硝化细菌的活性和硝化反应的进行,反而不利于石油烃的降解.因此只有添加适当类型和适当量的营养物质时才能加速强化微生物对石油烃的降解.

由于大多数石油烃进行好氧降解,因此,海洋中溶解氧的含量直接影响微生物的降解效率.通过物理或化学措施增加溶解氧含量,可有效改善海洋环境中微生物的活性,提高石油烃的微生物降解速度和程度.Boufadel 等[81]报道,向 4 个阿拉斯加威廉王子湾的海滩深处注入 H_2O_2 和营养物质进行生物修复后,来自 Exxon Valdez 油轮泄漏的多环芳烃的生物降解率提高至 13%—70%.Bellagamba 等[82]的研究结果指出,在被石油污染的沉积物中放入一定尺寸的阳极,利用 2 V 的低压电解海水驱动氧气的产生,增加溶解氧浓度,从而加快石油烃的生物降解.

2.4 应用微生物固定化技术

如图 2 所示,采用包埋法、吸附法和交联法等固定化技术将游离微生物限制在有限空间内形成的固定化微生物具有更高的聚集密度和降解活性[82-86].陈远以改性竹炭为载体固定从炼油厂曝气池回流污泥中筛选出的威尼斯不动杆菌(*Acinetobacter venetianus*),经过 96 h 的培养,该固定菌对柴油的降解率高达 86.35%[87].Liu 等[88]在含油海水修复研究中发现,固定石油烃降解菌的海藻酸钙微球对初始含油量为 2342 mg L^{-1}的海水的降解率为 78%,该降解率约为游离菌的 2 倍.Seaki 等[89]采用包埋法将生物表面活性剂生产菌 *Gordonia* sp. JE-1058 制成溢油修复剂,能在没有溶剂的情况下有效分散泄漏原油,不仅强化土著微生物的活性,而且还能加速海砂表面原油的清除.

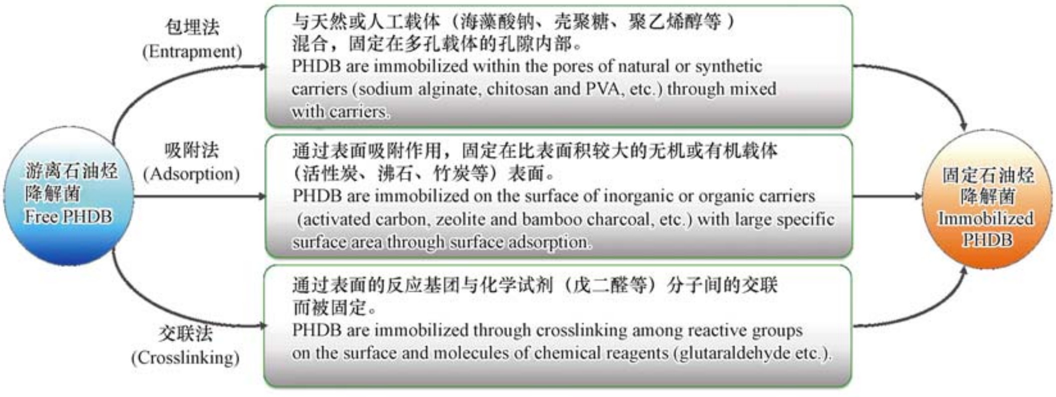

图 2 石油烃降解菌的固定化技术

Fig.2 Immobilization technology of petroleum hydrocarbon-degrading bacteria

将营养元素与微生物同时混合固定可进一步提高微生物对 pH 等环境条件的耐受力.王鑫等[90]报道,以 0.1 g 膨胀石墨为载体,将石油烃降解菌进行掺氮包埋制成 D3 菌剂,对柴油的去除率可达到 94.3%.微生物在固定化过程中会分泌大量含多糖和蛋白质的胞外聚合物.这些胞外聚合物对微生物在载体表面的附着固定和微生物间的聚集起到非常重要的作用.

3 结论和展望(Conclusions and prospects)

石油烃降解菌能快速适应受石油污染环境,但单一菌种对组分复杂的石油污染物的降解效率较低.通过引进外源石油烃降解菌、投加生物表面活性剂、施加营养物质和增加溶解氧浓度、采用石油烃降解菌固定化等强化技术,可有效提高石油烃降解菌对石油污染物的降解效率.

任何单一技术都难以达到理想的修复效果,研究发展高效降解和节能修复技术,并将其与微生物技术联合应用是今后石油污染环境修复的主要研究方向.芬顿氧化与微生物降解联合技术经试验室研究证明可以提高难降解石油烃的可生化性,提高微生物对石油烃的去除率.外加电场与微生物降解耦合的生物电化学技术通过阳极电极对细菌种群的选择性富集,提高石油烃的去除能力.生物电化学技术在修复受污染环境的同时又能产生额外电能,回收能量,实现废物资源化利用.然而,该技术中的产电菌和石油烃降解菌在修复过程中的协同作用机理,电子传递机制等还需要进一步研究.

微藻与细菌共同作用可使溢油与海洋中的矿物颗粒形成体积较大、沉降速率较快的石油—矿物颗粒聚集体,对石油污染物的纵向迁移和去除发挥重要作用,具有很好的海洋环境修复潜力.然而,在修复过程中,石油烃降解菌与微藻之间的相互作用,菌藻体系降解石油烃的机理,菌藻分泌的胞外聚合物对石油-矿物颗粒聚集体形成的作用等需要进一步研究.微生物修复技术在未来的发展中还需对石油污染物的降解性和能源的回收功效进行综合考虑.

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