Universidade Federal do Rio Grande do Sul
Instituto de Pesquisas Hidráulicas
Doutorado em Recursos Hídricos e Saneamento Ambiental

Marcos Henrique Gomes Ribeiro

APLICAÇÃO DE TRATAMENTO BIOLÓGICO ANAERÓBIO E FILTRAÇÃO POR MEMBRANA PARA REMOÇÃO DE CONTAMINANTES EMERGENTES DE ORIGEM FARMACÊUTICA E AGRÍCOLA DE ÁGUAS RESIDUÁRIAS

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Tese apresentada como requisito à obtenção do título de doutor em Recursos Hídricos e Saneamento Ambiental pelo Programa de Pós-Graduação em Recursos Hídricos e Saneamento Ambiental do Instituto de Pesquisas Hidráulicas da Universidade Federal do Rio Grande do Sul.

Orientador: Antônio Domingues Benetti
Coorientadora: Maria Cristina de Almeida Silva

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> “ Todos nascemos filhos de mil pais e de mais de mil mães, e a solidão é sobretudo a incapacidade de ver qualquer pessoa como nos pertencendo, para que nos pertença de verdade e se gere um cuidado mútuo. Como se os nossos mil pais e mais as nossas mil mães coincidissem em parte, como se fôssemos por aí irmãos, irmãos uns dos outros. Somos o resultado de tanta gente, de tanta história, tão grandes sonhos que vão passando de pessoa a pessoa , que nunca estaremos sós”
>
> Valter Hugo Mãe em “O Filho de Mil Homens”
>
> Edição: Biblioteca Azul, 2016

“Não entendo. Isso é tão vasto que ultrapassa qualquer entender. Entender é sempre limitado. Mas não entender pode não ter fronteiras. Sinto que sou muito mais completa quando não entendo. Não entender, do modo como falo, é um dom. Não entender, mas não como um simples de espírito. O bom é ser inteligente e não entender. É uma bênção estranha, como ter loucura sem ser doida. É um desinteresse manso, é uma doçura de burrice. Só que de vez em quando vem a inquietação: quero entender um pouco. Não demais: mas pelo menos entender que não entendo”

“Não entender”, crônica publicada em 01.02.69. Ibidem, p.172

Clarice Lispector, no livro “Cadernos de Literatura Brasileira”. (Edição Especial nº s.17 e 18. São Paulo: Instituto Moreira Salles, 2004)

RESUMO

RIBEIRO, Marcos Henrique Gomes. Aplicação de tratamento biológico anaeróbio e filtração por membrana para remoção de contaminantes emergentes de origem farmacêutica e agrícola de águas residuárias. 2026. Tese (Doutorado em Recursos Hídricos e Saneamento Ambiental) – Instituto de Pesquisas Hidráulicas, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2026.

Compostos de preocupação emergente (CPEs) ou contaminantes emergentes (CEs) são substâncias químicas de natureza complexa. Representam riscos severos à saúde humana e ao meio ambiente, com potencial de acúmulo na cadeia alimentar e toxicidade crônica, podendo causar mortalidade em organismos aquáticos, doenças e mutações de DNA. Entre os CPEs mais detectados em águas residuárias estão os fármacos e agrotóxicos. O uso de tratamento biológico anaeróbio e filtração por membrana se apresentam com alternativa para remover CPEs de águas residuárias. O objetivo geral desta tese de doutorado foi avaliar a eficiência do uso desses processos, com foco no biorreator anaeróbio de membrana dinâmica (AnDMBR), na remoção dos fármacos norfloxacino (NOR) e sulfametoxazol (SMX) e dos agrotóxicos atrazina (ATZ) e 2,4-D. A tese foi desenvolvida em forma de artigos. Os resultados do "Artigo 1", apontaram o interesse científico no tema, embora o número de publicações na última década tenha sido baixo (2013-2023). Os estudos concentraram-se na remoção de fármacos, com lacunas em relação à avaliação de pesticidas e aplicação de múltiplos CPEs simultaneamente. As configurações de reatores mais comuns foram em escala piloto, de bancada e com módulo de membrana externo, operados em temperaturas mesofílicas. Os resultados do " Artigo 2" apontaram que a classe e as concentrações dos fármacos influenciaram a produção de biogás e metano. A combinação do NOR e do SMX aumentou a produção acumulada de biogás (≈500 mL), mas resultou em um menor teor de metano (≈50%). A remoção do SMX não foi afetada pela concentração, enquanto a taxa de remoção de NOR diminuiu com o aumento de sua concentração no meio. A remoção de DQO seguiu padrões anaeróbios (>60%). Os achados sublinham os desafios na otimização do biogás e na remoção eficaz de fármacos em sistemas anaeróbios com múltiplos contaminantes. No "Artigo 3" foi observado que os agrotóxicos estimularam a produção de metano em todas as configurações testadas. O processo manteve uma estabilidade operacional satisfatória, atingindo boas eficiências de remoção de DQO (>80%). A presença simultânea de ATZ e 2,4-D ativou um mecanismo de co-metabolismo aumentando drasticamente a remoção do 2,4-D de 43,6% para mais de 90%. Sugerindo que a combinação de compostos é mais crítica do que a concentração individual para a sua remoção da fase aquosa. O processo se mostrou uma alternativa de tratamento viável e sustentável. De acordo com os resultados do "Artigo 4" a adição contínua de CPEs ao sistema AnDMBR2 não afetou a produção de biogás. Em ambos os sistemas testados houve alta remoção de

DQO (>95%) e redução gradual de cor e turbidez. O sistema AnDMBR2 apresentou defloculação do lodo causando *fouling* na membrana dinâmica e aumento da espessura do biofilme formado ao final da operação (1.07 mm). A maior diversidade microbiana apresentada pelo AnDMBR2 sugere a prevalência de organismos adaptados a adição contínua de CPEs e sua contribuição para degradação dos compostos.

Palavras-chave: tratamento de águas residuárias; digestão anaeróbia; filtração por gravidade; membrana dinâmica; biofilme

ABSTRACT

RIBEIRO, Marcos Henrique Gomes. Application of anaerobic biological treatment and membrane filtration for the removal of emerging pharmaceutical and agricultural contaminants from wastewater. 2026. Doctoral thesis (Water Resources and Environmental Sanitation) – Instituto de Pesquisas Hidráulicas, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2026.

Compounds of emerging concern (CECs) or emerging contaminants (ECs) are complex chemicals. They pose serious risks to human health and the environment, with the potential for accumulation in the food chain and chronic toxicity, and can cause mortality in aquatic organisms, disease, and DNA mutations. Among the CECs most frequently detected in wastewater are pharmaceuticals and pesticides. The use of anaerobic biological treatment and membrane filtration are alternatives for removing CECs from wastewater. The overall objective of this doctoral thesis was to evaluate the efficiency of these processes, focusing on the anaerobic dynamic membrane bioreactor (AnDMBR), in removing the drugs norfloxacin (NOR) and sulfamethoxazole (SMX) and the pesticides atrazine (ATZ) and 2,4-D. The doctoral thesis was developed in the form of articles. The results of "Article 1" pointed to scientific interest in the topic, although the number of publications in the last decade has been low (2013-2023). Studies focused on drug removal, with gaps in the evaluation of pesticides and the simultaneous application of multiple CECs. The most common reactor configurations were pilot scale, bench scale, and with an external membrane module, operated at mesophilic temperatures. The results of "Article 2" indicated that the class and concentrations of the drugs influenced biogas and methane production. The combination of NOR and SMX increased the cumulative biogas production (≈500 mL) but resulted in a lower methane content (≈50%). SMX removal was not affected by concentration, while the NOR removal rate decreased with increasing concentration in the medium. COD removal followed anaerobic patterns (>60%). The findings highlight the challenges in optimizing biogas and effectively removing drugs in anaerobic systems with multiple contaminants. In "Article 3," it was observed that pesticides stimulated methane production in all configurations tested. The process maintained satisfactory operational stability, achieving good COD removal efficiencies (>80%). The simultaneous presence of ATZ and 2,4-D activated a co-metabolism mechanism, dramatically increasing the removal of 2,4-D from 43.6% to over 90%. This suggests that the combination of compounds is more critical than individual concentration for their removal from the aqueous phase. The process proved to be a viable and sustainable treatment alternative. According to the results of "Article 4," the continuous addition of ECs to the AnDMBR2 system did not affect biogas production. In both systems tested, there was high COD removal (>95%) and a gradual reduction in color and turbidity. The AnDMBR2 system presented sludge deflocculation,

causing fouling in the dynamic membrane and an increase in the thickness of the biofilm formed at the end of the operation (1.07 mm). The greater microbial diversity presented by AnDMBR2 suggests the prevalence of organisms adapted to the continuous addition of ECs and their contribution to the degradation of compounds.

Keywords: wastewater treatment; anaerobic digestion; gravity-driven filtration; dynamic membrane; biofilm

LISTA DE FIGURAS

Figura 1 – Etapas do tratamento convencional de águas residuárias ……...……17
Figura 2 – Tipologias usadas no tratamento de águas residuárias....……...…….19
Figura 3 – Etapas do processo de digestão anaeróbia.....…….20
Figura 4 – Organização da tese por artigos.....……..…43

LISTA DE QUADROS

Quadro 1 – Tipos de membranas de filtração convencionais e exemplos de aplicações ……..23
Quadro 2 – Parâmetros operacionais de AnMBRs usados em estudos para diferentes fins……..25
Quadro 3 – Objetivos de cada um dos artigos desenvolvidos durante a tese.....43

SUMÁRIO

1. INTRODUÇÃO……..…….……14
1.1. MOTIVAÇÃO DA PESQUISA…...……..……..14
1.2. OBJETIVOS…...……..……...16
1.2.1. Objetivo Geral.....…….……16
1.2.2. Objetivos Específicos….....……...……...16
1.3. ORGANIZAÇÃO DO TRABALHO…...……..……..16
2. REFERENCIAL TEÓRICO……..……...…….17
2.1. TRATAMENTO DE ESGOTO……...…….……..17
2.2. TRATAMENTO ANAERÓBIO.....…….……...…….19
2.3. BIORREATOR ANAERÓBIO DE MEMBRANA……...……...……22
2.4. BIORREATOR ANAERÓBIO DE MEMBRANA DINÂMICA.....27
2.5. CONTAMINANTES DE PREOCUPAÇÃO EMERGENTE……...…….……..27
2.6. AGROTÓXICOS…...……..……29
2.6.1. 2,4-D.....…….……...30
2.6.2. Atrazina...……...…….……...……32
2.7. FÁRMACOS…….……..…33
2.7.1. Antibióticos..…….....…….……....34
2.7.2. Quinolonas.....35
2.7.3. Sulfonamidas…….....…….……....36
REFERÊNCIAS.....37
3. METODOLOGIA…….....……...43
4. RESULTADOS……...……....44
4.1. ANAEROBIC MEMBRANE BIOREACTOR TO REMOVE PESTICIDES AND PHARMACEUTICALS FROM WASTEWATER: A BIBLIOMETRIC REVIEW……...…...……44
4.1.1. Abstract…….....…….……..44
4.1.2. Introduction…….……...……...……..44
4.1.3. Material and Methods……...…….……46
4.1.4. Results and Discussion……...…….…..47
4.1.5. Conclusion……...……...…….…..66
4.1.6. References……...……...…….……..66
4.2. EVALUATION OF ANAEROBIC DIGESTION OF ANTIBIOTICS IN BATCH REACTORS: EFFECT ON METHANE AND BIOGAS PRODUCTION, OPERATING PARAMETERS, AND AQUEOUS PHASE REMOVAL EFFICIENCY…….....….71
4.2.1. Abstract……71
4.2.2. Introduction……...72
4.2.3. Material and Methods……....73
4.2.4. Results and Discussion……..77
4.2.5. Conclusion……84
4.2.6. References……85
4.3. REMOVAL OF ATRAZINE AND 2,4-D PESTICIDES FROM WASTEWATER BY ANAEROBIC BIOLOGICAL PROCESS USING BATCH REACT……..…90
4.3.1. Abstract…….....90
4.3.2. Introduction……...91
4.3.3. Material and Methods……92
4.3.4. Results and Discussion……..95
4.3.5. Conclusions……101
4.3.6. References……..……102
4.4. ANAEROBIC DYNAMIC MEMBRANE BIOREACTOR WITH EXTERNAL GRAVITY-DRIVEN FILTRATION MODULE REMOVING EMERGING

CONTAMINANTS……..106
4.4.1. Abstract……...…106
4.4.2. Introduction…….……107
4.4.3. Materials and Methods……109
4.4.4. Results and Discussion……112
4.4.5. Conclusion……...124
4.4.6. References……...125
5. CONSIDERAÇÕES FINAIS.....…….....…..…131
6. RECOMENDAÇÕES PARA TRABALHOS FUTUROS…..….....133
APÊNDICE A.....135
APÊNDICE B.....136
APÊNDICE C.....137

1. INTRODUÇÃO

1.1 MOTIVAÇÃO DA PESQUISA

Sinais da contaminação humana são encontrados desde a estratosfera até as profundezas dos oceanos, do polo Norte ao polo Sul, em muitas formas da vida selvagem, em todas as sociedades modernas, acumuladas na cadeia alimentar e na maioria dos indivíduos, incluindo recém-nascidos (Ncibi *et al.*, 2017). Novos poluentes são frequentemente encontrados na água devido ao uso ou à síntese de novas substâncias de forma legal ou ilegal. Esses poluentes são, em parte, chamados de poluentes ou contaminantes de preocupação emergente (CPEs), considerando sua diversidade e composição química geralmente complexa. A maioria desses poluentes é de origem orgânica, mas alguns autores também incluem nanopartículas inorgânicas nesse agrupamento (Vélez *et al.*, 2019).

Os CPEs são comumente provenientes de efluentes hospitalares e industriais, esgotos domésticos e atividades agrícolas. Incluem disruptores endócrinos, microplásticos, agrotóxicos, retardantes de chama, nanomateriais, produtos farmacêuticos e de cuidado pessoal (Dong *et al.*, 2023). Estão presentes no esgoto doméstico, tanto bruto quanto tratado, e a sua presença em águas causa danos à saúde humana e ao meio ambiente (Vélez *et al.*, 2019; Dong *et al.*, 2023). A maioria dos CPEs é conhecida pelos sérios riscos à saúde dos seres humanos e demais organismos (Ncibi *et al.*, 2017). Grande parte deles são disruptores endócrinos e causam toxicidade aguda e mortalidade de peixes, invertebrados aquáticos e outros animais (Singha & Shukla, 2022). Em termos de América Latina, Brasil e México são os países que apresentam maior cobertura ambiental no monitoramento de CPEs (De Oliveira *et al.*, 2020). Diferentes métodos químicos, físicos e biológicos de tratamento têm sido usados para remediar esses poluentes na água (Dong *et al.*, 2023).

Biorreatores anaeróbios de membrana (AnMBRs) são uma tecnologia promissora de tratamento de esgoto que integram degradação biológica e filtração por membrana. Dentre as principais vantagens dos AnMBRs pode-se citar: o aumento da qualidade do efluente tratado, o tamanho compacto da planta de tratamento, a menor geração de lodo, e a alta flexibilidade operacional, necessária para aumentar a eficiência de remoção de CPEs (Ncibi *et al.*, 2017). Em sistemas em que há o uso de biorreatores anaeróbios de membrana, o processo de catálise é orgânico, onde enzimas ou microrganismos são

conectados a um processo de membrana como micro ou ultrafiltração (Krishnan *et al.*, 2022). A combinação dos AnMBRs com processos de adsorção/biodegradação apresenta potencialidades de tratamento de CPEs como fármacos, agrotóxicos e compostos policlorados, entre outros (Ncibi *et al.*, 2017).

Apesar dos benefícios, o processo de fouling da membrana é um dos gargalos que limita a aplicação generalizada do AnMBR. O fouling leva ao aumento da pressão transmembranar e à redução do fluxo, afetando os custos operacionais e a eficiência energética (Vinardell *et al.*, 2020; Wang *et al.*, 2022; Nabi *et al.*, 2022). Tendo em vista que as membranas constituem uma parte significativa dos custos e despesas do AnMBR, uma alternativa que visa baratear o processo é o uso de membrana dinâmica (MD). A MD é uma camada de biofilme formada sobre um material de suporte, que atua como uma barreira para a remoção de poluentes e filtragem de partículas (Ersahin *et al.*, 2012; Zhang *et al.*, 2014). Uma camada DM pode ser auto depositada em um material de suporte barato (em vez de membranas de microfiltração/ultrafiltração, de maior custo) (Samaei *et al.*, 2023). Dessa forma, o biorreator anaeróbio de membrana dinâmica (AnDMBR) apresenta vantagens em relação aos AnMBRs, incluindo menor custo da membrana, menor demanda de energia, controle mais fácil do fouling e alta retenção de sólidos (Ersahin *et al.*, 2012; Hu *et al.*, 2016; Saleem *et al.*, 2018; Samaei *et al.*, 2023).

Entre os CPEs, os agrotóxicos são largamente usados na agricultura com o intuito de aumentar a produção agrícola. No entanto, o carreamento de agrotóxicos para corpos hídricos impacta negativamente o ambiente aquático. Alguns foram detectados em amostras de água de países europeus mais de vinte e cinco anos depois da proibição do seu uso (McGinley *et al.*, 2023). Além dos agrotóxicos, a presença de fármacos e outros compostos residuais, como hormônios, em águas de abastecimento geram preocupação do ponto de vista da saúde pública (De Oliveira *et al.*, 2020). Produtos farmacêuticos produzem efeitos fisiológicos adversos, mesmo em baixas concentrações, em humanos e animais (Bustos Bustos *et al.*, 2022). Como consequência do aumento do consumo e da baixa taxa de absorção do corpo, esses compostos são detectados em vários ambientes (Matsubara *et al.*, 2020). Efeitos danosos causados por fármacos incluem toxicidade aquática, desenvolvimento de resistência e multirresistência bacteriana, genotoxicidade e desordem endócrina (De Oliveira *et al.*, 2020). Estudos do uso de AnMBR para tratamento de metais, surfactantes, poluentes orgânicos emergentes (fármacos, disruptores endócrinos, produtos de higiene pessoal, microplásticos) e microrganismos patogênicos ainda permanecem escassos na literatura (Hu *et al.*, 2022).

1.2 OBJETIVOS

1.2.1 OBJETIVO GERAL

Avaliar o uso de tratamento biológico anaeróbio e filtração por membrana, com foco em biorreator anaeróbio de membrana dinâmica (AnDMBR), para remoção dos contaminantes de preocupação emergente atrazina (ATZ), ácido diclorofenoxiacético (2,4-D), norfloxacino (NOR) e sulfametoxazol (SMX).

1.2.2 OBJETIVOS ESPECÍFICOS

- Avaliar o impacto da presença de contaminantes de preocupação emergente ATZ, 2,4-D, NOR e SMX no processo de digestão anaeróbia;
- Avaliar a eficiência do uso de biorreator anaeróbio de membrana dinâmica (AnDMBR) para remoção dos contaminantes de preocupação emergente ATZ, 2,4-D, NOR e SMX;
- Avaliar o impacto da presença dos contaminantes de preocupação emergente durante a operação do AnDMBR;
- Caracterizar o biofilme formado pela membrana dinâmica ao longo do processo operacional;
- Caracterizar a comunidade microbiana desenvolvida na biomassa anaeróbia ao final da operação do AnDMBR;

1.3 ORGANIZAÇÃO DO TRABALHO

A tese está organizada em capítulos. O primeiro capítulo apresenta a motivação para a pesquisa e os seus objetivos, além de introduzir a estrutura do documento. O segundo capítulo é destinado a apresentação do referencial teórico usado como base para o desenvolvimento da tese. No terceiro capítulo é apresentada a estrutura metodológica usada para a construção da tese. O quarto é referente aos resultados de tese e é formado por subcapítulos apresentados em inglês, na forma de artigos. No quinto capítulo são apresentadas as conclusões gerais da tese, seguindo-se as referências bibliográficas usadas.

2. REFERENCIAL TEÓRICO

Este capítulo apresenta a base teórica usada como fundamentação para a tese de doutorado. Ele é dividido em tópicos e subtópicos de acordo com a grau de relevância para o tema estudado.

2.1 TRATAMENTO DE ÁGUAS RESIDUÁRIAS

O tratamento é um processo que visa a purificação de águas residuárias pela eliminação de matéria orgânica, patógenos e outras substâncias que causem risco potencial à saúde humana e ao meio ambiente. Ele é realizado em etapas, cada uma com finalidades específicas. A depender do tipo de água residuária a ser tratado e do grau de tratamento desejado, nem todas as etapas são necessárias. A Figura 1 apresenta um panorama geral das etapas de tratamento convencional de águas residuárias.

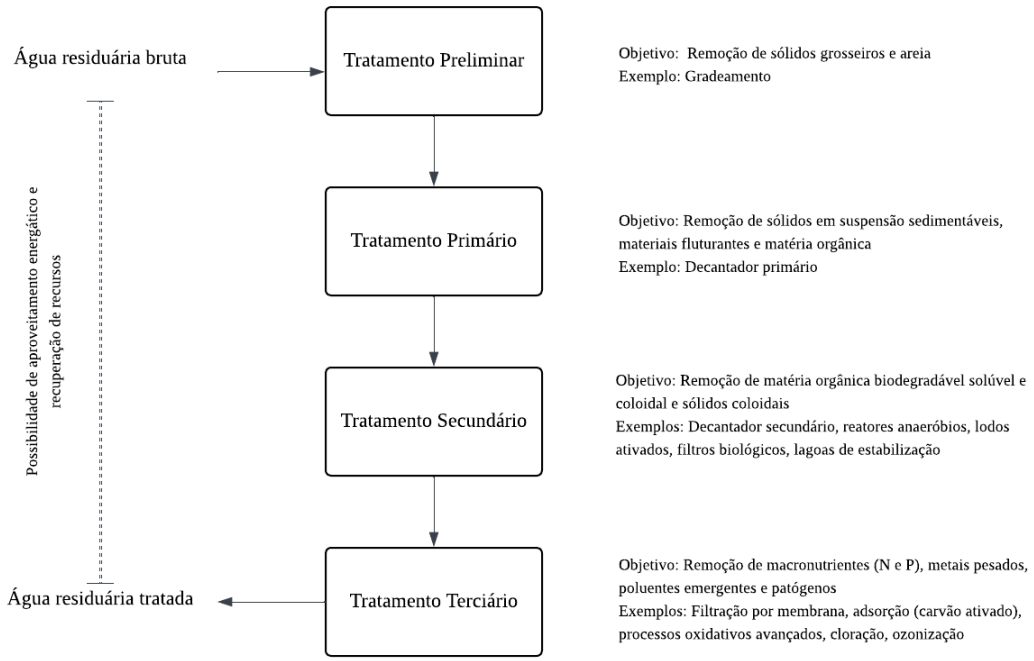


Figura 1: Etapas do tratamento convencional de águas residuárias
Adaptado de Krishnan *et al.*, 2022

Na etapa de tratamento secundário podem ser usadas tecnologias aeróbias ou anaeróbias. Nas aeróbias, os sistemas operam com o uso de oxigênio (O_2), e organismos

aeróbios, através de suas atividades metabólicas, degradam o material orgânico e oxidam a amônia. O oxigênio é injetado dentro dos sistemas, representando custo energético para a operação. Nos tratamentos anaeróbios, o O_2 não é necessário para os microrganismos, que utilizam outros elementos como aceptores de elétrons para os seus processos metabólicos. O custo energético é reduzido, podendo haver, ao contrário do consumo, a geração de energia. O tratamento de esgoto também pode ser realizado em ambiente anóxico. Nesse caso, durante o processo de desnitrificação, as bactérias heterotróficas usam o nitrato (NO_3^-) ou o nitrito (NO_2) como aceptores finais de elétrons durante o consumo da matéria orgânica (Santos *et al.*, 2020).

Em reatores, o tipo de fluxo e o padrão de mistura determinam o modelo hidráulico. Um reator pode ter fluxo contínuo, semicontínuo ou operar em batelada. Em reatores em batelada, a alimentação ocorre apenas no início do processo, não há escoamento entrando ou saindo. Para o fluxo semicontínuo, a alimentação e a saída ocorrem de forma intermitente (Von Sperling, 2016; Paulista, 2017; Baumguertner, 2018; Borzani, 2021).

A biomassa pode ser do tipo suspensa, aderida ou granular. Na biomassa suspensa, as células ou flocos microbianos estão em suspensão no reator. No caso da biomassa aderida, ou fixa, os agregados microbianos estão aderidos a um material suporte e imersos em uma matriz com alto grau de hidratação, constituída de polímeros extracelulares que eles mesmos excretam. O material suporte pode ser de leito fixo ou móvel, segundo as características do reator. A biomassa granular é constituída por partículas consideradas mais rígidas que os flocos, formadas por microrganismos e polímeros extracelulares sem suporte sólido. Os grânulos podem ser considerados um estágio mais avançado de agregação caracterizado por maior consistência que os flocos (Melo, 1996). A Figura 2 apresenta as tipologias comuns usadas no tratamento de águas residuárias.

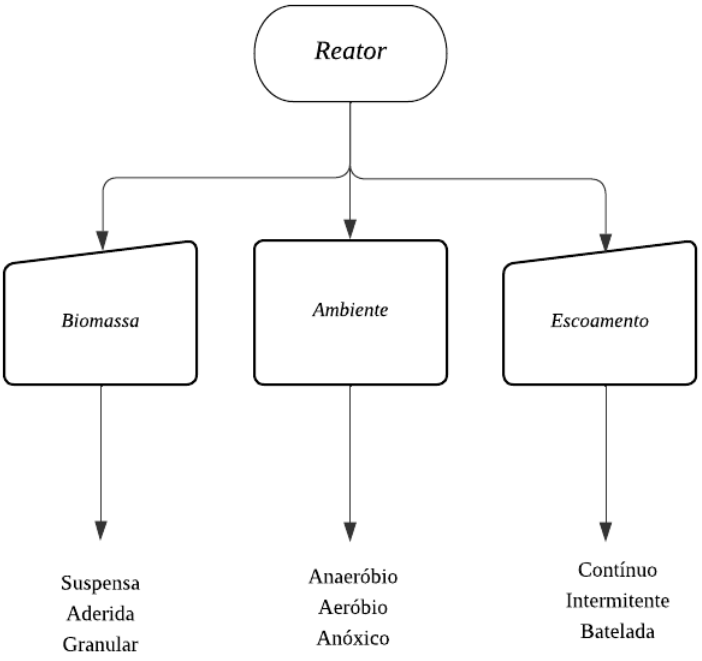


Figura 2: Tipologias usadas no tratamento de águas residuárias

Entre os parâmetros de operação de reatores pode-se citar a carga orgânica volumétrica (COV), o tempo de detenção hidráulica (TDH), o tempo de detenção de sólidos (TDS) e a temperatura. A COV se refere a massa de matéria orgânica que se aplica ao reator diariamente por unidade de volume. A quantidade de matéria orgânica pode ser expressa em termos de demanda química de oxigênio (DQO) ou demanda bioquímica de oxigênio (DBO). A COV, junto com a temperatura, influencia no consórcio de bactérias atuantes no processo de digestão anaeróbia (DA). Os TDH e TDS representam, respectivamente, os tempos médios em que o líquido e a biomassa (sólidos) permanecem no reator.

2.2 TRATAMENTO ANAERÓBIO DE ÁGUAS RESIDUÁRIAS

O tratamento biológico de águas residuárias por via anaeróbia acontece quando não há o uso do O_2 e NO_3^- pelos microrganismos durante a estabilização da matéria orgânica. No tratamento anaeróbio, microrganismos, durante a realização do processo metabólico de respiração celular, utilizam moléculas diferentes do O_2 (nitrato, sulfato, dióxido de carbono, fumarato, entre outros) como aceptores finais de elétrons. Devido à

falta de aceptores de elétrons externos no processo, os elétrons presentes nos esgotos acabam como produtos de valor agregado (H_2, CH_4, PHA…) (Lee & Liao, 2021). O tratamento do material biodegradável ocorre pela digestão anaeróbia (DA). A DA é um processo bioquímico complexo que envolve a conversão de matéria orgânica em metano (CH_4) e gás carbônico (CO_2) por uma grande variedade de microrganismos, especialmente bactérias sintróficas (Cheng *et al.*, 2019; Van Haandel & Lettinga, 1994). É considerada uma alternativa sustentável para o tratamento de esgotos, apresentando como benefícios, a geração de bioenergia, baixa geração de lodo, pouco consumo energético e emissões de gases de efeito estufa, como o CH_4 (Hu *et al.*, 2022). O processo ocorre, geralmente, em 4 etapas: (I) Hidrólise; (II) Acidogênese; (III) Acetogênese e (IV) Metanogênese. A Figura 3 apresenta as etapas da DA.

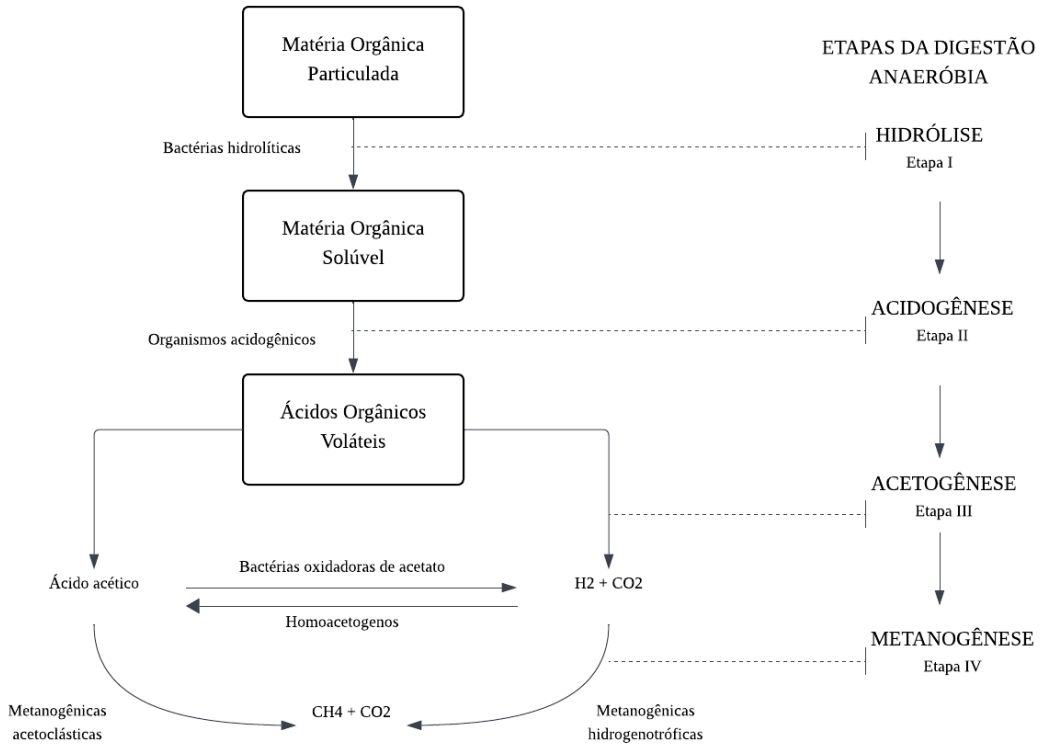


Figura 3: Etapas do processo de digestão anaeróbia
Adaptado de Hu *et al.*, 2022

A hidrólise é a primeira etapa da DA e consiste na quebra de moléculas orgânicas de tamanho maior (lipídios, proteínas e carboidratos) em moléculas menores. As bactérias responsáveis pela hidrólise podem ser anaeróbias estritas ou facultativas e seu desenvolvimento no meio é espontâneo quando em condições favoráveis (Tsagarakis &

Papadogiannis, 2006). Na acidogênese, as moléculas menores são absorvidas pela parede celular das bactérias acidogênicas ou fermentativas e decompostas internamente pelos processos metabólicos. Isto se reflete na produção de ácidos graxos voláteis e em hidrogênio como subproduto. No entanto, pressão parcial de hidrogênio menor que 10^{-4} atm durante a acidogênse resulta na formação de ácido acético como produto em desfavorecimento da geração de ácidos de cadeia longa (Harper & Pohland, 1986). Na terceira etapa da DA, a acetogênese, ocorre a formação de ácido acético a partir dos ácidos graxos voláteis formados na etapa de acidogênese. Dióxido de carbono e hidrogênio (H_2) também são gerados pelas bactérias acetogênicas por fermentação (Van Haandel & Lettinga, 1994). As bactérias homoacetoclásticas produzem o acetato com o uso do CO_2, H_2, e açucares. Esse processo é chamado de homoacetogênese e segue a seguinte reação estequiométrica Equação (1).

