

地下枯竭油藏残余原油生物气化研究进展

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丁恒武, 周卓, 承磊. 地下枯竭油藏残余原油生物气化研究进展[J]. 微生物学报, 2025, 65(6): 2338-2352.

DING Hengwu, ZHOU Zhuo, CHENG Lei. Research progress in methanogenic degradation of crude oil in depleted subsurface oil reservoirs[J]. *Acta Microbiologica Sinica*, 2025, 65(6): 2338-2352.

摘要: 我国对原油和天然气的需求量巨大。通过传统开采方式(如水驱、气驱、化学驱和微生物驱等), 仍有超过 1/2 的原油残留在地下, 无法通过“流动”的方式被开采利用。通过厌氧微生物代谢, 将液态原油生物转化为气态天然气, 进行“沼气化”开采, 有望成为枯竭油藏残余原油开发的未来技术。本文总结了原油生物气化的研究历史、研究进展以及下一步研究方向, 从而为原油生物气化的工业应用提供参考。

关键词: 枯竭油藏; 石油烃; 厌氧降解; 产甲烷

Research progress in methanogenic degradation of crude oil in depleted subsurface oil reservoirs

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Abstract: China has considerable demands for crude oil and natural gas. After oil recovery by conventional methods (such as water flooding, gas flooding, chemical flooding, and microbial enhanced oil recovery), more than 50% of the crude oil remains inaccessible in subsurface oil reservoirs, unable to be recovered by conventional flooding techniques. The residual crude oil

资助项目: 国家自然科学基金(92351301, 32325002); 中国农业科学院科技创新工程项目(CAAS-ASTIP-2021-BIOMA); 四川省科技计划(2024NSFTD0020)

This work was supported by the National Natural Science Foundation of China (92351301, 32325002), the Agricultural Science and Technology Innovation Project of the Chinese Academy of Agriculture Science (CAAS-ASTIP-2021-BIOMA), and the Science and Technology Program of Sichuan Province (2024NSFTD0020).

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Received: 2024-09-26; Accepted: 2024-11-12; Published online: 2024-12-26

could be converted into natural gas by anaerobic microorganisms, which makes it exploitable as biogas. This innovative approach shows promise as a microbial enhanced energy recovery technology for exploiting residual crude oil in depleted oil reservoirs. This review summaries the historical development and recent advancements in the methanogenic degradation of crude oil and makes an outlook on the future research directions to facilitate the industrial application of this approach.

Keywords: depleted oil reservoir; petroleum hydrocarbon; anaerobic degradation; methanogenesis

原油和天然气是我国重要的化石能源和化工原料, 2023 年的消费量分别占全球的 16.5% 和 10.1%, 对外依存度分别为 73.0% 和 40.5% (原油产量 2.09 亿 t, 进口量 5.64 亿 t; 天然气产量 2 343 亿 m³, 进口量 1 592 亿 m³)^[1]。过去 10 年, 我国原油年消费量从 5.24 亿 t 增至 8.23 亿 t, 增量高达 57.1%, 位居全球之首(根据日消费量换算); 天然气消费量从 1 719 亿 m³ 增加至 4 048 亿 m³, 增量高达 135.5%, 位居全球第四^[1]。截至 2020 年, 我国已探明的原油地质储量约 416 亿 t, 经济可采储量约 98 亿 t, 无经济开采效益的原油储量超过 300 亿 t^[2]。目前, 我国大庆、长庆、新疆、胜利、西北、渤海及延长油田等 7 个油田采出液的综合含水率超过 60%, 其中胜利、大庆油田综合含水率超过 90%^[3]。此外, 西北油田的采出程度接近 70%, 大庆、胜利、新疆油田的采出程度超过 80%^[3]。残余原油分布在地质条件复杂的油藏中, 通过传统原油剥离和流动的采油技术, 难以有效开采这些残余原油^[3-5]。因此, 如何最大限度地开发低品位油藏资源, 保障能源安全, 已成为我国石油行业的重大课题。

油藏是研究深部生物圈的一个特殊“窗口”, 其独特的缺氧、高温、高压和油气水共存一体的环境, 孕育了丰富多样的微生物资源^[6-8]。早在 1926 年, 科学家就报道了油藏中存在硫酸盐还原微生物^[9]。进入 21 世纪后, 又发现油藏微生物能够转化原油产生甲烷, 且这种现象在

全球的地下油藏环境中普遍存在^[10-11]。鉴于此, 科学家提出了一种新的原油开采思路: 利用厌氧微生物的生物代谢活性, 将残留在地下、难以通过“流动”开采的原油, 转化为甲烷(CH₄)和二氧化碳(CO₂), 进行“沼气化”开采^[12-16]。本文梳理总结了原油生物气化领域的研究进展。

1 原油生物气化的研究历史

在 20 世纪 40 年代, 科学家观察到了硫酸盐还原和产甲烷条件下石油烃的厌氧生物降解过程^[17-18]。当时厌氧操作技术不成熟, 这些生物降解过程是否真正发生在严格厌氧环境还存在很大争议。直到 1999 年, Zengler 等^[19]通过严格厌氧培养(双层厌氧密封), 首次在实验室证实了正十六烷烃降解产甲烷的生物学过程。2004 年, Aitken 等^[20]分析了 77 个生物降解油藏样品, 其中 40 个样本中检测到 5,6,7,8-四氢-2-萘甲酸和(或)十氢-2-萘甲酸, 这 2 种化合物是厌氧芳香烃降解的中间代谢产物, 表明地下油藏可以发生石油烃厌氧生物降解反应。2008 年, Jones 等^[11]通过实验室模拟原油降解产甲烷实验, 结合油藏环境的有机地球化学和微生物分子生态学特征分析, 进一步提出地下油藏原油降解产甲烷是一个常见的生化过程。同年, Dolfing 等^[21]通过热力学分析, 提出石油烃降解产甲烷是烃降解细菌和产甲烷古菌合作的互营代谢过程, 存在 5 条可能的产甲烷途径。随后, 国内外多个团队也先后从油藏采集样品, 培养

