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BRUNA KELLY DE OLIVEIRA SILVA

CARACTERIZAÇÃO GENÔMICA E FUNCIONAL DE MICRORGANISMOS COM POTENCIAL DEGRADADOR DE BTEX EM ESTAÇÕES DE TRATAMENTO DE EFLUENTES

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Área de concentração: Meio Ambiente

Orientador: Prof. Dr. Marcos Antônio de Morais Junior
Co-orientador: Prof. Dr. Fabrício Motteran

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“O importante é não parar de questionar. A curiosidade tem sua própria razão de existir.” - Albert Einstein

RESUMO

Em Pernambuco, Brasil, a ETE Multifabril, localizada em Jaboatão dos Guararapes, vem apresentando alta eficiência na remoção de matéria orgânica, alcançando taxas superiores a 90% de remoção, um desempenho incomum para sistemas que tratam efluentes industriais mistos. Esse comportamento sugere a possível atuação de comunidades microbianas especializadas, motivando a investigação conduzida neste estudo. Entre os contaminantes regularmente presentes em efluentes domésticos e industriais, destacam-se os compostos BTEX, monoaromáticos tóxicos e de reconhecido potencial carcinogênico. Assim, o objetivo desta pesquisa foi comparar a estrutura, a tolerância e o potencial metabólico dos microbiomas presentes na ETE Mangueira e na ETE Multifabril. Para este estudo, algumas metodologias como sequenciamento genômico de alto desempenho e avaliação de tolerância e biodegradação frente aos compostos BTEX foram realizadas. Com base nos resultados apresentados, foi possível observar que as comunidades microbianas das duas ETEs analisadas apresentam diferenças estruturais e funcionais marcantes, influenciadas pelo tipo de carga afluente. A ETE Mangueira exibiu maior tolerância aos compostos BTEX, suportando concentrações de até 200 mg/L, enquanto a ETE Multifabril tolerou apenas 50 mg/L, apesar de seu notável desempenho operacional na remoção de matéria orgânica, superior a 90%, mesmo tratando efluentes industriais com cerca de 4000 mg/L de DQO provenientes de 15 fábricas. Nos ensaios de degradação, os resultados obtidos por meio de Cromatografia Líquida de Alta Performance (HPLC), mostrou que ambos os consórcios reduziram aproximadamente 60% do BTEX inicialmente adicionado (Concentração inicial de 50 mg/L), sendo cerca de 40% dessa redução atribuída à atividade microbiana e 20% à volatilização ou adsorção. As análises de sequenciamento genético revelaram a presença de gêneros com potencial biodegradador, como é o caso do gênero *Fervidobacterium*, pertencente a família *Thermotogaceae*, associada na literatura por degradar hidrocarbonetos derivados de petróleo. Com base nas particularidades deste gênero, e em sua alta e consistente abundância relativa nas lagoas de tratamento da ETE Multifabril, nosso grupo de pesquisas publicou recentemente na *Anaerobe Journal*, uma revisão da literatura baseada no potencial biotecnológico das termoenzimas produzidas por este grupo bacteriano, que é capaz de degradar diversos substratos em altas temperaturas. Além disso, os resultados obtidos através da identificação taxonômica, proporcionou a predição enzimática de vias metabólicas associadas à degradação de compostos aromáticos, como os BTEX, reforçando que as diferentes pressões seletivas impostas pelos efluentes domésticos e industriais moldam microbiomas especializados com capacidades metabólicas distintas, mas

com capacidade em degradar compostos semelhantes. Esses achados reforçam ainda mais a importância de estudar o ambiente das ETEs, bem como as comunidades microbianas e funcionalidades ali presentes.

Palavras-chave: Biodegradação, Xenobióticos, Enzimas, Vias metabólicas, Bactérias, Efluentes Industriais e Domésticos.

ABSTRACT

In Pernambuco, Brazil, the Multifabril Wastewater Treatment Plant (WWTP), located in Jaboatão dos Guararapes, has shown high efficiency in removing organic matter, achieving removal rates exceeding 90%, an unusual performance for systems treating mixed industrial effluents. This behavior suggests the possible involvement of specialized microbial communities, motivating the investigation conducted in this study. Among the contaminants regularly present in domestic and industrial effluents, BTEX compounds stand out, which are toxic monoaromatic compounds with recognized carcinogenic potential. Thus, the objective of this research was to compare the structure, tolerance, and metabolic potential of the microbiomes present in the Mangueira WWTP and the Multifabril WWTP. For this study, some methodologies such as high-throughput genome sequencing and evaluation of tolerance and biodegradation against BTEX compounds were performed. Based on the results presented, it was possible to observe that the microbial communities of the two WWTPs analyzed present marked structural and functional differences, influenced by the type of influent load. The Mangueira wastewater treatment plant (WWTP) exhibited greater tolerance to BTEX compounds, withstanding concentrations up to 200 mg/L, while the Multifabril WWTP tolerated only 50 mg/L, despite its remarkable operational performance in organic matter removal, exceeding 90%, even when treating industrial effluents with approximately 4000 mg/L of COD from 15 factories. In degradation tests, the results obtained through High-Performance Liquid Chromatography (HPLC) showed that both consortia reduced approximately 60% of the initially added BTEX (initial concentration of 50 mg/L), with about 40% of this reduction attributed to microbial activity and 20% to volatilization or adsorption. Genetic sequencing analyses revealed the presence of genera with biodegrading potential, such as the genus *Fervidobacterium*, belonging to the *Thermotogaceae* family, associated in the literature with the degradation of petroleum-derived hydrocarbons. Based on the particularities of this genus, and its high and consistent relative abundance in the treatment lagoons of the Multifabril Wastewater Treatment Plant, our research group recently published a literature review in the Anaerobe Journal focusing on the biotechnological potential of thermoenzymes produced by this bacterial group, which is capable of degrading various substrates at high temperatures. Furthermore, the results obtained through taxonomic identification provided enzymatic prediction of metabolic pathways associated with the degradation of aromatic compounds, such as BTEX, reinforcing that the different selective pressures imposed by domestic and industrial effluents shape specialized microbiomes with distinct metabolic

capacities, but with the ability to degrade similar compounds. These findings further reinforce the importance of studying the environment of wastewater treatment plants, as well as the microbial communities and functionalities present there.

Keywords: Biodegradation, Xenobiotics, Enzymes, Metabolic Pathways, Bacteria, Industrial and Domestic Effluents.

SUMÁRIO

1. INTRODUÇÃO

As Estações de Tratamento de Esgoto (ETEs) desempenham um papel fundamental na proteção ambiental e na saúde pública, ao atuarem como barreiras essenciais contra a liberação de poluentes em corpos hídricos e solos (El-Naas et al., 2014; Kumar and Singh, 2025). Esses sistemas não apenas reduzem a carga orgânica e microbiológica dos efluentes domésticos e industriais, mas também representam um dos pilares para o cumprimento das metas globais de sustentabilidade, especialmente no contexto da Agenda 2030 da Organização das Nações Unidas (ONU). Em particular, as ETEs estão diretamente relacionadas ao Objetivo de Desenvolvimento Sustentável 6 (ODS 6), que estabelece a necessidade de assegurar disponibilidade e gestão sustentável da água e saneamento, reforçando a importância estratégica dessas infraestruturas em escala local e global (ONU, 2025).

Aqui em Pernambuco, Brasil, uma parceria público-privada entre a Companhia Pernambucana de Saneamento (COMPESA) e a BRK ambiental, administram cerca de 32 ETEs, dentre elas, está a ETE multifabril, localizada no Jaboatão dos Guararapes. Esta unidade vem chamando a atenção da COMPESA devido à sua elevada eficiência de remoção de matéria orgânica, quando comparada a outras estações administradas pela companhia, como é o caso da ETE Mangueira, também estudada nesta pesquisa. A ETE multifabril tem removido mais de 90% das 4000 mg/L de matéria orgânica afluente, medida em Demanda Química de Oxigênio (DQO), proveniente predominantemente dos resíduos industriais produzidos e descartados pelas 15 fábricas que ficam no entorno da estação.

Entretanto, além dos nutrientes e da matéria orgânica normalmente associada aos esgotos, estes sistemas recebem uma variedade crescente de contaminantes tóxicos, provenientes de atividades domésticas, comerciais e industriais. Entre eles, destacam-se os hidrocarbonetos monoaromáticos benzeno, tolueno, etilbenzeno e xilenos (BTEX), amplamente utilizados como solventes, combustíveis e matérias-primas em processos industriais (Kim et al., 2022; Zahed et al., 2024). Suas propriedades físico-químicas, incluindo volatilidade, estabilidade e lipofilicidade, favorecem tanto sua aplicabilidade industrial quanto sua mobilidade ambiental, permitindo que atinjam ETEs por diferentes rotas, que pode dar-se por infiltração de águas pluviais contaminadas, descargas industriais, efluentes domésticos contendo solventes e até migração subterrânea a partir de solos impactados (Ntsasa et al., 2023; Saeedi et al., 2024; Santurtún et al., 2024).

A presença de BTEX em sistemas de tratamento é motivo de preocupação, dada sua comprovada toxicidade e, no caso do benzeno, carcinogenicidade (de Almeida et al., 2021; Gao

et al., 2024). Esses compostos estão associados a efeitos neurotóxicos, hematotóxicos e imunotóxicos em seres humanos (Afrasiabi et al., 2024; Das et al., 2025), além de impactarem ciclos biogeoquímicos, comunidades microbianas e qualidade dos recursos hídricos (Vicente et al., 2015; Pena et al., 2020). Assim, compreender como as ETEs respondem à presença desses xenobióticos é essencial para melhorar sua eficiência e reduzir riscos ambientais.

Por outro lado, as ETEs são reconhecidas como verdadeiros hotspots de biodiversidade microbiana, abrigando consórcios complexos que evoluíram sob pressão seletiva imposta por elevada carga orgânica, condições de pH e redox extremas e presença recorrente de contaminantes recalcitrantes (Silva-Bedoya et al., 2016; Yang et al., 2020; Zhang et al., 2017). Atrelado a isto, a eficiência de remoção da matéria orgânica pela ETE Multifabril despertou interesse em identificar a microbiota presente nas lagoas de tratamento. Uma vez que, neste ambiente altamente desafiador, diversas linhagens microbianas desenvolvem capacidades metabólicas especializadas, incluindo a degradação de BTEX. Não menos importante, a ETE Mangueira, que recebe majoritariamente efluentes de origem doméstica, também apresenta remoção da matéria orgânica dentro dos padrões estipulados pela legislação vigente. Além disso sua biomassa microbiana apresentou notável tolerância aos compostos BTEX. Isso torna as ETEs não apenas unidades de tratamento, mas também reservatórios estratégicos para a bioprospecção de microrganismos degradadores de poluentes (Sharma, 2024).

A valorização biotecnológica do lodo de ETEs tem crescido, com aplicações que incluem produção de biogás, formulação de biofertilizantes, uso como material cimentício e, especialmente, sua utilização como inóculo microbiano em processos de biorremediação (Franus et al., 2015; Ahmad et al., 2016). Nesse sentido, investigar o potencial degradador de comunidades microbianas presentes em ETEs oferece ferramentas diretas para inovação em tecnologias limpas, formulação de políticas públicas e aprimoramento da operação das companhias de saneamento (El-Naas et al., 2014; Kumar & Singh, 2025).

Diante deste cenário a identificação dos fatores biológicos que contribuem para essa elevada eficiência motivou a presente pesquisa, desenvolvida pela Universidade Federal de Pernambuco em parceria com a COMPESA, visando o avanço científico, mas também reforçando o compromisso com práticas sustentáveis e com o desenvolvimento de soluções que atendam aos ODS. Testar essa hipótese permitiu identificar potenciais microrganismos-chave, elucidar vias metabólicas relevantes e fornecer informações valiosas para estratégias inovadoras de biorremediação e otimização operacional no setor de saneamento.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Esta pesquisa teve como objetivo geral identificar e avaliar o potencial de bactérias presentes nas ETE Mangueira (efluente doméstico) e ETE Multifabril (efluente industrial) como possível inóculo biorremediador, para tratamento de efluentes industriais variados, de alta complexidade e/ou áreas contaminadas com compostos recalcitrantes, como os BTEX.

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar os aspectos físico-químicos e biológicos das ETEs Mangueira e Multifabril;
- Identificar taxonomicamente os microrganismos presentes na biomassa microbiana (lodo) das ETEs;
- Avaliar o potencial de degradação das biomassas microbianas frente aos compostos BTEX;
- Identificar as prováveis enzimas envolvidas nas vias metabólicas de degradação de xenobióticos, incluindo compostos BTEX;
- Fornecer informações relevantes acerca da microbiota das ETEs e seu potencial biotecnológico.

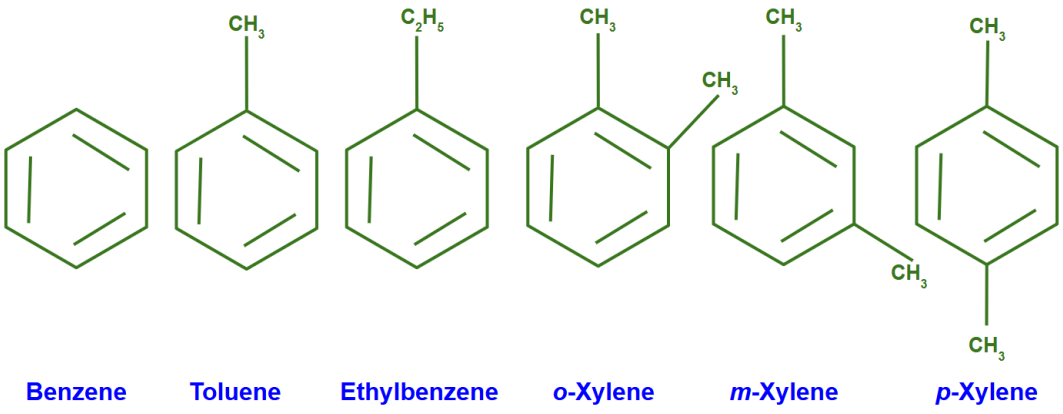
3. REFERENCIAL TEÓRICO

3.1 COMPOSTOS BTEX

Na segunda metade do século 18, o avanço da produção em grande escala ocasionados pela Revolução Industrial, desencadeou um aumento exponencial na demanda por insumos químicos, como os derivados de petróleo. Nesse contexto, a indústria petroquímica assumiu um papel central no desenvolvimento de combustíveis, solventes e inúmeros outros produtos essenciais aos processos de fabricação. Entre os compostos derivados de petróleo destacam-se os hidrocarbonetos monoaromáticos benzeno, tolueno, etilbenzeno e xilenos, conhecidos pelo acrônimo BTEX, amplamente utilizado como matéria-prima e aditivos industriais (Kim et al., 2022; Zahed et al., 2024).

Os BTEX são tóxicos, com alto potencial carcinogênico (Weelink et al., 2010), e são estruturalmente formados por um anel aromático (Figura 1). O benzeno possui a estrutura mais simples (C_6H_6) e é composto por apenas um anel aromático; o tolueno (C_7H_8) é um anel aromático com um grupo metil (CH_3) ligado ao anel; O etilbenzeno (C_8H_{10}) possui um grupo etila (C_2H_5) ligado ao anel aromático; e os xilenos (C_8H_{10}) possuem dois grupos metil (CH_3) ligados ao anel, mas em posições diferentes, que podem estar na posição *orto*, *meta* ou *para* (Silva, 2002).

Figura 1. Estrutura química dos compostos BTEX



Fonte: A autora (2025).

Estes compostos possuem baixo peso molecular e boa solubilidade em água, comparados a outros derivados de petróleo. Sua elevada solubilidade e volatilidade favorecem a

contaminação de solos e águas subterrâneas, exigindo estratégias de remediação eficientes e ambientalmente sustentáveis (Lima, 2010; Vicente et al., 2015; Weelink et al., 2010). Na tabela 1 faz-se possível observar as características físico-químicas e toxicológicas dos compostos BTEX. Estas informações são importantíssimas, pois permitem entender o comportamento dos compostos no ambiente, bem como os riscos à saúde que os mesmos possam vir a apresentar.

Na tabela 1, tem-se a classificação dos compostos BTEX quanto à sua carcinogenicidade, estes parâmetros são estipulados pelo IARC, agência internacional reconhecida mundialmente quanto a aspectos que englobam pesquisas relacionadas ao câncer. A agência organiza as substâncias químicas de acordo com o grau de evidência científica acerca do seu potencial carcinogênico. O grupo 1 consiste nos compostos comprovadamente cancerígenos para humanos, como por exemplo, o benzeno. O grupo 2 B inclui as substâncias possivelmente cancerígenas para animais, como o etilbenzeno. Já o grupo 3 reúne compostos para os quais não há evidências científicas suficientes para classificá-los quanto à carcinogenicidade, como os xilenos e tolueno.

Ainda na tabela 1, observa-se que o benzeno, com menor massa molar (78,11 g/mol) e maior solubilidade em água (1.790 mg/L a 25 ºC), apresenta elevada mobilidade hídrica e é classificado pela Agência Internacional de Pesquisa em Câncer (IARC), como carcinógeno do grupo 1, representando o maior risco toxicológico dentre os compostos. De todos os compostos, o benzeno é considerado o mais nocivo, pois mesmo em baixas concentrações seus metabólitos atacam a medula óssea e podem causar leucemia e outras doenças hematológicas (Liu et al., 2025; Zahed et al., 2024). Diversos estudos já demonstraram os efeitos severos que esses poluentes orgânicos causam à saúde humana. Alguns sintomas da exposição ocasional a esses compostos incluem tontura, falta de ar, náusea, dor de cabeça e insônia. Os efeitos da exposição crônica a esses contaminantes podem causar danos ao fígado, rins, pulmões, estômago e sistema nervoso central, além do risco de leucemia, anemia ou até mesmo morte (Afrasiabi et al., 2024; Das et al., 2025).

Todavia, vale ressaltar que o teor da toxicidade vai depender também do tempo de exposição, vias de exposição, e também do quadro clínico do indivíduo ou animal exposto. O tolueno e os isômeros de xileno apresentam solubilidade significativamente menor (130 - 515 mg/L a 25 ºC) e maior hidrofobicidade (Log Kow 2,13 - 3,15), o que indica maior tendência à bioacumulação e menor dispersão em meio aquoso. O etilbenzeno, embora menos solúvel (169 mg/L), é classificado como possivelmente carcinogênico, do grupo 2 B. Entender detalhadamente os aspectos físico-químicos desses compostos, permite relacionar o potencial de contaminação com a toxicidade de cada composto, auxiliando em estratégias de remediação

em áreas contaminadas com derivados de petróleo.

Tabela 1. Parâmetros físico-químicos e de toxicidade dos compostos BTEX

Composto	Fórmula química	Massa molar (g/mol)	Ponto de ebulição (°C)	Solubilidade em água (mg/L a 25°C)	Log Kow	Grupo IARC (Carcinogenicidade)
Benzeno	C_6H_6	78,11	80,1	1.790	2,13	Grupo 1 – Cancerígeno para humanos
Tolueno	C_7H_8	92,14	110,6	515	2,73	Grupo 3 – Não classificável
Etilbenzeno	C_8H_{10}	106,17	136,2	169	3,15	Grupo 2 B – Possivelmente cancerígeno
o-Xileno	C_8H_{10}	106,17	144,4	130	3,12	Grupo 3 – Não classificável
m-Xileno	C_8H_{10}	106,17	139,1	162	3,2	Grupo 3 – Não classificável
p-Xileno	C_8H_{10}	106,17	138,4	198	3,15	Grupo 3 – Não classificável

Fonte: Tabela criada com informações disponíveis em (Afrasiabi et al., 2024; Das et al., 2025; Davidson et al., 2021; Liu et al., 2014; Ntsasa et al., 2023; Saeedi et al., 2024; Santurtún et al., 2024; Silva, 2002; Weelink et al., 2010; Zahed et al., 2024).

Legenda: g/mol (massa molar); ºC (ponto de ebulição); Log Kow (coeficiente de partição octanol/água); IARC (Agência Internacional de Pesquisa em Câncer).

Os BTEX estão entre as principais referências de contaminantes ambientais dos últimos anos, chegando a se destacar na lista de poluentes prioritários da agência de proteção ambiental norte-americana *United States Environmental Protection Agency* (US EPA) devido ao seu elevado potencial carcinogênico e mutagênico (Zahed et al., 2024). Considerando o seu uso generalizado e descarte incorreto, estes compostos podem contaminar extensivamente solos e aquíferos subterrâneos, o que pode comprometer corpos hídricos e fontes de água potável, principalmente em locais próximo a postos de combustíveis , representando um sério problema ambiental e de saúde pública (Dou et al., 2008; Gao et al., 2024; Lima, 2010). Os BTEX são utilizados na indústria e em processos de fabricação de uma ampla gama de produtos, como tintas, vernizes, óleo, gasolina, lubrificantes e graxas, estando presentes no cotidiano de muitas fábricas e no dia-a-dia das pessoas (Ghobakhloo et al., 2023; Hosseinzadeh et al., 2023; Kim et

al., 2022). E por serem Compostos Orgânicos Voláteis (COVs), são também considerados importantes poluentes atmosféricos (Davidson et al., 2021; Kim et al., 2022; Yoshikawa et al., 2017).

Os BTEX são recalcitrantes e de difícil remoção quando em contato com a natureza. Tecnologias tradicionais para a remoção de BTEX demandam de custos elevados, por isso, o uso de biotecnologia para tratar desta problemática vem ganhando apoio (Farhadian et al., 2008; Mazzeo et al., 2010), principalmente após o derramamento de petróleo que ocorreu em 2019, atingindo 11 estados do litoral brasileiro, e considerado até hoje como o maior desastre ambiental envolvendo derramamento de óleo bruto no país (de Almeida et al., 2021; Pena et al., 2020).

O óleo bruto é extremamente tóxico; em contato com o ecossistema, desencadeia uma série de danos à saúde do homem, da fauna e da flora do local afetado. Durante o episódio, os estados se viram em meio a um déficit de métodos eficientes para a remoção destes contaminantes do ambiente (Pena et al., 2020). Por isso, faz-se necessária a pesquisa de novas tecnologias acessíveis e eficientes como o uso de microrganismos. No geral, os níveis de BTEX encontrados na natureza são de aproximadamente 200 mg/L, o que já vem a ser um problema ambiental e de saúde (Martiarena, 2016). Biotecnologias utilizando microrganismos já provaram eficiência na remoção de diversos contaminantes, incluindo os BTEX (Gómez-Acata et al., 2021; Capdevielle & Lopretti, 2011; Oliart-Ros et al., 2016; Martiarena, 2016). Na tabela 2, tem-se uma listagem de algumas tecnologias/métodos que podem ser utilizados para a remoção de BTEX de áreas contaminadas.

3.1.1 Métodos físicos, químicos e biológicos para a remoção de BTEX em áreas contaminadas

Levando em consideração o que foi apresentado anteriormente, vê-se que compostos aromáticos como os BTEX, são poluentes orgânicos prioritários devido à sua toxicidade, mobilidade e persistência no meio ambiente. Em função dos riscos ecotoxicológicos e carcinogênicos associados ao BTEX, diversos métodos têm sido desenvolvidos para remediação de solos e águas subterrâneas. Estes métodos podem ser agrupados em abordagens físicas, químicas e biológicas, cada qual com vantagens, limitações e contextos de aplicação específicos. A seguir, na tabela 2, apresenta-se uma síntese desses métodos.

Tabela 2. Métodos físicos, químicos e biológicos que podem ser empregados para a remoção

de BTEX em áreas contaminadas

Método	Tipo	Descrição	Vantagens	Limitações	Referências
Biorremediação (natural ou estimulada)	Biológico	Uso de microrganismos para degradar BTEX em condições naturais ou com adição de nutrientes/aceptores de elétrons.	Baixo custo, ambientalmente amigável, degrada completamente os compostos.	Lento, dependente das condições ambientais (pH, temperatura, oxigênio).	(Margesin et al., 2003)
Bioaumentação	Biológico	Introdução de cepas microbianas especializadas para aumentar a degradação.	Aumenta a taxa de remoção, útil para locais com baixa população degradadora.	Sucesso variável devido à competição microbiana e adaptação.	(Meckenstock et al., 2015)
Pump-and-Treat	Físico	Bombeamento da água subterrânea contaminada para tratamento externo (ex.: carvão ativado, oxidação química).	Bem estabelecido, rápido para redução inicial das concentrações.	Alto custo operacional, pode não remover completamente.	(Caetano et al., 2017)
Oxidação Química *In Situ* (ISCO)	Químico	Injeção de oxidantes (permanganato, peróxido, persulfato) para degradar BTEX no subsolo.	Rápida degradação, aplicável em locais de difícil acesso.	Pode gerar subprodutos tóxicos; risco de alteração do pH.	(Andrade, 2016)
Extração de Vapores do Solo (SVE)	Físico	Aplicação de vácuo para extrair vapores de BTEX do solo não saturado.	Rápido, eficiente para compostos voláteis.	Ineficaz em solos saturados ou com baixa permeabilidade.	(Lourenço, 2006)
Carvão Ativado (adsorção)	Químico	Uso de carvão ativado para remover BTEX da água ou ar.	Simples, custo moderado, regenerável.	Saturação rápida, necessidade de substituição.	(Lourenço et al., 2010)
Air Sparging	Físico	Injeção de ar na zona saturada para volatilizar BTEX, combinado com SVE.	Boa remoção de compostos voláteis, acelera a biodegradação.	Eficiência limitada para compostos pouco voláteis.	(Heidari et al., 2017)

Fitorremediação	Biológico	Uso de plantas para absorver, degradar ou volatilizar BTEX.	Sustentável, custo baixo, integração paisagística.	Lento, limitado a áreas rasas.	(Medeiros, 2015)

3.1.1.1 Estratégias biológicas

A biorremediação, seja natural ou estimulada, representa uma das abordagens mais amplamente empregadas para a remoção de BTEX, baseando-se na capacidade metabólica de microorganismos autóctones para oxidar ou reduzir compostos aromáticos. Em condições aeróbias, diversas bactérias utilizam BTEX como fonte de carbono e energia, promovendo sua mineralização completa em CO_2 e H_2O. Em ambientes anóxicos, aceptores alternativos de elétrons, como nitrato, ferro ou sulfato, podem sustentar a degradação, embora em taxas inferiores. As principais vantagens dessa abordagem incluem baixo custo, sustentabilidade e ausência de geração de resíduos perigosos. Entretanto, sua eficiência depende fortemente das condições ambientais, como disponibilidade de nutrientes, pH e temperatura, o que pode tornar o processo lento (Margesin et al., 2003).

A bioaumentação complementa essa estratégia ao introduzir cepas microbianas especializadas capazes de degradar BTEX de forma mais eficiente. Este método é particularmente útil em locais onde a comunidade nativa apresenta baixa diversidade ou pouca capacidade metabólica para biodegradar o BTEX. Contudo, a competição microbiana, predação e dificuldades de adaptação ao ambiente contaminado podem comprometer o sucesso da bioaumentação. Ainda assim, estudos têm demonstrado que consórcios microbianos anaeróbios ou aeróbios selecionados podem acelerar significativamente a remoção de BTEX quando adequadamente combinados com ajustes ambientais, como suplementação de nutrientes (Meckenstock et al., 2015).

Já a fitorremediação constitui outra abordagem biológica com apelo crescente, utilizando plantas para absorção, estabilização, dispersão ou degradação de BTEX. Suas vantagens incluem custo reduzido e benefícios ecossistêmicos. No entanto, suas aplicações são limitadas a áreas próximas à superfície e apresentam cinética lenta, tornando-a mais apropriada para tratamentos complementares ou áreas de baixa contaminação residual (Medeiros, 2015).

3.1.1.2 Tecnologias físicas

Métodos físicos são amplamente utilizados em função de sua robustez operacional e rapidez. Entre eles, o pump-and-treat é um dos mais consolidados, envolvendo o bombeamento de água subterrânea contaminada para tratamento *ex situ*. Técnicas como adsorção em carvão ativado ou oxidação externa permitem rápida redução inicial das concentrações de BTEX. Porém, o processo apresenta elevado custo operacional, além de poder exigir longos períodos de bombeamento devido à liberação lenta de contaminantes retidos no solo (Caetano et al., 2017).

Outro método físico utilizado consiste na Extração de Vapores do Solo (SVE), este método é eficiente para compostos voláteis em solos não saturados. Sua aplicação isolada ou associada ao *air sparging*, permite otimizar a remoção de contaminantes altamente voláteis, acelerando também a biodegradação aeróbia. No entanto, solos de baixa permeabilidade e zonas saturadas representam limitações importantes, reduzindo a eficiência do processo. Apesar dessas restrições, SVE e *air sparging* são amplamente empregados em áreas contaminadas por combustíveis fósseis devido ao baixo tempo de resposta inicial (Lourenço, 2006; Heidari et al., 2017).

Outra técnica amplamente utilizada é a adsorção em carvão ativado, aplicável tanto para água quanto para ar. O carvão ativado apresenta elevada afinidade por compostos orgânicos hidrofóbicos, permitindo a remoção eficiente de BTEX por mecanismos de adsorção física. Entretanto, ocorre saturação relativamente rápida, exigindo regeneração ou substituição periódica do material, o que pode aumentar os custos operacionais (Lourenço et al., 2010).

3.1.1.3 Tratamentos químicos

Entre as tecnologias químicas, a Oxidação Química *In Situ* (ISCO) destaca-se pelo uso de agentes como permanganato, peróxido de hidrogênio ou persulfato para promover a degradação direta dos compostos. A ISCO é particularmente indicada para regiões de acesso limitado e situações onde se deseja resposta rápida. No entanto, sua aplicação pode gerar subprodutos tóxicos, alterar significativamente o pH do meio e promover impactos geoquímicos indesejados. Além disso, a presença de matéria orgânica natural pode consumir os oxidantes, reduzindo a eficiência do processo (Andrade, 2016).

Além dos métodos descritos na tabela 2, existem alguns outros meios de remoção do BTEX, como os métodos biológicos denominados de *Landfarming* que pode ser utilizado para tratar solos superficiais (McCarthy et al., 2004), o método utilizando bioestimulação, que pode

ser utilizado em áreas com contaminação crônica e microbiota adaptada (Silva, 2020) e o método que faz uso de biorreator *ex situ*, que pode ser usado para remover BTEX de solos e águas profundas (Zílio et al., 2012). Existe também o método físico denominado de *air stripping* que se utiliza de sistemas de *pump-and-treat* de água subterrânea para tratar o BTEX (Abdullahi et al., 2015). Já o método físico-químico de barreira reativa permeável (BRP) é utilizado em locais onde a contaminação de águas subterrâneas é constante, como nas proximidades de postos de combustíveis (Lourenço et al., 2010). Por último, que não está listado na tabela 2, tem-se o método químico de oxidação química *in situ* (ISCR), que pode ser utilizado em locais com condição anaeróbica (Andrade, 2016).

A seleção de estratégias de remediação para BTEX depende da natureza da contaminação, características do ambiente atingido e objetivos do tratamento. Embora métodos físicos e químicos forneçam respostas mais imediatas, abordagens biológicas apresentam maior sustentabilidade e potencial para mineralização completa dos contaminantes. Na prática, sistemas híbridos combinando técnicas, por exemplo, air sparging associado a SVE, ou biorremediação estimulada após ISCO, tendem a atingir maior eficiência e estabilidade. Assim, a compreensão integrada das vantagens e limitações das diferentes tecnologias é essencial para o planejamento de estratégias eficazes de remediação em áreas contaminadas por BTEX, garantindo mitigação dos riscos ambientais e proteção à saúde humana (Gao et al., 2024; Mazzeo et al., 2010; Sharma, 2024).

Levando em consideração as vantagens ambientais das estratégias biológicas para a remoção do BTEX de ambientes, aposta-se amplamente na capacidade natural de microrganismos para degradar poluentes em produtos menos nocivos (Mazzeo et al., 2010). Assim, as ETEs oferecem um ambiente rico em microrganismos potencialmente adaptados a condições adversas, como a presença de compostos tóxicos, incluindo os BTEX. Utilizar a biomassa microbiana presente nas lagoas de tratamento para biorremediação, além de eficaz, é uma estratégia que agrega valor a um resíduo urbano e propõe uma solução de baixo custo, circular e ambientalmente correta para problemas de contaminação química (Kumar and Singh, 2025; Ruikar, 2022; Sharma, 2024). Dito isto, esta pesquisa teve como objetivo analisar o potencial para a degradação de BTEX por microrganismos advindos de ETEs.

3.2 ESTAÇÃO DE TRATAMENTO DE ESGOTO (ETE)

Estações de Tratamento de Esgotos (ETE) desempenham um papel essencial para a saúde pública, preservação ambiental e desenvolvimento sustentável. Uma vez que, a estrutura

e serviços prestados por uma ETE para as comunidades levam dignidade através do saneamento básico adequado e funcional. A Organização das Nações Unidas (ONU) estima que 3,6 bilhões de pessoas, cerca de 46% da população mundial, não têm acesso ao saneamento básico adequado. Dados do relatório da Organização das Nações Unidas para Educação, Ciência e Cultura (UNESCO), apontam que 44% do esgoto doméstico não é tratado de forma segura. Este verdadeiro retrato da desigualdade socioeconômica reflete na disseminação de doenças, compromete a subsistência e recaem sobre a segurança do meio ambiente, e saúde e bem-estar de humanos e animais (ONU, 2023).

No Brasil, a Agência Nacional de Águas e Saneamento Básico (ANA) e o Ministério das Cidades destacam que 38,6% dos esgotos do Brasil não são coletados e muito menos, tratados; 18,8% dos esgotos são coletados, mas não passam por tratamento, sendo lançados nos corpos hídricos de maneira inadequada e irresponsável; e 42,6% dos esgotos são coletados e tratados antes de voltarem ao meio ambiente, configurando assim o cenário ideal do saneamento, estabelecido pela lei nº 11.445/2007 e nº14.026/2020, conhecida como o Marco Legal do Saneamento Básico (ANA, 2017), porém este cenário considerado ideal só faz-se possível graças aos serviços das ETEs.

Em Pernambuco, 33,8% da população é assistida com a coleta de esgoto, em números, isto contabiliza 3.064.908 habitantes atendidos com o serviço. E do total de esgoto coletado, 68,2% passam por tratamento. Isto faz-se possível graças às 60 ETEs em operação no estado de Pernambuco (SNIS, 2022). Em um amplo contexto, as ETEs desempenham um papel importantíssimo em várias dimensões, como: proteção da saúde pública, preservação dos recursos hídricos, redução da poluição química, reaproveitamento e economia circular, mitigação das mudanças climáticas, cumprimento de legislações ambientais, desenvolvimento econômico e social, dentre outros aspectos.

No âmbito da proteção e saúde pública, o esgoto bruto possui patógenos, incluindo bactérias, vírus, protozoários, helmintos, e outros, que podem causar doenças como hepatite, cólera, febre e gastroenterite. Além de compostos tóxicos e recalcitrantes como os BTEX, considerados mutagênicos e carcinogênicos. O esgoto bruto, ao ser tratado através das ETEs, contribui para a remoção ou inativação desses microrganismos, além de tratar ou minimizar boa parte dos xenobióticos presentes no esgoto. Deste modo, o tratamento realizado pelas ETEs evita surtos de doenças e protege a população que faz uso de rios, lagos e aquíferos para abastecimento (Afrasiabi et al., 2024; ANA, 2017; ONU, 2023; Silva-Bedoya et al., 2016; Yang et al., 2020; Zhang et al., 2017). Quanto à preservação dos recursos hídricos, o processamento sofrido pelos esgotos por meio das ETEs reduz a carga orgânica lançada nos corpos hídricos,

evitando assim a eutrofização, que é o fenômeno causado pelo excesso de nutrientes, resultando na proliferação exacerbada de algas que degradam a qualidade da água. Assim, o tratamento do esgoto mantém o equilíbrio ecológico de rios e lagos, protegendo peixes, plantas aquáticas e outros organismos (Gadelha et al., 2022).