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad (1)$$

Na metanogênese, última etapa da digestão anaeróbia, ocorre a produção de gás metano pelas arqueas metanogênicas. Duas vias principais são responsáveis pela produção do CH_4, as reduções do ácido acético por bactérias acetotróficas e a de CO_2 pela via hidrogenotrófica. As reações para cada via, respectivamente, são apresentadas na Equação 2 (metanogênese acetotrófica) e na Equação 3 (metanogênese hidrogenotrófica).

$$C_2H_4O_2 \rightarrow CH_4 + CO_2 \qquad (2)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad (3)$$

No tratamento anaeróbio de esgoto doméstico, 8% do potencial energético é perdido durante o processo de conversão de compostos orgânicos complexos, como carboidratos, em compostos mais simples como o CH_4. Outros 7% são perdidos na conversão da porção orgânica em células necessárias pelos microrganismos para a manutenção das reações (McCarty *et al.*, 2011). A oxidação anaeróbia do metano ocorre pela transformação do CH_4 em CO_2 e água (H_2O) utilizando elementos redutores como sulfato (SO_4^{2-}), nitrito/nitrato ou componentes metálicos como aceptores de elétrons (Hu *et al.*, 2022).

O aproveitamento do potencial energético dos compostos orgânicos presentes nos esgotos domésticos é um grande desafio (McCarty *et al.*, 2011). Em reatores de mistura completa, cerca de 80% da fração biodegradável (DQO) presente no lodo pode ser convertido e capturado a biogás (Kehrein *et al.*, 2020). No que diz respeito a temperatura, os ambientes anaeróbios pode ser psicrofílicos (0 a 20°C), mesofílicos (20 a 45°C) ou

termofílicos (45 a 60°C). O regime mesofílico é o que alcança maior eficiência no tratamento anaeróbio, sendo a temperatura de 35°C a mais indicada para operação de reatores (Hoyos, 2021; Safley & Westerman, 1992; McCarty, 1964).

Além do potencial do uso do tratamento anaeróbio de esgoto como produtor de energia, a baixa geração de lodo representa uma grande vantagem econômica e energética (McCarty *et al.*, 2011). Entre as desvantagens dos processos anaeróbios podem-se citar a qualidade inferior do efluente em comparação aos aeróbios, necessitando posterior polimento, e o alto tempo de retenção hidráulica (Lee & Liao, 2021). Baixas temperaturas e cargas orgânicas são também comumente apontadas como barreiras comuns ao tratamento anaeróbio de esgotos domésticos (McCarty *et al.*, 2011).

2.3 BIORREATOR ANAERÓBIO DE MEMBRANA (AnMBR)

Biorreatores anaeróbios de membrana (AnMBR) são sistemas que funcionam de forma eficiente do ponto de vista energético e apresentam potencial para tratar esgotos domésticos e de demais origens. Por ser um tratamento do tipo anaeróbio, o processo produz energia na forma de CH_4, ao contrário de processos aeróbios que consomem energia. Além disso, são capazes de produzir efluentes com alto nível de qualidade, com padrões referentes a tratamento de nível secundário. O tratamento com AnMBR alcança baixas concentrações de sólidos suspensos e DBO, além de redução significativa na presença de bactérias totais e patógenos (Kim *et al.*, 2011; Li & Parker, 2021; McCarty *et al.*, 2011; Harb & Hong, 2017). Estudos recentes apontaram essa tecnologia é capaz de atingir cargas altas entre 25 e 40 kg DQO/m^3d, tratando esgoto facilmente biodegradável. Pesquisas anteriores sugeriam valores para COV máxima de 10 kg DQO/m^3d (Lee & Liao, 2021).

O potencial do sistema AnMBR para suprir limitações se deve a junção das vantagens do uso dos processos de DA e filtração por membrana. Além disso, pode-se citar vantagens únicas que incluem a retenção completa da biomassa, a maior eficiência na remoção de poluentes, a produção de bioenergia e flexibilidade. O uso da filtração por membranas ainda ajuda a evitar a perda de microrganismos, que é essencial no processo devido à baixa taxa de crescimento dos anaeróbios (Hu *et al.*, 2022). Outras vantagens são o controle da idade do lodo e do TDH, além do bom nível de mistura da concentração de sólidos suspensos no líquido, o que favorece o uso de reatores de tamanho menor.

Por outro lado, como desvantagens, se tem a complexidade do processo, a

sensibilidade aos choques de cargas e dificuldade no deságue do lodo. Também são citadas questões associadas com custos operacionais, equipamentos, manutenção e limpeza (Kehrein *et al.*, 2020). O entupimento das membranas (fouling) é umas das grandes desvantagens do processo. Ele resulta na diminuição da permeabilidade e aumento da necessidade de manutenção, afetando a operação a longo prazo. Pode ser causado pela formação de camadas ou entupimento dos poros.

O processo de separação em sistema AnMBR é a consequência da combinação do processo físico de peneiramento, baseado na exclusão segundo o tamanho das partículas, e de interações químicas com a membrana. A microfiltração (MF) é usada para separação de moléculas maiores como óleos, bactérias e coloides e pode ser realizada a baixa pressão. Vírus e macromoléculas podem ser separados por ultrafiltração (UF). Para peptídeos, compostos com baixa peso molecular e íons se aplica a nano filtração (NF) com pressão intermediária. A osmose reverse (RO) é o processo de filtração que exige maior pressão e é aplicada para purificação de água salobra ou salina (Agrawal *et al.*, 2023). O Quadro 1 resume características e aplicações dos tipos de membranas de filtração.

Quadro 1: Tipos de membranas de filtração convencionais e exemplos de aplicações

Membrana	**Tamanho de poro (µm)**	**Força motriz (bar)**	**Aplicações**
RO	0,0001 - 0,001	15 - 80	Separação de sais e microsolutos
NF	0,001 - 0,0015	5 - 25	Separação de sais polivalentes e pequenos solutos
UF	0,0015 - 0,1	1 - 7	Esterilização e separação de macromoléculas
MF	0,1 - 10	0,2 - 2	Remoção de bactérias e clarificação

Adaptado de Agrawal *et al.* (2023)

Em geral, processos de filtração baseados em membrana estão associados a altos gastos energéticos, no entanto, a baixa viscosidade da água em climas quentes é um fator que contribui para diminuir as necessidades de energia (Kehrein *et al.*, 2020). Entupimento das membranas, baixa eficiência na recuperação de energia e recursos, sensibilidade a condições extremas como temperaturas baixas, são citados como potenciais limitações para a comercialização em larga escala dos biorreatores anaeróbios de membrana (Hu *et al.*, 2022).

Entre os parâmetros importantes no processamento por membranas estão o fluxo do permeado, a pressão transmembrana, a rejeição e a recuperação. O fluxo do permeado (J) é a relação entre a vazão permeada e a área da membrana. No início da operação o

fluxo de permeado tende a ser alto já que não há deposito de sólidos na superfície da membrana que causem resistência ao fluxo. A pressão transmembrana (TMP) se refere a diferença de pressão entre o lado retido e o lado permeado. A rejeição e recuperação representam, respectivamente, a capacidade da membrana de reter o soluto e a razão entre a vazão do permeado e a vazão de alimentação (Agrawal *et al.*, 2023).

Através de separação por membranas, a matéria orgânica é majoritariamente removida por biossorção ou biodegradação, sendo os compostos retidos podendo ser posteriormente degradados com o aumento do tempo de retenção. Devido à falta de ambiente e condições favoráveis e microrganismos específicos para remoção de nutrientes, a remoção de nitrogênio e fósforo é limitada (Hu *et al.*, 2022). Isto pode ser uma vantagem caso haja intenção de reúso agrícola do efluente tratado.

Apesar das limitações, a remoção de fósforo em biorreatores anaeróbios de membrana pode ser feita com o uso de coagulantes. Já o controle do nitrogênio é um desafio devido à falta da etapa de nitrificação. Levando em conta a necessidade de remoção de nitrogênio, seria ideal o uso do metano dissolvido como doador de elétrons para simultaneamente remover CH_4 e nitrogênio do efluente (Lee & Liao, 2021). Outro fator que pode influenciar de forma negativa na operação dos reatores é a presença de SO_4^- na água residuária em concentrações altas, uma vez que as bactérias sulfetogênicas competem pela matéria orgânica com as metanogênicas. Também, a presença de enxofre reduzido no biogás é indesejável pelo seu efeito corrosivo. Considera-se que a razão DQO afluente/sulfato menor que 10 é prejudicial ao processo anaeróbio (Hu *et al.*, 2022).

O Quadro 2 apresenta um resumo das condições operacionais de AnMBR usados em estudos que tiveram objetivos diversos.

Quadro 2: Parâmetros operacionais de AnMBRs usados em estudos para diferentes fins

Objetivo do Estudo	Parâmetros Operacionais							CPEs Estudados	Ref.
	V[1]	**HRT[2]**	**SRT[3]**	**Tipo de Membrana**	**Fluxo Volumétrico**	**Pressão Transmembrana**	**Temp.[4]**		
Investigar a remoção de antibióticos e de genes resistentes utilizando AnMBR em esgoto farmacêutico real, com destaque para a ação positiva dos biofilmes	1.6 L	1.5 d	200 d	UF[5]	4.0 L/m^2.h	21 kPa 6 kPa	32-38°C	Mix de antibióticos incluindo levofloxacino	Wang *et al.* (2023)
Avaliar o efeito do tempo de retenção hidráulica no desempenho do AnMBR no tratamento de águas residuárias da pecuária, com destaque para a remoção de antibióticos	5.0 L	1 d 2 d	120 d	MF[6]	2.0 L/m^2.h 1.0 L/m^2.h	-	-	Sulfatiazol Sulfapiridina Sulfametazina Sulfametoxazol Norfloxacino Ciprofloxacino Ofloxacino Tetraciclina Oxitetraciclina Clortetraciclina	Liu *et al.* (2023)
Avaliar o efeito da diminuição da HRT, mantendo a taxa de carga orgânica, na remoção de contaminantes microbianos no AnMBR	35.0 L	12 h 10 h 8 h	-	-	-	4.0 kPa	35°C	Genes resistentes a antibióticos	Medina *et al.* (2023)
Investigar o desempenho do AnMBR tratando esgotos municipais de baixa carga orgânica, com adição de $FeCl_3$ para controle de *fouling* e remoção de fósforo	40.0 L	6 h	50 d	UF	13 L/m^2.h	-	15-25°C	Ibuprofeno Naproxeno Diclofenaco Cetoprofeno Bisfenol A Nonilfenol Triclosan	Plevri *et al.* (2023)
Avaliar um sistema AnMBR em escala laboratorial para o tratamento de águas residuárias de matadouros de aves, com	3.5 L	4 d	360 d	MF	6.49 L/m^2.h	-	35°C	Genes resistentes a antibióticos	Ramadan *et al.* (2023)

especial incidência na comunidade microbiana e na remoção de genes resistentes a antibióticos e agentes patogênicos									
Investigar a adição de carvão ativado granular na melhorar do desempenho do tratamento de esgoto farmacêutico e diminuição de *fouling* em AnMBR	4.0 L	-	-	NF[7]	-	20 kPa	-	Ampicilina Sultamicilina Tosilato	Balcioğlu *et al.* (2023)
Examinar a presença de genes resistentes a antibióticos e a diversidade da comunidade microbiana em esgotos municipais reais tratados com AnMBR	3.0 L	33 h	360 d	MF	6.7 L/m^2.h	-	35°C	Genes resistentes a antibióticos	Sawaya *et al.* (2022)
Avaliar a interação de coquetel antibacteriano com a comunidade microbiana na formação da camada de biofilme em AnMBR e remoção de bactérias resistentes a antibióticos	5.0 L	48 h	30 d	UF	-	25 kPa	-	Eritromicina Tetraciclina Sulfametoxazol	Aydin *et al.* (2022)
Avaliar o impacto da temperatura na performance biológica e da membrana em AnMBR com biomassa de crescimento suspenso e adição de carvão ativado em pó.	5.0 L	0.4 d	30 d	-	8.87 L/m^2.h	-	24°C 15°C 10°C	-	Li & Parker (2021)
Avaliar as vantagens do uso de AnMBR com leito fluidizado no pós-tratamento de efluentes quanto aos seus requisitos energéticos e capacidade de remoção de DQO e SS.	2.0 L	3.1 a 2.2 h	-	-	7 a 10 L/m^2.h	-	35°C	-	Kim *et al.* (2011)

V[1]: volume; HRT[2]: tempo de retenção hidráulica; SRT[3]: tempo de retenção de lodo; Temp[4]: temperatura; UF[5]: ultrafiltração; MF[6]: microfiltração; NF[7]: nano filtração

2.4 BIORREATOR ANAERÓBIO DE MEMBRANA DINÂMICA (AnDMBR)

Membrana dinâmica (MD), também chamada de membrana secundária ou biológica, é geralmente formada em um material inerte que funciona como suporte. Os poros podem variar de 10 a 200 µm e com exemplos de material suporte têm-se o poliéster, nylon, polipropileno, polietileno, algodão. O processo de formação da MD acontece pela deposição de partículas sólidas em suspensão como células ou flocos microbianos, onde um material poroso age como meio suporte para a formação da camada biologicamente ativa durante o processo de filtração (Chimuca *et al.*, 2023).

Biorreatores anaeróbios de membrana dinâmica (AnDMBRs) podem apresentar diferentes configurações a depender do tipo e posicionamento da MD adotada e do regime de alimentação. O módulo de MD pode ser localizado dentro ou fora do biorreator. Quando o módulo de MD é localizado fora do biorreator, a alimentação da membrana pode ser por bombeamento ou por gravidade. O módulo de MD pode ser submerso (interno ou externo) ou *side-stream*. Na configuração submersa, o módulo de MD é instalado diretamente no biorreator, com a operação da membrana sendo feita à vácuo. Para a configuração *side-stream*, a operação é feita sob pressão. As diferentes configurações adotadas resultam em diferentes processos de formação da MD e da performance de filtração. Poucos estudos adotaram o uso da configuração *side-stream* com modo de alimentação por gravidade (Ersahin *et al.*, 2017; Hu *et al.*, 2018).

Entre as vantagens do uso de MD em relação à processos como micro ou ultrafiltração estão maior fluxo de permeado, menor custo de investimento (já que o meio suporte é um material barato), alto potencial antipoluidor, baixa pressão de filtração (que permite o fluxo por gravidade e evita o uso de bombas, economizando energia) e capacidade de também reter ovos de helmintos. Além disso, a MD é adequada para processos anaeróbios devido ao lento processo de crescimento dos microrganismos e condição hidrodinâmica mais branda devido à falta de aeração. Bactérias essenciais para a degradação de matéria orgânica refratária, que geralmente apresentam crescimento lento, podem ser retidas e acumuladas na camada dinâmica, permitindo degradação posterior em substâncias mais facilmente biodegradáveis como ácidos graxos voláteis (Chimuca *et al.*, 2023).

2.5 CONTAMINANTES DE PREOCUPAÇÃO EMERGENTE

Poluentes emergentes, substâncias de preocupação emergente, contaminantes prioritários, ou CPEs, são termos usados para se referir a um grupo de substâncias químicas que causam efeitos danosos ao meio ambiente, não necessariamente tóxicos. No entanto, devido à falta de regulamentação, normas e estudos, ainda não são totalmente entendidos pela comunidade científica (Ncibi *et al.*, 2017). Com o desenvolvimento da indústria, junto aos poluentes convencionais, muitos poluentes emergentes começaram a ser gradualmente detectados no ambiente (Zhang *et al.*, 2023).

Os CPEs são compostos químicos sintéticos ou naturais detectados principalmente com o avanço de novas tecnologias analíticas. Esses compostos geralmente acabam no meio aquático através de drogas ou produtos excretados e/ou diretamente descartados na rede de esgotos sanitários, áreas de aquacultura/agricultura e lixiviados de aterros sanitários (Baudisch *et al.*, 2022). A acumulação destes químicos no meio ambiente é preocupante, já que pode resultar em acumulação na cadeia alimentar de acordo com o nível de contaminação e exposição (Singha & Shukla, 2022).

Entre os principais CPEs estão os microplásticos, os antibióticos (bactérias e genes resistentes a antibióticos), disruptores endócrinos, pesticidas, filtros UV, hormônios e aditivos alimentares (Zhang *et al.*, 2023; Vélez *et al.*, 2019). Antibióticos e disruptores endócrinos são particularmente preocupantes, pois estão relacionados a resistência bacteriana e modificações em rotas bioquímicas no corpo humano. Disruptores endócrinos também interferem nos hormônios mesmo em baixas concentrações (Vélez *et al.*, 2019).

Alguns CPEs são poluentes orgânicos persistentes que não são facilmente degradados e permanecem no ambiente aquático por longos períodos. Por apresentar essa característica de pseudo-resistência/persistência causam efeitos adversos no meio ambiente (Dong *et al.*, 2023). Contaminantes emergentes como microplásticos, bactérias e genes resistentes a antibióticos, material orgânico intracelular, material orgânico algal e íons de metais pesados têm sido objeto de intensa investigação por pesquisadores (Zhang *et al.*, 2023). O surgimento desses novos tipos de poluentes representa um grande desafio as técnicas de tratamento de esgoto (Ncibi *et al.*, 2017).

Métodos químicos, físicos e biológicos de tratamento têm sido usados para remediar esses poluentes na água (filtração por membrana, coagulação, adsorção, degradação fotocatalítica, biorreatores anaeróbios e lodos ativados). A aplicação de cada tipo de método depende da natureza do poluente e das condições ambientais (Dong *et al.*, 2023). Coagulação, adsorção, processos oxidativos avançados e ultrafiltração podem ser usados para a remoção de poluentes emergentes, no entanto nenhum processo sozinho é capaz de atingir nível ideal de tratamento. O uso de processo combinado, empregando um ou dois dos três primeiros métodos, seguidos da ultrafiltração, é considerado promissor para o tratamento desses poluentes (Zhang *et al.*, 2023). Além desses, processos de tratamento biológico, como fitoremediação, remediação microbiológica usando bactérias e fungos, assim como combinações como biodegradação/filtração, adsorção/biodegradação, e adsorção/ozonização, têm sido reportadas (Ncibi *et al.*, 2017).

O maior desafio na remoção de CPEs em sistemas de tratamento de água e de esgoto, bem como em ambientes aquáticos, está na sua alta reatividade e mobilidade, já que baixas concentrações desses compostos podem contaminar grandes volumes de água (Ncibi *et al.*, 2017). Poluentes

emergentes na fase aquosa não são tratados de forma efetiva por meio de processos e operações físico-químicas nas estações de tratamento convencionais. Isso corrobora para o aumento do risco de persistência desses compostos perigosos no meio ambiente. Enquanto este problema continuar, as estações de tratamento de esgoto estão se tornando fontes indiretas de poluição. Mesmo que os efeitos adversos dos CPEs ao meio ambiente ainda não tenham sido bem elucidados, é importante que soluções sejam apontadas (Ncibi *et al.*, 2017).

A taxa de transferência dos CPEs para a água, plantas e animais depende da polaridade do poluente e das características do meio ambiente. Quanto maior o conteúdo de lipídeos em plantas ou animais, maior é a taxa de sorção de poluentes emergentes não polares (Vélez *et al.*, 2019). Um importante fator que influencia na eficiência de remoção dos poluentes emergentes é a habilidade de interação dos contaminantes com as partículas sólidas, tanto naturais (argila, sedimentos, microrganismos) quanto adicionadas com a finalidade de tratamento (carvão ativado ou coagulantes). Em geral, compostos com baixo coeficiente de adsorção permanecem na fase aquosa, que favorece sua mobilidade. Poluentes emergentes não polares apresentam alta tendência de sorção no lodo, enquanto os polares permanecem dissolvidos na água. Compostos com boa afinidade com o material particulado podem ser eliminados por processos físico-químicos como sedimentação ou flotação, mas também através de tratamentos como a biodegradação. A maioria dos poluentes emergentes são polares e permanecem solúveis na água, onde são facilmente dispersos (Vélez *et al.*, 2019).

O estudo dos poluentes emergentes nas águas superficiais ajuda a entender a efetividade e necessidade do processo de tratamento de esgotos, além dos hábitos de consumo da população e os possíveis riscos à saúde humana e aos ecossistemas. No entanto, ao contrário do que já se observa em países europeus e outros, a falta de dados disponíveis sobre a ocorrência de poluentes emergentes nos efluentes em países do continente americano prejudica a comparação ou avaliação da eficiência dos processos de tratamento (Vélez *et al.*, 2019).

2.6 AGROTÓXICOS

Agrotóxicos são definidos como substâncias usadas para suprimir, erradicar ou evitar organismos considerados danosos para plantações. Os agrotóxicos podem ser categorizados pelo tipo de uso, pelo organismo alvo, pela origem de suas substâncias ativas ou pela sua periculosidade. A União Europeia classifica os agrotóxicos nas categorias de pesticida, herbicida, fungicida e inseticida, entre outros. O banco de dados sobre agrotóxicos da *Pesticide Properties Database* (PPDB), desenvolvido pela Universidade de Hertfordshire no Reino Unido, também inclui as categorias

relacionadas com propriedades físico-químicas, ecotoxicológicas e saúde humana (McGinley *et al.*, 2023).

Uma vez no ambiente, agrotóxicos se deslocam através do solo ou das águas superficiais para córregos e águas subterrâneas, onde podem provocar efeitos ecológicos não intencionais como a acumulação em organismos aquáticos e perda da biodiversidade do ecossistema. Além disso, na saúde humana, podem ter efeitos carcinogênicos, mutagênicos, neurotóxicos e/ou teratogênicos. A detecção de agrotóxicos em amostras de água tem sido geralmente atribuída a sua dessorção de solos ou sedimentos onde podem ter sido acumulados por aplicações prévias. Dependendo das suas propriedades (polaridade, coeficiente de partição n-octanol-água), podem ser adsorvidos no solo ou nas partículas de sedimentos, sendo os hidrofóbicos os mais sujeitos à adsorção (McGinley *et al.*, 2023). Concentrações de até 500 mg/L podem ser encontrados em águas de drenagem agrícolas (Islam *et al.*, 2018).

Agrotóxicos usados em atividades domésticas, como controle de pragas e ervas daninhas em jardins, podem ser transportados para o sistema de esgotamento sanitário por escoamento, aumentando a carga de tratamento nas ETEs (Li *et al.*, 2022). Inúmeros tipos de tratamentos físicos e químicos, incluindo a adsorção, filtração por membranas e processos oxidativos avançados, além de processos biológicos como biorremediação, lodos ativados e fito remediação, têm sido aplicados para a remoção de agrotóxicos de meios aquosos (McGinley *et al.*, 2023).

Segundo dados extraídos do Sistema Eletrônico de Relatórios Semestrais de Agrotóxicos e divulgados pelo IBAMA (2021), os ingredientes ativos mais vendidos no Brasil durante a série histórica de 2009 a 2021 foram, em ordem decrescente: glifosato (2.214,65 mil toneladas); 2,4-D (529,95 mil toneladas); mancozebe; acefato; óleo mineral; atrazina; enxofre; clorotalonil e clorpirifós. Foram comercializados 467 ingredientes ativos totais no país durante o período citado. Entre os herbicidas mais usados para fins domésticos estão o glifosato e o 2,4-D (Li *et al.*, 2022). Embora os agrotóxicos estejam entre os contaminantes orgânicos mais investigados no meio aquático, a ocorrência e destino deles durante as etapas de tratamento nas ETEs ainda não têm sido propriamente estudados no Brasil (Marson *et al.*, 2022).

2.6.1 2,4-D

O 2,4-D foi o primeiro agrotóxico do tipo herbicida comercial introduzido no mercado, na década de 1940. Pertence ao grupo dos herbicidas ácidos fenoxi e, desde seu lançamento, permanece como um dos mais usados mundialmente (Zipper *et al.*,1999; Elefsiniotis & Wareham, 2013). Isto deve-se, entre outras razões, ao seu baixo custo, seletividade, eficácia e controle de ervas daninhas (Cocco *et al.*, 2023; Islam *et al.*, 2018). O 2,4-D é registrado como ingrediente em aproximadamente

1500 herbicidas de uso agrícola e doméstico, tanto como composto ativo sozinho quanto em conjunto com outros. Fez parte do "Agente Laranja" usado durante a guerra do Vietnã como desfolhante químico (Zuanazzi *et al.*, 2020; Islam *et al.*, 2018).

Na formulação do 2,4-D estão inclusos ésteres, ácidos e sais com variadas propriedades químicas e comportamento ambientais, com maior ou menor grau de toxicidade. É uma molécula polar, e os ésteres e as aminas que o compõe são facilmente móveis em sistemas aquosos devido ao grupo ácido carboxílico (pKa = 2,8). Sua baixa adsorção no solo pode ser a razão da alta ocorrência no meio ambiente (Islam *et al.*, 2018).

Particularmente devido à sua solubilidade, 900 mg/L a 20°C, o potencial de contaminação do 2,4-D em cursos de água é extremamente alto (Elefsiniotis & Wareham, 2013). Está normalmente presente em águas e efluentes com concentrações que variam de ng/L a mg/L (Cocco *et al.*, 2023). Concentrações de 3 µg/L de 2,4-D foram observados no esgoto municipal de Queensland, na Austrália (Cardenas *et al.*, 2016). A estimativa de taxa de emissão do 2,4-D no ambiente, baseada em parâmetros do fabricante (área tratada, fator de diluição e porcentagem de ingredientes ativos) e uso mensal para controle de pragas e ervas daninhas, é de 3,27 mg/d.m^2 (Li *et al*., 2022). No Brasil, o limite máximo permitido para 2,4-D em corpos de água é de 30 µg/L (Zuanazzi *et al.*, 2020; BRASIL, 2021).

A aplicação direta do herbicida 2,4-D pelos sistemas agrícolas para controle de ervas daninhas é um dos fatores que contribui para sua distribuição no ambiente. Organismos não alvo são expostos ao 2,4-D de várias formas, o que pode causar efeitos tóxicos dependendo da dose, frequência e exposição, provocando até potenciais efeitos letais (Islam *et al.*, 2018). Além disso, existe a capacidade de bioacumulação desse herbicida em organismos não alvo expostos a um curto período. Em humanos, o 2,4-D é associado ao desenvolvimento da doença neurodegenerativa de Parkinson e autismo (Zuanazzi *et al.*, 2020).

O composto age imitando as auxinas naturais da planta, hormônio responsável pelo desenvolvimento, promovendo divisão, diferenciação e alongamento das células, agindo como regulador de crescimento (Zuanazzi *et al.*, 2020). Ele reduz as taxas de crescimento, induz a problemas reprodutivos, produz mudanças na aparência ou comportamento, além de causar a morte de espécies não alvo como plantas, animais e microrganismos. É disruptor endócrino, afetando processos de desenvolvimento mesmo em baixas concentrações (Pattanasupong *et al.*, 2004).

Mesmo com todas as informações sobre o comportamento do 2,4-D no ambiente, ainda existe uma lacuna sobre a biodegradação deste composto no tratamento de esgotos, particularmente na associação entre membranas e processo anaeróbio.

2.6.2 Atrazina

A atrazina (2-cloro-4-etilamino-6-isopropilamino-1,3,5-triazina) é um agrotóxico da classe dos herbicidas do grupo s-triazina de compostos nitro aromáticos. É introduzida no meio ambiente por usos industriais e agrícolas, sendo considerada uma das substâncias de maior preocupação monitoradas em águas europeias segundo diretivas da União Europeia (Boopathy, 2017; Nasseri *et al.*, 2014).

Sendo uma substância persistente no meio ambiente, causa sérios problemas. Devido à sua alta mobilidade no solo (com tempo de meia-vida entre 4 e 57 semanas), solubilidade moderada na água (33 mg/L à 20°C), baixa pressão de vapor e alto uso, é encontrada nas águas superficiais e subterrâneas (Boopathy, 2017; Nasseri *et al.*, 2014; Ghosh *et al.*, 2001). Apesar da relativa persistência no meio ambiente, sua bioacumulação não é significante (Boopathy, 2017).

Atua causando efeito inibitório reversível no processo de fotossíntese dificultando a fotofosforilação, no entanto, sem provocar letalidade ou dano permanente a célula vegetal a curto período. Além das plantas, pode apresentar efeito tóxico para espécies não alvo, sendo fitoplâncton, macrófitas, invertebrados bênticos, zooplâncton e peixes os organismos mais sensíveis (Boopathy, 2017). Também é potencial causador de câncer em seres humanos (Nasseri *et al.*, 2014).

A exposição humana a níveis considerados acima do limite máximo recomendado pode provocar problemas cardíacos, pulmonares, renais, baixa pressão arterial, espasmos musculares, perda de peso, deficiências no sistema nervoso central e imunológico (Nasseri *et al.*, 2014). A toxicidade, e persistência, da atrazina é associada à presença do grupo nitro nos anéis aromáticos. Sendo alguns de seus metabólitos também fito tóxicos, porém em menor grau que o composto original (Boopathy, 2017).

Embora seja um processo lento, a biodegradação é o único método pelo qual a atrazina pode ser mineralizada (Ghosh *et al.*, 2001). A biodegradação pode levar a atrazina a completa degradação ou mineralização, produzindo substâncias mais simples como dióxido de carbono, água, nitrogênio e compostos orgânicos. É considerado o meio mais efetivo para remoção desses poluentes do ambiente. O processo de biodegradação pode ocorrer em diversos ambientes como solo, sedimentos, águas superficiais e subterrâneas e lodo biológico (Nasseri *et al.*, 2014). Em ambiente anaeróbio e sob condições apropriadas de doadores e aceptores de elétrons, bactérias metanogênicas e redutoras de sulfato são capazes de metabolizar compostos nitro aromáticos, como a atrazina (Boopathy, 2017).

Estruturas aromáticas de lignina, benzoato ou outros ácidos aromáticos são metabolizados a produtos gasosos por culturas mistas em condições metanogênicas. No entanto, embora a taxa de

degradação desses compostos por microrganismos anaeróbios seja lenta, o requerimento energético para crescimento bacteriano não é suficiente para sustentar o metabolismo dos microrganismos. Assim, é necessário a adição de compostos orgânicos facilmente biodegradáveis para estimular o crescimento de bactérias anaeróbias, aumentando a taxa de degradação pela utilização dos compostos recalcitrantes como substrato secundário no processo de co-metabolismo (Ghosh & Philip, 2004).

2.7 FÁRMACOS

Aproximadamente 4000 fármacos estão incluídos entre as drogas de origem natural ou sintéticas biologicamente ativas usadas na medicina humana e veterinária. As mais usadas a nível mundial são os antibióticos, antidepressivos, analgésicos, anti-inflamatórios e anti-hipertensivos. Isto faz com que o monitoramento e o controle dessas substâncias sejam tópicos de grande interesse (Bustos Bustos *et al.*, 2022).

A contaminação ambiental causada por resíduos farmacêuticos pode ser dividida em três classes: as causadas por grandes geradores, como as indústrias, por pequenos geradores como os hospitais, instituições educacionais e de pesquisa, e por micro geradores, como as residências. A maior parte das ETEs atuais não são projetadas para remover fármacos, uma vez que o principal foco do tratamento convencional de esgoto é a remoção de matéria orgânica biodegradável e sólidos suspensos. Assim, vários medicamentos e seus subprodutos acabam chegando aos ambientes aquáticos (De Oliveira *et al.*, 2020).

A maioria dos fármacos são eliminados diretamente pelos pacientes sem metabolização. Sua contínua introdução no ciclo da água e no solo provoca toxicidade, bioacumulação e persistência no ambiente. As propriedades físico-químicas dos fármacos são fatores determinantes na facilidade de acumulação no ambiente aquático, solo ou na atmosfera. Fatores comuns que devem ser considerados são a hidrofobicidade e lipofílidade, que são indicadores toxicológicos que ajudam a prever sua permeabilidade ou acumulação no ambiente (Bustos Bustos *et al.*, 2022).

Uma importante característica dos fármacos é a falta de necessidade de serem persistentes para causarem efeitos negativos no ambiente. A velocidade de sua eliminação ou transformação é continuamente compensada pela introdução de novas drogas no meio. Uma vez no ambiente, os fármacos podem passar por diferentes processos, como mineralização a dióxido de carbono e água, biodegradação parcial e adsorção aos sedimentos. A característica lipofílica dificulta a degradação em estações de tratamento de esgotos (Bustos Bustos *et al.*, 2022).

Biorreator de membranas remove fármacos através de diferentes processos como biodegradação, sorção no lodo e retenção física nas membranas. As características físico-químicas dos compostos e as condições operacionais do biorreator são os responsáveis pelo processo e pela eficiência que ele pode alcançar. Além disso, os produtos de transformação dos compostos durante o processo de remoção podem ser formados de forma natural por degradação ou por processos foto ou bioquímicos, aos quais os compostos originais foram submetidos (Matsubara *et al.*, 2020).

2.7.1 Antibióticos

Antibióticos estão entre os fármacos mais detectados nos ambientes aquáticos. Devido a sua natureza antibacteriana, são raramente eliminados nas ETEs. Por seu consumo excessivo, algumas bactérias se tornaram resistentes a certos antibióticos, representando ameaça para a saúde e economia mundial. A composição dos antibióticos é complexa, no entanto, após eliminação nas fezes ou urina, mantém sua estrutura original, contaminando o solo e os recursos hídricos. A exposição contínua a essas substâncias mesmo em baixas concentrações, em corpos de água se tornou um sério problema para a saúde humana e de ecossistemas (Bustos Bustos *et al.*, 2022). A Organização Mundial da Saúde considera que a resistência a antibióticos é, atualmente, uma das maiores ameaças à saúde global, segurança alimentar e desenvolvimento (WHO, 2020).