获得了 15–55 °C 下的石油烃降解产甲烷培养物，并发现斯密斯氏互养菌属(*Smithella*)是中温条件下高频出现的互营微生物^[22–26]。Gray 等通过 qPCR 和产甲烷趋势相关性分析^[27]，本课题组通过 ¹³C-正十六烷烃培养和 DNA 稳定同位素探针^[28]，先后证实 *Smithella* 是降解石油烃的关键功能细菌。国内外多个团队先后通过单细胞测序或宏基因组技术，从石油烃降解产甲烷培养物中拼接获得了 *Smithella* 的基因组，并发现了烷基琥珀酸合酶的编码基因 *assA*，提出延胡索酸加成反应启动了烷烃的降解^[29–31]。本课题组也获得了 *Smithella* 的宏基因组组装基因组 (metagenome assembled genome, MAG)，并检出了正十六烷烃和正十五烷烃降解过程的关键中间代谢产物——甲基十五烷基琥珀酸和甲基十四烷基琥珀酸^[32]。科学家进一步应用宏基因组技术，从油藏等富烃环境中发现了其他的烷烃降解菌，如暗黑菌纲(*Atribacteria*)^[33]、*Candidatus Melinoarchaeum* (暂定种 *Candidatus*，简称为 *Ca.*)^[34] 和 *Ca. Syntrophaliphaticia*^[35]。2022 年，我们联合国内外多个研究团队，证实单一类型古菌 *Ca. Methanoliparum* 能够独立降解长链正构烷烃 ($\geq C_{13}$)、烷基环己烷和烷基苯 ($\geq C_{19}$) 产生甲烷^[36]。

2 原油生物气化的反应途径

原油成分复杂，含有数千甚至数万种不同类型的化合物^[37–38]。根据元素组成 (碳：84%–87%；氢：11%–16%；氧：0.1%–2.0%；氮：0.1%–2.0%；硫：0.06%–2.00%)，石油烃是原油的主要成分 ($>95\%$)^[39]。在石油烃中，不饱和和炔乙炔的 C–H 键离解能最高 (556 kJ/mol)，其次是苯、萘和乙烯 (460–475 kJ/mol)，随后是烷烃 (400–439 kJ/mol)，最后是烷基取代的芳烃化合物 (335–385 kJ/mol)^[40]。在有氧条件下，活性氧引入水分子产生的羟基自由基的键能为

497 kJ/mol，可以切断除乙炔外的绝大多数烃类化合物中的 C–H 键，从而启动有氧烃降解过程^[40]。在缺氧条件下，由于缺乏羟基自由基，微生物需通过一系列独特的生化反应机制克服 C–H 键较高的能障，才能启动石油烃的降解 (图 1)^[40]。

2.1 厌氧烃降解激活途径

目前已被证实的厌氧烃降解起始激活途径包括延胡索酸加成途径 (fumarate addition pathway)、羟基化途径 (hydroxylation pathway)、羧基化途径 (carboxylation pathway)、水合化途径 (hydration pathway) 和烷基辅酶 M 还原酶途径 (alkyl-coenzyme M reductase pathway)。

2.1.1 延胡索酸加成途径

在烷基/苄基琥珀酸合酶 (alkyl/benzylsuccinate synthase, Ass/Bss) 及其同源酶的催化作用下，石油烃 (烷烃、环烷烃、甲基取代芳烃) 与延胡索酸盐进行加成反应，生成相应的琥珀酸苄酯类物质，再通过碳骨架重排、脱羧、 β 氧化等步骤降解石油烃^[41–44]。含有 Ass/Bss 及其同源酶编码基因的微生物广泛分布于富烃环境中，如热液沉积物、油污污泥、河流等^[45–46]。延胡索酸加成也是油藏中常见的烃降解反应，Duncan 等^[47]在美国阿拉斯加北坡油田中观测到了延胡索酸加成产物烷基琥珀酸 (C_1 – C_4)。Bian 等^[48]在中国江苏油田、新疆油田和华北油田中检测到了烷烃 (C_1 – C_8)、甲苯和 2-甲基萘的延胡索酸加成产物及 *ass/bss* 基因。含有 Ass/Bss 及其同源酶的微生物主要分布于假单胞菌门 (*Pseudomonadota*) 和脱硫硫酸盐杆菌门 (*Desulfobacterota*) (数据已提交至国家微生物科学数据中心，登录号为 NMDCX0001713)。在油藏环境中，古生球菌 (*Archaeoglobus*)^[49–51]、脱硫硫酸盐杆菌 (*Desulfatibacillum*)^[52]、*Smithella*^[31,52] 是利用该途径进行厌氧烃降解的典型微生物。近

