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包气带-地下水石油烃污染及其原位生物修复潜力表征

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摘 要 包气带-地下水石油烃污染是全球普遍存在的环境问题,原位生物修复可同时有效修复包气带和地下水,对有效控制石油烃污染有重要意义。介绍了石油烃的组成、危害与分布形态,分析了石油烃原位生物修复过程,指出了通用型生物传感器可用于检测石油烃生物可利用性、表征石油烃生物修复潜力,并在总结研究进展的基础上,展望了深入的探索方向。

关键词 包气带-地下水 石油烃 原位生物修复 生物可利用性
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地下水是一种隐形的淡水资源,在解决水资源短缺问题中扮演重要角色[1]。石油是一种重要的化工原料,很大程度地促进了国民经济发展和人们生活水平提高[2],然而,伴随石油开采、加工、运输、储存和使用过程,大量石油烃通过包气带进入地下水系统[3,4],造成全球性普遍存在的包气带-地下水石油烃污染问题[4],对人类健康有直接危害或潜在威胁[5]。因此,亟需探索具有潜力的石油烃修复技术。

目前,包气带-地下水石油烃修复技术主要有物理、化学和生物 3 类,生物修复技术因具有高效性、生态友好性和经济可行性等优点而得以广泛应用[6]。石油烃生物修复,是利用自然界中普遍存在的、具有生物活性的微生物降解石油烃[7],可分为原位和异位生物修复。原位生物修复包括自然衰减监测与加强生物修复(生物刺激和生物强化),异位生物修复主要采用生物通气法、生物堆肥法、反应器等技术[8],选择适宜的生物修复技术是有效解决石油烃污染问题的关键。原位生物修复可以同时修复包气带和地下水,且不需要挖掘[9]、不对包气带-地下水系统造成扰动,成本更低[10],具有更高的研究价值。原位生物修复潜力受很多因素影响,主要包括①石油烃自身性质:组成成分、浓度、分布、残留时间和生物可利用性等;②场地因素:微生物多样性与活性、包气带介质、温度、氧气、污染历程、水文地质学和地球化学等[11]。

了解石油烃组成与分布形态特征、包气带-地下水石油烃原位生物修复潜力是制定相应生物修复方案的重要依据。因而,本文结合近年来国内外关于包气带-地下水石油烃污染与生物修复研究成果,主要从以下 3 个方面进行综述:①包气带-地下水石油烃组成与分布形态;②包气带-地下水石油烃原位生物修复过程分析;③石油烃生物可利用性与遗传毒性评估。

1 包气带-地下水石油烃组成与分布形态

1.1 包气带-地下水石油烃组成与危害

石油污染物是由多种水溶性低、黏滞性大的复杂碳氢化合物及其衍生物按一定数量结合而成的混合物[12],主要来源于原油、柴油、汽油、煤油、润滑油、加热燃料油、石蜡和沥青油[13],是包气带-地下水系统中最普遍的一类污染物,其主要成分为烷烃(正烷烃、支链烷烃、环烷烃)、芳香烃(单环芳烃、多环芳烃)、树脂(含有氮、磷、氧元素的化合物)、沥青质和少量其他有毒有机化合物[14],其中,烃类石油污染物称为石油烃(total petroleum hydrocarbon)芳

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香烃及其衍生物等污染物难以生物降解[15],且具有致癌、致畸和致突变的“三致”效应[16],一旦进入包气带-地下水系统,会直接威胁着人体健康和生态环境安全[17]。

1.2　包气带-地下水石油烃分布形态

石油污染物泄漏后,最先污染周边地表水和包气带[18],若其含量比较高,则会在降雨淋刷、地下水位波动等因素下[19],经包气带中垂向运移和地下水(含水层)中侧向运移而再分布[20],造成包气带-地下水石油污染。