A maioria dos antibióticos presentes nos esgotos é detectada na faixa de ng/L a 12.7 µg/L, chegando a 19 µg/L no caso de esgotos provenientes de indústria farmacêutica, com predominância das classes sulfonamidas e fluoroquinolonas. Antibióticos das classes sulfonamidas, quinolonas e macrolídeos são muito usados para aumento da produtividade animal, mas devido ao fato de não serem completamente metabolizados, permanecem nos esgotos (Mishra *et al.*, 2023).

A remoção de antibióticos em AnMBRs se dá por adsorção, biodegradação e processo de separação. A comunidade microbiana presente mantém a estabilidade no sistema pela aclimatação gradual das bactérias, com potencial de desenvolver resistência, devido ao maior tempo de retenção de lodo. Antibióticos não são retidos em membranas de micro ou ultrafiltração devido ao tamanho de suas moléculas. No entanto, a alta concentração de biomassa proporciona ambiente estável para o crescimento da comunidade microbiana (Zhu *et al.*, 2021; Zhou *et al.*, 2016; Trinh *et al.*, 2016).

Além da presença de antibióticos em esgotos domésticos de cidades, estudos em áreas rurais e menos povoadas são importantes, porque a criação animal também contribui para o descarte de antibióticos nos corpos d'água (Li *et al.*, 2023).

2.7.2 Quinolonas

Os antibióticos da classe quinolonas estão entre os cinco mais usados globalmente. São utilizados para o tratamento de infecções respiratórias e urinárias, especialmente devido ao poder antibacteriano, alta biodisponibilidade, características cinéticas e preço baixo (Meng *et al.*, 2023; Zou *et al.*, 2022). Quinolonas de segunda geração são conhecidos como fluoroquilonados e são muito efetivas contra bactérias gram-positivas e gram-negativas, além de micoplasmas (Orimolade *et al.*, 2023).

A modificação da estrutura das quinolonas para a síntese das fluoroquinolonas resultou na melhoria de características farmacológicas relacionadas à cinética e a dinâmica do composto e redução da toxicidade. Na maioria dos casos, sua ação se dá pela inibição da replicação ou transcrição do DNA bacteriano, responsável pelo funcionamento das células (Orimolade *et al.*, 2023).

Através do descarte nos sistemas de esgotamento sanitário, as quinolonas e seus metabólitos chegam nas ETEs. A adsorção no lodo é a principal forma de remoção desses compostos do esgoto sanitário. O processo de adsorção nas partículas de lodo pode ocorrer através de interações eletrostáticas, pontes de hidrogênio, hidrofobia ou outros mecanismos (Meng *et al.*, 2023; Zou *et al.*, 2022; Oberoi *et al.*, 2019). Um grande número de quinolonas são introduzidas no solo por via agrícola através da aplicação de lodo. Isso acarreta contaminação dos corpos d'água e da cadeia alimentar, levando a riscos para os seres humanos e para o meio ambiente (Meng *et al.*, 2023; Sun *et al.*, 2022). A presença de fluoroquinolonas no esgoto é problemática pela sua natureza recalcitrante e baixa solubilidade em água. Como mencionado, o desenvolvimento e a disseminação de genes resistentes a antibióticos são problemas que têm se agravado ao longo do tempo nas ETEs (Orimolade *et al.*, 2023).

O norfloxacino (NOR) é um antibiótico da classe fluoroquilonado usado em humanos e animais. Fluoroquinolonas podem ser encontradas nos esgotos domésticos em concentrações que variam entre 2 e 14 µg/L. Segundo Meng *et al.* (2023), as concentrações máximas desse composto em lodos de esgotos domésticos na China podem alcançar até 651,49 µg/kg, com média de 188,55 µg/kg. Li *et al.* (2023), em águas superficiais do rio Zijiang, na China, encontraram concentrações médias de 2,96 µg/L e 2,30 µg/L para as estações seca e chuvosa, respectivamente.

No estudo desenvolvido por Marson *et al.* (2022) foram encontrados valores para NOR em efluente municipal ao longo do Brasil entre 90 e 200 ng/L. Montagner *et al.* (2019) mediram valores médios de 2 ng/L para NOFX em águas superficiais do estado de São Paulo. Já Freitas *et al.* (2021) detectaram valores entre 8 e 18 ng/L para águas superficiais no mesmo estado. Na cidade de Porto

Alegre, Bisognin *et al.* (2021) não encontraram a presença de NOR nos afluentes ou efluentes de ETEs analisadas, entretanto, no lodo foi observado 0,271 mg/kg.

2.7.3 Sulfonamidas

Antibióticos da classe sulfonamida são drogas sintéticas usadas em infecções causadas por bactérias e protozoários em humanos e animais. São particularmente empregadas para fins veterinários devido ao baixo custo e alta eficiência (Montone *et al.*, 2023; Saha *et al.*, 2023).

Sulfonamidas podem ser submetidas a diversas rotas de transformação, sendo afetadas por fatores ambientais. É comum que o composto original e seus produtos de transformação alcancem esgotos e águas superficiais (Montone *et al.*, 2023). São frequentemente encontrados em efluentes e afluentes de ETEs ao redor do mundo devido à sua baixa suscetibilidade para hidrólise química e enzimática (Saha *et al.*, 2023; Jiang *et al.*, 2019; Xiong *et al.*, 2019). O baixo tempo de retenção hidráulica de ETEs convencionais limita a biodegradação das sulfonamidas, as quais acabam acumulando-se no lodo (Martín-Pozo *et al.*, 2019; Yang *et al.*, 2011).

Concentrações de sulfonamidas em esgotos variam entre poucos ng/L a dezenas de µg/L, com concentrações variando de 0,04 a 665 µg/kg em lodos. Em efluentes hospitalares e provenientes de criação de animais podem ser encontrados valores entre 5,03 e 142,90 µg/L (He *et al.*, 2019; Ouyang *et al.*, 2020; Wei *et al.*, 2019).

O sulfametoxazol (SMX) é um antibiótico da classe das sulfonamidas. Concentrações de SMX foram encontrados em efluentes hospitalares (0,01 e 710 µg/L), efluentes domésticos (0,03 a 55 µg/L) e efluentes de indústria farmacêutica 0,01 a 130 µg/L (Mishra *et al*., 2023). Montagner *et al.* (2019) encontraram valores médios de SMX em águas superficiais na Bacia Hidrográfica do Rio Atibaia, São Paulo, de 1 ng/L. Freitas *et al.* (2021) reportaram concentrações entre 20 e 120 ng/L para águas superficiais no Maranhão. Em esgoto bruto, Queiroz *et al.* (2012) mediram 13 ng/L em Belo Horizonte (MG). Marson *et al.* (2022) reportaram valores entre 100 e 1300 ng/L para amostras coletadas ao longo do país. Em Porto Alegre, Bisognin *et al.* (2021) detectaram concentrações de 0,980 e 0,301 µg/L em afluente e efluente de ETE, respectivamente.

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3. METODOLOGIA

A metodologia adotada foi construída a partir da escrita de artigos científicos com objetivos que se relacionam e que tem por intuito atender ao objetivo geral e aos objetivos específicos propostos por essa tese de doutorado. A Figura 4 apresenta os artigos escritos e suas relações. O Quadro 3 apresenta, de forma resumida, os objetivos de cada um dos artigos.

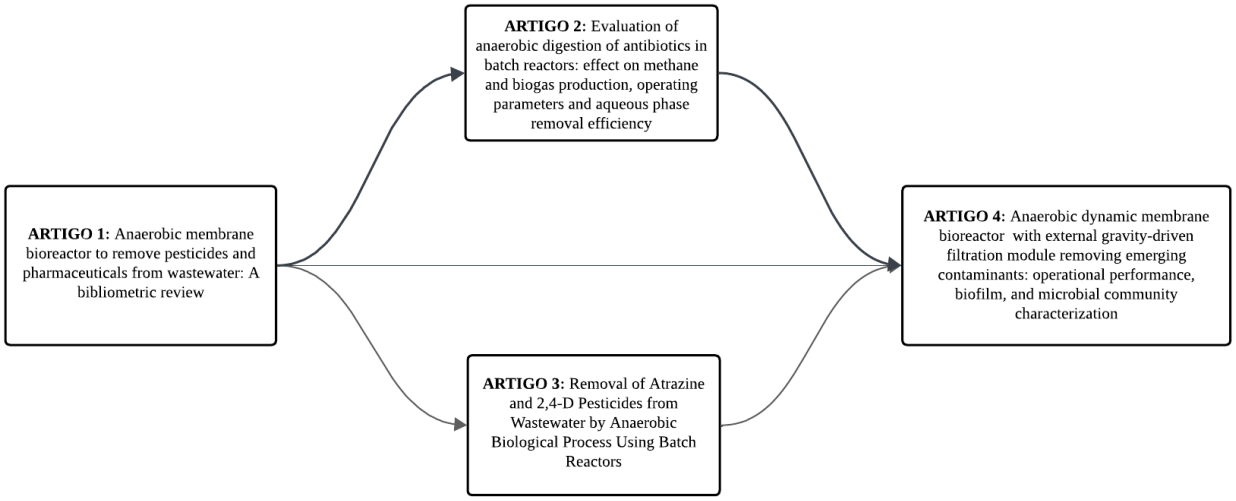


Figura 4: Organização da tese por artigos

Quadro 3: Objetivos de cada um dos artigos desenvolvidos durante a tese

Artigo	Objetivo
Artigo 1: Anaerobic membrane bioreactor to remove pesticides and pharmaceuticals from wastewater: A bibliometric review	Mapear a produção científica e avaliar as tendências de publicação sobre o uso de AnMBR para remover pesticidas e produtos farmacêuticos de águas residuárias.
Artigo 2: Evaluation of anaerobic digestion of antibiotics in batch reactors: effect on methane and biogas production, operating parameters and aqueous phase removal efficiency	Avaliar a remoção de antibióticos e seus efeitos na digestão anaeróbia de águas residuárias sintéticas.
Artigo 3: Removal of Atrazine and 2,4-D Pesticides from Wastewater by Anaerobic Biological Process Using Batch Reactors	Avaliar a remoção de atrazina e 2,4D considerando os parâmetros operacionais do processo de digestão anaeróbia para compreender interferências causadas pela presença dos compostos isoladamente ou em coexistência.
Artigo 4: Anaerobic dynamic membrane bioreactor with external gravity-driven filtration module removing emerging contaminants: operational performance, biofilm, and microbial community characterization	Avaliar o uso de um biorreator anaeróbico de membrana dinâmica de módulo externo operado por gravidade (AnDMBR) na remoção de contaminantes emergentes; sua influência no desempenho operacional, na formação de biofilme e na comunidade microbiana.

Esta tese de doutorado, com base nos artigos desenvolvidos, atende diretamente aos seguintes Objetivos de Desenvolvimento Sustentável (ODSs) da Organização das Nações Unidas (ONU): ODS 6 (água potável e saneamento), ODS 7 (energia limpa e acessível), ODS 13 (ação contra a mudança global do clima) e ODS 14 (vida na água).

4. RESULTADOS

Os resultados obtidos por esta tese de doutorada estão apresentados neste capítulo. Cada item corresponde a um artigo publicado e/ou submetido.

4.1 ANAEROBIC MEMBRANE BIOREACTOR TO REMOVE PESTICIDES AND PHARMACEUTICALS FROM WASTEWATER: A BIBLIOMETRIC REVIEW

Artigo publicado no periódico *ENVIRONMENTAL QUALITY MANAGEMENT*, da Editora Wiley, com a seguinte referência: RIBEIRO, Marcos Henrique Gomes; SILVA, Maria Cristina de Almeida; BENETTI, Antônio Domingues. Anaerobic membrane bioreactor to remove pesticides and pharmaceuticals from wastewater: A bibliometric review. *Environmental Quality Management*, v. 34, n. 2, p. e22261, 2024. https://doi.org/10.1002/tqem.22261. Vale ressaltar que o texto aqui apresentado pode diferir em relação ao publicado pelo periódico devido a correções realizadas. Para a leitura da forma mais atualizada, recomenda-se consulta ao site do respectivo periódico e/ou editora.

4.1.1 ABSTRACT

With the development of state-of-the-art analytical methods, new compounds have been detected in water and sewage. Due to their diverse and complex nature, these compounds have been classified as contaminants of emerging concern (CEC). Among the CEC, there are products of pharmaceutical origin and pesticides. Due to the damage caused by these contaminants to human health and the environment, there is a need to develop technologies for their removal in wastewater treatment. An anaerobic membrane bioreactor (AnMBR) is a technology that can potentially remove emerging compounds by combining biological treatment associated with membrane filtration. Thus, this article mapped literature describing the use of AnMBR for removing drugs and pesticides through a bibliometric review. A relatively low number of publications related to this subject was observed in the last ten years. Most of the research has been done in the Middle East, South, and Southeast Asia. The main themes addressed by the articles were the treatment of drugs, bacteria, and antibiotic-resistant genes. In particular, research on the removal of pesticides by AnMBR was scarce.

4.1.2 INTRODUCTION

Contaminants of emerging concern (CEC) are synthetic or natural chemical compounds detected in the water environment due to their increasing use and the development of new analytical technologies (Baudisch et al., 2022). Most contaminants pose serious health risks to humans and other organisms (Ncibi et al., 2017; Vélez et al., 2019). They commonly come from hospital and industrial

effluents, domestic sewage, and agricultural activities. CEC includes endocrine disruptors, microplastics, pesticides, flame retardants, nanomaterials, pharmaceuticals, and personal care products (Dong et al., 2023). They are present in raw and treated domestic sewage and may accumulate in the food chain according to the level of contamination and exposure (Singha & Shukla, 2023).

In systems operating with anaerobic membrane bioreactors, enzymes, and microorganisms catalyze the degradation of organic compounds associated with a micro or ultrafiltration membrane (Krishnan et al., 2023). They can produce high-quality effluents in addition to energy in the form of methane (Kim et al., 2011; Li & Parker, 2021; McCarty et al., 2011). In addition, using AnMBR can expand non-potable reuse wastewater practices safely (Sawaya et al., 2022). The combination of anaerobic membrane bioreactors with adsorption/biodegradation processes presents a potential for the treatment of contaminants of emerging concern, such as pharmaceuticals, pesticides, and polychlorinated compounds, among others (Ncibi et al., 2017).

Pesticides used in household activities, such as pest and weed control in gardens, can be carried into the sewage system and reach the wastewater treatment plants (Li et al., 2023). They can have carcinogenic, mutagenic, neurotoxic, and/or teratogenic effects (McGinley et al., 2023). Pesticides are among the most investigated organic contaminants in the aquatic environment. However, their occurrence and fate during wastewater treatment stages have yet to be thoroughly studied in Brazil (Marson et al., 2022). Brazil is among the highest users of pesticides in the world due to its large agricultural cultivation area. According to data from the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA; 2021), 467 active ingredients were commercialized in Brazil between 2009 and 2021. In 2021, the best-selling pesticides in Brazil, expressed in tons of active ingredients, were glyphosate (219,585.51), 2.4-D (62,165.70), mancozeb (50,340.24), chlorothalonil (38,320.40), atrazine (37,298.57), acephate (35,856.00), malathion (13,291.23), clethodim (9,750.70), sulfur (9,434.95), and s-metolachlor (9,374.02).

Most current wastewater treatment plants are not designed to remove pharmaceuticals or pesticides since conventional treatment focuses on suspended solids, biodegradable organic matter, and ammonia removals. For this reason, these chemicals are frequently discharged and found in the aquatic environment (de Oliveira et al., 2020). The continued introduction of pharmaceuticals into the water cycle and soil causes toxicity, bioaccumulation in organisms and plants, and environmental persistence (Bustos Bustos et al., 2022). Besides, the widespread use of antimicrobials contributes to the spread of antibiotic-resistant bacteria and genes. This topic is a significant concern in wastewater treatment plants (Zhu et al., 2021; Pu et al., 2017). Given their antibacterial nature and high consumption, antibiotics are hardly eliminated in wastewater treatment. According to data from the

Oswaldo Cruz Foundation (FIOCRUZ), Brazil ranks 17th in the world regarding antibiotic consumption. Inadequate prescribing and self-medication are among the causes that contribute to overconsumption (Assef et al., 2019).

Bibliometric analysis is a tool that allows quick and easy visualization of production indicators presented as networks and clusters. It also provides an overview of institutions, countries, research groups, journals, and research trends (Jiménez-Castro et al., 2020). Bibliometric studies can be divided into two categories. The first focuses on scientific production, and the second on understanding interrelationships between countries, institutions, authors, and keywords. In the first category, analyzing global, institutional, or individual scientific production facilitates increased knowledge about the studied topic, favouring the analysis of future trends. The second category allows for identifying degrees of interest in each region and the interrelationship between different research groups. Bibliometric analysis enables the identification of trends and technological advances. It makes it possible to analyze and compare different operating parameters and thus determine optimum operating conditions for the technology under development (Segredo-Morales et al., 2023).

Studies on using anaerobic membrane bioreactors to treat emerging contaminants are needed to control their discharge into the environment (Hu et al., 2022). Considering the discussion mentioned above, this article aimed to map the scientific production and evaluate the publication trends on using AnMBR to remove pesticides and pharmaceuticals from wastewater through bibliometric analysis.

4.1.3 MATERIAL AND METHODS

The Elsevier Scopus database was used to retrieve articles published on the subject. The search was performed on June 26, 2023, using the Boolean operators AND (restricts) and OR (extends) as a strategy. The unique character * (asterisk) was also used in the search term (retrieves terms with the prefix typed). The search expression used was: (“anaerobic membrane bioreactor* " OR " AnMBR* " OR " biorreator* anaeróbio de membrana ") AND (" emerging compound* " OR " emerging contaminant* " OR " emerging pollutant* " OR " contaminant* of emerging concern " OR " contaminante* emergente* " OR " poluente* emergente* " OR " contaminante* de preocupação emergente " OR " pesticide* " OR " herbicide* " OR “ insecticide* ” OR " pharmaceuticals " OR " antibiotic* " OR " agrotóxicos " OR " fármacos "). The search was made on the title, the abstract, and the keywords (TITLE-ABS-KEY). The VOSviewer software was used for cluster determination. It obtained the keyword co-occurrence, journals, cocitation, author, and countries’ network figures. VOSviewer allows the construction and visualization of bibliometric networks. This article used node

representation in which the node's size is proportional to the use of the term, whereas the thickness of the connection line between nodes refers to the strength of occurrence (Segredo-Morales et al., 2023). English, Portuguese, and Spanish documents were considered. However, only papers written in English could be retrieved. This article analyses the evolution of publications over the last ten years (2013 to 2023), presenting the principal authors, journals chosen for publications, collaboration networks, countries, and institutions. Finally, an evaluation was made of the current trends of the research topics that have been published.

4.1.4 RESERCH TENDENCIES ANALYSIS BY BIBLIOMETRICS

The search returned 90 documents. The first filter applied was the choice of "article" documents, resulting in 68 papers. The choice of publication period was restricted to the last ten years and resulted in 65 papers. Finally, the abstracts were read, and documents that applied the use of anaerobic membrane reactors associated with advanced oxidative processes, photochemical processes, and other processes that did not meet the purpose of this bibliometric review were eliminated. This selection left a total of 52 articles published between the years 2013 and 2023 (Table 1).

Table 1. Summary of the steps adopted for the selection of documents used in the bibliometric review

Scopus	Search expression	" anaerobic membrane bioreactor* " OR " AnMBR* " OR " biorreator* anaeróbio de membrana "	Total
		AND	
		" emerging compound* " OR " emerging contaminant* " OR " emerging pollutant* " OR " contaminant* of emerging concern " OR " contaminante* emergente* " OR " poluente* emergente* " OR " contaminante* de preocupação emergente " OR " pesticide* " OR " herbicide* " OR "insecticide* " OR " pharmaceuticals " OR " antibiotic* " OR " agrotóxicos " OR " fármacos "	90
	Filter 1	Article	68
	Filter 2	Publication period 2013-2023	65
	Filter 3	Records removed after abstract review	52
		Total	**52**

PUBLICATION EVOLUTION AND RESEARCH AREAS

Figure 1 shows the progress in the number of publications related to the search term in the last ten years. There was a substantial increase in publications between 2015 and 2018, followed by a decrease in stabilization during 2019-2021 and a new elevation in 2022.

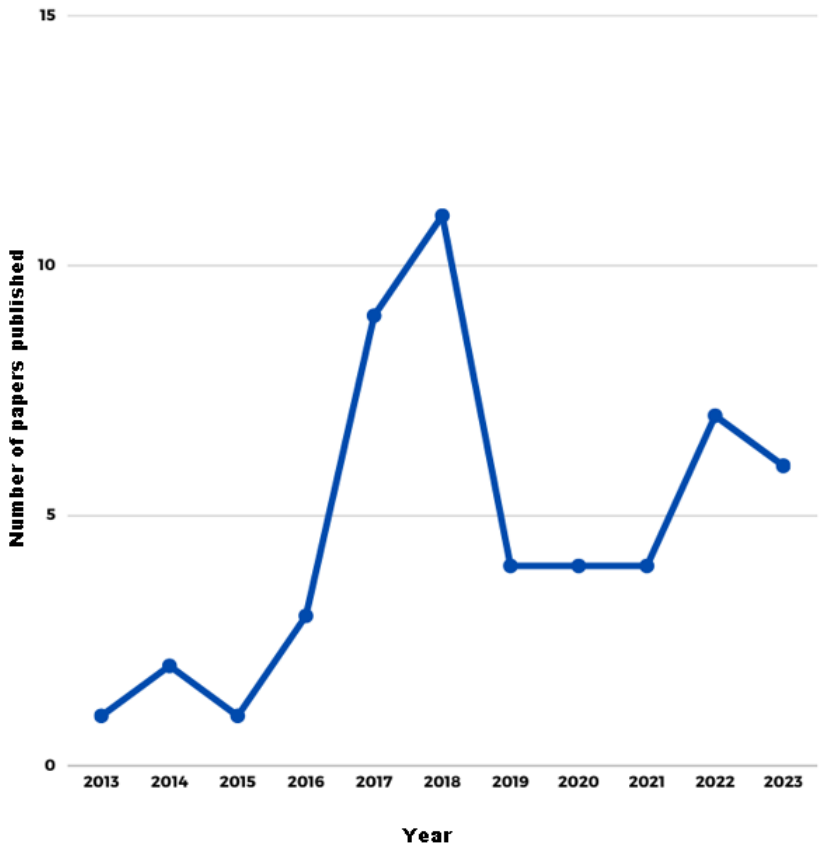


Fig. 1. Evolution of the number of publications related to pharmaceuticals, pesticides, and AnMBR over the last ten years (2013-2023).

Figure 2 shows the distribution of publications according to the ten most addressed areas. It was noted the predominance of the area of Environmental Sciences (36.2% of the publications), followed by the fields of Chemical Engineering (20.5%), Energy (11.8%) and Chemistry (10.2%). Health and environmental issues related to the detection of compounds of emerging concern were the main drivers in the choice of publications on environmental sciences. Publications in the areas related to energy and chemistry were selected for AnMBR articles, as the process generates biogas with potential for energy use. The main topics in the Chemical Engineering area are operating parameters, presence and removal of contaminants, and characterization of the microbial community were the main topics in the Chemical Engineering area. On the other hand, research focused on drugs and

antibiotics were published primarily on medicine and immunology.

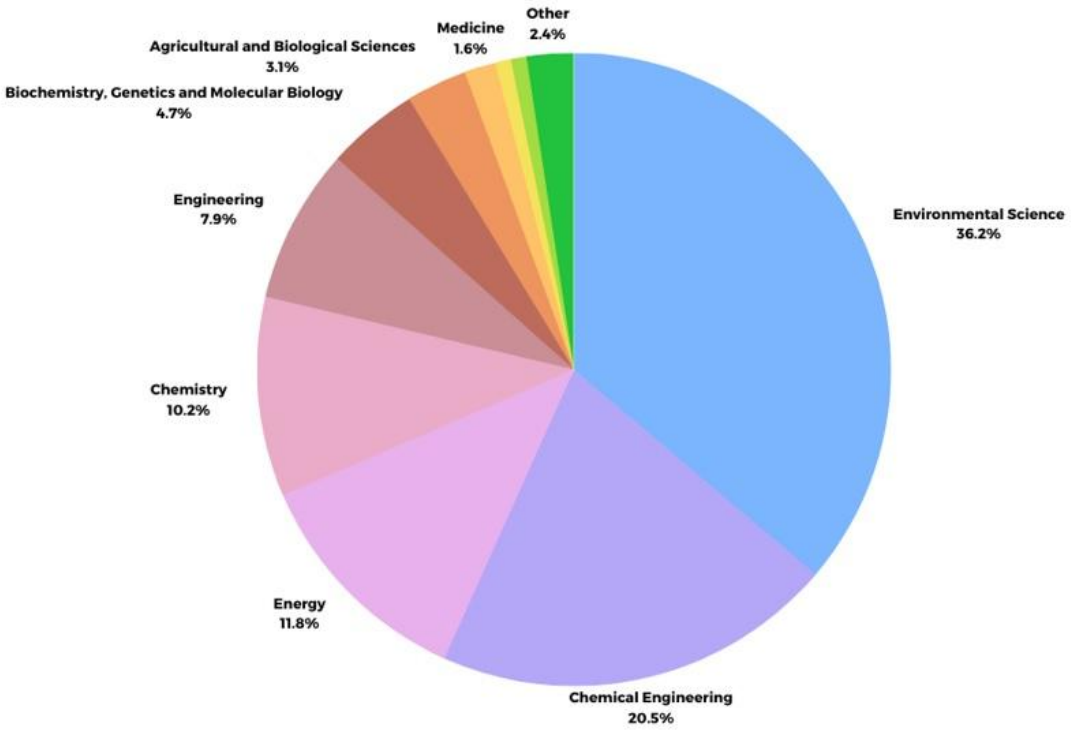


Fig. 2. Ranking of the 10 top publication areas over the last ten years of bibliometric analysis (2013-2023). Environmental Science (36.2%); Chemical Engineering (20.5%); Energy (11.8%); Chemistry (10.2%); Engineering (7.9%); Biochemistry, Genetics and Molecular Biology (4.7%); Agricultural and Biological Sciences (3.1%); Medicine (1.6%); Business, Management and Accountability (0.8%); Immunology and Microbiology (0.8%); Other (2.4%).

STUDY OF MAIN KEYWORDS AND JOURNALS

Figure 3 presents relations groups among the most cited keywords in the publications. It detected 896 keywords in the papers used in the bibliometric review. Of this total, 118 keywords appeared at least four times. The established relationships show four clusters centered on “anaerobic membrane bioreactor” and “wastewater treatment”. The yellow coloring refers to research on genes causing bacterial resistance and drug removal from wastewater in medicine and immunology (Figure 2). The group represented by red coloring presents keywords about energy production (i.e., biofuel, biogas, bacteria, *archaea*, *methanosaeta*). The other groups allude to biochemical and operational parameters associated with environmental sciences and chemistry. It was noticed an absence of keywords related explicitly to pesticides in AnMBR.

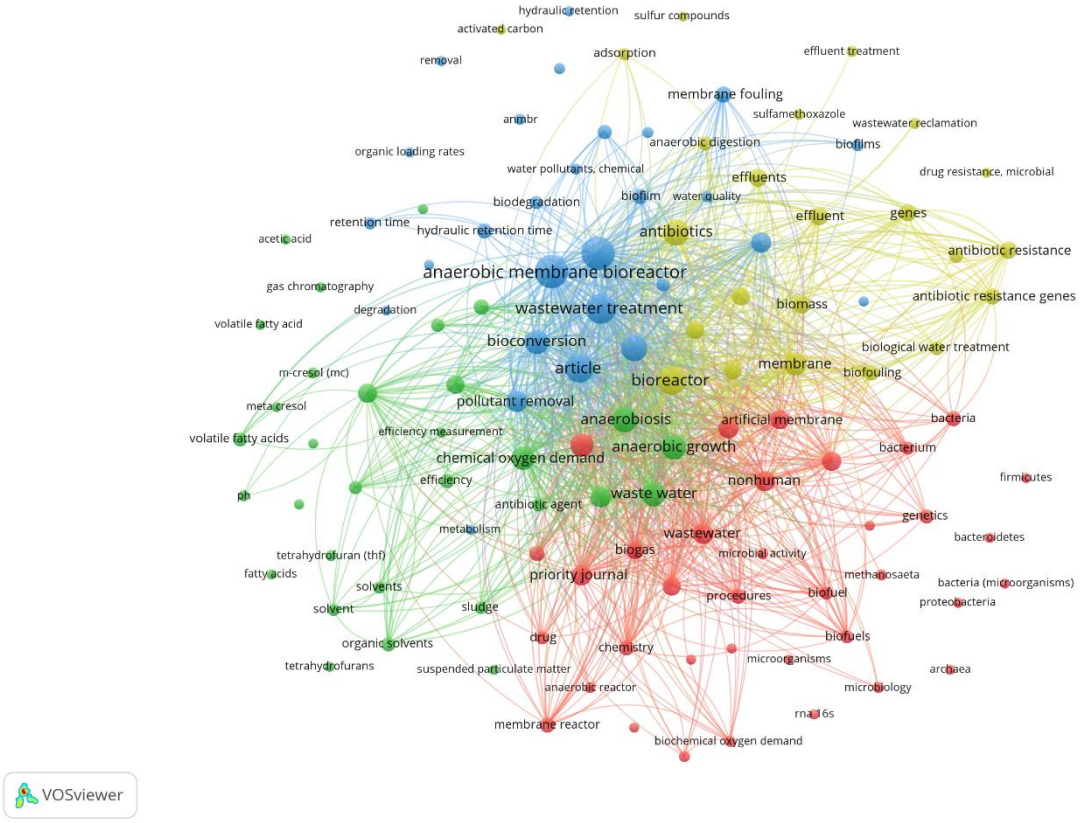


Fig. 3. Clustering of co-occurence of keywords used by authors over the last ten years in publications related to the bibliometric analysis (2013-2023).

Figure 4 shows the top 8 sources of publications of the bibliometric analysis papers in the last ten years. The papers used a total of 27 sources for research dissemination. Of this total, eight journals presented at least two publications. Bioresource Technology stands out with 12 publications. Next comes Chemical Engineering Journal and Environmental Science: Water Research and Technology with four publications each, followed by Chemosphere, Environmental Science and Technology, and Water Research with three publications each. Journal of Hazardous Materials and Science of the Total Environment had two publications each.

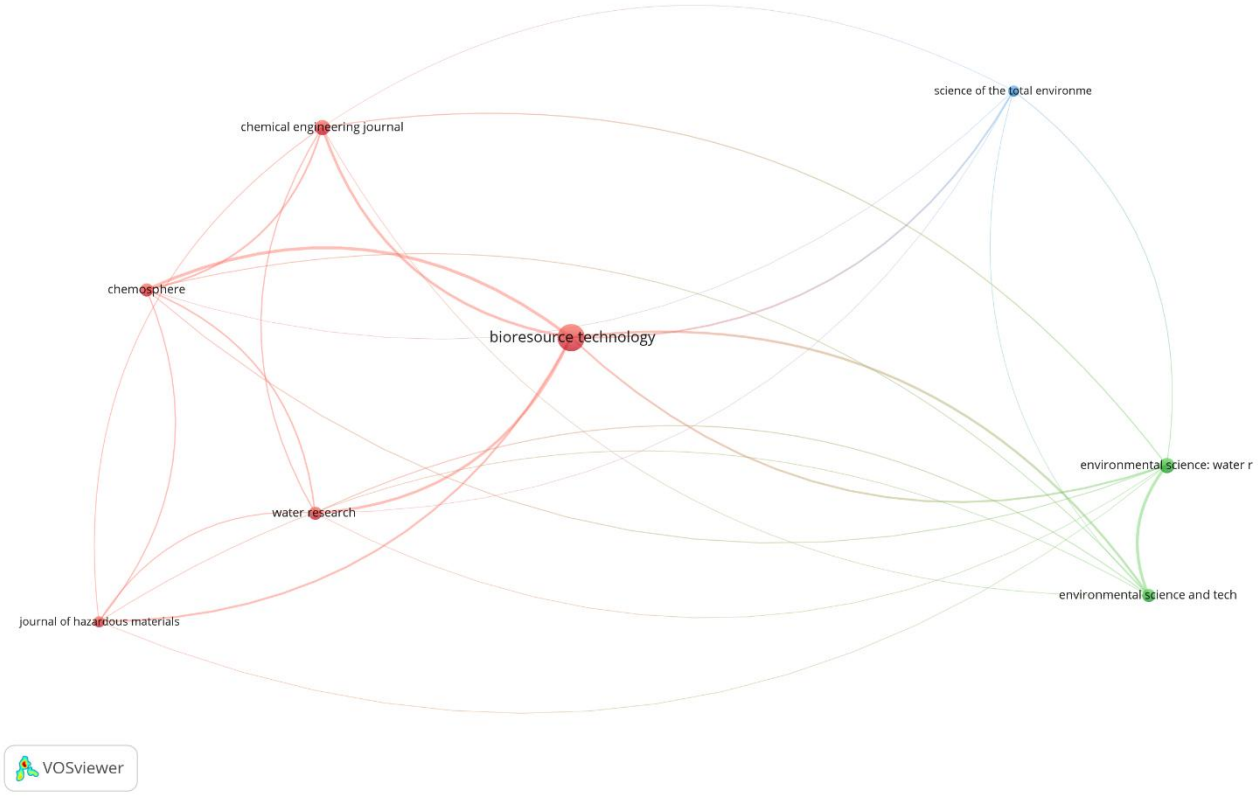


Fig. 4. Top 8 journals of publication related to the bibliometric analysis over the last ten years (2013-2023).

