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"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (BIOLOGIA CELULAR, MOLECULAR E MICROBIOLOGIA)

Biorremediação de Hidrocarbonetos do Petróleo em Água do Mar: Avaliação Comparativa de Bioaumentação, Bioestimulação e Atenuação Natural em Microcosmos

MIGUEL FELIPE COMPRI GONÇALVES

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Orientadora: Profª Drª Lara Durães Sette.
Coorientadora: Drª Patrícia Giovanella

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“*...Vi o camarão limpando o oceano,*
Enquanto muita gente ia só sujando...
...Acredito que um dia o homem vá mudar,
E as crianças do futuro vão poder brincar...
...É preciso paciência para ensinar,
É preciso consciência para poder mudar...
...Deixa, deixa as tartarugas!
Deixa, deixa os bichos do mar!
Deixa, deixa a natureza!”

Bichos do mar – Lenine / Chico Martins / Guy Marcovaldi

RESUMO

A saúde dos oceanos é vital para a subsistência humana. Contudo, os ambientes marinhos enfrentam crescente contaminação por produtos petrolíferos, evidenciada por eventos ao longo de décadas, como o derramamento do petroleiro Torrey Canyon em 1967, bem como o desastre de 2019 no litoral brasileiro, que poluiu 3 mil quilômetros de costa com 5 mil toneladas de óleo. Métodos eficazes e acessíveis são essenciais para remediar essa contaminação, dada a complexidade da composição do petróleo. O uso de microrganismos capazes de metabolizar carbono via enzimas (mono/dioxigenases, desidrogenases, citocromo P450) torna a biorremediação, especialmente com consórcios microbianos, uma técnica promissora e menos agressiva em relação a outros métodos de biorremediação. Isolados de locais contaminados, como a costa brasileira recentemente afetada, revelam microbiota apta e adaptada, daí a importância do avanço científico na avaliação de microrganismos do litoral brasileiro para a biorremediação de compostos relevantes. Desenvolvido no âmbito do projeto CNPq 440774/2020-9 (MicroBioMaR), este estudo objetivou aplicar diferentes estratégias de biorremediação: atenuação natural (AN), bioestimulação (BE), bioaumentação (BA) e BA+BE, em microcosmos de água do mar, visando identificar a abordagem mais eficaz na biorremediação dos hidrocarbonetos do petróleo, com base em análises funcionais (degradação de hidrocarbonetos policíclicos aromáticos e alcanos, fitotoxicidade, atividade das desidrogenases) e da comunidade microbiana, a partir de análises moleculares independentes de cultivo (*metabarcoding*), ao longo do tempo (0, 15, 45, 90 dias). Todos os consórcios demonstraram capacidade de degradação, porém o Consórcio 1 (*Microbacterium oleivorans*, *Alcanivorax* sp., *Sphingobium xenophagum*, *Acinetobacter beijerinckii* e *Paramarasmius palmivorus*) apresentou as maiores porcentagens de remoção de hidrocarbonetos e os melhores resultados no ensaio de fitotoxicidade com *Cucumis sativus*. Ao final do experimento, observou-se as maiores porcentagens de degradação de alcanos com BE (93,67%) e BA+BE (91,23%), enquanto BA+BE mostrou melhor remoção de fluoranteno e superior remoção quando comparado a BE de benzo(a)antraceno (BaA), além de maior NMP de degradadores (4,54 NMP g L^{-1}) e atividade de desidrogenase (7,22 µg de TFP mL^{-1} dia^{-1}). Nos ensaios de fitotoxicidade, BE e BA+BE apresentaram inibição aguda no crescimento da radícula e do hipocótilo, com BE exibindo maior toxicidade. Os resultados indicam que ambos os tratamentos foram eficazes na degradação: BE exibiu melhor desempenho para alcanos, enquanto BA+BE se destacou na degradação de HPAs específicos, além de melhores resultados enzimáticos, de NMP e fitotoxicidade. As análises de *metabarcoding* revelaram que a comunidade autóctone é altamente adaptável à remoção de hidrocarbonetos, com gêneros degradadores como *Acinetobacter* e *Exophiala*, mas apenas quando estimulada com nutrientes (BE) tornou-se capaz de remover ativamente os hidrocarbonetos, enquanto BA+BE mostrou maior especificidade para moléculas complexas como fluoranteno, sendo mais indicado para casos que exigem tal aplicação.

Palavras-chave: Biodegradação, biotecnologia ambiental, biobanco microbiano, petróleo, *metabarcoding*.