Abaixo, na tabela 3, pode-se visualizar os parâmetros de qualidade que um efluente tratado precisa ter para estar em conformidade antes de ser lançado em corpos hídricos. Estes padrões são estabelecidos pela legislação ambiental brasileira, conforme a Resolução do Conselho Nacional do Meio Ambiente (CONAMA), nº 430/2011 complementando a Resolução nº 357/2005 (CONAMA, 2011). Assim, todo efluente, seja de origem industrial ou doméstico, ao passar pelo tratamento, precisa estar de acordo com as diretrizes, como as dispostas na tabela 3.

Tabela 3. Parâmetros de conformidade para o lançamento de efluentes tratados em corpos hídricos

Parâmetro	**Limite / Condição**
pH	Entre 5 e 9
Temperatura	≤ 40 °C, com variação máxima de 3 °C na zona de mistura
Materiais sedimentáveis	Até 1 mL/L em 1 hora (cone Imhoff); quase zero em lagos/lagoas
Óleos e Graxas	Óleos minerais: até 20 mg/L; óleos vegetais/gorduras animais: até 50 mg/L
Ausência de materiais flutuantes	Obrigatória
Remoção mínima de DBO	≥ 60 % (em 5 dias a 20 °C); ou DBO ≤ 120 mg/L para esgoto sanitário, exceto com estudo de autodepuração
Benzeno	Máximo de 1,2 mg/L
Tolueno	Máximo de 1,2 mg/L
Etilbenzeno	Máximo de 0,84 mg/L
Xilenos (total)	Máximo de 1,6 mg/L
Ecotoxicidade	O efluente não pode causar efeitos tóxicos aos organismos aquáticos; exigência de testes com organismos de pelo menos dois níveis tróficos (CENO, CL50, CECR)

Legenda: pH (potencial hidrogeniônico); DBO (Demanda Bioquímica de Oxigênio); CENO (Concentração Efetiva Não Observada); CECR (Concentração do Efluente no Corpo Receptor); CL50 (Concentração Letal 50%).

A Resolução CONAMA nº 430/2011 detalha e complementa os requisitos listados na Resolução nº 357/2005. Este documento especifica parâmetros técnicos e de qualidade para o

lançamento de efluentes tratados; condições físicas como pH, temperatura, materiais flutuantes, visam proteger a fauna aquática e manter o equilíbrio dos corpos d'água (CONAMA, 2011). A análise da DBO, por exemplo, é essencial para avaliar a carga orgânica restante e assegurar que o receptor mantenha o oxigênio dissolvido necessário à saúde dos ecossistemas aquáticos. A determinação, também, de compostos recalcitrantes como os BTEX, vem para prevenir a contaminação por compostos tóxicos persistentes. E as exigências acerca dos ensaios ecotoxicológicos tornam a norma mais robusta, permitindo avaliar impactos agudos e crônicos no meio aquático.

Desta forma, as ETEs também contribuem para a redução da poluição química, removendo ou reduzindo compostos tóxicos, incluindo metais pesados, solventes, pesticidas e hidrocarbonetos. Assim, os serviços de uma ETE somam esforços para evitar a contaminação de solos e aquíferos, que poderiam vir a comprometer o uso da água para abastecimento humano e irrigação (Shchegolkova et al., 2016; Zhang et al., 2017). O tratamento de esgotos pelas ETEs também resultam no reaproveitamento desta água, e impulsionam a economia circular. A água tratada pode ser reutilizada em irrigação, processos industriais ou lavagem de ruas, reduzindo o consumo de água potável (Batista et al., 2008). Além disso, o lodo gerado nas ETEs pode ser estabilizado e utilizado como biofertilizante na agricultura, como fonte de energia para a produção de biogás, como produto cimentício na fabricação de tijolos ecológicos, e também como consórcios microbianos visando a biodegradação de xenobióticos em áreas contaminadas. Essa prática promove a valorização de resíduos e contribui para a economia circular (Franus et al., 2015; Ahmad et al., 2016).

Levando tudo o que foi apresentado em consideração, conclui-se que o funcionamento das ETEs é peça fundamental para que municípios e indústrias estejam em conformidade com a legislação e evitem penalidades (ANA, 2017; SNIS, 2022). Na esfera do desenvolvimento econômico e social, regiões com saneamento básico adequado atraem mais investimento e reduzem os gastos com saúde pública, fazendo com que esse montante financeiro possa ser aplicado em outras esferas, como educação e segurança. Além disso, comunidades com acesso à água limpa e esgotamento sanitário têm melhor qualidade de vida e maior produtividade econômica (Júnior et al., 2024; Libânio et al., 2005; Mendonça e Motta, 2005; Rossoni et al., 2020). Nesta pesquisa tratamos acerca de duas ETEs, uma que recebe efluentes de origem industrial, que é a ETE multifabril, localizada no bairro de Santo Aleixo em Jaboatão dos Guararapes, Pernambuco, Brasil; e uma ETE que trata água residuária de origem doméstica, a ETE Mangueira, localizada no bairro da Mangueira, Recife, Pernambuco, Brasil. Ambas são

administradas pela Companhia Pernambucana de Saneamento (COMPESA), em parceria com a BRK Ambiental, e terão seus aspectos e características explanados nos tópicos seguintes.

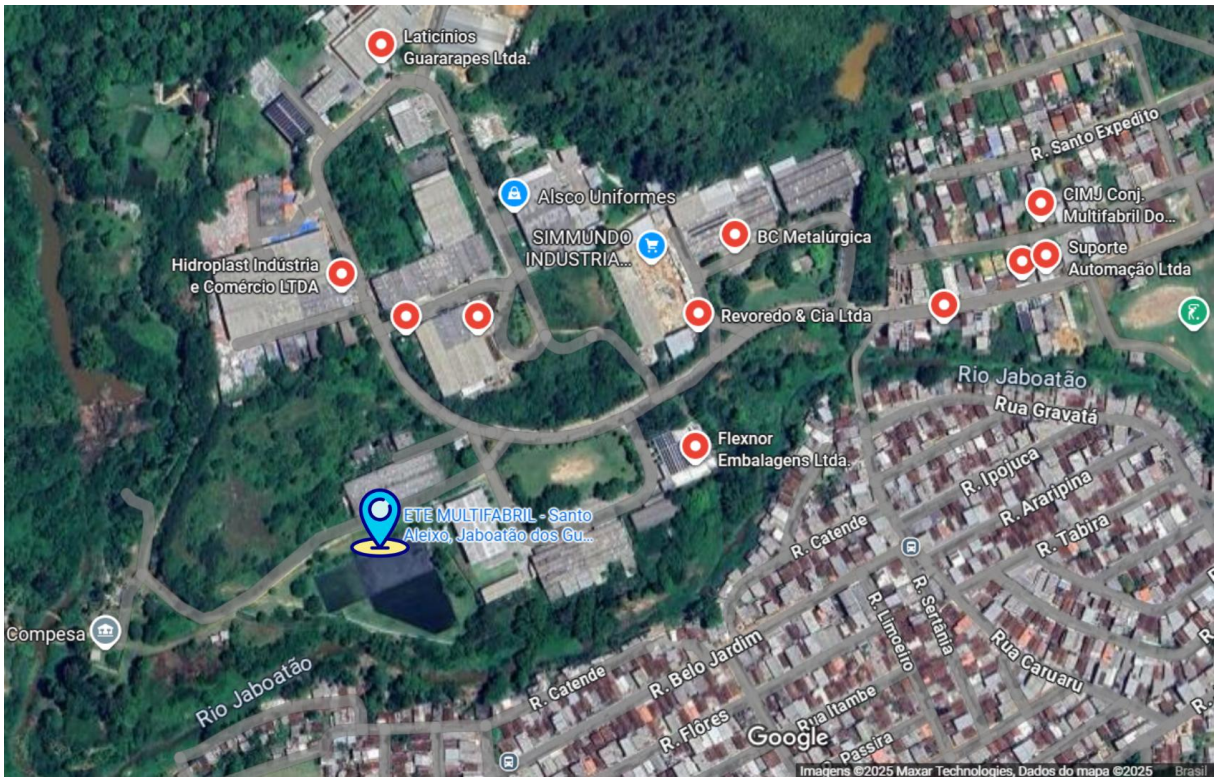
3.2.1 Estação de Tratamento de Esgotos de origem industrial - ETE Multifabril

A ETE multifabril (Figura 2), está localizada no bairro de Santo Aleixo, Jaboatão dos Guararapes, Pernambuco, Brasil (Figura 3). Esta unidade, como pode ser vista na figura 3, está inserida em um complexo fabril que contempla 15 fábricas das mais diversas categorias. Esta ETE foi construída com o objetivo de receber e tratar apenas efluentes domésticos, porém com a implementação das indústrias no entorno de suas dependências, a ETE passou a receber majoritariamente efluentes industriais. Contabilizando aproximadamente 35 anos de funcionamento, esta estação em todos esses anos de operação nunca passou pelo processo de limpeza, que consiste basicamente na remoção do sedimento/lodo das lagoas de tratamento, é recomendado que este processo em lagoas facultativas seja realizado num espaço entre 5 a 20 anos. Mesmo assim, esta unidade, quando comparada às demais ETEs administradas pela COMPESA, apresentou um resultado bastante satisfatório, no que diz respeito ao tratamento dos efluentes que passam por ela.

Figura 2. ETE multifabril do Jaboatão dos Guararapes

Fonte: A autora (2021).

Figura 3. Imagem de satélite da ETE multifabril e do complexo industrial ao qual está inserida



Legenda: Na região inferior esquerda da imagem o ícone em azul aponta a ETE multifabril; Os demais ícones de localização espalhados na imagem nas cores vermelha e azul apontam algumas das fábricas que fazem parte do complexo industrial.

Fonte: Google Maps (2025)

Esta ETE tem removido mais de 90% dos 4000 mg/L de matéria orgânica afluente, medida por meio da Demanda Química de Oxigênio (DQO), proveniente predominantemente de indústrias têxtil, de laticínio, embalagens, autopeças, entre outras, que apresenta em sua composição vários compostos orgânicos recalcitrantes, como óleos e graxas, hidrocarbonetos, corantes, metais, surfactantes, entre outros. Na tabela 4, faz-se possível visualizar a listagem das indústrias do entorno da ETE, bem como seus produtos e serviços, além das prováveis matérias-primas por elas utilizadas. Como resultado da bioacumulação, esses, representam problema à saúde pública porque muitos são tóxicos, mutagênicos e carcinogênicos (Afrasiabi et al., 2024; Das et al., 2025; Liu et al., 2025).

Esses efluentes são compostos principalmente de matéria orgânica degradáveis e xenobióticos, como corantes, hidrocarbonetos e surfactantes, além de metais pesados. Apesar da complexidade dessas águas residuárias, a ETE se manteve estável ao longo dos anos e, segundo dados fornecidos pela própria Compesa, a estação chega a remover mais de 80% da matéria orgânica disponível. A ETE multifabril é constituída por um sistema de grades que

realiza a triagem da água residuária que chega à estação, removendo possíveis resíduos sólidos, além de desarenadores. A unidade é composta por três lagoas de tratamento com profundidade de 2 m (Figura 4). A lagoa 1 tem 1.456 m² de superfície, caracterizada por uma espessa camada superficial preta composta predominantemente por óleos e graxas (Figura 5). Esta, recebe o efluente complexo com uma vazão de 14,4 L/h. A lagoa 2 tem 910 m^2 de área e apresenta pouca cobertura oleosa, enquanto a lagoa 3 de 1.740 m^2 de área e já conta com a presença de algumas microalgas. A ligação entre as três lagoas é realizada por tubos de 200 mm de diâmetro localizados desde a superfície até a camada de água. Deste último, a água tratada é lançada no rio Jaboatão (Figura 4).

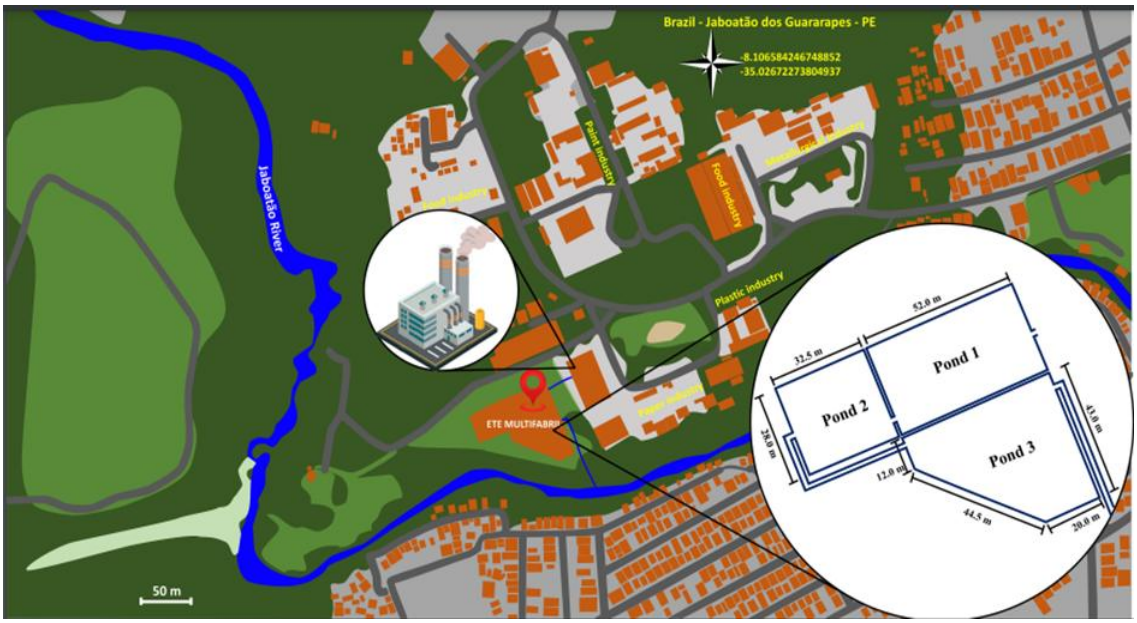
Tabela 4. Listagem das fábricas do complexo industrial do entorno da ETE multifabril, bem como seus produtos, serviços e prováveis matérias-prima utilizadas pelas mesmas

Fábrica	Produtos e Serviços	Ramo Industrial	Prováveis Matérias-primas / Insumos
1	Produtos para difusão e filtragem de ar, ventilação e eletrodomésticos	Metal-mecânico e eletroeletrônico	Chapas metálicas, plásticos técnicos, fios de cobre, motores elétricos, filtros sintéticos e de carvão ativado
2	Lavanderia industrial (uniformes, toalhas, EPI)	Serviços industriais e têxtil	Água, detergentes industriais, amaciantes, desinfetantes, energia térmica, EPIs, tecidos
3	Papel, cartolina, papel cartão, papelão ondulado	Papel e celulose	Polpa de celulose, amido, água, corantes, aditivos químicos, energia
4	Estruturas metálicas e construções	Construção metálica e civil	Aço carbono, aço galvanizado, eletrodos de solda, tintas anticorrosivas, cimento
5	Tintas, vernizes, esmaltes, lacas, solventes, argamassas, massas corridas	Químico (tintas e revestimentos)	Resinas, pigmentos, solventes orgânicos (BTEX, acetona), cargas minerais, aditivos químicos
6	Estruturas metálicas e montagem industrial	Metal-mecânico e construção	Perfis metálicos, aço carbono/inox, parafusos, chapas metálicas, equipamentos de soldagem
7	Batata palha, salgadinhos à base de milho	Alimentício (snacks)	Batata, milho, óleo vegetal, sal, temperos, embalagens plásticas
8	Tubos, conexões e peças especiais em PVC	Plástico (transformação)	Resina de PVC, plastificantes (ftalatos), estabilizantes térmicos, pigmentos, solventes
9	Derivados lácteos e sucos de	Alimentício (laticínios e	Leite, frutas, açúcar, estabilizantes,

	frutas	bebidas)	conservantes, embalagens PET/Tetra Pak
10	Transporte de cargas e operador multimodal (OTM)	Logística e transporte	Combustíveis (diesel), lubrificantes, pneus, paletes, embalagens, softwares de rastreamento
11	Móveis em madeira	Madeira e mobiliário	MDF, madeira maciça, colas à base de formaldeído, vernizes, parafusos, tintas
12	Serviços de pré-impressão	Gráfico/editorial	Tintas, papel fotográfico, solventes, emulsões, filmes, softwares gráficos
13	Embalagens plásticas e de papel (sacos, sacolas, copos, pratos, etc.)	Embalagens (papel e plástico)	Polietileno (PE), polipropileno (PP), papel kraft, tinta para impressão, adesivos
14	Máquinas e equipamentos para saneamento e meio ambiente	Metal-mecânico / engenharia ambiental	Aço inox, motores, bombas, válvulas, painéis elétricos, sensores, instrumentação
15	Impermeabilizantes, solventes e produtos afins	Químico (construção civil)	Betume, resinas, solventes orgânicos (BTEX, tolueno, xileno), aditivos, cargas minerais

Fonte: A autora (2025).

Figura 4. ETE multifabril de Jaboatão dos Guararapes (8°06'23.1"S 35°01'36.0"W), Brasil, composta por três lagoas de tratamento, que recebem efluentes de 15 segmentos industriais.



Fonte: A autora (2025).

Por não ter seu lodo removido ao longo dos anos, a primeira lagoa do sistema de tratamento tornou-se uma lagoa semi-anaeróbia, devido a falta de limpeza, uma camada densa, basicamente formada por óleos e graxas criou uma espécie de cobertura na superfície da lagoa, ocasionando a conformação de várias zonas mortas, ou seja, zonas que não há circulação/movimentação da água residuária. Na figura 5, é possível observar a circulação do efluente apenas no momento da entrada na lagoa, seguida depois pela visualização apenas de uma espessa camada de matéria escura.

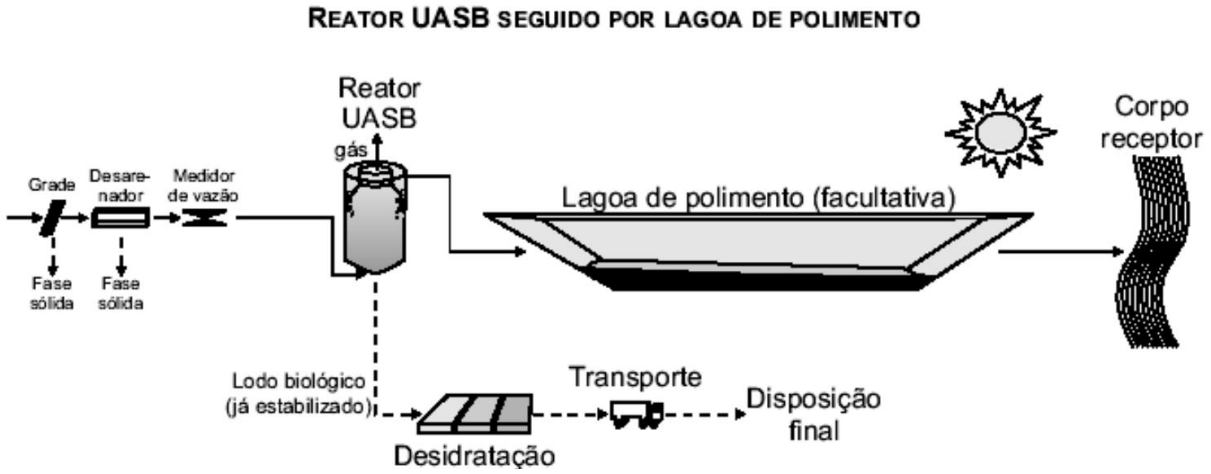
Figura 5. Superfície da lagoa de tratamento 1 apresentando espessa camada de matéria orgânica, óleos e graxas em sua superfície, além de zonas mortas

Fonte: A autora (2021).

3.2.2 Estação de Tratamento de Esgotos de origem doméstica - ETE Mangueira

A ETE Mangueira, está localizada no bairro da Mangueira, Recife, Pernambuco, Brasil (8°04'40.22" S e 34°55'29.39" W). Esta ETE foi projetada para tratar os esgotos domésticos de aproximadamente 50 mil habitantes, abrangendo também os bairros de San Martin, Mustardinha e regiões adjacentes. A estrutura da ETE Mangueira compreende um sistema preliminar de grades e desarenadores, seguido de um sistema biológico composto por oito reatores anaeróbios de fluxo ascendente (UASB), leitos de secagem de lodo e uma lagoa de polimento, com capacidade para tratar 32 litros de esgotos por segundo. Na figura 5, faz-se possível observar um modelo esquemático que se aproxima muito da infraestrutura da ETE Mangueira, divergindo apenas na quantidade de reatores UASB, no qual a ETE possui um total de oito reatores.

Figura 6. Modelo esquemático de uma ETE que utiliza reator UASB e lagoa de polimento como mecanismo de tratamento de esgotos

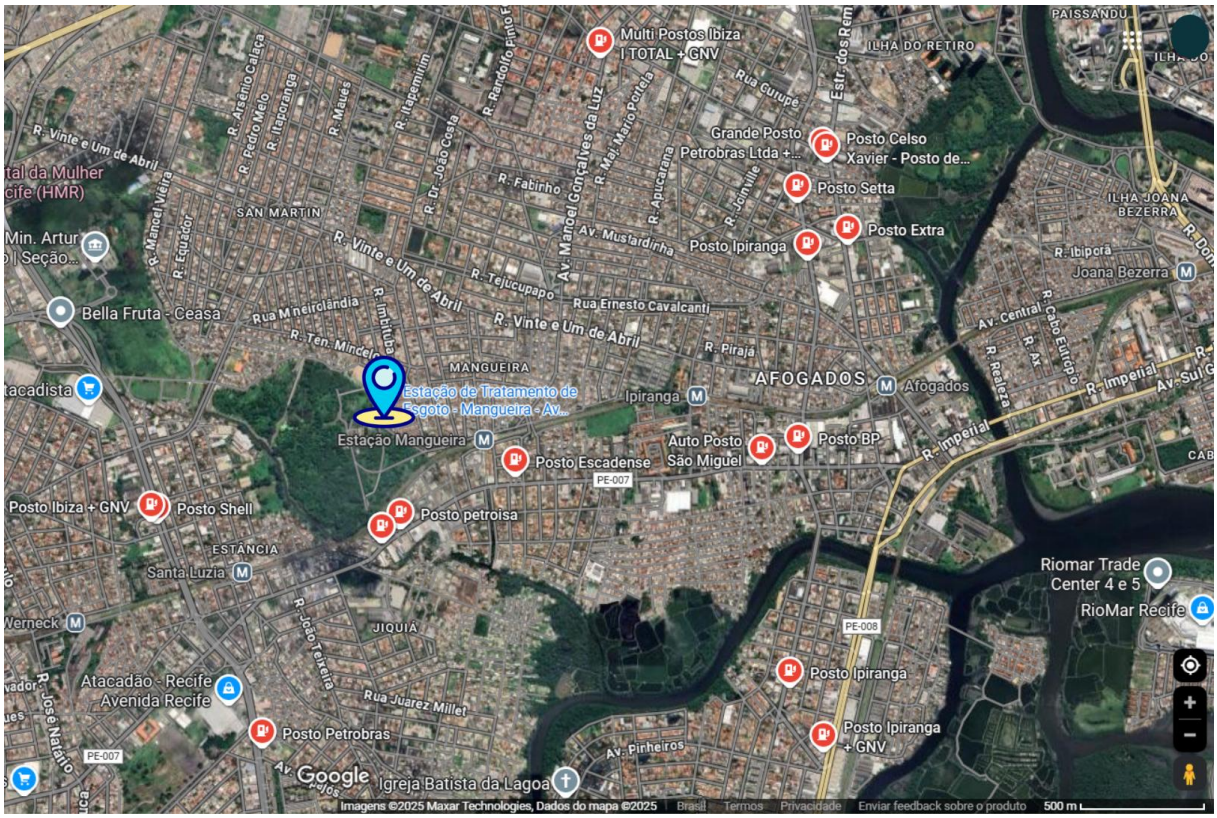


Fonte: (Gonçalves, 2016).

Devido a ações antrópicas, o BTEX pode se tornar um poluente bastante comum em grandes centros urbanos, uma vez que estes compostos estão presentes como matéria-prima principal ou secundária de uma série de produtos, como solventes, tintas, vernizes e combustíveis (Ghobakhloo et al., 2023; Kim et al., 2022; Zahed et al., 2024). Esses compostos, muitas das vezes de maneira acidental, acabam contaminando o ambiente e indo parar nos esgotos (Lima, 2010; Vicente et al., 2015; Zhang et al., 2023).

Na figura 7, é possível observar que a ETE Mangueira possui em seu entorno aproximadamente 16 postos de gasolinas (ícones de localização na cor vermelha). Vários estudos, como os realizados por (Figueiredo et al., 2021; Lima, 2010; Moraes and Oliva, 2019; Vicente et al., 2015), falam justamente acerca da contaminação de esgotos e águas subterrâneas por BTEX e outros xenobióticos em regiões próximas a postos de gasolina, uma vez que estes poluentes vão parar na rede de esgoto convencional ou no meio ambiente com pouco ou nenhum tipo de tratamento prévio, causando sérios problemas para a saúde pública e preservação ambiental.

Figura 7. Imagem de satélite da ETE Mangueira cercada por postos de combustíveis



Legenda: Ícone de localização azul com amarelo no centro da imagem aponta a ETE Mangueira, os demais ícones de localização em vermelho apontam os postos de combustíveis do entorno da ETE.

Fonte: Google Maps (2025).

A contaminação ambiental por compostos BTEX ao redor de postos de combustível é um problema recorrente, geralmente oriundos de vazamentos em tanques, falhas no sistema de contenção e manejo inadequado de resíduos oleosos. Esses compostos, presentes na gasolina e em outros derivados de petróleo, possuem alta mobilidade no solo e podem atingir aquíferos, comprometendo a qualidade da água subterrânea e superficial, além de representar riscos à saúde humana devido ao seu potencial tóxico e, no caso do benzeno, reconhecido caráter carcinogênico (Figueiredo et al., 2021; Lima, 2010; Moraes and Oliva, 2019; Vicente et al., 2015).

No Brasil, a prevenção e controle dessa contaminação são regidos por normas como a Resolução CONAMA nº 273/2000, que estabelece diretrizes para o licenciamento ambiental de postos de combustível, exigindo sistemas de contenção, monitoramento e manutenção preventiva (CONAMA, 2000). A Resolução CONAMA nº 396/2008, que define padrões de qualidade para águas subterrâneas também precisa ser respeitada pelos postos de gasolina.

Além disso, a Portaria GM/MS nº 888/2021 fixa limites máximos de BTEX na água para consumo humano, reforçando a necessidade de controle rigoroso. O cumprimento dessas legislações é essencial para evitar a contaminação ambiental, proteger os recursos hídricos e reduzir os riscos à saúde (CONAMA, 2008).

Por exemplo, a Resolução CONAMA nº 396/2008 estabelece alguns limites para os compostos BTEX em águas subterrâneas, são eles: benzeno 5 µg/L; tolueno 170 µg/L; etilbenzeno 200 µg/L e xilenos 300 µg/L. Já a Resolução CONAMA nº 273/2000 não estabelece limites quantitativos específicos para os compostos BTEX, mas exige que postos de combustíveis previnam e investiguem possíveis vazamentos e contaminações. Já a Portaria nº 888/2021 serve mais como uma norma geral que trata assuntos referentes à potabilidade da água para consumo humano, fixando os Valores Máximos Permitidos (VMP) dos BTEX em 5 µg/L para benzeno, 30 µg/L para tolueno, 300 µg/L para etilbenzeno e 500 µg/L para xilenos (Ministério da Saúde, 2021).

Apesar de todos os esforços cobrados legalmente, o que ocorre está bem longe da perspectiva estipulada pelas legislações vigentes. A contaminação por BTEX em áreas urbanas é real, e boa parte desta contaminação é oriunda dos postos de combustíveis (Figueiredo et al., 2021; Lima, 2010; Moraes and Oliva, 2019; Silva, 2002; Vicente et al., 2015). Deste modo é de se esperar que a ETE Mangueira além de receber efluentes de origem doméstica, acabem também por receber e tratar compostos recalcitrantes como os BTEX. Assim, não é de se admirar que a microbiota existente na ETE Mangueira possua certa adaptabilidade e tolerância aos compostos BTEX.

3.3 ESTAÇÕES DE TRATAMENTO DE ESGOTO COMO FONTE DE BACTÉRIAS DEGRADADORAS DE BTEX

A bioprospecção consiste na busca e exploração de organismos ou componentes do mesmo, como enzimas, genes e metabólitos, visando o desenvolvimento de um produto economicamente viável (Vadlja et al., 2022; Soccol et al., 2022). As ETEs constituem um ponto estratégico para pesquisas que visem avaliar a aplicação e potencial de microrganismos como degradadores de poluentes orgânicos persistentes, como os compostos BTEX (Janssen et al., 2005; Kumar and Singh, 2025; Sharma, 2024). As lagoas de tratamento são responsáveis pela remoção de matéria orgânica, nutrientes e contaminantes do efluente doméstico e industrial, operando como verdadeiros ecossistemas, mantendo comunidades microbianas diversificadas e metabolicamente adaptadas a condições ambientais extremas (Rajarshi, 2023; Tapadar et al.,

2021). A presença de compostos recalcitrantes no efluente das ETEs cria uma espécie de seleção natural, favorecendo a proliferação de microrganismos capazes de utilizar contaminantes tóxicos e potencialmente cancerígenos como fontes de carbono e energia (Ruikar, 2022).

Assim, o interesse científico e tecnológico em torno das ETEs, como fontes de bactérias degradadoras de BTEX é crescente, não apenas devido a abundância e diversidade desses organismos, mas devido a facilidade de acesso e utilização do lodo. O sedimento das ETEs é rico em biomassa microbiana, com gêneros bacterianos adaptados à presença de xenobióticos, e muitas vezes com vias metabólicas especializadas na degradação de hidrocarbonetos (Yang et al., 2020). Alguns gêneros como *Enterococcus*, *Citrobacter*, *Desulfovibrio*, *Alcaligenes*, dentre outros identificados nesta pesquisa, também possuem relatos na literatura reconhecendo a capacidade desses organismos em degradar hidrocarbonetos aromáticos, incluindo os BTEX (Gupta et al., 2022; Jin et al., 2023; Motteran et al., 2024; Pandolfo et al., 2023; Ruikar, 2022; Toth et al., 2021).

A literatura aponta que bactérias presentes em ETEs desenvolvem estratégias para sobreviver e prosperar nesses ambientes (Rajarshi, 2023). Um dos mecanismos utilizados por esses microrganismos está relacionada a maquinaria enzimática que envolvem monooxigenases, dioxigenases, desidrogenases e transferases (Yesankar et al., 2023). A degradação microbiana de BTEX nas ETEs pode ocorrer por vias aeróbias ou anaeróbias. Em sistemas de tratamento que possuem concentrações elevadas de oxigênio dissolvido, os microrganismos podem utilizar enzimas como tolueno dioxigenase, catecol 2,3-dioxigenase ou fenol hidroxilase para catalisar reações iniciais de oxidação de compostos aromáticos, convertendo-os em intermediários mais hidrossolúveis, como catecol e ácido benzoico (El-Naas et al., 2014; Hocinat et al., 2020).

Já em sistemas de tratamento majoritariamente anaeróbios, ou em zonas parcialmente anóxicas como o reator UASB da ETE Mangueira e a lagoa 1 da ETE multifabril, a degradação do BTEX pode vir a ocorrer mediada por consórcios microbianos que utilizam nitrato ou sulfato, esses aceptores substituem o O_2 na respiração celular. Estes consórcios geralmente envolvem bactérias redutoras de sulfato, metanogênicas e fermentativas, que degradam compostos aromáticos em condições de baixa disponibilidade de oxigênio (Dhar et al., 2023; Dou et al., 2008; Lueders, 2017; Palma and Costa, 2021; Toth et al., 2021). Estudos recentes aplicando metagenômica para desvendar o sedimento de ETEs revelaram a presença de genes codificadores de enzimas consideradas essenciais para estas vias metabólicas como benzaldeído desidrogenase e catecol 1,2-dioxigenase, indicando que estes ambientes são verdadeiros bancos

genéticos para aplicações biotecnológicas na remediação de poluentes (Fan et al., 2023; Haernvall et al., 2018; Hernández-Ospina et al., 2024; Schmid et al., 2015)

Estudos como os realizados por Dhar et al., (2023), Hocinat et al., (2020) e Jin et al., (2023) identificaram os filos *Desulfobacterota*, *Actinobacteriota* e *Pseudomonadota* como principais grupos envolvidos na degradação de hidrocarbonetos aromáticos. Estes estudos reforçam a relevância de investigações para compreender a dinâmica microbiana e otimizar processos de tratamento. Pois, a utilização de microrganismos advindos de ETEs visando a biodegradação de BTEX oferece inúmeras vantagens, como alta capacidade de adaptação a ambientes contaminados, elevada disponibilidade de biomassa microbiana e diversidade genética que podem ser bem aproveitadas e empregadas em sistemas de biorreatores (Dou et al., 2008; Mello, 2007; Yoshikawa et al., 2017).

Além disso, a integração entre dados taxonômicos e funcionais, obtidos por análise de microbioma ou metagenômica, permite não apenas identificar microrganismos de interesse, como também mapear vias metabólicas e prever a expressão de genes envolvidos na degradação de BTEX. Essa abordagem permite o uso de estratégias de manejo e melhoramento de consórcios microbianos, onde esses consórcios otimizados podem ser inseridos dentro das ETEs visando aumentar a eficiência do sistema de tratamento para a remoção de poluentes (Langille et al., 2013; Wasmund et al., 2024).

Por fim, conclui-se que a bioprospecção direcionada de bactérias degradadoras de BTEX em ETEs não se restringe apenas ao isolamento de cepas, mas se insere em um contexto amplo de inovação tecnológica e sustentabilidade. Ao valorizar e compreender o potencial microbiano dessas unidades, cria-se uma base sólida para o desenvolvimento de soluções de baixo custo e alto impacto para a remediação ambiental, alinhadas aos princípios que permeiam a preservação ambiental e economia circular (Nwankwo et al., 2021; Pandolfo et al., 2023).

4. RESULTADOS E DISCUSSÃO

Os resultados desta tese estão apresentados em forma de artigos científicos.

4.1 ARTIGO 1 (ARTIGO SUBMETIDO PARA PUBLICAÇÃO NA ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH)

CHARACTERIZATION OF THE TREATMENT UNITS AND THEIR MICROBIAL COMMUNITIES IN A WASTE STABILIZATION POND SYSTEM TREATING WASTEWATER FROM AN INDUSTRIAL COMPLEX LOCATED IN NORTHEASTERN BRAZIL

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ABSTRACT

The Multifactory Wastewater Treatment Plant (MF-WWTP) in Recife, Brazil, receives industrial and domestic effluents from 15 different industries and unlike other facilities, MF-WWTP has never undergone sludge removal, allowing for the establishment of a highly adapted microbial community. This study investigates the physico-chemical characteristics of the treatment process and the microbial composition that contributes to its efficiency. Results indicate a COD removal of 84% and BOD removal of 83%, primarily occurring in Pond 1 (P1, anaerobic), while Pond 3 (P3, aerobic with high algal activity) aided in heavy metal removal. Despite the removal efficiencies, toxicity persisted in the final effluent, evidenced by mitotic index values (9.20–11.44% vs. 6.79% control) and chromosomal alterations (1.49–2.20%), which could be a result of toxin-producing algae in P3. The microbial analysis identified *Fervidobacterium* (18–38% relative abundance) as the dominant bacterial genus, alongside methanogenic archaea (*Methanolinea* and *Methanosaeta*), suggesting their importance for the organic substrate conversion and degradation of industrial pollutants. Additionally, the cyanobacteria *Cyclotella* and the microalgae *Planktothrix* were highly abundant in P3 (10 $mm^3.L^{-1}$ and 9 $mm^3.L^{-1}$, respectively), which might have contributed to treatment system but also potential toxin production in the final effluent. These findings suggest that the combination of anaerobic, microaerobic, and aerobic ponds, along with additional polishing units, can be a viable approach for industrial wastewater treatment. Furthermore, both the sludge and final effluent from MF-WWTP could serve as valuable inoculum sources for other treatment units dealing with complex industrial contaminants, aiding in establishing resilient microbial communities for optimized wastewater treatment.