AUTHOR'S NETWORK AND MOST ACTIVE AUTHORS

Figure 5 presents the ten most active authors according to the number of published papers. M. Harb published ten papers, followed by Z. Chen and D. Hu with nine papers each.

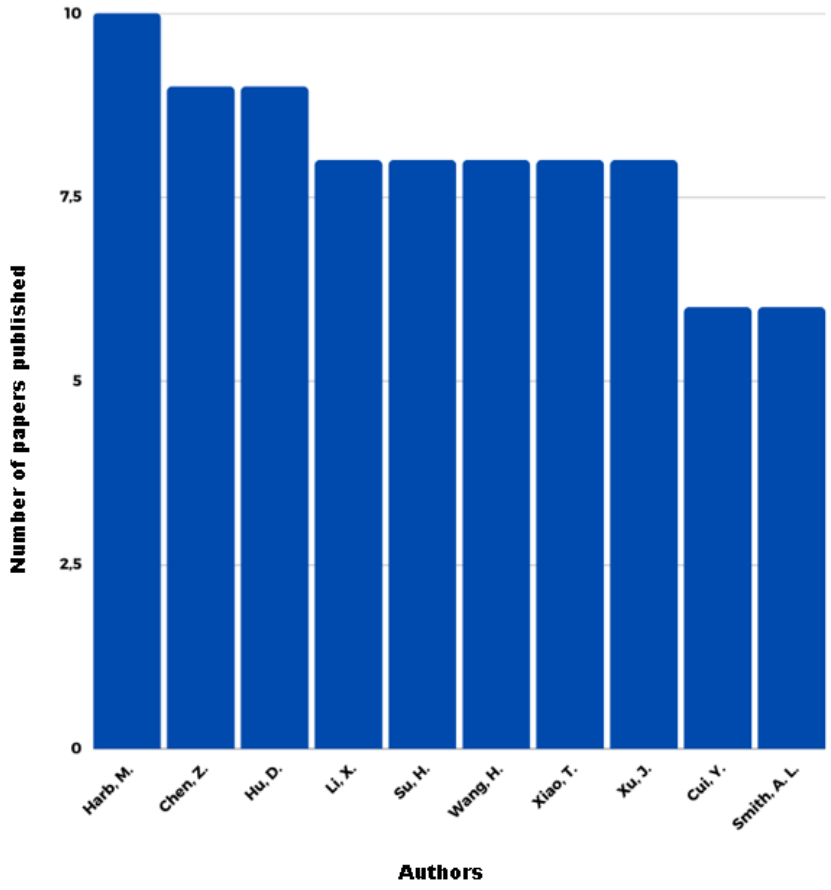


Fig. 5. Top 10 most productive authors over the last ten years in publications related to the bibliometric analysis (2013-2023).

Figure 6 shows the co-citation network among authors. According to the data collected by the bibliometric review, 19 references (out of 2393 total) are cited at least four times in the published papers. Among the most productive authors (Figure 5), two appear in the co-citation network: M. Harb and A.L. Smith. While the former seems restricted to a single co-citation group, A.L. Smith breaks the barrier with the theme of antibiotic-resistant genes. More specific research themes addressed by the clusters are microbial community characterization (yellow group), membrane fouling (green group), and the use of activated carbon in an anaerobic membrane bioreactor (red group). Pharmaceutical wastewater treatment and the removal of antibiotic-resistant genes are common themes in all clusters.

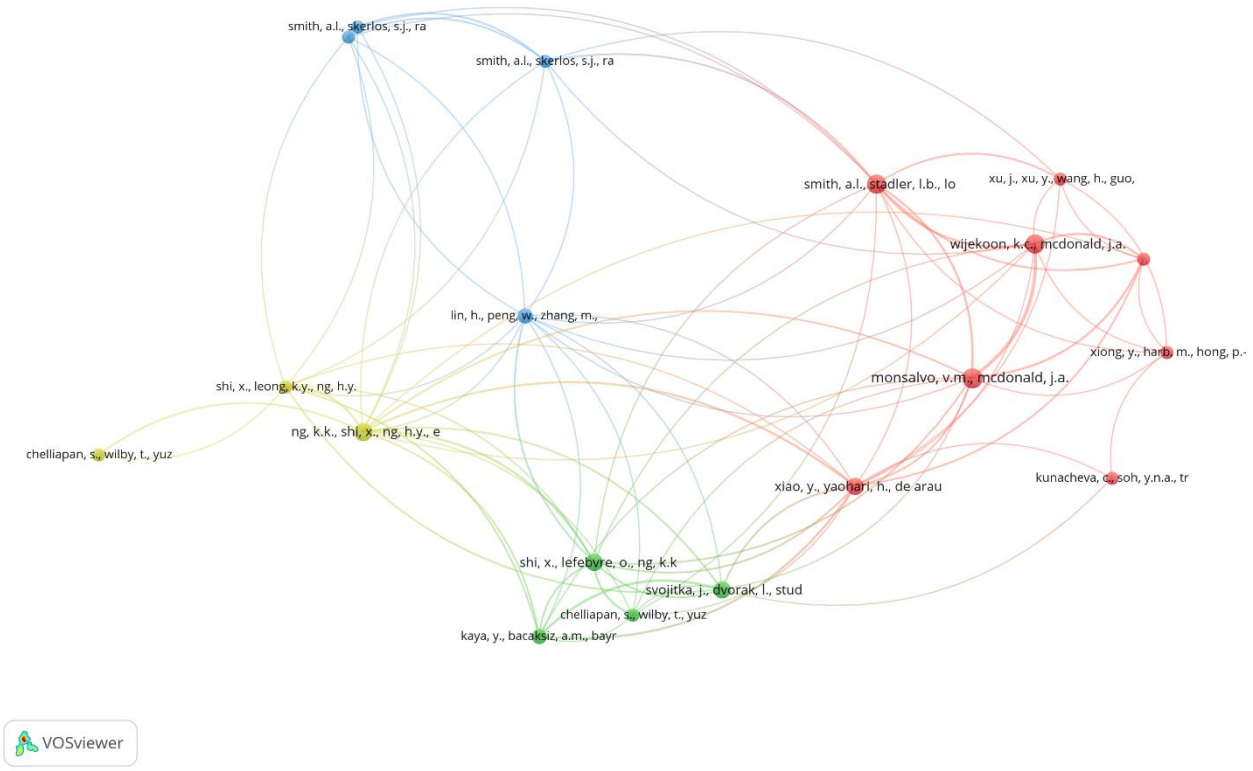


Fig. 6. Cocitation network over the last ten years in publications related to the bibliometric analysis (2013-2023).

Figure 7 presents the connection between the authors. Of the 178 authors publishing the articles surveyed in the bibliometric review, 17 with at least two publications showed some connection. Two groups were formed, mainly by the most productive authors (Figure 5). The author P. Wang appears as the link between the two different groups.

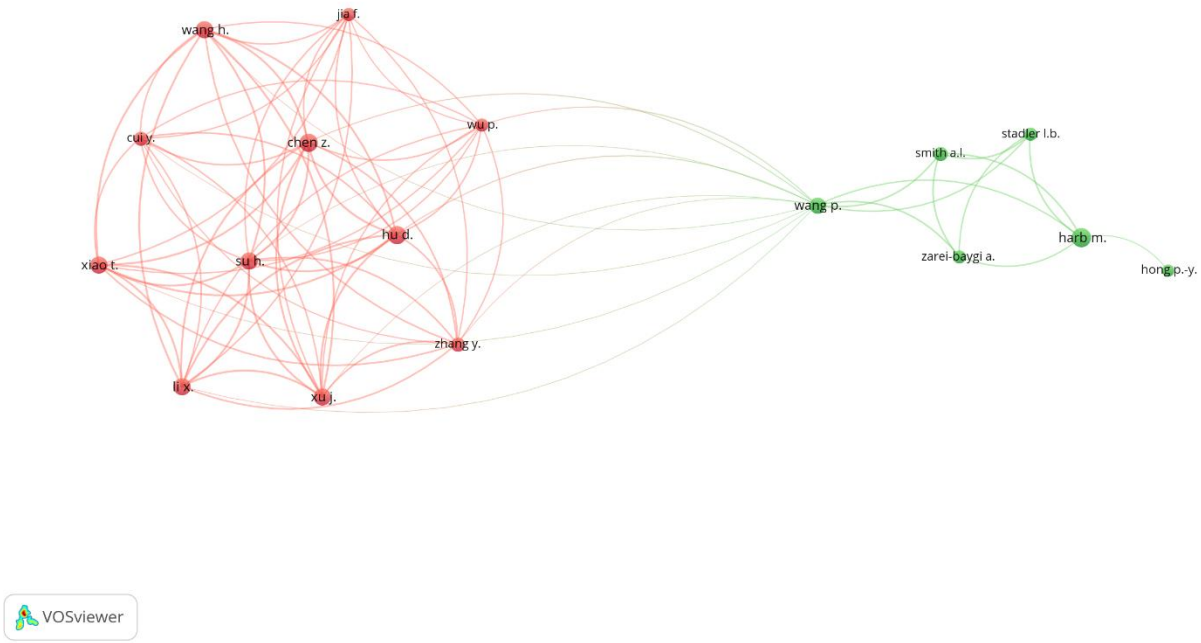


Fig. 7. Most expressive author's network over the last ten years in publications related to the bibliometric analysis (2013-2023).

BIBLIOMETRIC STUDY OF INSTITUTIONS AND COUNTRIES

Figure 8 shows the 12 leading countries with more publications on the bibliometric review in the last ten years. China stands out with 16 publications out of the 52 totals evaluated. Then come the United States (8 publications), Lebanon and Singapore (7 publications each), and the United Kingdom (6 publications). The South and Southeast Asian regions and the Middle East represent half of the countries in the top 12 publications.

Removal of antibiotic-resistant genes and microbial diversity were the topics of most significant interest in Saudi Arabia, Lebanon, and the United States. China and Singapore focused their research on drug removal and treatment. In addition to pharmaceuticals, the UK is studying the impact and treatment of herbicides on the operation of AnMBR.

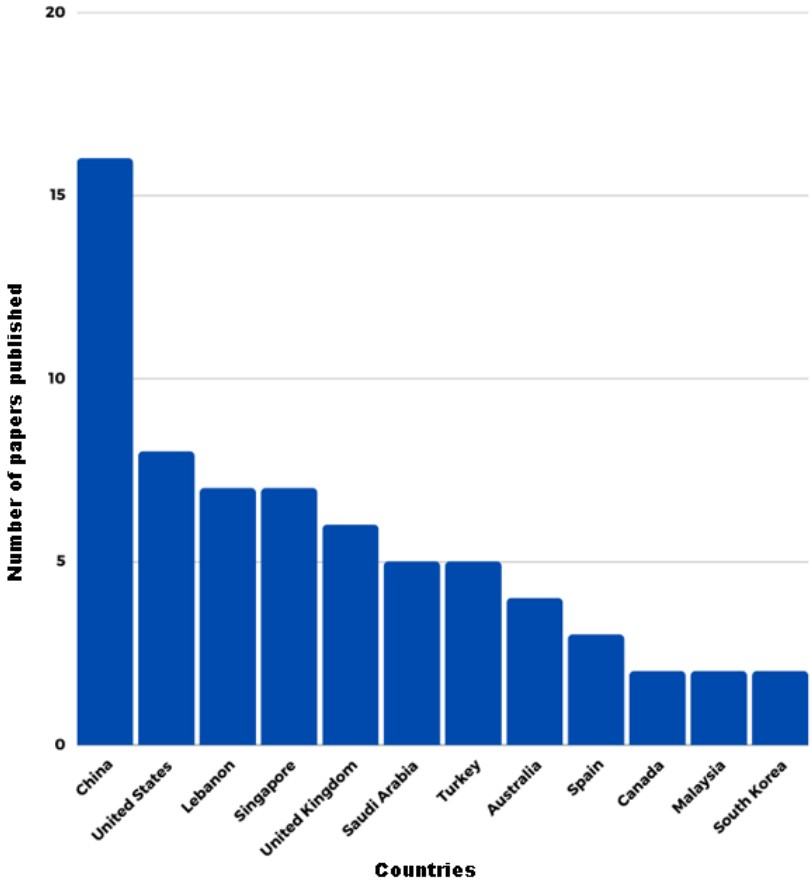


Fig. 8. Top 12 main publication countries related to the bibliometric analysis over the last ten years (2013-2023).

Figure 9 presents the top 10 institutions of affiliation of the authors of the papers related to the bibliometric analysis. Among the institutions, only three were outside the South and Southeast Asia and Middle East regions: the University of Southern California, Rice University, both in the USA, and Imperial College London (UK).

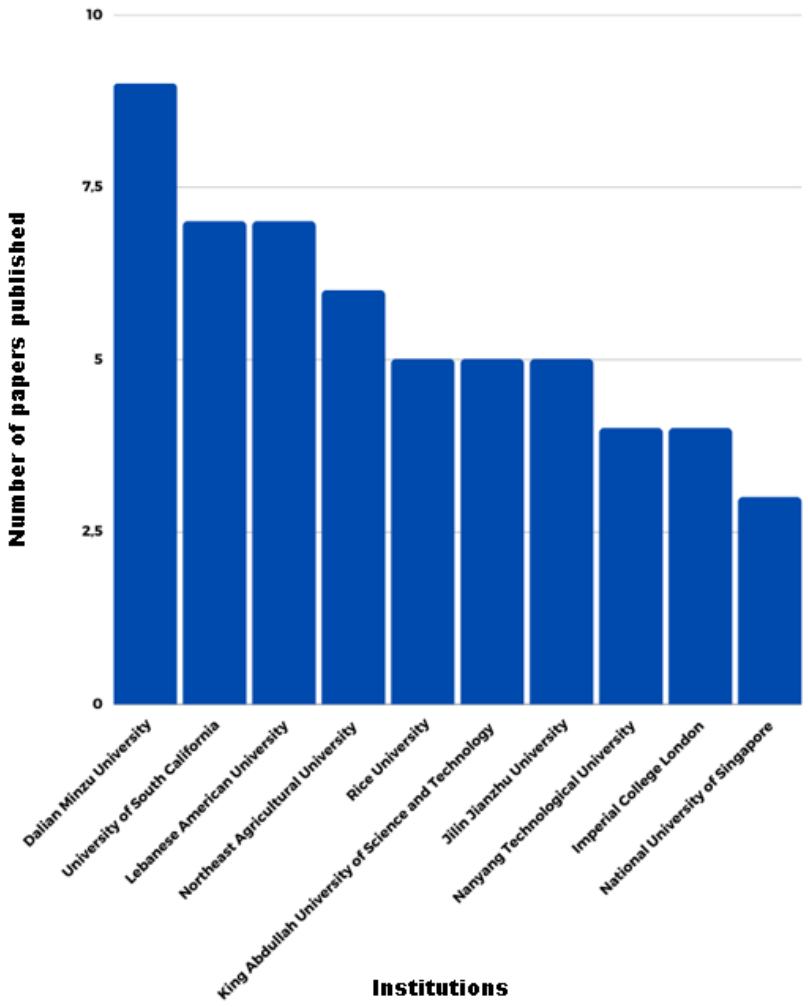


Fig. 9. Top 10 main institutional authors's affiliation related to the bibliometric analysis over the last ten years (2013-2023).

The collaboration network among countries is shown in Figure 10. In all, publications from 19 countries were evaluated in the bibliometric review. Of these, 12 had two or more publications (Figure 8). Among them, nine countries presented some connection (Figure 10). It was observed the presence of 3 clusters of countries: Singapore, United Kingdom, and Malaysia (green coloring); Australia, Saudi Arabia, China, and South Korea (red coloring); and finally, Turkey and Spain (blue coloring).

The cluster formed by the most significant number of countries (China, Saudi Arabia, Australia, and South Korea) collaborated with research on removing sulfonamide-type antibiotics using AnMBRs. In addition to drug removal, Singapore, the UK, and Malaysia collaborated on microbial diversity in reactors and herbicide treatment. Turkey and Spain focused their collaboration on increasing methanogenic activity in AnMBRs, leading to higher biogas production.

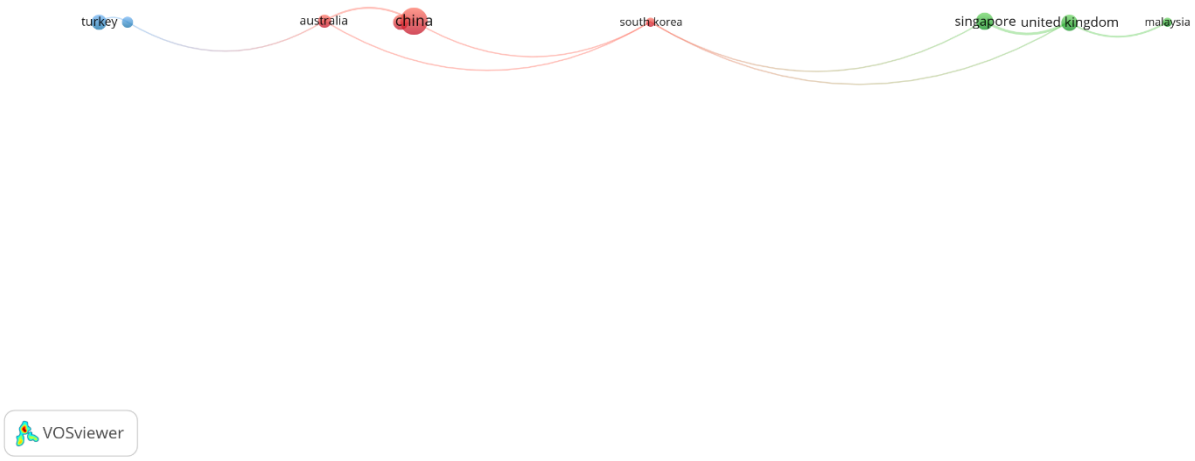


Fig. 10. Collaboration network strength between the countries according to co-authorship related to the bibliometric analysis over the last ten years (2013-2023).

PUBLISHED CONTENTS AND TENDENCIES

Table 2 summarizes the contents of the top 10 cited papers in 2019-2023, excluding self-citation, related to the bibliometric analysis. Treatment of pharmaceutical wastewater, natural or synthetic, is the most common topic among the most cited papers analyzed. The removal of bacteria and antibiotic-resistant genes and the characterization of the microbial community are also among the issues usually addressed. Operational parameters and comparisons between different reactor configurations are other themes the most cited papers address. There needed to be more emphasis on the removal of pesticides in AnMBR compared to the number of studies dedicated to pharmaceuticals.

Table 2. Top 10 cited papers related to the bibliometric analysis over last five years (2019-2023)

Ranking	Title	Issue	Key Findings	Total Citations	Publication Year	Reference
1	Removal of trace organics by anaerobic membrane bioreactors	To study the biological removal of 38 organic compounds, including pharmaceuticals, endocrine disruptors, and pesticides, in an AnMBR[1]	Only 9 out of 38 compounds achieved removal rates above 90%, and 23 reached removal lower than 50%. Removal occurred in a biological path, while some compounds were partially adsorbed, retained on the flocs, or deposited on the membrane.	126	2014	Monsalvo et al.
2	Evaluation of system performance and microbial communities of a bioaugmented anaerobic membrane bioreactor treating pharmaceutical wastewater	To evaluate the performance of an AnMBR in treating pharmaceutical wastewater, focusing on membrane fouling, biogas yield, and microbial community characterization. Two configurations of AnMBR, conventional and bioaugmented, were compared.	The bioaugmented AnMBR achieved higher total COD[2] removal efficiency and methane yield, attributed to lower inhibition of microorganisms by the saline environment and the presence of recalcitrant compounds. It also achieved longer membrane operating time. The predominant methanogenic species were *Methanosaeta* and *Methanolobus* in the conventional and bioaugmented AnMBR, respectively.	89	2015	Ng et al.
3	Removal of selected pharmaceuticals in an anaerobic membrane bioreactor (AnMBR) with/without powdered activated carbon (PAC)	Investigate the removal of five pharmaceutical compounds (trimethoprim, sulfamethoxazole, carbamazepine, diclofenac, and triclosan) in laboratory-scale AnMBR using synthetic wastewater.	Adding powdered activated carbon increased the removal of the five drugs studied. Adsorption onto the sludge was the significant contributing factor to the initial removal of the compounds.	89	2017	Xiao et al.

4	Performance of an anaerobic membrane bioreactor for pharmaceutical wastewater treatment	To test the treatment efficiency and identify inhibitory factors of sewage and organic solvent waste from the chemical and pharmaceutical industry with AnMBR on a pilot scale.	The low concentration and varying composition of the industrial wastewater decreased the treatment efficiency. Adding organic solvents caused acidification, low methane production, and a drop in the methanogenic population.	83	2017	Svojitka et al.
5	A novel application of anaerobic bio-entrapped membrane reactor for the treatment of chemical synthesis-based pharmaceutical wastewater	Investigating the treatment performance and stability of conventional and bio-entrapped AnMBR with high organic load using synthetic pharmaceutical wastewater	Bio-entrapped AnMBR showed higher COD removal (5-10% higher) and higher methane yield (15% higher) than conventional AnMBR. Decreasing HRT[3] resulted in decreasing both factors. Membrane fouling was higher in the bio-entrapped AnMBR.	78	2014	Ng et al.
6	Treatment of pharmaceutical wastewater containing B-lactams antibiotics by a pilot-scale anaerobic membrane bioreactor (AnMBR)	Treat real pharmaceutical wastewater using AnMBR with different organic loadings, evaluating pollutant removal, VFAs[4] accumulation, alkalinity, and biogas. Developed a mathematical model for antibiotics and COD removals.	The increased organic load resulted in increased COD removal, with increased accumulation of VFAs, biogas, and methane production. An increase in the removal of antibiotics (amox, ceft, cefo, and ampi) was also observed. The developed mathematical model fitted well with the experimental results.	74	2018	Huang et al.
7	Removal of antibiotic-resistant bacteria and antibiotic resistance genes affected by varying degrees of fouling on anaerobic microfiltration membranes	To evaluate the removal efficiency of ARBs[5] and ARGs[6] in AnMBR with different transmembrane pressures (20, 40 and 60 kPa) and fouling stages.	Increasing transmembrane pressure decreased ARB's removal for the subcritical fouling state. Both transmembrane pressure and fouling state synergistically affected	71	2017	Cheng & Hong

			ARB removal. However, the foulant layer was the most determining factor for ARG removal.			
8	Effects of salinity build-up on the performance of an anaerobic membrane bioreactor regarding basic water quality parameters and removal of trace organic contaminants	To investigate the effect of salinity on the performance of AnMBR in removing 33 trace organic contaminants (representing four groups: pharmaceuticals, pesticides, personal care products, and industrial chemicals).	High salinity negatively affects the treatment performance of AnMBR. The removal of COD, TOC[7] and biogas production was reduced with increasing salinity. This also reduced the removal of trace organic contaminants.	61	2016	Song et al.
9	Evaluating antibiotic resistance gene correlations with antibiotic exposure conditions in anaerobic membrane bioreactors	To study the effect of antibiotics at various concentrations on the presence and abundance of antibiotic resistant genes in AnMBR system.	Different ARG profiles were observed in the biomass and effluent. Genes conferred resistance to the biomass's macrolides and sulfonamides in the effluent.	56	2019	Zarei-Baygi et al.
10	Biological performance and fouling mitigation in the biochar-amended anaerobic membrane bioreactor treating pharmaceutical wastewater	To achieve AOX[8] removal and membrane fouling mitigation simultaneously, using a control AnMBR system without biochar and a biochar-amended AnMBR treating pharmaceutical wastewater	The biochar-amended AnMBR enhanced the performance of treating the pharmaceutical wastewater with higher COD and AOX removal efficiencies.	55	2020	Chen et al.

AnMBR[1]: Anaerobic Membrane Bioreactor; COD[2]: Chemical Oxygen Demand; HRT[3]: Hydraulic Retention Time; VFAs[4]: Volatile Fatty Acids; ARBs[5]: Antibiotic Resistent Bacterias; ARGs[6]: Antibiotic Resistent Genes; TOC[7]: Total Organic Carbon; AOX[8]: Adsorbable Organic Halogens.

Table 3 shows the last ten papers published and retrieved by this bibliometric analysis regarding operational parameters and contaminants of emerging concern studied. Among the most published studies, it was observed that the type of anaerobic reactor mainly used to make up the AnMBR system was the continuously stirred-tank reactor (CSTR) or other types of reactors that also use continuous mixing. This may happen because, in complete mixing reactors, around 80% of the biodegradable fraction in the sludge can be converted and captured as biogas (KEHREIN et al., 2020) since enhanced biogas generation is very desirable in the AnMBR operating process.

The use of fluidized bed reactors in AnMBRs, as observed in expanded granular sludge bed (EGSB) configuration and experiment developed by Chyoshi et al. (2022), can improve mass transfer and prevent the loss of biomass (microorganisms) when the reactor is operated with a low hydraulic retention time. Keeping the biomass in the reactor makes it possible to maintain an adequate cell detention time for the degradation of organic matter (MCCARTY *et al.*, 2011).

Besides aiding the process of removing CEC, granular activated carbon can be used as a fluidized support medium for biological growth in reactors (KIM et al., 2011). Activated carbon before the membrane filtration process increases treatment performance (KEHREIN et al., 2020). Besides improving CEC removal, Balcıoğlu et al. (2023) used powdered activated carbon to minimize membrane fouling. Pleyri et al. (2023) studied iron addiction as a strategy to control membrane fouling along with phosphorous recovery.

In all the experiments, the use of an external membrane module was adopted, with a balance between the use of micro and ultrafiltration membranes. In anaerobic bioreactors, the membrane module can be submerged inside the reactor, but installing an external membrane module makes maintenance easier. Through membrane separation, organic matter is removed mainly by biosorption or biodegradation, and the retained compounds can be further degraded with increased retention time (HU et al., 2022). Microfiltration (MF) is used to separate larger molecules such as oils, bacteria, and colloids and can be carried out at low pressure. Viruses and macromolecules can be separated by ultrafiltration (UF) (AGRAWAL et al., 2023).

Table 3. Last 10 papers published retrieved by the bibliometric analysis.

Title	Issue	Initial Operational Parameters							CEC[5]	Removal Performance	Ref.[16]
		Reactor	V[1] (L)	HRT[2] (h)	SRT[3] (d)	Membrane (Area)	Flux ($Lm^{-2}h^{-1}$)	Temp.[4] (°C)			
Anaerobic membrane bioreactor for real antibiotic pharmaceutical wastewater treatment: Positive effect of fouling layer on antibiotics and antibiotic resistance genes removals	Evaluate the effect of biofilm in the presence of antibiotics and ARGs in real pharmaceutical wastewater treated by AnMBR[10]	EGSB[11] (LS[12])	1.6	36	200	UF[13] 112.5 cm^2	4.0	32-38	Pharmaceuticals; ARG[14]	59.2-60.2%	Wang et al. (2023)
Antibiotic removal from swine farming wastewater by anaerobic membrane bioreactor: Role of hydraulic retention time	Evaluate the impact of HRT on the performance of AnMBR treating animal wastewater regarding antibiotic removal	CSTR	5.0	24-48	120	MF NA	2.0-1.0	NA	Pharmaceuticals	21-99%	Liu et al. (2023)
Decreasing hydraulic retention time of anaerobic membrane bioreactor: Effect on core genera and microbial contaminants removal	Study the effect of decreasing the HRT on the core genera associated with AnMBR operating with constant organic load and contaminant removal	Reactor following an attached-growth configuration (PS[15])	35	12-10-8	Infinite	MF 1.4 m^2	NA	35	ARG	NA	Medina et al. (2023)
Enhancing the performance of AnMBR treating municipal	Investigating the performance of AnMBR treating low organic load municipal	Fully mixed reactor (LS)	40	6	50	NA 0.5 m^2	13.0	15-25	Endocrine-disrupting chemicals; Pharmaceuticals	10-40%	Plevri et al. (2023)

wastewater at a high organic loading rate with iron addition	sewage with the addition of iron chloride for fouling control and phosphorus removal										
Anaerobic membrane bioreactor-based treatment of poultry slaughterhouse wastewater: Microbial community adaptation and antibiotic resistance gene profiles	Examining the adaptation of the microbial community in an AnMBR system treating poultry slaughterhouse wastewater	CSTR	3.5	1728-840	360	MF 57 cm^2	6.49	35	ARG	NA	Ramadan et al. (2023)
Effect of powdered activated carbon addition on membrane performance and fouling in anaerobic membrane bioreactor	Evaluate the increase in treatment performance and decrease in fouling of an AnMBR system treating pharmaceutical wastewater with the addition of activated carbon	The reactor consisted of the fermenter control unit, peristaltic pumps, mechanical mixer, pH probe, temperature and level sensor, and heating jacket	4.0	NA	NA	UF 66 cm^2	NA	NA	Pharmaceuticals	≤ 96%	Balcıoğlu et al. (2023)
Metagenomic analysis of the antibiotic resistance risk between an aerobic and anaerobic membrane bioreactor	Evaluating the microbial community and antibiotic resistance in the treatment of synthetic sewage using anaerobic and aerobic membrane bioreactors operated in parallel	CSTR[6] (BS[7])	5.0	16	300	MF[8] 0.015 m^2	NA[9]	25	Pharmaceuticals	NA	Wang et al. (2022)

Effects of influent municipal wastewater microbial community and antibiotic resistance gene profiles on anaerobic membrane bioreactor effluent	Examining the microbial community profile and the presence of antibiotic-resistant genes in real municipal wastewater treated by AnMBR	CSTR	3.0	33	360	MF 0.057 m^2	6.7	35	ARG	NA	Sawaya et al. (2022)
Bacteriophage cocktail as a promising bio-enhancer for methanogenic activities in anaerobic membrane bioreactors	Evaluate interactions between a pyophage cocktail and the indigenous microbial community of a biofilm layer in AnMBR regarding to antibiotic resistant bacteria	Reactor with continuous mixing (LS)	5.0	48	30	UF 0.125 m^2	NA	35	Pharmaceuticals; ARG	86.1-99%	Aydin et al. (2022)
Fate and removal of emerging contaminants in anaerobic fluidized membrane bioreactor filled with thermoplastic gel as biofilm support.	Evaluating the performance of a hybrid membrane-based fluidized anaerobic bioreactor using polyurethane support media in the removal of emerging contaminants	Hybrid membrane-based fluidized anaerobic bioreactor (LS)	0.2	12.9-12.7-6.5	15	UF 62 cm^2	2.0-3.0-5.6	NA	Hormones; Pharmaceuticals	5-93%	Chyoshi et al. (2022)

V[1]: Volume; HRT[2]: Hydraulic retention time; SRT[3]: Sludge retention time; Temp[4]: Temperature; CEC[5]: Contaminant of emerging concern; CSTR[6]: Continuously stirred-tank reactor; BS[7]: Bench-scale; MF[8]: Microfiltration; NA[9]: Not available; AnMBR[10]: Anaerobic membrane bioreactor; EGSB[11]: Expanded granular sludge bed; LS[12]: Lab-scale; UF[13]: Ultrafiltration; ARG[14]: Antibiotic resistance genes; PS[15]: Pilot-scale; Ref[16]: Reference

The use of membrane filtration helps to avoid the loss of microorganisms, which is essential in the process due to the low growth rate of anaerobes (HU et al., 2022). Although membrane-based filtration processes are associated with high energy costs, the low viscosity of water in hot climates is a factor that contributes to lower energy requirements (KEHREIN et al., 2020).

Another operational characteristic commonly observed in the studies was adopting the mesophilic regime (20 to 45°C). The mesophilic regime is the one that achieves the most outstanding efficiency in anaerobic treatment, with a temperature of 35°C being the most suitable for reactor operation (SAFLEY & WESTERMAN, 1992; MCCARTY, 1964). Low temperatures are commonly pointed out as barriers to anaerobic sewage treatment (MCCARTY et al., 2011).