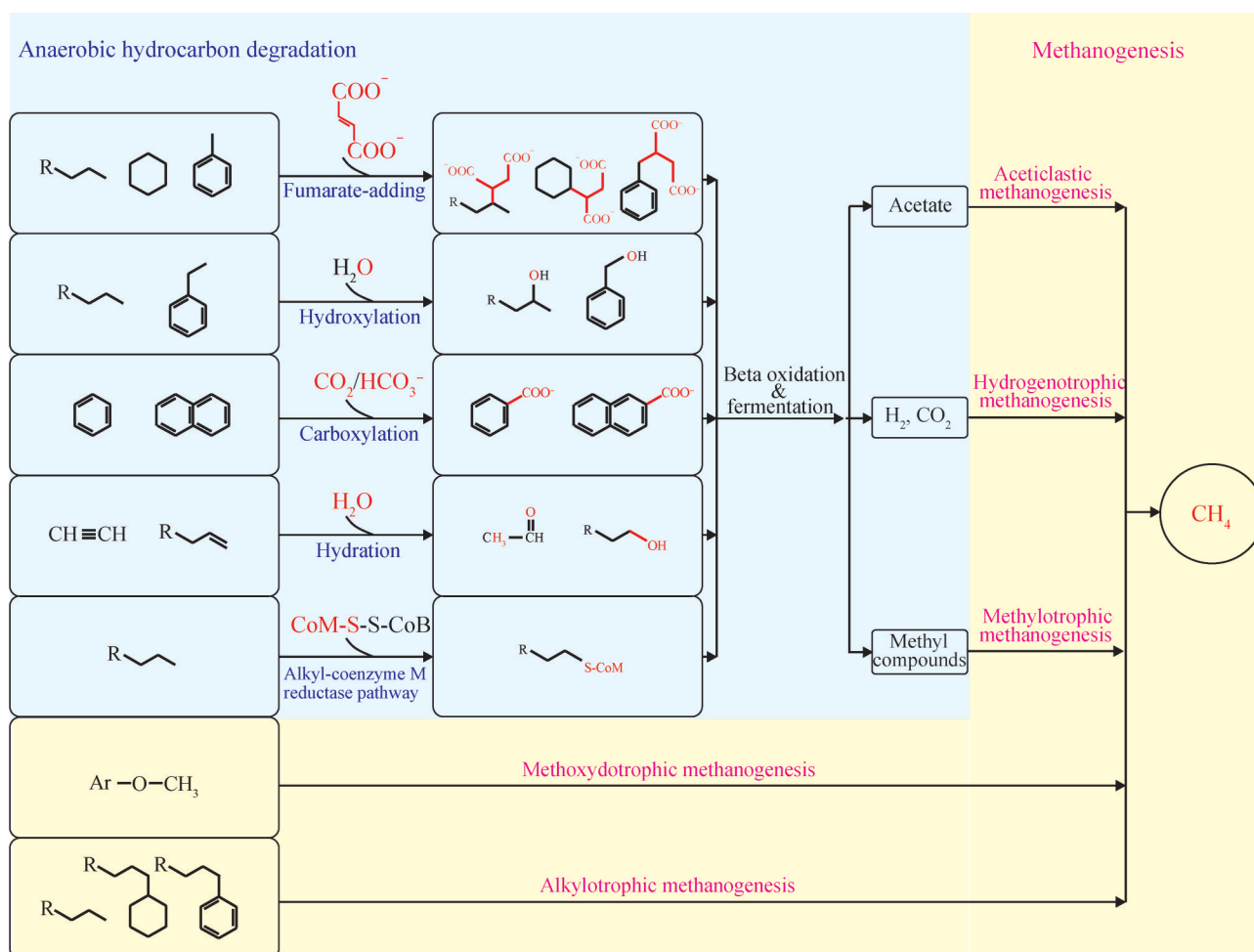


图1 石油烃降解产甲烷示意图

Figure 1 Schematic diagram of methanogenic degradation of petroleum hydrocarbon.

期, Liu 等^[35]从胜利油田采出水样本中富集培养获得了烷烃降解(C_{22} 、 C_{24} 、 C_{26} 、 C_{28} 和 C_{30})产甲烷的富集培养物, 放线菌 *Ca. Syntrophatricia* 是其中的优势菌群, 宏基因组和宏转录组分析表明 *Ca. Syntrophatricia* 的 MAGs 包含完整的 *ass* 基因, 且高丰度表达, 由此推测 *Ca. Syntrophatricia* 可以利用延胡索酸加成途径降解长链烷烃(登录号为 NMDCX0001713)。此外, Liu 等^[33]重新组装了加拿大 MHGC 油田、加拿大米尔德里德湖油泥砂、美国阿拉斯加北坡油田样本的宏基因组数据, 发现 *JS1* 类群 *Atribacteria* 的 MAGs 含有 *ass/bss* 相关基因。最

近, 本课题组联合中山大学从胜利油田中富集了一种名为 *Phoenicimicrobium oleiphilum* HX-OS.bin.34^{TS} 的 *JS1* 类群暗黑菌, 通过培养实验、宏基因组和宏转录组分析, 推测该菌株具备降解长链烷烃(C_{16} 、 C_{20} 和 C_{30})的能力(登录号为 NMDCX0001713)^[53]。

2.1.2 羟基化途径

在乙苯脱氢酶(ethylbenzene dehydrogenase, Ebd)、烷烃羟化酶(alkane hydroxylation, Ahy)或 4-异丙基甲苯脱氢酶(*p*-cymene dehydrogenase, Cmd)的催化作用下, 水分子中的氧原子被引入底物烃分子中(乙苯、烷烃、4-异丙基甲苯), 生

成相应的醇类物质，激活烃分子厌氧降解^[44,54-55]。从美国旧金山炼油厂处理池沉积物分离的固氮弯曲菌(*Azoarcus* sp.) EB1 (降解芳烃)^[54]，以及从德国汉堡港油田石油生产设备分离的食石油脱硫棍状菌(*Desulfosudis oleivorans*) Hxd3 (降解烷烃)^[56]是利用该途径进行厌氧降解的代表菌株(登录号为 NMDCX0001713)。近期，Shou 等^[57]在胜利油田和江苏油田中均发现了羟基化途径的次级降解产物(2-乙酰烷酮酸)。