KEYWORDS: industrial wastewater; waste stabilization ponds; *Fervidobacterium*; *Cyclotella*; *Planktothrix*; microbial community; algal community

RESEARCH HIGHLIGHTS

- Serial anaerobic-microaerobic-facultative ponds provided good treatment performance.
- *Fervidobacterium* was the most abundant genus and might be a key for the treatment.
- Abundance and diversity of microalgae and cyanobacteria in ponds 2 and 3, with *Cyclotella* and the cyanobacteria *Planktothrix* with greatest biovolume in P3

1 INTRODUCTION

The increase of urban sprawl, deforestation, and agricultural land area as a result of the increase of anthropogenic land use is, nowadays, the main driver for the global decline of water quality (Mello et al. 2020; Giri & Qiu 2016). According to the census performed in 2022, Brazil has over 203 million inhabitants, making the country the most populous in South America and the 7th in the world and almost 80% of the Brazilian population are living in urban areas, of which only 63% of Brazilian households have access to sanitary sewer or septic tanks (IBGE 2023). The Environmental Quality Report published in 2020 by the Brazilian Institute of Environment and Renewable Natural Resources (Instituto Brasileiro de Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA) indicated that the dumping of raw or partially treated domestic and industrial wastewater in the environment is the major source of contamination of water sources in Brazil.

In regards to the domestic wastewater treatment in Brazil, in 2017, there were 2768 operational urban wastewater treatment plants, responsible for treating 51% of the produced wastewater, and the main focuses of the treatment plants are organic matter and pathogens removal with most of them showing average BOD removal ranging from 60 - 80% (ANA 2017; IBGE 2023; SNIS 2022). Thus, there is an urgent need for the expansion of treatment capacity, with the installation of new plants and also the expansion of the current working plants to implement nutrient removal units and for the improvement of their performances. Studying the treatment plants that show good performances is also important, because it would give important information for the design of new plants and improvement of non-well performed installed ones.

Waste stabilization ponds (WSP) are the most used treatment processes in Brazil, representing 36% of the operational plants (anaerobic, aerobic, facultative, maturation ponds and their combination). While they are high in number, they only account for the removal of 21% of the equivalent population, indicating that while they are spread throughout the country, they are not operating in highly dense urban areas (ANA 2017). WSP became popular in Brazil, especially in rural areas, because of the favorable environmental conditions, land area availability, and the low maintenance required (Von Sperling 2017).

Stabilization ponds are designed to receive domestic or industrial wastewater where they stay for days, allowing for bacteria (aerobic in facultative and aerated ponds, or anaerobic bacteria in anaerobic ponds) to degrade the soluble organic compounds and fine particulate matter suspended in the liquid, while the large particulate organic matter settles and are

stabilized by anaerobic bacteria. The oxygen required for the aerobic bacteria in facultative ponds are supplied by algae, through photosynthesis. In some cases the ponds are designed to be shallow, allowing for enough light penetration that aids in the combat against pathogens. This type of treatment represents a viable economic alternative since environmental conditions, such as high temperature and long periods of light intensity, are favorable to the biological treatment of wastewater in tropical countries (Espinosa et al. 2017; Bressani-Ribeiro et al. 2019; Mahapatra et al. 2022).

In WSP, a consortium of bacteria, archaea, and microalgae is established to work in synergy to biodegrade the domestic and industrial wastewater (Peil et al. 2016; Chai et al. 2021). These organisms harbor molecular tools, such as biodegradation enzymes, that produce a gradient of metabolization in which the product of an enzyme from a microorganism might be the substrate for the next one. It creates a flux of nutrients that feeds the trophic chain and that favors their adaptation to such hazardous environments (Tatta et al. 2022).

The Pernambuco State Sanitation Company (Companhia Pernambucana de Saneamento - Compesa) operates a Multifactory Wastewater Treatment Plant (MF-WWTP), located in the municipality of Jaboatão dos Guararapes, Recife metropolitan area, Pernambuco (Brazil), that is responsible for the treatment of a complex effluent wastewater of the Multifactory Industrial Complex (MIC), from baking, dairy, metallurgy, laundry, ink, among other industrial sectors. The MF-WWTP project was originally designed to work with two facultative ponds, followed by a maturation pond, and to solely receive domestic sewage. However, over the time, following the industrialization of the area, industrial wastewater was also generated and mixed to the effluent.

Nowadays, this wastewater is mainly composed of organic degradable compounds (carbonaceous organic matter) and recalcitrant industrial products such as dyes, hydrocarbons and surfactants, as well as different types of metals. Despite this complexity, its ponds remained stable over years and according to Compesa they are capable of removing more than 80% of the organic matter, measured as carbon oxygen demand (COD), which stands as a highly efficient treatment unit regarding the pond system.

In terms of the legislation, the output of the MF-WWTP has to comply with the parameters of current resolution of the Environmental Nacional Council (CONAMA 430/2011) with monthly measurement of the influent and the final effluent (COD, ammonium, solids, and pathogens). Considering the good overall performance of the MF-WWTP in terms of COD removal (80%), this work aimed to provide a deeper comprehensive evaluation of such a unique system in terms of (1) physico-chemical evaluation of each treatment unit (2) microbial

community structure throughout the treatment process, (3) and the ecotoxicity and cyto-genotoxicity of the system, in order to understand how the system works and how it could be replicated or transferred to other treatment units.

2 MATERIALS AND METHODS

2.1 SITE DESCRIPTION

This research was conducted at the MF-WWTP, which started operating in the early 1990's and is currently co-managed under a public-private agreement involving the public company Compesa and the private company BRK Ambiental. This unit is located in the municipality of Jaboatão dos Guararapes (8°06'23.1"S 35°01'36.0"W), within the Recife metropolitan area, state of Pernambuco, Brazil. It is part of the Jaboatão industrial complex encompassing 15 segments, including food industry, metallurgy, machining, refrigeration, stationery, carpentry, graphic products, inks and solvents, galvanized products, polymers, among others (Fig 1). In addition, the domestic sewage of the industries is also integrated into the collection network, making the effluent that arrives at the MF-WWTP a complex and diverse substrate.

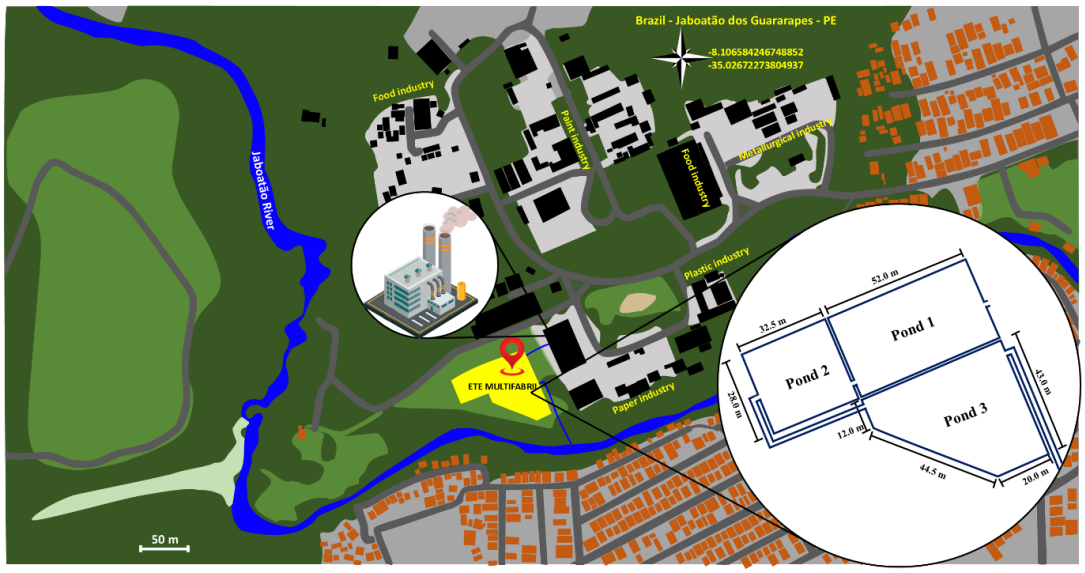

Fig 1. Multifactory Effluent Treatment Plant (MF-WWTP) in the municipality of Jaboatão dos Guararapes (8°06'23.1"S 35°01'36.0"W), Brazil, consisting of three stabilization ponds, which receive effluents from 15 industrial segments. In an enlarged view, it is possible to see the floor plan of the MF-WWTP, with its respective dimensions.

The unit comprises three stabilization ponds (P1, P2 and P3) with 2 m deep in average (Fig 1): P1 has 1,456 m^2 of surface area, characterized by a thick black surface layer composed predominantly of oils and greases. It receives the complex effluent in a highly varying flow rate, with averages of 3,4 ± 3,8 $L.s^{-1}$ but with peaks reaching 44 $L.s^{-1}$; P2 has 910 m^2 of area and presents little oily coverage; while P3 has 1,740 m^2 of surface area and already has the presence of some aquatic plants. The connection between P1 and P2 and between P2 and P3 is carried out by pipes of 200 mm in diameter located from the surface downwards of the water layer. From the latter, treated water is discharged into the Jaboatão river in that municipality (Fig 1). For this study, samples were collected at the inlet and the outlet of each pond and at internal points, as marked in Fig. S1 (Supplementary material).

2.2 SAMPLING PROCEDURE

Samples were collected during the spring (November) of 2021 and summer (February) of 2022 according to the scheme in Fig S1 (Supplementary material), both during the dry season with monthly precipitation averages of 28.0 ± 21.9 mm and 90.6 ± 28.7 mm, respectively (Table S1, Supplementary material). For physicochemical analysis, wastewater samples were collected in four points, corresponding to the untreated wastewater (Sampling point 13, located in the Parshall flume at the entrance of the treatment plant, Fig S1, and treated wastewater after P1 (located in the pipe that connects P1 and P2, Sampling point 14, Fig S1), after P2 (in the canal that connects P2 and P3, Sampling point 15, Fig S1), and after P3 (located in the outfall pipe, Sampling point 16, Fig S1).

The mean color of each pond was defined (Table S2, Supplementary material). For each sampling point, four 1L polyethylene (PE) bottles were filled, kept on ice and transported to the laboratories, where some analyses were performed on the same day as recommended by the American Public Health Association - APHA (2017) and an aliquot of the samples was preserved for further analyses. For algal and bacterial community analysis, liquid and sludge samples were collected from P1, P2, and P3. In P1, samples were taken from four points: two near the entrance, one in the middle near the edge, and one near the pipe connecting P1 and P2. A 3-meter-long extension arm was used since the thick top layer in this pond prevented the use of an inflatable boat.

Liquid samples were collected from the surface, while sludge samples were taken from the bottom. In P2, samples were collected from two points: one in the middle of the pond and

one near the effluent channel. In P3, samples were taken throughout the pond using an inflatable boat and a Van Dorn sampler (Fig S1, Supplementary material). Liquid samples for algal community, ecotoxicity, and cyto-genotoxicity analysis were collected from the surface and at a depth of 60 cm, mixed, homogenized, and stored in 500 mL PE bottles on ice. Samples intended for algal community analysis were preserved in 4% formaldehyde before being sent to the laboratory. Sludge samples for bacterial and archaeal community analysis were also stored in PE bottles, kept on ice, and transported to the laboratory for DNA extraction within 24 to 36 hours after collection.

2.3 PHYSICOCHEMICAL ANALYSIS

Conductivity, pH, Dissolved Oxygen (DO) and Redox Potential (ORP) were performed during the sampling. Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), alkalinity (total and partial) and heavy metals and semi-metals were performed in the laboratories on the same day of sampling. For COD and BOD, samples were analysed raw and filtered in a 1.2 µm glass fiber membrane (Satorius, Gottingen, Germany). Statistical analyses were performed using RStudio to assess normality and homogeneity of variance. Statistical analysis was performed to evaluate the data distribution, either parametric tests (t-test, ANOVA followed by Tukey's post-hoc test) or non-parametric tests (Kruskal-Wallis and Mann-Whitney) were applied as appropriate. All statistical analyses were conducted using RStudio (Posit Team, 2025).

After sample fractionation, 2 L of the filtered samples were acidified with H_2SO_4 (40% v/v) to pH 2 and kept in amber glass flasks at 4°C for ammoniacal-nitrogen ($N\text{-}NH_3$) and Total Kjehdal Nitrogen (TKN), while another 2 L of filtered samples were stored for maximum of 14 days at 4°C for nitrate ($N\text{-}NO_3$), nitrite ($N\text{-}NO_2$), color, total and suspended solids analysis. For VFA analysis, aliquots of 10 mL were stored in 10 mL glass flasks without headspace and kept refrigerated at 4°C. The VFA analysis was performed by gas chromatography (Agilent Technologies 7890A, Agilent Technologies Inc., Wilmington, DE, USA) coupled with a FID detector, and is described in the Supplementary Materials.

2.4 BACTERIAL COMMUNITY ANALYSIS

Sludge samples (Fig S.1, Supplementary Materials) were subjected to total DNA extraction using the PowerSoil DNA extraction kit™ (Qiagen, Hilden, Germany), following

the protocol recommended by the manufacturer. Total DNA was quantified using the NanoDrop 2000 equipment (Thermo Fisher Scientific, Walthan, USA) and then sent for its 16S rRNA amplicon sequencing at Neoprospecta company (Florianopolis, Brazil), which used MiSeq Illumina device using metabarcoding applied to the V3/V4 domain of the 16S rRNA gene. Data were processed using Metagenomics Rapid Annotation Using Subsystems Technology (MG-RAST), and the SILVA SSU database was selected for analysis.

Relative abundances of bacteria and archaea were calculated separately, and only taxa with a relative abundance greater than 1% were considered relevant to this study. Alpha (α) and beta (β) diversity analyses were performed using the phyloseq package in R, executed via RStudio (McMurdie and Holmes, 2013; McMurdie and Holmes, 2015). Statistical differences in the relative abundances of taxa between sample pairs were assessed using STAMP (Parks et al., 2014), employing a two-sided G-test with Yates' correction and Fisher's exact test, along with the Newcombe-Wilson method for calculating confidence intervals. Taxonomic classification data were used for functional inference using FAPROTAX (Louca et al., 2016).

2.5 IDENTIFICATION AND COUNTING OF PHYTOPLANKTON

Samples for phytoplankton identification were preserved in 4% formaldehyde and stored at 4 °C for quantitative and qualitative analysis by optical microscopy (model DMLB, Leica Microsystems GmbH, Germany) following APHA (2017). The identification was performed at the genus level using the parameters in the specialized literature: Komárek (1983) and Prescott and Vinyard (1982) for Chlorophyta; Komárek and Anagnostidis (2005, 1989), Komárek and Komáková (2004) and Komárek and Cronberg (2001) for Cyanophyta; Popovský and Pfiester (1990) for Dinophyta; (Krammer, 1991a, 1991b) for Heterokontophyta and (John et al., 2002) for other phytoflagellates, such as Euglenophyta and Chryptophyta.

A minimum of 400 cells of each sample were counted in Sedgwick-Rafter chambers to determine phytoplankton density (cel/mL), following (APHA 2017). The mean volume of each species was calculated considering the cellular measurements of 30 individuals, according to the geometric models suggested by (Hillebrand et al. 1999), and biomass was expressed in mm^3/L. The taxonomic classifications were based on the specialized literature (Komárek and Komáková 2004; Oliveira et al. 2015).

2.6 ECOTOXICITY USING *Aliivibrio fischeri*

The acute toxicity of pond samples was assessed using lyophilized bioluminescent marine bacteria *Aliivibrio fischeri* (A. fischeri NRRL B-11177, synonym Vibrio fischeri), based on the inhibition of light emission when bacterial cells are exposed to toxic conditions. The method followed the Brazilian standardized procedures (ABNT NBR 15411-3) for untreated effluents. *A. fischeri* cells were exposed to untreated samples from three ponds and a series of dilutions (1:512; 1:256; 1:128; 1:64; 1:32; 1:16; 1:8; 1:4; 1:2; 1:1).

Label K represents the reference where samples were replaced with 2% (w/v) NaCl. The positive control included the addition of 3,5-dichlorophenol (3,5-DF; Sigma Aldrich, No. LRAC5200) at 4.5 mg/L. After 15 minutes of exposure, the suspensions were analyzed for bacterial bioluminescence at the Hydrobiology and Toxicology Laboratory of the State Environment Agency (CPRH). The inhibitory effect of the aqueous samples was evaluated by the reduction in bioluminescence of the suspensions, which defined the Toxicity Factor (TF) according to the criteria established by the standardized procedure.

2.7 TOXICITY AND CYTOGENOTOXICITY *Allium cepa* test-system

The toxicity and cyto-genotoxicity assays were performed using the *Allium cepa* test-system according to Fiskesjö (1988), Fernandes et al. (2007) and Leme et al. (2008). Seeds from *A. cepa cv*. Vale Ouro IPA-11 variety were germinated on a layer of sterile cotton in Petri dishes covered with filter paper discs moistened with 15 mL of each MF-STS sample. Fifty seeds were placed per dish with three dishes per sample. For negative control, discs were moistened with sterile mineral water. For the genotoxicity analysis positive controls, the discs were moistened with the herbicide Trifluralin (0.84 ppm of active principle) or with the mutagenic agent Methyl Methanesulfonate (MMS, 400 μM).

After 72 h of germination, root tips were collected, fixed in Carnoy (ethanol:acetic acid, 3:1) for 24 h at room temperature and stored at -20 ºC. For the preparation, fixed roots were washed three times in distilled water, 5 min each, hydrolysed in 1 mol.L^{-1} HCl at 60 °C for 10 min, washed again and stained with Schiff's Reagent (1.090033, Sigma-Aldrich) for 1 h in a dark room. After this period, the roots were washed in distilled water and the meristem was separated, placed in a slide with a drop of 2% acetic carmine, covered with a coverslip and lightly crushed. After coverslip removal using liquid nitrogen, the slides were air dried and mounted with Entellan™ solution (Sigma-Aldrich, St Louis, USA). Cytological analyses were carried out using an optical light microscope at a magnification of 400x. Cell images were captured with a Leica DFC 340FX camera, using Leica's CW 4000 program. Images were

optimized for brightness and contrast using Adobe Photoshop CS3 (Adobe Systems Incorporated).

The toxic potential of the samples was evaluated based on the average Germination percentage (G), calculated by the ratio between the number of seeds germinated after 48 h, 72 h and 20 days of incubation and the total number of incubated seeds multiplied by 100. On the other hand, the cytotoxic potential of the samples was evaluated by the Mitotic Index (MI) and genotoxicity by the Chromosomal Alteration Index (CAI) in meristematic cells of *A. cepa*. MI and CAI were obtained from the analysis of 500 meristematic cells/slide, with 10 slides/treatment, totalling 5000 cells/treatment. The MI was calculated as the ratio between the number of cells in division by the total number of surveyed cells. CAI was obtained by the ratio between the number of observed cellular alterations (C-metaphases, nuclear buds, micronuclei, multipolar anaphases, polyploid metaphases, and chromosomal adhesion, losses, delay, breaks and bridges) and the total number of surveyed cells.

For Statistical analysis, seven samples were evaluated for the bioassays with *A. cepa* four samples from the MF-WWTP, one negative control and two positive controls. For the toxicity test, three repetitions were used for each treatment, consisting of a Petri dish with 50 seeds. For cytotoxicity and genotoxicity tests, the experimental unit consisted of one slide (500 cells analysed), 10 slides from each treatment. Data normality and homogeneity were verified by Shapiro-Wilk and Kolmogorov-Smirnov tests, respectively. The data obtained from the MI and the CAI tests did not present a normal distribution nor were they homogeneous. Therefore, the non-parametric Kruskal-Wallis test was used ($p < 0.05$). On the other hand, the G data that showed normal distribution and homogeneity was submitted to Tukey's parametric test ($p < 0.05$). The three parameters were further tested using the statistical program GraphPad Prism (version 8.4.2). G and MI values were transformed using the formula arcsine(√frequency), while the CAI was transformed using the formula x+1. The data were loaded on an Excel™ worksheet and plotted as graphical representation.

3 RESULTS AND DISCUSSION

3.1 CHARACTERISTICS OF THE WASTEWATER TREATMENT UNIT

Fig 2 shows the results of the measured physicochemical parameters from the treatment units. The ORP values indicate that P1 was operating as an anaerobic system with the redox potential values of -300 mV, while P2 can be considered as a microaerobic system with -70.5 mV, according to the classification reported in the literature (Nguyen and Khanal, 2018). The low dissolved oxygen concentration measured for P1 and P2 effluent corroborates this consideration (Fig 2). The industrial effluent reached the treatment station slightly alkaline, but after the treatment from Pond 1 the effluent became neutral. This could be caused by the conversion of organic matter from the raw wastewater into organic acids, from the anaerobic processes by the microbial population in P1 (Fig 3).

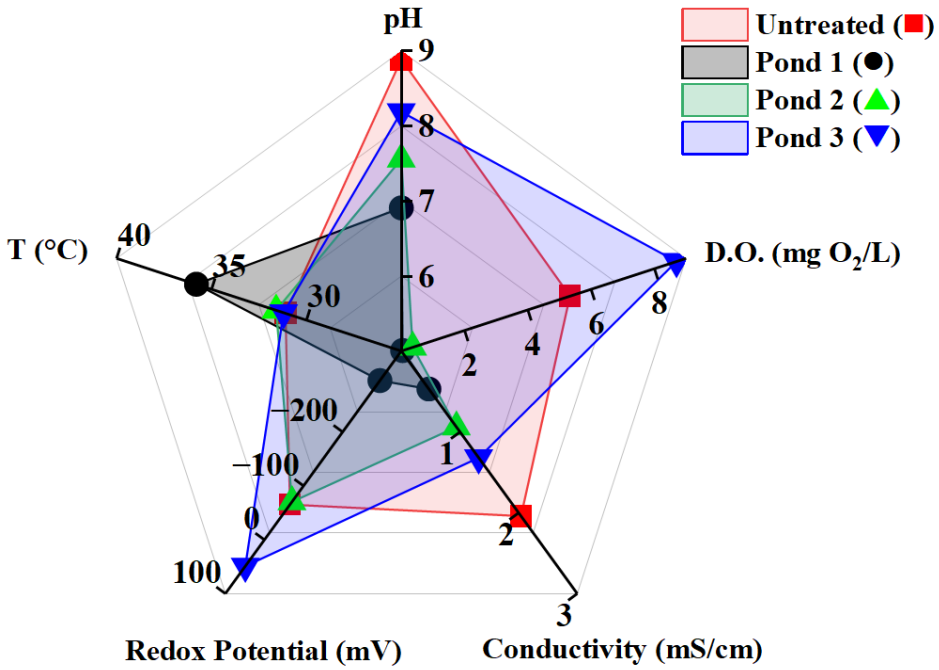

Fig 2. Multiparameter plot of the physicochemical variables of the influent (industrial effluent) and the effluent of the Multifactory Wastewater Treatment Plant (MF-WWTP).

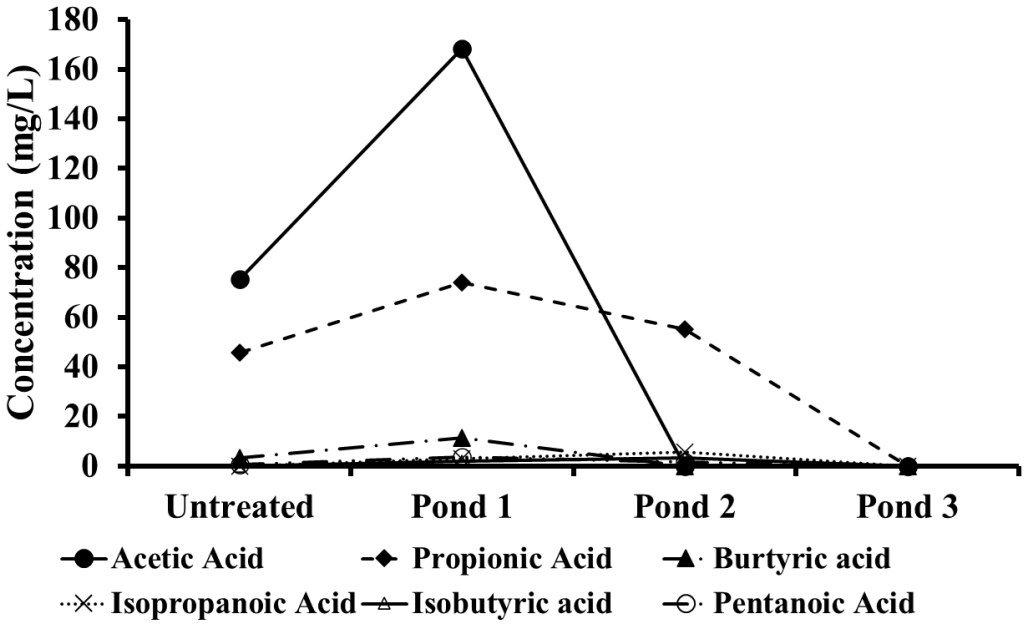

Fig 3 Primary organic acids detected and their concentration after each unit treatment of the Multifactory Wastewater Treatment Plant (MF-WWTP).

Acetic and propionic acid, which were the major organic acids found in P1, are found as the most common organic acids produced in anaerobic digesters (Gujer and Zehnder 1983; Harirchi et al. 2022). These initial results showed that the Pond 1 which was first designed to act as a facultative pond, is now working anaerobically. The high dissolved oxygen concentration, positive redox potential and alkaline pH from the final effluent indicated that P3 was operating as a facultative stabilization pond (Fig 2). The photosynthesis in stabilization ponds with high algal activity can increase the dissolved oxygen concentration and carbon dioxide consumption, resulting in an alkaline environment (Moghazy et al. 2022), which was observed after the treatment from Pond 3.

The high COD and BOD concentration from the raw untreated wastewater, and low BOD from the filtered untreated shown in Table 1 indicated a high presence of soluble suspended organic matter in the untreated wastewater and the high COD/BOD ratio from the filtered untreated wastewater suggests the presence of recalcitrant dissolved compounds, which is expected from a mix of industrial wastewater (Tchobanoglus et al., 2003; Vítězová et al., 2020). Alves et al. (2022) and Florencio et al. (2001) reported an average COD from 206 to 290 mg $O_2.L^{-1}$ for the raw domestic wastewater from a location nearby, much lower than the raw COD from the untreated wastewater measured in this study.

Table 1. Chemical demand for oxygen (COD) and Biochemical Oxygen Demand (BOD) analysis and removal efficiencies of the untreated sample and pond 1, 2 and 3 samples from the Multifactory Wastewater Treatment Plant (MF-WWTP).

Parameter (mg O_2/L)	Untreated	Pond 1	Pond 2	Pond 3
Raw COD	1569.8 ± 439.5	1000.9 ± 529.7	728.7 ± 335.7	826.6 ± 563.8
Filtered COD	1301.8 ± 136.5	491.3 ± 544.2	347.0 ± 268.0	250.7 ± 159.3
Raw $BOD_{5,20}$	875.0 ± 530.3	475 ± 530.3	550.0 ± 565.6	375.0 ± 388.9
Filtered $BOD_{5,20}$	225.0 ± 247.4	500.0 ± 0.0	345.0 ± 275.7	150.0 ± 0.0
Raw COD/$BOD_{5,20}$	1.79	2.11	1.32	2.20
Filtered COD/$BOD_{5,20}$	5.79	0.98	1.01	1.67

The wastewater treatment system demonstrated distinct removal patterns for particulate and dissolved organic matter across the sequential ponds. Statistical analysis revealed that while all three treatment ponds significantly reduced filtered COD compared to the untreated wastewater ($p < 0.001$), the majority of this removal occurred in Pond 1, with subsequent ponds contributing little additional reduction ($p > 0.38$ between ponds). In contrast, raw COD showed a different behavior, where significant reductions only became apparent after Pond 2 and were further enhanced in Pond 3 ($p < 0.05$), with Pond 1 showing no statistically significant removal. These findings suggest that dissolved organics are effectively treated in the initial stage, while particulate matter requires extended treatment through multiple ponds.

The system's performance indicates that Pond 1 plays a crucial role in dissolved organic removal but may benefit from optimization to improve particulate matter reduction, whereas Ponds 2 and 3 are essential for particulate removal but show diminishing returns for dissolved organics. Statistical analysis showed no significant reduction in either raw or filtered BOD across ponds, despite some numerical changes, indicating consistent treatment limitations likely influenced by variability and small sample size. The high raw COD and BOD concentrations after the final treatment from P3 might be a consequence of the presence of algae, which could be visually observed in the liquid samples collected from each pond and also in the high presence of volatile suspended solids (Fig S2 and Table S3, Supplementary material).

While the MF-WWTP showed small COD removal (47%) for raw untreated wastewater and raw P3 effluent, when considering the filtered COD of P3 to remove the effect caused by the presence of algae, then the removal reaches 84% for COD and 83% for BOD, with results comparable to similar plants treating industrial wastewater (Alves et al. 2020; da Silva et al. 2011; Mahapatra et al. 2022; Veeresh et al. 2010). These findings indicate the necessity of a filtering unit after P3 to remove the algae from the final effluent and achieve good performance.

The nitrogen series presented in Table 2 offers relevant complementary insights into the performance of the MF-WWTP. The untreated wastewater had lower ammonia ($N\text{-}NH_3$) and higher nitrate ($N\text{-}NO_3^-$) concentrations compared to typical values reported for domestic wastewater in the region, which range from 32.5 to 42.0 mg $N\text{-}NH_3$/L and 0.65 to 1.00 mg $N\text{-}NO_3^-$/L (Espinosa et al., 2016; Silva et al., 1995; Soares et al., 1996). No statistically significant differences in $N\text{-}NH_3$ and TKN concentrations were observed across the treatment stages, which can be caused by the solubilization of organic matter via anaerobic metabolism in reactors P1 and P2 that may have contributed to the release of ammonia into the effluent (Haandel & Lubbe, 2012; Chernicharo, 2007; Von Sperling, 2007b).

Table 2. Physico-chemical parameters of the untreated and ponds 1, 2 and 3 samples from the Multifactory Wastewater Treatment Plant (MF-WWTP).

Parameter	Untreated	Pond 1	Pond 2	Pond 3	Upper limit*
Raw ammonia (mg N-NH_3/L)	8.4 - 17.2	9.4 - 21.1	8.4 - 17.4	9.9 - 11.1	20.0
Filtered ammonia (mg N-NH_3/L)	7.3 - 11.3	7.5 - 19.6	7.1 - 15.7	6.9 - 9.8	-
Raw TKN (mg N/L)	23 - 50.2	17.5 - 67.4	23.3 - 26.3	28.2 - 51.5	-
Nitrate (mg N-NO_3/L)	4.65 ± 0.03	0.39 ± 0.02	0.35 ± 0	0.50 ± 0	-
Nitrite (mg N-NO_2/L)	0.12 ± 0.01	<0.01	<0.01	<0.01	-

* Defined by the Brazilian legislation CONAMA 430/2011 and 357/2005

Additionally, the presence of algae in P3 may have further influenced the elevated concentrations of N-NH_3 and TKN. The removal of nitrate and nitrite observed in reactor P1 (Table 2) is likely attributable to microbial denitrification processes, facilitated by the anaerobic conditions and availability of organic substrates. The treated wastewater from the MF-WWTP achieves N-NH_3 concentrations in compliance with the standards established by the discharge conditions and standards of effluents in Brazilian legislation (CONAMA 430/2011).

In addition to organic matter and minerals, heavy metals are commonly present in industrial effluent treatment systems in Brazil (Souza and Siqueira, 2023). Industries have the largest number of processes in which heavy metals can be originated, as they are used from incorporation into the product to washing machinery, pipes, floors, for cooling and steam generators. In the present work, 18 metals and semimetals were quantified in the MF-WWTP, since the incoming effluent receives waste from the metallurgical and metal-mechanic industries (Table 3).

Table 3. Metal and semimetal data of the untreated sample and pond 1, 2 and 3 samples from the Multifactory Wastewater Treatment Plant (MF-WWTP).

Metal (mg/L)	Untreated	Pond 1	Pond 2	Pond 3	Upper limit*
Aluminium	7.5	<0.01	<0.01	<0.01	-
Barium	0.097	0.061	0.065	0.056	5.0
Beryllium	<0.04	<0.04	<0.04	<0.04	-
Boron	<0.1	<0.1	<0.1	<0.1	5.0
Cadmium	<0.001	<0.001	<0.001	<0.001	0.2
Calcium	29.33	25.97	29.41	22.43	-
Lead	<0.01	0.013	0.017	0.015	0.5
Cobalt	<0.005	<0.005	<0.005	<0.005	-
Copper	0.048	0.027	0.028	0.023	-
Iron	<0.01	<0.01	<0.01	<0.01	15.0
Lithium	0.2	<0.1	<0.1	<0.1	-
Magnesium	<1	<1	<1	<1	-

Manganese	<0.02	<0.02	<0.02	<0.02	1.0
Nickel	0.018	<0.01	0.013	0.01	2.0
Silver	<0.005	<0.02	<0.02	<0.02	0.1
Vanadium	<0.02	<0.02	<0.02	<0.02	-
Zinc	0.13	<0.05	<0.05	<0.05	5.0

* Defined by the Brazilian legislation CONAMA 430/2011 and 357/20

Out of the 18 metals/metalloids analyzed, only nine have specific effluent disposal limits defined (CONAMA 430/2011): lead (0.5 mg/L), cadmium (0.2 mg/L), boron (5.0 mg/L), barium (5.0 mg/L), iron (15.0 mg/L), manganese (1.0 mg/L), nickel (2.0 mg/L), silver (0.1 mg/L), and zinc (5.0 mg/L). All samples from the MF-WWTP system were in compliance with the Brazilian legislation for these nine compounds (Table 3). Additionally, in P3 effluent, complete removal of aluminum and a reduction level of barium, calcium, lithium, copper, nickel, and zinc were observed, most probably by the microalgae population (see below).

The results show that a combination of sequential anaerobic, microaerobic and aerobic or facultative ponds can show good treatment performance in treating a mix of industrial and domestic wastewater, provided a final filtering unit responsible for the removal of algae from the final effluent. Pond 1 plays a crucial role in dissolved organic conversion and nitrate removal, while particulate matter required extended treatment across multiple ponds. The presence of algae in P3 influenced final COD, BOD and TKN oncentrations, suggesting that a post-treatment unit is nececssary to improve effluent quality and ensure more stable removal efficiencies.

3.2 TOXICITY OF THE WASTEWATER SAMPLES AFTER TREATMENT

The ecotoxicity test with *A. fischeri* used in this work is widely used for the evaluation of wastewater and industrial effluents because it provides a quick and economical response for monitoring effluents (Abbas et al. 2018) and lies in its ability to provide comprehensive insights into the biological effects of the treatment process. These tests are crucial for understanding how the system works and determining the degree of toxicity reduction at various stages of

treatment (Boehler et al. 2017). The results revealed the toxicity of all samples tested in the *A. fischeri* test, with the highest values for samples collected from the effluents of P1 and P3 (Table S4, Supplementary material).

Additionally, the *A. cepa* test system allowed the analysis of toxicity, cytotoxicity, and genotoxicity based on seed germination (Table 5, Table S5, Supplementary material). After 48 hours of exposure, a significant reduction in germination values was observed in the presence of the untreated sample (16%) and samples from P1 (20%), P2 (11.33%), and P3 (31.33%), compared to the negative control (72%). At 72 hours, a significant reduction in the G72 value was observed only for the influent (66%) and P2 (59.33%) samples (Table 5) ($p<0.05$). At the end of 20 days, the germination values of the four points sampled from the MF-WWTP were similar to the negative control.

Despite the toxicity observed in all three treatment ponds, the *A. cepa* data indicates that P2 presented higher toxicity, probably related to the presence of biodegradation byproducts observed in the first two ponds. This understanding is vital for replicating or transferring the system to other treatment units, ensuring that the same efficiency and effectiveness in reducing toxicity can be achieved elsewhere. Despite some reduction in toxicity compared to P2, the final effluent from P3 still exhibited persistent toxicity, likely influenced by the presence of algal toxins.

