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THUANE MENDES ANACLETO

AVALIAÇÃO DA EFICIÊNCIA DE PRÉ-TRATAMENTOS PARA MAXIMIZAÇÃO DA PRODUÇÃO DE BIOGÁS E POTENCIAL AGRONÔMICO DO DIGESTATO

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Thuane Mendes Anacleto

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Orientador: Dr. Alex Enrich Prast

Co-orientadora: Drª Érika Flavia Machado Pinheiro

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Este documento representa o fim de uma longa linhagem de trabalhadoras domésticas em minha família. A partir de agora, geramos doutoras.

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RESUMO

ANACLETO, Thuane Mendes. **Avaliação da eficiência de pré-tratamentos para maximização da produção de biogás e potencial agronômico do digestato**. Rio de Janeiro, 2024. Tese (Doutorado em Biotecnologia Vegetal e Bioprocessos) – Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2024.

A digestão anaeróbica (DA) é uma tecnologia que enfrenta desafios globais como gerenciamento de resíduos, geração de energia renovável, produção de biofertilizantes e mitigação de emissões de gases de efeito estufa, contribuindo significativamente para os objetivos globais de descarbonização. Esta tese investiga o efeito de diversos métodos de pré-tratamento aplicados a uma variedade de resíduos orgânicos na produção de biogás. Foram realizadas quatro revisões sistemáticas seguidas por meta-análises para avaliar a eficiência dos pré-tratamentos em relação à produção de biogás, medida pelo rendimento de metano (CH_4). Os resultados mostram que, embora os pré-tratamentos possam aumentar significativamente a produção de biogás, seu desempenho em diferentes fontes de matéria orgânica ainda é pouco explorado. A meta-análise demonstrou que a eficiência do pré-tratamento depende fortemente da composição química predominante dos resíduos orgânicos. Estas descobertas podem impactar significativamente a demanda global de energia. Por exemplo, com uma seleção mais assertiva de pré-tratamentos, mesmo resíduos menos investigados para aplicação na DA, como a biomassa algal, podem ser otimizados, aumentando seu potencial de geração de biogás em 125%. Em contraste, resíduos amplamente utilizados, como o esterco, podem não alcançar seu potencial energético máximo devido à aplicação incorreta de pré-tratamentos, reduzindo o rendimento de CH_4 em aproximadamente 110%. Além disso, a combinação de DA com pré-tratamentos mostrou-se altamente vantajosa para o tratamento de resíduos industriais. Quando pré-tratamentos adequados são aplicados a resíduos da indústria têxtil, a segunda maior poluidora do mundo, a DA reduz o potencial poluidor enquanto gera energia limpa, tornando a cadeia produtiva mais sustentável. Com base nessas descobertas, é possível inferir configurações ideais para explorar novas fontes de resíduos ou otimizar as já utilizadas, maximizando sua eficiência energética. A escolha do pré-tratamento adequado aumenta o lucro econômico, reduz custos operacionais e riscos ambientais, e eleva o potencial energético gerador desses resíduos. Além dos benefícios energéticos, esta tese também avaliou a contribuição da DA no setor agrícola. Ensaios experimentais avaliaram o valor agronômico do digestato, produto da DA amplamente reconhecido pelo seu potencial fertilizante e/ou condicionador de solo, uma alternativa promissora para reduzir a dependência de fertilizantes minerais e promover a reciclagem de nutrientes dentro do modelo de economia circular. Investigou-se a fitotoxicidade de diferentes tipos de digestato (*e.g.*, lodo de esgoto, resíduos alimentares, biomassa agrícola e esterco) de digestores de biogás em escala industrial, avaliando sua influência na germinação de sementes, através do índice de germinação (IG). Os resultados mostram que a melhoria do IG do digestato depende fortemente da fração do digestato (i.e, sólida, líquida ou total). A separação líquido-sólido reduziu a fitotoxicidade na fração líquida, resultando em IG de 99,31 ± 32,67%, enquanto aumentou na fração sólida, com IG menor que 0,1%. Além disso, a fonte do digestato também teve um impacto significativo na determinação da sua fitotoxicidade, uma vez que, dependendo da sua origem, pode conter contaminantes como metais pesados, herbicidas e resíduos farmacêuticos, que podem prejudicar o desempenho do digestato como fertilizante, reduzindo seu IG.

Palavras-chave: digestão anaeróbica, digestato, pré-tratamento, nutrientes, metano

ABSTRACT

ANACLETO, Thuane Mendes. **Assessing pretreatment efficiency to maximize biogas production and agronomic potential of digestate.** Rio de Janeiro, 2024. Tese (Doutorado em Biotecnologia Vegetal e Bioprocessos) – Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2024.

Anaerobic digestion (AD) is a technology that addresses global challenges such as waste management, renewable energy generation, biofertilizer production, and greenhouse gas emission mitigation, significantly contributing to global decarbonization goals. This thesis investigates the effect of various pretreatment methods applied to a variety of organic wastes on biogas production. Four systematic reviews followed by meta-analyses were conducted to assess the efficiency of pretreatments concerning biogas production, measured by methane (CH_4) yield. The results show that although pretreatments can significantly increase biogas production, their performance on different organic matter sources is still poorly explored. The meta-analysis demonstrated that the efficiency of pretreatment heavily depends on the predominant chemical composition of the organic wastes. These findings can significantly impact the global energy demand. For example, with a more assertive selection of pretreatments, even less-studied wastes for AD application, such as algal biomass, can be optimized, increasing their biogas generation potential by 125%. In contrast, widely used wastes, such as manure, may not reach their maximum energy potential due to the incorrect application of pretreatments, reducing CH_4 yield by approximately 110%. Furthermore, the combination of AD with pretreatments proved to be highly advantageous for treating industrial wastes. When adequate pretreatments are applied to wastes from the textile industry, the second-largest polluter in the world, AD reduces the pollutant potential while generating clean energy, making the production chain more sustainable. Based on these findings, it is possible to infer ideal configurations to explore new waste sources or optimize those already used, maximizing their energy efficiency. The appropriate choice of pretreatment increases economic profit, reduces operational costs and environmental risks, and elevates the energy-generating potential of these wastes. In addition to the energy benefits, this thesis also evaluated the contribution of AD in the agricultural sector. Experimental trials assessed the agronomic value of digestate, a product of AD widely recognized for its potential as a fertilizer and/or soil amendment, a promising alternative to reduce dependence on mineral fertilizers and promote nutrient recycling within the circular economy model. The phytotoxicity of different types of digestate (e.g., sewage sludge, food waste, agricultural biomass, and manure) from industrial-scale biogas digesters was investigated, evaluating their influence on seed germination through the germination index (GI). The results show that the improvement of the digestate GI heavily depends on the digestate fraction (i.e., solid, liquid, or whole). Liquid-solid separation reduced phytotoxicity in the liquid fraction, resulting in a GI of 99.31 ± 32.67%, while it increased in the solid fraction, with a GI of less than 0.1%. Furthermore, the source of the digestate also had a significant impact on determining its phytotoxicity, as it may contain contaminants such as heavy metals, herbicides, and pharmaceutical residues depending on its origin, which can impair the digestate's performance as a fertilizer, reducing its GI.

Keywords: anaerobic digestion, digestate, pretreatment, nutrients, methane

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AGV	Ácidos graxos voláteis
ANOVA	Análise de variância
ANP	Agência Nacional do Petróleo
CG-DIC	Cromatógrafo gasoso com detector por ionização de chama
COP 21	21ª Conferência das Partes
COT	Carbono orgânico total
DA	Digestão anaeróbia
DL	Digestato líquido
DS	Digestato sólido
DT	Digestato total
ETR	Elementos terras raras
GEE	Gases de efeito estufa
IC	Intervalo de confiança de 95%
ICP-MS	Espectrometria de massa com plasma indutivamente acoplado
IG	Índice de germinação
NAT	Nitrogênio amoniacal total
N_{org}	Nitrogênio orgânico
NDT	Nitrogênio dissolvido total
NOT	Nitrogênio orgânico total
ODS	Objetivos de Desenvolvimento Sustentável
ONU	Organização das Nações Unidas
PCA	Análise de componentes principais
PHA	Polihidroxialcanoatos
PLA	Ácido polilático
PRISMA	Itens de relatório preferenciais para revisões sistemáticas e meta-análises
RR	Razão de resposta logarítmica natural
RRG	Porcentagem relativa do comprimento da raiz
RSG	Porcentagem relativa de sementes germinadas
SDM	Diferença média padronizada
ST	Sólidos totais

SV	Sólidos voláteis
TCO	Taxa de carregamento orgânico
TRH	Tempo de retenção hidráulica
UE	União Europeia

Sumário

1 INTRODUÇÃO

1.1 Cenário global de segurança energética e alimentícia

A Agência Internacional de Energia define segurança energética como a oferta e disponibilidade de serviços energéticos a todo momento, em quantidade suficiente e a preços acessíveis (IEA, 2005). A energia é frequentemente caracterizada como um recurso essencial e, portanto, um foco estratégico e político, além de ser um objeto de crescente mercantilização (HÖYSNIEMI, 2022). Historicamente, os combustíveis fósseis, especialmente o carvão, dominaram o cenário energético, desempenhando um papel crucial na Revolução Industrial, explicando pelo menos 60% do crescimento das populações urbanas de 1750 a 1900 (FERNIHOUGH; O'ROURKE, 2021).

O aumento da demanda energética global e os efeitos negativos do uso de combustíveis fósseis, como a emissão de gases de efeito estufa (GEE), o desequilíbrio dos ecossistemas, a distribuição geográfica desigual e a natureza finita das reservas (FARIAS; SELLITTO, 2011; THOMBS, 2022) têm impulsionado a exploração de fontes alternativas de energia. Segundo o IPCC (2023), as emissões globais de GEE precisam ser significativamente reduzidas para limitar o aumento da temperatura global a 1,5 °C acima dos níveis pré-industriais, conforme estabelecido no Acordo de Paris. O relatório do IPCC enfatiza a necessidade urgente de transições sistêmicas em larga escala nas áreas de energia, uso do solo, transporte, construção e indústria para alcançar essas metas. Políticas ambientais, como o Protocolo de Kyoto (1997) e o Acordo de Paris da Conferência das Partes (COP 21), juntamente com os Objetivos de Desenvolvimento Sustentável (ODS) da Agenda 2030 da Organização das Nações Unidas (ONU), têm como principais objetivos a mitigação das emissões de GEE, o aumento da eficiência energética, a promoção de energias renováveis, a erradicação da fome, a proteção dos ecossistemas e a garantia de um futuro mais sustentável e equitativo. Esses acordos incentivam os países signatários a implementarem políticas internas que favoreçam a transição para uma economia de baixo carbono, promovendo inovações tecnológicas e mudanças estruturais que visem à sustentabilidade ambiental e à justiça climática.

Apesar das políticas de incentivo, a dependência de combustíveis fósseis continua sendo um desafio, especialmente devido às mudanças climáticas e à necessidade urgente de reduzir as emissões de GEE. A crise energética global de 2022, exacerbada pela invasão da Ucrânia pela Rússia, evidenciou a vulnerabilidade dos sistemas energéticos baseados em combustíveis fósseis e a urgência de diversificar as fontes de energia (JING, 2023). Isso levou muitos países a reconsiderarem suas políticas energéticas, acelerando a transição para fontes renováveis e reforçando a importância da segurança energética.

A transição para uma matriz energética global mais sustentável é essencial para alcançar os objetivos dos acordos internacionais e limitar o aumento da temperatura média global. Não apenas técnicas, mas políticas, conceitos e práticas desenvolvidas como parte de sistemas energéticos movidos a combustíveis fósseis precisam ser reconsiderados no mundo de baixo carbono (HÖYSNIEMI, 2022).

Avanços tecnológicos e a diminuição dos custos de produção de energias renováveis, como a solar e a eólica, tornaram essas fontes mais competitivas (GLENK; REICHELSTEIN, 2022). Segundo o Relatório Global de Eletricidade 2023, as energias renováveis representaram quase 30% da geração de eletricidade global em 2022, um aumento significativo em comparação com a década anterior (WIATROS-MOTYKA *et al.,* 2023). Novas fontes de energia limpa, como a energia nuclear avançada, biometano e o hidrogênio verde, estão emergindo como alternativas promissoras para complementar a matriz energética e garantir a estabilidade e a segurança do fornecimento (KANWAL; TORRIERO, 2022; TIN; SWARUP; KUMAR, 2021).

A segurança alimentar global é outro desafio crítico, com cerca de 795 milhões de pessoas sofrendo de fome crônica, apesar de uma diminuição na proporção de indivíduos subnutridos de 23,3% em 1990-1992 para 12,9% em 2014-2016 (TEICHMANN, 2015). A segurança alimentar abrange disponibilidade, acesso, utilização e estabilidade dos alimentos, exigindo esforços colaborativos de organizações internacionais, governos, academia e população (HENNEBERRY; CARRASCO, 2014). Tentativas históricas dentro do sistema da ONU para abordar a segurança alimentar destacaram os desafios persistentes, como a complexidade da integração dos sistemas alimentares com outros sistemas sociais, o impacto dos sistemas alimentares corporativos na saúde, os limites ambientais do uso de recursos agrícolas, e a necessidade de soluções abrangentes para garantir um abastecimento alimentar sustentável e seguro para todos (CLAY, 2008).

O uso regular de fertilizantes é essencial para a segurança alimentar global (LORICK *et al.*, 2020). De 2000 a 2019, o uso global de fertilizantes aumentou de 79 milhões de toneladas para 125 milhões de toneladas, com maior consumo observado em regiões economicamente desenvolvidas, como América do Norte e Europa, que registraram o maior uso total de fertilizantes químicos (SANE *et al.*, 2021).

Para atender às necessidades da produção de alimentos, reservas minerais finitas têm sido exaustivamente exploradas para extração de fósforo, e altos consumos energéticos são investidos na produção de nitrogênio sintético (LORICK *et al.*, 2020). Fertilizantes inorgânicos, produzidos a partir de rochas e minerais processados, fornecem nutrientes de forma imediata e concentrada (MANNING; THEODORO, 2020). Em contraste, os fertilizantes orgânicos, originados de materiais naturais como esterco, compostagem e resíduos vegetais, liberam nutrientes de maneira mais lenta e sustentável (BADAGLIACCA *et al.*, 2024). O Regulamento de Fertilizantes da União Europeia (UE), instituído em julho de 2019, visa promover uma competição igualitária entre fertilizantes orgânicos e inorgânicos no mercado interno da UE (ECN, 2021), viabilizando, desta forma, uma alternativa para suprir a demanda nutricional alinhada às políticas ambientais.

A digestão anaeróbia (DA) é uma tecnologia que combina o tratamento de resíduos orgânicos com a produção de bioprodutos valiosos, sendo os mais comuns o biogás e o digestato. O biogás, que pode ser refinado para biometano ($\leq$ 90% CH_4), é aplicável como fonte de energia elétrica, térmica e automotiva (ANGELIDAKI *et al.*, 2019). O digestato, por sua vez, é utilizado como biofertilizante e condicionador de solo. A DA se destaca por seus baixos custos operacionais e de capital em comparação com outras fontes de energia renovável, como solar, eólica e hidrelétrica, tornando-se uma opção economicamente viável (KUSHWAHA *et al.*, 2022). Além de ser uma excelente fonte de bioenergia, a DA é particularmente vantajosa na redução das emissões de carbono proveniente do descarte incorreto de resíduos, como despejo a céu aberto ou queima, contribuindo significativamente para a neutralidade de carbono e a economia circular (SUBBARAO *et al.*, 2023). A DA não só contribui para a segurança energética ao produzir bioenergia, mas também se alinha à segurança alimentar ao produzir o digestato, rico em nutrientes, que melhora a fertilidade do solo e reduz a necessidade de fertilizantes sintéticos, promovendo assim uma agricultura mais sustentável *(MANYI-LOH et al.*, 2019).