ABSTRACT

The health of the oceans is vital for human subsistence. However, marine environments face increasing contamination from petroleum products, evidenced by events over decades, such as the 1967 Torrey Canyon oil spill, as well as the 2019 disaster on the Brazilian coast, which polluted 3,000 kilometers of coastline with 5,000 tons of oil. Effective and accessible methods are essential to remedy this contamination, given the complex composition of crude oil. The use of microorganisms capable of metabolizing environmental pollutants through enzymatic pathways (mono/dioxygenases, dehydrogenases, cytochrome P450) makes bioremediation, especially with microbial consortia, a promising and less aggressive technique, compared to other remediation methods. Isolates from contaminated sites, such as the recently affected Brazilian coast, reveal a suitable and adapted microbiota, highlighting the importance of scientific advancement in evaluating microorganisms from the Brazilian coast for the bioremediation of relevant compounds. Developed under the CNPq project 440774/2020-9 (MicroBioMaR), this study aimed to apply different bioremediation strategies: natural attenuation (NA), biostimulation (BS), bioaugmentation (BA), and BA+BS, in seawater microcosms, seeking to identify the most effective approach for petroleum hydrocarbon bioremediation. This identification was based on functional analyses (degradation of polycyclic aromatic hydrocarbons and alkanes, phytotoxicity, dehydrogenase activity) and microbial community structure through culture-independent molecular analyses (metabarcoding) over time (0, 15, 45, 90 days). All consortia demonstrated degradation capacity, but Consortium 1 (*Microbacterium oleivorans*, *Alcanivorax* sp., *Sphingobium xenophagum*, *Acinetobacter beijerinckii*, and *Paramarasmius palmivorus*) presented the highest hydrocarbon removal percentages and the best results in the *Cucumis sativus* phytotoxicity assay. At the end of the experiment, the highest alkane degradation percentages were observed with BS (93.67%) and BA+BS (91.23%), while BA+BS showed better removal of fluoranthene and superior removal of benzo(*a*)anthracene (BaA) compared to BS. Furthermore, BA+BS yielded the highest MPN of degraders (4,54 NMP g L^{-1}) and dehydrogenase activity (7,22 µg de TFP mL^{-1} dia^{-1}). In the phytotoxicity assays, both BS and BA+BS exhibited acute inhibition of radicle and hypocotyl growth, with BS displaying greater toxicity. The results indicate that both treatments were effective in degradation: BS exhibited better performance for alkanes, while BA+BS excelled in the degradation of specific PAHs, in addition to better enzymatic, MPN, and phytotoxicity results. Metabarcoding analyses revealed that the autochthonous community is highly adaptable to hydrocarbon removal, featuring degrading genera such as *Acinetobacter* and *Exophiala*, but only when stimulated with nutrients (BS) did it become capable of actively removing hydrocarbons. In contrast, BA+BS showed greater specificity for complex molecules like fluoranthene, making it more suitable for cases requiring such application.

Keywords: Biodegradation, environmental biotechnology, microbial biobank, petroleum, metabarcoding.

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LISTA DE ABREVIATURAS

AN	Atenuação Natural
ANOVA	Análise de Variância
ANP	Agência Nacional do Petróleo, Gás Natural e Biocombustíveis
BA	Bioaumentação
BaA	Benzo(a)antraceno
BE	Bioestimulação
BTEX	Benzeno, Tolueno, Etilbenzeno e Xilenos
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CONAMA	Conselho Nacional do Meio Ambiente
EJ	Exojaules
EPA	*Environmental Protection Agency*
EPS	Substâncias poliméricas extracelulares
HPA	Hidrocarbonetos Policíclicos Aromáticos
HTP	Hidrocarbonetos Totais de Petróleo
IA	Inteligência Artificial
LQ	Limite de Quantificação
NMP	Número Mais Provável
PCoA	Análise de Coordenadas Principais
QS	*Quorum Sensing*

SUMÁRIO

1 INTRODUÇÃO

"*Homo homini lupus*" (tradução do latim "O homem é o lobo do próprio homem") é uma afirmação atemporal, presente na peça Asinaria do dramaturgo romano, Tito Mácio Plauto, a qual ecoa fortemente ao longo dos séculos, mantendo sua ressonância em diversos momentos da sociedade. A despeito de ter sido proferida há milhares de anos, ela permanece profundamente relevante. A busca incessante pelo progresso, no século XIX, levou ao início da exploração do chamado "Ouro Negro", o petróleo, um grande recurso da sociedade moderna, o qual tem sido essencial em diversos setores, desde a geração de energia até a produção de inseticidas (FRANCO et al., 2021). Entretanto, junto com o grande avanço que o petróleo trouxe à humanidade, veio sua exploração descontrolada, no início do século XX, que tem levado a inúmeros problemas ambientais, incluindo emissões globais de gases poluentes e destruição dos ecossistemas marinhos e terrestres (BANG & LAHN, 2019).