Similar toxic potential was found previously for effluents from electroplating, paper and dye industries (Abbas et al. 2018). In the present work, the differences in toxic potential for *A. fischeri* and in *A. cepa* seed germination can be explained by changes in the characteristics of the ponds and the bioavailability of products/substances present in the collected samples. Wastewater from paint manufacturing, for instance, contribute to the increment of the chemical oxygen demand and turbidity, besides the organic and toxic chemicals levels, such as surfactants, bactericides, oils, solvents, preservatives and heavy metals which can cause environmental damages (Nair K et al. 2021; Verma et al. 2012).

It is worth noting that the multifactory complex under study includes two paint manufacturing companies. Additionally, the results suggested that some non-bioavailable products in P1 underwent chemical transformation, possibly generating toxic by-products in Pond 2. On the other hand, these products appear to have undergone additional transformations in P3, resulting in a reduction of toxicity towards the *A. cepa* test system. Xylene, used in paints, rubber cleaning products, among others (Niaz et al. 2015), for instance, is considered a toxic compound.

When not properly treated, it can be transformed into benzene, which is also a toxic environmental pollutant (Rana and Verma 2005). Moreover, previous study with biotransformation of a tetra-azo dye showed a high acute ecotoxicity in anaerobic *A. fischeri* assay due to aromatic amines accumulation. Its ecotoxicity was only eliminated after aromatic amines removal in micro-aerated condition (Menezes et al. 2019).

Additionally, cytotoxicity and genotoxicity were evaluated by mitotic and chromosomal alteration indexes (Table 4). The samples at the exit of each pond showed a significant increase of the mitotic index between 9.20% and 11.44% in relation to the negative control (6.79%), revealing a cytotoxic potential in their composition ($p<0.05$). Additionally, a genotoxic effect was evidenced for all evaluated samples, attributed to the significant increase in the rate of chromosomal alterations, which ranged between 1.49% and 2.20% to untreated and P3 samples, respectively (Table 4).

Table 4. Evaluation of toxicity, cytotoxicity and genotoxicity of samples collected from the ponds of the Multifactory Wastewater Treatment Plant using the *Allium cepa* test system, through analysis of the Germination index (G), Mitotic Index (MI) and Chromosome Alteration Index (CAI) in meristematic cells. Sampling points: untreated sample (SP13), pond 1 (SP14), pond 2 (SP15) and pond 3 (SP16).

Sample	Toxicity			Cytotoxicity	Genotoxicity
	G_{48} (%)	G_{72} (%)	G (%)	MI (%)	CAI (%)
NC	72.00 ± 2.86	81.33 ± 4.11	94.67 ± 1.89	6.79 ± 1.51	0.62 ± 0.30
MMS	-	-	-	-	1.36 ± 0.27*
TRI	-	-	-	-	6.77 ± 0.76*
Untreated	16.00 ± 2.83*	66.00 ± 6.53*	91.33 ± 7.36	7.75 ± 1.37	1.49 ± 0.65*
Pond 1	20.00 ± 4.90*	74.00 ± 4.32	93.33 ± 9.43	9.83 ± 1.97*	1.60 ± 0.58*
Pond 2	11.33 ± 4.11*	59.33 ± 9.29*	93.33 ± 6.80	9.20 ± 1.31*	1.88 ± 0.85*
Pond 3	31.33 ± 5.73*	82.67 ± 7.54	98.67 ± 1.89	11.44 ± 1.47*	2.20 ± 0.92*

NC = Negative Control; MMS = Methyl MethaneSulfonate; TRI = Trifluralin; G_{48} = Partial germination at 48 h; G_{72} = Partial germination at 72 h; G = Total germination; MI = Mitotic Index; CAI = Chromosomal Alteration Index. Statistically significant values different from NC ($p < 0.05$) are followed by an asterisk.

The following alterations showed a significant increase when compared to the negative control: micronuclei and chromosomal bridges in all three ponds; chromosomal breaks in P2 and P3, and chromosomal losses and C-metaphases in P1. Additionally, nuclear buds, chromosomal delays, chromosomal adhesions and polyploid cells were also observed (Table S5; Fig S5). The nuclear and chromosomal alteration types suggest both the clastogenic (chromosome break) and the aneugenic (mitotic fibre problem) potentials of the molecules present in the ponds. Thus, the cytogenotoxicity data show that, despite the improvement in the physicochemical aspects at the end of the biological treatment process, there is maintenance of the cytogenotoxic potential at the exit of P3.

Products and by-products present in the effluents generated by the several industries of the complex and released into the MF-WWTP also seem to have acted as inducers of cytogenotoxicity in *A. cepa*. According to Leme and Marin-Morales (2009), chemical compounds can influence the mitotic index, which can trigger uncontrolled cell growth leading to the formation of cellular and genetic alterations. The significant increase in the mitotic index observed in the present study in P1, P2, and P3 may be associated with the presence of cytotoxic substances that may have induced uncontrolled cell division in tissues (Grippa et al. 2010).

Furthermore, different authors have associated chromosomal alterations with the presence of heavy metals (Sabeen et al. 2020), aromatic amines (Bomhard 2003), detergents (Pedrazzani et al. 2012), pesticides (Camilo-Cotrim et al. 2022), among others. The unique characteristic of the MF-WWTP, where a variety of products from textile cleaning, industrial washing, paint, varnish, enamel, lacquer, waterproofing, solvents, and related products, as well as domestic sewage, are collected, can also explain the significant genotoxic potential observed in the samples from all three ponds.

The phenolic metabolites of benzene, for instance, can cause DNA strand breaks, chromosomal damage, sister chromatid exchange, inhibition of topoisomerase II and damage to mitotic spindle (Rana and Verma 2005). Benzene itself showed cytotoxic and genotoxic effects in *A. cepa*, by stimulating cell division and increasing chromosomal alterations, respectively (Barbhuiya et al. 2018). Taking into account all the experimental data obtained for toxicity, we understand that, although toxicity has decreased at the P3 outlet, some methodologies can be implemented as an alternative to mitigate toxicity rates. For example, the

installation of a primary coagulation treatment unit for some effluents could reduce toxicity and increase BOD.

3.3 BACTERIAL COMMUNITY IN THE SLUDGE

High-performance sequencing of the V3/V4 regions of the 16S rRNA identified 582 bacterial genera and 15 Archaea genera in the three treatment ponds. The α-diversity and b-diversity analysis of the microbial community composition (Table 5) showed that all ponds exhibited similar microbial diversity, with only minor variations in index values, indicating no substantial differences in community composition. Pond 1 showed the highest observed species richness (417 OTUs) and the greatest diversity according to both the Shannon (2.988) and Simpson (0.853) indices, indicating not only a larger number of genera but also a more even distribution compared to P2 and P3. In contrast, P2 had the lowest diversity values across all metrics (385 observed OTUs, Shannon = 2.340, Simpson = 0.768), suggesting a less diverse community potentially dominated by a few competitive or stress-tolerant genera. P3 while slightly lower in observed richness (412 OTUs) compared to P1, showed the highest estimated total richness (Chao1 = 542.6, ACE = 523.6), implying a substantial pool of rare, low-abundance species that were not fully captured in the sequencing.

Table 5. Alpha diversity indices from the (Observed, Shannon, Simpson, Chao1, and ACE) calculated using the phyloseq package. These metrics describe the richness and evenness of microbial communities across the analyzed samples.

Sample	Observed	Shannon	Simpson	Chao1	ACE
Pond 1	417.000	2.988	0.853	516.000	515.191
Pond 2	385.000	2.340	0.768	513.775	498.507
Pond 3	412.000	2.865	0.831	542.634	523.645

Previous studies have reported observed OTUs ranging from 530 to over 2000, and Chao1 estimates ranging from approximately 800 to over 1600, depending on the type of industrial wastewater and treatment systems. Shannon indices in those studies typically ranged

between 2.6 and over 9.0, and Simpson values ranged from 0.10 to above 0.99 in some cases, indicating very high evenness and richness in those systems. In contrast, the Shannon diversity values for the ponds in this study ranged from 2.3 to 2.9, and Simpson indices ranged from 0.77 to 0.85. Nevertheless, the diversity observed in the three ponds falls toward the lower end of the spectrum when compared to values reported in the literature for sludge from treatment plants dealing with industrial and domestic wastewater. Nevertheless, the diversity observed in the three ponds falls toward the lower end of the spectrum when compared to values reported in the literature for sludge from treatment plants dealing with industrial and domestic wastewater, indicating that the microbial community from the MF-WTTP is less diverse and could be dominated by a few taxa (Wu et al., 2018; Calderón et al., 2019; Wu et al., 2021).

The β-diversity analysis (Fig. 4), which evaluated microbial community similarity among the ponds, revealed that while all three ponds shared highly similar compositions (Bray-Curtis distances ranging from 0.234–0.281), P2 and P3 exhibited the greatest similarity, whereas P1 and P3 showed the least similar communities. This is in accordance with their physicochemical profiles: P1 was considered an anaerobic, fermentative system, while P3 was a facultative pond with high algal activity. Furthermore, P2, an intermediate, facultative/microaerobic pond, the absence of a sediment top-layer, which enhanced the algal activity likely explains its stronger compositional resemblance to P3 than to P1.

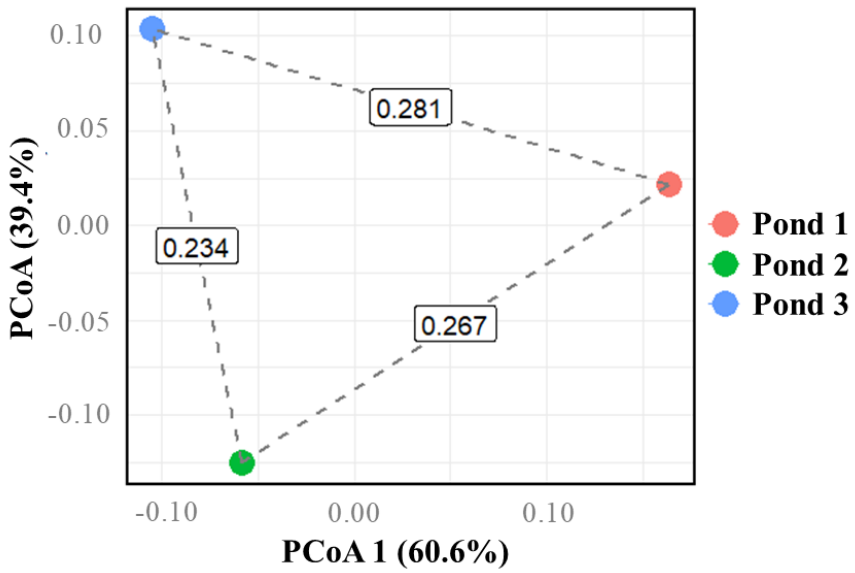

Fig 4. Principal Coordinates Analysis (PCoA) of microbial communities based on Bray-Curtis distances, showing compositional dissimilarity between samples (Pond1, Pond2, Pond3). Closer points indicate more similar communities, while farther points reflect higher

dissimilarity. Values on connecting lines represent pairwise Bray-Curtis distances (range: 0 = identical, 1 = maximally distinct).

The functional composition of microbial communities across the three treatment ponds (Fig. 5) was inferred using FAPROTAX, based on taxonomic profiles obtained from 16S rRNA gene sequencing. In Pond 1, functions associated with chemoheterotrophy (30.4%) and fermentation (19.9%) predominated, consistent with the accumulation of volatile fatty acids (VFAs), suggesting active anaerobic conversion of the organic matter entering the system. Organisms with their function related to nitrate reduction (3.7%) were also present, corresponding to the nitrate removal, observed in this pond. In P2, the abundance of organisms classified with fermentation functions decreased to 15.1%, while phototrophy (6.0%) and photoautotrophy (5.8%) increased, suggesting a transition toward oxidative and photoautotrophic activity.

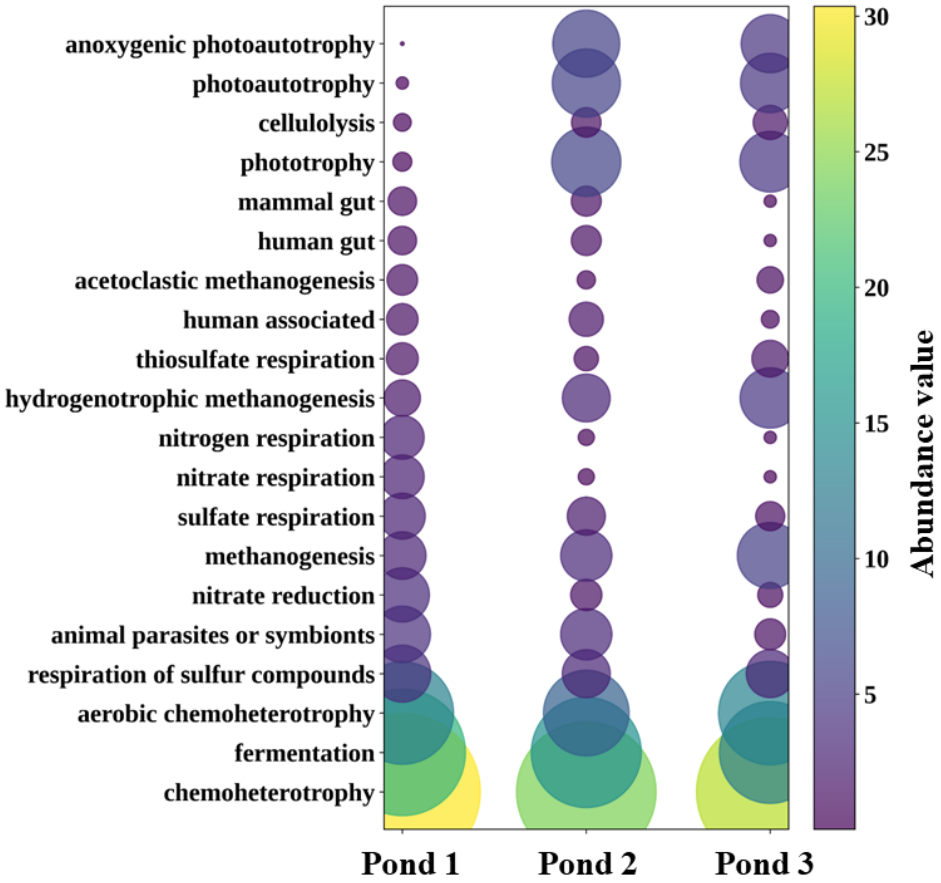

Fig 5. Relative abundance of microbial functions predicted by FAPROTAX based on 16S rRNA gene sequencing data from three sequential wastewater treatment ponds (P1 to P3). Only functions with a relative abundance >1% in at least one pond are shown.

P3, the largest pond, characterized by substantial algal activity, functional potentials for methanogenesis (5.4%) and hydrogenotrophic methanogenesis (4.5%) were identified alongside sustained levels of chemoheterotrophy (27.1%) and aerobic chemoheterotrophy (13.3%), reflecting a mixed community structure capable of both aerobic and anaerobic metabolism. The physicochemical profile of P3, including the consumption of VFAs, and slight increase in the nitrate concentration, supports the predominance of aerobic mineralization of organic matter and the presence of organisms linked to the nitrogen cycling processes. Overall, the predicted functional profiles correspond with measured environmental gradients and highlight the shift from anaerobic to increasingly aerobic and phototrophic microbial processes along the treatment sequence.

Fig. 6 shows the microbial community composition of archaea (Fig. 6.a) and bacteria (Fig. 6.b) from the sludge of each pond, considering only the organisms with relative abundance over than 1%. Regarding to the archaeal population, the three ponds were dominated by the Archaea classified as *Methanolinea* (P1 with 34%, P2 with 39% and P3 with 33%), *Methanosaeta* (P1 with 40%, P2 with 11% and P3 with 15%), and *Methanoregula* (P1 with 9%, P2 with 33% and P3 with 30%). All these genera have been reported as having either hydrogenotrophic or acetoclastic methanogenic metabolism, typical of anaerobic digestion reactors, and are extremely resilient to extreme survival conditions (Mori et al. 2012; Weerakoon et al. 2023). Other archaeal genera are also present with relative abundance higher than 1% in at least one of the ponds, such as *Methanobacterium* (hydrogenotrophic methanogen, 10% in P1 and 3% in P2), *Methanofollis* (hydrogenotrophic methanogen, 1.6% in P1), *Methanosphaerula* (hydrogenotrophic methanogen, 1.4% in P1) and *Methanospirillum* (hydrogenotrophic methanogen, 3.3% in P2 and 1.6% in P3).

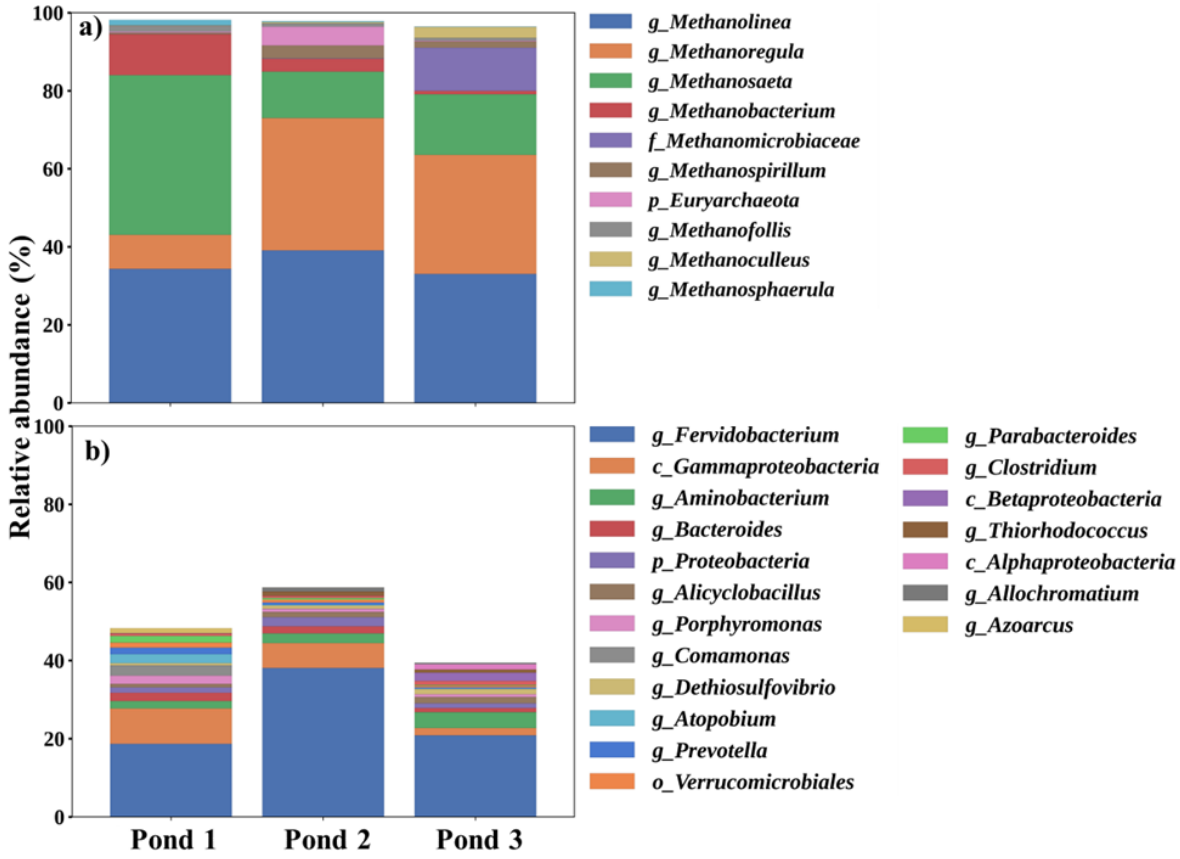

Fig 6. Diversity of archaea (a) and bacteria (b) in the ponds of the MF-WWTB. The Y-axis indicates the relative abundance (RA) of organisms with an RA above 1% in at least one pond. The prefixes denote the most refined taxonomic classification level: p (phylum), c (class), o (order), f (family), and g (genus). Organisms classified solely at the kingdom level were excluded from the analysis.

The bacterial population of organisms with relative abundance higher than 1% (Fig. 6.b) shows that most of the genera detected corresponds to extremophile bacteria, characteristic of inhospitable environments with high organic load, high temperatures and acidic pH, which corroborates the results of the physicochemical parameters found in the ponds (Tables 1 and 2). From all identified genera, *Fervidobacterium* stood as the most prominent in the treatment station with relative abundance of 18%, 38% and 21% in the bacterial population from P1, 2 and 3, respectively (Fig 6.b). This genus includes a set of Gram-negative bacterial species, in the form of motile rods, strictly anaerobic and thermophilic, isolated for the first time from an Icelandic hot spring (Kanoksilapatham et al. 2016).

Some species are reported as keratinase producers (Dhanasingh et al. 2021; Dhanasingh and Lee 2019; Kang et al. 2020; La et al. 2020). In wider terms, *Fervidobacterium* belongs to the *Thermotogaceae* family, widely distributed in nature and frequently found in salt flats, oil and petroleum contaminated environment, and biodigesters treating refinery effluents

(Schaechter 2010). Besides the use of carbohydrates, many representatives of the *Thermotogaceae* family are known for the use of petroleum-derived compounds as carbon source to lactic and acetic acids, ethanol, CO_2 and H_2 (DiPippo et al. 2009). The presence of this bacteria in a high abundance in the population of P1 (Fig 5.b) could be associated with the presence of xenobiotic compounds and with the production of acetic acid in P1 (Fig S3, Supplementary materials). Obviously, the production of acetic acid must not be due only to the activity of *Fervidobacterium* species, but mainly because of it.

The second most abundant organism (9% in P1 and 6% in P2) belongs to the *Gammaproteobacteria* class, which comprises aerobic and facultative organisms. This class includes some of the most significant and widely recognized hydrocarbon-degrading bacteria, frequently enriched in marine environments and playing a crucial role in the breakdown of petrochemical and other xenobiotic compounds. Additionally, organisms from this class can perform denitrification, a process observed in P1 (Gutierrez, 2019). The decrease in its relative abundance from P1 to P2 and P3 (Fig S4, Supplementary material) suggests that the primary compounds degraded by this organism were largely broken down in the first two ponds.

In P1, the genera *Comamonas*, *Atopobium*, *Bacteroides* and *Porphyromonas* compose a group of organisms with low but significant relative abundance (2.1 – 2.6%), suggesting functional importance. They are all associated with the fermentation of complex organic compounds and may also participate in the degradation of xenobiotic substances. *Comamonas* is particularly known for its ability to degrade aromatic hydrocarbons (e.g., phenols, benzoate) and alkanes under anaerobic conditions, and being able to perform nitrate reduction (Kwon, Kwon, and Kim 2019; Cummings and Branch 1986; Goyal and Zylstra 1996; Martinez-Burgos et al. 2020; Neal, Thiruppathy, and Zengler 2023; Willems and De Vos 2006; Wu, Zaiden, and Cao 2018; Xu et al. 2024).

Fermentative genera such as *Prevotella* and *Atopobium* likely contribute to volatile fatty acid (VFA) production through polysaccharide or amino acid fermentation, while *Porphyromonas*, with its proteolytic capabilities, may aid in breaking down nitrogen-rich substrates like proteins and mucins. The significant decrease of their relative abundance in P2 and P3 (Fig S4, Supplementary material) can also indicate their major role with the fermentation of organic compounds in P1.

The genus *Aminobacterium* (Fig 4) also showed high relative abundance (1.9% in P1, 2.5% in P2 and 4.1% in P3). This genus belongs to the *Synergistaceae* family and is composed of Gram-negative bacterial species that degrade amino acids, being isolated mainly in ponds

that receive dairy waste (Baena et al. 2000), which is also the case of MF-WWTP. This indicates that there is high potential for amino acid metabolizing activity in the bacterial community.

The genus *Azoarcus* was detected mainly in P1 (relative abundance of 1%), a pond with a surface layer of oil and grease residues. It is a genus of anaerobic, nitrogen-fixing bacteria, often with an endophytic lifestyle and that degrades a large number of aromatic compounds. Among these compounds, some are considered extremely toxic and carcinogenic, such as toluene and xylene, components of BTEX compounds (benzene, toluene, ethylbenzene and xylene) (Fernandes et al., 2014; Kato et al. 2019; Patil et al. 2021; Pournia et al. 2019; Wushke et al. 2018).

A few genera that were not significantly present in P1 and P2 increased their relative abundance in P3. Among them, members of the classes *Betaproteobacteria*, *Gammaproteobacteria*, and *Alphaproteobacteria*, suggesting an important role in the terminal stages of organic matter conversion. These classes include metabolically versatile genera capable of utilizing diverse carbon substrates, including residual volatile fatty acids (VFAs), algal metabolites, and more recalcitrant compounds derived from earlier fermentation and partial oxidation processes. For instance, *Betaproteobacteria* are known for their ability to thrive in nutrient-depleted, oxygen-rich environments and contribute to heterotrophic nitrification and biofilm formation (Zhang et al., 2019). Likewise, *Gammaproteobacteria* include organisms that specialize in degrading xenobiotics and algal-derived dissolved organic matter (Alves et al., 2022).

The presence of members from the class *Alphaproteobacteria*, many of which form close associations with algal cells, further points to the ecological importance of algae-bacteria interactions in this pond. Some genera can metabolize aromatic compounds and polysaccharides, particularly those derived from algal exudates, and are also known for their capacity to produce extracellular polymeric substances (EPS), enhancing floc stability and nutrient capture (Landa et al., 2017).

Interestingly, several taxa typically associated with anaerobic metabolism, such as *Clostridium*, *Bacteroides*, and *Dethiosulfovibrio* were also detected in this aerobic pond. Their persistence likely reflects the presence of anoxic microenvironments within flocs, sediments, or algal mats, where fermentative degradation of complex organics such as proteins, amino acids, and polysaccharides can happen. *Clostridium* and *Bacteroides* are well-documented anaerobes that play significant roles in polysaccharide and protein fermentation, while *Dethiosulfovibrio* is associated with sulfur metabolism and amino acid degradation in anaerobic niches (Thomas et al., 2011; Ravot et al., 1999; Wiegel et al., 2006).

The detection of *Alicyclobacillus* in P3 also merits attention. Although typically associated with thermophilic and acidophilic environments, some species have demonstrated the ability to degrade lignin-derived compounds, such as those found in pulp and paper wastewater (Aston et al., 2016). Their presence in the third pond may indicate the persistence of recalcitrant aromatic compounds that resisted degradation in upstream units.

The high relative abundance of *Fervidobacterium* across all ponds suggests its potential as a key player in treatment performance in the MF-WWTP. As a thermophilic anaerobe linked to hydrocarbon metabolism, including petroleum-derived compounds, this genus may contribute significantly to the breakdown of soluble organic substrates and xenobiotic contaminants in all ponds. Moreover, the sludge from these ponds can serve as a viable inoculum source for other systems treating recalcitrant pollutants, facilitating the remediation of industrial effluents.

The microbial communities in three sequential ponds exhibited slight functional shifts which matched with the physicochemical analysis. The *Fervidobacterium* genus was notably abundant across all ponds, highlighting its potential role in the biodegradation of industrial effluents. Pond 1, dominated by anaerobic fermenters and xenobiotic degraders facilitated initial organic matter breakdown. Pond 2 transitioned toward oxidative metabolism, with declining fermenters and rising phototrophic organisms.

Pond 3 was characterized by aerobic processes and algal interactions, featuring abundant *Betaproteobacteria* and *Alphaproteobacteria* for nitrogen cycling, though the presence of many facultative organisms indicated the presence of anoxic microenvironments. Despite lower α-diversity, each pond maintained specialized taxa adapted to the degradation of industrial wastewaters. The sludge's microbial richness, particularly *Fervidobacterium*, positions it as a promising inoculum for degrading recalcitrant pollutants. This study underscores how structured microbial networks enable efficient wastewater treatment through phased anaerobic, phototrophic, and aerobic processes, offering insights for optimizing industrial bioremediation.

3.4 CYANOBACTERIA AND MICROALGAE COMMUNITY

In the analysis of 12 points of the MF-WWTP, a total of 27,183 organisms belonging to cyanobacteria and microalgae were observed under the optical microscope, which were classified into 17 genera: nine genera of cyanobacteria (*Oscillatoria*, 14.22%; *Geitlerinema*, 13.58%; *Phormidium*, 0.10%; *Pseudanabaena*, 0.001%; *Arthrospira*, 11.55%; *Planktorhrix*,

9.21%; *Planktolyngbya*, 0.53%; *Chroococcus*, 14.91%, and *Merismopedia*, 2.55%) and eight genera of microalgae (*Fragillaria*, 7.84%; *Navicula*, 0.001%; *Aulacoseira*, 0.02%; *Cyclotella* 10.32%; *Chlorella*, 13.92%; *Trachelomonas*, 0.28%; *Oocystis*, 15.15%, and *Choricystis*, 11.14%) (Fig 7 and Fig S6, Supplementary material).

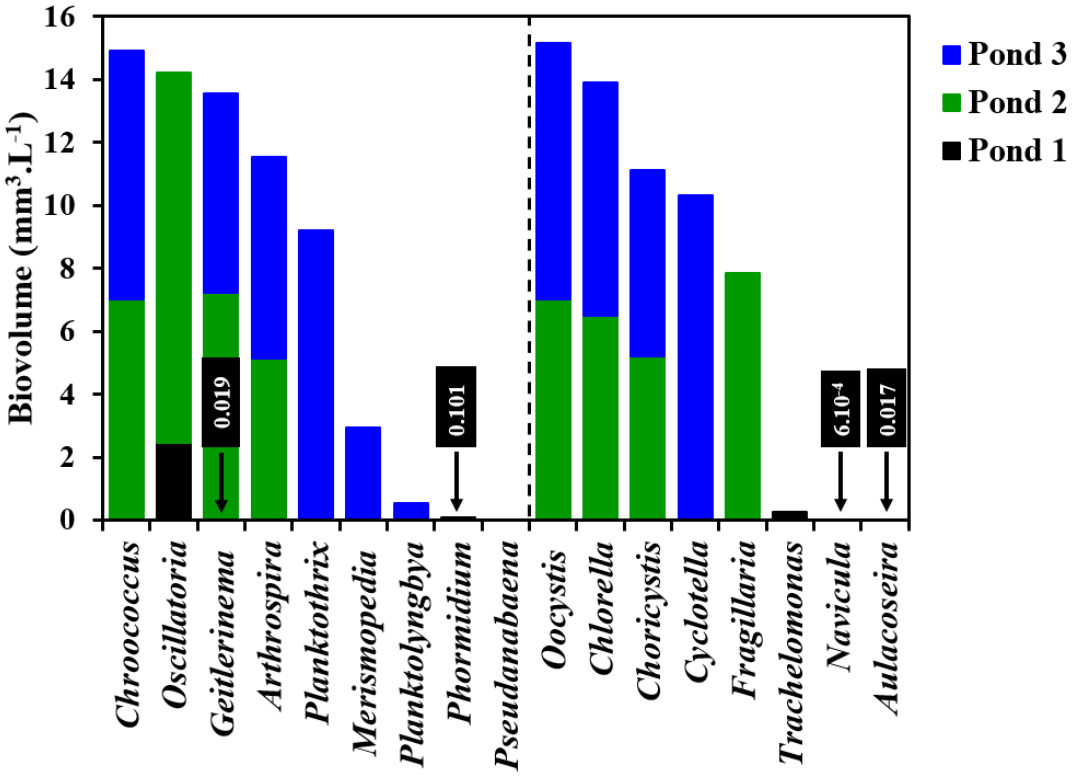

Fig 7. Diversity of cyanobacteria (left) and microalgae (right) in the ponds 1, 2, and 3 of the MF-WWTP.

Most of these microorganisms were observed in P2 and P3, right in the ponds with higher values of redox potential, conductivity, pH and DO. This indicates that, in fact, most of the organic load of the industrial effluent is consumed by the microbial population in P1. Thus, the very low content of organic matter coming from P1 makes possible the establishment of cyanobacteria and microalgae populations in the further ponds (Fig 7). In the ponds of the Multifabril Wastewater Treatment Plant, cyanobacteria such as *Phormidium* and *Pseudanabeana* were exclusively found in P1; *Merismopedia*, *Planktolyngbya*, and *Planktothrix* were exclusively found in P3, while the remaining ones were observed in two or three ponds simultaneously. Regarding microalgae, *Trachelomonas*, *Navicula* and *Aulacoseira* were exclusively found in P1, while *Fragillaria* was exclusive of P3. The remaining ones were observed in P2 and P3 (Fig 7).

Oscillatoria was the most observed cyanobacteria/microalgae in P1 (85%) (Fig S6, Supplementary material), indicating how inhospitable this environment is for this type of microorganism. This is the pond with the lowest amount of dissolved oxygen, as well as the highest concentration of ammonia and phosphorus, highest temperature, lowest redox potential and highest concentration of pollutants. In addition, this pond is covered with a layer of oil that prevents the passage of sunlight to its interior. *Oscillatoria* comprises a group of mixotrophic cyanobacterial species capable of colonizing eutrophic environments characterized by an excess of nitrogen and phosphorus (Reynolds et al. 2002), and these species are also known for their ability to fix nitrogen and carry out chemosynthesis (O'Farrell et al. 2003). Their presence is associated with excessive pollution with an organic load and also indicates environments with little availability of dissolved oxygen (França et al. 2022), stratified environments with the presence of chemical and thermal gradients and even nutrient circulation (Li 2021), parameters that characterises P1.

The green microalgae *Chlorella*, *Oocystis* and *Choricystis*, as well as the cyanobacteria *Choococcus* were frequently observed in P2. In this pond dissolved oxygen was higher than in P1, with lower temperature and absence of oil agglomerations on the surface. Consequently, this pond has a higher incidence of sunlight and allows the development of green microalgae. These organisms are associated with aerobic, illuminated and eutrophic environments (Reynolds et al. 2002), in addition to being resistant to several chemical compounds in rivers (Chen et al. 2022). However, the presence of *Oscillatoria* in P2 with the highest value in biovolume among the microorganisms observed (Fig 7) is an indication that this pond still has a considerable organic load and mixotrophic activity.

Given the presence of four unique microorganisms, P3 was the most diverse pond (9/17). The largest biovolume measured was that of the genus *Cyclotella* followed by *Planktothrix*. The first corresponds to a group of diatoms related to eutrophic environments with excess nitrogen and phosphorus (Arumugham et al. 2023; Wu 1999). These are two elements that reappear from P2 and are found in high concentration in P3 (Table 2). As highlighted above, P3 has the highest conductivity and pH values and the lowest temperature value among the lakes, and these are precisely variables that positively impact the abundance of *Cyclotella* (Heneash et al. 2022).

Planktothrix is a quite diverse group of cyanobacteria that inhabit from freshwater to turbid mixed lakes (Komárek and Komáková 2004). It means that this microorganism inhabits environments with high levels of dissolved oxygen as is the case of P3 (Fig 2). However, blooms of *Planktothrix* are associated with problems regarding human health and negative

impact to agriculture due to the production of cyanotoxins by this group of cyanobacteria (Christiansen et al. 2003). The presence of this type of microorganism in large proportions in P3, from which the treated effluent should be discharged into the nearby hydrographic basin, may constitute an alert for the MF-WWTP treatment unit to monitor the release of cyanotoxins in the environment.

4 CONCLUSIONS

Unlike others treatment units from Compesa, the MF-WTTP, in addition to receiving a mix of industrial effluents, the unit has never undergone the process of removing its sludge. The MF-WWTP which allowed for the enrichment of bacteria, archaea, and algae adapted to extreme conditions, making them well-suited for breaking down industrial wastewater. Notably, the microbial diversity includes extremophilic organisms with high relative abundance, such as the Fervidobacterium (18–38%) genus, as well as methanogenic archaea (Methanolinea and Methanosaeta), inticading their importance on the conversion of organic substrates and recalcitrant compounds from the industrial effluents.