2.1.3 羧基化途径

在类辅酶 Q 羧化酶(ubiquinone decarboxylase-like, UbiD-like)的催化作用下，无取代芳烃(苯、萘、菲等)与 CO₂ 或 HCO₃⁻ 反应，生成相应的甲酸类芳烃化合物，激活芳烃厌氧降解^[58-60]。Aitken 等^[20]在油藏环境中检测到了萘的羧基化代谢产物(2-萘酸)，暗示了油藏中存在羧基化途径。在厌氧条件下，无取代芳烃的降解周期长达数月至数年(如苯并蒽、芘和萘 338 d 仅降解 9%、13% 和 22%)^[61]，羧基化途径的研究进展相对缓慢。近期，Zhang 等^[62]从新疆克拉玛依油田土壤中分离的需盐反硝化枝芽孢杆菌(*Virgibacillus halodenitrificans*) PheN4 是利用该途径降解石油烃的典型菌株(登录号为 NMDCX0001713)。

2.1.4 水合化途径

在乙炔水合酶(acetylene hydratase, Ach)等的催化作用下，水分子中的氢和氧原子均被加成到不饱和烃中(乙炔、1-烯烃)，生成相应的醇类物质，激活不饱和烃厌氧降解^[63-65]。乙炔共养杆菌(*Syntrophotalea acetylenica*)是降解乙炔的代表菌^[64]。此外，*Desulfatibacillum* 属内一些烷烃降解菌也可以通过水合途径降解 1-烯烃(登录号为 NMDCX0001713)^[56,65-66]。值得注意的是，*Desulfatibacillum* 参与催化 1-烯烃水合反应的关键酶尚未得到解析^[40]。

2.1.5 烷基辅酶 M 还原酶途径

在烷基辅酶 M 还原酶(alkyl-coenzyme M reductase, Acr)的催化作用下，异二硫化物 CoM-S-S-CoB 中的 CoM-S-基团被引入烷烃的碳链上，生成相应的烷基-辅酶 M，激活烷烃厌氧降解^[67-68]。该催化机制目前仅在古菌类群中发现^[69]。利用该途径进行厌氧降解的类群被称为厌氧烷烃降解古菌(alkane-oxidizing archaea, ANKA)^[69-70]。目前，已被实验证明的 ANKA 有 *Ca. Alkanophaga* (降解中长链烷烃)^[71]、*Ca. Argoarchaeum* (降解乙烷)^[72]、*Ca. Cerberiarchaeum* (降解正十六烷)^[73]、*Ca. Ethanoperedens* (降解乙烷)^[74]、*Ca. Melinoarchaeum* (降解正十六烷)^[34]、*Ca. Methanoliparia* (降解长链烷烃)^[36] 和 *Ca. Syntrophoarchaeum* (降解丙烷和丁烷)^[67,75] (登录号为 NMDCX0001713)。近期，本课题组联合深圳大学从国内 15 个油田中采集了 209 个油藏样本，通过 qPCR 检测、16S rRNA 基因测序、宏基因组分析、宏转录组分析和代谢产物检测，发现 85% 的油藏样品存在 *Ca. Methanoliparia*，52% 的样本存在多种烷基辅酶 M 代谢物，表明烷基辅酶 M 还原酶途径是油藏环境中普遍存在的烃降解途径^[10]。

2.2 产甲烷途径

根据底物营养类型，产甲烷代谢途径可以分为 5 种类型：氢营养型产甲烷途径(hydrogenotrophic methanogenesis)、乙酸营养型产甲烷途径(acetoclastic methanogenesis)、甲基营养型产甲烷途径(methylotrophic methanogenesis)、甲氧基营养型产甲烷途径(methoxydotrophic methanogenesis)和烷基营养型产甲烷途径(alkylotrophic methanogenesis)。

在标准状况下，且无外源电子受体时，石油烃厌氧降解反应为吸热反应，无法自发进行

$[\Delta G^0 > 0]$, 表 1, 反应式(1)–(3)], 而产甲烷反应可以自发进行 $[\Delta G^0 < 0]$, 表 1, 反应式(4)–(5)]。当产甲烷古菌不断消耗烃降解菌产生的 H_2 , 使其浓度维持在 Pa 级时, 烃降解反应才能启动; 这种通过烃降解菌-产甲烷菌合作产甲烷的方式被称为“互营产甲烷”^[76-77]。传统观点认为, 互营体系只能依赖于“细菌-古菌”的互作, 然而本课题组联合上海交通大学从云南热泉富集培养获得了高温正十六烷烃降解产甲烷富集物, 发现古菌 *Ca. Melinoarchaeum* 起始了烷烃降解, 产甲烷古菌甲烷热杆菌属(*Methanothermobacter*)和产甲烷丝菌属(*Methanothrix*)参与了甲烷产生, 从而提出了古菌-古菌互营烃降解产甲烷的新模式^[34]。此外, 值得注意的是, 整个“烃降解-产甲烷”过程是放能反应 $[\Delta G^0 < 0]$, 表 1, 反应式(6)–(8)], 这意味着微生物可以直接代谢石油烃产生甲烷。本课题组培养发现的新型古菌 *Ca. Methanoliparum* 可以直接将烷基烃转化为甲烷[表 1, 反应式(8)], 这种产甲烷方式被称为“烷基型产甲烷”^[36]。