1.1.1 Cenário no Brasil: Desafios e Oportunidades

A matriz energética brasileira é uma das mais diversificadas do mundo, o que posiciona o país como um potencial líder na transição energética global. Enquanto a média mundial de fontes renováveis na matriz energética é de 15%, no Brasil, esse número é de 48,9% (Figura 1) (EPE, 2023). A diversificação da matriz energética nacional começou na década de 70 com o Programa Nacional do Álcool (Proálcool – 1975), que visava aumentar a produção de etanol por meio de incentivo ao desenvolvimento e aprimoramento de tecnologias capazes de atender a demanda interna e assim reduzir a dependência ao petróleo (STOLF; DE OLIVEIRA, 2020).

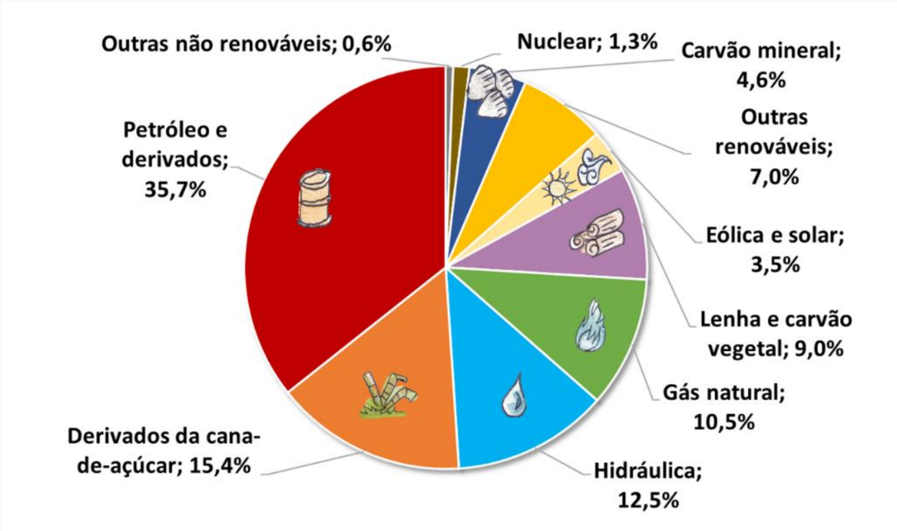

Figura 1. Matriz energética brasileira.
Fonte: Adaptado de EPE (2023).

O Brasil é um dos maiores produtores de biocombustíveis do mundo, destacando-se na produção de etanol, a partir da cana-de-açúcar, e do biodiesel, a partir de óleos vegetais e gorduras animais (STOLF; DE OLIVEIRA, 2020). Em 2023, a produção de etanol no Brasil foi de 8.260 milhões de galões, representando 28% do volume global, consolidando o país como o segundo maior produtor mundial, atrás apenas dos Estados Unidos (RFA, 2023). Além disso, a implementação da Política Nacional de Biocombustíveis (RenovaBio), lançada em 2017, visa apoiar os compromissos do Brasil sob o Acordo de Paris, aumentando o consumo de biocombustíveis, reduzindo as emissões de GEE e além de promover a segurança energética e um mercado de carbono regulamentado (GRANGEIA; SANTOS; LAZARO, 2022).

Apesar desse potencial, o setor energético brasileiro enfrenta desafios significativos, como a dependência da hidroeletricidade, vulnerável a variações climáticas, como secas prolongadas (EPE, 2018). A crise hídrica de 2021 destacou essa vulnerabilidade, levando ao aumento do uso de usinas termoelétricas, que são mais caras e poluentes. Para mitigar esses desafios, o Brasil tem investido em outras fontes de energia renovável, como a eólica e a solar. Em 2022, a capacidade instalada de energia eólica no Brasil ultrapassou 21 GW, enquanto a solar atingiu 13 GW, refletindo um crescimento substancial nos últimos anos (ABEEÓLICA, 2022; ANEEL/ABSOLAR, 2022). Além disso, a tecnologia da DA tem ganhado força no cenário nacional, contando com 936 plantas de biogás no país e a produção de 2,9 bilhões Nm³/ano de biogás em 2023 (CIBIOGAS, 2023). Em relação a produção de biometano, atualmente há 6 usinas de biometano autorizadas pela Agência Nacional do Petróleo (ANP), com capacidade total de produção de 417,1 mil Nm³ por dia de biometano (CIBIOGAS, 2023).

Apesar do status do Brasil como um dos maiores produtores e exportadores de alimentos do mundo, o país enfrenta problemas significativos de insegurança alimentar e nutricional. A ineficiência na implementação de políticas públicas contribuiu para a persistência e até mesmo o crescimento da fome, com os níveis de insegurança alimentar aumentando notavelmente entre 2013 e 2022 (DA SILVA BATISTA; LINS; ESPINOZA, 2023). De acordo com o IBGE (2023), cerca de 27,6% (21,6 milhões) dos domicílios brasileiros estavam em situação de insegurança alimentar. A pandemia de COVID-19 amplificou as vulnerabilidades existentes, particularmente na Região Norte, onde uma alta prevalência de insegurança alimentar foi encontrada entre famílias com crianças menores de cinco anos, destacando as disparidades geográficas e sociais que contribuem para esta crise (DA MATA; NEVES; DE MEDEIROS, 2022).

Atualmente, o Brasil é o quarto consumidor mundial de fertilizantes, mas apenas 15% dos alimentos cultivados são produzidos com fertilizantes nacionais (BUENO *et al.*, 2023). Uma alternativa para garantir a segurança alimentar no país seria focar na produção de biofertilizantes. Esses fertilizantes podem se originar de várias fontes, incluindo resíduos agrícolas, esterco animal e composto, com potencial para serem mais ecológicos e sustentáveis em comparação com os fertilizantes minerais. No entanto, a produção de fertilizantes orgânicos no Brasil ainda é limitada, necessitando de incentivos e estudos para aumentar sua viabilidade econômica e adoção pela indústria agropecuária (MDIC, 2023).

1.2 Digestão anaeróbia

A DA é um processo biológico no qual a matéria orgânica complexa é transformada, na ausência de oxigênio, em digestato e biogás, sendo este último composto principalmente por metano (CH_4) e dióxido de carbono (CO_2) (CAZAUDEHORE *et al.*, 2022). Quando aplicada como tecnologia de tratamento de resíduos sólidos e líquidos (efluentes), a DA desempenha um papel crucial na gestão sustentável de resíduos orgânicos, convertendo-os em bioprodutos valiosos. O biogás gerado tem aplicações que variam desde o uso doméstico até industrial, sendo utilizado para aquecimento, geração de eletricidade e como combustível automotivo (ANGELIDAKI *et al.*, 2019). Além disso, o digestato, a fração não degradada rica em nutrientes, possui alto valor agronômico (CAZAUDEHORE *et al.*, 2022).

A DA é um processo complexo, conduzido por microrganismos, e dividido em quatro fases bioquímicas: hidrólise, acidogênese, acetogênese e metanogênese (Figura 2) (YADAV *et al.*, 2022).

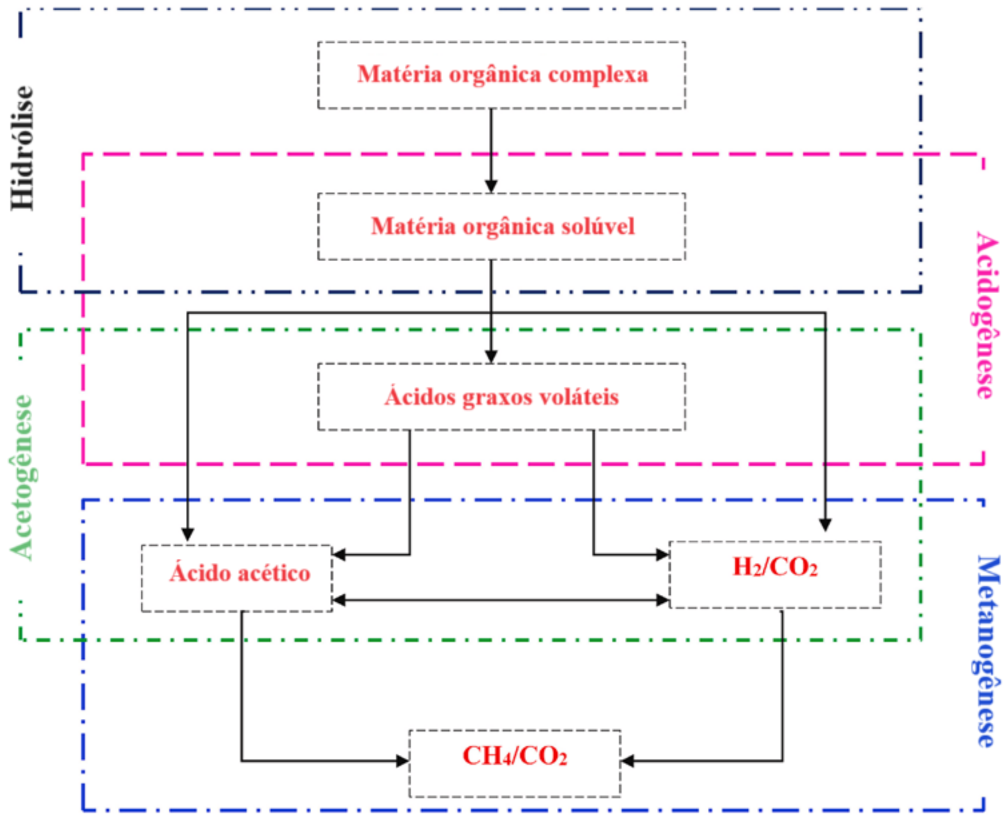

Figura 2. Etapas da digestão anaeróbica.
Fonte: Adaptado de Yadav *et al.* (2022).

Na hidrólise, microrganismos hidrolíticos liberam hidrolases que quebram moléculas complexas, reduzindo-as a moléculas simples (YADAV *et al.*, 2022; ZHENG *et al.*, 2014). A fragmentação do substrato é necessária para convertê-lo a moléculas aptas a atravessarem as paredes celulares microbianas, sendo utilizadas como fontes de energia ou nutrientes (KIM *et al.*, 2003).

A conversão em monômeros solúveis possibilita a fermentação na etapa seguinte, a acidogênese, realizada por vários microrganismos anaeróbios e anaeróbios facultativos. Durante essa etapa, ocorre a degradação dos compostos hidrolisados, resultando na produção de CO_2, H_2, álcoois, ácidos orgânicos, compostos de nitrogênio orgânico e compostos orgânicos com enxofre (GERARDI, 2003). Os compostos formados na acidogênese são convertidos em acetato, CO_2 e H_2 na etapa de acetogênese (STEGER *et al.*, 2022).

Por fim, na metanogênese, há a formação de metano através de moléculas de acetato, CO_2 e H_2. Essa transformação é mediada por arqueias metanogênicas, que são organismos estritamente anaeróbios (ZHANG *et al.*, 2019).

1.2.1 Fatores que afetam a eficiência da digestão anaeróbica

A eficiência da DA é influenciada por vários fatores interrelacionados. Os principais fatores incluem a composição e a qualidade da matéria-prima, que afetam diretamente a comunidade microbiana responsável pelo processo de digestão (KOSTOPOULOU *et al.*, 2023; ZAMRI *et al.*, 2021). Parâmetros operacionais como temperatura, regime de alimentação, tipo de reator, pH, tempo de retenção hidráulica (TRH) e taxa de carregamento orgânico (TCO) também são cruciais. As faixas de temperatura incluem: psicrófila (>25 °C), mesofílica (25 – 45 °C), termofílica (50 – 60 °C) e hipertermofílica (>60 °C). O fluxo de alimentação pode ser em batelada ou contínuo. O pH ideal geralmente varia entre 7,3 e 8,5, com valores abaixo de 6 sendo menos favoráveis. O TRH ideal varia entre 30 e 60 dias, dependendo do substrato e das condições operacionais (AJAYI-BANJI; RAHMAN, 2022; SRISOWMEYA; CHAKRAVARTHY; NANDHINI DEVI, 2020). Por exemplo, manter temperaturas termofílicas pode melhorar a digestão de substratos com alto teor de sólidos, como a fração orgânica dos resíduos sólidos urbanos (YADAV KUMAR; SINGH KUMAR, 2018; ZAMRI *et al.*, 2021).

Outro fator significativo é a relação carbono/nitrogênio (C/N). Um equilíbrio adequado dessa relação, idealmente entre 20 e 30, é essencial para evitar o acúmulo de compostos inibitórios, afetando adversamente a atividade microbiana, especialmente NH_3 e NH_4 (PAUL; DUTTA, 2018; YADAV KUMAR; SINGH KUMAR, 2018). A intensidade da mistura também desempenha um papel vital; velocidades de mistura mais altas (100 rpm) podem reduzir as zonas mortas e melhorar o rendimento de biogás, garantindo uma distribuição mais homogênea de substratos e populações microbianas (SINGH *et al.*, 2021).

Além disso, a presença de compostos inibitórios, seja como componentes inerentes à matéria-prima (e.g., metais pesados, pesticidas e antibióticos) ou como subprodutos do metabolismo microbiano (e.g., ácidos graxos voláteis (AGV) em excesso, amônia e sulfeto de hidrogênio), pode inibir o processo de digestão, exigindo monitoramento e manejo cuidadosos (YADAV KUMAR; SINGH KUMAR, 2018).

1.2.2 Pré-tratamento das matérias primas

O pré-tratamento é uma etapa crucial para otimização da DA, atuando, principalmente, na fase de hidrólise. Sob condições ideais, o pré-tratamento visa aumentar a eficiência do processo ao quebrar estruturas orgânicas complexas e reduzir e/ou remover contaminantes (ALVIRA *et al.*, 2010). A influência do pré-tratamento no rendimento total de metano é amplamente descrita na literatura, demonstrando que diferentes tipos de pré-tratamentos aplicados ao mesmo substrato podem resultar em impactos distintos no rendimento final de metano (ANACLETO *et al.*, 2022, 2024; TAHERZADEH; KARIMI, 2008).

As diversas técnicas de pré-tratamento, incluindo métodos físicos, químicos, biológicos e suas combinações, podem ser aplicadas aos resíduos orgânicos para modificar suas estruturas físico-químicas e melhorar sua biodegradabilidade, de acordo com a severidade do pré-tratamento ou da energia consumida no processo (Tabela 1) (ALVIRA *et al.*, 2010; ANACLETO *et al.*, 2024).

Tabela 1. Métodos de pré-tratamento para otimização da digestão anaeróbica.

Classificação	Métodos	Vantagens	Desvantagens
Físico	• Térmicos (autoclavagem, explosão de vapor); • Mecânicos (maceração, moagem, corte, trituração, fresagem).	• Redução do tamanho das partículas; • Aumento da área superficial; • Quebra da matéria orgânica e aumento da sua exposição na degradação enzimática; • Pasteurização efetiva.	• Alto consumo de energia; • Pode gerar desgastes mecânicos; • Necessidade de equipamentos especializados.
Químico	• Alcalinos; • Ácidos; • Solvente orgânicos; • Suplementos minerais.	• Efetivo na quebra de ligações complexas; • Pode eliminar patógenos; • Efetivo na quebra de materiais recalcitrantes (lignina).	• Necessidade de produtos químicos; • Mais suscetível à geração de compostos inibidores/ tóxicos; • Alto custo operacional; • Maior corrosão de equipamentos.
Biológico	• Adição de enzimas (comerciais, fungos, bactérias); • Cultura de fungo; • Cultura com consórcio microbiano.	• Menor impacto ambiental; • Mais econômico.	• Tempo de processamento mais longo; • Sensível a condições ambientais.
Combinado	• Enzima + térmico; • Alcalino + térmico; • Ácido + térmico.	• Sinergia de múltiplos métodos; • Potencial para melhores resultados.	• Maior complexidade operacional; • Custos mais elevados; • Requer investimento em infraestrutura adequada.