Physico-chemical analyses confirmed that the combination of an anaerobic pond (P1) followed by a microaerobic (P2) and a final aerobic (P3) pond, significantly removed COD and BOD (84% and 83%, for COD and BOD respectively, not considering the presence of algae), which mainly happened in P1. However, toxicity persists in the final effluent, as indicated by mitotic index values (9.20–11.44% vs. 6.79% control) and chromosomal alterations (1.49–2.20%). It is likely that the presence of toxin-producing algae in the final effluent contributed to these effects. The findings highlighted the need for a post-treatment unit to reduce the presence of algae and the remaining toxicity from the effluent of P3.

The combination of anaerobic, microaerobic, and aerobic ponds, along with additional polishing units, could serve as an effective strategy for other industrial wastewater treatment facilities in the region. This study revealed that *Fervidobacterium* bacterial genus, the microalgae *Cyclotella* and the cyanobacteria *Planktothrix* were highly abundant in this long-term, highly adapted system, suggesting that these microorganisms play a key role in degrading industrial effluents. Additionally, both the sludge and treated effluent from this system could act as valuable inoculum sources for other wastewater treatment units handling complex industrial contaminants, helping establish similarly resilient microbial communities.

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ETHICAL APPROVAL

The authors would like to declare that no ethical approval was required for this study.

CONSENT TO PARTICIPATE

The authors declare that they consent to participate in this study.

CONSENT TO PUBLISH

The authors declare that they consent to the publication of this study.

AUTHOR CONTRIBUTION

Luiz Pereira da Silva Júnior, Bruna Kelly de Oliveira Silva, Nathália Bandeira Carvalho dos Santos, Natercia Correa de Araújo, Amaral, Fernanda Magalhães Amaral: conceptualization, methodology, validation, formal analysis, investigation and writing the original draft. Bartholomeu Siqueira Júnior and Fábio Henrique Portella Corrêa de Oliveira: conceptualization, review and project administration. Bruna Soares Fernandes, Fabrício Motteran, Kyria Cilene de Andrade Bortoleti, Ana Christina Brasileiro Vidal, Marcos Antônio de Morais Júnior and Sávia Gavazza dos Santos Pessoa: conceptualization, review and editing, project administration and supervision.

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COMPETING INTERESTS

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DATA AVAILABILITY STATEMENT

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Materials files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

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SUPPLEMENTARY MATERIALS

Table S1. Monthly average rainfall from 2015-2021 from pluviometry stations in the neighbourhood of the Multifactory Wastewater Treatment Plant (MF-WWTP): station 268 (Barragem Duas Unas) and station 604 (Cidade da Copa).

Station		Year								
Station 268 - J.G. (Barragem Duas Unas)		2015	2016	2017	2018	2019	2020	2021	Average	Std. Dev.
	January	40.6	172.3	22.2	174.7	153.5	36.3	64.9	94.9	68.8
	February	58.3	62.4	77.0	126.3	109.6	57.9	112.0	86.2	29.0
	March	275.4	178.9	203.5	157.2	231.9	100.1	174.2	188.7	55.9
	April	14.2	220.6	225.7	484.7	174.3	227.6	427.1	253.5	157.9
	May	180.9	327.8	300.2	178.3	247.4	259.4	476.0	281.4	102.3
	June	352.8	112.0	425.6	99.8	458.8	248.0	138.3	262.2	151.7
	July	386.9	77.4	345.1	98.3	403.6	177.4	198.6	241.0	136.3
	August	96.0	30.7	150.8	67.7	183.2	118.8	328.8	139.4	97.6
	September	19.0	43.0	98.4	32.8	115.6	67.0	70.8	63.8	35.0
	October	30.6	17.7	41.0	15.2	49.7	17.2	46.9	31.2	14.8
	November	16.7	19.1	29.7	68.1	0.7	39.4	30.5	29.2	21.2
	December	103.5	45.1	32.2	81.3	27.3	64.5	158.7	73.2	46.5
Station 604 - J.G. (Cidade da Copa)										
	January	63.2	129.8	37.6	194.5	145.8	44.2	50.6	95.1	61.3
	February	64.4	71.0	63.2	137.2	124.0	93.0	111.6	94.9	30.1
	March	356.3	217.4	203.2	208.2	225.0	207.0	220.0	233.9	54.5
	April	47.8	275.0	216.8	511.4	199.6	245.2	477.6	281.9	162.4
	May	97.0	441.2	376.4	211.6	215.4	255.6	0.0	228.2	151.6
	June	475.4	107.8	437.0	139.8	450.2	335.6	0.0	278.0	192.6
	July	424.8	124.4	496.8	113.8	423.2	194.0	0.0	253.9	192.1
	August	111.4	55.0	106.8	97.4	221.0	118.8	0.0	101.5	67.3
	September	25.2	33.0	105.2	54.8	110.8	81.0	0.0	58.6	42.1
	October	31.4	20.6	49.6	16.6	54.0	16.8	0.0	27.0	19.3
	November	17.8	24.6	21.0	59.4	4.2	60.6	0.0	26.8	24.3
	December	62.0	63.6	41.4	74.6	33.6	67.0	200.6	77.5	56.2
Average of stations 268 and 604										
	January								95.0	62.6
	February								90.6	28.7
	March								211.3	58.0
	April								267.7	154.6
	May								254.8	127.2
	June								270.1	166.8
	July								247.5	160.2
	August								120.5	82.9
	September								61.2	37.3
	October								29.1	16.7
	November								28.0	21.9
	December								75.4	49.6

Source: data publicly available at APAC - Agência Pernambucana de Águas e Climas. Monitoramento pluviométrico, available at <http://old.apac.pe.gov.br/meteorologia/monitoramento-pluvio.php>. Last accessed: August 1st, 2023.

Volatile Fatty Acids (VFAs) Quantification Methodology

VFAs were quantified by gas chromatography (Agilent Technologies 7890A, Agilent Technologies Inc., Wilmington, DE, USA) coupled with a FID detector. Samples first received an ethyl ether organic acid extraction, following the protocol:

1) Addition of 1 g of sodium chloride, in a 10 mL glass tube with a screw cap;
2) Addition of reagents the glass tube:
    a) 2 mL of sample;
    b) 100 µL of crotonic acid as standard for evaluation of recovery;
    c) 200 µL of sulfuric acid;
    d) 600 µL of ethyl ether;
3) Vortex at maximum speed for 2 minutes;
4) Sample collection with syringe pre washed with ethyl ether.

The chromatographic conditions were as follows:

Injection volume:	2 µL
Injection mode:	Split 1:10
Injector temperature:	250 °C
Column:	J&W 122-7332: 260°C: 30 m x 250 µm x 0,25 µm
Temperature ramp:	100 °C for 1 min, followed by a temperature increase ramp of 8 °C/min until 150 °C, kept for 1 min, followed by a temperature increase ramp of 35 °C/min until 200 °C, kept for for 1 min
Run time:	10,7 minutes
Carrier gas:	N_2
Detector temperature:	300 °C
Flow rate:	1 mL/min
Detector:	Flame Ionization Detector (FID)

Table S2. Apparent and real colour of the untreated and treated wastewater collected on November 27, 2021.

Analysis	Sampling point			
	Untreated	Pond 1	Pond 2	Pond 3
Apparent color (PtCo)	383	246	393	419
Real color (PtCo)	31	36	82	61

Table S3. Total and suspended solids series from untreated sample and from Ponds 1, 2 and 3 of the Multifactory Wastewater Treatment Plant.

Solids series (mg/L)	Sampling point			
	Untreated	Pond 1	Pond 2	Pond 3
Total solids (TS)	1989.64 ± 0.51	1580.62 ± 41.54	1679.63 ± 0.52	1411.25 ± 8.84
Total volatile solids (TVS)	745.33 ± 82.62	595.63 ± 11.49	503.55 ± 17.4	404.38 ± 25.63
Total fixed solids (TFS)	1244.32 ± 82.12	985 ± 30.05	1176.08 ± 17.91	1006.88 ± 34.47
Total suspended solids (TSS)	396.11 ± 2.36	280 ± 28.28	308.86 ± 19.61	289.52 ± 52.53
Volatile suspended solids (VSS)	296.11 ± 13.36	230.19 ± 20.94	249.32 ± 50.46	282.14 ± 48.82
Suspended fixed solids (VFS)	100 ± 15.71	49.81 ± 49.23	59.55 ± 70.07	7.38 ± 3.7

Table S4. Analysis of acute toxicity to *Aliivibrio fischeri* (Beijerinck, 1889) Uranczyk et al., 2007 (synonym *Vibrio fischeri*) for samples from the Multifactory Wastewater Treatment Plant.

Parameter	Sample			
	Untreated	Pond 1	Pond 2	Pond 3
TF	64	512	64	512

TF: Toxicity factor, minimum dilution of the sample with no quantifiable inhibition of luminescence.

Table S5. Chromosomal alterations found in meristematic cells of *Allium cepa* L. after exposure to samples collected from the ponds of the Multifactory Wastewater Treatment Plant. Sampling points: untreated sample (SP13), pond 1 (SP14), pond 2 (SP15) and pond 3 (SP16).

Alteration	Sample						
	CN	MMS	TRI	Untreated	P1	P2	P3
MN	0 ± 0	0.38 ± 0.21*	0.56 ± 0.41*	0.18 ± 0.16*	0.14 ± 0.18*	0.19 ± 0.19*	0.04 ± 0.08*

NB	0.04 ± 0.07	0.08 ± 0.13	0.06 ± 0.09	0.06 ± 0.13	0.02 ± 0.06	0.04 ± 0.08	0.06 ± 0.12
CL	0.10 ± 0.13	0.34 ± 0.18	0.36 ± 0.20	0.14 ± 0.15	0.31 ± 0.12	0.27 ± 0.25	0.59 ± 0.40*
CB	0 ± 0	0.14 ± 0.18*	0.10 ± 0.13*	0.16 ± 0.19*	0.19 ± 0.20*	0.14 ± 0.31*	0.21 ± 0.29*
CD	0.04 ± 0.08	0.30 ± 0.64	0.10 ± 0.30	0.08 ± 0.13	0.04 ± 0.08	0.02 ± 0.06	0.02 ± 0.06
B	0.13 ± 0.13	0.24 ± 0.20	0.20 ± 0.20	0.37 ± 0.39	0.39 ± 0.33	0.43 ± 0.41*	0.9 ± 0.27*
CA	0.10 ± 0.30	0.30 ± 0.64	0.20 ± 0.60	0.48 ± 0.88	0.57 ± 0.88	0.47 ± 0.75	0.30 ± 0.90
PC	0.12 ± 0.10	0.22 ± 0.20	0.04 ± 0.08	0.33 ± 0.35	0.45 ± 0.48	0.26 ± 0.35	0.39 ± 0.38
CM	0.10 ± 0.10	0.06 ± 0.09	0.04 ± 0.08	0.14 ± 0.18	0.10 ± 0.10	0.14 ± 0.18	0.06 ± 0.18*

NC = Negative Control; MMS = Methyl MethaneSulfonate; TRI = Trifluralin; MN = Micronucleus; BN = Nuclear Bud; CL = Chromosomal Loss; CB = Chromosomal Bridge; CD = Chromosomal Delay; B = Chromosomal Break; CA = Chromosomal Adhesion; CP = Polyploid Cell; CM = C-metaphase. Statistically significant values different from NC ($p < 0.05$) are followed by an asterisk in bold.

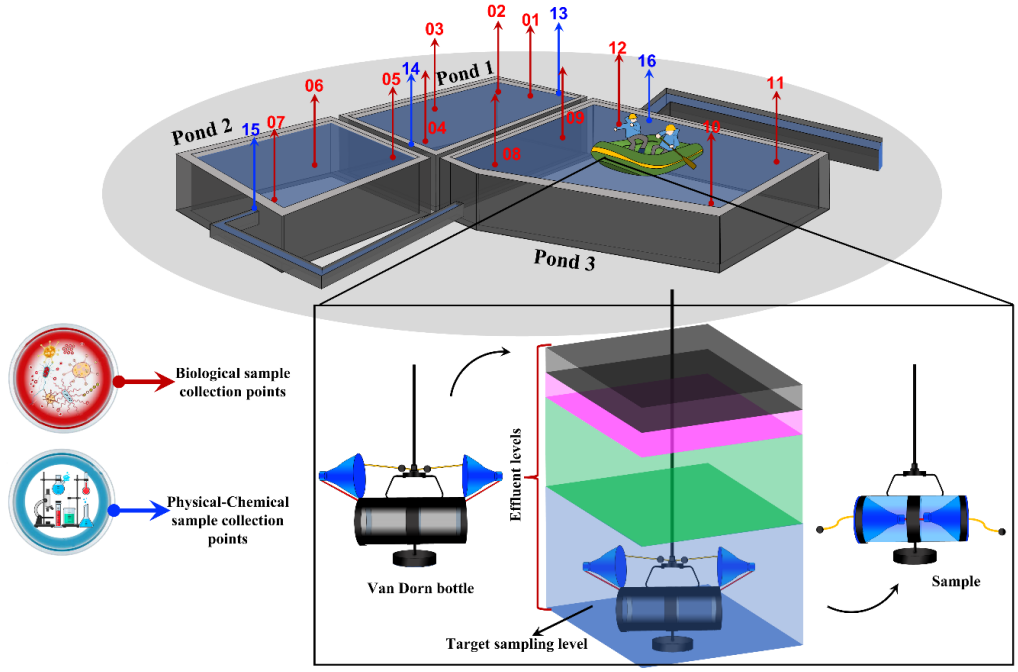

Fig S1. Sampling scheme in the three sequential stabilization ponds of the Multifactory Wastewater Treatment Plant (MF-WWTP), showing the collecting points for physicochemical and toxicity (blue), and biological (red) analysis in each pond. In the bottom, an amplification image shows the sampling approach using Van Dorn bottles.

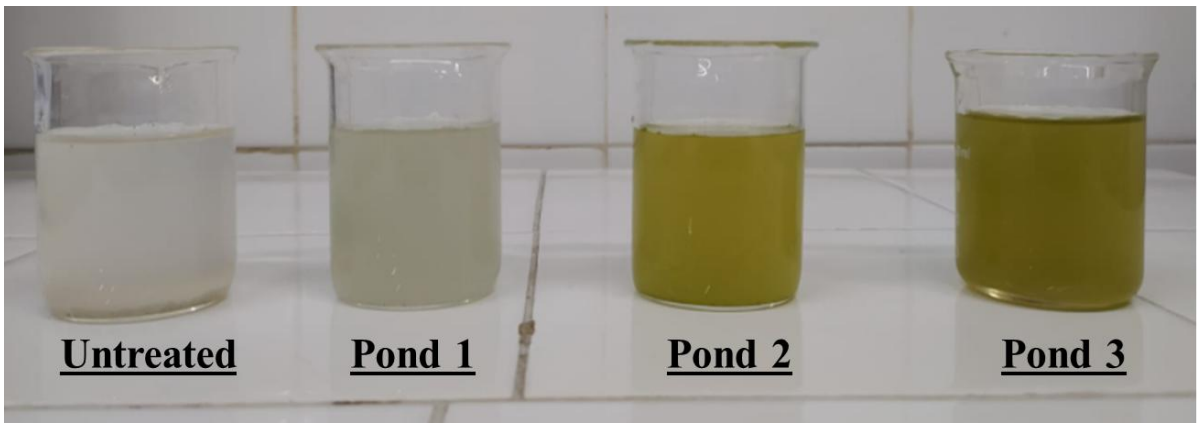

Fig S2. Samples for visual characterization from the first sampling campaign, on November 27, 2021.

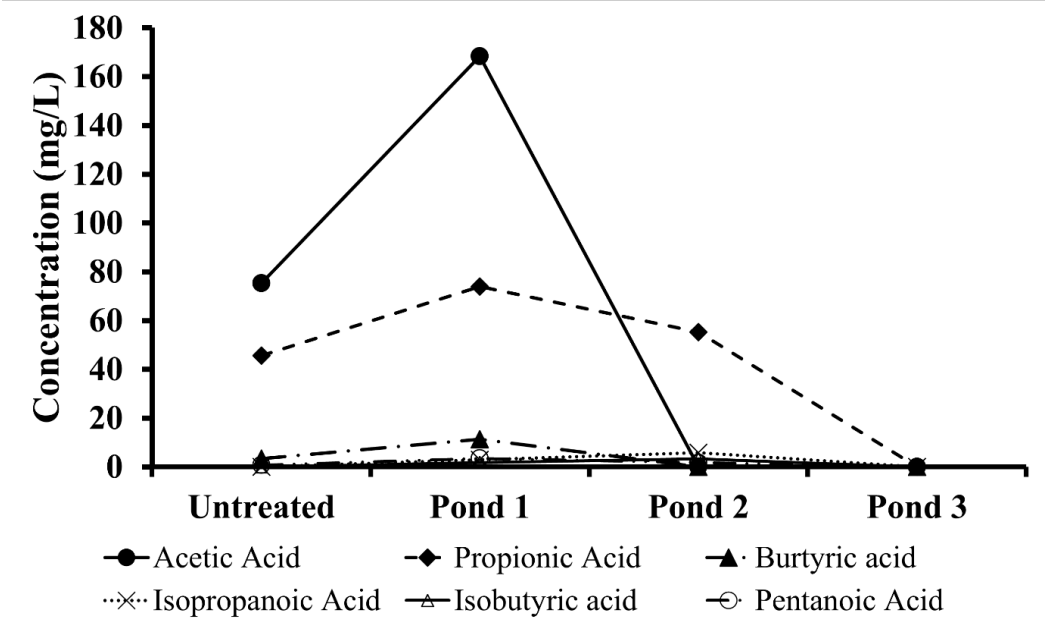

Fig. S3. Organic acids detected in the untreated influent (industrial effluent) and in the samples of different ponds of the Multifactory Wastewater Treatment Plant (MF-WWTP).

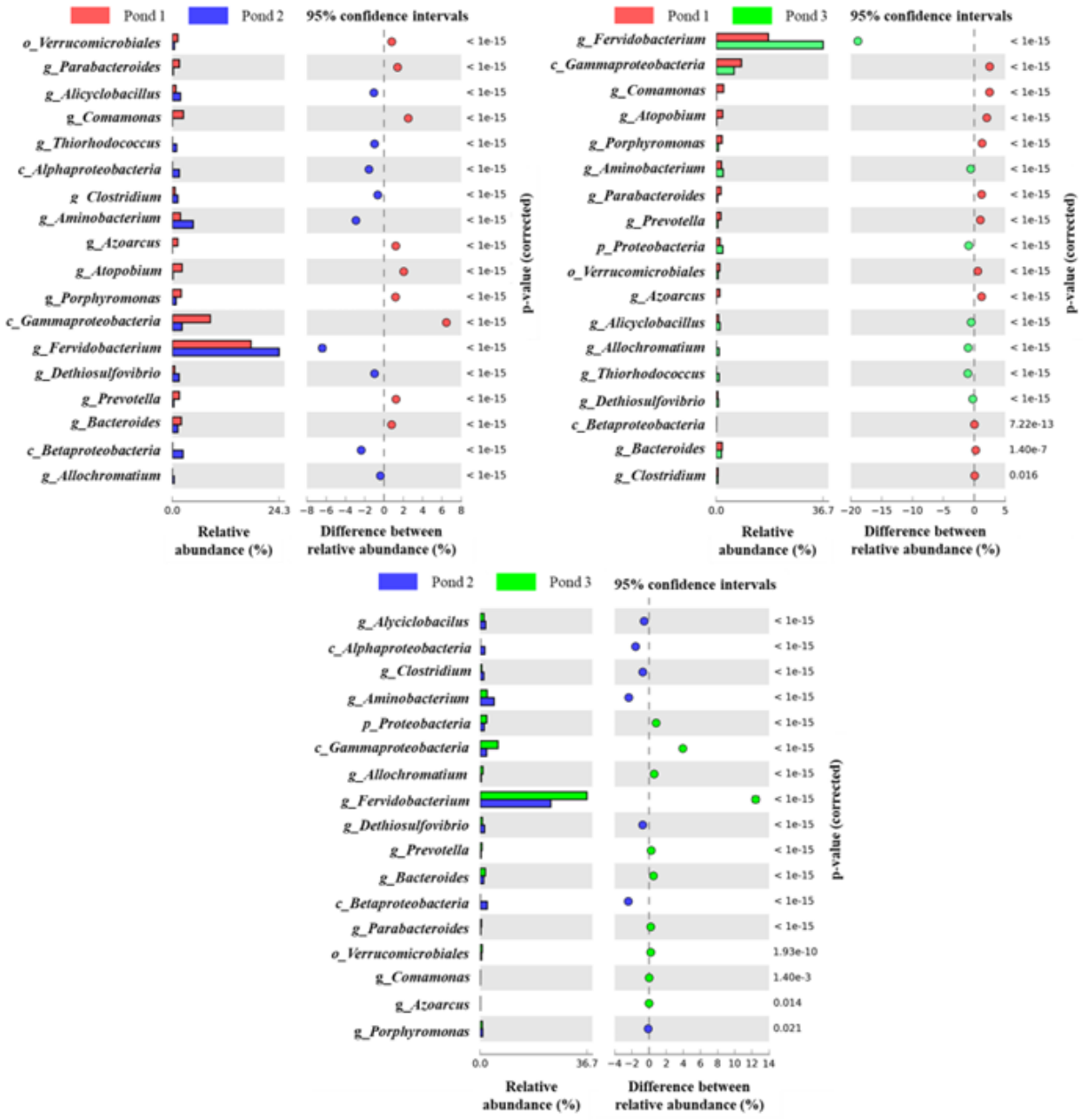

Fig S4 Statistical comparison of microbial community composition among three pond samples (P1, P2, P3) using STAMP software. of organisms with an RA above 1% in at least one pond. The prefixes denote the most refined taxonomic classification level: p (phylum), c (class), o (order), f (family), and g (genus).

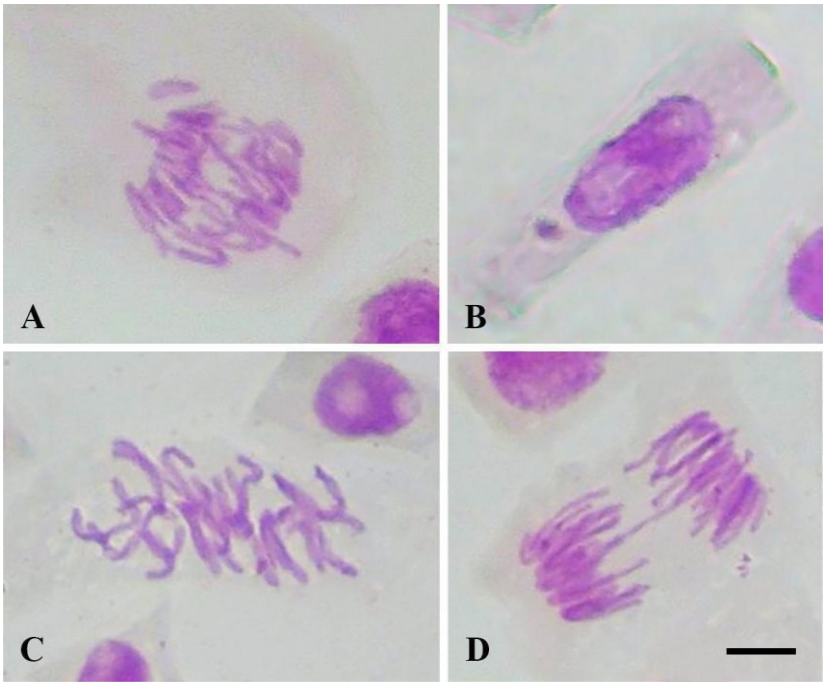

Fig S5 Chromosomal alterations found in meristematic cells of *Allium cepa* L. after exposure to samples from the Multifactory Wastewater Treatment Plant (MF-WWTP). (A) chromosomal loss (untreated sample); (B) Micronucleus (Pond 1); (C) C-metaphase (Pond 3); (D) chromosomal bridge (Pond 3). Bar in (D) corresponds to 10 µm.

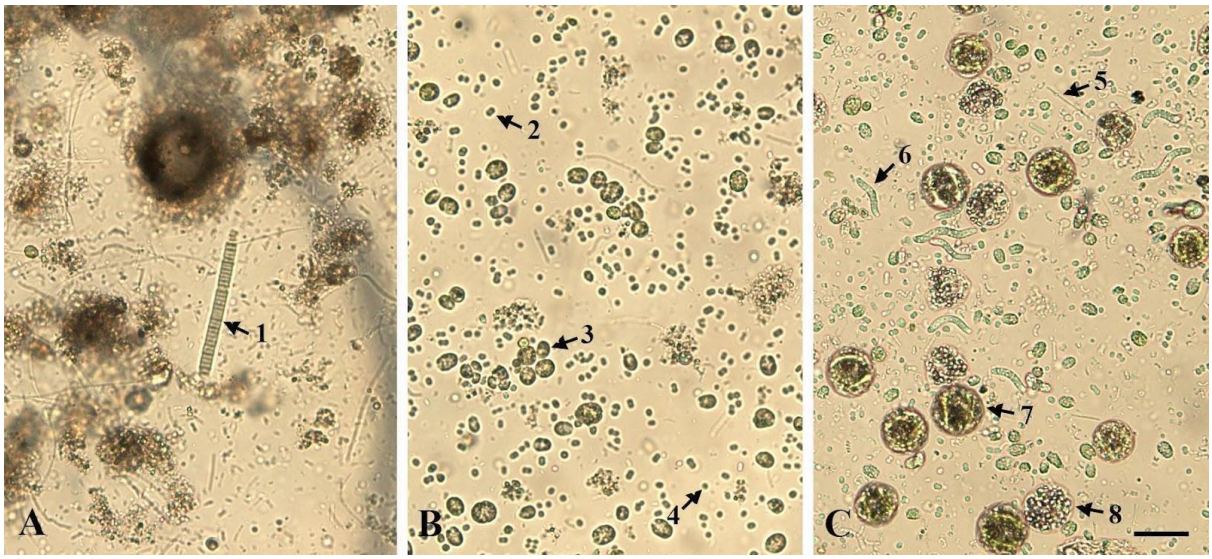

Fig S6 Images of the community of cyanobacteria and microalgae of the Ponds 1 (A), 2 (B), and 3 (C). Arrows indicate cyanobacteria: (1) *Oscillatoria*, (5) *Geitlerinema*, (6) *Arthospira*, (8) *Merismopedia*, and microalgae: (2) *Chroococcus*, (3) *Choricystis*, (4) *Oocystis*, (7) *Chlorella*. Bar in (C) corresponds to 50

4.2 ARTIGO 2

O artigo “The genus Fervidobacterium, its thermoenzymes and biotechnological potencial: an integrative review” foi publicado no Anaerobe Journal, volume 93, 2025, link para acesso: https://doi.org/10.1016/j.anaerobe.2025.102967

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Anaerobes in the environment

The genus *Fervidobacterium*, its thermoenzymes and biotechnological potential: an integrative review

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ABSTRACT

The genus *Fervidobacterium* has only seven described species to date, namely *F. changbaicum*, *F. gondwanense*, *F. islandicum*, *F. nodosum*, *F. pennivorans*, *F. riparium* and *F. thailandense*. These species were first isolated from places with high thermal amplitude, such as hot springs and sites that originated from volcanic activity. With temperatures ranging from 40 °C to 90 °C, they are characteristically considered hyperthermophilic. This genus belongs to the phylum Thermotoga, composed of families of extremophilic organisms, that is, adapted to inhospitable environments. The genus is considered strictly anaerobic and heterotrophic and is widely reported in the literature regarding its biochemical machinery, as it produces a wide variety of enzymes capable of metabolising the most diverse carbon sources. It stands out for the degradation of mostly keratinolytic substrates, such as those composed of feathers of native birds. Therefore, this literature review aimed to gather information about the genus and its enzymology. There are few recent studies that outline the genus *Fervidobacterium* and the biotechnological and commercial potential added to the genus. The enzymes produced by these species are resistant to several detergents and organic solvents and have a high and remarkable thermal stability. Consequently, these microorganisms hold significant biotechnological potential across various industrial sectors and are also promising candidates for environmental remediation efforts.

1. Introduction

The genus *Fervidobacterium* is a group of extremophilic anaerobic Gram-negative rod-shaped bacteria that belongs to the domain Bacteria, phylum Thermotogae, class Thermotogae, order Thermotogales and the family Fervidobacteriaceae. Overall, the phylum Thermotogae contains approximately 47 species distributed in its phylogenetic branches, which have been recently revisited by comparative genomic analysis [1]. The class Thermotogae is divided into orders: Thermotogales, Kosmotogales and Petrotogales. The order Thermotogales includes 2 families: Thermotogaceae and Fervidobacteriaceae. The family Thermotogaceae comprises the genera Thermotoga and Pseudothermotoga, whereas the family Fervidobacteriaceae includes the genera Fervidobacterium and Thermosipho. The order Kosmotogales contains the family Kosmotogaceae, which includes the genera Mesotoga and Kosmotoga, and the order Petrotogales encompasses the family Petrotogaceae, which includes the genera Defluviitoga, Petrotoga, Geotoga, Oceanotoga and Marinitoga [2].

The organisms of the phylum Thermotogae are rod-shaped organisms with a “toga”, shaped envelope, which gives the phylum its name. They are Gram-negative, strictly anaerobic, fermentative and thermophilic, withstanding temperatures that can vary between 40 °C and 100 °C. The organisms belonging to this phylum are commonly found in geothermal basins, oil wells, pipelines, refineries and biodigesters, as these places share favourable characteristics such as the absence of oxygen, abundant organic matter and high temperatures [3–5]. The genera belonging to the phylum Thermotogae have an extensive history of reports on proteins and enzymes produced by them. Their metabolic and biochemical machinery is associated with the degradation of petroleum derivatives, keratin, as well as the most diverse substrates [6–8].

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THE GENUS *Fervidobacterium*, ITS THERMOENZYMES AND BIOTECHNOLOGICAL POTENTIAL: AN INTEGRATIVE REVIEW

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ABSTRACT

The genus *Fervidobacterium* has only seven described species to date, namely: *F. changbaicum*, *F. gondwanense*, *F. islandicum*, *F. nodosum*, *F. pennivorans*, *F. riparium* and *F. thailandense*. These species were first isolated from places with high thermal amplitude, such as hot springs and sites that originated from volcanic activity. With temperatures ranging from 40°C to 90°C, they are characteristically considered hyperthermophilic. This genus belongs to the phylum *Thermotoga*, composed of families of extremophilic organisms, that is, adapted to inhospitable environments. The genus is considered strictly anaerobic and heterotrophic, and is widely reported in the literature regarding its biochemical machinery, as it produces a wide variety of enzymes capable of metabolizing the most diverse carbon sources. It stands out for

the degradation of mostly keratinolytic substrates, such as those composed of feathers of native birds. Therefore, this literature review aimed to gather information about the genus and its enzymology. There are few recent studies that outline the genus *Fervidobacterium,* and the biotechnological and commercial potential added to the genus. The enzymes produced by these species are resistant to several detergents and organic solvents, and have a high and remarkable thermal stability. Consequently, these microorganisms hold significant biotechnological potential across various industrial sectors and are also promising candidates for environmental remediation efforts.

KEYWORDS:

Bacteria, Anaerobic microorganisms, Enzymes, Keratins, Biotechnology, Environmental remediation.

1 INTRODUCTION

The genus *Fervidobacterium* is a group of extremophilic anaerobic Gram-negative rod-shaped bacteria that belongs to the domain Bacteria, phylum *Thermotogae*, class *Thermotogae*, order *Thermotogales* and the family *Fervidobacteriaceae*. Overall, the phylum *Thermotogae* contains approximately 47 species distributed in its phylogenetic branches, which have been recently revisited by comparative genomic analysis [19]. The class *Thermotogae* is divided into three orders: *Thermotogales, Kosmotogales* and *Petrotogales*. The order *Thermotogales* includes two families: *Thermotogaceae* and *Fervidobacteriaceae*. The family *Thermotogaceae* comprises the genera *Thermotoga* and *Pseudothermotoga*, while the family *Fervidobacteriaceae* includes the genera *Fervidobacterium* and *Thermosipho*. The order *Kosmotogales* contains the family *Kosmotogaceae,* which includes the genera *Mesotoga* and *Kosmotoga,* and the order *Petrotogales* encompasses the family *Petrotogaceae*, which includes the genera *Defluviitoga*, *Petrotoga*, *Geotoga*, *Oceanotoga,* and *Marinitoga* [4].

The organisms of the phylum *Thermotogae* are rod-shaped organisms with a “toga”, shaped envelope, which gives the phylum its name. They are Gram-negative, strictly anaerobic, fermentative and thermophilic, withstanding temperatures that can vary between 40°C and 100°C. The organisms belonging to this phylum are commonly found in geothermal basins, oil wells, pipelines, refineries and biodigesters, this is due to the fact that these places share favourable characteristics such as the absence of oxygen, abundant organic matter and high temperatures [12, 16, 27]. The genera belonging to the phylum Thermotogae have an extensive history of reports on proteins and enzymes produced by them. Their metabolic and biochemical

machinery is associated with the degradation of petroleum derivatives, keratin, as well as the most diverse substrates [22, 40, 42].

Regarding the genus *Fervidobacterium*, it comprises the species *F. changbaicum, F. gondwanense, F. islandicum, F. nodosum, F. pennivorans, F. riparium* and *F. thailandense*. All of them have common morphological and physiological characteristics, which are cells in the form of short rods, the presence of a spheroid toga at their end, they are tolerant to high temperatures, and they use various carbon sources, which allows them to explore different substrates. These species are widely reported in terms of enzyme production, with remarkable thermostability, which arouses biotechnological interest in the genus. Since the bioprospecting behind enzymes has a high added value [20, 21, 22].

The ecological diversity of the genus *Fervidobacterium* has already been identified in samples of water, soil, animals and even plants; however, its greatest abundance is reported in aquatic samples [34]. For example, our research group, in a recent metagenomic analysis, identified the genus *Fervidobacterium*, with relative abundance above 60%, in sludge samples from a Multifactory Wastewater Treatment Plant (MF-WWTP), located in an industrial complex in the municipality of Jaboatão dos Guararapes, state of Pernambuco, Brazil (8°06'23.1"S 35°01'36.0"W) (manuscript in preparation).

In addition to the genus *Fervidobacterium*, our metagenomic analysis also identified the genera *Kosmotoga, Petrotoga*, *Thermosipho*, and *Thermotoga*. Since *Fervidobacterium* is the genus with the greatest abundance in our analyses, we understand the importance of conducting a literature search on it. Hence, this literature search aims to perform a literature review on the genus *Fervidobacterium*. This study will objectively address the global distribution of the 16S rRNA gene sequences found for the genus, as well as the environment where it was isolated, its morphological characteristics, and the thermoenzymes produced by the species belonging to this genus, indicating its biotechnological potential and applications.

2 METHODOLOGY

This bibliographic review on the genus *Fervidobacterium* was conducted through searches of the main online literary databases, such as: PubMed, Scopus, Research Gate, Scientific Electronic Library online (SciELO) and Science Direct. All articles that met the inclusion and exclusion criteria below were selected: (i) Not being a review article; (ii) In English, Portuguese or Spanish; (iii) Not being an academic work such as a dissertation or thesis; and (iv) Having been published in a journal of scientific relevance.

The year of publication criterion was not taken into consideration for the preparation of this review article. Since we aim to address all the nuances already published about the genus *Fervidobacterium*. Thus, this literary research covers everything from the first articles published on the genus, which deal with its unprecedented isolation and taxonomy, to the most recent articles that discuss the thermoenzymes produced by this group of microorganisms, as well as their biotechnological applications.

In accordance with the Health Sciences Descriptors/Medical Subject Headings (DeCS/MeSH), database searches were conducted using the following keywords: *Fervidobacterium*, *Thermotogaceae*, bacteria, anaerobic, enzymes, keratins and biotechnology. Following the inclusion and exclusion criteria, the selected articles underwent an initial reading of the title, abstract and keywords. When they demonstrated relevance for this review, the second stage was the reading of the introduction and conclusion. The third stage consisted of reading the entire article and the main points were categorized in a tabulated spreadsheet.