石油污染物在包气带孔隙中垂向运移过程中,会因自身性质及其与包气带介质的相互作用[21]而改变分布形态、影响生物修复潜力[22]。一般情况下,包气带-地下水石油污染物分布形态主要有 3 种:①残留态:不易溶于水,可被基质颗粒吸附,难于排出毛细带[23];②溶解态:可溶于水,随水流动[24];③自由态:可自由流动的非水相流体 NAPL(non-aqueous phase liquid),按照密度大小,又可分为轻质非水相流体(LNAPL)和重质非水相流体(DNAPL)。其中,以烷烃、芳香烃等为代表的石油烃属于 LNAPL 类,主要存在于地下水位附近,形成透镜体,可随水位波动而改变垂向分布状态[25]。此外,溶解态和自由态的石油污染物会在水动力驱动下发生侧向运移。

2　包气带-地下水石油烃原位生物修复过程分析

2.1　氧气时空变化

石油烃污染生物修复过程中,包气带-地下水系统逐渐从污染状态变为健康状态,并伴随着一系列环境指标的变化[26]。氧气是一个重要的环境条件,会改变微生物群落结构、影响石油烃生物降解效率[27]。一般情况,包气带具有足够的氧气作为电子受体,微生物群落主要为好氧微生物,具有较高的生物活性,而地下水(含水层)一般缺乏氧气,厌氧微生物处于优势地位,不利于地下水石油烃的生物修复[28]。

然而,由于包气带异质性[29]、毛细带形态等特征[30]、气候性、季节性条件改变,地下水位波动等原因,会改变氧气的传递与分布。在线监测氧气时空变化,可以从一定程度上反映石油烃的生物降解途径、效率与生物修复潜力[31]。目前,氧气在线监测主要采用接触式探针、非接触式光学方法等[32],Haberer 等[33]提出了一种基于光极、高分辨率、非侵入式氧气监测方法,研究包气带-地下水界面的氧气分布,并模拟了氧气二维流通实验,发现地下水毛细带边缘的垂直流体动力学分散是限制氧气传递的重要因素,而毛细带形态对氧气传递没有任何影响。

2.2　石油烃动态变化

石油烃组分具有各自特殊的性质[34],在包气带-地下水系统中迁移、生物降解过程中存在很大差异[35]。石油烃含量和组成的动态变化是对其生物降解的反馈[36],可采用气相色谱、高效液相色谱、气质联用仪、光谱等方法[37]分析石油烃的再分布与含量[38]、组分的动态变化,并此作为环境效应的标志物[39]来指示石油烃的来源和所处环境条件。杨明星等[40]在石油烃污染场地设置监测井长期取样,并通过气质联用仪(GC-MS)定量分析石油烃含量、定性分析石油烃组成,来掌握地下水石油烃动态变化。Abbas O 等[41]采用红外和紫外光谱来分析石油烃动态变化,测定被生物降解和未被生物降解的石油烃组成情况,并发现不同的生物降解阶段有其相应的降解速率。

2.3　石油烃生物降解性能

好氧生物降解是包气带-地下水石油烃生物修复的主要途径,其代谢最终产物为二氧化碳,二氧化碳含量可以反映石油烃好氧生物降解性能[42,43]。然而,包气带-地下水系统存在异质性,常规侵入式原位监测技术仅可提供离散位置上监测井处的信息[44],无法真实表征石油烃原位生物修复潜力,且成本高昂、存在一定的技术挑战[45]。

将地球物理学技术与同位素标记二氧化碳分析联用,可以实时探测石油烃污染及其好氧生物修复过程[46],是目前具有较好应用前景的技术。Guimbaud 等[47]研发了一个同位素比红外光谱仪,用于鉴定石油烃好氧生物降解过程中释放的二氧化碳的生物化学来源,研究发现该技术可以快速、精准地监测出大量具有稳定同位素信号的二氧化碳及其所占全部二氧化碳释放量的比例。Noel 等[48]开发了一种光谱诱导极化法,利用与甲苯具有相同稳定同位素信号的二氧化碳生成演变过程来表征微生物活性,发现光谱诱导极化紧随微生物活性变化而产生位相移动,表明该方法可以应用于监测甲苯好氧生物降解过程。