In addition to energy recovery, nutrients are also a point of interest among published works. However, nitrogen and phosphorus removal tends to be limited due to the need for a favorable environment and conditions and specific microorganisms for nutrient removal (HU et al., 2022). Despite this, phosphorus removal in AnMBRs can be achieved using coagulants. Nitrogen control, on the other hand, is a challenge due to the need for a nitrification step (LEE & LIAO, 2021).

Regarding the scale of the reactors, those with a volume of around 5 L are the most used. Control of sludge age and hydraulic retention times, as well as a good level of mixing of the concentration of suspended solids in the liquid, can favor using smaller reactors. On the other hand, issues associated with operating costs, equipment, maintenance, and cleaning should also be considered when choosing a reactor (KEHREIN et al., 2020).

The predominance of drugs, especially antibiotics, and ARG as the most studied CECs in research is notable. Regarding removal rates, a high variability (5 to 99%) between the papers is observed. It happened especially due to differences in the chemical composition of CEC or operational parameters, such in Liu et al. (2023) and Medina et al. (2023), which found high-performance removal with increased HRT.

AnMBR removes drugs through different processes, such as biodegradation, sorption in the sludge, and physical membrane retention. The physicochemical characteristics of the compounds and the operating conditions of the bioreactor are responsible for the process and the efficiency it can achieve. In addition, the transformation products of the compounds during the removal process can be formed naturally by degradation or by photochemical or biochemical processes to which the original compounds have been subjected (MATSUBARA et al., 2020).

Antibiotics are removed by adsorption, biodegradation, and the separation process. The microbial community present maintains stability in the system by gradually acclimatizing bacteria with potential for antibiotic resistance due to the longer sludge retention time. Antibiotics are not retained in micro or ultrafiltration membranes due to the size of their molecules. However, the high biomass concentration provides a stable environment for the growth of the microbial community

(ZHU et al., 2021; ZHOU et al., 2016; TRINH et al., 2016).

Biological treatment can increase the abundance of ARGs instead of removing them due to the accumulation generated in the sludge during the process. Removal routes and mechanisms in biological and combined treatments are not yet fully elucidated (ZHU et al., 2021; PU et al., 2018). Papers focus on quantification or the presence of ARG in AnMBR instead of removal to verify treatment efficiency.

4.1.5 CONCLUSION

This bibliometric review broadened the knowledge about publications on "removing pharmaceuticals and pesticides in anaerobic membrane bioreactors". According to the data retrieved by the bibliometric search and analysis, the topic has a relevant scientific interest. However, the number of publications during the last ten years was relatively small, and some research gaps, such as the lack of studies with pesticides or using different types of contaminants of emerging concern at the same time and the use of pilot-scale AnMBR, were identified.

A prevalence of research topics related to removing pharmaceuticals and genes, and antibiotic-resistant bacteria in AnMBR was observed. On the other hand, there is a scarcity of studies about the fate and the removal of pesticides. Countries, institutions, and authors from the Middle East, South, and Southeast Asia were the most active in publishing. Still, authors from Europe, the USA, Canada, and Australia also have made relevant contributions to the subject.

The results retrieved by this bibliometric review revealed a need for more Brazilian articles and research on pharmaceuticals and pesticide removal in anaerobic membrane bioreactors. Brazil is one of the leading countries in the use of pesticides and pharmaceuticals, and their presence in the water environment is a significant concern. Anaerobic membrane bioreactors might be an alternative to produce high-quality effluents, especially considering the warmer average temperatures in most of the country.

Continuous mixing and bench-scale reactors with an external membrane module operated at mesophilic temperatures were the most common configurations among the published works. Among the CECs studied, drugs and resistant genes stand out from the other types. The characterization of the microbial community is a common theme related to biogas generation in AnMBR. Measures to control fouling and recover nutrients during the process are trends observed in the papers.

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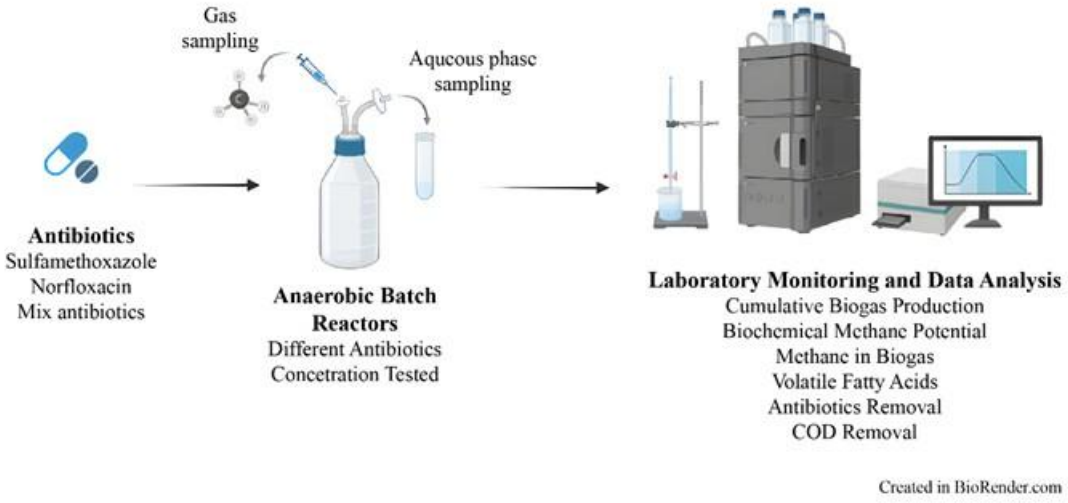
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4.2 Evaluation of anaerobic digestion of antibiotics in batch reactors: effect on methane and biogas production, operating parameters, and aqueous phase removal efficiency

Artigo publicado no periódico *International Journal of Environmental Research*, da Editora Springer Nature, com a seguinte referência: Gomes Ribeiro, M. H., Duarte Soroka, V., Konrad, O., de Almeida Silva, M. C., & Domingues Benetti, A. (2026). Evaluation of Anaerobic Digestion of Antibiotics in Batch Reactors: Effect on Methane and Biogas Production, Operating Parameters and Aqueous Phase Removal Efficiency. *International Journal of Environmental Research*, *20*(1), 27. https://doi.org/10.1007/s41742-025-00986-2. Vale ressaltar que o texto aqui apresentado pode diferir em relação ao publicado pelo periódico devido a correções realizadas. Para a leitura da forma mais atualizada, recomenda-se consulta ao site do respectivo periódico e/ou editora. O Apêndice A contém imagens referentes ao experimento em questão.

Graphical Abstract



4.2.1 ABSTRACT

Antibiotics pose a challenge to conventional wastewater treatment processes, which generally have limited capacity to remove them. High and indiscriminate consumption combined with improper disposal are how these compounds reach the environment. Anaerobic digestion (AD) is a biological process that can be used as an alternative for removing antibiotics from wastewater. In addition to eliminating the

compounds, other advantages of AD are the recovery of biogas produced during the process for energy purposes, along with a reduction in greenhouse gas emissions into the atmosphere. Despite its potential, there is still a need for further studies on the removal of antibiotics through biological processes such as AD. The objective of this study was to evaluate the AD for the degradation of the antibiotics sulfamethoxazole (SMX) and norfloxacin (NOR). Batch reactors were used under controlled operating conditions. Reactors containing different concentrations of antibiotics, as well as mixed antibiotics, were evaluated. Operational parameters of cumulative biogas production, biochemical methane potential, percentage of methane in biogas, volatile fatty acids, chemical oxygen demand, and removal of antibiotics from the aqueous phase were analyzed. Results indicated that the type and concentration of antibiotics influenced cumulative biogas production. The highest values for cumulative biogas production and biochemical methane potential were for reactors with mixed antibiotics. The percentage of methane in biogas remained within the range considered normal for all samples. The concentration of SMX did not influence the removal rate, while for NOR, a reduction in the removal rate from the aqueous phase was observed with increasing concentration.

4.2.2 INTRODUCTION

Contaminants of emerging concern (CECs) are chemical substances that can potentially cause metabolic damage to living beings. They are considered emerging because of their diversity and generally complex chemical composition, as well as the lack of regulations, standards, and studies. Their potential risks are not fully understood, even though they are already widely used (Sauvé & Desrosiers, 2014; Ncibi et al., 2017; Vélez et al., 2019). Examples of widely used CECs include pharmaceutical products such as antibiotics.

Factors such as their complex nature and antibacterial properties, in addition to their high consumption, make antibiotics difficult to eliminate in wastewater treatment plants (WWTPs). Antibiotics are divided into classes based on their chemical structure and functionality. Antibiotics of the sulfonamide class, such as sulfamethoxazole (SMX), are the most worrying detected in WWTPs (Mishra et al., 2023). Due to their characteristics, such as their low susceptibility to chemical and enzymatic hydrolysis, these substances are often found in wastewater (Saha et al., 2023; Jiang et al., 2019; Xiong et al., 2019). Antibiotics of the quinolone class, such as norfloxacin (NOR), are among

the five most used globally (Meng et al., 2023; Zou et al., 2022). Their presence in wastewater represents a potential danger to the environment due to their recalcitrant nature and low solubility in water (Orimolade et al., 2023).

Anaerobic digestion (AD) has the potential to effectively remove antibiotics from the environment (Aziz et al., 2021). AD is a complex process that involves the conversion of organic matter into methane (CH4), carbon dioxide (CO2), and other gases by a wide variety of microorganisms, especially syntrophic bacteria (Cheng et al., 2019). During the last stage, methanogenesis, the carbon contained in the biomass, is transformed into CO2, hydrogen (H2), and methane gas by methanogenic archaea (Kunz et al., 2022). AD is a biological process carried out by microorganisms that interact with each other through multiple interdependent processes, in which the substrate from one stage will play a role in the next. As a substrate for growth, microorganisms can use antibiotics, for example, as carbon and energy sources. However, as these compounds are usually present in low concentrations, another primary substrate is used to sustain microbial abundance (Azizan et al., 2021). In this context, primary substrates can be understood as substances with simpler chemical structures that are easily assimilated by microorganisms, such as simple sugars. According to Gaballah et al (2024), the removal and effects of antibiotic mixes in the AD process, such as their interference in the production of biogas and CH4, have not yet been fully explored.

Therefore, this study aimed to evaluate the anaerobic digestion of antibiotics. Experiments were carried out with batch anaerobic reactors considering different concentrations of SMX and NOR antibiotics. Mixed antibiotics were also assessed. Attention was given to the effects of antibiotics on parameters related to biogas and methane production, as well as evaluating their removal from the aqueous phase. Thus, the study intended to contribute to the generation of knowledge and elucidation of the effects that antibiotics can cause on the AD process and its efficiency in removing them from wastewater.

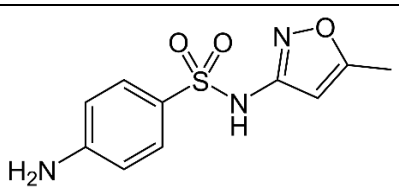
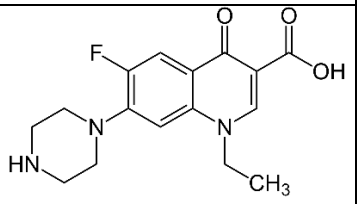
4.2.3 MATERIAL AND METHODS

Antibiotics

Two veterinary antibiotics purchased for research purposes were used: sulfamethoxazole (purity >99%, lot. 21890817, origin: India) and norfloxacin (purity

>99%, lot. 210415-1, origin: China). The physicochemical properties of the antibiotics sulfamethoxazole (SMX) and norfloxacin (NOR) are shown in Table 1.

Table 1. Antibiotics' physical-chemical properties.

Physical-chemical properties	SMX	NOR
Class	Sulfonamide	Quinolone
Molecular formula	$C_{10}H_{11}N_3O_3S$	$C_{16}H_{18}FN_3O_3$
Core structure		
Peso molecular (g/mol)	253.28	319.33
Solubility in water (mg/L)	610 at 37 ºC	280 at 25 ºC
Half-life (days)	0.42	0.15
pKa	1.85;5.70	6.34;8.75
logKow	0.89	0.46
Kd (L/kg)	280;370	1242;4930

Source: PubChem (2024); Note: pKa = dissociation constant; logKow = octanol-water partition coefficient; Kd = sludge adsorption coefficient

Operational conditions of anaerobic batch reactors

Laboratory-scale glass reactors with a working volume of 500 mL were used and operated at a controlled mesophilic temperature (35°C) for 15 days. The period for conducting the experiments (15 days) was established based on the point when daily biogas production was equivalent to 1% of the total volume of biogas produced during the entire experiment (Guedes et al., 2024; VDI 4630, 2006). The contents of the reactors were made up of a combination of inoculum, substrate, and different concentrations of antibiotics, which were calculated based on the amount of organic matter inserted into the reactor according to the total volatile solids (VS) content. After adding inoculum, substrate, and antibiotics, the reactors were sealed to ensure anaerobic conditions. The sealing system had the function of preventing oxygen entry, and the reactors remained closed until the end of the experiment. In case of unexpected oxygen presence, the detection would be indicated by the monitoring results.

Anaerobic sludge from a UASB reactor utilized to treat agro-industrial waste was used as inoculum. The substrate was a mix of glucose, sodium acetate, and peptone in the

proportions 16:9:3, adapted from Tadkaew et al. (2011). Each reactor had 6.87 g/VS, with 4.60 g/VS referring to the inoculum and 2.27 g/VS to the substrate, i.e., the inoculum/substrate ratio used for all reactors was 0.5. Thus, each reactor had an organic loading rate (OLR) equal to 13.8 gVS/L.

SMX concentrations were 20, 100, and 200 mg/L, while 10, 40, and 80 mg/L were used for NOR. Reactors containing samples with the two antibiotics mixed (co-digestion) were operated at concentrations of 100 and 40 mg/L for SMX and NOR, respectively. Reactors containing inoculum and substrate samples, without adding antibiotics, were used as controls. The experiments were carried out in quintuplicate, totaling 40 reactors.

Determination and quantification of biogas and methane

The volume of biogas produced was quantified using equipment based on a U-shaped fluid displacement system. For every 40 mL of biogas produced, the IFODI-3180-11P sensor (Ace Schmersal) recorded the "event" and stored the volume quantification information on the server. The volume produced individually was calculated and corrected according to the ideal gas equation. For this purpose, the measuring equipment had a temperature sensor (ΔTmin = 0.0625°C, TEMP-WM sensor, Novus) and an atmospheric pressure sensor (ΔPmin = 0.24 hPa, TP-ST18 sensor, Acros) (Konrad et al., 2023).

The percentage of CH_4 present in the biogas produced by the AD process was determined using an Advanced Gasmitter sensor by PRONOVA Analysentechnik GmbH & Co. The injection was performed using 20 mL of the biogas produced in each reactor directly into the device with the aid of a syringe (Guedes et al., 2024).

To determine biochemical methane potential (BMP), the accumulated volume of biogas and methane in the sample was divided by the amount of organic material added to the reactor, expressed in g/VS (Steinmetz et al., 2016). The BMP was expressed according to Equation 1.

$$BMP = (Vs - Vb \times (mSVis / mSVib)) / mSVs \quad \text{Equation 1}$$

Where:

Vs: Accumulated methane volume of the samples

Vb: Accumulated methane volume of the blank (inoculum only)

mSVis: mass of volatile solids corresponding to the inoculum in the sample

mSVib: mass of volatile solids corresponding to the inoculum in the blank
mSVs: mass of volatile solids corresponding to the content in the sample

Determination and quantification of antibiotics in aqueous phase

The removal of antibiotics from the aqueous phase was assessed at the end of the operation using high-performance liquid chromatography (HPLC Shimadzu LC20A) with automatic sampler SIL-20A, a C18 column (Shim-pack XR-ODS, 3.0 mm ID x 50 mm, Shimadzu) and DAD SPD-20AV detector. 0.025 M phosphate buffer (pH 2.5; 2.5 g $NaH_2PO_4.2H_2O$ Merck P.A; 0.65 mL H_3PO_4 Synth P.A. 85 %) was used as mobile phase A and acetonitrile (Merck, HPLC grade) as mobile phase B with a 1 $mL.min^{-1}$ flow. For detection in DAD, wavelengths (λ) equal to 277 and 269 nm and retention times of 3.62 and 5.07 min were used for NOR and SMX, respectively. Calibration curves using known concentrations of the antibiotics were made to validate the method. The removal efficiency was calculated according to Equation 2.

$$\text{Removal efficiency (\%)} = (1 - C_{final}/C_{initial}) \times 100 \qquad \text{Equation 2}$$

Where:
C_{final}: Final concentration of the antibiotic detected in the aqueous phase
$C_{initial}$: Initial concentration of the antibiotic added to the aqueous phase

Determination and quantification of volatile fatty acids

Volatile fatty acids (VFAs) were determined and quantified using a Dani Instrument Master GC gas chromatograph with a flame ionization detector (FID), a GC Nukol 24107 capillary column, and an oven temperature of 105ºC. An automatic sampler with a volume of 1 µL was used to inject the samples. The method used was validated using standard solutions of known concentrations of acetic (99.8%), propionic (99.5%), butyric (99%), and valeric (99%) acids. The acids used for validation were of high purity from Sigma Aldrich, Merck, and Neon. Acetic, butyric, propionic, and valeric acids in the samples were determined and quantified at the end of the experiment. Detection and quantification of VFAs was performed at the end of the batch experiments.

Determination of chemical oxygen demand (COD) and removal efficiency

The final COD value was determined using the 5220D colorimetric method

(APHA, 1989). The theoretical oxygen demand (ThOD), calculated according to OECD (1992), was used as the initial value, considering the amount of substrate and antibiotic added to the reactors (Equation 3). The use of ThOD as an estimate of the initial COD was due to the accuracy of computing the values for the quantities of substrate and antibiotic added to the samples during the reactor setup. The removal efficiency was calculated according to Equation 4.

$$ThOD = \{16[2c + ½(h\text{-}cl\text{-}3n) + 3s + 5/2p + 1/2na\text{-} o]\}/MW \quad \text{Equation 3}$$

Where:

ThOD: $C_cH_hCl_{cl}N_nNa_{na}O_oP_pS_s$ (mg O_2/mg substrate)

MW: molecular weight

$$\text{Removal efficiency (\%)} = (1 - COD_{final}/COD_{initial}) \times 100 \quad \text{Equation 4}$$

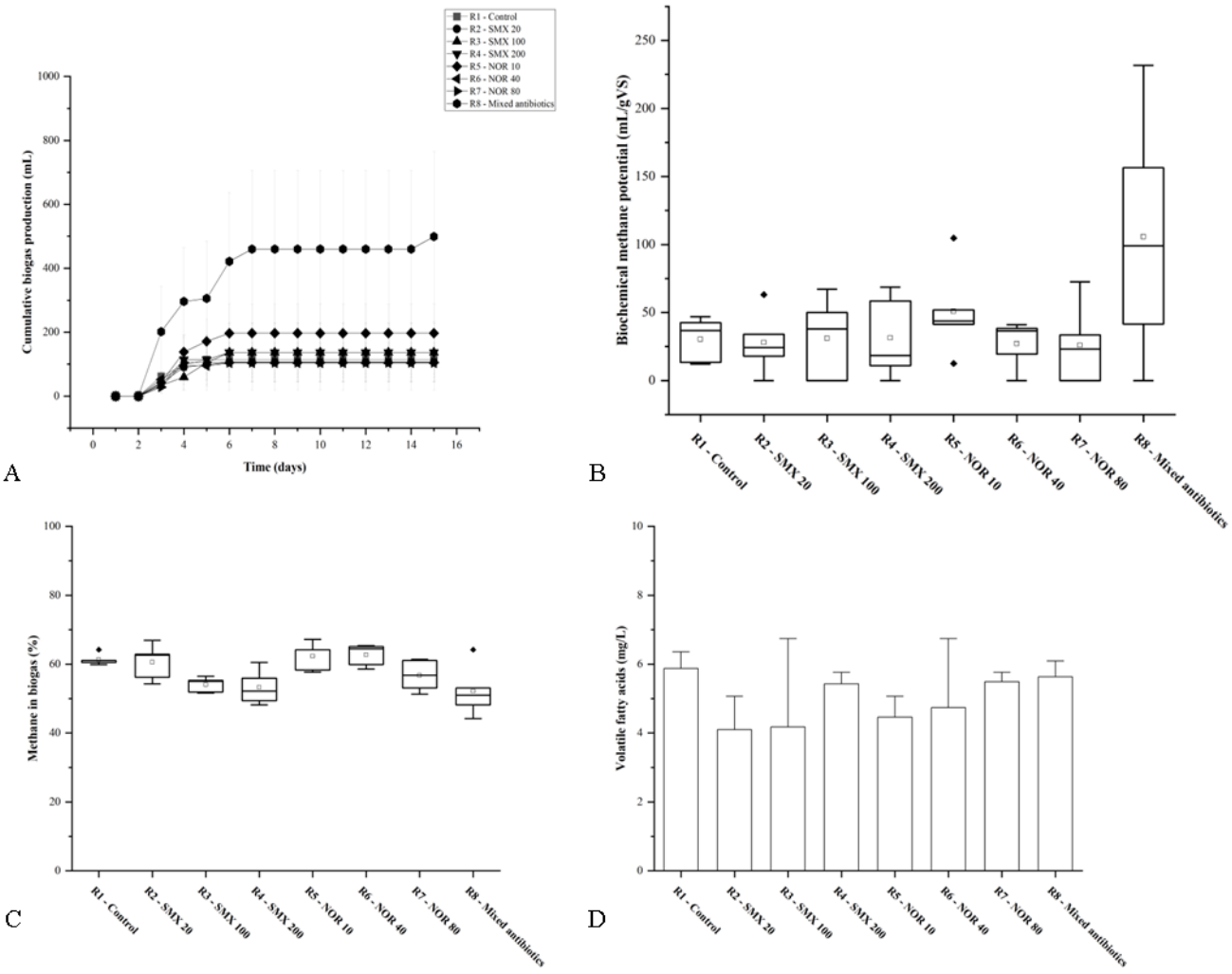
Statistical analysis

The normality of the data was verified using the Shapiro-Wilk test. Since the data showed a non-normal distribution, and considering the sample size, it was decided to use a non-parametric statistical test. Kruskal-Wallis test was applied, followed by Dunn's post hoc test with Bonferroni corrected p values. PAST software was used to carry out statistical tests (Hammer et al., 2001).

4.2.4 RESULTS AND DISCUSSION

Figure 1 shows the results for cumulative biogas production (A), biochemical methane potential (B), percentage of methane in biogas (C), and concentration of volatile fatty acids (D). Parameters related to biogas and methane production and yield during the anaerobic digestion process.

Fig.1: (A) Cumulative Biogas Production (mL); (B) Biochemical Methane Potential (BMP) (mL/gVS); (C) (%) Methane in biogas; (D) VFAs concentration



The peak biogas production occurred between the sixth and seventh days of operation for all samples, including R1-Control. This period probably indicates the highest rate of consumption of organic matter present, whether substrate, antibiotics, or endogenous consumption. After that, production stabilized. Fig. 1A shows that R8 - Mixed antibiotics presented considerably higher cumulative biogas production than the other samples tested. Considering this fact, it appears that the combination of antibiotics was favorable to the AD process. The different samples presented values numerically close to those offered by R1-Control, except for R5-NOR 10, which had a slightly higher value. These results indicate that, under the experimental conditions tested, the antibiotics did not cause toxicity or imbalance in the stages of AD.

The CBP values showed statistically significant differences between the samples ($p<0.05$). R8 – Mixed antibiotics was statistically different from R1 – Control, R2 – SMX 20, R3 – SMX 100, R6 – NOR 40, and R7 – NOR 80. This result corroborates the hypothesis that mixed antibiotics were beneficial for AD, boosting biogas production. In addition, there were differences between samples R4 – SMX 200 and R6 – NOR 40, indicating a possible effect of the different classes of antibiotics used. R5 – NOR 10 was statistically different from R6 – NOR 40 and R7 – NOR 80, indicating a possible effect of the concentration of NOR used in biogas production.

Biochemical methane potential (BMP), presented in Fig. 1B, refers to the maximum amount of gas obtained in the AD process under standard conditions. It is expressed as the volume of methane per unit mass of organic matter (volatile solids) (Steinmetz et al., 2016). R8 – Mixed antibiotics, showed a BMP value (105.75±91.90 mL/gVS) more than three times higher than that observed for R1-Control (30.33±16.49 mL/gVS). However, there were no statistically significant differences between the samples ($p>0.05$). It is important to note the high variability of the results for sample R8 – Mixed antibiotics. This may have been caused naturally, since AD is a complex process involving factors that may or may not be controlled. For example, there may be differences in the composition and abundance of microorganisms present in samples subjected to the same operating conditions. The number of replicates used in the study should also be considered.

Antibiotics can have varying effects on methane production, inhibiting it, having no effect, or even stimulating it, depending on their type, concentration, and the operating conditions applied to the anaerobic digestion process (Ali et al., 2025). According to Zhi et al. (2019), antibiotics such as SMX and NOR stimulate methane production in the anaerobic digestion process at high solids contents (≥15%), improving the system's processing capacity and the microbial community. Zeng et al. (2023) found that increased NOR concentrations did not significantly affect methane production during AD. Concentrations as high as 100 mg/L of antibiotics strongly stimulate methane yield. SMX drives methane production by stimulating the stages of acetogenesis and homoacetogenesis (Tang et al., 2022). For Tang et al. (2024), SMX also promoted the acetoclastic and hydrogenotrophic routes, increasing methane production during AD. Cetecioglu et al. (2015) found that higher concentrations of SMX resulted in higher

percentages of methane in biogas. This was attributed to the sensitivity of homoacetogenesis and hydrogenotrophic methanogenesis to SMX. Showing that there is a chance of recovery and acclimatization of the system to the compound, even at higher concentrations.

Although some published studies indicate positive effects of antibiotics on AD, others point to toxicity and a decrease in biogas and methane production parameters. Zhu et al. (2021a) obtained a reduction in methane production as the concentration of NOR increased, suggesting an imbalance in the AD stages due to the inhibitory effects of the antibiotic. Gaballah et al. (2024) stated that sulfonamide antibiotics (such as SMX) could be the primary contributor to biogas inhibition when mixed with quinolones (such as NOR).

The average methane content in the biogas (%) (Fig. 1C) ranged from 50 to just over 60% for all the samples tested. Values lower than those presented for R1-Control possibly indicate a probable difficulty or delay in the degradation process to convert antibiotics into methane during the methanogenic stage. This may have occurred due to the time required for the microorganisms to acclimatize to the compounds. Despite this, all samples showed methane percentages in biogas within the range considered normal for anaerobic digestion. Biogas mainly consists of methane (50-75%), carbon dioxide (25-50%), hydrogen sulfide, hydrogen, ammonia (1-2%), trace elements, and other gases such as oxygen and nitrogen (Atelge et al., 2020). There were no statistically significant differences between the samples ($p>0.05$). Moreover, all samples tested presented CH_4 concentrations within the range considered ideal for energy utilization (50-65%) from biogas (Li et al., 2017).

According to the results obtained and data from the literature, there is still no consensus on the effect of antibiotics on the AD process. Therefore, it is essential to consider specific concentrations and combinations of antibiotic classes, in addition to the operational parameters adopted in the process, when assessing the favorable or adverse effects. In this study, the operating conditions applied together with the classes and concentrations of antibiotics used were favorable for biogas production. This probably favored the development and maintenance of a microbial community capable of adapting to the imposed conditions. Zhi et al (2019) also obtained stimulation in parameters related to methane yield during anaerobic digestion of antibiotics.

Regarding volatile fatty acids (VFAs) (Fig. 1D), the low VFAs values at the end of the batch operation for all samples (did not exceed 10 mg/L) may have been due to

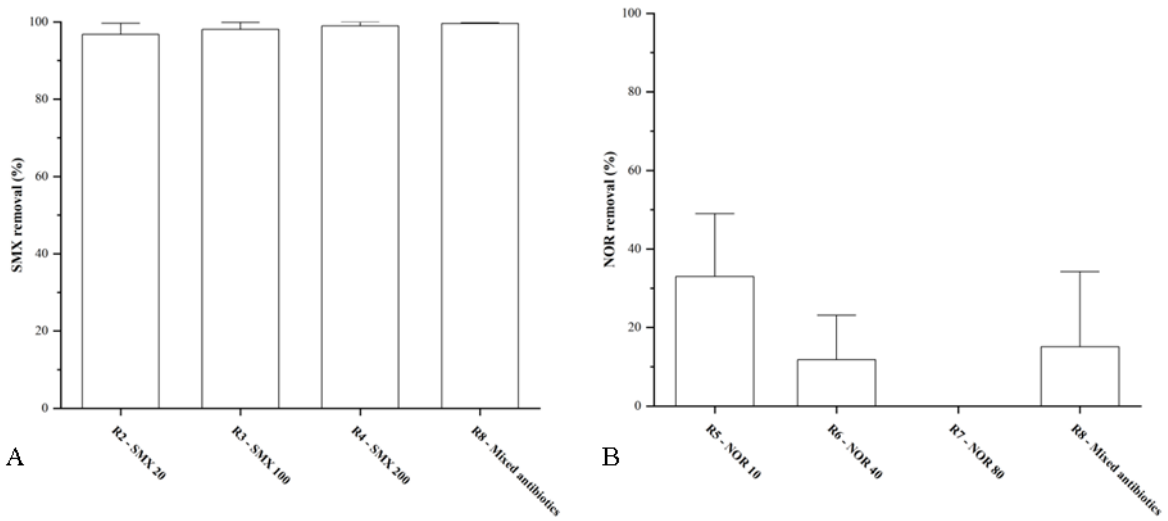
insufficient organic content to maintain production or difficulty in degrading the compounds present. The available organic content was quickly consumed by microorganisms during the first days of the experiment, which resulted in the peaks of biogas and methane production. At the end, however, the content was insufficient to maintain output, leading to low VFAs production in the process. A similar pattern of VFA distribution was observed between the samples tested, with a predominance of acetic acid, followed by valeric, propionic, and butyric acid, respectively. The results for VFAs showed no statistical differences ($p>0.05$).

In addition, the pH remained in the neutral range (6-7) throughout the experiment, indicating that antibiotics did not unbalance the acidogenic and methanogenic stages and did not inhibit microbial action. Low concentrations can also suggest that the hydrolytic stage was favored due to the poor conversion rate of the chemical compounds into VFAs (Jiménez-Castro et al. 2020).

VFAs are inevitable intermediaries during the biogas production in AD (Beschkov et al., 2025). They are indicators of imbalance in the process, with butyrate production being excellent, as its accumulation does not cause toxicity or methane production (Ahring et al., 1995). VFAs originate from acid-producing bacteria in the initial stages of AD (Feito et al., 2025).

The removal rates of antibiotics are shown in Figure 2. All samples containing SMX showed removal rates greater than 90%, regardless of the compound concentrations (Fig. 2A). About NOR (Fig. 2B), the removal rates obtained decreased with increasing concentrations added to the reactors. The lowest concentration of NOR (R5–NOR 10) resulted in the highest removal rate (35%), compared to the highest concentration for sample R7–NOR 80, which resulted in a zero-removal rate. The removal rates for sample R8 – Mixed antibiotics showed similar behavior to those of samples containing antibiotics in individual concentrations. Despite the differences observed for NOR, there were no significant differences ($p>0.05$) between the results obtained for any of the conditions tested.

Figure 2. Removal of antibiotics from the aqueous phase



Once in the environment, antibiotics can undergo different processes, such as mineralization into carbon dioxide and water, physical or biological degradation (biodegradation), and adsorption into sediments. These processes can affect the substances completely or partially. Throughout the removal processes, there is also the possibility of forming intermediate compounds, resulted from the metabolic routes.

The transfer rate of CECs to water depends on both the compound's polarity and the environment's characteristics. Their removal efficiency is influenced by their ability to interact with solid particles, such as sediments and microorganisms, or those used for treatment, such as coagulants or activated carbon. Generally, compounds with a low adsorption coefficient remain in the aqueous phase, which favors their mobility. Non-polar compounds tend to sorb onto the sludge, while polar ones stay dissolved in the water (Vélez et al., 2019). Regarding pharmaceuticals, such as antibiotics, common factors to consider are hydrophobicity and lipophilicity, which are toxicological indicators that help predict their permeability or accumulation in aquatic systems (Bustos Bustos et al., 2022). Mixtures of antibiotics generate competition for biological degradation and adsorption in sludge during AD (Gaballah et al., 2024).

Considering the antibiotic characterization data presented in Table 1, SMX has a low adsorption affinity in sludge, with low values for the sludge adsorption coefficient (Kd) and the octanol-water partition coefficient (log Kow). Furthermore, considering the pKa values of the compound and the neutral pH value presented by the samples throughout the experiment, it is plausible to state that SMX possibly acted as an anionic species, being repelled by the sludge. This fact tended to prevent its adsorption in the

sludge, causing the removal rates obtained (Fig. 2A) to be associated with biodegradation. SMX is biodegradable under anaerobic conditions, with biodegradation being the predominant form of removal (Cetecioglu et al., 2015; Li et al., 2010).