Fonte: Adaptado de Anacleto *et al.* (2024).

A redução no tamanho das partículas, o aumento da área superficial e a solubilidade da matéria orgânica particulada aumentam a acessibilidade dos microrganismos ao substrato, melhorando sua degradação e, consequentemente, a produção de biogás. O pré-tratamento pode atuar através da alteração da estrutura física do substrato, como na redução de tamanho da matéria orgânica particulada e na quebra de estruturas complexas em moléculas mais simples (Figura 3).

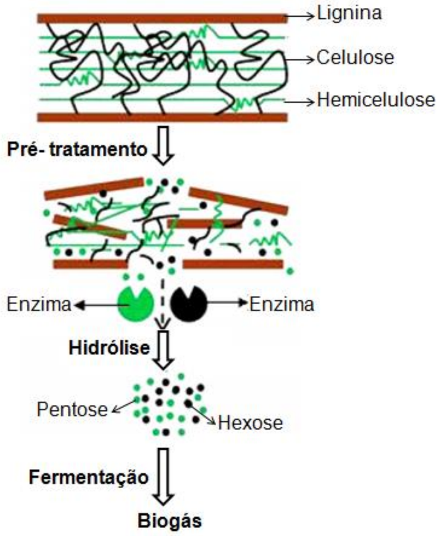

Figura 3. Participação do pré-tratamento no processo de hidrólise, primeiro estágio da digestão anaeróbica.
Fonte: Adaptado de Asgher *et al.* (2014).

Contudo, esses pré-tratamentos também elevam o custo do processo de DA, pois aumentam o consumo de energia, exigem a compra de aditivos e geralmente dependem de investimentos capitais e operacionais para adequação de equipamentos ao pré-tratamento (ANACLETO *et al.*, 2024). Além disso, os pré-tratamentos podem ter efeitos adversos na DA e resultar em menores rendimentos de CH_4 se o pré-tratamento selecionado não for adequado para um determinado resíduo orgânico (ANACLETO *et al.*, 2024).

Diversos parâmetros operacionais podem afetar o desempenho dos pré-tratamento, tais como: pH, razão C/N, teor de sólidos, AGV, temperatura e TRH (ATELGE *et al.,* 2020; SCHNÜRER; JARVIS, 2012). No entanto, apenas esses fatores não são suficientes para estimar o comportamento do pré-tratamento em uma dada matéria-prima. É necessária a avaliação da eficiência de degradação cada tipo de matéria orgânica quando exposta a diferentes metodologias de pré-tratamentos, permitindo compreender e delinear as configurações mais adequadas para o processo de pré-tratamento.

1.3 Geração de bioprodutos a partir da digestão anaeróbica

A implementação da tecnologia de DA para converter resíduos orgânicos em produtos de valor econômico enquanto reduz seu potencial poluidor tem sido apontada mundialmente como uma das estratégias mais promissoras para o gerenciamento de resíduos (ZHANG *et al.*, 2019). Comparada a outras tecnologias de tratamento de resíduos, como compostagem e incineração, a DA é a mais eficiente tanto no aspecto ambiental quanto econômico, devido à elevada taxa de recuperação de energia e de nutrientes (SURENDRA *et al.*, 2014).

Além do biogás e do digestato já mencionados, a DA pode produzir outros bioprodutos valiosos, como biochar (HUNG *et al.*, 2017a), bio-óleo (BARBANERA *et al.*, 2018) e biometano (STEPHAN, 2013). Esses bioprodutos têm ampla aplicação nos setores energético, agropecuário e industrial (Figura 4). No setor energético, o biogás é utilizado tanto na geração de eletricidade quanto no aquecimento de ambientes domésticos, comerciais e industriais. Esse uso refletiu um crescimento global de aproximadamente 90% na última década, atingindo 120 GW em 2019, comparado a 65 GW em 2010 (ABANADES *et al.*, 2021). No Brasil, a capacidade instalada de geração de energia elétrica a partir do biogás é de 486 MW, posicionando o país como o sétimo maior produtor do mundo (IRENA, 2023).

No setor agropecuário, o digestato é um valioso suplemento nutricional para solos agrícolas. Suas aplicações incluem o uso como recuperador de nutrientes, biofertilizante, fertilizante orgânico, suplemento mineral, estabilizante de solo, biochar, hidrochar e biossólido (ALBURQUERQUE *et al.*, 2012; GŁAB; SOWIŃSKI, 2019; HUNG *et al.*, 2017b; PRASK *et al.*, 2018; REZA; MUMME; EBERT, 2015; SLEPETIENE *et al.*, 2020; VANEECKHAUTE *et al.*, 2017). Esses produtos melhoram a qualidade física, química e biológica do solo e, consequentemente, aumentam a produtividade agrícola.

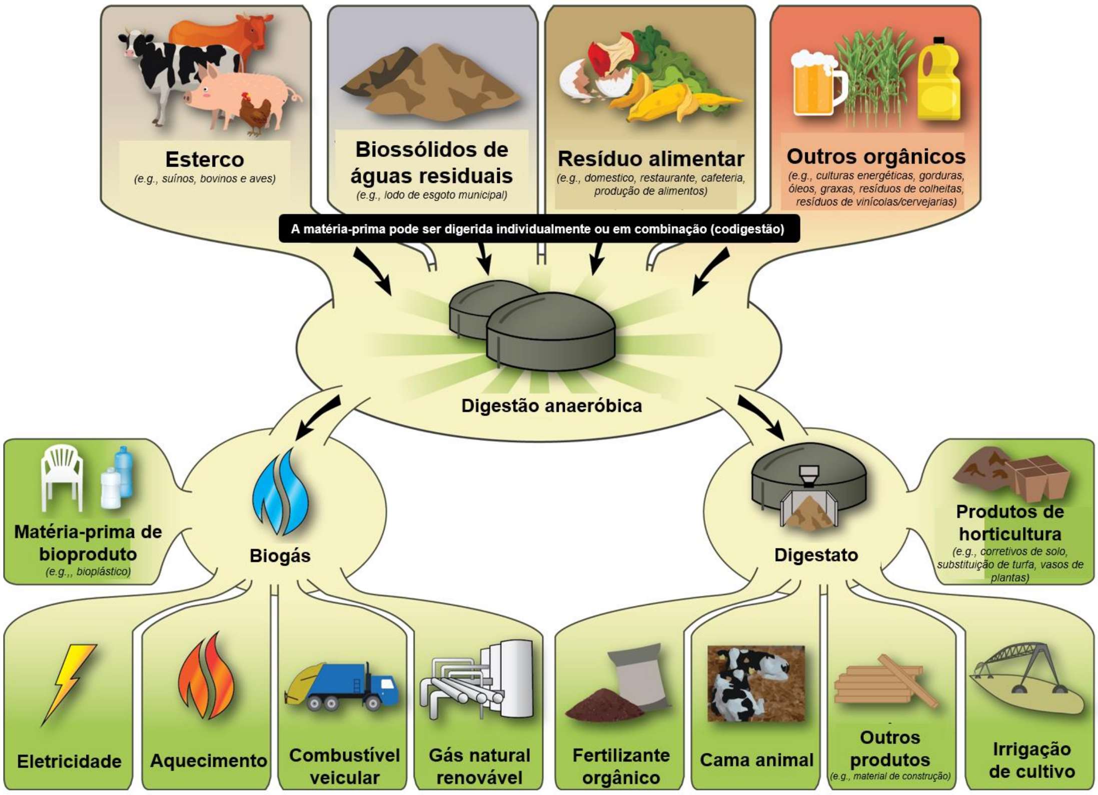

Figura 4. Principais bioprodutos produzidos a partir da digestão anaeróbia de resíduos orgânicos.

United States Environmental Protection Agency, 2024. Disponível em: <https://www.epa.gov/agstar/how-does-anaerobic-digestion-work>. Acesso em: 02 de julho de 2024.

Além disso, bioplásticos compostos por ácido polilático (PLA) e polihidroxialcanoatos (PHA), podem ser produzidos durante a DA (SELVAMURUGAN MUTHUSAMY; PRAMASIVAM, 2019). O digestato desidratado também pode ser usado na fabricação de cama animal e materiais de construção, como tijolos, agregados leves e concreto (SØRENSEN; NØRSKOV, 2019).

1.3.1 Produção e uso do biogás

O biogás é uma fonte de energia renovável obtida através da DA, composto principalmente por CH_4 (60-80% v/v) e CO_2 (20-40% v/v), além de pequenas quantidades de outros gases como sulfeto de hidrogênio (H_2S), nitrogênio (N_2) e hidrogênio (H_2) (SOUZA *et al.*, 2010). O teor de metano é o principal determinante da qualidade do biogás, correspondendo a 90% do seu valor energético (KOTHARI *et al.*, 2010). A presença de impurezas como CO_2, H_2S e vapor de água pode reduzir seu poder calorífico e causar problemas de corrosão em equipamentos *(*AFISNA *et al.*, 2022*)*.

Para melhorar a qualidade do biogás, métodos de purificação como adsorção utilizando materiais como hidróxido de cálcio, óxido de ferro, zeólita e carvão ativado podem ser empregados. Esses métodos aumentam significativamente o teor de metano e reduzem as impurezas *(*AFISNA *et al.*, 2022*)*. Outras tecnologias de purificação incluem membranas de separação e criogenia, que também têm mostrado eficácia na melhoria da qualidade do biogás (KAMATH; MEDA, 2023).

As vantagens do biogás não se limitam apenas ao seu potencial de produção energética, mas também incluem efeitos indiretos decorrentes da sua utilização. A produção e utilização de biogás possuem uma ampla capacidade de descarbonização, com potencial para reduzir entre 10-13% das emissões globais de GEE e substituir até 10% do consumo mundial de energia primária ou 23-32% do consumo global de carvão (WORLD BIOGAS ASSOCIATION, 2019), com aplicabilidade em diversos setores.

Além disso, o biogás/biometano se destaca positivamente dos demais biocombustíveis devido à sua contribuição para a economia circular. Ele valoriza os resíduos sólidos por meio da conversão em recursos úteis, fechando o ciclo de produção e consumo e promovendo a sustentabilidade (CHANG *et al*., 2011). A utilização de resíduos orgânicos na produção de biogás também contribui para a gestão eficiente dos resíduos, reduzindo a quantidade de resíduos destinados à aterros e minimizando a emissão de GEE associados à decomposição em ambientes não controlados.

A implementação de tecnologias de biogás pode gerar benefícios socioeconômicos, como a criação de empregos, o desenvolvimento rural e a promoção de tecnologias verdes *(*GEETHATHANUJA *et al.,* 2023*)*. A produção descentralizada de biogás permite que comunidades rurais se tornem mais autossuficientes em termos energéticos, ao mesmo tempo que contribui para a segurança energética nacional.

1.3.2 Aproveitamento do digestato como biofertilizante

O digestato é um subproduto da DA rico em nutrientes essenciais, como nitrogênio (N), fósforo (P) e potássio (K), o que permite sua utilização como biofertilizante em diversos tipos de cultivos (ROMERO GÜIZA *et al.*, 2016). Estima-se que uma usina de biogás com potência de 500 kW produza mais de 10.000 toneladas de digestato por ano, com aproximadamente 10% de matéria seca (KRATZEISEN *et al.*, 2010). Embora o incentivo à instalação de usinas de biogás seja benéfico em termos socioeconômicos e ambientais, ele traz preocupações quanto ao destino do montante de digestato gerado. Dependendo da fonte de biomassa, o digestato pode causar graves problemas ambientais e sanitários, exigindo necessariamente a aplicação de pós-tratamentos para reduzir a toxicidade e eliminar patógenos (TÖRNWALL *et al.*, 2017).

Apesar desses nutrientes valiosos, o digestato pode apresentar características que prejudiquem sua reutilização como biofertilizante. Uma preocupação significativa é a alta concentração de nitrogênio amoniacal (NH_4^+/NH_3) resultante do processo de mineralização do nitrogênio orgânico (N_{org}), que pode ser fitotóxico (ZENG; DE GUARDIA; DABERT, 2016). Embora o NH_3 em si não seja um gás de efeito estufa, ele pode levar à emissão de outros compostos de nitrogênio, como o óxido nitroso (N_2O), que tem um potencial de aquecimento global aproximadamente 298 vezes maior do que o CO_2 em um período de 100 anos (BERTAGNI *et al.*, 2023). Além disso, metais pesados como arsênio, cadmio e chumbo quando presentes nas fontes orgânicas digeridas podem se acumular no digestato. Esses metais podem prejudicar o crescimento de plantas e resultar em contaminação, tornando-as impróprias para o consumo animal e humano (XU *et al.*, 2022).

Para facilitar sua reutilização, o digestato é comumente separado em fração sólida e líquida, facilitando a escolha da melhor estratégia de manejo. As técnicas de separação sólido-líquido incluem centrifugação, filtração, e decantação, cada uma com suas vantagens e desvantagens e, termos de eficiência e custo. Métodos de alta eficiência, como a centrifugação e o uso de coagulantes, floculantes ou polímeros, são particularmente eficazes na redução de sólidos suspensos totais e no aumento da recuperação de nutrientes na fração líquida (AKHIAR *et al.*, 2021; BEGGIO *et al.*, 2022).

A composição química da fração líquida do digestato é influenciada por vários fatores, incluindo o tipo de matéria-prima, o processo de separação sólido-líquido e os métodos de tratamento subsequentes. As concentrações de nutrientes na fração líquida podem variar amplamente, com níveis de fósforo variando de 230,9 a 649,1 mg PO_4^{3-}/L e níveis de nitrogênio de 1363 a 3211 mg N/L, predominantemente na forma de nitrogênio amoniacal (60% a 90% do nitrogênio total) (TUSZYNSKA; WILINSKA; CZERWIONKA, 2021). Essa fração pode ser utilizada como água de irrigação (ŚWIĄTCZAK; CYDZIK-KWIATKOWSKA; ZIELIŃSKA, 2019), fornecendo nutrientes ao solo e atuando como nematicida, suprimindo nematoides parasitas que prejudicam o desenvolvimento das plantas (EBERLEIN *et al.*, 2020). No entanto, devido à alta concentração de amônia, tratamentos adicionais, como a diluição ou a remoção de amônia, podem ser necessários para evitar a toxicidade para as plantas.

A fração sólida, por sua vez, é rica em matéria orgânica e nutrientes que melhoram a estrutura do solo e sua capacidade de retenção de água. A separação sólido-líquido quimicamente aprimorada usando cloreto de polialumínio, epicloridrina-dimetilamina com etilendiamina e poliacrilamidas pode melhorar significativamente a eficiência da separação. As frações sólidas resultantes possuem maiores concentrações de alumínio (até 20 g kg−1 ST), carbono orgânico (até 324 mg kg^{-1} ST) e nitrogênio (44,1 mg TKN kg^{-1} ST) (BEGGIO *et al.*, 2022). A digestão termofílica pode enriquecer ainda mais a fração sólida com elementos como Fe, Co, Cu, Zn, Cr, As, Cd, Pb, Ge e elementos de terras raras (ETRs) em relação a condições mesofílicas (ZAFFAR *et al.*, 2023).

O Índice de Germinação (IG) é uma métrica crucial para avaliar a qualidade e a fitotoxicidade do digestato, medindo a germinação das sementes e o crescimento precoce das plântulas. O IG é calculado multiplicando a porcentagem relativa de sementes germinadas (RSG) pela porcentagem relativa do comprimento da raiz (RRG) das plântulas (MULYATI *et al.*, 2022). Em estudos ambientais, o IG é usado para avaliar a maturidade e a fitotoxicidade de compostos feitos de lodo de esgoto, com valores mais altos de IG indicando menor fitotoxicidade e maior maturidade do composto (JAKUBUS; BAKINOWSKA, 2018). No contexto da utilização do digestato como biofertilizante, um IG elevado indica que o digestato possui baixo nível de fitotoxicidade e é adequado para uso agrícola, contribuindo para o crescimento saudável das plantas (LOGAN; VISVANATHAN, 2019). Estudos têm demonstrado que o digestato pode apresentar valores de IG variáveis dependendo da sua composição e dos processos de tratamento aplicados. Um IG abaixo de 50% geralmente indica que o substrato é fitotóxico e, portanto, não pode ser utilizado com segurança na agricultura (LOGAN; VISVANATHAN, 2019).