2.4　微生物活性

微生物活性,是指一段时间内微生物所有代谢活动的总和,可以通过微生物代谢速率、放射性同位素标记和微生物细胞内 RNA 浓度来间接表征微生物活性大小[49]。微生物代谢速率主要是通过 O_2 消耗率、CO_2 生成率和代谢过程诱导的温度条件变化来测定[50];放射性同位素标记是利用痕量放射性同位素标记前体物质,通过微生物体内放射性同位素

的活度来测定生长速率,生长速率大的微生物则具有较高的生物活性[51];微生物细胞内 RNA 浓度主要指核糖体 RNA(rRNA)浓度,因 rRNA 是指导细胞合成酶(蛋白质)的核心信息,而微生物生命活动直接依赖于蛋白质,rRNA 浓度则可反映微生物的活性信息[52],且与微生物生长速率有很高的相关性[53],通过现代分子生物学方法,分析微生物细胞内 rRNA 的存在与丰度大小,可以实现微生物活性的表征[54],此外,信使 RNA(mRNA)作为合成酶的模板,也可以在一定程度上反映微生物的活性[55]。

2.5 酶活性

酶作为环境中物质转换和能量代谢的"催化剂",在石油烃生物修复过程中起关键性作用[56]。许多石油烃生物修复相关研究[57,58]表明,酶活性可以从一定程度上反映微生物多样性程度及污染物浓度等环境压力条件对石油烃代谢活性的影响,即酶活性是监测、描述和评价石油烃污染生物修复的一项指标[59],也是石油烃生物降解潜力的重要依据,且具有测定便捷、简单,结果准确的优势[60]。陈凯丽等[61]研究了微生物修复陕北地区石油污染土壤过程中,过氧化氢酶、脱氢酶、脂肪酶和多酚氧化酶的活性,以及微生物群落对烷烃和多环芳烃的降解活性,发现生物修复过程会提高过氧化氢酶和脂肪酶活性与烷烃和多环芳烃降解活性,而脱氢酶和多酚氧化酶活性则表现出先升高后降低的变化趋势。Rosa 等[62]研究了柴油浓度、生物刺激(无机肥料和有机肥料)和生物修复时间对石油烃去除、酶活性和微生物群落结构组成的影响,发现生物刺激可以在很大程度上提高脂肪酶的活性和石油烃去除率,石油烃初始浓度与肥料加入量则与微生物数量和群落结构相关,而生物修复时间引起的各项差异均很小。

2.6 微生物群落结构与功能基因

包气带-地下水中微生物在石油烃的迁移、转化和生物降解过程中占有重要地位[63],携带功能基因的微生物能够以石油烃作为营养物质,在降解石油烃的同时实现自身的生长繁殖、新陈代谢和微生物群落结构动态变化[64]。刘雨佳等[65]以石油烃为唯一碳源,运用传统分离培养法,从石油烃污染场地中筛选出 9 株具有石油烃降解功能的菌株,通过观察各菌株的形态与生理生化特点来确定其隶属 Pseudomonas 等 5 个菌属,鉴定其分子生物学特征发现功能基因为 *GSTs*、*alkB* 和 *LmPH* 基因。然而,自然环境中只有少于 1% ~10% 的微生物可以被培养,大量石油烃降解微生物不能利用纯培养法分离纯化[66],极大地限制了该方法的研究深度。

随着现代分子生物学的飞速发展,聚合酶链式反应-变性梯度凝胶电泳(PCR-DGGE)、Illumina 高通量测序、定量聚合酶链式反应(qPCR)、克隆文库法、稳定同位素标记(SIP)和原位荧光杂交(FISH)等大量基于核酸的非培养技术手段[67],为揭示环境中石油烃降解微生物群落结构和功能基因提供了新的支持,并可在多个层面探索石油烃生物修复的生物学过程和机制[68]。微生物 16S rDNA 序列相对稳定且具有高度保守性[69],将提取的 16S rDNA 序列进行 PCR-DGGE 技术分析,可以鉴定微生物种属与多样性[70];Illumina 高通量测序可通过测定 DNA 分子序列来揭示温度、pH、污染物浓度等环境条件变化所引起的微生物群落结构和功能基因的动态变化[71];qPCR 可通过定量分析功能基因数量和表达强度来建立生物可利用石油烃与微生物群落及功能基因之间的相互关系[72];SIP 可利用微生物降解稳定性同位素标记的石油烃,通过提取、分离和鉴定 DNA 等生物标志物来比对分析降解微生物的相关信息[73];FISH 可通过荧光标记的特异寡聚核苷酸片段作为探针与微生物基因组中的 DNA 分子杂交,在微米尺度研究特定环境微生物[74],建立起石油烃降解微生物种属和降解功能基因之间的联系[75],并进一步探索石油烃降解微生物的多样性和赋存状态[76]。Davis 等[77]研究发现 SIP 和 FISH 在石油烃的定源、示踪和演化等方面也可以起到重要作用,是进行地下水石油烃污染取证的方法。