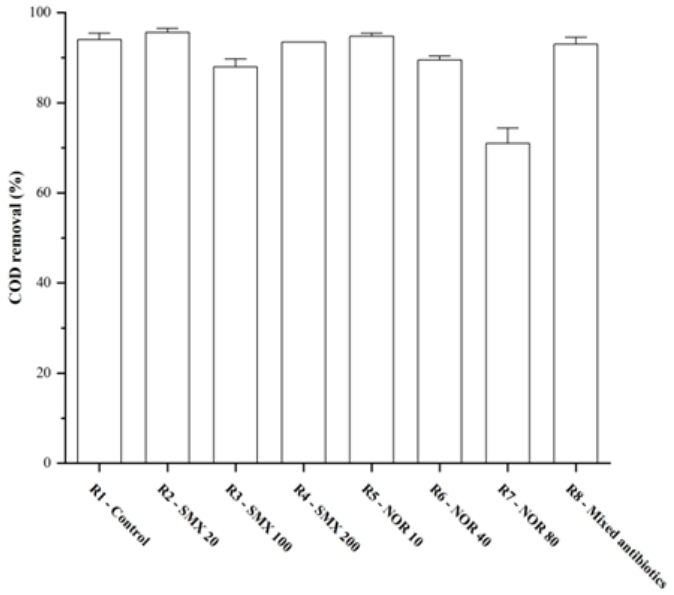
NOR, on the other hand, has a recalcitrant nature and low water solubility (Orimorale et al., 2023). It is not easy to degrade, even in longer cycles (Zhu et al., 2021). Adsorption in sludges is the primary way of removing NOR from wastewater. It can occur through electrostatic interactions, hydrogen bonds, hydrophobicity, or other mechanisms (Meng et al., 2023; Zou et al., 2022; Oberoi et al., 2019). Still, contrary to expectations due to its high K_d value and to what is reported in the literature, NOR remained in the liquid, especially in R7–NOR 80, resulting in lower removal efficiency.

Although quinolone antibiotics, such as NOR, may be biodegradable, the low rate of biodegradation means that adsorption is their main route of removal. The sorption of NOR is a process controlled by reactions that occur on the surface. Experimental conditions (such as pH, redox conditions) can impact sorption affinity because independent mechanisms linked to hydrophobicity are closely related to the speciation properties of the compounds and sludge (Wang et al., 2017). The experimental conditions adopted in this present study may not have been sufficient to promote its adsorption, favoring its permanence in the aqueous phase. The decrease in NOR removal efficiency with increasing concentration may be related to factors such as inhibition of the microbial community or saturation of adsorption surfaces (Xu et al., 2020; Ji et al, 2023).

It is essential to highlight the importance of characterizing and determining the microbial community and the formation of secondary metabolites and transformation products to more thoroughly and accurately assess the processes and rates of antibiotic removal. In addition, there is a need to invest in environmental monitoring to mitigate damage caused by the improper or inefficient disposal of antibiotics, whether through their eventual persistence in the aquatic environment or adsorption in sludge.

Figure 3 presents COD removal rates. Analysis of the results shows that R7-NOR 80 had the lowest COD removal value. This is consistent with the results presented above (Fig. 2B), given that this same sample had a zero-removal rate for the added antibiotic. This corroborates the hypothesis that NOR remained in the liquid phase with little or no adsorption in the sludge. However, from a statistical point of view, sample R7 - NOR 80 differed significantly only from sample R2 - SMX 20.

Figure 3. COD removal from the aqueous phase



The behavior, routes, and mechanisms of CECs removal in biological and combined treatments still need to be fully elucidated, and research should continue on these topics (Zhu et al., 2021b; Pu et al., 2018). It is crucial to delve into the ability of anaerobic digestion to effectively eliminate antibiotic mixtures (Gaballah et al., 2024). Understanding of how antibiotics impact AD can help develop more robust and efficient biogas systems (Saady et al., 2024). How anaerobic microorganisms react with certain drugs and the consequent impact on methane production still needs to be evaluated, especially in situations where the contaminant inhibits or reduces the microorganisms' activity.

4.2.5 CONCLUSION

Antibiotic class and concentration interfered with anaerobic digestion outcomes influencing biogas and methane parameters results. Mixed antibiotics enhanced cumulative biogas production and biochemical methane potential, however the lower methane content in biogas suggests increased production of other gases, such as CO_2. Low VFAs concentration at the end of the experiment indicates rapid depletion of available organic matter content by microorganisms in the first days and explains the early peaks in biogas production. The concentration of SMX did not influence its removal rate. Regarding NOR, the removal rate decreased with antibiotic concentration. SMX was

likely biodegraded, while NOR persisted in the aqueous phase due to unfavorable conditions for either degradation or adsorption. COD removal followed expected ranges for anaerobic processes, with lower COD efficiency linked to reduced compound removal in the liquid phase. Findings suggest challenges for biogas optimization and antibiotic removal in anaerobic systems when multiple compounds coexist. Studies are still needed to clarify the antibiotics removal processes more effectively. Future research should employ larger sample sizes, integrate microbial community characterization, and track transformation products to better understand degradation pathways. In a broader perspective, coupling laboratory findings with environmental monitoring and improved disposal practices are also essential to mitigate ecological risks.

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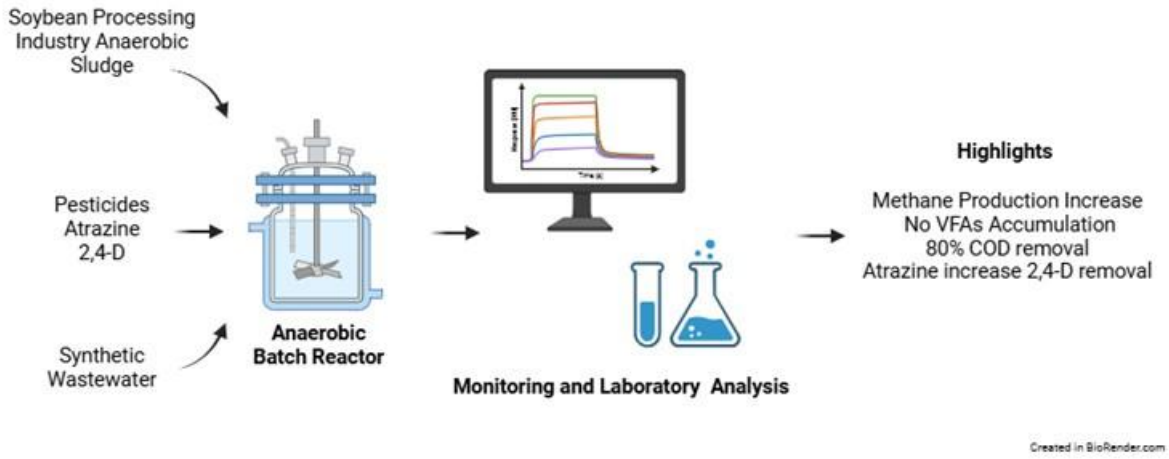
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4.3 Removal of Atrazine and 2,4-D Pesticides from Wastewater by Anaerobic Biological Process Using Batch Reactors

Artigo publicado no periódico *Journal of Environmental Science and Health, Part B, Pesticides, Food Contaminants, and Agricultural Wastes,* da Editora Taylor and Francis, com a seguinte referência: Ribeiro, M. H. G., Machado, M. L., Silva, M. C. D. A., & Benetti, A. D. (2025). Removal of atrazine and 2, 4-D pesticides from wastewater by anaerobic biological process using batch reactors. *Journal of Environmental Science and Health, Part B*, 1-8. https://doi.org/10.1080/03601234.2025.2608542. Vale ressaltar que o texto aqui apresentado pode diferir em relação ao publicado pelo periódico devido a correções realizadas. Para a leitura da forma mais atualizada, recomenda-se consulta ao site do respectivo periódico e/ou editora. O Apêndice B contém imagens referentes ao experimento em questão.

GRAPHICAL ABSTRACT



4.3.1 ABSTRACT

Biological treatment via anaerobic digestion (AD) represents a sustainable alternative for removing persistent organic contaminants, such as pesticides, from aqueous matrices while simultaneously enabling bioenergy production. This research aimed to evaluate the use of AD in anaerobic batch reactors to remove the pesticides atrazine (ATZ) and 2,4-D from wastewater at mesophilic temperatures (35 ºC). The results demonstrated that the presence of the pesticides stimulated methane production and did not inhibit the overall anaerobic process. Total Chemical Oxygen Demand (COD) removal efficiencies exceeded 80% for all tested concentrations, with stable operational parameters including optimal pH and total alkalinity, and no accumulation of volatile fatty

acids (VFAs). Pesticide removal kinetics revealed that ATZ achieved a maximum removal of 43.6% when applied alone. Conversely, the presence of ATZ in the mixed reactors served as a co-metabolic stimulant, significantly increasing the removal of 2,4-D to over 90% for all combined concentrations. This synergistic effect suggests that the presence of mixed pesticides can induce the necessary enzymatic activity (co-metabolism) required for the effective degradation of 2,4-D, thereby eliminating the need for a long acclimatization period. AD is thus presented as an efficient, stable, and sustainable approach for managing wastewater contaminated with these specific co-occurring pesticides.

4.3.2 INTRODUCTION

Pesticides are substances used to suppress, eradicate, or prevent organisms considered harmful or nuisance to crops. They can be categorized by the type of use, target organism, origin of their active substances or dangerousness. Pesticides can be classified as herbicides, fungicides, and insecticides, with herbicides used to combat weeds in gardens or plantations. Pesticides are widely used in agriculture to increase crop production (McGinley et al., 2023). Brazil, which stands out worldwide for its food production and exports, is one of the most significant users of these substances. According to data from the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA, 2021), 467 active ingredients were commercialized between 2009 and 2021. Among the five best-selling pesticides, there are substances considered dangerous to human health and the environment, such as dichlorophenoxyacetic acid (2,4-D) and atrazine, the latter of which is already banned in European Union countries.

The application of pesticides varies in dosage rate, time, and space, resulting in a heterogeneous distribution (Schipper et al., 2008). After use, they move through the soil or surface water to streams and groundwater, where they can cause unintended ecological effects such as accumulation in aquatic organisms and loss of ecosystem biodiversity. In addition, they can have carcinogenic, mutagenic, neurotoxic, and/or teratogenic effects on human health (McGinley et al., 2023). Pesticides used in domestic activities, such as pest and weed control in gardens, can be transported to the sewage system by runoff, increasing the treatment load on wastewater treatment plants (WWTPs) (Li et al., 2022).

The runoff of these substances through the urban rainwater drainage system also contributes to their accumulation in WWTPs. Although they are among the most investigated organic contaminants in the aquatic environment, the occurrence and fate of

pesticides during wastewater treatment stages have not yet been adequately studied in Brazil (Marson et al., 2022).

Numerous types of physical and chemical treatments have been used to remove these substances from the aqueous environment, including adsorption, membrane filtration and advanced oxidative processes, as well as biological processes such as bioremediation, activated sludge, and phytoremediation (McGinley et al., 2023). Aerobic and anaerobic biological treatments are considered effective methods for removing pesticides from wastewater (Yan et al., 2024). Anaerobic biological treatment occurs via anaerobic digestion (AD) when microorganisms do not use oxygen and nitrate during the stabilization of organic matter. AD is a complex biochemical process that involves the conversion of organic matter into methane (CH_4) and carbon dioxide (CO_2) by a wide variety of microorganisms (Cheng et al., 2019). It is seen as a sustainable alternative for wastewater treatment, with the benefits of generating bioenergy, low sludge generation and low energy consumption and greenhouse gas emissions (Hu et al., 2022). Therefore, anaerobic biological treatment is an alternative for removing these substances from aqueous matrices.

Microorganisms can destroy pollutants such as pesticides, to survive, doing so only under environmentally favorable circumstances to meet their demands. They are also capable of adapting themselves to degrade pesticides more quickly in restricted periods. Exposure to an optimal concentration of these pollutants can initiate a metabolic pathway for the digestion of that compound by microorganisms (Chia et al., 2024). The presence of organic micropollutants in wastewater is a significant environmental challenge, and for the development and application of effective removal strategies, it is necessary to understand the mechanism of co-metabolic biodegradation (Lyu et al., 2025).

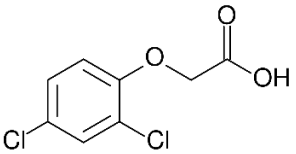
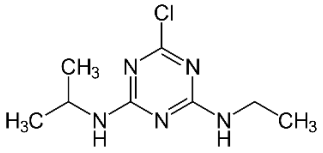
Different types of pesticides often coexist in aqueous solutions. Therefore, it is important to explore efficient methods for their removal (Wang & Hou, 2024). Thus, the general objective of this research was to evaluate the use of anaerobic biological treatment to remove the pesticides atrazine and dichlorophenoxyacetic acid (2,4D) from aqueous matrices. In addition to removal efficiency, the operational parameters of the anaerobic digestion process were also evaluated to understand possible negative interferences caused by the presence of the compounds alone or in coexistence.

4.3.3 MATERIAL AND METHODS

Experimental Setup and Operation

Batch experiments were carried out in 300 mL glass reactors. A thermostat controlled the temperature and kept it in the mesophilic range (35 ºC). The total operating time of the batch was 15 days. The reactors contained inoculum and synthetic wastewater contaminated with different compositions and concentrations of atrazine and 2,4-D. Table 1 shows the physicochemical properties of atrazine and 2,4-D.

Table 1. Pesticides physicochemical properties

Physicochemical properties	2,4-D	Atrazine
Class	Herbicide	Herbicide
Molecular formula	$C_8H_6C_{l2}O_3$	$C_8H_{14}ClN_5$
Core structure		
Molecular weight (g/mol)	221.03	215.68
Solubility in water (mg/L)	677	33
pKa	2.73	1.60
logKow	2.81	2.61

Adapted from PubChem (2024)

Anaerobic sludge from a soybean processing industry was used as inoculum. An inoculum/substrate (I/S) ratio of 0.5 gTVS/g COD was adopted to ensure high microbial activity and low risk of overloading (Angelidaki & Sanders, 2004). Synthetic wastewater was used as a substrate, adapted from Tadkaew et al. (2011). It consisted of glucose (400 mg/L), peptone (75 g/L), KH_2PO_4 (17.5 mg/L), $MgSO_4$ (17.5 mg/L), $FeSO_4$ (10 mg/L) and sodium acetate (225 mg/L). The operating conditions adopted resulted in an estimate organic load rate (OLR) of 8.41 g/L COD in each reactor.

Pesticides 2,4-D (DMA® 806 BR, MAPA registration no. 02108604) and atrazine (ACLAMADOBR®, MAPA registration no. 00712) were purchased for research purposes. The concentrations of pesticides used in the experiment aimed to evaluate their effects on the anaerobic process. In addition to facilitating the detection and quantification of compounds by the analytical methods used. Thus, it was decided to use concentrations higher than those usually found in aqueous matrices. In addition to different

concentrations for the two compounds evaluated.

The experiments were carried out in duplicates using 12 reactors in a single batch. Reactors without the addition of pesticides were used as a control. Table 2 shows the experimental design used in the assays.

Table 2. Experimental design according to the pesticide's concentrations tested in the experiment

Reactor	Pesticide concentrations in the reactors	
	Atrazine	**2,4-D**
Control	-	-
ATZ	2.5 mg/L	-
2,4D	-	10 mg/L
Mix 1	2.5 mg/L	10 mg/L
Mix 2	5 mg/L	20 mg/L
Mix 3	10 mg/L	40 mg/L

Analytical Methods

Total chemical oxygen demand (COD_t mg/L), pH and total alkalinity (mg CaCO3/L) were evaluated according to APHA (2012). Methane production was quantified using direct volume measurement via gravimetry, from the displacement of 3% m/v NaOH solution according to Aquino et al. (2007).

A Shimadzu LC20A high-performance liquid chromatograph (HPLC) with an SPD-20AV UV-VIS diode array detector (DAD) and a SIL-20A automatic sampler, was used to evaluate the removal of the pesticides. The compounds were separated using a Shim-pack XR-ODS C18 column (3.0 mm ID × 50 mm, Shimadzu). Phosphate buffer 0.025 M (pH 2.5; 2.5 g $NaH_2PO_4.2H_2O$ Merck P.A; 0.65 mL H_3PO_4 Synth P.A. 85 %) was used as mobile phase A and acetonitrile (Merck, HPLC grade) as mobile phase B. The analysis was carried out in isocratic mode (60 % mobile phase A and 40 % mobile phase B) with a flow rate of 1 mL min^{-1}, an injection volume of 20 µL and a temperature of 20 °C. For detection in DAD, λ = 210 nm was used for 2,4D and λ = 230 nm for atrazine, with retention times of 7.1 and 6.5 min, respectively.

Volatile fatty acids (VFAs) were measured using a DANI Instrument Master GC gas chromatograph equipped with a flame ionization detector (FID) and a GC Nukol 24107 capillary column. The injector and oven temperatures were 250ºC and 80ºC, respectively, with an injection equivalent to 1 µL using an automatic sampler.

Statistical Analysis

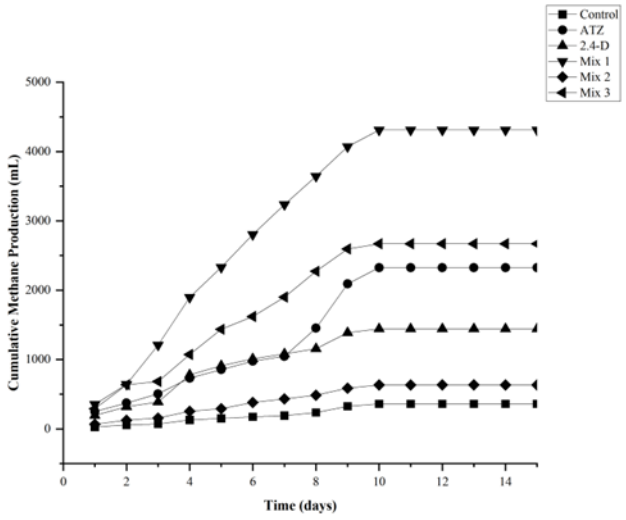
Considering the sample size used in the experiment and the number of replicates considered for each case, descriptive statistics were used for data analysis.

4.3.4 RESULTS E DISCUSSION

Methane Production

Figure 1 shows the reactors' cumulative methane production (CMP) over time. The pesticides increase CMP for all the configurations evaluated. The reactors with the addition of the mixed pesticides, Mix 1 and Mix 3, showed the highest numerical values, with Mix 1 standing out, reaching a cumulative value of 4.3 L. Regarding single pesticides, the reactors with added 2,4-D showed higher CMP values than those with ATZ. This was likely due to the greater ease with which microorganisms degrade and utilize 2,4-D. The delay in the methane peak observed for the reactors with added ATZ (9th day of operation), compared to the others (4th day of operation), points to the need for more time for microorganisms to adapt to assimilate the compound. Thus, the results indicate that 2,4-D was the most responsible for the increase in CMP obtained in the mix reactors, requiring a longer incubation time to account for the effect of atrazine.

Fig. 1 Cumulative Methane Production (CMP) according to the reactor´s content.



Considering the maximum theoretical methane production, where 1g/L of COD produces around 350 mL of CH_4, volumes of around 9 L of methane would be expected in the reactors (Arias et al., 2020; Lesteur et al., 2010). However, the much lower values

obtained experimentally are justified by factors such as the fraction of the substrate used to synthesize bacterial biomass, the presence of compounds that cannot be biodegraded anaerobically, or nutritional limitations. In addition, for highly particulate organic matter, as observed in the sludge used as inoculum, the bonding between the particles or structure causes their unavailability, resulting in a conversion rate of 30-60% (Angelidaki & Sander, 2004).

The complexity of atrazine's molecular structure (Table 1) is a possible cause of its difficulty in the biodegradation process and the consequent delay in the methane production. Nitro or chlorinated groups inhibit gas generation (Bettersby et al., 1989). Ghosh et al. (2001) state that the biodegradation process of atrazine is slow, but it is the only way to mineralize it.

There is still no consensus on the effect of pesticides on the anaerobic digestion process, while some studies report inhibitory effects, others point to an increase in methane production. Avila et al. (2021) stated that pesticides had no inhibitory effect on AD, without harming methane production yield. This result is like that obtained in the present study. In which, the higher methane production in reactors containing pesticides may have occurred due to the greater availability and variety of compounds present. Since in the control reactors the substrate was consumed more quickly and cellular endogeny was not sufficient to maintain high methane production for a long time. The opposite was reported by García-Mancha et al. (2017), who observed significant inhibition of methanogenesis, especially acetoclastic, caused by pesticides.

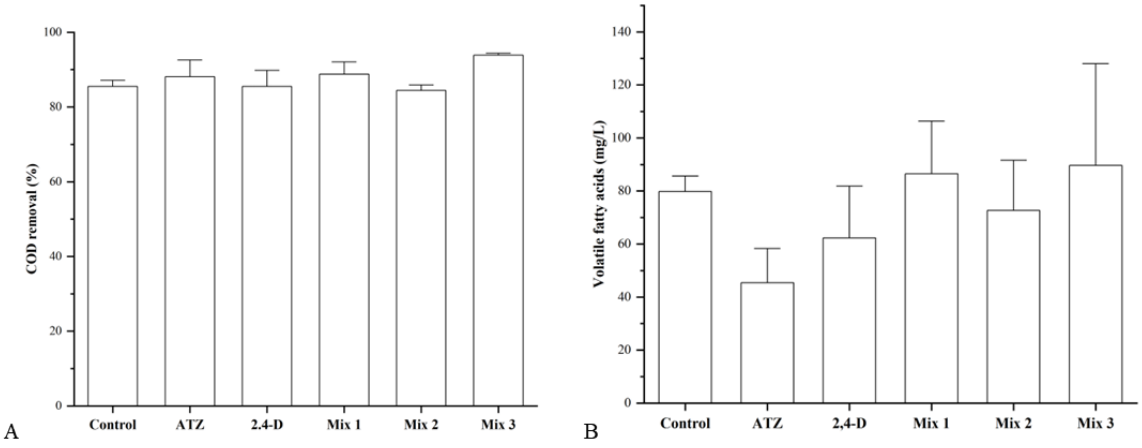
COD Removal

Figure 2A shows the percentages (%) of organic matter removal, in terms of COD_t, achieved by the reactors after the anaerobic process. All the reactors reached COD_t removal of more than 80%, with Mix 2 being the configuration that numerically showed the highest percentage (94%). The results indicate that there was no interference from the initial concentrations of the compounds, or even the presence of the compounds, in the efficiency of organic matter removal by the anaerobic digestion process. In addition, during the process of organic matter degradation through anaerobic digestion, which results in COD removal, various gaseous by-products are produced, including methane. Thus, the difference in methane production (Fig. 1), even with similar COD rates (Fig. 2A), was possibly due to the production of other gases, such as CO_2, H_2, and N_2.

Lin (1990), in a study of the anaerobic digestion of pesticides in WWTP, found

COD removal to be between 87 and 91.5 percent, with an optimum detention time of 15 days. This result is in line with what was obtained in the present study.

Fig. 2 Organic matter (COD) removal rates (A) and final concentration of volatile fatty acids (B) according to reactor's content



VFAs, pH and Total Alkalinity

Figure 2B shows the final composition of volatile fatty acids. For all the reactors, the predominance of compounds formed by acetic and propionic acids was observed, followed by valeric acid. Only the reactors containing atrazine alone showed the presence of butyric acid. Elefsiniotis & Li (2008) achieved a similar result, finding a predominance of acetic and propionic acids during the acidogenic stages.

Under methanogenic conditions, using VFAs leads to the formation of gaseous products, mainly methane and carbon dioxide. The authors concluded that the presence of 2,4-D did not have a marked effect on the production of VFAs. Still, it did reduce their biodegradation, indicating a potential inhibitory impact on the methanogenic stage (Elefsiniotis & Li, 2008). The concentration of VFA is an important indicator for controlling and optimizing the anaerobic digestion process (JIN et al., 2016). However, excessive accumulation of these acids, mainly caused by high organic loads, inhibits AD, with acetic acid being the primary inhibitor in methanogenesis (XU et al., 2014).

Despite what is reported in the literature, the results found for methane production (Fig 1), which were higher than the control, suggest no inhibition of the methanogenic stage, with the herbicides having a positive impact on gas generation. This may have been due to the inoculum used in the reactors, and anaerobic sludge from the soybean processing industry. There is a possibility that the microorganisms present are already

adapted to receiving pesticides, given their everyday use in crops in Brazil. This facilitates the metabolization of the compounds and the generation of methane gas.

pH and total alkalinity (Table 3) remained within the expected range, 6.5-7.5 (Chernicharo, 2016) and 1000-5000 mg/L (Metcalf & Eddy, 2014), indicating that the process took place without harm or toxicity to methanogenic microorganisms due to the addition of herbicides. These results were like those obtained by Elefsiniotis & Li (2008), who found that the presence of pesticides did not trigger any apparent long-term effect on the anaerobic digestion process.

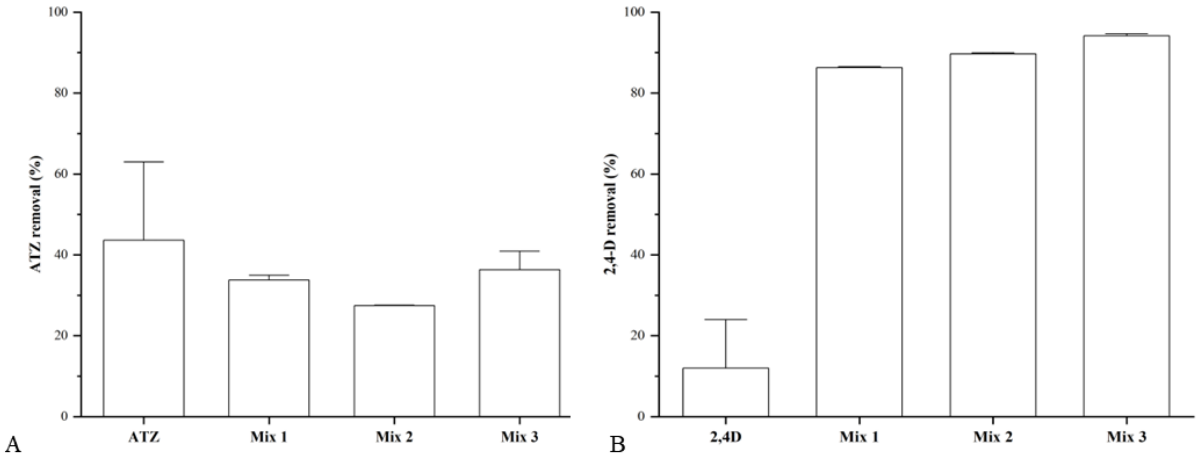
Table 3 Total alkalinity and pH

Reactor	Initial pH	Final pH	Total alkalinity (mg/L $CaCO_3$)
Control	7.66±0.01	7.28±0.03	3221.03±475.92
ATZ	7.75±0.01	7.18±0.02	4855.58±475.92
2,4D	7.72±0.03	7.11±0.95	4903.65±747.87
Mix 1	7.69±0.06	7.27±0.01	4519.05±271.95
Mix 2	7.77±0.01	7.79±0.76	4807.50±951.84
Mix 3	7.71±0.01	7.92±0.16	3942.15±543.91

Pesticide Removal from Aqueous Phase

Figures 3A and 3B show the percentages of pesticide removal from the aqueous phase for atrazine and 2,4-D, respectively. When considering the reactors containing the pesticides alone, atrazine (ATZ) showed a higher rate (43.6%) than 2,4-D (0%). This result was contrary to expectations, since, considering the results obtained for CMP (Fig. 1), it was expected that 2,4-D would achieve a higher removal rate than the atrazine. Furthermore, it was not expected that atrazine, even though it is a more recalcitrant compound than 2,4-D, would degrade in the same period.

Fig. 3. Atrazine and 2.4-D removals from aqueous phase



Zipper et al. (1999) stated that the concentration of 2,4-D in anaerobic conditions decreases exponentially, reaching complete removal after an incubation period of 17 days. Elefsiniotis & Li (2008) observed complete biodegradation of 2,4-D after an acclimatization period. The authors state that acclimatization of biomass to 2,4-D is essential to allow bacteria involved in the process to develop appropriate enzymes that induce biodegradation of the compound. Celis et al. (2008), also in batch anaerobic reactors, reported degradation of up to 40% of 2,4-D after an acclimatization period of 70 days.

Considering the reports in the literature, the acclimatization period adopted for this experiment (15 days) may not have been sufficient to result in the biodegradation of 2,4-D. However, this is not the case regarding the results for atrazine, which, due to its chemical structure, presents more significant obstacles to biodegradation than 2,4-D. According to Kumar et al. (2025), the stability of persistent organic pollutants can be attributed to the chemistry of C-halogen bonds (such observed in the 2,4-D and atrazine molecular structure in Table 1), which are among the strongest types of bonds, with carbon having lower electron density and halogen being electronically dense. They exhibit a high dipole moment, conferring ionic characteristics and resulting in high electrostatic interaction between C-halogen bonds.

Biodegradation can lead atrazine to complete degradation or mineralization, producing simpler substances such as carbon dioxide, water, nitrogen, and organic compounds. This is the most effective way of removing these pollutants from the environment (NASSERI et al., 2014). In an anaerobic environment and under appropriate electron donors and acceptors conditions, methanogenic and sulfate-reducing bacteria can

metabolize aromatic nitro compounds such as atrazine (BOOPATHY, 2017). Aromatic structures of lignin, benzoate, or other aromatic acids are metabolized to gaseous products by mixed cultures under methanogenic conditions. However, the degradation rate of these compounds by anaerobic microorganisms is slow (GHOSH & PHILIP, 2004).

In the reactors containing the combined pesticides (Mix 1, Mix 2 and Mix 3), atrazine seemed to act as a co-metabolic stimulant of 2,4-D, indicating the benefit of combining the compounds for the biodegradation process. According to Ghosh & Philip (2004), in mixed cultures, adding a more easily biodegradable organic compound stimulates anaerobic bacteria's growth, increasing the recalcitrant compound's degradation rate as a secondary substrate. In the mixed reactors, a biological mechanism may have occurred whereby the stress caused by the presence of ATZ induced the production of specific enzymes such as dechlorinases or hydrolases that degraded 2,4-D. In addition, the intermediate products of co-metabolism may have served as an additional substrate, resulting in increased methane production in the mixed reactors.

Microbial degradation is a process that involves the oxidation of a compound, resulting in the formation of carbon dioxide, water, and secondary products. This process is essential as it provides the carbon and energy required for microbial growth and reproduction. Each degradation step is catalyzed by a specific enzyme (cellular or extracellular). The lack of an appropriate enzyme can lead to the compound's persistence in the environment (Chia et al., 2024). Microorganisms facilitate the transformation of organic micropollutants through direct metabolic pathways or, more often, through co-metabolic (indirect) processes. Given that the concentration of micropollutants in wastewater is typically at trace levels, it is not high enough to meet the primary metabolic demands of microorganisms. For this reason, co-metabolic transformation tends to be the dominant route. In this mechanism, microbial enzymes, which are normally used for a primary substrate, catalyze the transformation of structurally similar pollutants. Despite the importance of the process, knowledge regarding the specific enzymes, metabolic pathways, and the microbial community involved in the degradation remains limited (Lyu et al., 2025).

It is crucial to acknowledge that the removal efficiencies documented in this study must be interpreted as the cumulative outcome of both adsorption onto the sludge biomass and the concomitant biological degradation. Specifically, the high removal rate observed for 2,4-D in the presence of atrazine strongly implicates a biologically mediated co-metabolic mechanism. Nevertheless, the potential contribution of adsorption, especially

for the more hydrophobic compound, atrazine, cannot be conclusively excluded.

Results found indicated that for the assimilation of compounds by microorganisms, the type and combination of pesticides were more important than the concentrations tested. In general, the presence of pesticides alone or in combination did not seem to impact on the anaerobic digestion process, but high removal rates were observed for 2,4-D in the reactors combined with atrazine. This does not mean that the presence of pesticides in wastewater is positive, given the danger these substances pose to human and environmental health.

Limitations and Future Work

To refine the current understanding of the results and overcome the limitations in experimental methodology, the authors propose several suggestions for future research. It is recommended that future experiments utilize an increased number of replicates (reactors) to allow for the application of more robust statistical tests to the resultant data. Furthermore, the incorporation of killed-control experiments (e.g., using atrazine-treated sludge) and longer incubation time is suggested to quantitatively distinguish between abiotic sorption and true biodegradation. Other critical areas for future work include the determination of transformation products, the characterization of the present microbial community, and the identification of specific enzymes. This comprehensive approach is essential to establish a more definitive mechanistic understanding of the observed interactions and address current limitations.