A valorização do digestato é crucial para reduzir ao máximo o potencial poluidor dos resíduos tratados pela DA. Sua inserção no ciclo produtivo atende ao conceito de valorização da biomassa “em cascata”, onde a saída de um processo se torna a entrada do seguinte, um princípio que ganhou relevância na última década (TAYIBI *et al.*, 2021). Isso está alinhado à meta de *zero waste*, finalizando o ciclo no contexto de economia circular (BARAMPOUTI *et al.*, 2020). Além disso, a reintrodução desses nutrientes recuperados no solo reduz a pressão nos ciclos biogeoquímicos promovidas para exploração exacerbada de nutrientes minerais (MACURA *et al.*, 2019).

1.4 Meta-análise

A meta-análise é uma ferramenta estatística que combina diversos dados já publicados na literatura para conduzir uma síntese quantitativa. A seleção e coleta de dados é realizada por uma revisão sistemática, onde o resultado geral da busca de literatura é submetido a uma triagem rigorosa a partir de critérios de exclusão/inclusão definidos. A abordagem sistemática visa identificar, avaliar, sintetizar e combinar resultados de estudos já disponíveis na literatura (STROUP *et al.*, 2000), desta maneira, a soma de vários dados experimentais gera um N amostral robusto, garantindo maior confiabilidade na resolução de hipóteses.

A síntese quantitativa conduzida pela meta-análise considera (i) o tamanho da amostra, (ii) o peso de cada estudo, (iii) as diferenças nas condições experimentais e (iv) as variações das amostras dentro do mesmo estudo (LOVATTO *et al.*, 2007). Assim, os erros estatísticos gerados devido ao manuseio de dados de diferentes origens são minimizados, além de diminuir os riscos de enviesamento.

Os Itens de Relatório Preferenciais para Revisões Sistemáticas e Meta-análises (PRISMA) são usados como guia para condução de revisão sistemática e meta-análise, fornecendo um conjunto mínimo de itens necessários que devem conter nos artigos para execução do projeto experimental (Figura 5), além de ajudar na determinação da estratégia de busca, definição de palavras-chave e critérios de exclusão/ inclusão para avaliar a qualidade dos artigos (MOHER *et al.*, 2016).

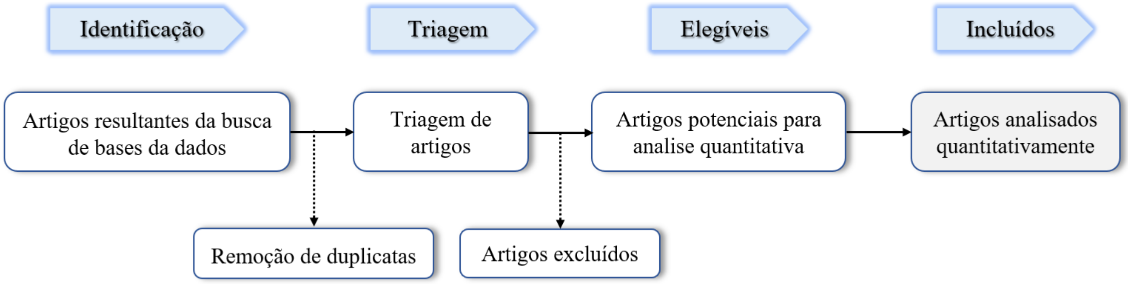

Figura 5. Diagrama Itens de Relatório Preferenciais para Revisões Sistemáticas e Meta-análises (PRISMA) das etapas de revisão sistemática e meta-análise.
Fonte: Elaboração própria (2024).

2 OBJETIVO

Investigar o efeito de tratamentos aplicados a distintos resíduos orgânicos na produção e qualidade de biogás e biofertilizante em digestores anaeróbios.

2.1 Objetivos específicos

- Avaliar o efeito da composição química dos resíduos orgânicos na resposta aos pré-tratamentos e seu impacto na maximização da produção de biogás, utilizando uma revisão sistemática e meta-análise.
- Identificar, por meio de revisão sistemática e meta-análise, pré-tratamentos apropriados para maximizar a produção de biogás de esterco de animais distintos.
- Examinar, com base em uma revisão sistemática seguida de meta-análise, o efeito dos pré-tratamentos na produção de biogás a partir de biomassa algal.
- Investigar o efeito de diversos métodos de pré-tratamento na redução do potencial poluidor e aumento de produção de biogás de resíduos industriais, utilizando uma revisão sistemática e meta-análise.
- Avaliar quais resíduos orgânicos produzem os digestatos mais promissores para uso como biofertilizante.
- Analisar o desempenho do digestato e de suas diferentes frações (i.e., líquido, sólido e total) em relação à sua qualidade e fitotoxicidade através do índice de germinação de sementes.

3 MATERIAL E MÉTODOS

O estudo desenvolvido nesta tese foi realizado em duas etapas: uma teórica, baseada em revisões sistemáticas e meta-análises com foco na maximização da produção de biogás no processo de DA a partir da aplicação de pré-tratamentos; e uma experimental, focada na avaliação do valor agronômico do digestato resultante do processo de DA.

3.1 Etapa teórica

3.1.1 Revisão sistemática e estratégia de busca

Foram conduzidas quatro revisões sistemáticas distintas (Artigo I, II, III e IV), seguindo as orientações recomendadas pelo PRISMA (MOHER *et al.*, 2016). Além disso, foi estabelecido um protocolo de triagem (seção 3.1.2), obedecendo a critérios pré-definidos que garantem a qualidade dos dados coletados e utilizados.

As buscas foram realizadas na base de dados Web of Science e Scopus, abrangendo artigos originais publicados em inglês no período de 1945 a 2021. Os estudos foram conduzidos utilizando combinações de palavras-chave relevantes, com o auxílio de operadores booleanos (AND, OR) e curingas (*). Foram elaborados códigos de busca específicos para cada revisão, utilizando diferentes palavras-chave:

- Artigo I: “hydrolysis”, “anaerobic digestion”, “methane yield” and “pretreatment”.
- Artigo II: “hydrolysis”, “anaerobic digestion”, “manure”, “dung”, “livestock”, “cow”, “cattle”, “pig”, “hog”, “chicken”, “poultry”, “hen”, “slurry”, “methane” and “pretreatment”.
- Artigo III: “anaerobic digestion”, “textil” and “pretreatment”.
- Artigo IV: “anaerobic digestion” “pretreatment” “methane” and “algae”.

3.1.2 Triagem de estudos

A etapa inicial da triagem de estudos consistiu em selecionar os artigos aptos a serem incluídos na revisão sistemática. Nesta etapa, os títulos e resumos dos artigos foram examinados para verificar sua relevância com os temas em questão. Os artigos que passaram por essa primeira avaliação foram então submetidos à leitura completa. Quando considerados elegíveis após a triagem, os dados relevantes foram coletados e organizados em uma tabela do Excel, seguindo categorias pré-definidas garantindo consistência e padronização.

Os critérios de elegibilidade para inclusão dos artigos nas meta-análises foram: i) descrição do valor médio, desvio padrão e número de réplicas para o rendimento de metano com e sem pré-tratamento (controle); ii) descrição do pré-tratamento aplicado; e iii) número de réplicas maior que 2. Esse último critério foi incluído porque, embora seja recomendado um mínimo de 3 réplicas para testes de potencial bioquímico de metano, especialmente para frascos de tratamento, o número de réplicas em reatores de escala laboratorial raramente ultrapassa 2.

3.1.3 Coleta de dados

Os dados coletados incluíram informações gerais, como: nome do primeiro autor, título do artigo e ano de publicação. Além disso, foram coletados detalhes específicos sobre o substrato utilizado, sua composição química, o tipo de inóculo empregado, a configuração operacional do experimento (incluindo temperatura, tempo de retenção hidráulica, agitação, tipo e escala do reator, volume total e volume de trabalho). Também foram extraídas as informações sobre o método de pré-tratamento aplicado, as condições específicas desse pré-tratamento e os resultados de rendimento de metano (média, desvio padrão e número de réplicas).

As técnicas de pré-tratamento, como autoclave, mecânica, alcalina, ácida e enzimática, foram agrupadas em categorias mais amplas, como métodos físicos, químicos, biológicos e combinados (ANACLETO *et al.*, 2024). Essa categorização foi realizada devido às semelhanças nas transformações alcançadas na matéria orgânica dentro de cada grupo de técnicas. Para as categorias de pré-tratamento estatisticamente significantes, todas as técnicas individuais que compõem a categoria foram avaliadas separadamente. Além disso, as diferentes matérias-primas foram agrupadas com base na predominância de sua composição química para facilitar a análise comparativa dos resultados.

Todos os dados foram coletados a partir dos artigos elegíveis na triagem. Para os artigos que disponibilizaram os dados exclusivamente em gráficos, foi utilizado o programa *Web Plot Digitizer v4.2* para extração dos dados (automeris.io, 2017).

3.1.4 Meta-análise

A meta-análise multinível ponderada foi utilizada nesse estudo devido a sua habilidade em combinar e comparar dados de diferentes estudos contendo diferentes tratamentos (WHITE *et al.*, 2019).

No artigo I, a diferença média padronizada (SMD) estimada pelo Hedges' g foi aplicada como medida de tamanho do efeito para quantificar os dados de produção de metano (Equação 1) (GUO *et al.*, 2020). Este tamanho de efeito é considerado menos tendencioso do que outras abordagens de cálculo e é recomendado para amostras pequenas (LIN, 2018).

$$g = \frac{Mean_T - Mean_C}{\sqrt{(n_1-1)s_1^2 + (n_2-1)s_2^2}} \times 1 - \frac{4}{4(n_1+n_2)-9} \qquad 1$$

Onde $Mean_T$ é o rendimento de metano médio do grupo tratado e $Mean_C$ é o rendimento de metano médio do grupo controle, n_1 e n_2 são o tamanho de amostra tratada e controle, respectivamente, e s_1^2 and s_2^2 são a variância da população estimada para o grupo tratado e para o grupo controle, respectivamente (GUO *et al.*, 2020).

Uma meta-análise multinível foi realizada, seguida por uma análise de subgrupos, uma vez que os dados foram agrupados em categorias de pré-tratamento (ASSINK; WIBBELINK, 2016). Além disso, foi considerada a dependência dos tamanhos de efeito, uma vez que um determinado estudo pode comparar vários tratamentos a um único grupo controle, o que significa que os dados não são independentes. Assumimos também o modelo de efeito aleatório, considerando a diferença na metodologia dos experimentos realizados em cada estudo incluído na análise (ASSINK; WIBBELINK, 2016; GUO *et al.*, 2020).

Nos artigos II, III e IV, foi conduzida uma meta-análise não ponderada. A produção de metano foi quantificada utilizando a razão de resposta logarítmica natural (RR), baseada em (HEDGES; GUREVITCH; CURTIS, 1999), conforme a Equação 2:

$$RR = \ln(\overline{X}_T/\overline{X}_C) \qquad 2$$

Onde, $\overline{X}_T$ é o rendimento de metano médio do grupo tratado, e $\overline{X}_C$ é o rendimento de metano médio do grupo controle.

Em ambas as análises, os tamanhos médios de efeito, intervalos de confiança de 95% (IC) com correção de viés e valores p foram calculados no *software* R, utilizando o pacote "metafor" para cada pré-tratamento, bem como para as técnicas específicas dos métodos de pré-tratamento significativos (ASSINK; WIBBELINK, 2016; GUO *et al.*, 2020). Os pré-tratamentos foram considerados significativos ($p \leq 0,05$) quando seus valores médios e IC não transpuseram à linha RR = 0. Valores médios e IC localizados abaixo dessa linha indicaram uma resposta negativa (pré-tratamento < controle), enquanto valores médios e IC acima da linha zero indicaram uma resposta positiva ao pré-tratamento (pré-tratamento > controle), indicando uma maior produção de CH_4 em relação ao controle.

3.2 Etapa experimental

3.2.1 Coleta do digestato

O digestato foi coletado em 23 digestores de biogás de escala industrial localizadas na Suécia, Noruega e Dinamarca (Tabela 2). Essas amostras são parte das 33 amostras coletadas durante a Campanha de Biogás Nórdica, realizada de agosto a dezembro de 2022. Os digestores possuíam quatro tipos principal de matéria-prima: A biomassa agrícola, alimentícios, esterco e esgoto.

Tabela 2. Características iniciais do digestato coletado de digestores de escala industrial.

Digestor	Temperatura (°C)	Fonte orgânica principal	TRH (d)	TCO (kg SV/m³.d)	País
3	40	Esterco	N.D.	N.D.	Suécia
4	37	Lodo de esgoto	22	N.D.	Suécia
5	36	Lodo de esgoto	20	N.D.	Suécia
6	41	Biomassa agrícola	N.D.	N.D.	Suécia
7	41	Resíduos de alimentos	40	N.D.	Suécia
8	38	Esterco	41	3,7	Suécia
9	52	Resíduos de alimentos	41,5	2,56	Suécia
10	38	Resíduos de alimentos	30	4	Suécia
11	40	Resíduos de alimentos	32,5	2,4	Noruega
12	53	Resíduos de alimentos	N.D.	N.D.	Suécia
13	53	Resíduos de alimentos	23	5,4	Suécia
14	44	Resíduos de alimentos	N.D.	N.D.	Suécia
15	40	Biomassa agrícola	N.D.	N.D.	Suécia
16	56	Resíduos de alimentos	43	2,6	Suécia
17	53	Resíduos de alimentos	N.D.	N.D.	Suécia
18	52,5	Esterco	N.D.	N.D.	Dinamarca
19	51	Esterco	N.D.	N.D.	Suécia
20	51	Esterco	N.D.	N.D.	Dinamarca
21	50	Esterco	N.D.	N.D.	Dinamarca
22	41	Resíduos de alimentos	25	3,3	Suécia
23	40	Esterco	30	N.D.	Suécia
24	42	Resíduos de alimentos	35	4,5	Suécia
25	38	Lodo de esgoto	20	2	Suécia

TRH: tempo de retenção hidráulica; TCO: taxa de carregamento orgânico; DA: digestão anaeróbia; SV: sólidos voláteis; N.D.: não disponível. Fonte: Elaboração própria (2024).

O material digerido foi coletado em triplicata em recipientes de 10 L. Após a coleta, as amostras foram imediatamente transportadas para o laboratório e mantidas em banho-maria na temperatura operacional do digestor de origem.

3.2.2 Caracterização química do digestato

Após a coleta, amostras do material foram imediatamente submetidas a análises de pH, sólidos totais (ST), sólidos voláteis (SV) e AGV, todas realizadas em triplicata. As amostras destinadas às análises de carbono, nitrogênio e elementos inorgânicos foram congeladas a -20 °C e analisadas durante o experimento.

3.2.2.1 Análise do pH

O pH foi analisado por um medidor de pH (InoLab 7310, WTW, Alemanha). A calibração do equipamento foi realizada semanalmente com soluções tampão de pH 4 e 7, sendo considerada confiável uma calibração com variação inferior a 0,15 usando a solução tampão pH 8 como referência.