2.2.1 氢营养型产甲烷途径

氢营养型产甲烷途径是指古菌利用 H_2 作为电子供体还原 CO_2 产生 CH_4 的途径, 也称为 CO_2 还原途径^[78]。根据基因组分类数据库

(GTDB-R220)的分类信息, 目前至少有 4 门 10 纲的古菌可以利用该途径进行产甲烷代谢: (1) *Halobacteriota* (*Archaeoglobi*、*Bog-38*、*Methanocellia*、*Methanoliparia*、*Methanomicrobia*、*Methanosarcinia*); (2) *Methanobacteriota* (*Methanobacteria*、*Methanopyri*); (3) *Methanobacteriota_A* (*Methanococci*); (4) *Thermoproteota* (*Methanomethylicia*) (登录号为 NMDCX0001713)。Mayumi 等^[79]从 Yabase 油田高温石油储层中提取了环境样本的总 DNA, 并通过 PCR 扩增及测序, 分析了 16S rRNA 基因序列, 发现来自甲烷热杆菌属(*Methanothermobacter*)和产甲烷卵石状菌属(*Methanocalculus*)的氢营养型产甲烷古菌是该环境中的优势类群。

2.2.2 乙酸营养型产甲烷途径

古菌将乙酸分解为 CO_2 和 CH_4 的途径称为乙酸发酵途径^[78]。目前, 只有来自甲烷八叠球菌纲(*Methanosarcinia*)的 3 个属的古菌(登录号为 NMDCX0001713)可以利用该途径进行产甲烷代谢: 甲烷八叠球菌属(*Methanosarcina*)、产甲烷丝菌属(*Methanothrix*) (原属于 *Methanosaeta*)以及去年新提出的 *Methanocrinis* 属 (部分 *Methanothrix* 物种被重新划分到该属中)^[80-81]。其中, *Methanothrix* 和 *Methanocrinis* 属古菌是

表 1 石油烃降解产甲烷的化学计量学和吉布斯自由能值的变化(修改自文献[76])

Table 1 Stoichiometry and Gibbs free energy for reactions involved in methanogenesis via petroleum hydrocarbon degradation (modified from literature [76])

过程 Process	反应式 Reaction	自由能 ΔG^0 (kJ/mol)
Hydrocarbon degradation	(1) Toluene: $C_7H_8 + 7H_2O \rightarrow 3.5CH_3COO^- + 3.5H^+ + 4H_2$	+113.6
	(2) Naphthalene: $C_{10}H_8 + 10H_2O \rightarrow 5CH_3COO^- + 5H^+ + 4H_2$	+101.1
	(3) Hexadecane: $C_{16}H_{34} + 16H_2O \rightarrow 8CH_3COO^- + 8H^+ + 17H_2$	+470.8
Methanogenesis	(4) Hydrogenotrophic: $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-135.6
	(5) Acetotrophic: $CH_3COO^- + H_2O \rightarrow HCO_3^- + CH_4$	-31.0
Overall	(6) Toluene: $C_7H_8 + 7.5H_2O \rightarrow 4.5CH_4 + 2.5HCO_3^- + 2.5H^+$	-130.5
	(7) Naphthalene: $C_{10}H_8 + 10H_2O \rightarrow 6CH_4 + 4HCO_3^- + 4H^+$	-189.5
	(8) Hexadecane: $C_{16}H_{34} + 16H_2O \rightarrow 12.25CH_4 + 3.75HCO_3^- + 3.75H^+$	-353.5

低温油藏环境中常见的产甲烷古菌类群^[82-84]。乙酸是油藏有机物降解过程中的常见中间代谢产物,在高温常压下,乙酸通过乙酸互营氧化耦联 CO₂ 还原途径进行产甲烷代谢^[79];而在高分压 CO₂ 条件下,乙酸营养型产甲烷古菌通过乙酸发酵途径将乙酸转化为 CH₄ 和 CO₂^[84]。

2.2.3 甲基营养型产甲烷途径

甲基营养型产甲烷途径是古菌将简单的甲基类化合物(甲醇、二甲基硫、甲胺、二甲胺、三甲胺等)分解为 CH₄ (甲基还原型)或 CO₂ 和 CH₄ (甲基裂解型)的途径^[78]。目前有 5 门 9 纲的古菌可以利用该途径进行产甲烷代谢: (1) *Halobacteriota* (*Archaeoglobi*、*Methanonatronarchaeia*、*Methanosarcinia*); (2) *Methanobacteriota* (*Methanobacteria*); (3) *Methanobacteriota_B* (*Thermococci*); (4) *Thermoproteota* (*Methanomethylicia*、*Nitrososphaeria*、*Korarchaeia*); (5) *Thermoplasmata* (*Thermoplasmata*) (登录号为 NMDCX0001713)。最近,本课题组从胜利油田中分离出了 *Thermoproteota* (原属于 *Verstraetearchaeota*) 古菌 *Methanosuratincola petrocarbonis* LWZ-6,证实了它具有依赖 H₂ 还原甲醇产甲烷的能力,但不具备发酵生长的潜力,从而直接通过生理实验证实了产甲烷古菌可以分布在非广古菌门以外的类群^[85]。此外,本课题组联合国外团队研究发现,甲基营养型产甲烷古菌通过与产甲醇细菌合作,以种间甲醇转移的方式参与了产甲烷代谢,丰富了种间互营产甲烷代谢功能的认知(Huang et al., 原则接受)。这些新型的甲基营养型产甲烷古菌在湿地、动物胃肠道、厌氧消化器、深部地下环境、深海沉积物等环境中广泛分布^[86],它们的生理生态学功能有待进一步研究。