4.3.5 CONCLUSIONS

The addition of both individual and combined pesticides stimulated methane production across all configurations, demonstrating that the presence of the compounds did not exert an inhibitory effect on the methanogenic stage, with the maximum cumulative methane production (4.3 L) achieved in the Mix 1 reactor (2.5 mg/L ATZ and 10 mg/L 2,4-D). The process maintained satisfactory operational stability, achieving total COD removal efficiencies exceeding 80% for all conditions. Furthermore, pH and total alkalinity remained within optimal ranges, and no accumulation of inhibitory volatile fatty acids was observed. Notably, the simultaneous presence of pesticides facilitated a co-metabolic mechanism that significantly enhanced the removal of 2,4-D. While the

ATZ-only reactor achieved a moderate removal of 43.6% , the presence of ATZ in the mixed reactors increased 2,4-D removal to over 90% for all tested concentrations. This result suggests that the compounds' combination is more critical than their individual concentration in promoting biodegradation, potentially by inducing the enzymatic activity required for 2,4-D degradation. Anaerobic digestion proved to be an effective and stable process for the removal of 2,4-D from the aqueous phase and for simultaneous methane production. Given the observed efficiency and operational stability, AD represents a viable and sustainable alternative for the treatment of wastewater containing these contaminants.

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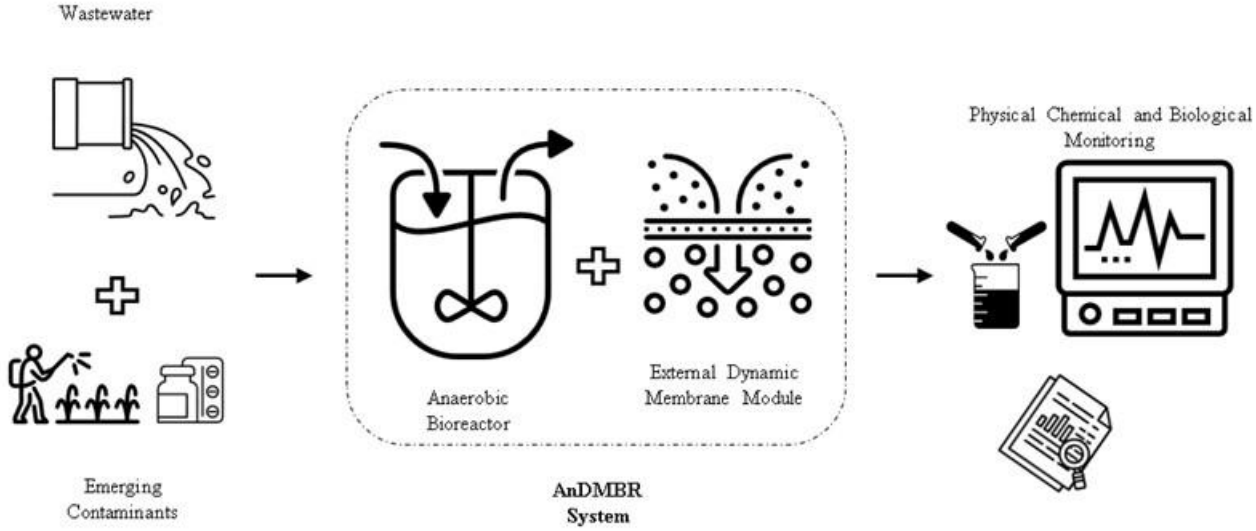
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4.4 Anaerobic dynamic membrane bioreactor with external gravity-driven filtration module removing emerging contaminants

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GRAPHICAL ABSTRACT



4.4.1 ABSTRACT

Emerging Contaminants (ECs) are chemical substances known to harm human health and the environment. This research evaluated the use of an Anaerobic Dynamic Membrane Bioreactor (AnDMBR) for EC removal from wastewater. Two parallel AnDMBR systems, fed with synthetic wastewater, were operated to assess EC impact and microbial adaptability: one received a single contamination (AnDMBR1), while the other received continuous contamination (AnDMBR2). The studied ECs were the antibiotics norfloxacin (NOR) and sulfamethoxazole (SMX), and the pesticides atrazine (ATZ) and 2,4-D. Filtration was achieved using a dynamic membrane (DM) in a semi-continuous,

gravity-driven mode. Both AnDMBRs showed a significant reduction in apparent color and turbidity and high COD removals (over 95%). The DM contributed to lower color/turbidity values but did not significantly affect COD. Sludge deflocculation was observed in AnDMBR2. SMX achieved high removal rates in both AnDMBRs. For the other ECs, AnDMBR1 showed higher values than AnDMBR2. However, AnDMBR2 exhibited greater microbial diversity, suggesting that the continuous addition of ECs favored the development of specialized microorganisms for EC removal.

4.4. 2 INTRODUCTION

Emerging contaminants (ECs) or contaminants of emerging concern (CECs) refer to chemical substances of a generally complex nature found more frequently in the environment. ECs are generally divided into various categories, such as pharmaceutical and personal care products (PPCPs), endocrine disrupting chemicals (EDCs), flame retardants, pesticides, microplastics (MPs) and antibiotic resistance genes (ARGs) (Campo and Picó, 2015; Ji et al., 2020).These contaminants are released into the environment, often from industrial, agricultural, and urban activities and are present in a variety of different water bodies, including surface water, groundwater, drinking water, and precipitation (Khan et al., 2022; Rathi et al., 2021).

The damage from ECs is relatively hidden because their effects on the environment are often chronic and cumulative over time. ECs have complex chemical structures, strong stability, and are often difficult to degrade naturally in the short-term (Ji et al., 2020). Besides, many of these contaminants are not properly eliminated in conventional Wastewater Treatment Plants (WWTPs). Hence, they are discharged to surface water, eventually reaching groundwater, soil, sediments, and oceans (Gogoi et al., 2018). Thus, novel technologies are needed to remove these contaminants. The presence of complex mixtures of ECs with different physical and chemical properties in the environment emphasizes the challenge of their complete removal (Gomes et al., 2020).

Anaerobic membrane bioreactor (AnMBR) is a technology that removes pollutants through physical retention and microbial biodegradation (Ahmed et al., 2021). AnMBR exhibits high system stability, a diverse microbial community, a long sludge retention time, a rapid start-up time, and a smaller footprint (Robles et al., 2018; Ji et al., 2020). This technology stands out in terms of higher biogas production and EC

biodegradation efficiency than traditional AD systems and has excellent potential in wastewater treatment (Wandera et al., 2018). Despite the benefits of this technology, membrane fouling is a bottleneck to the widespread application of AnMBRs, leading to increased transmembrane pressure and reduced flux, which in turn impact operational costs and energy efficiency (Vinardell et al., 2020; Wang et al., 2022; Nabi et al., 2022).

Another possible and innovative technology for membrane bioreactor (MBR) systems is the use of dynamic membrane (DM) (Cayetano et al., 2019). It is a highly permeable layer (cake layer) of biofilm formed on top of a support material, and which acts as a barrier for pollutant removal and particle filtration (Ersahin et al., 2012; Zhang et al., 2014). A DM layer can be self-deposited on an inexpensive support material instead of expensive microfiltration/ultrafiltration (MF/UF) membranes (Samaei et al., 2023). The formation and colonization of the surface of the membrane is linked to the attachment of microorganisms, followed by reproduction and growth, leading to the development of the biofilm. The process of attachment to the surface is the key to biofilm formation, which can directly affect biological and physiological functions, as well as the start-up and operational cycle of biofilm-based reactors (Li et al., 2022).

The anaerobic dynamic membrane bioreactor (AnDMBR) brings several advantages over conventional MBRs including lower membrane cost, lower energy demand, easier fouling control, high solids retention and efficient treatment performance (Ersahin et al., 2012; Hu et al., 2016; Saleem et al., 2018; Samaei et al., 2023). In the side stream model, the membrane module is external to the bioreactor and the treated wastewater is pumped into the external membrane module. Although the tangential flow rate in the filtration area promotes fouling of the membrane, it can be easily removed for maintenance and cleaning (Ji et al., 2020). As a way of reducing energy consumption and making the process more sustainable, there is the possibility of operating the system by gravity.

This research aimed to evaluate an AnDMBR operated by an external semi-continuous filtration module driven by gravity removing ECs. Antibiotics norfloxacin (NOR) and sulfamethoxazole (SMX) and pesticides atrazine (ATZ) and 2,4-D, frequently detected in WWTPs and wastewater, were the ECs investigated. It was evaluated the ECs impact on operational performance, biofilm formation, and the microbial community. The experimental configuration adopted is under-explored and beneficial, as it establishes a new paradigm for wastewater treatment by reducing the energy consumption of the filtration process, directly addressing the global need for more sustainable and cost-

effective water technologies. Furthermore, the experimental approach comparing the effects of single versus continuous contamination of ECs including antibiotics and pesticides provides essential insights into microbial adaptability under chronic stress.

4.4.3 MATERIALS AND METHODS

EXPERIMENTAL SETUP AND OPERATION

The experiment was formed by two anaerobic dynamic membrane bioreactors (AnDMBR) systems consisting of a CSTR-type bioreactor and a side stream membrane module configuration. The side-stream membrane module was fed by gravity with the effluent coming out from the CSTR bioreactor. Before receiving the contaminants , the AnDMBR systems underwent a 28-day stabilization period. The contaminants were then added in sequence, each with an interval space of one week . The order of addition was ATZ - NOR – 2,4-D - SMX on the 28th, 35th, 42nd and 49th day of operation, respectively. As a way of assessing the possible impact of contaminants on the system and its ability to adapt, the AnDMBR1 system was contaminated only once, while AnDMBR2 received continuous contamination throughout the experiment. The total operation time was 102 days.

The CSTR-type bioreactors were operated continuously. They were made of glass with a total volume of 7.5 L and a useful volume of 5 L. The hydraulic retention time (HRT), sludge age and organic load rate (OLR) were, respectively, 10 d, 102 days and 0.147 $kg.m^{-3}.d^{-1}$. The reactors were operated at mesophilic temperature (35 ºC) and flowrate of 0.5 L.d-1. The external filtration modules were operated semi-continuously, with a feed flow rate of 0.5 L.d-1, fed twice a week. The support material used to develop the dynamic membrane was polyester/nylon 77 fabric, with a wire diameter of 48 μm, opening of 77 μm, thickness of 80 μm, open area of 35% and an effective filtration area of 0.01 m^2. Backwashes were carried out weekly for maintenance and to avoid total blockage of the flow over time.

INOCULUM, FEEDWATER AND CONTAMINANTS CHARACTERIZATION

Anaerobic sludge from a soybean processing industry was used as inoculum. An inoculum/substrate (I/S) ratio of 0.5 gVS/g COD was adopted to ensure high microbial activity and a low risk of overloading (Angelidaki & Sanders, 2004). The AnDMBR reactors were fed with synthetic wastewater with a composition adapted from Tadkaew

et al. (2011). It was composed of glucose (400 mg/L), peptone (75 g/L), KH_2PO_4 (17.5 mg/L), $MgSO_4$ (17.5 mg/L), $FeSO_4$ (10 mg/L), and sodium acetate (225 mg/L).

The ECs used in the study were chosen because they are among the most widely used and detected in effluents worldwide. For instance, according to the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA, 2021), 2,4-D and atrazine are among the ten most widely sold active ingredients in Brazil during the historical series from 2009 to 2021. 2,4-D ranks second on the list, while atrazine, although banned in several countries, and the European Union, ranks sixth among the most widely used active ingredients in the country. Norfloxacin belongs to one of the five most used classes of antibiotics worldwide, the quinolones (Meng et al., 2023; Zou et al., 2022). Sulfamethoxazole is often found in wastewater and WWTPs around the world due to its low susceptibility to chemical and enzymatic hydrolysis (Saha et al., 2023; Jiang et al., 2019; Xiong et al., 2019). The contaminants were added to the AnDMBR systems using a solution with the target desired concentrations: 2.5 $mg.L^{-1}$ for ATZ; 4.0 $mg.L^{-1}$ for NOR; 6.0 $mg.L^{-1}$ for 2.4-D and 10 $mg.L^{-1}$ for SMX. Higher concentrations than those commonly found in wastewater and WWTPs were chosen to evaluate their impact on the AnDMBR systems and facilitate the analytical detection methods.

ANALITICAL METHODS

Analytical methods used to evaluate AnDMBRs operating parameters followed APHA (2012). Total chemical oxygen demand (COD), apparent color, turbidity, pH, total alkalinity and total volatile solids (TVS) were monitored in the systems permeates. The removal efficiency of COD was calculated based on the difference between the initial concentration and the final values in permeate of the AnDMBRs. The methane gas produced in the CSTR bioreactor was quantified by direct volume measurement via gravimetry, based on the displacement of a 3% m/v NaOH solution, according to Aquino et al. (2007).

Detection and quantification of ECs in the systems permeates was carried out using high-performance liquid chromatography (HPLC). The chromatograph used was a Shimadzu LC20A, with a UV-VIS SPD-20AV diode array detector (DAD) and a SIL-20A autosampler. The compounds were separated using a Shim-pack XR-ODS C18 column (3.0 mm ID × 50 mm, Shimadzu). A 0.025 M phosphate buffer (pH 2.5; 2.5 g $NaH_2PO_4.2H_2O$ Merck P.A; 0.65 mL H_3PO_4 Synth P.A. 85 %) was used as mobile phase A and acetonitrile (Merck, HPLC grade) as mobile phase B. The analysis was carried out

in isocratic mode (60% mobile phase A and 40% mobile phase B) at a flow rate of 1 mL min^{-1}, an injection volume of 20 µL and a temperature of 20 °C. For detection in DAD, wavelengths (λ) equal to 277; 230; 269 and 220 nm and retention times of 3.62; 4.66; 5.07 and 5.85 min were used for NOR, 2,4-D, SMX and ATZ, respectively. The removal efficiency of the ECs was calculated based on the difference between the initial ECs concentration and the final permeate of the AnDMBRs.

MEMBRANE FLUX

The permeate flow throughout the side-stream membrane was calculated using Eq. 1. Where: *J* is the permeate flow ($Lm^{-2}h^{-1}$); *M*: mass filtered (g); *A*: membrane area (m^2); *t*: filtration time (h); *ρw*: specific mass of the liquid (gL^{-1}).

$$J = \frac{M}{A\,.t.\rho w} \qquad \text{Eq. 1}$$

BIOFILM CHARACTERIZATION

The biofilm formed on the membranes was characterized by scanning electron microscopy (SEM). The samples were prepared following the protocol of fixation with 25% glutaraldehyde and 0.2M phosphate buffer, washing with 1:1 phosphate buffer and distilled water, dehydration with ethanol (sequence of 100, 90, 70, 50 and 30% solutions), desiccation in a Balzers CPD030 critical point dryer and metallization with a golden target. The samples were then scanned using a Zeiss EVO MA10 electron microscope to determine the density, degree of development and groups of microorganisms present in the biofilm.

MICROBIAL COMMUNITY CHARACTERIZATION

The microbial community present in the anaerobic biomass of the CSTR reactors was determined. Samples were collected throughout the experiment and sent for DNA extraction, sequencing and analysis to Neoprospecta Microbiome Technologies (Florianópolis, Brazil). The microorganisms present were identified using the 16S rRNA gene in the V3/V4 regions. Primers 314F and 806R were used for amplification and the MiSeq Sequencing System from Illumina Inc was used for sequencing.

STATISTICS

Mann-Whitney test was used to compare means and check for significant

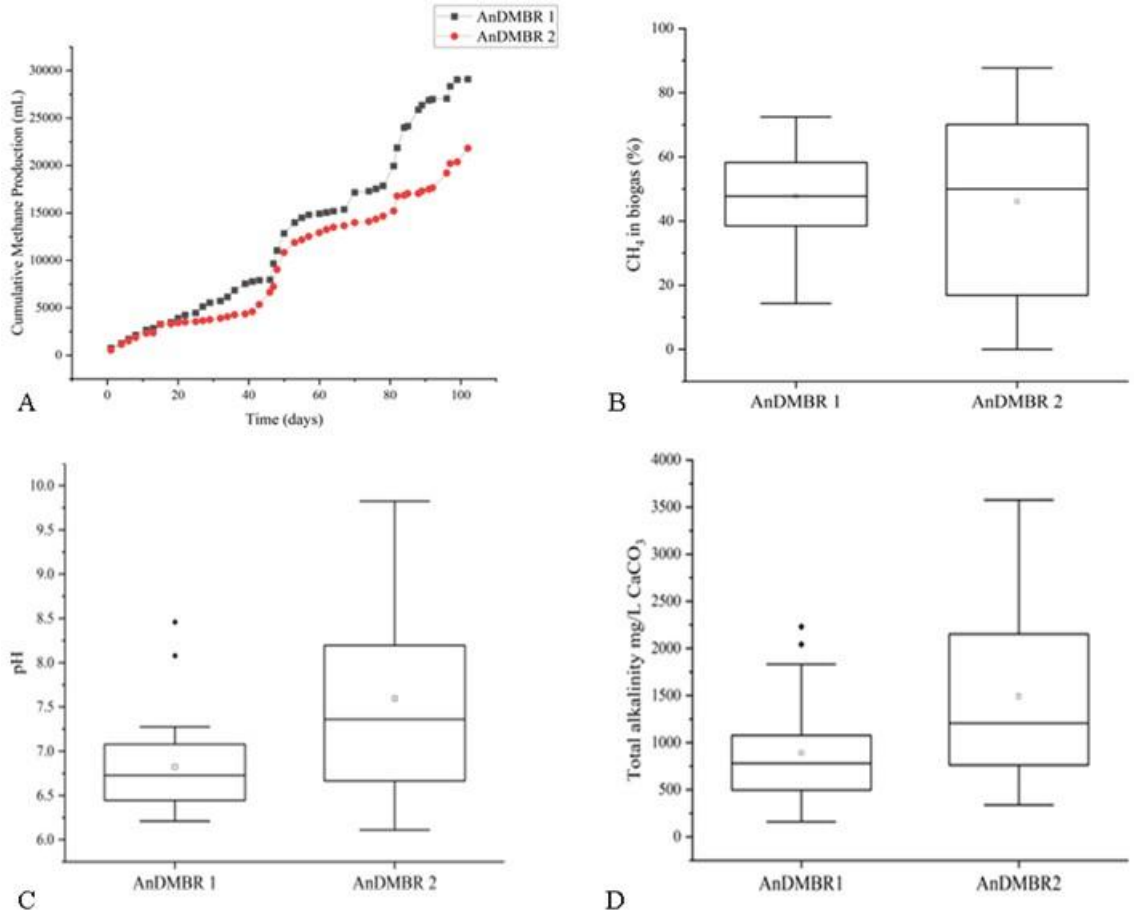
differences between the samples, considering a 95% confidence level ($p<0.05$). The statistics were made using software PAST version 4.17 (Hammer et al. 2001).

4.4.4 RESULTS AND DISCUSSION

Methane production

Results for cumulative methane production (CMP) and percentage of CH4 in biogas are shown in Figure 1 (1A and 1B, respectively). CMP showed an upward trend throughout the operation in both systems, even after the start-up period and the addition of ECs (32nd day). However, from then on, there was a greater distance between the curves, suggesting that the continuous contamination in AnDMBR2 may have harmed CH4 production. Despite this, the average percentage of methane in the biogas was similar for both AnDMBRs, with 47.8% for AnDMBR1 and 46.2% for AnDMBR2, with no significant differences ($p = 0.0829$). Since the only difference in the reactor's operation was the continuous input of EC in reactor 2, it is possible that the chemicals influenced the stabilization of the reactors.

Figure 1. (A) Cumulative methane production (mL); (B) methane in biogas (%) in the AnDMBRs systems; (C) pH and (D) total alkalinity (mg/L CaCO3) values in AnDMBRs systems permeate.



Ersahin et al. (2017), treating synthetic wastewater without ECs in AnDMBR, obtained 63% methane in biogas. Alibardi et al. (2016) achieved methane between 55 and 65% in biogas for wastewater treatment with AnDMBR. Chen et al., (2018) using AnMBR to treat pharmaceutical wastewater obtained average methane production of 78.5%. It suggests that in the present study the mixed ECs added to the systems (antibiotics and pesticides) caused a disturbing effect on the systems. Probably they do not have enough anaerobic biodegradability to enhance methane production. However, it does not seem to be highly negative for the systems, since CH_4 production remained around 50% of biogas.

pH and Total alkalinity

Figure 1C and 1D illustrate, respectively, the pH and total alkalinity values in the permeates from the AnDMBR systems. AnDMBR 1 exhibited lower values for pH and total alkalinity compared to AnDMBR 2. As stated previously, the continuous addition of

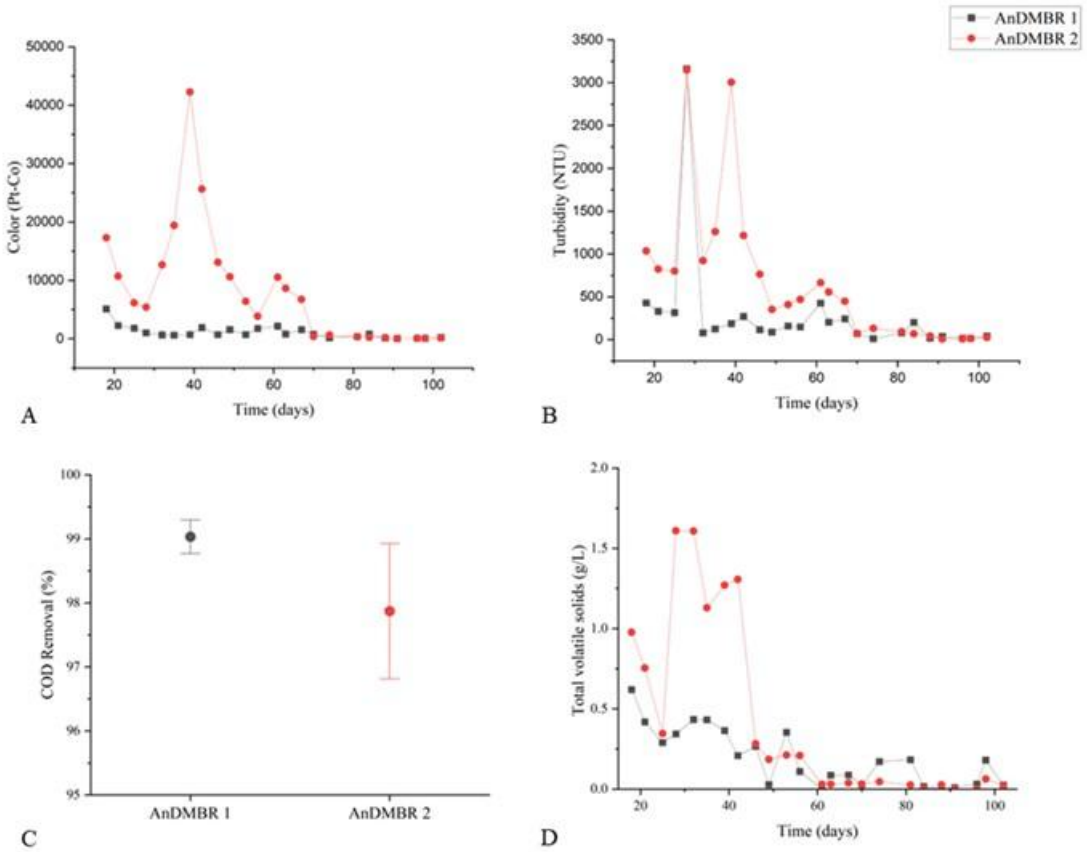
ECs in the AnDMBR2 system is one possible cause of instability in these parameters. However, it was expected to rise in total alkalinity in AnDMBR2 values since it is related to protein degradation, not necessarily indicating inhibition of the process. However, statistically significant differences ($p<0.05$) were observed between the systems for the evaluated parameters. The process of degradation of the ECs with the formation of intermediate transformation products in ionic forms was possibly responsible for the increase in pH values between the systems.

The performance of an AnMBR treating wastewater containing ECs is reduced under acidic and alkaline pH conditions. Under these conditions, the system produces a large amount of soluble microbial products (SMP) and extracellular polymeric substances (EPS), which accelerate membrane fouling (Ji et al., 2020). Inhibition of methanogenic activity occurs when the pH drops below 6.2, with optimum values in the range above 7. Moideen et al. (2023) reported a significant reduction in biogas production attributed to the inhibitory activity caused by a decrease in pH. Considering that the parameter values remained within the range considered ideal by literature, no damage to the process was caused by these parameters.

Color and turbidity

The results measured for apparent color and turbidity are presented in Figure 2A and 2B. It indicated that the formation of the biofilm layer in the dynamic membrane had a positive impact on the treatment efficiency. AnDMBR1 showed lower values, indicating a faster membrane stabilization process. The operational instability of AnDMBR2, characterized by significantly higher initial color and turbidity values in the permeates compared to AnDMBR1, especially observed between days 25 and 35, may have contributed to the difficulty in forming and stabilizing the biological membrane. There were significant differences between the results ($p<0.05$). At the end of the operation, the results between the two systems converged. After the 80th day of operation, the filtration process stabilized in both AnDMBRs, indicating the formation of a stable membrane.

Figure 2. (A) Color; (B) Turbidity values in AnDMBRs systems permeate; (C) COD removals efficiency; (D) Total volatile solids concentrations in AnDMBRs systems permeate.



It was found that in both AnDMBRs, the membrane contributed to the removal of color and turbidity, being more effective for AnDMBR 1. For AnDMBR 2, the contribution of the membrane was approximately 37% and 18% for the removal of color and turbidity, respectively. For AnDMBR 1, the values increased to around 71% and 25% in the reduction of the respective parameters. This demonstrates the differences in the stabilization process of the membranes and supports the hypothesis that the ECs interfere with the stabilization of the AnDMBR 2 system, causing deflocculation of the sludge by increasing the number of particles present in the medium.

Pore size and the type of membrane material affect the rate of biofilm layer formation and the quality of the final effluent. Smaller pores generally lead to faster formation of the filter layer and better removal of parameters such as color and turbidity (Fonseca et al., 2022). Studying landfill leachate treatment with AnDMBR, Saleem et al. (2018) reported that lower turbidity (less than 10 NTU) was achieved with the smallest pore sizes (52 µm) compared to larger sizes (200 and 85 µm). Also using AnDMBR with an external filtration module, Ersahin et al. (2017) obtained high turbidity removal

efficiency, with final values in the permeate of 30 NTU.

COD and Total volatile solids

Efficiencies of COD removals and total volatile solids concentrations in the permeates are presented in Figure 2C and 2D. As shown, it took longer for AnDMBR2 to achieve stability compared to AnDMBR1. However, around the 60th day of operation, a downward trend in the results is observed, indicating stabilization. This trend continued until the end of the experimental period. A statistically significant difference was obtained for the COD results ($p=0.0002$), while the total volatile solids (TVS) results did not differ statistically ($p=0.9157$).

The composition of the feed water and operating conditions influence the characteristics of the suspended biomass. Initial deflocculation of the sludge from reactor AnDMBR2 was observed. The only difference between the affluent and the reactors was the constant flux of EC to reactor 2. It is possible that this difference impacted on the stabilization period of AnDMBR2, which was longer. Deflocculation causes a loss of biomass and reduces treatment efficiency. This leads to an increase in the reactor's turbidity values, as the sludge flocs are broken down into smaller particles. Although deflocculation can increase the stabilization of organic matter by boosting hydrolysis, its excess can destabilize the reactor by releasing a vast number of fine particles and affecting the structure of the microbial community (Ebenezer et al., 2015; Wilén et al., 2000).

The reduction in TVS values over time achieved by the membranes is evidence of the biofilm formation process, resulting from the deposition of particulate material and microbial biomass. The greater contribution observed in AnDMBR 2 (around 0.5%) corroborates the formation of a thicker biofilm layer. While in AnMBR 1, the membrane only contributed around 0.2% to reducing the TVS value.

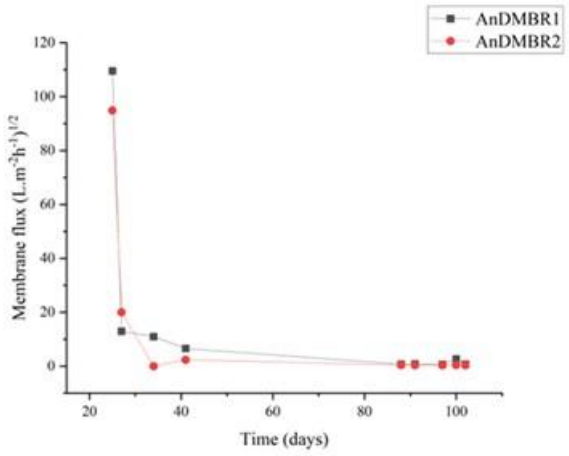
Total COD removal of 87% was achieved by Alibardi et al. (2016) after 122 days of AnDMBR operation. Moideen et al. (2023) completed a maximum COD removal of 99.54%. The authors attributed the high efficiencies to the combination of anaerobic metabolism treatment and the physical barrier provided by the membrane. The high retention efficiency of the membrane explains the system's ability to absorb variations and maintain low levels of COD in the permeate. The better COD removal performance can be attributed to the presence of highly specialized biomass. When intercepted by the membrane, bacteria and specific organic macromolecules have had their development favored, causing an increase in the removal efficiency of recalcitrant compounds (Chyoshi et al., 2022). Despite the rise, the membrane made a negligible contribution to

total COD removal, with values of around 0.5% and 2.4% for AnDMBR1 and AnDMBR2, respectively.

Membrane flux

Figure 3 shows the flow of permeates through the membranes over time. Despite some differences, both systems exhibit a similar flow behavior, indicating that a stable dynamic membrane had formed. The operation of the AnDMBR 2 was hampered by the clogging of the pores, which impaired the filtration process throughout the experiment. This resulted in very low permeate volumes at the end of the operation, even with periodic backwashing. Typical behavior of gravity-operated membranes, as also observed by de Souza et al., 2023. Clogging was also observed for AnDMBR 1, but not as intensely. This suggests that the clogging of the pores in AnDMBR 2 may have occurred due to the accumulation of ECs during the filtration process.

Figure 3. Membrane flux in the AnDMBRs systems



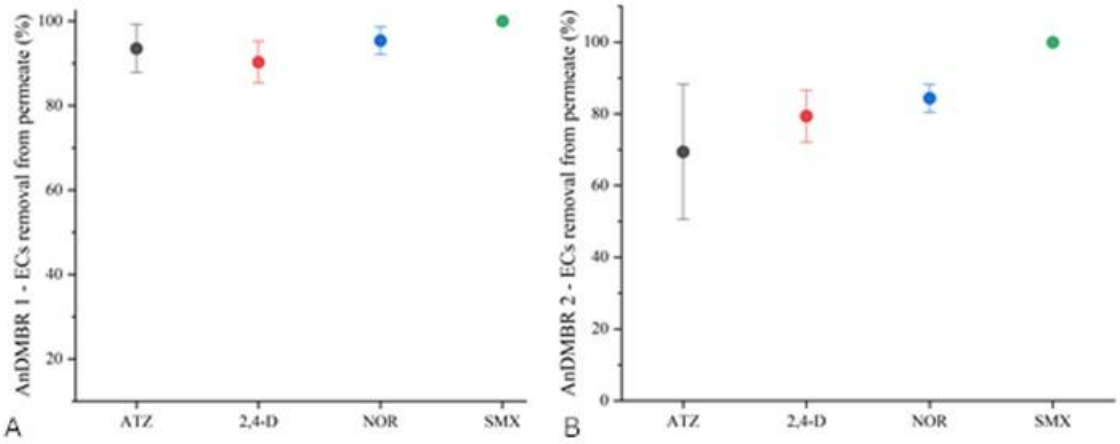
The characteristics of wastewater, reactor configurations and the conditions applied to the filtration module influence the formation, consolidation and filtration resistance of DM (Alibardi et al., 2016). Suspended solids, dissolved organic matter, and soluble microbial products are the main contributors to fouling membrane bioreactors. The formation of the cake layer, which controls the applied flux and transmembrane pressure, is the main contributor to membrane fouling and can be partially removed by backwashing (Yang et al., 2024). Exposure to ECs caused a decrease in specific surface area and total pore volume. Decreasing the particle size of DM can form a dense structure and consequently increase the specific resistance during filtration. Smaller particles may contain more EPS than larger ones, contributing to the formation of a dense structure (Sun

et al., 2025).

Emerging contaminants removal

The continuous addition of ECs resulted in a decrease in removal efficiency, as shown in Figure 4. AnDMBR1 achieved removal percentages greater than 85% for all ECs evaluated, with the lowest value for 2,4-D at 89 % and the highest for SMX, achieving total removal. On the other hand, AnDMBR2 achieved lower removal efficiency for all ECs compared to AnDMBR1. ATZ achieved the lower rate with 70% removal. SMX was the only contaminant to achieve total removal for both AnDMBRs, probably due to its high biodegradability. However, statistically significant differences were observed only for the ATZ and NOR results ($p<0.05$) between the AnDMBRs.