3 RESULTS AND DISCUSSION

3.1 ECOLOGY AND DISTRIBUTION OF THE GENUS *Fervidobacterium*

From an ecological point of view, the genus *Fervidobacterium* tends to prefer anoxic aquatic locations, with high temperatures that can range from 40°C to 90°C. They are commonly associated with locations that originated from volcanic activity, with their first reports on islands or hot springs [21]. The first records for the genus are concentrated in a specific area of the globe, such as Asia and Europe; however, it is currently observed through the Microbe Atlas Project that the genus has been recorded all over the planet, including in South America [34, 36].

The genus *Fervidobacterium* is commonly associated with aquatic environments, even though it is isolated in the most diverse habitats, where it appears in greater diversity (Table 1). Chronologically, the species of this genus were identified as: *F. nodosum* from a hot spring in New Zealand [31], *F. islandicum* from a hot spring in Iceland [17], *F. gondwanense* was first isolated from a geothermal basin in Australia [1], *F. pennivorans* from a hot spring in the Azores Islands of Portugal [13], *F. changbaicum* was from a hot spring in China [6], *F. riparium* isolated from a hot spring on Kunashir Island, Russia [33] and *F. thailandense* first isolated from Fang Hot Spring, Thailand [21]. Figure 1 shows the locations where sequences for the genus *Fervidobacterium* were identified and reported for the first time in the literature, in blue.

In red, sequence for the genus identified in 2022 by our research group during metagenomic analysis of sludge from a Multifactory Sewage Treatment Plant, located in Recife, Pernambuco, Brazil. In particular, this treatment plant receives effluents from an industrial complex, making its treatment lagoons rich in nutrients and compounds of the most varied categories, including oil and grease residues (manuscript in preparation).

Table 1
Environmental origin of isolates of the different species of the genus *Fervidobacterium*.

Species	16S Sequences	Sequence Database	Aquatic Samples	Soil Samples	Animal Samples	Plant Samples	Total Samples	Nº Projects	Distinct Geographical Location
Fervidobacterium changbaicum	AY878719	NCBI, ENA, SILVA	109	14	30	8	161	108	81
Fervidobacterium gondwanense	Z49117	NCBI, ENA, SILVA	54	2	1	11	68	43	32
Fervidobacterium islandicum	M59176	NCBI, ENA, SILVA	–	–	–	–	–	–	–
Fervidobacterium nodosum	M59177	NCBI, ENA	–	–	–	–	–	–	–
Fervidobacterium pennivorans	HE582750	NCBI, ENA, SILVA	320	19	62	9	410	168	136
Fervidobacterium riparium	EU851047	NCBI, ENA, SILVA	431	10	17	6	464	154	136
Fervidobacterium thailandense	KJ473436	NCBI, ENA, SILVA	23	6	1	–	30	33	26
Fervidobacterium sp.	FN556062	NCBI, ENA, SILVA	869	103	144	16	1132	434	375

Caption. Table prepared with data obtained through the Bac Dive platform and Microbe Atlas Project, a database that provides information on the biodiversity of bacteria and archaea, linked to strain [35] or (doi: 10.1093/nar/gkab961).

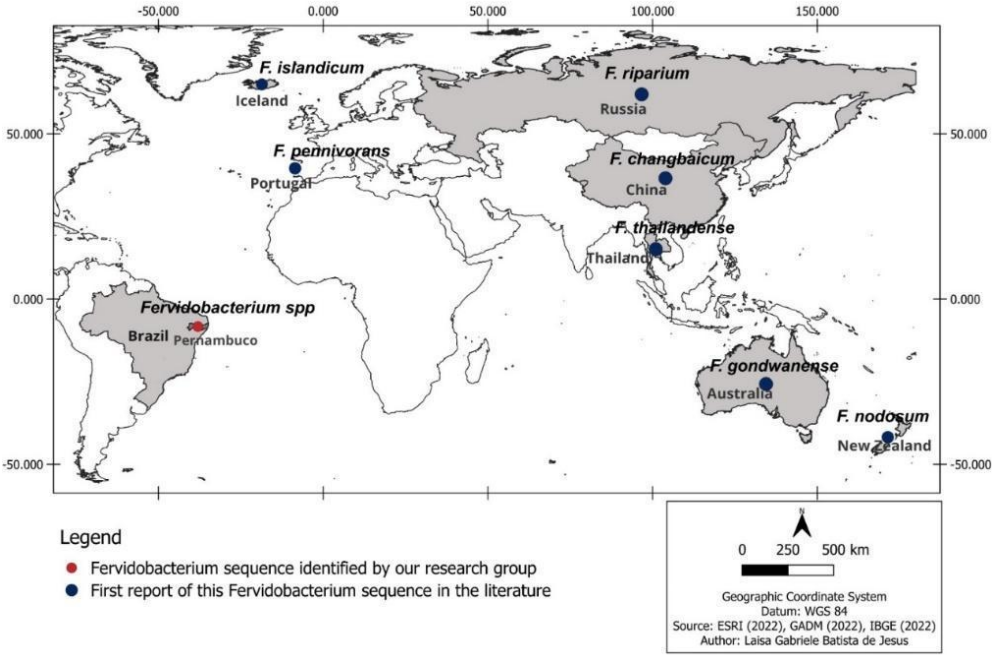

Figure 1. Map of the distribution of the first *Fervidobacterium* sequences reported in the literature (Blue), and sequence identified by our research group in metagenomic analysis of the microbial community of a WWTP (Red).

Despite the first records for the species mentioned above were in aquatic environments, it is possible to verify through the Bac Dive and Microbe Atlas Project, a database that provides information on the biodiversity of bacteria and archaea, linked to the strain, that sequences of some of these species have already been recorded for the most diverse environments (Table 1). This platform gathers information from sequences recorded in different databases such as the National Center for Biotechnology Information (NCBI), Gen Bank, also in the European Nucleotide Archive (ENA) and the Silva RNA Database. Based on this information, in which was created the table, where it is possible to observe for some of the species the 16S rRNA gene sequence identifier, sample type, number of projects registered for each species, as well as the number of distinct geographic locations where the sequence was identified [9, 38]. The median genome of the genus is 2 Mb of sze, harbouring the average of 2 k open reding frames, and median G+C content of 40 mol% [19].

Although in lower abundance, 16S rRNA gene sequences for the genus *Fervidobacterium* were also found in soil, animal and plant samples. The preference for geothermal aquatic environments may be linked to the metabolic preferences of the genus. Aquatic environments are more conducive to the multiplication and development of the species,

since they tend to be more nutritious and stable than other environments, presenting carbon sources from the most diverse origins [21, 36].

3.2 MORPHOLOGY AND METABOLISM OF THE GENUS *Fervidobacterium*

3.2.1 *Fervidobacterium nodosum*

F. nodosum Rt17-B1, an eubacteria first described in 1985 [31], was isolated from a hot spring in New Zealand. This species is heterotrophic, hyperthermophilic, Gram-negative, obligately anaerobic, and its DNA content is 33,7% (guanine/cytosine). Its cells are rods, measuring 0.5-0.55 x 1-2.5 μm, and can occur individually or in chains of up to 17 cells, surrounded by an external membrane. Something common for this species is the formation of a structure called a 'spheroid', which sprouts from the external membrane of the cell wall [24]. The generation time of *F. nodosum* is approximately 105 minutes, using glucose, sucrose, maltose, lactose, fructose, galactose, mannose, sorbitol and starch as carbon sources. The optimal conditions for the cultivation of this strain include an optimum temperature of 70°C, ideal pH around 7.0 and low salinity 0-0.5% NaCl. The products of its metabolism result in lactate, acetate, hydrogen and carbon hydroxide [21, 26, 31].

Some thermostable enzymes have been described for this species. In study of Wang et al. [39] cited the endoglucanase cellulase, with a molecular mass of 37.6 kDa. The gene that encodes this protein is FnCel5a, the cellulase was overexpressed in *Escherichia coli* cells that contained the FnCel5a plasmid. In the Wang's study, the authors tested the enzymatic activity in four different substrates, namely: the sodium salt of carboxymethyl cellulose (CMC), which showed a specific activity of 440 IU/mg of protein, and a relative activity of 100%; the second substrate tested was regenerated amorphous cellulose (RAC), which showed a specific activity of 402 IU/mg protein and a relative activity of 91%; the third test substrate was barley β-d-glucan, with a specific activity of 1360 IU/mg protein and a relative activity of 309%; the last substrate tested was galactomannan, with a specific activity of 895 IU/mg protein and a relative activity of 203%. The optimum temperature for enzymatic activity was in the range of 80-83°C, the optimum pH range was 5.0-5.5, and the half-life of cellulase in an incubation at 70-75°C did not decrease for 5 days, and when tested at 70-83°C, it had stimulated activity.

It was also reported about a lipase from the metabolism of *F. nodosum* [41] The gene encoding this lipase is Fond_1333, which was expressed in *E. coli* BL21 (DE3) Codon Plus cells under the control of the T7 promoter and inducible with isopropyl-β-D-

thiogalactopyranoside (IPTG). The test substrate in this assay was p-nitrophenyl-β-D-cellobioside (pNPC). The lipase obtained a specific activity of 52,00 IU/mg at 70°C and pH 8.0. Furthermore, according to the authors, this enzyme showed high thermal stability and high tolerance to organic solvents. Another enzyme described was 2,4-diaminopentanoate dehydrogenase [15], with a molecular mass of 38 kDa, encoded by the gene Fn_1646. The enzymatic activity was evaluated using the test substrate was 2,4-diaminopyridine at pH 8.5 with an optimum temperature of 55°C; however, the half-life of this enzyme was 38 minutes at 90°C and 2 minutes at 100°C.

F. nodosum has the ability to produce enzymes that are tolerant to various substrates, with high thermal stability, which is ideal for industrial applications. This species has biotechnological potential for the degradation of hemicellulosic substrates. Since at high temperatures, enzymes produced by this species present hydrolytic activity and thermal stability [39, 41]. It is not surprising that this species has already been isolated in effluents from paper and pulp mills [26].

3.2.2 *Fervidobacterium islandicum*

The first report in the literature about the species *F. islandicum* H21T was made by Huber and collaborators [17]. The species was isolated from a hot spring in Iceland and is a thermophilic, fermentative eubacteria. They are Gram-negative, their cells are rod-shaped and measure between 0.6 x 1-4 µm. They are similar to *F. nodosum*, as they also have a structure called a 'spheroid' that sprouts from their external cell wall. Their DNA content is 41% (guanine/cytosine), slightly higher than that presented by *F. nodosum*. The ideal conditions for the growth of *F. islandicum* include low salinity, an optimum temperature of 65°C and a pH of 7.2. The main carbon sources for the cultivation of the species include glucose, maltose, fructose, arabinose, galactose, mannose, starch and cellulose. The generation time of the species is 150 minutes [17, 21].

Some thermoenzymes have also been described for *F. islandicum*. A keratinolytic serine protease was described by Nam et al. [29]. The author discusses a new enzyme produced by the species *F. islandicum* AW-1. In this study, the strain was isolated from a geothermal stream in Indonesia. The enzyme described has a molecular weight of >200 kDa, a half-life of 90 minutes at 100°C, and an optimum pH of around 9.0. The enzyme was able to degrade feathers of native birds, showing high specific activity on keratinolytic substrates. The author also highlights that after the disulfide bridges of the keratins found in the feathers were minimized with the use of

10 mM dithiothreitol, the enzyme catalysed the cleavage of the peptide bonds more quickly and efficiently. The research also indicates that the balance of the metabolism of the degradation of bird feathers resulted in the production of cysteine, lysine, histidine, methionine and tryptophan, the last two of which are considered rare in bird feather keratins.

Another thermoenzyme described for *F. islandicum* AW-1 is the carboxypeptidase M32 (FisCP) encoded by the gene FIAW1_1600. This gene was overexpressed in *E. coli* BL21 (DE3) cells, resulting in an heterologous carboxypeptidase with activity at high temperatures (70°C) and slightly alkaline pH of 8.0, which can completely degrades bird feathers in just two days of incubation [25]. In addition, a thermostable β-glucosidase with optimal activity at 90 °C at pH at 6.0 to 7.0 was described by Jabbour et al. [18]. The correspondent gene Bgl1A was successfully overexpressed in *E. coli* cells and the author argues that this enzyme has a high biotechnological value due to its resistance to denaturing and reducing agents, in addition to its thermostability [18].

Another relevant highlight is the 'Islandisin', a serine protease produced by *F. islandicum* (DSMZ-5733). This enzyme is similar to subtilisin in the thermitase subgroup, has a molecular weight of 76,1 kDa and a half-life of 4 hours at 90°C and 1.5 hours at 100°C. The gene encoding this enzyme is Fis, and was overexpressed in *E. coli* BL21 (DE3) pLysS cells. 'Islandisin' is approximately 40% homologous to 'fervidolisin', which is produced by the species *F. pennivorans*, in addition to being thermosTable 1nd resistant to solvents and detergents [15].

More enzymes have been described for this species, such as an ATP-independent DNA topoisomerase, which has a molecular weight of 75 kDa and thermal stability at 75 kDa [11]. Furthermore, the literature also reports the recombinant FIG protein (rFIG), produced by *F. islandicum* AW-1 (KCTC-4680), capable of degrading cellulosic and hemicellulosic substrates. This enzyme has a half-life of 113 hours at 85°C, and an optimal pH of around 5.0. In that research, the author reports that the enzyme was tested on three different substrates, namely: barley β-D-glucan, presenting a specific activity of 367,0 IU/mg of protein; galactomannan, with a specific activity of 174,0 IU/mg of protein; and 4-nitrophenyl-cellobioside, with a specific activity of 66,1 IU/mg of protein, which can be used in the hydrolysis of substrates for biofuel production [20]. Furthermore, it is worth mentioning that the species *F. islandicum* AW-1 has 2.259 protein coding sequences with distinct functions, which include degradation, metabolism, folding, biosynthesis, and protein processing and modification. Genomic studies also reveal that this species has more than 50 proteolytic enzymes that can be used in the biodegradation of keratins [25].

3.2.3 *Fervidobacterium gondwanense*

F. gondwanense AB39 was first reported by Andrews and Patel [1]. The species is considered anaerobic and thermophilic, and was isolated from an artesian geothermal basin located in Australia. Its cells range from 0.5-0.6 x 4-40 µm, its DNA content is 35% (G+C mol%), and its generation time is 79 minutes. This species can utilize glucose, maltose, xylose, lactose, fructose, cellobiose, starch, and mannose as preferred carbon sources. The optimum temperature for its development is 65°C to 68°C, its optimum pH is around 7.0, and its cells are sensitive to NaCl [21]. One of the thermoenzymes reported for this species is a xylose isomerase (XylA), cloned and overexpressed in *E. coli*, with a specific activity of 15,0 IU/mg for the conversion of glucose into fructose, at an optimum temperature of 70°C and an optimum pH of 7.3. Furthermore, this enzyme resembles counterparts found in the species *Thermotoga maritima* and *Thermotoga neapolitana* [23]. The Type II restriction endonuclease Fgo I was reported from *F. gondwanense* AB39T [2]. This enzyme has activity at an optimum temperature between 60-70°C and optimum pH between 6.5-8.5. The thermoenzyme was identified as an isoschizomer of the Type II restriction endonucleases Mae I and B*f*a I.

3.2.4 *Fervidobacterium pennivorans*

F. pennivorans is a strict anaerobic bacterium, extremely thermophilic, that was first isolated in hot springs of the Azores Islands, islands of volcanic origin located in the Republic of Portugal. This species is widely reported regarding the enzymes it produces, being the first known thermophilic bacterium with the capacity to completely degrade bird feathers [13]. The DNA content (guanine + cytosine) of this strain is 40 mol%, its cells measure around 0.5 x 2-20 µm, its generation time is 126 minutes, with an optimum pH of 6.5 and a temperature range of 60-80°C. This bacterium grows well in the presence of xylose, maltose, glucose, fructose, mannose, galactose and starch [21].

Some thermoenzymes have been reported for this species that will be listed henceforth. A serine protease with a molecular mass of 130 kDa, with ideal activity at pH 10 at a temperature of 80°C, was showed an efficient degradability over bird feathers flour, converting it into amino acids and peptides extract [13]. The Type I pullulanase reported for this species is a debranching enzyme with 93 kDa and a half-life of 2 hours at 80°C at pH 6.0 [3]. The pulA gene was overexpressed in *E. coli* FD748 and encodes a protein containing approximately 850

amino acids with a 28-residue signal peptide. Furthermore, the authors also highlighted that the substrate specificity of pulA for pullulanase, starch, amylopectin and glycogen, coupled with its thermostability, enhances the biotechnological profile of this enzyme in the starch and its derivatives processing industry [3].

Canganella [8], also reported amylase and pullulanase from *F. pennivorans* that presented good activity in the temperature range of 90°C to 100°C, at pH of 5.5 to 6.5, evidencing their thermal stability and highlighting their biotechnological applicability. Another enzyme also described for this species is fervidolysin, a keratin-degrading enzyme with 73 kDa, associated with the degradation of substrates composed of resistant fibrous polypeptides [22]. All these enzymes with the potential to hydrolyse keratin could become a common and effective solution for degrading waste from the poultry industry, which is considered an environmental health problem.

Since Brazil is among the main players in the poultry meat, with the production of 15 million tons of chicken meat in 2022 according to the Brazilian Agricultural Research Corporation (EMBRAPA), only below the United States of America [37]. Thus, a large amount of bird feathers is generated by this industry annually. Feathers account for approximately 7% of the weight of birds, and the handling of this waste basically consists of burning it, generating carbon dioxide emissions, which is considered a major impact on the environment [28, 29].

3.2.5 *Fervidobacterium changbaicum*

F. changbaicum was first isolated from a hot spring in China, located in the Changbai Mountains. It is a strictly anaerobic and thermophilic bacterium. Its rod-shaped cells measure approximately 0.5-0.6 x 1-8 µm. The DNA content of this species is 31,9 mol% Guanine-Cytosine. The generation time of this strain is 99 minutes, with glucose, sucrose, maltose, lactose, fructose, galactose, sorbitol, trehalose, starch and cellobiose as its preferred carbon sources [6; 21]. For this species, we have described a recombinant enzyme called *F. changbaicum* Lipase 1 (FcLip1), with thermal stability at 70°C and an optimum pH of around 7.8. This type of enzyme is linked to the hydrolysis of oils and fats. This enzyme in question is part of the lipase V family, which is little known but has high commercial potential [7]. At the same time, the degradation of oils and fats by *Fervidobacterium* is interesting. The fact that these lipids serve as carbon sources for the metabolism of this organism is reflected in the high abundance of the genus in the MF-WWTP, the sampling unit of our research group, which has

one of the lagoons with a thick layer composed of oils and greases, originating from the industrial complex's manufacturing activities.

3.2.6 *Fervidobacterium riparium*

The species was isolated from a hot spring on Kunashir Island, Kuril Islands, Russia. Its cells are motile rods, measuring between 0.4-0.5 x 1-3 µm. This species metabolizes a wide range of carbon sources, such as xylose, fructose, maltose, sucrose, glucose, starch, cellobiose and cellulose. Its generation time is 55 minutes, with an optimum temperature of 65°C and pH of 7.8. The DNA content of this species is 31 mol% Guanine-Cytosine [21, 33]. Our literature search did not find any other bibliography about this species or about the enzymes it produces. However, the above authors Podosokorskaya et al., [33] reports that this isolate degraded a wide variety of substrates rich in starch, cellulose and their derivatives, which suggests that this species must produce some type of amylase and cellulase with potential for biodegradation of these substrates resulting from the processing of starch and cellulose.

3.2.7 *Fervidobacterium thailandense*

This species was first reported in 2016 [21]. The authors reports that the temperature of the sampling site at the time of collection was 90°C, reinforcing the affinity and adaptation of this genus to places with high temperatures. The strain *F. thailandense* FC2004T was isolated from the Fang hot spring in Thailand, its cells measuring between 0.5-0.6 x 1.1-30 µm. The generation time of this strain was 85 minutes, at an optimum temperature of 78-80°C and an optimum pH of 6.0-8.5. The species in question preferentially metabolizes glucose, sucrose, maltose, fructose, starch and cellobiose. Its Guanine-Cytosine DNA content in mol is 45.8%. The author also highlights that *F. thailandense* hydrolyzed feathers from native birds, which suggests that the species has an enzymatic apparatus capable of degrading keratinolytic substrates.

In our search for literature, we found a study of Pheungphasutadol et al. [32] in which the authors report the characterization and purification of a keratinolytic protease produced by *Fervidobacterium sp.* strain FA004 isolated from a stream in Thailand, which was further phylogenetically related to *F. thailandense* strain ATCC BAA-2483T. In this research, the authors describe a protease with a molecular weight of 67 kDa, a specific activity of 54,98 IU/mg, at an optimum pH between 7.0-9.0 and an optimum temperature between 70-90°C. Its

half-life lasted >12 hours at 70°C, using feathers from native birds as a substrate. These studies strengthen the use of these enzymes in the degradation of keratin-rich substrates, making them commercially attractive due to their thermal stability and their ability to degrade bird feathers.

Thus, it is no coincidence that we found the genus *Fervidobacterium* so well established in the MF-WWTP. When taking a look at the industrial complex in which the unit is located, we noticed the presence of an animal feed industry; these factories usually use poultry carcasses and feather meal as raw materials for their feed, which are rich keratinolytic substrates [25, 28, 30].

3.3 BIOTECHNOLOGICAL APPLICATIONS AND BIOREMEDIATION

Bioprospecting behind enzymology is of attracting industrial interest, as it is a growing niche that moves the global stock market and generates high annual returns. According to Data Bridge Market Research, the enzyme market is only growing, heading towards profits that could reach US$19.86 billion by 2029 [10]. Thus, there is a high biotechnological interest in microorganisms with the potential to produce enzymes, such as *Fervidobacterium*, whose enzymes stand out for being biocatalysts capable of degrading the most diverse substrates, in addition to being resilient to high temperature ranges [7, 20].

Some enzymes such as carboxypeptidase M32 [25], endoglucanase cellulase [39], lipase [41], keratinolytic protease [32], keratinolytic serine protease [13, 29], pullulanase type I [3], among many others are described for the genus in the sections above. These enzymes have biotechnological advantages and potential applications in industry. Pullulanases have important industrial applications and are highly sought after by the starch processing market, as they can convert starch into glucose, fructose and maltose, in addition to being widely used in the production of syrups and food sweeteners [3]. Carboxypeptidase, as well as keratinolytic proteases and serines, may become important and common players in feed production factories, as these enzymes can be used in the hydrolysis of keratinolytic substrates, converting bird feathers into products for the manufacture of animal feed [13, 25, 29, 32].

Catalases are applicable in catalytic reaction processes, converting cellulosic substrate into fuel and energy, which is advantageous, since this type of biomass is usually very dense and abundant [39]. As for lipases, they can act in several processes, meeting various industrial demands. Lipases can act on oily substrates and can also be used in the processing of paper, leather, and cleaning. In addition, they can act in the production of cheese, bread, and detergents [41].

Some substrates are mentioned by the authors, but the main emphasis is on substrates rich in keratin, obtained mainly from bird feathers. Keratinolytic substrates are difficult to degrade, and the poultry meat industry discards tons of feathers every year, which become an environmental problem [13, 21, 25, 29, 32]. In their experiments, these authors demonstrated that the enzymes produced by *F. islandicum*, *F. thailandense* and *F. pennivorans* presented high performance in the degradation of bird feathers, converting this substrate into amino acids and peptides. In addition, some amino acids such as lysine, histidine, casein, methionine and tryptophan were identified as the balance of the metabolism of bird feather degradation. These amino acids are considered important, as they are nutritionally essential.

By consulting the NCBI database for enzymes and proteins, we identified several enzymes associated with the 16S rRNA gene sequences of the species presented in this review. Thus, based on what is presented in scientific articles for the genus, we structured a list that gathered the main enzymes described for the *Fervidobacterium* species, with their respective relevant characteristics (Table 2). It is worth mentioning that, during our research in the literature, we did not find any reports of the use of these enzymes in the bioremediation of xenobiotic and recalcitrant compounds. However, it has to take in account the environments in which these genera are constantly identified, as well as their preferences for inhospitable places that receive organic loads considered toxic to many forms of life. In addition to associating their tolerance to acidic pH and high temperatures, the enzymes show broad spectrum of substrates and tolerance to solvents and detergents, qualities that are difficult to find in enzymes currently commercialized. Hence, it can be concluded that the genus *Fervidobacterium* could be very valuable in the bioremediation of contaminants.

That said, the biotechnological importance of the enzymes produced by *Fervidobacterium* is notable. This points to the fact that it becomes more and more important to investigate the enzymology of microorganisms adapted to extreme conditions, such as high temperatures and pH [5, 7, 8, 18, 41].

Table 2. Enzymology of the genus *Fervidobacterium*

SPECIES	ENZYME	MOLECULAR WEIGHT	GENE	EXPRESSION	GREAT PH	OPTIMUM TEMPERATURE (°C)	SUBSTRATE	SPECIFIC ACTIVITY (UL/MG OF PROTEIN)	HALF-LIFE	REFERENCE
Fervidobacterium nodosum	Endoglucanase cellulase	37.6 kDa	FnCel5a	*Escherichia coli*	5.0-5.5	80-83 °C	Carboxymethyl Cellulose (CMC) sodium salt	440 Ul/mg	The half-life of cellulase in an incubation at 70-75 °C did not decrease for 5 days, and when tested at 70-83 °C, its activity was stimulated	[39]
							Regenerated Amorphous Cellulose (RAC)	402 Ul/mg		
							Barley β-d-glucan	1360 Ul/mg		
							galactomannan	895 Ul/mg		
	Lipase	-	Fond_1333	*E. coli* BL21 (DE3) Codon Plus	8.0	70 °C	p-nitrophenyl-β-D-cellobioside (pNPC)	52.000 U/mg	-	[41]
Fervidobacterium islandicum	2,4-diamino pentanoate dehydrogenase	38 kDa	Fn_1646	-	8.5	55 °C	2,4-diaminopyridine	-	the half-life of this enzyme was 38 minutes at 90 °C and 2 minutes at 100 °C	[15]
	keratinolytic serine protease	>200 kDa	-	-	9.0	100 °C	native bird feathers	-	half-life of 90 minutes at 100 °C	[29]
	carboxypeptidase M32 (FisCP)	-	FIAW1_16 00	*E. coli* BL21 (DE3)	8.0	70 °C	native bird feathers	-	-	[25]
	β-glucosidase	-	Bgl1A	*E. coli*	6.0-7.0	90 °C	-	-	-	[18]
	'Islandisin', a serine protease	76.1 kDa	Fis	*E. coli* BL21	-	90 °C	-	-	half-life of 4 hours at 90°C and 1.5 hours at 100°C	[15]

				(DE3) pLysS						
	ATP-independent DNA topoisomerase	75 kDa	-	-	-	75 °C	-	-	-	[11]
	recombinant FIG protein (rFIG)	-	-	-	5.0	85 °C	Barley β-D-glucan galactomannan 4-nitrophenyl-cellobioside	367.0 Ul/mg 174.0 Ul/mg 66.1 Ul/mg	half-life of 113 hours at 85 °C	[20]
Fervidobacterium gondwanense	xylose isomerase (XylA)	-	-	*E. coli*	7.3	70 °C	conversion of glucose to fructose	15.0 U/mg	-	[23]
	Type II restriction endonuclease (Fgo I)	-	-	-	6.5-8.5	60-70 °C	-	-	-	[2]
	serine protease	130 kDa	-	-	10.0	80 °C	flour made from bird feathers	-	-	[13]
Fervidobacterium pennivorans	Type I pullulanase	93 kDa	pulA	*E. coli* FD748	6.0	80 °C	pullulan, starch, amylopectin and glycogen	-	half-life of 2 hours	[3]
	amylase and pullulanase	-	-	-	5.5-6.5	90-100 °C	resistant fibrous polypeptides	-	-	[8]
	fervidolysin	73 kDa	-	-	-	-	-	-	-	[22]
Fervidobacterium changbaicum	Lipase 1 (FcLip1)	-	-	-	7.8	70 °C	oil and fat	-	-	[7]
Fervidobacterium thailandense	keratinolytic protease	67 kDa	-	-	7-9	70-90 °C	native bird feathers	54.98 U/mg	half-life withstood >12 hours at 70 °C	[32]

4 CONCLUSION

Bibliographic research on anaerobic and extremophilic bacterial genera, such as *Fervidobacterium*, is of considerable scientific relevance. Since these microorganisms are subjected to extreme situations, they tend to adapt and develop an entire metabolic and enzymatic apparatus that makes them successful in inhospitable places. This results in the production of enzymes such as those mentioned in this review, enzymes that present a series of excellent biotechnological characteristics, which arouse and create industrial interest and stimulate bioprospecting in the inputs generated. The species that comprise the *Fervidobacterium* genus produce several enzymes, with affinity for multiple substrates and with exceptional thermal stability. Thus, it will not be surprising to come across future descriptions of many other enzymes and metabolites described for this genus of microorganism. We understand that this review may also be a starting point for generating scientific interest in the genus, with regard to the creation of new projects and experimental research on these enzymes.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Bruna Kelly de Oliveira Silva: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. José do Carmo Barbosa Neto: Formal analysis, Data curation. Laisa Gabriele Batista de Jesus: Formal analysis, Data curation. Fabrício Motteran: Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition. Marcos Antonio de Morais Junior: Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing financial or personal interests.

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DATA AVAILABILITY

Data will be made available on request.

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4.3 ARTIGO 3

IDENTIFICATION OF THE MICROBIAL COMMUNITY AND ENZYMATIC PREDICTION INVOLVED IN BTEX DEGRADATION BY MICROORGANISMS FROM WASTEWATER TREATMENT PLANTS: A SHORT COMMUNICATION

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ABSTRACT

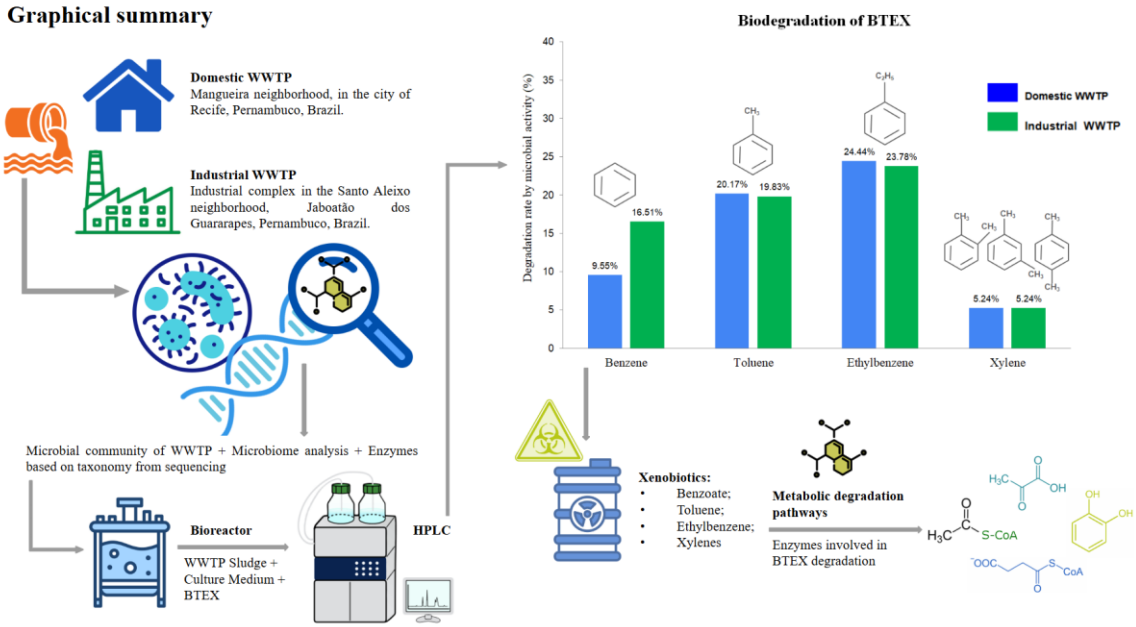
Wastewater treatment plants (WWTP) have a diversity of bacteria capable of biodegrading xenobiotics. Understanding the microbiome, as well as the enzymes and metabolic pathways used by these microorganisms, is essential for understanding these environments and of fundamental importance in the development of new biotechnologies. Thus, this research aimed to characterize and compare the microbial communities present in a WWTP that receives domestic sewage and a WWTP that receives and treats industrial effluents, focusing on comparing the microbial genera and metabolism of both involved in the degradation of BTEX compounds. The results of this research revealed subtle differences between the treatment plants. In the tolerance test to the BTEX compound, the microbiome of the domestic WWTP was tolerant to 200 mg/L of BTEX, while the microbiome of the industrial WWTP tolerated only 50 mg/L of the compound. In the degradation test, the microbial consortia showed a 60% reduction in the BTEX initially added to the test reactors, which, when compared to the control reactors with only culture medium and BTEX compounds, presented approximately 20% of the compound loss occurring through volatilization or adsorption, while 40% of the loss was due to microbial activity. In addition, the results obtained through high-throughput genetic sequencing and data inference allowed the verification of the probable enzymes and metabolic pathways associated with BTEX degradation for the identified microorganisms. These insights are particularly relevant to support the development of biotechnological strategies for the remediation of environments contaminated by BTEX, whether urban or natural.

KEYWORDS: Biodegradation, Xenobiotics, Enzymes, Metabolic pathways, Bacteria, Industrial and Domestic Effluent.

HIGHLIGHTS

- Use of taxonomy to identify enzymes and metabolic degradation pathways
- Degradation of 40% of BTEX by WWTP microbial consortia
- Bacteria from the domestic WWTP are tolerant to 200 mg/L of BTEX
- Use of WWTP microorganisms as possible hydrocarbon bioremediators

GRAPHICAL SUMMARY



1 INTRODUCTION

Benzene, toluene, ethylbenzene, and xylenes (BTEX) are monoaromatic hydrocarbons widely used in industry and in the manufacturing processes of various products, such as paints, varnishes, oil, gasoline, lubricants, and greases, and are present in the daily lives of many factories and in the daily lives of the community. They are also considered Volatile Organic Compounds (VOCs), which makes them important air pollutants. Due to their widespread use and improper disposal, these compounds are among the main environmental contaminants, mainly affecting matrices such as soil and water (Gao et al., 2024).

The BTEX are structurally formed by an aromatic ring (Figure 1). Among the compounds, benzene is considered the most toxic and carcinogenic of the group, and has the simplest structure (C6H6), being formed by only one aromatic ring; Toluene (C7H8) is an aromatic ring with a methyl group (CH3) attached to the ring; ethylbenzene (C8H10) has an ethyl group (C2H5) attached to the aromatic ring; and xylenes (C8H10) have two methyl groups (CH3) attached to the ring, but in different positions, which can be ortho, meta, or para (Silva, 2002). The compounds are colorless liquids with a strong odor, toxic, flammable, and highly volatile. Among the pollutants found in petroleum or gasoline, they are considered the most soluble, which means they have good mobility and a greater potential for contamination, since they move easily through contaminated areas (Weelink et al., 2010).

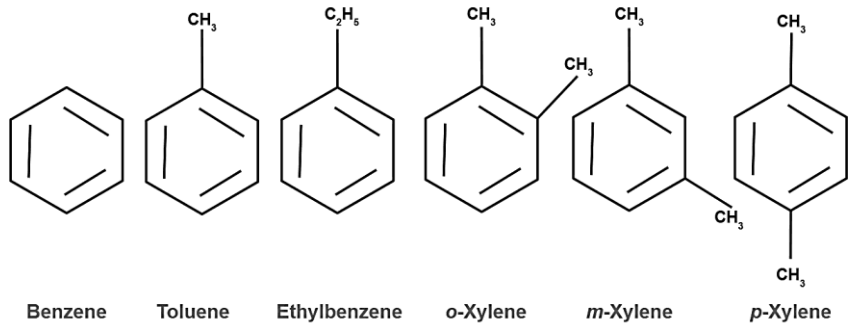


Figure 1. Chemical structure of BTEX compounds

The World Health Organization (WHO) considers BTEX a carcinogenic and mutagenic agent for humans. However, the toxicity of the compounds depends on their chemical characteristics, concentration, and the time of exposure to which the individual, whether human or animal, is exposed to the compound. In addition, considering health status, age, genetics, and exposure routes, the effects of BTEX can vary among individuals. Organizations such as the International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (EPA), as well as the European Union (EU), have established groups, parameters, and some limits for these hydrocarbons (Afrasiabi et al., 2024).