2.2.4 甲氧基营养型产甲烷途径

甲氧基营养型产甲烷途径是指古菌将甲氧

基芳烃类化合物(ArO-CH₃)分解为酚类物质(ArOH)并产生 CO₂ 和 CH₄ 的途径^[87-88]。本课题组从胜利油田中分离并鉴定了产甲烷古菌新物种,并命名为胜利油田热甲烷微球菌(*Methermicoccus shengliensis*) ZC-1^T,过去认为它只能利用甲醇、甲胺等甲基类化合物产生甲烷^[89]。10 年后,Mayumi 等^[87]发现该物种可以直接利用烟煤、褐煤和无烟煤等不同类型的煤炭产生甲烷,进一步研究发现 *M. shengliensis* 可以通过去甲基化的方式利用超过 30 种不同的甲氧基芳香化合物来产甲烷,并提出了第 4 条产甲烷途径——甲氧基营养型产甲烷途径。近期,Kurth 等^[88]通过转录组、蛋白质组和酶学实验,鉴定了去甲基化系统(demethoxylation system, Mto)的关键基因——MtoABCD。

2.2.5 烷基营养型产甲烷途径

古菌将长链烷基烃(烷烃、烷基环己烷、烷基苯)分解为 CO₂ 和 CH₄ 的途径为烷基营养型产甲烷途径^[10,36]。*Ca. Methanoliparum* 是利用该途径进行产甲烷的代表类群(登录号为 NMDCX0001713)^[10,36]。传统观点认为原油生物气化需要烃降解菌和产甲烷菌的互营代谢才能完成(图 2A)^[36,90],而 *Ca. Methanoliparum* 基因组存在烷基辅酶 M 还原酶基因、 β 氧化途径、Wood-Ljungdahl 途径和甲基辅酶 M 还原酶途径,可以直接代谢 C₁₃ 及以上烷烃、C₁₉ 及以上烷基环烷烃和烷基苯产生甲烷(直接利用原油产甲烷)^[36]。这突破了产甲烷古菌只能利用 C₁ 和 C₂ 简单化合物产甲烷的传统认知,是一条全新的甲烷产生途径,将其命名为烷基型产甲烷代谢途径(图 2B)^[36]。

3 原油生物气化的产气性能

3.1 产甲烷潜量

单位质量原油产生甲烷气的总体积

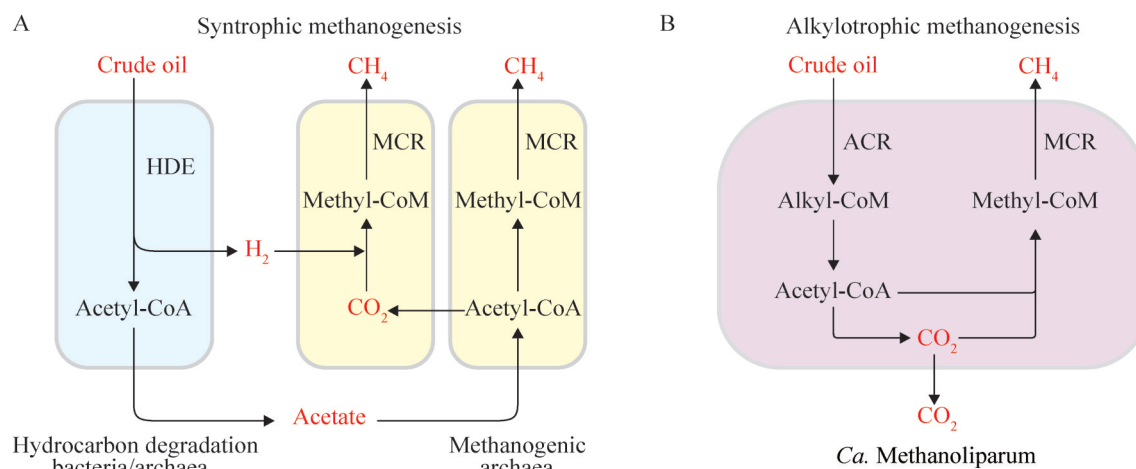


图2 原油生物气化的反应模式(修改自文献[90])。A: 互营产甲烷; B: 烷基型产甲烷。HDE: 烃降解酶; MCR: 甲基辅酶M还原酶; ACR: 烷基辅酶M还原酶。

Figure 2 Reactive mode of crude oil biogasification (modified from literature [90]). A: Syntrophic methanogenesis; B: Alkylotrophic methanogenesis. HDE: Hydrocarbon degrading enzyme; MCR: Methyl-coenzyme M reductase; ACR: Alkyl-coenzyme M reductase.

(单位: m^3/t)可以衡量原油生物气化的产甲烷潜量^[12,91-92]。20 世纪 90 年代, 本课题组对辽河稠油开展了厌氧微生物降解实验, 测得甲烷的产气潜量为 $162.70\text{--}176.75 \text{ m}^3/\text{t}$ ^[12]。随后, 本课题组又采集了国内数个油田的稠油样本, 并开展了生物气模拟试验, 测得甲烷的产气潜量为 $8.78\text{--}9.92 \text{ m}^3/\text{t}$ ^[93]。通过物模实验, Gieg 等^[23]测得甲烷的产气潜量为 $67.2 \text{ m}^3/\text{t}$ (根据每克原油产气量换算)。刘岩等^[91]通过物模实验, 测得甲烷的产气潜量可达 $150 \text{ m}^3/\text{t}$ 。林军章等^[92]针对林樊家地区稠油开展了原油生物气化模拟实验, 测得甲烷的产气潜量为 $67 \text{ m}^3/\text{t}$ 。本课题组联合中国石油大学从胜利油田孤岛区块富集培养获得了稠油降解产甲烷富集物, 测得产甲烷潜量为 $29.14\text{--}42.59 \text{ m}^3/\text{t}$ (根据每克原油产气量换算)^[94], 而利用 L801 原油在 $35\text{ }^\circ\text{C}$ 和 $55\text{ }^\circ\text{C}$ 培养条件下, 其产甲烷潜力分别达到了 $76.2\text{--}90.5 \text{ m}^3/\text{t}$ 和 $56.0\text{--}70.1 \text{ m}^3/\text{t}$ ^[37]。虽然不同组分的原油降解潜力有差异, 但综合国内外的模拟培养实验, 估计原油生物气化的产甲烷潜量约为 $30\text{--}150 \text{ m}^3/\text{t}$ 。