3.2.2.2 Análise de sólidos totais e sólidos voláteis

Os teores de sólidos foram determinados de acordo com o Standard Methods (APHA, 2005). Os cadinhos de porcelana foram levados à mufla por pelo menos 1 h à 550 ºC. Após o resfriamento em dessecador até atingirem massa constante, alíquotas das amostras, contendo 10-15 g, foram adicionadas aos cadinhos e a massa inicial do conjunto amostra e cadinho foi registrada. Os cadinhos foram levados à estufa à 105 ºC por pelo menos 20 h para evaporação da água. A massa seca do conjunto do cadinho e da amostra foi obtida após resfriamento no dessecador até massa constante. A concentração de ST (em %) foi calculada a partir da Equação 3.

$$ST\ (\%) = \frac{Peso\ seco\ após\ 105\ °C}{Peso\ úmido} \times 100 \qquad 3$$

Para a análise de sólidos voláteis, os cadinhos contendo o material seco foram levados à mufla à 550 °C, por 2 h. Após o resfriamento em dessecador até a massa constante, a massa foi registrada. O teor de SV (em % de ST) foi calculado a partir da Equação 4.

$$SV\ (\%\ de\ ST)\ =\ \frac{(Peso\ seco\ após\ 105\ °C) - (Peso\ após\ 550\ °C)}{\text{Peso seco após 105 °C}} \times 100 \qquad 4$$

3.2.2.3 Ácidos graxos voláteis

Os AGV (*i.e.*, acetato, propionato, butirato, isobutirato, valerato, isovalerato, caproato e isocaproato), foram determinados de acordo com Jonsson e Boren (2002), utilizando um cromatógrafo gasoso com detector por ionização de chama (sistema 8860 CG-DIC, Agilent, EUA). Para a injeção no cromatógrafo, as amostras foram preparadas a partir da centrifugação em microtubos de 2 mL a 12.000 rpm por 10 minutos. Após a centrifugação, 400 µL do sobrenadante foram transferidos para o frasco de vidro e acidificado com 40 µL do padrão interno (solução de ácido fórmico a 25%, contendo 52 µg de ácido crotônico). As amostras de AGV foram armazenadas a 4 °C por até 5 dias até a realização da análise.

3.2.2.4 Análise dos teores de carbono e nitrogênio

Para a análise de nitrogênio amoniacal total (NAT), as amostras foram centrifugadas à 10.000 rpm, por 10 minutos, à 20 °C em uma centrífuga de alta velocidade (Beckman Avanti J-E) de acordo com o método Tecator para Kjeltec ISO 5664. Em seguida, o sobrenadante resultante foi filtrado utilizando filtros de seringa de polietileno sulfonado de 0,45 µm e armazenadas a -20 °C até a realização da análise. As amostras foram descongeladas e diluídas de 100 a 7900 vezes, dependendo da concentração de nitrogênio amoniacal, para adequação à faixa de calibração do AutoAnalyzer (SEAL Analytical, EUA). Duplicatas analíticas foram realizadas para cada triplicata. A concentração de amônia livre foi calculada conforme a Equação 5.

$$NH_3 - N\ =\ \frac{NAT}{1 + \frac{10^{-pH}}{10^{-\left(0{,}09018+\frac{2729{,}92}{T}\right)}}} \qquad 5$$

Para as análises de carbono e nitrogênio orgânicos totais (COT e NOT, respectivamente), as amostras foram descongeladas antes da análise. Entre 3 e 5 g de lodo úmido foram secos à 60 °C em frascos de vidro até peso constante. O material seco foi triturado em um moinho de bolas (IKA-ULTRA TURRAX, UT TD S1). O pó fino (0,5 mg) foi pesado em cápsulas de estanho utilizando uma balança com precisão de 6 casas decimais (Sartorius, ME 36S), e os teores de carbono e nitrogênio foram quantificados utilizando um analisador elementar CHN (Thermo Fischer, Flash 2000).

3.2.2.5 Elementos inorgânicos

Os elementos inorgânicos foram analisados utilizando espectrometria de massa com plasma indutivamente acoplado (ICP-MS, Agilent 8900) (VANHAECKE, 2012). As amostras foram preparadas por meio de digestão ácida, adicionando-se 8 mL de ácido nítrico 7 M e 2 mL de H_2O_2 a 0,3 g da amostra. A mistura foi submetida a um forno de digestão assistida por micro-ondas, com a temperatura gradualmente elevada a 180 °C durante 25 minutos e mantida na temperatura máxima por 15 minutos. As amostras foram então diluídas até 50 mL e posteriormente mais 15 vezes antes de serem analisadas pelo ICP-MS.

Para a análise de lítio (Li), berílio (Be) e boro (B), não foi utilizado nenhum gás de reação. O gás hélio (He) foi empregado como gás de reação para a detecção de sódio (Na), magnésio (Mg), alumínio (Al), potássio (K), cálcio (Ca), titânio (Ti), vanádio (V), cromo (Cr), manganês (Mn), ferro (Fe), cobalto (Co), níquel (Ni), cobre (Cu), zinco (Zn), estrôncio (Sr), molibdênio (Mo), prata (Ag), cádmio (Cd), antimônio (Sb), bário (Ba), tálio (Tl), chumbo (Pb) e bismuto (Bi). Para a análise de arsênio (As) e selênio (Se), foi utilizado oxigênio (O_2) como gás de reação.

3.2.2.6 Teste de fitotoxicidade

A avaliação da fitotoxicidade foi conduzida in vitro utilizando sementes comerciais de tomate (*Solanum lycopersicum*). As sementes foram armazenadas em temperatura ambiente (25 °C) em um ambiente seco até o dia do experimento. Os testes de fitotoxicidade foram conduzidos utilizando três frações orgânicas do material digerido: digestato sólido (DS), digestato líquido (DL) e digestato total (DT). A separação sólido-líquido do digestato foi realizada utilizando uma centrífuga a 10.000 rpm por 10 minutos à 20 °C. Para a preparação da solução que foi adicionada às sementes, 1 g de material fresco foi misturado com 9 mL de água deionizada (LI *et al.,* 2020). Como controle, as sementes foram incubadas apenas com água deionizada (10 mL).

O ensaio foi montado em placas de *Petri* (diâmetro de 9 cm) contendo 2 folhas de papel de filtro Whatman Nº 1. O experimento foi realizado em triplicata, com 10 sementes por placa de *Petri* (QUINA *et al.,* 2015). As sementes foram embebidas com 10 mL de solução e mantidas no escuro em temperatura ambiente (23,9 ± 0,7 °C), por 72 h. IG foi determinado pela Equação 6 (QUINA *et al.*, 2015).

$$\text{IG (\%)} = \frac{RSG\ (\%)\ \times\ RRG\ (\%)}{100} \qquad 6$$

Onde RSG é a porcentagem relativa de sementes germinadas e RRG é a porcentagem relativa do comprimento da raiz, calculada usando as Equações 7 e 8, respectivamente:

$$\text{RSG (\%)} = \frac{N_{SG,T}}{N_{SG,B}} \times 100 \qquad 7$$

$$\text{RRG (\%)} = \frac{L_{R,T}}{L_{R,B}} \times 100 \qquad 8$$

Onde $N_{SG,T}$ e $N_{SG,B}$ são o número médio de sementes germinadas no extrato (solução contendo digestato) e no controle (água deionizada), respectivamente; $L_{R,T}$ e $L_{R,B}$ são o comprimento médio das raízes no extrato e no controle, respectivamente.

3.2.3 Análise estatística

A distribuição normal foi analisada utilizando os testes de normalidade de D'Agostino-Pearson e Shapiro-Wilk para os dados experimentais. Posteriormente, análise de variância (ANOVA) de dois fatores, seguida pelo teste de comparação múltipla de Tukey. Os valores de $p \leq 0{,}05$ indicaram diferenças estatisticamente significativas. As análises estatísticas foram realizadas utilizando o software GraphPad Prism 6.01.

Análise de componentes principais (PCA) com elipses de confiança de 95% foi realizada usando os pacotes FactoMineR, factoextra e ggplot2 para comparar propriedades químicas em cada matéria-prima. A análise foi executada utilizando o *software* R versão 4.2.3, com os pacotes ggplot2 e corrplot.

4 RESULTADOS E DISCUSSÃO

A seção de resultados e discussão está organizada em subseções, cada uma contendo artigos elaborados a partir dos resultados obtidos nesta tese.

O primeiro artigo, apresentado na seção 4.1, é uma meta-análise intitulada “Methane yield response to pretreatment is dependent on substrate chemical composition: a meta-analysis”, publicada na revista Scientific Reports. Os resultados deste estudo destacam a importância da predominância química do resíduo orgânico para a escolha apropriada do pré-tratamento a ser aplicado antes da digestão anaeróbia.

O segundo artigo, na seção 4.2, intitulado “Boosting manure biogas production with the application of pretreatments: A meta-analysis”, publicado no Journal of Cleaner Production, é também uma meta-análise que discute como a escolha do pré-tratamento pode aumentar a produção de biogás e contribuir para a demanda energética global a partir de fontes já comumente utilizadas, como o esterco.

O terceiro artigo, na seção 4.3, intitulado “Comprehensive Meta-Analysis of Pathways to Increase Biogas Production in the Textile Industry”, publicado na revista Energies, trata da aplicação da digestão anaeróbia em uma fonte pouco utilizada para a geração de energia. Além de tornar a cadeia produtiva mais sustentável, essa aplicação teria grande impacto, considerando que a indústria têxtil é a segunda maior poluidora do mundo. O artigo também aborda como a digestão anaeróbia combinada a pré-tratamento pode reduzir o potencial poluidor desses resíduos e, ao mesmo tempo, maximizar a geração de energia.

O quarto artigo, na seção 4.4, intitulado “Maximizing biogas production from algal biomass”, está em fase de revisão na revista Biofuels, Bioproducts & Biorefining. Este artigo avalia a maximização da produção de biogás a partir de biomassa algal e como essa produção pode contribuir globalmente com a demanda energética.

O quinto artigo, na seção 4.5, intitulado “Assessing phytotoxicity of anaerobic digestate: Effect of feedstock composition and liquid-solid separation”, a ser submetido, analisa a influência da composição química da fonte orgânica do digestato e a separação líquido-solido no seu valor agronômico.

4.1 Artigo I

Anacleto, T.M., Kozlowsky-Suzuki, B., Björn, A. et al. Methane yield response to pretreatment is dependent on substrate chemical composition: a meta-analysis on anaerobic digestion systems. Sci Rep 14, 1240 (2024). https://doi.org/10.1038/s41598-024-51603-9

Methane yield response to pretreatment is dependent on substrate chemical composition: A meta-analysis on anaerobic digestion systems

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Abstract

Proper pretreatment of organic residues prior to anaerobic digestion (AD) can maximize global biogas production from varying sources without increasing the amount of digestate, contributing to global decarbonization goals. However, the efficiency of pretreatments applied on varying organic streams is poorly assessed. Thus, we performed a meta-analysis on AD studies to evaluate the efficiencies of pretreatments with respect to biogas production measured as methane yield. Based on 1374 observations our analysis shows that pretreatment efficiency is dependent on substrate chemical dominance. Grouping substrates by chemical composition e.g., lignocellulosic-, protein- and lipid-rich dominance helps to highlight the appropriate choice of pretreatment that supports maximum substrate degradation and more efficient conversion to biogas. Methane yield can undergo an impactful increase compared to untreated controls if proper pretreatment of substrates of a given chemical dominance is applied. Non-significant or even adverse effects on AD are, however, observed when the substrate chemical dominance is disregarded.

Introduction

Anaerobic digestion (AD) is a successful and robust waste treatment biotechnology converting organic waste into clean energy in the form of biogas[1] and recovering nutrients as fertilizers and soil conditioners[2]. AD plays a crucial role in achieving the ambitious goal of the European Climate Law, aiming for climate neutrality by 2050[2]. An estimated increase from 0.3 EJ to 8.3 EJ by 2050 from biogas upgraded to biomethane (90% methane) makes it the non-fossil source with the greatest potential to be carbon neutral[2]. AD systems mitigate the emission of greenhouse gases (GHG), by recovering methane (CH_4) from organic wastes, and, when used as a combustion fuel, release carbon-neutral carbon dioxide (CO_2)[3]. About 60 to 80% of GHG emissions from transportation can be reduced by replacing gasoline with biomethane produced from AD[4]. Currently, the global potential for energy generation from biogas is estimated to be 10,000 to 14,000 TWh, with the potential to replace up to 10% of the world's primary energy consumption[5] of electric power, heat and automotive fuel. Unlike other sources of non-fossil energy, organic residues are the raw primary source for biogas production, which is relatively less sensitive to seasonality or scarcity.

Due to integrated socioenvironmental benefits[1] e.g., the replacement of energy resources such as firewood by biogas can improve quality of life, and promote gender equality, and higher educational levels[6]. AD surpasses several other renewable energy sources[7] representing the major technological pathway for the implementation of the United Nations Sustainable Development Goals (SDGs)[4]. Besides expanding local employment opportunities[6], AD promotes energy decentralization, with electricity supply to remote areas, e.g., rural communities by the implementation of small-scale biogas plants or by direct injection into the existing natural gas grid[4,8,9].

AD follows 4 steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis[9]. Hydrolysis by microbial extracellular enzymes converts complex biopolymers (i.e., protein, lipid, polysaccharides) into smaller compounds (i.e., sugar, amino acids, fatty acids)[10], which in turn are converted into volatile fatty acids (VFA), CO_2 and H_2 in the acidogenesis step[11]. Subsequently, acetate is produced in the acetogenesis step, providing the product for the generation of mainly CH_4 and CO_2 in the methanogenesis step[10,11]. Studies have exhaustively identified hydrolysis as the bottleneck for biogas production from recalcitrant biomass[12,13] usually leading to low AD efficiency upon application in, for example, agricultural sectors[14].

Substrates are often subjected to pretreatment prior to AD, and the potential of pretreatments to improve hydrolysis has been extensively reported in the literature. Several chemical, physical and biological pretreatments (Fig. S1) are applied to organic wastes to modify their physical-chemical structures and improve their biodegradability[15–17]. The resulting reduction in particle size and increase in surface area, porosity, and solubility of particulate organic matter[18] enhances the accessibility by microorganisms, improving hydrolysis and biogas production[19]. However, all of those pretreatments also increase the cost of the AD process, as they lead to increased energy consumption, require the purchase of additives, and usually depend on operational investments to adapt equipment to suit the pretreatment[13,20]. In addition, pretreatments may even have adverse effects on AD and result in lower CH_4 yields[1,21] if the selected pretreatment is not suitable for a given substrate. The proper choice of pretreatment is crucial to achieving viable and cost-effective conversion of recalcitrant feedstocks and to increasing biogas production[22]; therefore, the effects of pretreatment on organic wastes must be evaluated with respect to the chemical composition of the biomass.

Grouping substrates by origin (e.g., agricultural, municipal, industrial wastes, and aquatic biomass) is a widespread and common strategy applied in the industry to lower logistics costs and to promote the digestion of the greatest amount of waste available in a given geographic area. This has led to the application of pretreatments disregarding the heterogeneity of the biomass chemical composition or even to the implementation of co-digestion. Co-digestion is a strategy applied for simultaneous management of different waste streams by AD where two or more types of feedstocks are combined[23]. Since in co-digestion the substrate is mixed as a strategy to optimize the AD process[9,24] (e.g. balancing macro and micronutrients supply, and the moisture content or diluting inhibitory compounds), interventions such as pretreatment may lead to adverse process performance due to organic matter overload. For instance, co-digestion of (30% primary sludge and 70% sewage sludge) and glycerol (1% v/v) decreased CH_4 yields from 500 to 70 mL/gVS_{added} after alkaline pretreatment application[9]. Se**Erro! Indicador não definido.**veral studies [e.g.,[15,17,25–27]] have tested the application of specific pretreatments to specific substrates, but to the best of our knowledge, not a single study has yet consistently quantified the efficiencies of different pretreatments with varying types of substrates sorted by predominant chemical composition. Identifying proper pretreatments by substrate chemical predominance may open an opportunity for the management of new organic streams (individual or in combination) via AD. Also, it prevents unnecessary costs as the pretreatment implementation comprises a substantial proportion (up to ca 20%) of the total biomethane production cost[28].