One of the stipulated parameters is the concentration of these pollutants in drinking water, being 5 µg/L for benzene, 1000 µg/L for toluene, 700 µg/L for ethylbenzene, and 10000 µg/L for xylenes. Taking into account their potential for toxicity, these compounds have also been allocated into groups: benzene belongs to group 1 and is considered a human carcinogen; ethylbenzene belongs to group 2 and is considered an animal carcinogen; while toluene and xylenes belong to group 3 and have negative toxic effects, but are not carcinogenic. Of all the compounds, benzene is the most harmful, since even at low concentrations its metabolites attack the bone marrow and can cause leukemia and other hematological diseases (Liu et al., 2025; Zahed et al., 2024).

Several studies have already demonstrated the serious effects that these organic pollutants cause to human health. Some symptoms of occasional exposure to these compounds include dizziness, shortness of breath, nausea, headache, and insomnia. The effects of chronic exposure to these contaminants can cause damage to the liver, kidneys, lungs, stomach, and central nervous system, in addition to the risk of leukemia, anemia, or even death (Afrasiabi et al., 2024; Das et al., 2025). Due to its economic appeal and widespread use by industries, BTEX can be easily found in urban or natural environments. Furthermore, accidents such as oil spills can favor the contamination of natural environments by these compounds (Zahed et al., 2024).

One of the most publicized cases was the accident that occurred in 2019 on the Brazilian coast, with the spill of crude oil that extended along the coastal region of 11 states, between the Northeast and Southeast regions. This accident led to the contamination of a large coastal strip, making the water unsuitable for bathing. It has also contaminated corals, marine animals and birds, and adhered to vegetation in mangrove areas, harming the fauna and flora of these locations (de Almeida et al., 2021; Pena et al., 2020). Accidents like these generate alerts and a search for technologies that enable the safe and effective removal of these hydrocarbons from contaminated environments. Some physical and chemical techniques are already used, but most are expensive and laborious (Gao et al., 2024). Therefore, sustainable approaches, such as bioremediation using microorganisms as degradation agents, emerge as a promising solution to address this problem, converting these contaminants into byproducts that are less harmful to the environment (Hernández-Ospina et al., 2024).

In this context, Wastewater Treatment Plants (WWTPs) represent strategic environments for prospecting for degrading microorganisms, as they receive varied organic loads, including compounds xenobiotic factors, which favor the selection of metabolically versatile communities adapted to extreme conditions (Kumar et al., 2022; Motteran et al., 2025, 2024; Sharma, 2024). Thus, this research sought to test the biodegradation potential of BTEX compounds by microorganisms from Wastewater Treatment Plants (WWTPs). These extremophilic microorganisms adapt to inhospitable environments such as WWTPs and develop degradation mechanisms for various compounds (Rajarshi, 2023; Tapadar et al., 2021). In this study, two Wastewater Treatment Plants (WWTPs), one domestic and one industrial, were evaluated. The analyses, performed using high-throughput genomic sequencing, allowed the identification of microorganisms previously described in the literature as involved in hydrocarbon degradation. Additionally, sequencing combined with the prediction of functional genes allowed for the inference of several enzymes in the analyzed reactors.

2 METHODOLOGY

2.1 SAMPLING UNIT

The samples used in this research were collected from a domestic WWTP (8°04'40.22" S and 34°55'29.39" W), located in the Mangueira neighborhood, in the city of Recife, Pernambuco, Brazil; and from an industrial WWTP (8°06'23.1" S and 35°01'36.0" W), located in a manufacturing complex in the Santo Aleixo neighborhood, Jaboatão dos Guararapes, Pernambuco, Brazil.

The domestic WWTP was designed to serve the population of the Mangueira, San Martin, Mustardinha neighborhoods and surrounding areas, totaling approximately 50.000 inhabitants. It consists of a preliminary system of grates and desanders, a biological system consisting of eight upflow anaerobic reactors (UASB), sludge drying beds and a polishing pond. The industrial WWTP consists of a grid system, followed by three anaerobic lagoons in series, all with 2 m deep. This WWTP is located in an area that houses approximately 15 industries from various segments, including food, galvanized steel, paper, paints and even laundries.

For the experiments, 500 mL of sludge (microbial biomass) from the domestic WWTP was used, from a sample that had been previously collected and stored in the Laboratory of Molecular Biology and Environmental Technology and which served as a standard sample for experiments by the laboratory's research group. The sample from the industrial WWTP was collected in the first lagoon of the treatment plant with the aid of a collection arm. Four equidistant and representative points were collected, which were mixed, forming a blend, from which 500 mL of microbial biomass was obtained. This biomass was stored in a polyethylene bottle and properly packaged in a thermal box containing ice, and taken to the Environmental Sanitation Laboratory of the Federal University of Pernambuco, where it was stored in a refrigerator at a temperature of 4 °C.

2.2 ADAPTATION OF MICROBIAL BIOMASS FROM WWTP SLUDGE TO BTEX

The bacteria present in the sludge from the treatment plant samples were cultivated and enriched in Nutrient Broth (peptone (5 g/L), yeast extract (5 g/L), meat extract (5 g/L), sodium chloride (9 g/L) and distilled water (1 L)). Then, the enriched microbial biomass from both WWTPs underwent an acclimatization period in Minimum Mineral Medium ($CaCl_2$ (0.0074 mg/L), $MgSO_4H_2O$ (0.463 mg/L), $ZnSO_4.7H_2O$ (0.02 mg/L), $MnSO_4H_2O$ (0.01 mg/L), $NH_4Fe(SO_4)_2.12H_2O$ (0.197 mg/L), KH_2PO_4 (0.5 mg/L), K_2HPO_4 (0.5 mg/L), $(NH_4)_2SO_4$ (0.5 mg/L), NaCl (9 g/L), Peptone (2 g/L) and distilled water (1 L)), containing monoaromatic hydrocarbons (BTEX) at 50 mg/L. The Minimum Mineral Medium was composed based on information from the work of Mello (2007). These microbial biomasses were fed weekly with 50 mg/L of BTEX for a period of 20 days. After this period of adaptation and selection, the biomass were stored in a refrigerator at 4 ºC, to be used as inocula in the test reactors. This final methodology, regarding culture medium, BTEX compound concentration, and acclimation period, was established based on multiple pilot tests previously performed.

2.3 BTEX TOLERANCE TEST

The previously enriched and adapted microbial biomass was subjected to tolerance tests for BTEX compounds, using four different concentrations: 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L. These concentrations were selected based on literature data and BTEX levels frequently detected in contaminated environments (Martiarena and Jesus, 2016). The experiments were performed in amber glass flasks (reactors) with a useful volume of 50 mL, hermetically sealed with stoppers and sealing caps. Each reactor was filled with 30 mL of experimental solution, composed of 100 µL of biomass, BTEX compounds at the established concentrations, and a complementary volume of Minimum Mineral Medium until reaching 30 mL. The reactors were incubated at 37°C for a period of 24 hours. After incubation, a 100 µL aliquot was removed from each reactor and diluted in a ratio of 10^{-2}. The samples were then seeded on the surface of Petri dishes containing Minimal Mineral Agar Medium. The plates were incubated at 37 °C for 96 hours. The experiment was performed in triplicate. The samples were considered tolerant to BTEX compounds when the Petri dishes showed growth greater than 30 Colony Forming Units per milliliter (CFU/mL), according to the criterion established by Martiarena and Jesus (2016).

2.4 BTEX DEGRADATION ANALYSIS

For the degradation test, a 10 g/L BTEX stock solution was used, of which the equivalent of 50 mg/L was added to each test reactor. The enriched and adapted microbial biomass was used as inoculum, with 4.25 mL of this biomass being added to each experimental reactor. The culture medium used was the Minimum Mineral Medium, and each reactor received 45 mL of medium. For this methodology, it was chosen to use sacrificial reactors with a useful volume of 50 mL. Amber glass bottles were used for the reactors and properly sealed with a stopper, sealing caps and parafilm, to minimize the volatilization of BTEX. The use of sacrificial reactors is valid for this type of compound, since a reactor is used for each reading, which is disassembled and discarded immediately afterwards, to avoid losses due to volatilization at the time of collection and reading. For this experiment, we used test reactors (medium + BTEX + inoculum), control reactors with reagent (medium + BTEX), control reactors with inoculum (medium + inoculum) and, finally, the medium sterility control reactors (only culture medium). The experiment was performed in triplicate, with reactors that were initially aerobic, but that became intentionally anaerobic over the incubation time, due to the

limited free space in each flask. The results were organized in spreadsheets in Microsoft Office Excel, using the equation below (Equation 1), the removal rate of BTEX compounds was calculated for each time. The results were plotted as a graph for better visualization (Figure 4). Equation 1:

$$BTEX\ Removal\ Rate\ (\%) = \left(\frac{Initial\ Concentration - Final\ Concentration}{Inicial\ Concentration}\right) \times 100$$

2.5 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS

Monoaromatic hydrocarbons (BTEX) were analyzed by high performance liquid chromatography (HPLC) (Waters Corporation, Milford, MA, USA) with a C18 column (5 µm) in reverse phase (Merck), diode array detector (DAD) with a wavelength of 210 nm and an injection volume of 20 µL. The mobile phase used was composed of 40% H_2O and 60% acetonitrile as organic solvent. A calibration curve for the compound was performed using HPLC standard BTEX solution; the curve contemplated the concentrations of 5, 10, 25, 50, 75, 100 and 125 mg/L of the compound. The chromatographic run time was 13 min, with retention times of 5.6 min (benzene), 7.5 min (toluene), 9.9 min (ethylbenzene), and 10.3 min (xylenes) (Figure 2). For HPLC reading, 1.5 mL of sample was withdrawn from each reactor, which was immediately filtered with a syringe through a 0.22 µm polyethersulfone (PES) membrane filter. To avoid losses by volatilization, the sample was read immediately. HPLC readings were performed at 0 h, 48 h, 72 h, and 96 h.

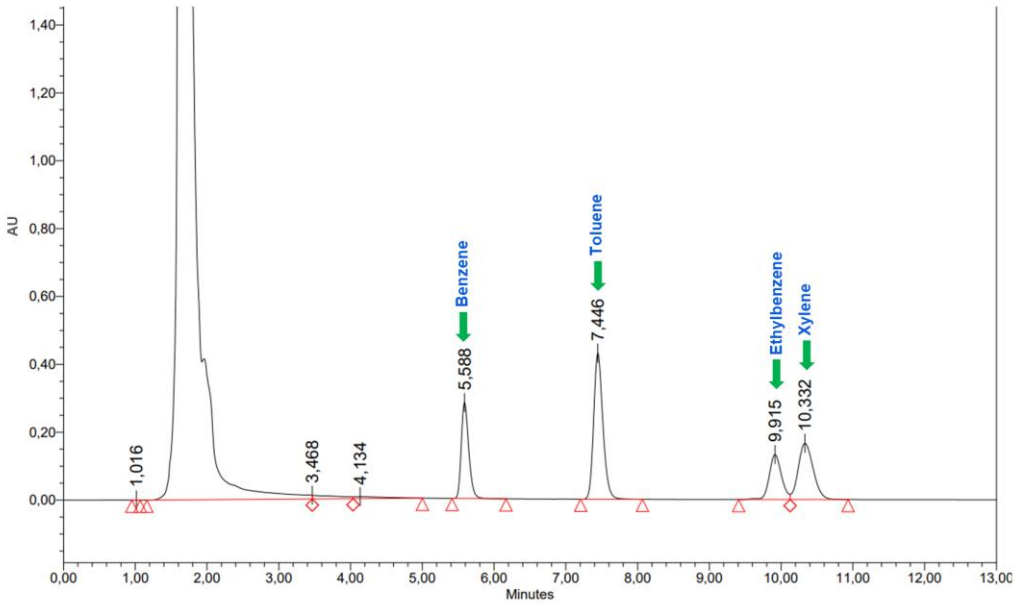


Figure 2. Chromatographic peaks of BTEX compounds

2.6 DNA EXTRACTION AND SEQUENCING

A sample from each of the biomasses adapted to BTEX exposure at 50 mg/L for 20 days was subjected to total genomic DNA extraction using the Power Soil® DNA Isolation Kit (Qiagen, Hilden, Germany), following the protocol established by the manufacturer. DNA quantification was performed by spectrophotometry using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, USA), ensuring integrity and adequate concentration for sequencing. The samples were sent to GoGenetic facility (Curitiba, Brazil), where the hypervariable regions V3-V4 of the 16S rRNA gene were amplified and sequenced on the Illumina NextSeq platform, following the respective protocols. The generated sequences were processed and analyzed using the QIIME2 pipeline (Bolyen et al., 2019), which allowed the taxonomic identification of the microorganisms present and the determination of relative abundances. Only high-quality reads were included in the bioinformatic processing.

Sequence analysis was conducted in RStudio, using DADA2 (Callahan et al., 2016), Bioconductor (Gentleman et al., 2004), and Phyloseq (McMurdie and Holmes, 2013), with definition of Amplicon Sequence Variants (ASVs) based on 97% similarity between reads. For taxonomic assignment, the SILVA database was used (Quast et al., 2013). Subsequently, the relative abundance of ASVs was used in the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) program (Douglas et al., 2020), for functional prediction based on annotations from the Kyoto Encyclopedia of Genes and Genomes (KEGG) banks, using EC (Enzyme Commission) and KO (KEGG Orthology) identifiers (Kanehisa et al., 2016; Kanehisa and Goto, 2000; Langille et al., 2013). This approach allowed the inference of the main microbial enzymes involved in metabolic pathways associated with the degradation of aromatic hydrocarbons, including BTEX, enabling a functional view of the microbiome in response to exposure to these xenobiotics.

The genetic sequencing data were deposited in the National Center for Biotechnology Information (NCBI) database, with the following accession numbers: SAMN49797721 and SAMN49797722 (Domestic WWTP – B1), and SAMN49797723 and SAMN49797724 (Industrial WWTP – B2). The corresponding experimental records are available under accession numbers SRX29563704 (Domestic WWTP – B1), and SRX29563705 (Industrial WWTP – B2), all associated with project PRJNA1286782.

3 RESULTS AND DISCUSSION

3.1 ADAPTATION, TOLERANCE AND BIODEGRADATION OF BTEX COMPOUNDS

The initial stage of the experiments aimed to promote the adaptation of the sludge from the WWTP to the BTEX compounds. This acclimatization period lasted 20 days and was essential for the selection of the most resistant microorganisms present in the microbial biomass. The biomass from the domestic WWTP showed tolerance to all BTEX concentrations evaluated (50, 100, 150 and 200 mg/L). In contrast, the biomass from the industrial WWTP showed tolerance only to a concentration of 50 mg/L. This result was unexpected, considering that this plant treats industrial effluents, characterized by the presence of recalcitrant compounds, which would presumably select a more resistant microbiota.

Based on these data, the BTEX degradation tests were conducted with a standardized initial concentration of 50 mg/L for both microbial biomasses. The chromatographic analyses showed well-defined peaks, allowing the precise quantification of the areas corresponding to the concentrations of BTEX remaining after the reactor incubation process. When comparing the initial and final times of the experiment, the reduction of the compounds in the reaction medium is noticeable (Figure 3).

The peaks detected between 1 and 4 minutes in the HPLC chromatogram (Figure 3) can be attributed to the presence of more polar or volatile compounds, which have lower affinity for the column's stationary phase and therefore elute more quickly. It is plausible that these signals are related to the presence of ethanol used as a solvent in the BTEX stock solution, since ethanol has a characteristically short retention time of between 1 and 2 minutes (Sari Erkan et al., 2018). Furthermore, other constituents of the medium or metabolites derived from WWTP sludge biomass may also contribute, including short-chain alcohols, volatile organic acids, and other soluble compounds produced by the microbiota (Gottardo et al., 2024).

Figure 3 shows the results for industrial WWTP, which demonstrates that the microbial consortium in question showed a reduction in the concentrations observed in the chromatographic analyses. The same occurred for the biomass of domestic WWTP. To ensure that this reduction was attributed exclusively to microbial activity, the degradation values were corrected based on the volatility control reactors, in which there was no biological activity (Table 1). This correction allowed the exclusion of losses caused solely by the volatilization of the compounds, resulting in an estimate of the actual microbial degradation rate (Figure 4). In this way, it was possible to accurately distinguish the fraction of BTEX removed by abiotic

processes from that effectively degraded by the metabolic action of the microorganisms present in the experimental reactors.

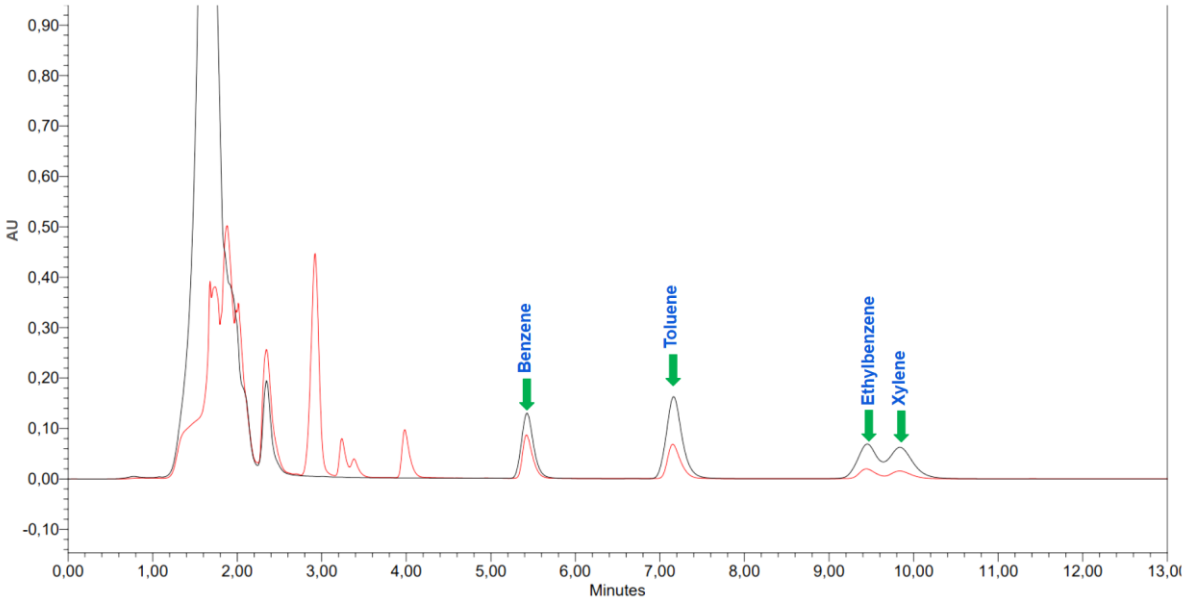


Figure 3. Chromatographic peaks representing the biodegradation of BTEX compounds by the microbial biomass of the industrial WWTP, comparing the initial time (0 h - black line) with the final time (96 h - red line). The reduction of the peaks over time indicates the removal of the compounds, evidencing the degradative activity of the microbial consortium.

Table 1. Table representing the actual quantity of each compound removed by volatilization and biodegradation, in mg/L, allowing a more precise quantitative analysis of the efficiency of treatment systems.

Compound	Volatilization control reactor (BTEX abiotic loss in mg/L)	Domestic WWTP reactor (Total BTEX loss in mg/L)	Industrial WWTP reactor (Total BTEX loss in mg/L)	Domestic WWTP reactor (BTEX biotic loss in mg/L)	Industrial WWTP reactor (BTEX biotic loss in mg/L)
Benzene	23.71	28.49	31.97	4.78	8.26
Toluene	37.52	47.61	47.43	10.09	9.92
Ethylbenzene	37.78	50.0	49.67	12.22	11.89
Xylenes	47.38	50.0	50.0	2.62	2.62

Caption: Table showing abiotic and biotic degradation values in mg/L for domestic WWTP and industrial WWTP. Note that the initial concentration of BTEX compounds in each reactor was 50 mg/L.

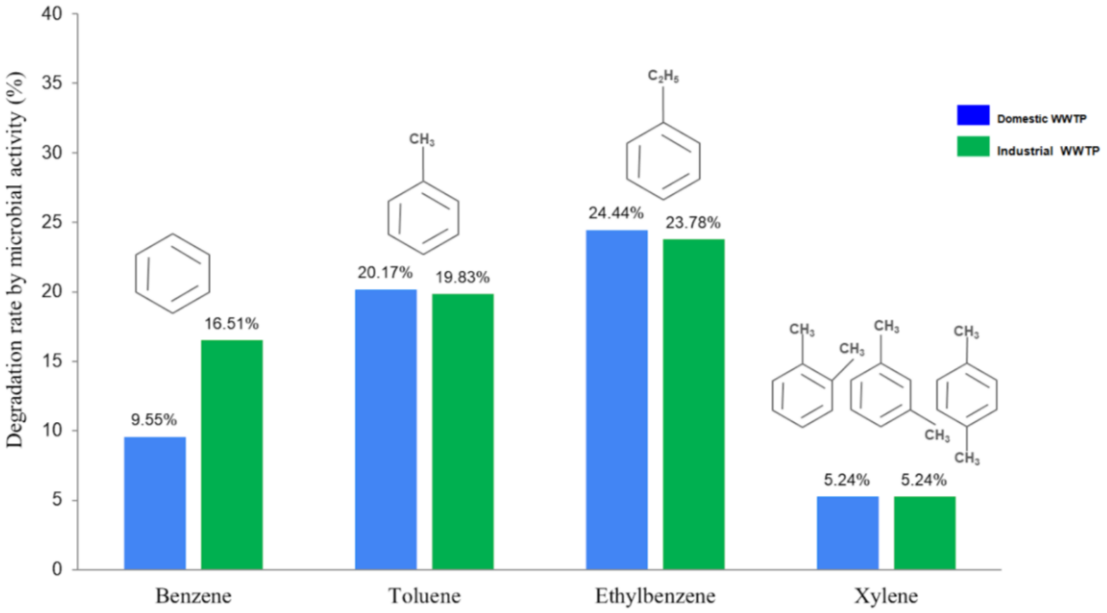


Figure 4. Degradation rate of BTEX compounds by microbial activity

Considering that most natural and urban environments contaminated by BTEX tend to consist of aqueous systems, the degradation tests performed in this research used reactors containing liquid media. Despite the low solubility of BTEX, aqueous systems increase the bioavailability of the compounds in the environment, facilitating the absorption and metabolization of BTEX by the microorganisms present in the microbial biomasses tested. The microorganisms present in WWTP have adaptations and enzymatic machinery that allow the metabolization of recalcitrant compounds, turning these pollutants into sources of carbon and energy (Silva et al., 2025).

After correcting the values for volatilization, it was observed that the microbial contribution to BTEX removal varies significantly among the compounds (Figure 4). Benzene biodegradation was relatively low, especially in the domestic WWTP (9.55%), suggesting that volatilization is the main removal mechanism for this compound, with limited microbial activity, although still noticeable in the industrial WWTP (16.51%). Toluene and ethylbenzene showed high biological removal (over 19% and 23%, respectively), after accounting for volatilization. These compounds are more readily metabolized by various aerobic and anaerobic bacteria, which explains the high rate of active degradation. The values are similar for both sludge types, indicating microbial robustness in both WWTPs for these compounds. In contrast, xylenes showed a limited biological contribution to degradation (5.24%), with most of the loss attributed to volatilization. Despite this, the fact that 100% removal was achieved in both

WWTPs (Table 1) indicates that the system is able to completely eliminate the compound, but mainly due to abiotic factors.

The degradation process by microorganisms is considered one of the best means of removing BTEX from contaminated environments, taking into account that the percentage that is not removed by volatilization is metabolized by microorganisms, transforming these compounds into intermediate byproducts, such as catechol, benzoic acid, salicylic acid, carboxylic acids, alcohol and aldehydes (El-Naas et al., 2014; Harirchi et al., 2022b; Weelink et al., 2010). Some of these byproducts can be monitored in the metabolic pathway model proposed in this research for the degradation of these target compounds (Figure 6). The data presented so far can be used in future studies that aim to detail the metabolic pathways of the microbial biomasses tested, as well as quantify the intermediate byproducts generated by them.

In the initial phase of the degradation test, the reactors are partially aerobic; under these conditions, taking into account the findings in the literature, it is assumed that the intermediate by-products mentioned above can be mineralized, resulting in carbon dioxide (CO_2) and water (H_2O) (Aydin et al., 2025; Lueders, 2017; Yoshikawa et al., 2017). Over time, oxygen is expected to be consumed by aerobic microorganisms, creating an anaerobic environment, making room for the true actors of biodegradation (Costa et al., 2025). Therefore, it is understood that in the absence of oxygen (O_2), the degradation of these byproducts can, in turn, generate volatile fatty acids (Aburto-Medina and Ball, 2015; Harirchi et al., 2022b). Thus, depending on the microbial composition and environmental conditions, the biodegradation potential of BTEX compounds by microorganisms becomes a favorable and efficient alternative for the development of biotechnologies aimed at the partial or complete degradation of these contaminants.

3.2 TAXONOMIC AND MICROBIAL DIVERSITY

Microbiome analysis performed on the samples yielded a total of 81.295 (domestic WWTP) and 88.356 (industrial WWTP) quality reads. The domestic WWTP yielded a total of 348 identified taxa, 76.822 classified sequences, and 4.473 unclassified sequences, representing approximately 5.5% of the unclassified sequences for this WWTP. The industrial WWTP yielded a total of 329 taxa, 79.542 classified sequences, and 8.814 unclassified sequences, totaling 10% unclassified sequences. In terms of alpha diversity, the Shannon (3.45 for domestic WWTP and 2.81 for industrial WWTP), Simpson (0.93 and 0.79), and Evenness (0.80 and 0.69)

indices demonstrate that domestic WWTP have a more diverse and equitable microbial community, suggesting a functionally generalist ecosystem.

Similar results were described by Rodrigues et al., (2020). In both studies, the authors also address microorganisms present in Brazilian WWTP that primarily treat domestic wastewater and that showed potential for xenobiotic biodegradation and similar diversity indices. Another study, this time in China, carried out by Yang et al., (2020) presented similar results regarding microorganisms found in WWTP that treat industrial effluents, where more specialized communities stand out in environments with high toxic loads, as well as the bacteria found in the data of this study.

When analyzing the data, focusing on phyla and families with biotechnological relevance in the degradation of BTEX and other xenobiotics, the five phyla detected were: *Pseudomonadota*, with a relative abundance of 36.1% in the domestic WWTP and 78.3% in the industrial WWTP; *Clostridiota* with 53.8% and 21.2%; *Bacteroidota* with 6.01% and 0.38%; *Desulfobacterota* with 3.2% in the domestic WWTP, but not evidenced in the industrial WWTP; and *Actinobacteriota* with 0.85% in the domestic WWTP and 0.09% in the industrial WWTP.

The phylum *Pseudomonadota* is the most representative phylum in the industrial WWTP, which can be explained by the influence of the characteristics of the effluent entering the WWTP. The phylum is relevant in the degradation of BTEX and Polycyclic Aromatic Hydrocarbons (PAHs), in addition to being the phylum with the greatest functional diversity for the degradation of these compounds (Jin et al., 2023). Another highly significant phylum in both samples is the phylum *Clostridiota*. The presence of microorganisms from this phylum in environments such as WWTPs is essential, as they act in the mineralization of recalcitrant compounds and are potentially active in the degradation of BTEX and xenobiotics (Harirchi et al., 2022b).

The *Bacteroidota* phylum is also relevant because, in addition to participating in the BTEX degradation process, it also participates in the secondary degradation of byproducts, resulting in the complete mineralization of recalcitrant compounds (Liu et al., 2024). The fourth phylum detected was *Desulfobacterota*, bacteria belonging to this phylum that can degrade benzene, toluene, and phenol in the absence of oxygen (Dhar et al., 2023). Finally, the *Actinobacteriota* phylum has robust metabolic potential, with complex enzymatic pathways involving oxygenases and peroxidases, essential for the breakdown of aromatic rings. These bacteria are capable of metabolizing recalcitrant compounds such as BTEX and PAHs,

converting them into intermediates such as catechol, benzoic acid, or acetyl-CoA (Hocinat et al., 2020; Wongbunmak et al., 2017).

Regarding the number of bacterial genera identified for domestic WWTP, a total of 52 were found, while for industrial WWTP, a total of 43 were found. Figure 5 shows the genera that presented relative abundance above 0.5% for at least one of the microbiomes tested. Table 2 shows the relationship between the identified bacterial genera and the metabolic pathways and possible enzymes used by them.

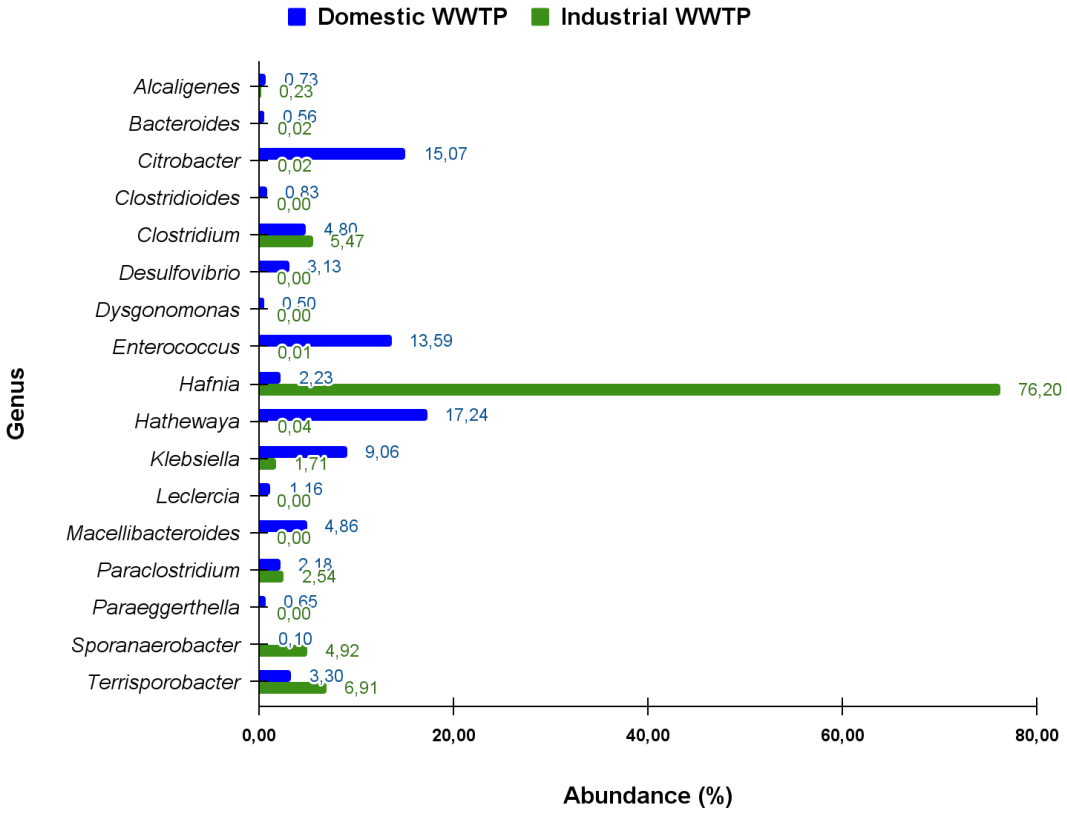


Figure 5. Relative abundance of the genera of the microbial community of the domestic and industrial WWTP that presented abundance above 0.5% in at least one of the WWTP

Table 2. Functional profile of identified microorganisms and mechanisms associated with biodegradation

Genus	Degraded Compounds	Main Metabolic Pathways	Related Enzymes	References
Alcaligenes	Aromatics, industrial solvents	Nitrification, denitrification, ortho/meta pathway	Oxidases, nitrate reductase	(Chen et al., 2022; Motteran et al., 2024; Ruikar, 2022)

Bacteroides	Polysaccharides, drugs	Saccharolytic fermentation, via succinate-propionate	Glycosidases, fermentation enzymes	(Wexler and Goodman, 2017)
Citrobacter	BTEX, pharmaceuticals, industrial solvents	Monooxygenation, fermentation, coupled denitrification	Monooxygenases, GSTs, NADH dehydrogenases	(Gupta et al., 2022; Jin et al., 2023; Liu et al., 2024; Sharma, 2024; Sumathi and Manian, 2024)
Clostridioides	Nitrogen and phenolic compounds	Reductive fermentation, proteolysis	Proteases, aminotransferases	(Janezic and Rupnik, 2015)
Clostridium	Complex organic compounds, solvents	Anaerobic fermentation, acetogenesis	Hydrogenases, acetyl-CoA synthetases	(Harirchi et al., 2022a; Liu et al., 2024; Nwankwo et al., 2021)
Desulfovibrio	Sulfur compounds, hydrocarbons	Sulfate reduction, anaerobic β-oxidation	Sulfate reductases, hydrogenases	(Dhar et al., 2023; Toth et al., 2021)
Dysgonomonas	Aromatic compounds, complex carbohydrates	Fermentation, anaerobic degradation	Glycosidases, dehydrogenases	(Liu et al., 2024)
Enterococcus	Phenol, dyes, aromatic compounds	Decarboxylation, lactic fermentation	Phenol hydroxylases, dehydrogenases	(Pandolfo et al., 2023; Ruikar, 2022)
Hafnia	PAHs, nitrogenous compounds	Deamination, aerobic oxidation	Amine oxidases, nitroreductases	(Kumar et al., 2022)
Klebsiella	BTEX, catechol, pesticides	Ortho and meta pathway	Catechol-1,2-dioxygenase, monooxygenases	(Rodriguez et al., 2023; Zhang et al., 2023)
Macellibacteroides	Amino acids, complex sugars	Anaerobic fermentation, polysaccharide degradation	Glycosidases, fermentation enzymes	(Palma and Costa, 2021)

The table shows the genera *Hafnia* (2.23% in domestic WWTP and 76.20% in industrial WWTP), *Klebsiella* (9.06% and 1.71%) and *Citrobacter* (15.07% and 0.02%), these genera belong to the family *Enterobacteriaceae* (35.16% and 78.05%), phylum *Pseudomonadota*, this family, in addition to degrading aromatic compounds, is also tolerant to heavy metals and solvents, which illustrates why a family with such specificity and adaptability is so present in industrial WWTP (Jin et al., 2023). The industrial wastewater treatment plant is located within

a complex of approximately 15 factories, processing products from a wide range of categories, such as paint and varnish manufacturing, galvanized steel, laundry, paper manufacturing, polyvinyl chloride (PVC), animal feed, dairy products, citrus juices, and others. The manufacturing and processing of these products and services releases a variety of recalcitrant compounds into the effluent, including BTEX, which are involved in the manufacturing process of a variety of products (Ghobakhloo et al., 2023; Hosseinzadeh et al., 2023; Kim et al., 2022).

The genus *Hafnia* is commonly associated with the degradation of PAHs and nitrogenous compounds (Kumar et al., 2022). The *Klebsiella* genus has the ability to degrade BTEX and pesticides and participates in the catechol transformation process (Zhang et al., 2023). The *Citrobacter* genus can act in the degradation of benzene, phenol, and derivatives, as well as pharmaceuticals (Gupta et al., 2022; Liu et al., 2024; Sharma, 2024). Another family belonging to this phylum and identified in the sequencing was the *Alcaligenaceae* family (0.73% and 0.23%), represented in the samples by the *Alcaligenes* genus (0.73% and 0.23%). This genus can act in the degradation of toluene, benzoate, and phenanthrene (Motteran et al., 2024; Ruikar, 2022). In general, the mentioned genera can degrade benzene, toluene, xylenes and PAHs using metabolic pathways that involve enzymes such as monooxygenases and dioxygenases (Table 2).