3 石油烃生物可利用性与遗传毒性

现代分子生物学方法相结合可用于石油烃降解微生物群落结构和功能基因研究,为石油烃生物修复机制研究提供科学依据,却无法表征石油烃生物修复潜力[78]。石油烃在包气带-地下水系统迁移、转化过程中,仅有一部分可被微生物降解利用[79],为快速检测可被微生物降解的石油烃含量,可以构建通用型石油烃细胞生物传感器,检测石油烃生物可利用性、评估石油烃遗传毒性[80]。

生物传感器是由固定化的生物成分(如酶、蛋白质、生物膜)或生物体本身(如细胞、微生物、组织等)作为敏感材料的分子识别元件与信号转换器相结合的一种检测装置[81],具有选择性好、灵敏度高、价格低廉、可实现复杂体系中在线连续检测的特点[82]。Li 等[83]通过 3 步筛选程序开发了一种多环芳烃在线通用型生物传感器,用于定量检测水环境中 3-5 环多环芳烃,并与 GC-MS 所测样品的含量进行对比分析,发现大多数样品的两种检测结果具有良好的相关性。

近年来,随着分析技术的微型化发展,使得以细胞作为敏感元件的细胞生物传感器成为研究热点[84],细胞生物传感器是由具有生物活性的细胞与其他转换器组合而成的生物传感器,所测得污染物为生物可利用部分[85],是其他方法难以实现的。Hu 等[86]以 HEK-293 细胞作为敏感元件,开发了水杨苷细胞生物传感器,发现此细胞生物传感器对于水杨苷具有很高的响应,可完成水杨苷生物可利用性检测。

4 结论与展望

综述了石油烃的组成、危害与分布形态,分析了包气带-地下水石油烃原位生物修复过程,并指出通用型生物传感器的建立可以检测石油烃的生物可利用性、评估石油烃的遗传毒性、表征石油烃的生物修复潜力。基于目前的研究进展,可以从以下几个方面深入探索:①好氧生物降解是石油烃原位生物修复的主要途径,氧气是好氧生物降解的重要条件,需进一步研发氧气实时监测技术,建立氧气-石油烃生物降解预测模型,为制定相应生物修复方案提供理论依据;②加强对石油烃原位生物修复过程分析,识别功能微生物与功能基因的核心作用,重点考察微生物活性与石油烃生物修复效能的相关关系;③构建特异性石油烃细胞生物传感器,完善石油烃生物修复潜力表征。

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Pollution of Petroleum Hydrocarbon and Potential Characterization of Its In-situ Bioremediation in Vadose-groundwater System

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[**Abstract**] Petroleum hydrocarbon pollution in the vadose-groundwater system has become a globally ubiquitous environmental problem, in-situ bioremediation can successfully remediate the vadose and groundwater at the same time, which has great significance to control the pollution of petroleum hydrocarbon effectively. Introduction of the composition, including dangers and distribution of petroleum hydrocarbon, was presented, the process of petroleum hydrocarbon bioremediation was analyzed, in addition, the bioavailability of petroleum hydrocarbon and the potential of its bioremediation, which could be detected and characterized by the universal biosensor, were also pointed out. At last, the further exploration directions were prospected on the basis of summarizing the research progress.

[**Key words**] vadose-groundwater petroleum hydrocarbon in-situ bioremediation bioavailability