Here we conducted a systematic review and a comprehensive meta-analysis to quantify the performance of different pretreatments according to the predominant chemical composition of the organic waste. Despite inherent limitations of performing a meta-analysis in AD systems, e.g., encompassing variations in operating conditions and feedstock characteristics across studies, the application of meta-analysis in AD systems offers substantial advantages. The outcomes derived from meta-analysis play a pivotal role in steering research efforts, shaping best practices, and advancing the knowledge base in AD systems. A comprehensive synthesis of the existing research allows for the identification of trends and overarching insights that may not be apparent in individual studies. Here, we evaluated 192 studies from which 1374 individual effect sizes were calculated from peer-reviewed scientific articles over the past 45 years (Table S1) and provide a comprehensive decision-making guideline for the choice of appropriate pretreatment based on the predominant organic chemical composition of the substrates.

Substrate chemical composition affects pretreatment efficiency

The effect and magnitude of the different pretreatments were assessed by calculating the standardized mean difference (SMD), which is the CH_4 yield difference between the treated and untreated (control) substrate groups. SMD Hedges' g ≤ 0.2 represents a small effect, 0.3–0.5 a medium effect, and ≥ 0.6 a large effect[29]. CH_4 yield is significantly improved by a given pretreatment if SMD is higher than zero and the lower limit of the confidence interval (CI) does not cross zero, while significantly depressed by a given pretreatment if SMD is lower than zero and the upper limit of the CI does not cross zero. Our findings indicate that to reach higher efficiencies for biogas production, classification based on chemical predominance rather than on the origin of the waste, prior to the choice of proper pretreatment is fundamental (Fig. 1).

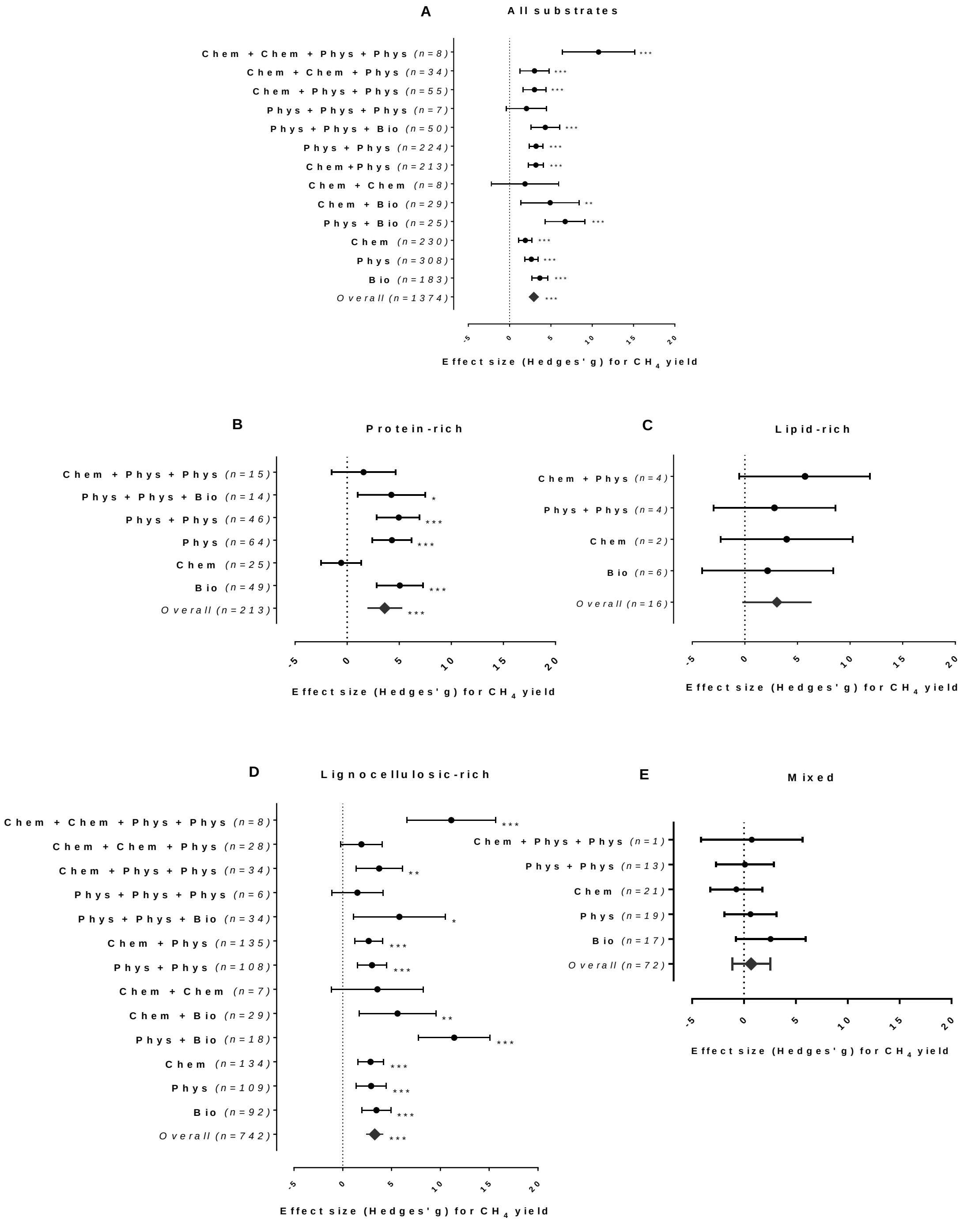

Figure 1. Mean effect size (Hedges' g) and 95% confidence intervals for CH_4 yield from protein-, lipid- and lignocellulosic-rich substrates subjected to different pretreatments. Phys= physical, Chem= chemical and Bio= biological (Figs. S3-5); these abbreviations denote the treatments and combinations applied to different substrates. A: All substrates were sorted by pretreatment regardless of their chemical composition. B: Protein-rich substrates were predominantly composed of animal waste, microalgae, or high protein content (≥40% dry matter). C: Lipid-rich substrates were predominantly composed of agricultural oil residues, swine slaughterhouse wastewater, or any source with high lipid content (≥40% dry matter). D: Lignocellulosic-rich substrates were predominantly composed of crop residues, cattle manure, or high lignocellulose content (≥50% dry matter). E: Mixed substrates included only food waste. Detailed information on the substrate categories can be found in the Supplementary material (Figs. S6-8). Significance level: $p \leq 0.001$ (***); $p \leq 0.01$ (**); $p \leq 0.05$ (*). n = number of effect sizes per treatment type.

Protein-rich substrates

About 1 million tons of protein-rich waste is produced globally every year[12]. Although protein-rich substrates have high theoretical methane potential, ca 0.5 Nm^3/kg volatile solid (VS), AD can be severely affected by ammonia accumulation from protein breakdown[12,30]. High concentrations of ammonia can particularly inhibit acetoclastic methanogenesis[18], leading to VFA accumulation, a lower biomethane yield, and process disturbances[3].

Our literature search demonstrated that microalgae, meat processing waste, slaughterhouse waste, and swine and chicken manure are those substrates that have been reported as protein-rich feedstock of AD[31]. Microalgae were the most common feedstock studied among protein-rich substrates (Fig. S6), which can be explained by their rapid growth rates and cultivation viability without requiring arable lands[16].

The outcomes of the meta-analysis resulted in 213 effect sizes from pretreatment of protein-rich substrates (Fig. 1B). Biological (SMD= 5.061, 95% CI: 2.839 to 7.282) and physical (SMD= 4.301, 95% CI: 2.405 to 6.197) pretreatments applied alone or in combination led to the highest CH_4 yields from protein-rich substrates (Fig. 1B), while chemical pretreatments (SMD= -0.573, 95% CI: -2.520 to 1.374) had no significant effect. Biological pretreatments (e.g., enzymatic pretreatment), which increase protein hydrolysis and solubilization[16]. Some biological pretreatments such as bacteria flocculation (flocs) increase methanogens tolerance to NH_3 concentration and toxic compounds (i.e., furfural)[12]. At full-scale, biological pretreatments have proven to further reduce substrate viscosity and the energy demand for mixing[32]. In particular, the application of protease as enzymatic pretreatment led to a significant increase in CH_4 yield (SMD= 5.132, 95% CI: 1.178 to 9.085, Fig. 2), which can be attributed to the specificity of protease in hydrolyzing proteins. The application of protease is associated with low pollution risk to the environment and low energy demand, making it more suitable than energy-intensive options such as thermal pretreatments at the laboratory or full-scale[32]. The overall advantages of biological pretreatments are their reaction specificity (in case of enzymatic pretreatment), low operating and energy costs, and a lack of toxic end products[15].

Pretreatments that involve heat application, including thermal (SMD= 3.655, 95% CI: 0.748 to 6.561), steam explosion (SMD= 7.386, 95% CI: 4.851 to 9.922), and hydrothermal (SMD= 13.144, 95% CI: 6.693 to 19.595) were those exhibiting the best performance for protein-rich substrates (Fig. 2). These pretreatments are effective in breaking down organic matter and increasing its exposure to enzymatic degradation during the hydrolysis step[19]. Heat pretreatments are one of the most applied in full-scale biogas plants[9], which may be a result of the mandatory pasteurization requirement for some substrates. However, the relatively high cost:effectiveness ratio of these pretreatments discourages their use, especially when compared with biological pretreatments, which are relatively inexpensive to implement.

Homogenization is a promising physical pretreatment at the industrial scale, as it disrupts substrate structure and decreases particle sizes, consequently improving the substrate accessibility for microbial degradation[25]. Homogenization significantly increased the CH_4 yield (SMD= 8.339, 95% CI: 3.798 to 13.001) of protein-rich substrates. Similarly, ultrasonication (SMD= 5.421, 95% CI: 3.434 to 7.407, Fig. 2) promotes organic waste degradation via hydromechanical stress, reducing hydrolysis time and increasing the production of biogas[17]. Although homogenization requires high pressure (>800 bar) to increase up to 15% of the protein solubilization, the energy balance of the pretreatment is positive[9], as energy costs are covered by biomethane production, and has been successfully applied on a full-scale[25]. Ultrasonication is equally successful at practical levels, producing 3–10 kW in CH_4 yield for every kilowatt of ultrasonic energy applied[17].

Chemical pretreatments applied to protein-rich substrates led to an overall reduction, though non-significant, in CH_4 yield (Fig. 1B). This can be attributed to the generation of secondary degradation products from complex molecular bonds of proteins in addition to the formation of inhibitory compounds such as ammonia[12].

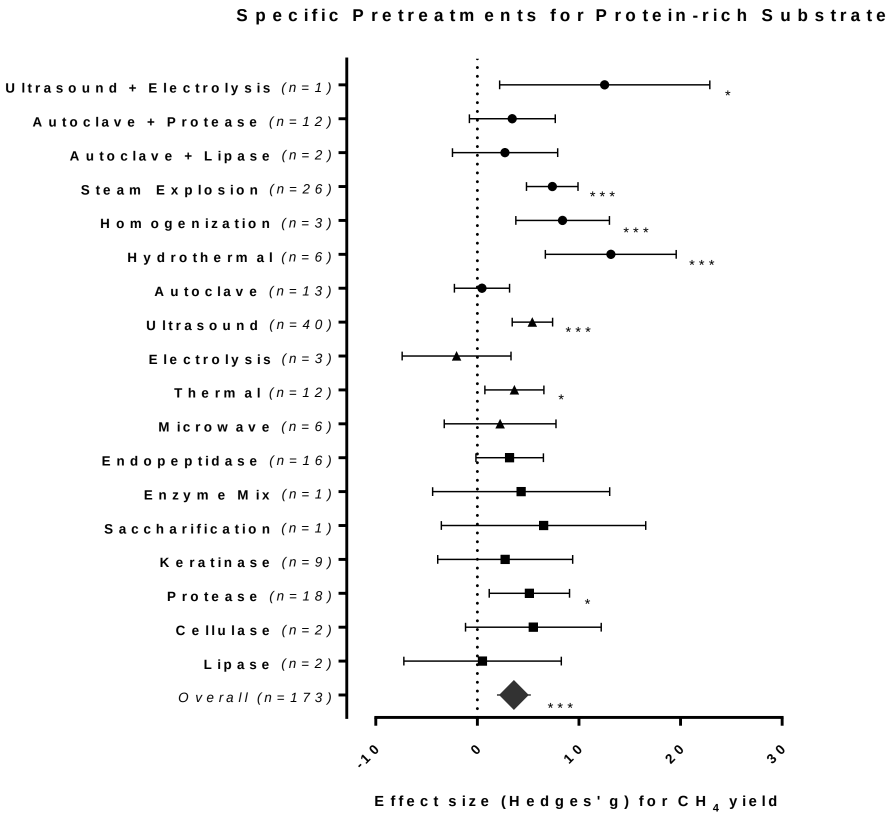

Figure 2. Mean effect size (Hedges' g) and 95% confidence intervals for CH_4 yield for the most efficient pretreatment methods (biological= squares, physical= triangles and combinations thereof= circles) applied to protein-rich substrates; the plot depicts 95% confidence intervals of the Hedges' g effect size for CH_4 yield. Significance level: $p \leq 0.001$ (***); $p \leq 0.01$ (**); $p \leq 0.05$ (*). n= number of effect sizes per treatment type.

Lipid-rich substrates

Milk and meat processing waste, oilseeds, and kitchen waste are examples of lipid-rich substrates (Fig. S7)[33]. Lipid-rich substrates can exhibit greater biogas production than protein- and carbohydrate-rich substrates[34], with the theoretical methane potential of ca 1.0 Nm^3/Kg VS[10]. Lipids consist of long-chain fatty acids (LCFAs) linked to glycerol, alcohols or other groups by ester or ether linkages[33]. However, high concentrations of LCFAs are harmful to AD and cause severe inhibition to microorganisms, especially in the acetogenesis and methanogen stages[33].

As shown in Fig. 1C, 16 effect sizes were calculated for lipid-rich substrates. Pretreatments had marginal positive effects, and none of the tested categories yielded a higher efficiency than those of the non-pretreated controls (Fig. 3C). However, this result should be interpreted with caution, as the number of observations was considerably lower than the number reported for other substrates.

The use of lipid sources as a sole substrate is not a common practice for biogas production due to the need for nutrient balance (C:N:P:S ratio) to achieve optimal microbial activity. Thus, substrates with high lipid content (>60% of wet weight) achieve the highest production of biogas in co-digestion[35]. Nevertheless, biogas production can be hampered by excessive loads of lipids due to the hydrophobic nature of lipid-rich materials[34] and by disturbances such as foaming that inhibit microbial activity[33].

Appropriate pretreatment can mitigate the AD instability associated with high loads of waste lipids by improving the dispersion and solubilization of lipids in the sludge matrix[19]. Nevertheless, our results suggest that optimizing the balance of substrates and nutrient ratios via co-digestion could be more promising than investments in pretreatments. LCFAs from the lipid-rich substrate are usually stabilized when co-digested with low biodegradability co-substrates[10], improving overall biogas production. Alternative operational approaches such as effluent solid recirculation or pulse feeding has also shown promising results on increasing the capacity of AD for handling high loads of lipids[36,37].

Lignocellulosic-rich substrates

Lignocellulosic biomass is one of the most abundant sources globally for biofuel production[22]. Approximately 181.5 billion tons of lignocellulosic biomass are generated worldwide every year[38]. It is classified by its molecular organization consisting of crystalline cellulose, organized into macrofibrils firmly attached by intermolecular hydrogen bonds, combined with amorphous chains of hemicelluloses, all immersed in a lignin matrix[39]. However, the broad chemical heterogeneity of this organic source prevents the application of a single operational condition that meets all requirements of this feedstock[40]. The biogas production of its widely heterogeneous composition decreases dramatically if treated under equal operating conditions[40]. Although feedstocks e.g., hardwoods, soybeans, sugar beets, manure, and sugarcane bagasse have been treated under the same classification, their distinct content of biopolymers sorts them apart.