The phylum *Clostridiota* is most prevalent in domestic WWTP and is well represented taxonomically in the analyzed biomasses, with the families *Peptostreptococcaceae* (16.73% in domestic WWTP and 9.46% in industrial WWTP) and *Clostridiaceae* (21.76% and 6.63%). The predominance of this phylum in domestic WWTP may be related to the nature of the sample, since the biomass from this WWTP was collected directly from a UASB reactor. These families are important in anaerobic environments, as they participate in the initial and final stages of BTEX degradation in oxygen-poor environments. Furthermore, genera such as *Clostridium* (4.80% and 5.47%) act in the anaerobic reduction of aromatic compounds, being able to transform benzene and toluene into organic acids, such as acetate or butyrate (Harirchi et al., 2022a).

The *Bacteroidota* phylum has some representative families in the tested biomasses, namely: *Bacteriodaceae* (0.57% and 0.03%), this family is tolerant to toxic environments and participates in the fermentation of aromatic intermediates; and the *Dysgonomonadaceae* family (0.57% and 0.34%), represented in the domestic WWTP by the genus *Dysgonomonas* (0.50%), which has the potential to degrade PAHs such as phenanthrene and pyrene, and is also capable of hydrolyzing complex compounds with aromatic rings (Liu et al., 2024); another relevant family for this phylum is *Tannerellaceae* (4.87% and 0.01%), represented in the domestic

WWTP by the genus *Macellibacteroides* (4.86%), a genus that participates in the fermentation route of intermediates in the degradation of BTEX (Palma and Costa, 2021). The *Desulfobacterota* phylum is represented by the *Desulfovibrionaceae* family, identified in domestic WWTP with 3.20% relative abundance, represented by the *Desulfovibrio* genus (3.13%). Bacteria belonging to this genus have the ability to degrade BTEX and PAHs anaerobically, through sulfate reduction (Dhar et al., 2023; Toth et al., 2021).

In general, most of these genera contain enzymes such as oxygenases and dehydrogenases, which are considered crucial in the initial phase of aromatic ring degradation, according to bibliographic data (Table 2). Furthermore, the presence of decarboxylases, transferases, monooxygenases, and dioxygenases may enable the conversion of toxic compounds into intermediates of the central metabolism of pyruvate, acetyl-CoA, and others. These genera, in addition to exhibiting metabolic versatility, are capable of utilizing recalcitrant compounds as a source of carbon and energy, even in adverse situations. They exhibit adaptability through natural selection, as observed in their high levels of abundance after 20 days of exposure to 50 mg/L of BTEX compounds in the reactors. Furthermore, BTEX degradation by the tested biomasses can result in byproducts such as organic acids, namely acetic acid, succinic acid, and oxaloacetic acid. They can also generate intermediates from central pathways, such as catechol, benzoate, and succinyl-CoA.

An ecological analysis of the dynamics of microbial communities reveals that domestic WWTP have a more diverse microbiome, which may be an advantage in more comprehensive bioremediation processes. On the other hand, industrial WWTP has a specialized microbial community adapted to specific contaminants, thus presenting potential for applications focused on the degradation of industrial compounds. Based on the findings, it can be concluded that the microbial communities present in the tested microbiomes represent a functional network, with degradation pathways ranging from initial oxidation to fermentation and mineralization of toxic intermediates.

3.3 ENZYMES INVOLVED IN THE METABOLIC PATHWAYS OF BTEX DEGRADATION

A total of 1.597 functional genes (KO - KEGG Orthology) were inferred in the domestic WWTP sample. By cross-referencing the data with what was available in the Kyoto Encyclopedia of Genes and Genomes (KEGG), it was found that, of this total, 32 KO were involved in the benzoate degradation pathway, 5 in the degradation of toluene, 1 in the

degradation of ethylbenzene and 10 in the degradation of xylenes. For the industrial WWTP, a total of 1.581 KO were highlighted, 28 for benzoate, 6 for toluene, 1 for ethylbenzene and 10 for xylenes (Table 3). Table 3 shows some relevant information recorded in the KEGG for each enzyme involved in the degradation of BTEX, as well as the number of times each of them was recorded for each microbial biomass.

It was noted that the domestic WWTP had a higher enzymatic abundance and diversity than the industrial WWTP. When comparing the treatment plant, KO related to enzymes such as acetaldehyde dehydrogenase (EC: 1.2.1.10) were found, which appeared 70 times in the biomass of the industrial WWTP and 101 times in the domestic WWTP, indicating greater diversity of this dehydrogenase in this specific microbiome. Finding the KO for this enzyme is important, as its function is to transform acetaldehyde, a toxic compound, into acetate, a compound that is not toxic to the environment (Corella, 2007). Some KO were exclusively present in only one microbiome, such as KO related to enzymes maleylacetate reductase (EC: 1.3.1.32) and hydroxyquinol 1,2-dioxygenase (EC: 1.13.11.37), detected only in the industrial WWTP microbiome. These enzymes are directly involved in key steps in the degradation of aromatic compounds, such as benzoate and toluene, components of the BTEX group (Table 3).

In contrast, the KOs regarding the enzymes 2-hydroxy-4-carboxymuconate semialdehyde hemiacetal dehydrogenase (EC: 1.1.1.312), protocatechuate 4,5-dioxygenase (1.13.11.8), 2,3-dihydroxybenzoate decarboxylase (EC: 4.1.1.46) and 4-oxalomesaconate tautomerase (EC: 5.3.2.8) were detected only in the domestic WWTP microbiome (Table 3). The presence or absence of a KO in the microbiome of a given WWTP may indicate functional specialization of the local microbiota, which tends to be influenced by the type of pollutant predominant in each system (Sharma, 2024).

Table 3. KO and respective Enzymes that act in the BTEX degradation pathway and that were identified for domestic and industrial WWTP

KO – KEGG Orthology	Gene	Enzyme name	EC	Pathway	Reaction	Nº EC in WWTP	
						Industrial	Domestic
K01782	fadJ	3-hydroxyacyl-CoA dehydrogenase	EC:1.1.1.35	Benzoate degradation	3-hydroxypimeloyl-CoA:NAD+ oxidoreductase	23	32
K00055	-	aryl-alcohol dehydrogenase	EC:1.1.1.90	degradation of xylene and toluene	4-methylbenzyl alcohol:NAD+ oxidoreductase	3	8

K00074	paaH, hbd, fadB, mmgB	3-hydroxybutyryl-CoA dehydrogenase	EC:1.1.1.157	Benzoate degradation	(S)-3-hydroxybutanoyl-CoA:NADP+ oxidoreductase	56	75
K10219	ligC	2-hydroxy-4-carboxymuconate semialdehyde hemiacetal dehydrogenase	EC:1.1.1.312	Benzoate degradation	4-carboxy-2-hydroxymuconate semialdehyde hemiacetal:NADP+ 2-oxidoreductase	-	1
K04073	mhpF	acetaldehyde dehydrogenase	EC:1.2.1.10	degradation of xylene and benzoate	acetaldehyde:NAD+ oxidoreductase (CoA-acetylating)	70	101
K00141	xylC	benzaldehyde dehydrogenase (NAD)	EC:1.2.1.28	degradation of xylene and toluene	p-methylbenzaldehyde:NADP+ oxidoreductase	3	5
K18366	bphJ, xylQ, nahO, tesF	propanal dehydrogenase	EC:1.2.1.87	degradation of xylene and benzoate	propanal:NAD+ oxidoreductase (CoA-propanoylating)	18	22
K05783	benD-xylL	dihydroxycyclohexadiene carboxylate dehydrogenase	EC:1.3.1.25	degradation of xylene and benzoate	(1R,6S)-1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate:NAD+ oxidoreductase (decarboxylating)	5	8
K00217	-	maleylacetate reductase	EC:1.3.1.32	degradation of benzoate and toluene	3-oxoadipate:NAD+ oxidoreductase and 3-oxoadipate:NADP+ oxidoreductase	1	-
K00252	GCDH, gcdH	glutaryl-CoA dehydrogenase	EC:1.3.8.6	Benzoate degradation	glutaryl-CoA:electron-transfer flavoprotein 2,3-oxidoreductase (decarboxylating)	4	5
K03381	catA	catechol 1,2-dioxygenase	EC:1.13.11.1	degradation of benzoate and	catechol:oxygen 1,2-	5	8

				toluene	oxidoreductase(decyclizing)		
K00446	dmpB, xylE	catechol 2,3-dioxygenase	EC:1.13.11.2	degradation of xylene and benzoate	catechol:oxygen 2,3-oxidoreductase (decyclizing)	4	12
K00448	pcaG	protocatechuate 3,4-dioxygenase, alpha subunit	EC:1.13.11.3	Benzoate degradation	protocatechuate :oxygen 3,4-oxidoreductase (decyclizing)	5	7
K04100	ligA	protocatechuate 4,5-dioxygenase, alpha chain	EC:1.13.11.8	Benzoate degradation	protocatechuate :oxygen 4,5-oxidoreductase (decyclizing)	-	1
K04098	chqB	hydroxyquinol 1,2-dioxygenase	EC:1.13.11.37	Benzoate degradation	benzene-1,2,4-triol:oxygen 1,2-oxidoreductase (decyclizing)	1	-
K05549	benA-xylX	benzoate 1,2-dioxygenase subunit alpha	EC:1.14.12.10	degradation of xylene and benzoate	2-fluorobenzoate, NADH:oxygen oxidoreductase (1,2-hydroxylating)	6	9
K00481	pobA	p-hydroxybenzoate 3-monooxygenase	EC:1.14.13.2	Benzoate degradation	4-hydroxybenzoate,NADPH:oxygen oxidoreductase (3-hydroxylating)	5	8
K00626	ACAT, atoB	acetyl-CoA C-acetyltransferase	EC:2.3.1.9	Benzoate degradation	acetyl-CoA:acetyl-CoA C-acetyltransferase	63	90
K00632	fadA, fadI	acetyl-CoA acyltransferase	EC:2.3.1.16	degradation of benzoate and ethylbenzene	succinyl-CoA:acetyl-CoA C-acyltransferase	23	32
K07823	pcaF	3-oxoadipyl-CoA thiolase	EC:2.3.1.174	Benzoate degradation	succinyl-CoA:acetyl-CoA C-acyltransferase	10	11
K01031	pcaI	3-oxoadipate CoA-transferase, alpha subunit	EC:2.8.3.6	Benzoate degradation	succinyl-CoA:3-oxoadipate CoA-transferase	6	9

K01055	pcaD	3-oxoadipate enol-lactonase	EC:3.1.1.24	Benzoate degradation	4-carboxymethylbut-3-en-4-olide enol-lactonohydrolase	6	9
K01061	-	carboxymethylenebutenolidase	EC:3.1.1.45	Toluene degradation	dienelactone hydrolase	20	26
K10221	ligI	2-pyrone-4,6-dicarboxylate lactonase	EC:3.1.1.57	Benzoate degradation	2-pyrone-4,6-dicarboxylate lactonohydrolase	1	-
K01075	-	4-hydroxybenzoyl-CoA thioesterase	EC:3.1.2.23	Benzoate degradation	4-hydroxybenzoyl-CoA hydrolase	1	2
K01607	pcaC	4-carboxymuconolactone decarboxylase	EC:4.1.1.44	Benzoate degradation	4-carboxymuconolactone carboxy-lyase	34	56
K14333	DHBD	2,3-dihydroxybenzoate decarboxylase	EC:4.1.1.46	Benzoate degradation	2,3-dihydroxybenzoate carboxy-lyase	-	4
K10218	ligK, galC	4-hydroxy-4-methyl-2-oxoglutarate aldolase	EC:4.1.3.17	Benzoate degradation	4-hydroxy-4-methyl-2-oxoglutarate pyruvate-lyase (pyruvate-forming)	1	2
K01666	mhpE	4-hydroxy 2-oxovalerate aldolase	EC:4.1.3.39	degradation of xylene and benzoate	4-hydroxy-2-oxopentanoate pyruvate-lyase (acetaldehyde-forming)	9	21
K01692	paaF, echA	enoyl-CoA hydratase	EC:4.2.1.17	Benzoate degradation	(S)-3-hydroxybutanoyl-CoA hydro-lyase	57	76
K02554	mhpD	2-keto-4-pentenoate hydratase	EC:4.2.1.80	degradation of xylene and benzoate	4-hydroxy-2-oxopentanoate hydro-lyase (2-oxopent-4-enoate-forming)	4	12
K10220	ligJ	4-oxalmesaconate hydratase	EC:4.2.1.83	Benzoate degradation	2-hydroxy-4-oxobutane-1,2,4-tricarboxylate 2,3-hydro-lyase	1	2

K01821	praC, xylH	4-oxalocrotonate tautomerase	EC:5.3.2.6	degradation of xylene and benzoate	2-hydroxy-5-methyl-cis,cis-muconate 2-oxo-5-methyl-cis-muconate isomerase	25	38
K16514	galD	4-oxalomesaconate tautomerase	EC:5.3.2.8	Benzoate degradation	4-oxalomesaconate keto---enol-isomerase	-	2
K03464	catC	muconolactone D-isomerase	EC:5.3.3.4	Benzoate degradation	(S)-5-oxo-2,5-dihydrofuran-2-acetate delta3-delat2-isomerase	5	8
K01856	catB	*muconate cycloisomerase*	EC:5.5.1.1	degradation of benzoate and toluene	2,5-dihydro-5-oxofuran-2-acetate lyase (decyclizing)	5	8
K01857	pcaB	3-carboxy-cis,cis-muconate cycloisomerase	EC:5.5.1.2	Benzoate degradation	2-carboxy-2,5-dihydro-5-oxofuran-2-acetate lyase (decyclizing)	8	15

Caption: KO - KEGG Orthology; EC - Enzyme Commission; Columns 7 and 8 refer to the number of times each listed enzyme was identified in the biomass of domestic WWTP and industrial WWTP.

Many of the KO listed in Table 3 participate in redox reactions and the breakdown of aromatic compounds, which are involved in bioremediation processes. The KO related to enzymes 3-hydroxybutyryl-CoA dehydrogenase (EC: 1.1.1.157), catechol 2,3-dioxygenase (EC: 1.13.11.2]), and acetyl-CoA C-acetyltransferase (EC: 2.3.1.9) are examples of dehydrogenases, oxygenases, and transferases, respectively. These enzyme categories indicate an active microbiome that is metabolically capable of degrading complex organic compounds (Sharma, 2024; Yesankar et al., 2023). Other authors have already reported the presence of these enzymes in microbiomes of environments contaminated by BTEX. Catechol 2,3-dioxygenase is a key enzyme in the oxidative opening of the aromatic ring of toluene and xylenes, in metabolic pathways described for microorganisms belonging to the phylum Proteobacteria (Wu et al., 2023). This phylum was identified with an abundance of 36.11% for domestic WWTP and 78.31% for industrial WWTP.

The KO presented in Table 3 highlights the broad functional diversity of WWTP. For example, oxygenases and dehydrogenases participate in the initial steps of oxidation of aromatic rings and the breakdown of chemical structures of recalcitrant compounds (Janssen et

al., 2005). The domestic WWTP microbiome had a greater potential for xenobiotics degradation , with a greater absolute number of KO relating to enzymes involved in biodegradation pathways.

The lower complexity and low organic load of the effluent feeding domestic WWTPs favor a more diverse and metabolically versatile microbial community, capable of adapting to different substrates. In contrast, the microbial community of industrial WWTPs is exposed to more complex and recalcitrant contaminants, which limits its adaptability to diverse substrates. This is reflected in the presence KO associated with enzymes relevant to the degradation of compounds characteristically resulting from industrial activities. Other studies, such as those described by Shchegolkova et al., (2016) and Zhang et al., (2017), which analyzed the microbiota of activated sludge from WWTPs treating domestic and industrial effluents, observed results similar to those observed in the microbial community data and enzymatic inference of this study. This demonstrates that each WWTP has a microbiome shaped by the unique and distinct conditions of each system.

Industrial WWTP were inferred KO related to enzymes capable of degrading compounds of greater chemical complexity such as Acetaldehyde dehydrogenase (EC: 1.2.1.10), 3-hydroxybutyryl-CoA dehydrogenase (EC: 1.1.1.157) and enoyl-CoA hydratase (EC: 4.2.1.17) stand out for participating in the central steps of the oxidation of aromatic compounds and toxic intermediates, converting them into simpler and more water-soluble molecules (Schmid et al., 2015). Figure 6 shows the grouping of the total occurrence of the KO listed in Table 3 by functional category, comparing the WWTP. It is clear that KO related to oxidoreductases enzymes were predominant in both wastewater treatment plants, reflecting their relevance in the degradation processes of xenobiotics. Table 4 shows the distribution of KO relating to enzymes by metabolic function, with possible contribution of the different types of enzymes in the biodegradation processes, both in domestic and industrial WWTP. The predominance of dehydrogenases and oxygenases emphasizes the role of these enzymes in the oxidation and breakdown of complex organic compounds (Haernvall et al., 2018; Silva-Bedoya et al., 2016; Wasmund et al., 2024).

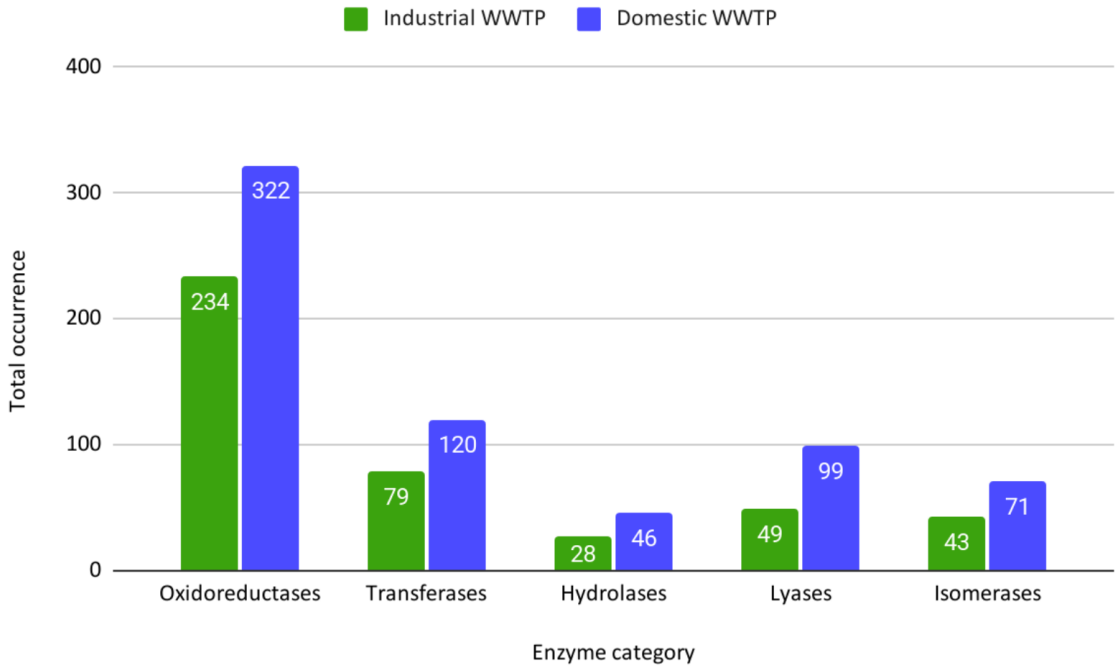


Figure 6. Graph of total occurrence (absolute numbers) of KO of the respective enzyme groups by functional category, comparing domestic and industrial WWTP

Table 4. Distribution of enzyme groups according to their metabolic functions, based on corresponding KEGG Orthology (KO) annotations

Metabolic function	**Occurrence count of each Enzymatic Group based on annotated KOs**	
	Industrial WWTP	**Domestic WWTP**
Dehydrogenases	179	239
Oxygenases/Dioxygenases	21	38
Acyl group transferases	96	133
Lyases	49	99
Ester hydrolases	27	46
Isomerases	43	71
Sulfur group transferases	6	9

Caption: KO - KEGG Orthology: WWTP – Wastewater treatment plant

In general, the KO found in the KEGG database for this research, in addition to being involved in the metabolic pathways of BTEX degradation, are also KO related to enzymes that act in microbial metabolism in different environments and in the degradation of various types of aromatic compounds, such as protocatechuate 3,4-dioxygenase (EC: 1.13.11.3), in the biosynthesis of secondary metabolites, such as 4-hydroxybenzoyl-CoA thioesterase (EC:

3.1.2.23), in the degradation of polycyclic aromatic hydrocarbons, such as protocatechuate 4,5-dioxygenase (EC: 1.13.11.8), among others (Figure 7) (Fuchs et al., 2011; Harwood and Parales, 1996; Kanehisa and Goto, 2000).

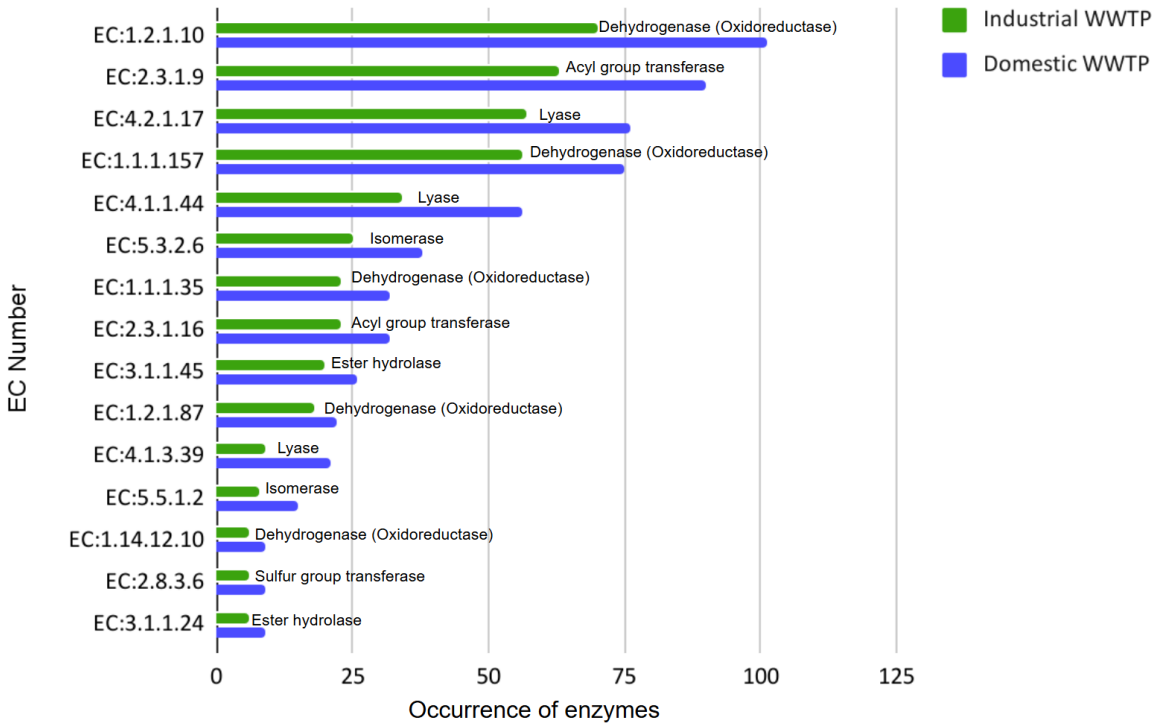


Figure 7. Comparison of the frequency of occurrence of enzymes (in absolute numbers) based on inferred KEGG Orthologies (KOs) in microbiomes of domestic and industrial WWTP

In Figure 8, a model of the degradation pathway of benzoate, and consequently of toluene and xylenes, can be observed. Based on KO inferences from the metabolic pathways of the KEGG database, it was possible to trace some of the possible routes used by microorganisms present in the microbiome of industrial and domestic WWTP for the BTEX biodegradation. When observing this metabolic pathway, it was evident that the presence of oxidoreductases, transferases, lyases, hydrolases and isomerases in the microbiota of WWTP indicate diverse and complementary metabolic routes, capable of converting complex pollutants into central intermediates such as catechol, succinyl-CoA, acetyl-CoA and pyruvate, key molecules that feed universal metabolic pathways such as the citrate cycle and glycolysis.

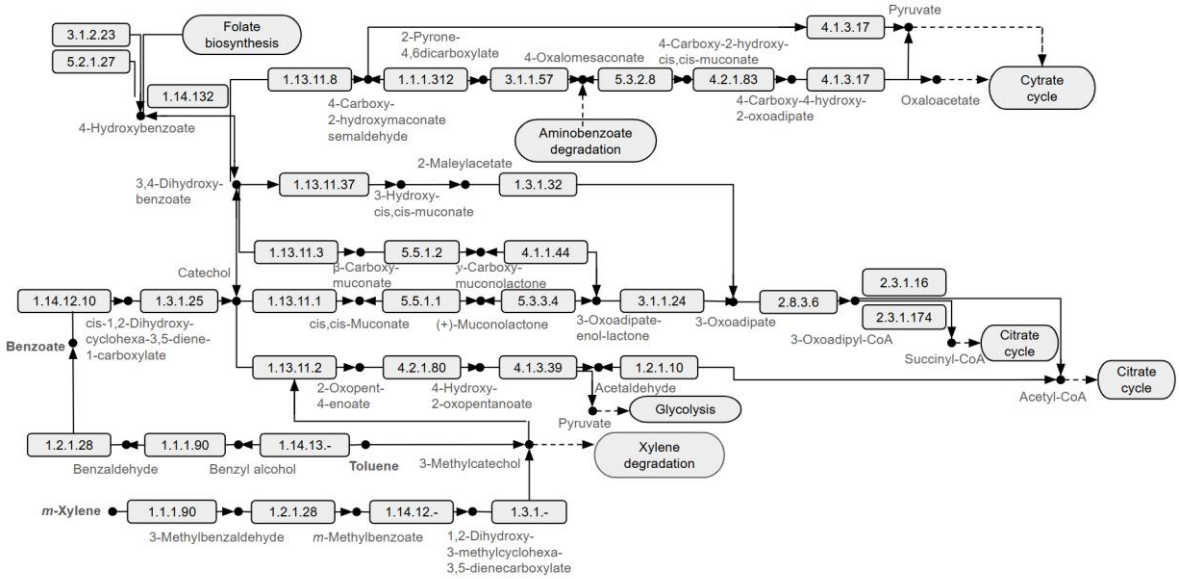


Figure 8. Inference of the metabolic pathways that may occur in WWTP microbiomes for the degradation of BTEX compounds, based on the inferred KEGG Orthologies (KOs) and their respective related enzymes, see Table 3.

The prediction of these enzymes indicated that several of them participate in metabolic pathways associated with the degradation of benzoate, toluene, ethylbenzene and xylenes. These results indicate that the microorganisms present in the studied WWTP have significant potential as biodegrading agents of BTEX compounds and other xenobiotics. Finally, it was concluded that despite the structural and functional differences inferred, both WWTP have the capacity to integrate efficient bioremediation processes. This demonstrates not only the metabolic adaptation of the microbial communities, but also their biotechnological potential for use in strategies aimed at treating waste and environmental pollutants.

4 CONCLUSION

The biodegradation of xenobiotics such as BTEX compounds through microbial activity emerges as a sustainable and effective strategy to mitigate the impacts of these toxic and carcinogenic hydrocarbons on the environment. Microorganisms previously adapted to BTEX have demonstrated their ability to use these compounds as a source of carbon and energy, resulting in the transformation into less harmful metabolites and even in the complete mineralization of the compounds. Chromatographic analyses confirmed the biodegradative activity of biomasses from domestic and industrial WWTP. In addition, the taxonomic profile

and functional inference revealed the presence of important bacterial genera such as *Clostridium*, *Klebsiella*, *Alcaligenes* and *Enterococcus*, all reported in the literature regarding their potential in the degradation of recalcitrant compounds. The tested biomasses exhibited tolerance to BTEX toxicity, probably due to adaptive mechanisms, such as responses to oxidative stress, resulting in metabolic activity even in the presence of contaminants. Functional predictions identified crucial enzymes that catalyze reactions that convert BTEX into intermediates such as catechol. Thus, the combination of microbial resilience and enzymatic versatility reinforces the applicability of microbial consortia such as those in WWTP in the remediation of BTEX contaminated environments and in the development of environmentally friendly technologies.

AUTHORS' CONTRIBUTIONS

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Marcos Antonio de Morais Junior: Writing – review and editing, Writing – original draft, Supervision, Resources, Fundraising.

DECLARATION OF CONFLICTS OF INTEREST

The authors declare that they have no competing financial or personal interests.

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5. CONCLUSÃO

As diferenças estruturais e operacionais marcantes entre as ETEs Mangueira e Multifabril, influenciaram de forma significativa a composição microbiana e o potencial de degradação de compostos BTEX em cada sistema. As duas ETEs abrigam comunidades microbianas robustas e metabolicamente versáteis, porém com perfis taxonômicos e funcionais distintos, refletindo as especificidades do tipo de efluente tratado e das condições ambientais impostas por cada unidade. A integração dos dados taxonômicos e funcionais revelou a presença de genes (KO) associados a enzimas essenciais para a degradação de benzoato, tolueno, xilenos e etilbenzeno, embora o conjunto e a abundância relativa dessas funções apresentem diferenças claras entre os sistemas.

Na ETE Multifabril destaca-se a elevada abundância relativa do gênero *Fervidobacterium*, um táxon termofílico reconhecido pela produção de termoenzimas de interesse industrial e associado à decomposição de compostos recalcitrantes, incluindo derivados de petróleo. Essa predominância sugere uma adaptação funcional particular da comunidade industrial a cargas xenobióticas elevadas. Embora ambas as biomassas tenham demonstrado capacidade efetiva de biodegradação de BTEX, os resultados indicam níveis distintos de tolerância e atividade metabólica, refletindo a influência direta das diferenças ambientais e operacionais entre as duas ETEs.

Em síntese, os resultados obtidos reforçam a relevância de investigar de forma aprofundada as comunidades microbianas de ETEs, considerando não apenas sua ecologia e funcionalidade, mas também suas particularidades metabólicas e adaptativas. O conhecimento gerado oferece bases sólidas para o desenvolvimento de estratégias inovadoras e tecnologias limpas, capazes de otimizar o tratamento de efluentes e promover soluções eficientes, economicamente viáveis e ambientalmente sustentáveis para a mitigação da poluição por BTEX e outros xenobióticos.

6. SÚMULA CURRICULAR

Certificado

Certificamos que **BRUNA KELLY DE OLIVEIRA SILVA** apresentou o trabalho 'IDENTIFICAÇÃO TAXONÔMICA E FUNCIONAL DA COMPOSIÇÃO BACTERIANA DA ESTAÇÃO DE TRATAMENTO DE ESGOTO MULTIFABRIL DA COMPESA E DETERMINAÇÃO DO SEU POTENCIAL PARA BIORREMEDIAÇÃO' durante o evento **''I Encontro do PPGCB: A ciência como uma ferramenta da AGENDA 2030 para o desenvolvimento sustentável''**, promovido pela Diretoria do(a) Centro de Biociências, no período de 20 a 21 de setembro de 2022, registrado no SIGPROJ - Sistema de Informação e Gestão de Projetos sob o n° 384523.2130.134786.28072022.

Recife, 28 de novembro de 2022.

Oussama Naouar
Pró-Reitor de Extensão e Cultura

PROExC
Pró-Reitoria de Extensão e Cultura

Universidade Federal de Pernambuco

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32° CONGRESSO DA ABES
COBESA - Congresso Brasileiro de Engenharia Sanitária e Ambiental
FITABES 2023 Feira Internacional de Tecnologias de Saneamento Ambiental

Certificamos que

Bruna Kelly de Oliveira Silva

participou como autor(a) do trabalho TRATAMENTO BIOLÓGICO DE EFLUENTES CONTAMINADOS POR COMPOSTOS MONOAROMÁTICOS DERIVADOS DE PETRÓLEO durante o 32º Congresso Brasileiro de Engenharia Sanitária e Ambiental, realizado em Belo Horizonte-MG, no período de 21 a 24 de maio de 2023, com carga horária de 26 horas.

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Nº do Protocolo: 23076.063818/2025-74

Recife-PE, 31 de julho de 2025.

Certificamos que a aluna **Laisa Gabriele Batista de Jesus** apresentou o Trabalho de Conclusão do Curso de Bacharelado em Ciências Biológicas com ênfase em Ciências Ambientais, intitulado "**BIODEGRADAÇÃO DE HIDROCARBONETOS MONOAROMÁTICOS: POTENCIAL BIOTECNOLÓGICO DA MICROBIOTA PRESENTE NO LODO DA ETE MANGUEIRA**", sob orientação do **Prof. Dr. Fabrício Motteran** e coorientação da **MSc. Bruna Kelly de Oliveira Silva**. A apresentação pública ocorreu em **31 de julho de 2025**, sendo o trabalho avaliado pela banca examinadora composta por **MSc. Bruna Kelly de Oliveira Silva**, **MSc. Luiz Pereira da Silva Junior** e **MSc. Nathália Bandeira Carvalho dos Santos**.

(Assinado digitalmente em 31/07/2025 14:36)
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Curso de Biomedicina
Disciplina Estágio Curricular (BC406)

Recife-PE, 19 de novembro de 2024

DECLARAÇÃO

Declaramos para os devidos fins que o estudante **JOSÉ DO CARMO BARBOSA NETO** apresentou o Trabalho de Conclusão de Curso - TCC intitulado: "AVALIAÇÃO DO POTENCIAL DE *Enterobacter ludwigii* e *Citrobacter freundii* NA DEGRADAÇÃO DE HIDROCARBONETOS MONOAROMÁTICOS", orientado pelo Dr. Marcos Antônio de Morais Junior e coorientado pela Msc Bruna Kelly de Oliveira Silva. O TCC foi apresentado no dia 08 de outubro de 2024, às 08:00 horas, com a participação dos seguintes membros da banca:

1º membro: Dr. Fabricio Motteran

Local :Departamento de Engenharia Civil e Ambiental da UFPE

2º membro: Msc Luiz Pereira da Silva Junior

Local: Laboratório de Saneamento Ambiental – LSA

Suplente: Msc Tiago Luiz Santana Calazans

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APÊNDICE

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ORIGINAL PAPER

Microbial diversity, dynamics, and metabolic analysis of microorganisms present in wastewater treatment plants in urban areas

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Abstract
Wastewater treatment plants (WWTPs) are essential for improving water quality and protecting the environment by reducing organic pollutants and harmful microorganisms. However, challenges in densely populated areas can affect their efficiency. The performance and stability of microbial communities are key to the effectiveness of WWTPs, making it crucial to understand them for optimizing treatment processes and improving sanitation efforts. This study aimed to analyze the development, diversity, and dynamics of microbial communities in WWTPs in the Recife metropolitan region, Pernambuco, Brazil, and their response to different treatment processes and toxic compounds. Samples were collected from various WWTPs in Recife. The genetic material was extracted from the WWTP and sent to the facility for metabarcoding sequencing of the 16S rRNA gene using the Illumina MiSeq platform. Bioconductor, DADA2, PhyloSeq, and PICRUSt2 were used for processing the sequenced samples. Microbial diversity was examined by identifying different genera, with their development analyzed under aerobic and anaerobic conditions, alongside the presence of industrial and domestic effluents. Metabolic analysis was conducted to explore the link between microbial pathways and xenobiotic compound degradation. The analysis revealed diverse microbial genera influenced by treatment processes (aerobic or anaerobic) and toxic compounds. Key microorganisms included *Brevundimonas*, *Clostridium*, *Pseudomonas*, and *Methanolinea*. Industrial effluents increased microbial diversity compared to domestic wastewater. The discovery of microorganisms with potential antibiotic resistance in aerobic systems raised public health concerns. The study highlighted the diversity and dynamics of microbial communities in WWTPs and their role in treatment efficiency. The presence of antibiotic-resistant microorganisms in aerobic systems calls for further investigation, while the link between microbial metabolic pathways and xenobiotic degradation offers opportunities for improving treatment processes.

Keywords Aerobic and anaerobic processes · Antibiotic resistance · Domestic and industrial effluents · Environmental sanitation · Operational stability · Prediction of functional genes