Figure 4. ECs removal in AnDMBRs systems permeates (%)



The removal of ECs in AnDMBRs is related to several processes that can occur simultaneously and individually in the bioreactor and in the dynamic membrane. These include biodegradation, sorption and biosorption. For AnDMBR1, it was observed that the DM increased the removal of 2,4-D and NOR by 22% and 12.5%, respectively. In AnDMBR2, DM only had a substantial effect on NOR removal, with a 28% increase. For 2,4-D it was observed the role of DM in the removal increase was a 5% increase. For ATZ and SMX, the membrane did not contribute to removal in any of the systems.

Polar substances, such as the emerging contaminants used in this research, are mainly removed by biodegradation, while non-polar substances can undergo adsorption or microbial degradation. Thus, it can be said that for polar substances, biodegradation is the mechanism that contributes most to their removal, with other mechanisms, such as

membrane filtration, being auxiliary (Wang et al., 2023). The presence of electron acceptors or donor groups can also influence removal by biodegradation. Most electron-donating groups (hydroxyls and amines) achieve high removal efficiencies (Langehn et al., 2021). The proteins in EPS have a high affinity for binding to antibiotics, this process is spontaneous and contributes to the removal of these compounds by DM (Sun et al., 2025).

Arcanjo et al., (2022) obtained an overall removal rate of over 96.2% for the drugs studied. Chyoshi et al. (2022) measured rates of over 60%, even after reducing the HRT used from 13 to 6.5h. The authors attributed the high rates obtained mainly to biodegradation. Wang et al., 2023 also observed the dominance of microbial degradation although membrane filtration and adsorption had an auxiliary role on the removal of the micropollutants studied. The authors obtained removals of around 20% for 2,4-D after two months of operation at a mesophilic temperature of 35°C. Tadkaew et al. (2011) observed less than 5% removal of ATZ in MBR.

SRT is one of the most important parameters in the removal of recalcitrant compounds from wastewater. Generally, high SRT values help the development of slow-growing microorganisms, increasing the diversity of the reactor's microbial community. SRTs greater than 30 days, as in this study, increase the removal efficiency of antibiotics and promote the growth of bacteria groups associated with their removal (Langehn et al., 2021).

It is also necessary to consider that in the current study, higher concentrations of ECs were used than those normally found in the environment, as described in the Materials and Methods section. The concentration of ECs is a key factor in the degradation process. High concentrations can either increase degradation due to greater availability of carbon and energy sources, or inhibit it due to toxicity, depending on the compound and microorganisms involved (Mqambalala et al., 2025; Liu et al., 2024). Anaerobic biodegradation generally results in the accumulation of intermediate compounds. Co-metabolism process, which is generally favored by high concentrations, may result in partial degradation or the formation of intermediate products (Mqambalala et al., 2025).

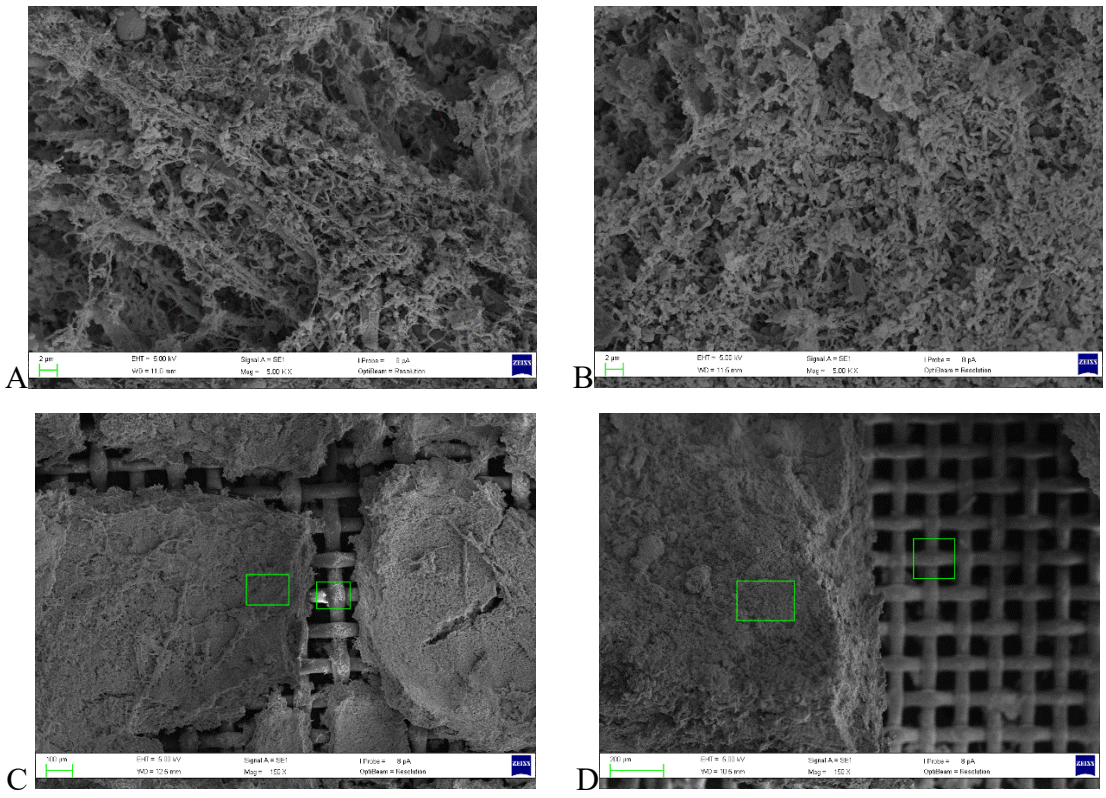
The combination of high concentrations of ECs and anaerobic conditions creates a challenging scenario for the degradation process. Among the challenges are reduced metabolic capacity, as microorganisms are more susceptible to toxicity, as well as slow degradation rates of compounds (Liu et al., 2025).Overall, in this study, the removal of

ECs using AnDMBR proved to be promising, as it offers an alternative for eliminating these contaminants from wastewater.

Biofilm

Figure 5 shows the characterization of the biofilm formed in the reactors. The reactors revealed the presence of bacterial colonies, primarily composed of cocci and bacilli. However, AnDMBR 1 had a greater presence of bacterial microfilaments (5A) than AnDMBR2 (5B). The high presence of solids observed throughout the operation of the AnDMBR2 reactor may have contributed to the formation of a thicker biofilm, 1.07 mm, with a more continuous surface (5D). AnDMBR1, on the other hand, had a biofilm 0.28 mm thick, with a more irregular and brittle surface (5C).

Figure 5. Biofilm characterization for the AnDMBR1 (5A and 5C) and AnDMBR2 (5B and 5D) systems.



Biofilms are complex structures formed by microbial cells irreversibly associated with a surface and wrapped in a polysaccharide matrix. The presence of microorganisms of various species with diverse metabolic activities enables the degradation of contaminants (Donlan, 2002; Mahto & Das, 2022). The solid-liquid interface between the surface and the aqueous medium provides an ideal environment for the attachment and

growth of microorganisms, however the colonization process is always affected by factors in the medium, such as pH, nutrient availability and temperature (Donlan, 2002; Li et al., 2022). Physicochemical properties of the surface can also have a strong influence on the efficiency and extent of attachment, with research indicating that microorganisms tend to attach more quickly to non-polar hydrophobic surfaces (Donlan, 2002).

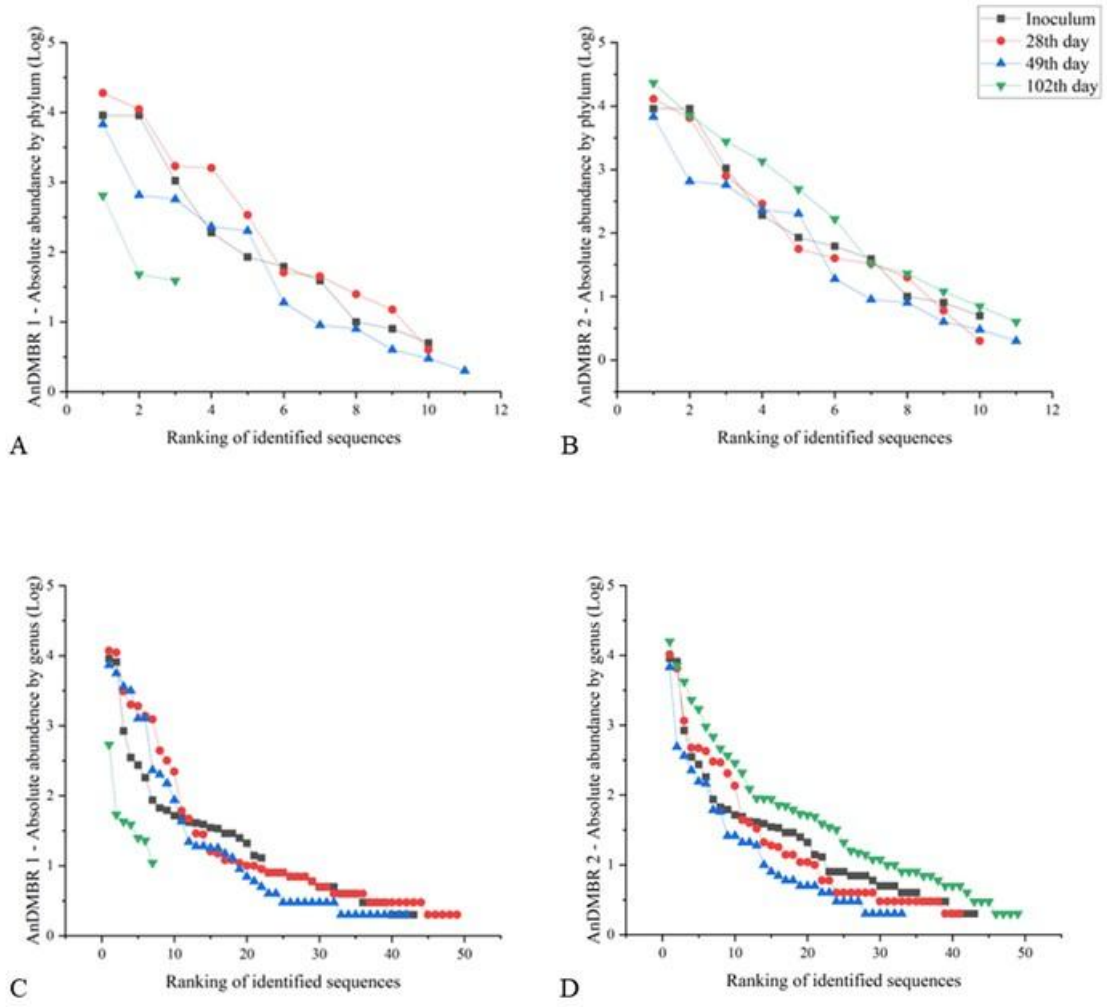
When contaminated wastewater passes through biofilm, EPS act as a sorption agent for the pollutants. The metabolically active cells present in the biofilm rapidly degrade or detoxify dangerous contaminants (Mahto & Das, 2022). In addition, EPS contributes to antibacterial resistance by preventing the transport of antibiotics through the biofilm, possibly due to the direct link with them (Donlan, 2002). The access to nutrients from the biofilm medium by the microorganisms in the anaerobic layer helps in the degradation of sewage pollutants (Li et al., 2022). The use of antimicrobial agents before biofilm development can be an effective strategy to prevent the adhesion of microorganisms. However, continuous use of the same chemicals often results in the occurrence of microorganisms that are tolerant to them (Hori & Matsumoto, 2010).

The microbial community of the cake layer is likely to be found in the sludge mass in classification and function. Specific local conditions in the deposition layer tend to favor microorganisms that are better adapted to the development of the biofilm (Yang et al., 2024).

Microbial community abundance

Figure 6 (6A and 6B) shows the abundance ranking (Whittaker Plot) for the microorganism sequences identified in terms of phylum at different times during the operation of the AnDMBR systems (28th, 49th, and 102nd day). It can be inferred that the diversity and richness of the microbial community were maintained over time for AnDMBR2. AnDMBR1, on the other hand, showed a notable change in its microbial dynamics. At the end of the operation (102nd day), AnDMBR1 showed a microbial community with few abundant individuals and little uniformity in distribution. This may be associated with the fact that the 49th day of operation was when the AnDMBR1 system received the last contaminant (SMX, which was the last to be added to both systems). From then on, it did not receive another dose of contamination. The decrease in the diversity of available carbon sources may have negatively impacted the microbial community in AnDMBR1, leading to its shrinkage.

Figure 6. Change in ranking of absolute abundance (Whittaker Plot) of the identified sequences in terms of the phylum (6A and 6B) and genus (6C and 6D) of microorganisms during the operation of AnDMBR systems.



The main classified microbial phyla found in the inoculum were *Euryarchaeota* (46.39%) and *Proteobacteria* (5.37%). At the end of the operation (102nd day), in both AnDMBRs, *Proteobacteria* dominated, followed by *Euryarchaeota*, and the emergence of *Bacteroidetes*. The phylum *Firmicutes* were also detected in the inoculum (1.81%) and in AnDMBR2 (1.39%) but were absent in AnDMBR1.

The Proteobacteria phylum is often cited as one of the main phyla capable of degrading various types of ECs, including drugs such as SMX (Mishra et al., 2021; Shah et al., 2024). According to Fang et al. (2019), SMX and ATZ degraded rapidly under sulfate-reducing conditions, indicating that they react with sulfate-reducing species such as microorganisms from the phylum Bacteriodetes. Species within this group use sulfate as a final electron acceptor and can initiate the ATZ dechlorination process.

The abundance ranking (Whittaker Plot) for the microorganism sequences identified in terms of genus at different times during the operation of the AnDMBR systems (28th, 49th, and 102nd day) is presented in Figure 6 (6C and 6D). In terms of genus, AnDMBR 1 showed low abundance and little uniformity in the distribution of organisms. AnDMBR2, on the other hand, had a richer and more diverse community, with greater uniformity in the distribution of abundances. The results followed the same trend as those presented for the phyla (Figure 8). In terms of genus, the main ones detected in the inoculum were *Methanothrix* (41.59%), *Methanobacterium* (4.31%), and *Rhizobium* (1.80%). For AnDMBR1, on the 102nd day, the genus *Pseudomonas* (73.43%) and *Brachymonas* (7.36%) stood out. For AnDMBR2, the genus *Janthinobacterium* (45.12%) dominated, followed by *Pseudomonas* (11.89%), *Methanothrix* (6.54%), and *Brachymonas* (4.85%).

The genus Pseudomonas was common to both reactors. Pseudomonas have been reported to be efficient at biodegrading ECs in contaminated environments, indicating exceptional potential for bioremediation. The biodegradation ability of these microorganisms is strongly influenced by interactions with various factors, including salinity, temperature, carbon and nitrogen source availability, pH, and inoculum concentration (Mishra et al., 2021). They are among the organisms involved in the SMX biodegradation process, using it as a carbon and energy source (Shah et al., 2024). Pseudomonas are among the key genera for the degradation of 2,4-D (Chen et al., 2024; Kumar et al., 2014). They also participate in the degradation of NOR, causing the cleavage of the piperazine ring and displacement of the fluorine, resulting in intermediate products with reduced antibacterial potential (Amorim et al., 2013).

The genus Janthinobacterium, the dominant one in AnDMBR2, contributes to denitrification, dechlorination, and benzene ring cleavage processes, and is thus active in the removal of ECs. Together with other genera, such as Pseudomonas, they can mineralize antibiotics and their metabolites (Liu et al., 2022). Brachymonas, on the other hand, not only removes ECs but also contributes to increasing and strengthening the structure of the microbial community, thereby enhancing reactor stability (Zheng et al., 2020).

Microorganisms degrade ECs using them as a carbon or nitrogen source. The efficiency and type of degradation depend on environmental conditions (such as the presence or absence of oxygen), the properties of the contaminants, and the structure of the microbial community. A diverse microbial community generally gives better results

due to cooperation and metabolic interactions between microorganisms (Mishra et al., 2021; Shah et al., 2024; Biel-Maeso et al., 2019; Fang et al., 2019). Most common microorganisms involved in the biodegradation of ECs remain undefined because not all microorganisms present in nature can be cultivated under in vitro conditions, but rather in viable-but-non-culturable (VBNC) environments (Mishra et al., 2021).

Microbial activity is affected by the conditions imposed by the process (Ersahin et al., 2017). High levels of ECs can cause changes in microbial structure and function, affecting respiration, EPS production, and gene expression, thereby affecting the ability to degrade compounds (Mqambalala et al., 2025; Gomes et al., 2020). In general, AnDMBR 2 showed greater microbial diversity than AnDMBR 1. This was likely due to the increasing diversity of carbon sources over time, which enabled the development of a more diverse community of microorganisms. According to Mishra et al. (2021), a diverse community of microorganisms can survive in highly contaminated environments, exhibiting significant potential for biodegrading and transforming pollutants.

4.4.5 CONCLUSION

The two AnDMBR systems showed similar values for methane in the biogas produced, indicating that the continued ECs added to AnDMBR2 did not affect the process. A significant reduction in apparent color and turbidity was observed in both AnDMBRs, with COD removals of over 95%. Sludge deflocculation was observed in AnDMBR2, possibly due to the continuous addition of ECs, resulting in a higher solids content throughout the operation and increased membrane fouling. The higher solids resulted in a thicker and more uniform membrane for AnDMBR2.

In terms of microbial community, AnDMBR 1 exhibited low abundance and limited uniformity in the distribution of organisms at both the phylum and genus levels. AnDMBR2, on the other hand, had a richer and more diverse community, with greater uniformity in the distribution of abundances. The microorganisms identified at the end of the operation in the AnDMBRs indicate that anaerobic biodegradation was among the processes responsible for the degradation of ECs.

The dynamic membrane proved to be effective in retaining solids, resulting in a considerable reduction in apparent color and turbidity values, as well as a small contribution to COD removal. For the removal of ECs, the dynamic membrane showed different performances depending on the compound evaluated. Future studies are needed

to elucidate further its role in removing ECs from wastewater.

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5. CONSIDERAÇÕES FINAIS

A análise bibliométrica confirmou o elevado interesse científico pelos biorreatores anaeróbicos de membrana (AnMBR) para o tratamento de contaminantes de preocupação emergente (CPEs), porém revelou que o campo ainda apresenta lacunas. A pesquisa demonstrou a prevalência de estudos sobre a remoção de produtos farmacêuticos e genes resistentes a antibióticos. No entanto, foi constatada escassez de estudos sobre agrotóxicos e a aplicação de AnMBR em escala piloto, limitando a sua validação em escala real. Países do Oriente Médio, Sul e Sudeste Asiático foram os mais ativos em publicações, e a pesquisa global se concentrou em reatores de bancada e temperatura mesofílica. Como tendências futuras se observou enfoque na caracterização microbiana para geração de biogás e no desenvolvimento de medidas de controle do *fouling* e recuperação de nutrientes. Dada a posição do Brasil como um dos líderes mundiais no uso de pesticidas e fármacos, o desenvolvimento de estudos nacionais sobre a remoção desses CPEs é fundamental para soluções de saneamento de alta qualidade e mitigação do risco ambiental.

Em relação à digestão anaeróbia (DA) dos fármacos, os resultados demonstraram que a classe e a concentração dos antibióticos sulfametoxazol (SMX) e norfloxacino (NOR) exerceram interferência no processo de DA. A presença dos antibióticos misturados impulsionou a atividade microbiológica, resultando em um aumento na produção cumulativa de biogás e no potencial bioquímico de metano. Contudo, essa aceleração não se traduziu em melhor qualidade, pois o menor teor de metano no biogás sugeriu um aumento na produção de dióxido de carbono. A baixa concentração final de ácidos graxos voláteis (AGVs), ao final do experimento, indicou que a matéria orgânica foi rapidamente esgotada nos primeiros dias, justificando os picos iniciais de produção de biogás. Em relação à remoção dos antibióticos, o processo foi seletivo. A taxa de remoção do SMX não foi afetada pela sua concentração, sugerindo que o composto foi provavelmente biodegradado pelos microrganismos. Em contraste, a remoção da NOR diminuiu com o aumento da sua concentração, com o composto persistindo na fase aquosa, indicando condições desfavoráveis para sua degradação ou adsorção. Embora a eficiência de remoção de DQO geral tenha se mantido dentro das faixas esperadas, essa persistência da NOR contribuiu para uma menor eficiência de DQO ligada à redução de compostos na fase líquida.

Se tratando da DA dos agrotóxicos, os resultados dos experimentos demonstraram

que a adição do 2,4-D e da atrazina (ATZ) foi bem tolerada pelo sistema e estimulou positivamente a produção de metano. O resultado mais notável foi o valor máximo de 4,3 L de metano alcançado nos reatores contendo a combinação de 2,4-D (10 mg/L) e ATZ (2,5 mg/L). A eficiência geral do processo foi alta, com a remoção total de DQO superior a 80% em todos os reatores. Além disso, a estabilidade do sistema foi mantida, com os valores de pH e alcalinidade total permanecendo na faixa operacional esperada, e a predominância dos ácidos acético e propiônico sem sinais de acúmulo de AGVs, indicando um processo metabólico equilibrado. O sistema anaeróbico provou ser um meio eficiente para a produção de metano e para a remoção do 2,4-D. Contudo, a biodegradação da ATZ se mostrou incompleta e menos eficaz em condições mistas.

Os resultados do experimento com os dois biorreatores anaeróbicos de membrana dinâmica (AnDMBR), sendo um de controle (AnDMBR1) e outro com adição contínua de CPEs (AnDMBR2), revelou um processo robusto, mas com interferências físicas e biológicas causadas pelos CPEs. Ambos os sistemas apresentaram um desempenho satisfatório na qualidade do efluente final, com remoções de DQO superiores a 95% e redução significativa na cor aparente e na turbidez. Além disso, os CPEs adicionados continuamente ao AnDMBR2 não afetaram a qualidade do biogás, pois os valores de metano no gás produzido foram semelhantes nos dois sistemas. No entanto, a interferência física causada pelos CPEs foi visível no AnDMBR2. A adição contínua de CPEs resultou na defloculação do lodo, levando a um maior teor de sólidos e, consequentemente, a um aumento do *fouling* na membrana dinâmica (MD). O maior teor de sólidos formou uma MD mais espessa e uniforme no AnDMBR2. Em relação à comunidade microbiana, o AnDMBR1 apresentou baixa diversidade e uniformidade de organismos, enquanto o AnDMBR2 desenvolveu uma comunidade mais rica e diversificada, indicando que a pressão seletiva dos CPEs pode ter estimulado a proliferação de microrganismos adaptados. A identificação de organismos ao final da operação confirmou que a biodegradação anaeróbica foi um dos processos responsáveis pela degradação dos CPEs. Por fim, a MD mostrou-se eficaz na retenção de sólidos, mas sua contribuição para a remoção de CPEs variou dependendo do composto, indicando a necessidade de estudos futuros para elucidar melhor o seu papel específico nos mecanismos de remoção de CPEs.

6. RECOMENDAÇÕES PARA TRABALHOS FUTUROS

Com base nos resultados desta tese, sugere-se que as pesquisas futuras na área se concentrem em três eixos principais para aprofundar o conhecimento e promover a sustentabilidade dos sistemas de tratamento: avaliação de mecanismos de degradação, análise de riscos microbianos e viabilidade de reúso de efluentes.

- Avaliação de mecanismos de degradação: recomenda-se o emprego de amostras experimentais significativamente maiores para aumentar a robustez estatística dos resultados. Além disso, também se sugere a integração da caracterização da comunidade microbiana com o rastreamento dos produtos da transformação dos contaminantes (ex: metabólitos), a fim de elucidar as vias de degradação.
- Análise de riscos microbianos: recomenda-se a avaliação da presença e determinação de genes resistentes a antibióticos (ARGs) na biomassa anaeróbia ao final da operação do sistema. Esta análise é importante para compreender o papel potencial do sistema no ciclo de disseminação da resistência.
- Viabilidade de reúso de efluentes: Como foco de pesquisas futuras, é prioritário avaliar a qualidade físico-química e microbiológica do efluente tratado e do lodo gerado, verificando sua adequação para reúso em diferentes finalidades (urbana, industrial, agrícola), conforme as legislações estaduais e nacionais vigentes (ex: Resolução CONSEMA/RS nº 419/2020).

Tendo em vista a lacuna regulatória no Brasil, sugere-se a realização de estudos de caso e análises comparativas que possam subsidiar a criação de normas nacionais mais abrangentes para o reúso de efluentes, inspiradas em referências internacionais como Organização Mundial da Saúde (OMS), a Agência da ONU para Agricultura e Alimentação (FAO) e a Agência Americana de Proteção Ambiental (USEPA), mas adaptadas ao contexto e necessidades brasileiras. Recomenda-se aprofundar a relação entre as técnicas de reúso e a presença/destino de CPEs nos efluentes e lodos tratados.

Como forma de contribuir para o desenvolvimento da relação entre as técnicas de reuso e a presença de CPEs, o então aluno de doutorado realizou um período de mobilidade acadêmica, na Università degli Studi di Roma "La Sapienza" (Itália) através do projeto intitulado: “REUSE OF TREATED WASTEWATER FOR AGRICULTURAL IRRIGATION: INFLUENCE OF THE PRESENCE OF CONTAMINANTS OF EMERGING CONCERN”, fomentado com auxílio proveniente do Edital Mobility

Confap Italy MCI 2025 pela Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

APÊNDICE A

As imagens retratadas mostram registros fotográficos que apresentam o sistema de reatores anaeróbios em batelada que foi usado no experimento com fármacos referente ao “Artigo 2”. O sistema foi o mesmo utilizado por adaptado de Guedes *et al.*, (2024) e Konrad *et al*. (2023).



a) Reatores anaeróbios montados para início do experimento em batelada com os fármacos.



b) Reatores posicionados em compartimento com temperatura controlada e controle automatizado de produção de biogás

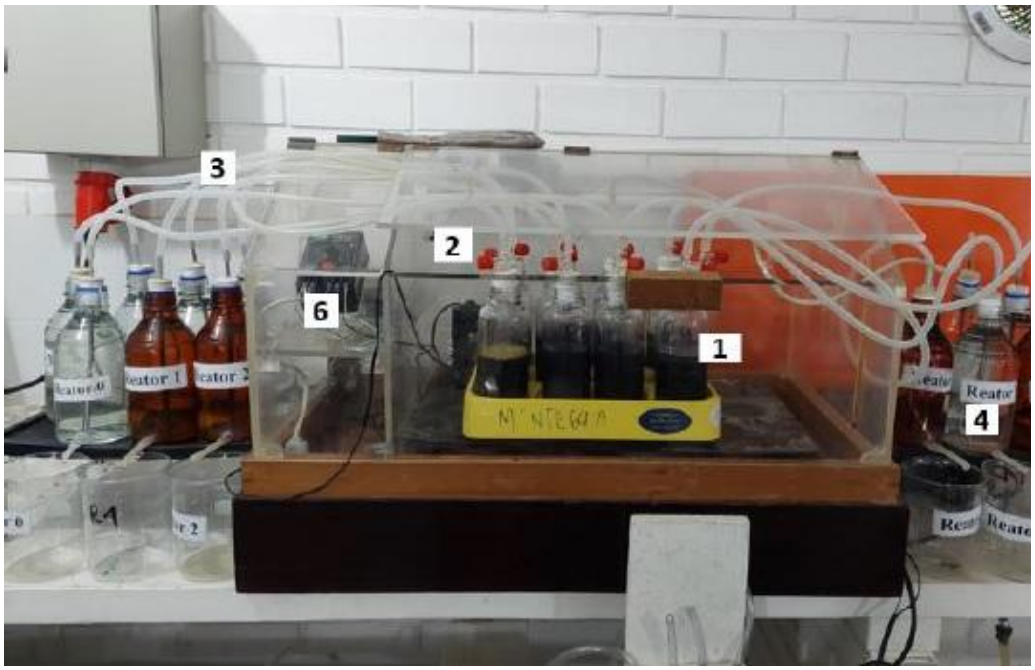


c) Aparelho usado para quantificação da quantidade de metano presente no biogás ao longo do experimento

APÊNDICE B

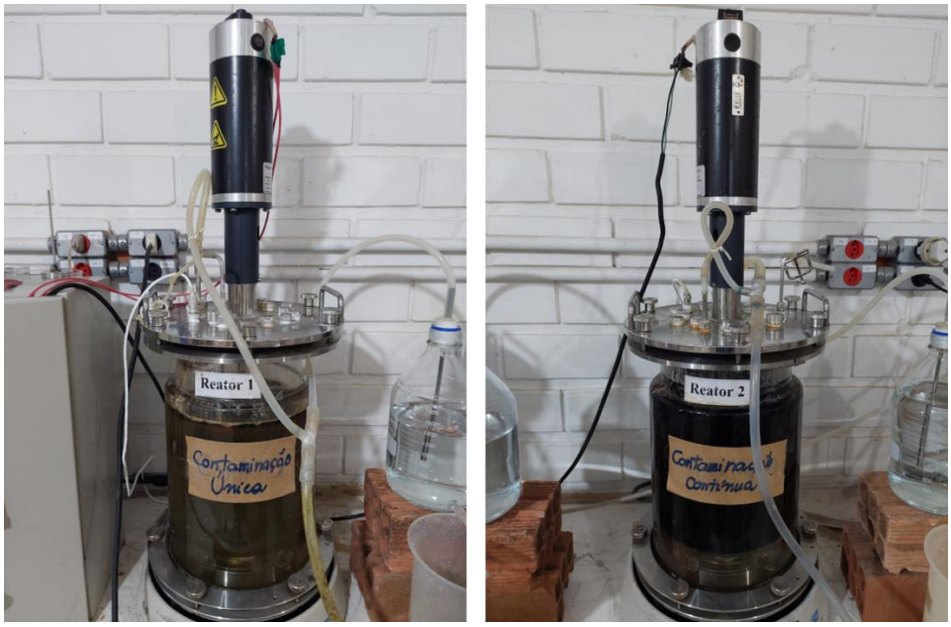
A imagem retratada mostra registro fotográfico que apresenta o sistema de reatores anaeróbios em batelada que foi usado no experimento com agrotóxicos referente ao “Artigo 3”. O sistema é adaptado de Guedes *et al.*, (2024) e apresenta as seguintes partes:

1. Bandeja agitadora.
2. Reatores anaeróbios.
3. Tampa e dispositivos de borracha para vedação e coleta de biogás.
4. Termostato para ajuste e controle de temperatura.
5. Mangueiras de borracha para transporte do biogás produzido.
6. Frascos de Boyle-Mariotte preenchidos com água para quantificação do volume de biogás produzido.



APÊNDICE C

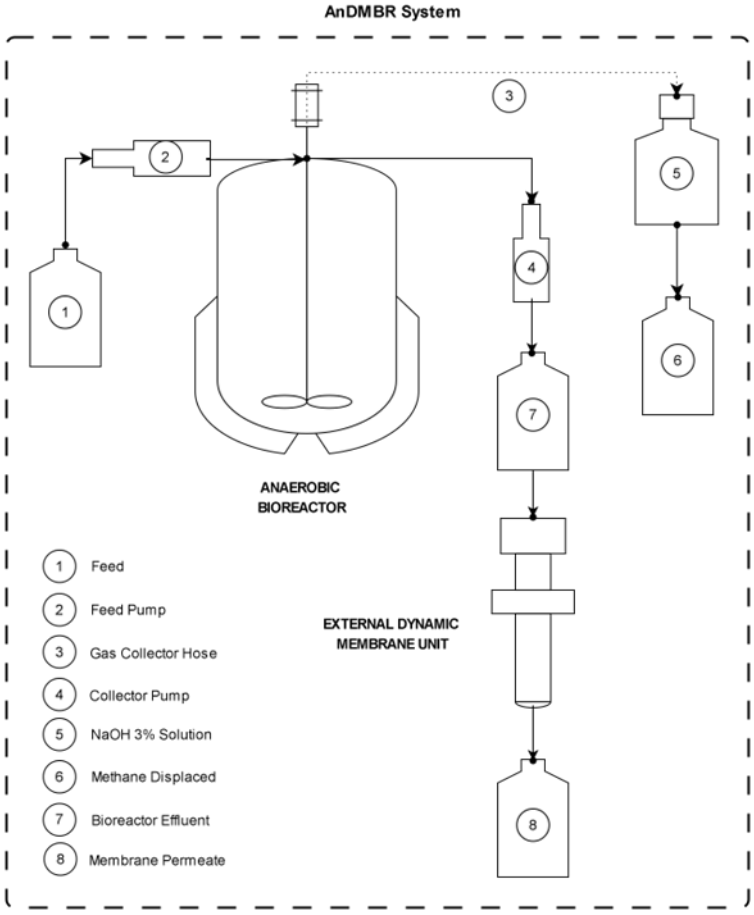
As imagens retratadas mostram registros fotográficos do experimento de remoção dos fármacos e agrotóxicos com o uso dos sistemas de biorreatores anaeróbios de membrana dinâmica (AnDMBR) referentes ao “Artigo 4”.



a) Biorreatores anaeróbios do tipo Bioflo usados como componentes dos sistemas AnDMBR



b) Módulos de filtração externa desenvolvidos e usados como componentes dos sistemas AnDMBR



c) Desenho esquemático da configuração completa usada no experimento.