A total of 742 effect sizes were calculated for lignocellulosic substrate, more than the sum of all other substrates (Fig. 1D). With a few exceptions, pretreatments applied to lignocellulosic-rich biomasses had positive effects on CH_4 yields, despite an unclear response towards specific pretreatments (Fig. 1D). This was probably a result of a large number of different biomass sources that were merged into this group implying large variations in the substrate chemical composition. Lignocellulosic biomass e.g., wood, energy crop, and plant residues are primarily comprised of cellulose, hemicellulose, and lignin, and the composition of these components determines the recalcitrance nature and biodegradability of their chemical structure[27,39].

Lignin in plants mainly provides structural support, impermeability, and resistance against microbial attack and oxidative stress[27]. Despite the difficulty in degrading lignin, the application of appropriate pretreatment resulted in a CH_4 yield increase of almost 40%[41]. Lignin content has been identified as one of the main barriers to the AD of lignocellulosic biomass[11] and can be used as an independent variable to assess the effects of pretreatments on lignocellulosic-rich substrates[14]. Therefore, lignocellulosic-rich substrates were divided into three categories according to their lignin content (<10%, 10–25%, and >25% lignin dry weight (DW), Fig. 3).

Chemical pretreatments degrade lignin very efficiently and are commonly applied to overcome the recalcitrance of lignocellulosic-rich organic residues[28]. Chemical additives (such as sulfuric acid, hydrochloric acid, sodium hydroxide, potassium hydroxide, lime, and hydrogen peroxide) remove the protective barrier created by lignocellulosic fibers, increasing cellulose exposure and facilitating its degradation during AD[28]. However, chemical addition implies an increase in operational costs when applied at full-scale[42] related to chemical reagents and construction of corrosion-resistant reactors[43]. Generation of toxic compounds[4] that can disturb biogas production is also identified as a drawback of using chemical pretreatments[4]. Nevertheless, the overall effect of various chemical pretreatment applied on lignocellulosic-rich substrates resulted in an increase in CH_4 yield based on the outcomes of our meta-analysis (Fig. 3A, B and C).

Interestingly, at low and medium lignin content (<25% lignin DW), combined physical and biological pretreatments were more efficient than the addition of chemicals and should be used preferentially if the main reason for pretreatment is to increase CH_4 yield. As an exception, biogas production from the lignocellulosic substrate at medium lignin content (Fig. 3B), dropped dramatically when subjected to a combination of temperature, pressure and enzymatic pretreatment, in contrast to the high performance of the physical+biological combination[9]. The adverse effect possibly occurred in response to multiple interventions generating a highly bioavailable organic matter, overloading the AD system negatively affecting biogas production[9].

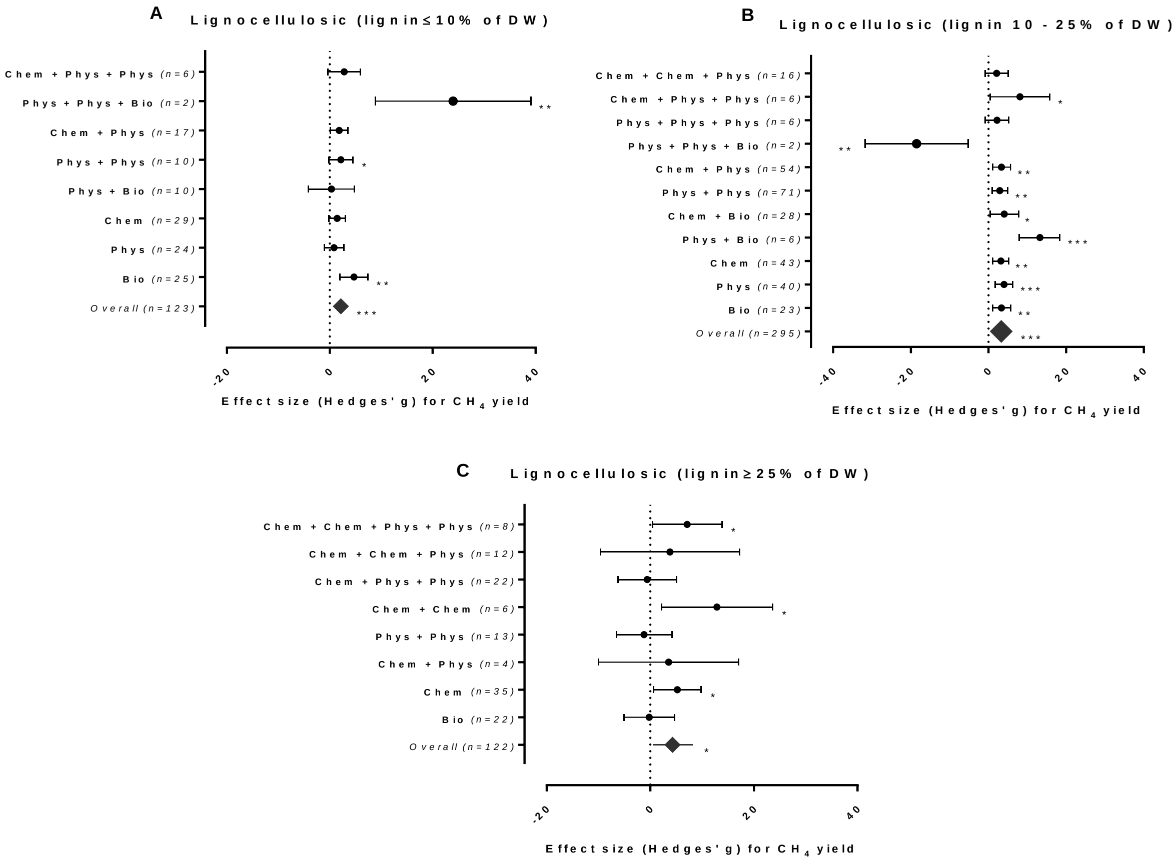

Figure 3. Mean effect size (Hedges' g) and 95% confidence intervals of CH_4 yield for lignocellulosic-rich substrates subjected to different pretreatments. Phys= physical, Chem= chemical and Bio= biological; these abbreviations denote the treatments and their combinations applied to substrates with different lignin contents. A: lignin<10%, B: lignin 10–25% and C: lignin>25% DW. Significance level: p≤0.001 (***); p≤0.01 (**); p≤0.05 (*). n= number of effect sizes per treatment type.

Lignocellulosic substrates with low lignin contents (≤10% DW) have less of a protective barrier and are therefore more susceptible to biodegradation; hence, pretreatment may have no effect or even an inhibitory effect on CH_4 yield due to the accumulation of toxic compounds such as phenolic substances, 5-hydroxymethylfurfural (HMF) furfurals and aldehydes[1,44]. Our results suggest that substrates with low lignin content require only milder interventions, including the application of biological pretreatments, e.g., enzymes. Enzymatic pretreatment alone (SMD=11.390, 95% CI: 1.169 to 21.610) or combined with autoclavation (SMD= 25.941, 95% CI: 10.998 to 40.884) or rumen fluid addition (SMD= 8.525, 95% CI: 4.368 to 12.682) led to the highest CH_4 yields from substrates at low lignin content (Fig. 4). Up to 83% increase in CH_4 yields of low-lignin substrates was achieved after biological pretreatment (Table S3).

Sugar beet pulp and Napier grass are examples of lignocellulosic sources with low lignin content that were subjected to biological pretreatment (Table S2; Fig. 4). The addition of microbial consortia (bacteria and fungi) and enzymes for pretreatment, not only preserved the weight of cellulose for the hydrolysis step but also increased ca 84% of the total sugar yield which serves as methanogenic substrate in AD systems[45]. Also, enzymes from fungi have been reported as a strategy for the optimization of AD on full-scale, where its addition increased CH_4 yield by 8% and reduced the AD operational costs by 10%[32]. Thus, indicating that, the use of biological pretreatments of lignocellulosic substrates with lignin content <10% should be prioritized over the use of chemicals.

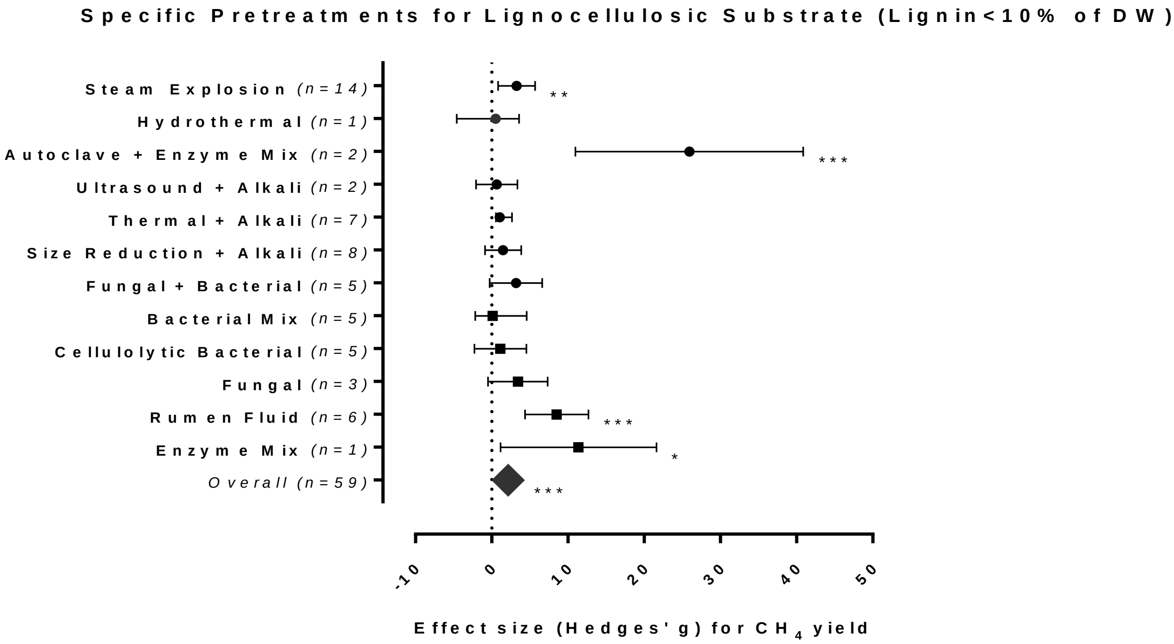

Figure 4. Methane yields for the most efficient pretreatment methods (biological= squares, combinations= circles) applied to lignocellulosic-rich substrates (lignin<10% DW). The plots depict 95% confidence intervals of Hedges' g effect size for CH_4 yield. Significance level: $p \leq 0.001$ (***); $p \leq 0.01$ (**); $p \leq 0.05$ (*). n= number of effect sizes per treatment type.

Most agricultural residues have intermediate levels of lignin content (10–25% DW)[41] and comprised the majority of the lignocellulosic substrates used for biogas production (Fig. 3) with 295 individual effect sizes. The overall effect of all pretreatments applied to lignocellulosic substrates with intermediate lignin contents was positive and significant (SMD= 3.331, 95% CI: 2.055 to 4.607, Fig. 5).

A common strategy used in the agricultural sector to deal with intermediate lignin content is to apply physical pretreatment to reduce particle sizes; this process alone has a small positive effect. However, combining particle size reduction with fungal (SMD= 12.734, 95% CI: 7.520 to 17.948) or alkaline (SMD= 2.426, 95% CI: 0.082 to 4.771) addition significantly enhanced CH_4 yields (Fig. 5) and led to increases of up to 170% compared to the untreated substrate (Table S4). Particle size reduction increases surface area and facilitates microbial access to biodegradable cellular compounds[13]; furthermore, when this approach was combined with the application of ligninolytic enzymes excreted by fungi, a highly delignified biomass was obtained, and the benefits of this combined approach surpassed the positive effect of fungal addition alone (SMD= 4.377, 95% CI: 1.050 to 7.703, Fig. 5).

Alkaline addition decreases the recalcitrance of lignocellulosic materials by enhancing lignin and hemicellulose solubilization, thus reducing the crystallinity of the cellulose[39]. It also promotes the removal of acetyl groups and uronic acid substitutions in hemicelluloses, increasing access to carbohydrates during hydrolysis, being more favorable for biomass with low/medium lignin content[46]. Alkaline pretreatments alone had positive effects (SMD= 3.936, 95% CI: 0.594 to 7.277) on CH_4 yield and can be considered for application as the only pretreatment since this approach is cost-effective even at full-scale[13].

Thermal (SMD= 4.675, 95% CI: 0.498 to 8.852) and autoclave (SMD= 4.920, 95% CI: 1.468 to 8.372) are physical pretreatments that resulted in significant increases in CH_4 yields when applied to substrates with moderate lignin contents. The increase in temperature promotes cell lysis making intracellular material available for microbiological degradation[43]. Autoclaving is a combined pretreatment method involving high temperatures and pressures and leads to a steam explosion when applied to organic matter.

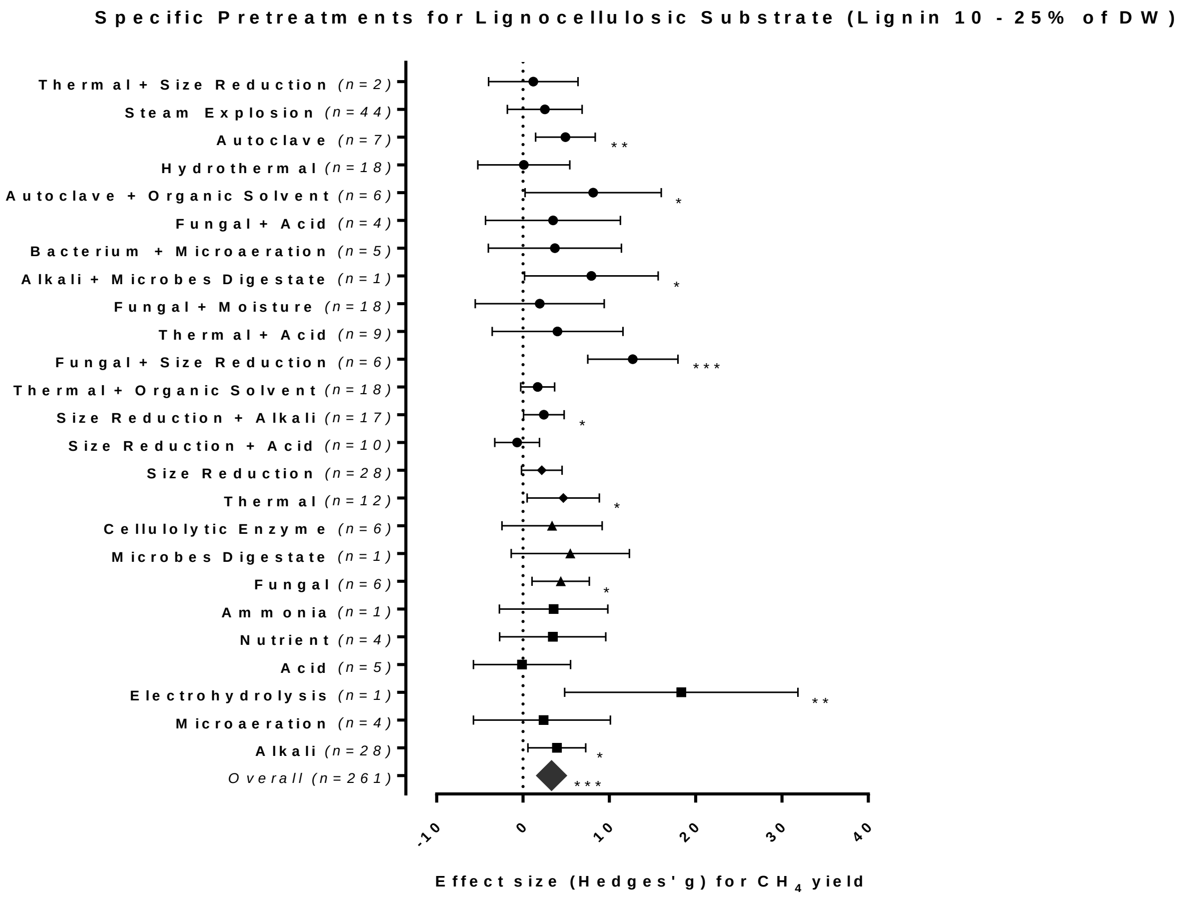

Figure 5. Methane yield effects for the most efficient pretreatment methods (chemical= squares, biological= triangles and combined methods= circles) applied to lignocellulosic-rich substrates (lignin 10–25% DW). The plots depict 95% confidence intervals of Hedges' g effect size for CH_4 yield. Significance level: $p \leq 0.001$ (***); $p \leq 0.01$ (**); $p \leq 0.05$ (*). n= number of effect sizes per treatment type.