3.2 产甲烷速率

单位质量原油在单位时间内产生的甲烷摩尔量[单位: $\mu\text{mol}/(\text{d}\cdot\text{g})$]可以衡量原油生物气化的产甲烷速率^[23]。通过物模实验, Gieg 等^[23]初步评估原油生物气化的产甲烷速率约为 $11\text{--}31 \mu\text{mol}/(\text{d}\cdot\text{g})$ 。Berdugo-Clavijo 等^[95]测得产甲烷速率为 $5.8 \mu\text{mol}/(\text{d}\cdot\text{g})$ 。在先前的研究中, 本课题组评估了不同富集培养体系下原油降解产甲烷的速率(根据原油添加量、甲烷产量和培养时间换算)分别为 $7.5\text{--}8.8 \mu\text{mol}/(\text{d}\cdot\text{g})$ ^[37]、 $6.4\text{--}8.0 \mu\text{mol}/(\text{d}\cdot\text{g})$ ^[37]、 $12.5 \mu\text{mol}/(\text{d}\cdot\text{g})$ ^[26] 和 $2.9\text{--}8.8 \mu\text{mol}/(\text{d}\cdot\text{g})$ ^[94]。近期, 本研究团队富集了以 *Ca. Methanoliparum* 为主的烷基型产甲烷菌群, 其产甲烷速率可达 $41.7\text{--}102.3 \mu\text{mol}/(\text{d}\cdot\text{g})$ (根据油泥砂含油量换算)^[36]。

3.3 最小甲烷倍增时间

产甲烷古菌只能通过甲烷产生途径获取能量进行生长, 其最小甲烷倍增时间(单位: d), 可以客观地指示产甲烷菌(群)的代谢活性^[96-98]。近期, 本研究团队比较了不同温度梯度下传统

发酵体系(以互营产甲烷为主)与新型产甲烷体系(以烷基型产甲烷为主)的甲烷倍增时间(图 3),结果显示新型产甲烷途径的甲烷倍增时间的中位数为 11.3 d (范围: 6.9–23.3 d), 低于传统互营烃降解产甲烷倍增时间的中位数 67.3 d (范围: 10.6–231.0 d), 表明新型产甲烷途径具有更高效的原油生物气化性能^[36]。

4 原油生物气化的应用策略

油藏环境中富含碳氢化合物, 碳氮比和碳磷比过高, 影响微生物的生长和繁殖, 通常需要添加无机氮和磷源^[99]。此外, 油藏微生物的生长需要易于利用的碳水化合物作为其碳源。当无机盐不足以有效激活微生物时, 添加少量的有机营养物质(如葡萄糖、糖蜜、酵母粉等)能够明显地增强激活效果^[99]。原油生物气化的应用策略未来可分为“内源激活”和“外源注入”

◦ Traditional fermentation system (syntrophic methanogenesis)
 • Novel methanogenic system (alkylotrophic methanogenesis)

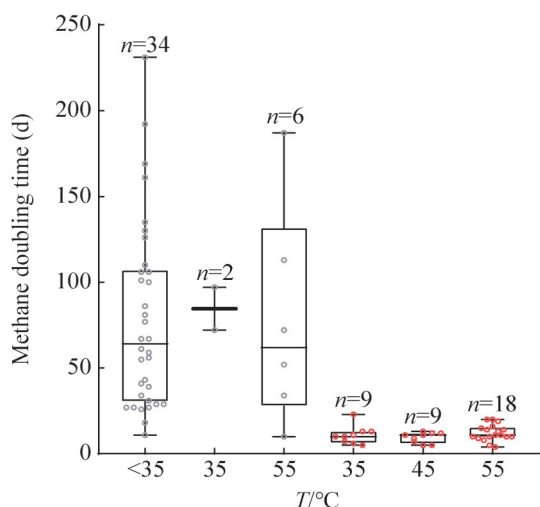


图3 传统发酵体系与新型产甲烷体系甲烷倍增时间的比较(数据源于文献[36]的统计)

Figure 3 Comparison of methane doubling time between traditional fermentation pathway and novel methanogenic pathway (data retrieved from the statistical results of literature [36]).

2 种。目前, 这 2 种应用策略仍处于实验室模拟试验阶段, 场地试验还有待进一步深入研究。

4.1 内源激活

内源激活是指向油藏中注入营养物质, 以激活油藏中存在的原油生物气化菌群, 从而增强原油生物气化速率的一种方法。Gao 等^[100]的研究表明, 在不加入营养激活剂的条件下, 实验体系几乎无法产生甲烷(表 2, 实验方案 1); 而添加营养激活剂后, 实验体系才具备一定的产甲烷活性(产甲烷 11.6 mL, 表 2, 实验方案 2)。李彩凤等^[101]的研究结果同样表明, 营养激活剂对于内源激活原油生物气化菌群是必要的(表 2, 实验方案 3 和 4)。