Dentre os impactos causados pela exploração do petróleo e derivados, destacamos a contaminação de solos, sedimentos marinhos e água do mar por esses compostos (AMBAYE et al., 2023). A contaminação por esses poluentes tem sido alvo de preocupação ambiental. Exemplos recentes atestam a dimensão da problemática ambiental causada pelo petróleo em ambientes marinhos, como é o caso do derramamento de petróleo (óleo bruto) ocorrido em agosto de 2019 que atingiu a costa brasileira e alcançou estados do Nordeste e Sudeste (CABRAL et al., 2022).

Como em diversas outras aplicações, os microrganismos são solução para contaminações ambientais com poluentes orgânicos, através de enzimas capazes de metabolizar essas moléculas, em um processo denominado biorremediação (BALA et al., 2022). A grande diversidade microbiana e seu metabolismo sinérgico e diversificado, quando aplicados em conjunto por meio de consórcios microbianos, vêm demonstrando alta eficácia na degradação desses contaminantes (GIOVANELLA et al., 2023; REZAEI, AMOOZEGAR & MOGHMI, 2025). Nesta técnica, espera-se a mitigação do contaminante com a degradação dos poluentes, atingindo uma mineralização completa dessas moléculas, tendo como vantagem sobre os métodos físicos e químicos o custo inferior e menor agressividade ao ambiente (UGRINA & JURIC, 2023; HOSSEINI, SHARIFI & HABIBI, 2025).

O presente projeto de mestrado está associado ao projeto CNPq 440774/2020-9 intitulado "Biorremediação de petróleo em sedimentos e água do mar: estrutura e função de comunidades microbianas (MicroBioMaR)", sob a coordenação da Profa. Dra. Lara D. Sette (UNESP/Rio Claro) e teve como objetivo avaliar a capacidade de biodegradação de consórcios de fungos e bactérias isolados de ambientes marinhos da costa brasileira (previamente selecionados pela capacidade de degradar hidrocarbonetos e/ou apresentar propriedades emulsificantes) visando a aplicação em estudos em microcosmos, bem como estudar diferentes tratamentos de biorremediação (atenuação natural - AN, bioestimulação - BE, bioaumentação – BA e BA+BE) de água do mar contaminada com petróleo bruto em experimentos de microcosmos. Para tanto, foram realizadas análises de degradação de alcanos e hidrocarbonetos policíclicos aromáticos (HPAs), fitotoxicidade, desidrogenases e número mais provável (NMP) de microrganismos degradadores. Também foram realizadas análises da estrutura das comunidades microbianas (*metabarcoding*) nos melhores tratamentos, em comparação com a atenuação natural. Espera-se que a aplicação dos tratamentos inoculados com o consórcio elaborado e bioestimulados, resulte nos resultados mais eficientes de remoção dos hidrocarbonetos do petróleo. Os resultados desse estudo irão subsidiar a seleção do melhor tratamento para aplicação em estudos de mesocosmos, como parte dos objetivos do projeto MicroBioMaR.

6 CONCLUSÕES FINAIS

A etapa de estruturação e avaliação dos consórcios, demonstrou que todos possuem potencial de degradação dos hidrocarbonetos. Entretanto, o Consórcio 1 destacou-se como o mais eficiente na degradação de hidrocarbonetos, sendo selecionado para aplicação em microcosmos, embora não tenham sido observadas diferenças significativas nos testes de fitotoxicidade entre nenhum dos consórcios.

Os resultados obtidos no tratamento de bioestimulação (BE) indicam a presença de uma comunidade autóctone com alta capacidade hidrocarbonoclástica, caracterizada pela identificação de microrganismos degradadores amplamente reconhecidos, como *Thalassospira*, *Acinetobacter*, *Cladosporium* e *Exophiala* e pelas melhores taxas de remoção da maior parte dos alcanos.

Todavia, a combinação da bioaumentação e bioestimulação (BA+BE) apresentou os melhores resultados nas análises de NMP, atividade da desidrogenase e na degradação de poluentes específicos e recalcitrantes, como moléculas de benzo(a)antraceno (BaA) e fluoranteno.

Em conclusão, a bioestimulação da comunidade autóctone da água do mar foi efetiva para a biodegradação dos alcanos. No entanto, para a biodegradação de moléculas específicas como BaA e fluoranteno, a estratégia de bioaumentação/bioestimulação apresentou maior eficiência, o que evidencia o potencial do consórcio para ser utilizado na biorremediação de ambientes marinhos contaminados com petróleo.

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