4.2 外源注入

外源注入是指向地下油藏中注入营养物质和发酵浓缩的微生物, 发挥外源微生物优良的烃降解能力或(和)产甲烷能力, 从而提高原油生物气化速率的一种方法。李彩凤等^[101]的研究结果发现, 仅向反应体系外源性添加嗜热嗜脂肪地芽孢杆菌(*Geobacillus stearothermophilus*) SL-1, 而不添加营养激活剂的情况下, 反应体系不具备产气能力(表 2, 实验方案 5), 表明了营养物质对于原油生物气化过程的重要性。当同时添加外源菌 SL-1 和营养物质后, 反应体系表现出了优秀的产甲烷能力(产甲烷 140.79 mL, 实验方案 6), 并且产量是内源激活的 4.8 倍(产甲烷 29.22 mL, 表 2, 实验方案 4)^[101]。王晓^[102]发现外源添加地芽孢杆菌(*Geobacillus* sp.) K4 的反应体系同样具有较强的产甲烷性能(产甲烷 48.73 mL, 表 2, 实验方案 8)。最近, 丁明山等^[103]通过物模实验, 发现外源性引入新型产甲烷古菌 *Ca. Methanoliparum* 的反应体系拥有较强的产甲烷能力(产甲烷 54.36 mL, 表 2, 实验方案 14), 其产甲烷性能是内源性激活油藏微生物(产甲烷 1.51 mL, 表 2, 实验方案 12)的 36 倍。

表2 原油生物气化模拟试验

Table 2 Simulation experiments for methanogenic degradation of crude oil

类型 (周期) Type (period)	营养激活剂配方 Nutrient activator formula	实验方案 Experimental scheme	总产气量 Total gas yield (mL)	甲烷产量 Methane yield (mL)	参考文献 References
Cultivation (90 d)	6 g/L NaNO ₃ +2 g/L (NH ₄) ₂ HPO ₄ +6 g/L molasses	(1) Formation water (2) Formation water+nutrient activator	— 186	— 11.60	[100]
Cultivation (30 d)	0.3 g/L glucose+ 0.3 g/L peptone+ 0.2 g/L yeast extract+ 0.27 g/L K ₂ HPO ₄ +0.5 g/L NaCl	(3) Formation water (4) Formation water+nutrient activator (5) Formation water+SL-1 (6) Formation water+nutrient activator+SL-1	— 117 — 277	— 29.22 — 140.79	[101]
Cultivation (35 d)	A: 0.3 g/L glucose+ 0.3 g/L peptone+ 0.2 g/L yeast extract+ 0.27 g/L K ₂ HPO ₄ +0.5 g/L NaCl B: 0.1 g/L urea+0.5 g/L NaCl+ 0.27 g/L K ₂ HPO ₄	(7) Formation water (8) Formation water+nutrient activator (A+B)+K4 (9) Formation water+nutrient activator (A+B)+methanogenesis inhibitor (10) Formation water+nutrient activator (A+B)+methanogenesis inhibitor+K4	65 185 — 150 124	0.08 48.73 — 0.42 0.05	[102]
Physical model (90 d)	0.3 g/L NH ₃ Cl+ 0.2 g/L K ₂ HPO ₄ + 0.15 g/L cysteine	(11) Formation water+nutrient activator (12) Formation water+nutrient activator+ crude oil (13) Formation water+nutrient activator+ <i>Ca. Methanoliparum</i> (14) Formation water+nutrient activator+ crude oil+ <i>Ca. Methanoliparum</i>	— 15.68* — 70.05*	— 1.51* — 54.36*	[103]

Formation water: Water was collected from oil reservoirs, and contained crude oil and indigenous microorganisms; SL-1: *Geobacillus stearothermophilus* SL-1; K4: *Geobacillus* sp. K4; —: Below detection limit; *: Data conversion according to molar quantities.

5 总结与展望

油藏是一个大型地质生物反应器。假设全国 10% 的残余原油可用于生物气化, 预计可增产甲烷气 954–4 770 亿 m³。目前, 地下枯竭油藏残余原油生物气化仍处于实验室研究阶段。为进一步发展和完善原油生物气化理论和技术, 建议重点关注以下 3 个方面的研究工作。

(1) 加强基础研究。地下油藏目前仍是一个“黑箱子”, 大部分油藏微生物仍处于未培养分离的状态。我们对它们的生理生化功能以及代谢催化机制的认识还不清楚; 特别是它们在地

下可以通过哪些途径、功能酶来催化石油烃的代谢, 我们的认知仍然有限。甚至它们在地下油藏的生存边界(80 °C是否是一个门槛)也有待进一步研究。这些基础油藏微生物学的研究会给我们带来新的启发和思路。

(2) 油藏厌氧功能菌的工业放大。油藏微生物对氧气十分敏感且生长缓慢, 因此在工业发酵过程中, 其最佳生长条件(如温度、pH 值及培养基成分等)仍有待探究。此外, 通过人工智能等新技术, 模拟、评估和优化发酵工艺, 可以为厌氧微生物稳定的工业放大提供理论基础。

(3) 油藏环境的勘探评估。油藏地质条件复

杂,在开展场地实验之前,必须先深入研究相应油藏的理化性质、地貌特征、内源微生物群落组成、注入微生物生理生化特征,以保证注入微生物与油藏环境的匹配性。另外,通过构建物理模型和先导试验,可以评估和监测原油生物气化在不同地质构造油藏中的效果,为大规模工业化应用提供工艺支撑。

致谢

感谢农业农村部成都沼气科学研究所的巫可佳、黄越义、刘伟和钟佳来在本文撰写过程中所提供的帮助。

作者贡献声明

丁恒武:论文撰写;周卓:论文撰写;承磊:论文构思、设计和修改。

作者利益冲突公开声明

作者声明不存在任何可能会影响本文所报告工作的已知经济利益或个人关系。

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