The lignin content in lignocellulosic-rich substrates is proportional to the ability of the substrate to withstand microbial hydrolysis[13]. Accordingly, lignocellulose substrates with lignin contents above 25% e.g., woods, stalks, processed bagasse, and silage (Fig. S8) are less effectively biodegraded and exhibit limited potential for methane production. Substrates with this high lignin content have been more rarely tested leading to only 122 individual effect sizes (Fig. 6), for which chemical pretreatments applied alone or in combination are the only viable strategy for increasing the CH_4 yield.

Acid pretreatments are the most commonly applied to such substrates with a CH_4 yield increase in up to 500% (Table S5). The addition of acid can accelerate the sugar conversion rate over 90%, by promoting the breakdown of glycosidic bonds of long chains of cellulose and hemicellulose into sugar monomers[47]. However, the use of acids requires extra care, as high concentrations of reagents can cause serious damage and corrosion of the operational system in addition to causing imbalances in the AD process[40]. At a practical level, chemical addition handled with accuracy and caution is supported techno-economically[13] despite the requirement of high investments for operation and final safe environmental disposal via the digestate[11].

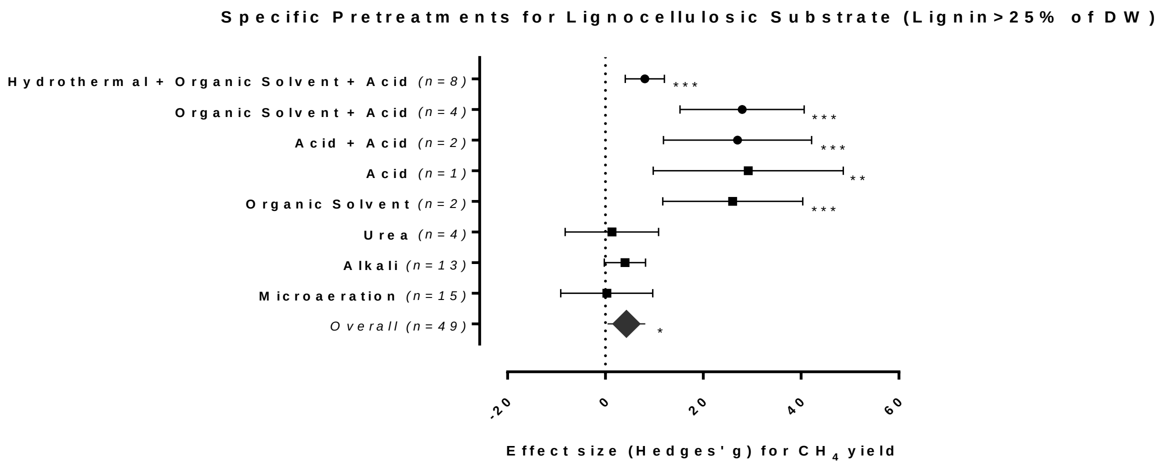

Figure 6. Methane yield effects for the most efficient pretreatment methods (chemical= squares and combinations= circles) applied to lignocellulosic-rich substrates (lignin>25% DW). The plot depicts 95% confidence intervals of Hedges' g effect size for CH_4 yield. Significance level: $p \leq 0.001$ (***); $p \leq 0.01$ (**); $p \leq 0.05$ (*). n= number of effect sizes per treatment type.

Mixed substrates

As mentioned earlier, substrate mixing is a very common practice, either to treat all organic waste from a given location in a single operation or to perform co-digestion. However, except for co-digestion, chemical predominance and nutrient balance are not often considered for mixed substrates. Here, mixed substrates are those in which carbohydrates, lipids, and proteins are roughly equal without major disproportions between their contents. Although food waste, sewage, and co-digestion comprise a mixture of several organic sources, food waste seems to be the most suitable to be used as a model, since co-digestion prioritizes geographical location and stabilization of organic matter without the addition of pretreatment[48] while the sewage is often pointed out as lipid-rich[19].

Food waste constitutes a complex organic matrix where the final composition depends on eating habits and varies between countries, regions and periods of the year[49], preventing a unified characterization of food wastes. From the 72 individual estimated effect sizes, there were no significant differences among pretreatments applied to food waste with an overall effect of SMD = 0.693, 95% CI: -1.132 to 2.518 (Fig. 1E). The outcomes highlight that the application of pretreatments might even have a negative marginal effect on CH_4 yield of food waste. Therefore, the appropriate pretreatment should be identified on a case-by-case basis depending on the chemical predominance of the analyzed substrate[50]. If no chemical component predominates, targeted pretreatment cannot be advised, and therefore, positive effects on substrate degradation might be drastically reduced. Therefore, the selection of pretreatments applied to mixed substrates with undefined chemical compositions should consider other factors, such as decreased costs or the need to meet legal requirements (i.e., pasteurization).

Conclusions

Lack of cost-effective pretreatment options or the application of suboptimum pretreatments to specific substrates are among the factors that currently limit the global potential for biogas production. Our meta-analysis showed that the choice of pretreatment should be defined by the predominant chemical composition of the targeted organic waste. For example, major global crop residues including corncob, rice husk, rice straw, sugarcane bagasse, and wheat straw with a combined annual generation of ca 1.3 billion tones by the key producing countries are all grouped as lignocellulosic substances with intermediate lignin content based on our categorization (<25% lignin). Most of the studies (87%) utilize laboratory batch conditions using a Biochemical Methane Potential (BMP) assay for pretreatment evaluation. Despite concerns of upscaling results to the industry, BMP assays are the first step applied by researchers and industrial biomethane producers for the evaluation of the feasibility of biomass as a feedstock for AD. Thus, the outcomes reported based on BMP quantifications can aid the selection of suitable pretreatments for laboratory- or pilot-scale simulations of AD processes for the industry. Our outcomes suggest that the current methane potential of these substrates could be enhanced by up to 170% if appropriate pretreatment methods are applied. This would add up to 1800 TWh of the global renewable energy potential assuming roughly 90% dry matter content and a conservative methane potential of 220 m^3 CH_4 per dry weight of the untreated feedstock. The guideline provided in this study assists selection of proper pretreatment methods based on the knowledge generated in past 45 years to boost economic gains and promote the contribution of AD to societal sustainability and decarbonization.

Methods

Search strategy and study selection

We performed a systematic review and meta-analysis of studies published in the Web of Science database between 1975 and July 2020 based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, http://www.prisma-statement.org/) checklist. The search was performed using the following keywords: “hydrolysis”, “anaerobic digestion", "methane yield" and "pretreatment". The search was restricted to only articles (document type) and only publications in English (language) (Fig. S9).

The eligibility criteria for inclusion of articles in the meta-analysis were as follows: i) description of the average value, standard deviation (SD) and number of replicates for methane yield with and without pretreatment (control); ii) description of the pretreatment applied; and iii) methane yield provided separately from the total biogas production rate. We included studies with replicates ranging from 2 to 5, recognizing that, despite the general recommendation of a minimum of 3 replicates for Biochemical Methane Potential (BMP) tests, particularly for treatment bottles, the number of replicates of larger lab-scale reactors are seldom above 2.

Data collection

Articles eligible after screening by the inclusion criteria had their data collected in an Excel spreadsheet. The data extracted from each article includes general information (e.g., first author's name, article title and year of publication), substrate type, substrate chemical composition, inoculum description, operational configuration (e.g., temperature condition, hydraulic retention time (HRT), stirring (i.e., RPM), reactor type, operational scale, total volume and working volume), pretreatment method, specific pretreatment conditions and methane yield data (mean, standard deviation (SD) and number of replicates).

Pretreatment techniques (e.g., autoclave, mechanical, alkaline, acid and enzyme) were grouped into methods (e.g., physical, chemical, biological and combined) since the transformations achieved in organic matter are rather similar within techniques belonging to the same group[51]. Once the effect of each pretreatment method is significant in the quantitative synthesis, all the techniques that compose it are individually evaluated. Also, the different feedstocks were grouped by the predominance of the chemical composition.

Substrate classification by predominant chemical composition

The substrates tested in the studies included in the meta-analysis were grouped into categories according to their predominant chemical composition in dry weight (DW). Based on the chemical characterization reported in the articles from the systematic review, the substrates were divided into 4 main categories: protein-rich, lipid-rich, lignocellulosic-rich and mixed.

As the AD literature does not present a range of protein content for protein-rich substrates[12,32,34], data from the articles included in the systematic review were screened in order to assess their chemical composition. Protein-rich substrates were then considered those with an average protein content of ≥40% DW.

Due to operational limitations mono-digestion of lipid-rich substrates is rare[34,35], and so is the chemical characterization. Based on the classification of lipid-rich substrates from previous studies in the literature, the average lipid content of lipid-rich substrates was ≥40% DW.

Lignocellulosic substrates have at least >50% lignocellulose content per DW. The chemical composition of lignocellulosic biomass is composed of three main biopolymers: cellulose, hemicellulose and lignin[41]. Lignin was selected as the independent variable due to its widespread description in the literature as one of the main barriers to the degradation of lignocellulosic content[11]. Lignocellulosic substrates were here divided into three lignin concentration ranges. The choice of lignin content range was based on the difficulty in converting crop residues into biogas in the range of 10–25% DW of lignin applied as mono-digestion, either due to the complexity of the structure of the material or the generation of phenolic compounds that inhibit AD[38]. In addition, most crop residues applied to energy generation are in this range of lignin content, which requires high attention to optimize the digestion[38]. Lignocellulosic substrates were then classified into 0–10%, 10–25% and >25% DW lignin relative to the total lignocellulosic content. The lignin content (%DW) in lignocellulosic biomass (LB) was calculated with the equation used by Thomsen et al. (2014), where LB is composed of cellulose (X_C), hemicellulose (X_H) and lignin (X_L) (Equations 1 and 2).

$$LB = (X_C + X_H + X_L) \quad (1)$$

$$Lignincontent(\%DW) = \frac{X_L*100}{\mathrm{LB}} \quad (2)$$

Mixed substrates consisted of highly variable biomass sources that did not show any pattern of chemical predominance. For instance, the chemical compositions of food waste and sewage are often affected by culture, season, social class and holidays[49], making it impossible to precisely determine their chemical composition over time.

Data analysis

We applied the standardized mean difference (SMD) estimated by Hedge's g as the effect size with which to quantify methane yield data. Following the formula[29]:

$$g = \frac{Mean_T - Mean_C}{\sqrt{(n_1-1)s_1^2 + (n_2-1)s_2^2}} * 1 - \frac{4}{4(n_1+n_2)-9} \quad (3)$$

Where the $Mean_T$ is the treated group and $Mean_C$ is the control group, n_1 and n_2 are the sample size while s_1^2 and s_2^2 are the estimated population variance for the treated and control group, respectively[29]. This effect size is considered less biased than other calculation approaches and is recommended for small sample sizes[53].

Mean effect sizes (Hedges' g), 95% confidence interval (CI) with bias correction and p-value were calculated in R software (R Core Team, 2021) using the "metafor" package (version 3.0-2) for each pretreatment as well as for the specific techniques of significant pretreatment methods[29,54]. Pretreatments were considered significant ($p < 0.05$) when their mean value and CI did not overlap the zero line. Mean and CI values below the zero line indicated a negative response (pretreatment < control), while mean and CI values above the zero line indicated a positive response (pretreatment > control).

A multilevel meta-analysis was performed followed by a subgroup analysis as the data were grouped into pretreatment categories for analysis[54]. Also, the dependence of effect sizes was considered since a given study can compare several treatments to a single control group, which means that the data are not independent. Furthermore, we assumed the random effect model considering the difference in methodology of experiments performed in each study included in the analysis[29,54].

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4.2 Artigo II

Anacleto, T. M., Oliveira, H. R., Diniz, V. L., de Oliveira, V. P., Abreu, F., & Enrich-Prast, A. (2022). Boosting manure biogas production with the application of pretreatments: A meta-analysis. Journal of Cleaner Production, 362(May), 132292. https://doi.org/10.1016/j.jclepro.2022.132292

Boosting manure biogas production with the application of pretreatments: A meta-analysis

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Highlights

- CH_4 production from different manure sources varies with different pretreatments.
- Chemical and biological pretreatments increase CH_4 production from omnivore manure.
- Physical and chemical pretreatments increase CH_4 production from herbivore manure.
- The appropriate pretreatment can increase CH_4 yield up to ten times.

Abbreviations[1]

Abstract

Anaerobic digestion (AD) is a versatile manure management approach that can combine waste treatment, energy generation and nutrient recovery, thus playing a central role in circular economy. The AD process is highly influenced by manure composition which, depending on the source, may contain high loads recalcitrant materials (e.g., lignocellulosic and fibers) or lead to the formation of toxic compounds (e.g., NH_3), decreasing the energetic potential of the waste and requiring specific pretreatments to increase its degradability and biogas production. Although there are distinctions in the chemical composition of manure according to animal diets, different manure sources are usually grouped together, leading to a suboptimal performance of both the pretreatment and the AD process. Here, we performed a meta-analysis of 54 studies to evaluate the effects of different pretreatments on different manure types and their effect on methane (CH_4) yield and we estimated the energy potential if the appropriate pretreatment is applied to largest manure producing countries. The results showed that chemical and/or biological pretreatments were more effective for omnivore manure (e.g., swine, chicken), while physical and a combination of chemical and physical pretreatments negatively affected CH_4 production. Physical and/or chemical pretreatments had a positive effect on CH_4 yield from herbivore manure (e.g., cattle, horses), while biological pretreatments had a negative effect. The application of the adequate pretreatment can more than double the energy recovered from manure, allowing for an important substitution of fossil fuels, while decreasing operational costs and environmental risks and ultimately improving profitability. The development of pretreatment technologies and their application are strongly related to public policies for sustainable manure management and biogas use and production.

Keywords

Anaerobic digestion, pretreatment, manure, biogas, methane, meta-analysis

[1] AAS: Aqueous Ammonia Soaking; AD: Anaerobic Digestion; BS: Biological Supplements; CI: Confidence Intervals; COD: Chemical Oxygen Demand; EU: European Union; GHG: Greenhouse Gas; LMC: Lignocellulolytic Microbial Consortium; MG: Manure Generation; NBW: Nano-bubble Water; OM: Organic Matter; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis; RR: Response Ratio; SMG: Specific Manure Generation; TAN: Total Ammonia Nitrogen; TS: Total Solids; USA: United States of America; VS: Volatile Solids

1. Introduction

The global consumption of animal products per capita has doubled over the last 40 years, boosting the growth of the livestock sector especially in developing countries (Shober et al., 2018; Zhang et al., 2019). Consequently, huge amounts of waste and manure are produced, which lead to a growing concern over environmental issues. Inadequate manure disposal leads to several environmental problems, such as greenhouse gas (GHG) emissions, boosting climate change; acidification; particulate matter formation caused by NH_3 e NO_x; and eutrophication of soils, waterbodies and groundwaters (De Vries et al., 2012; Petersen et al., 2013).

Anaerobic digestion (AD) is one of the most versatile strategies for manure management. Based on the biochemical degradation of organic matter by a consortium of different organisms in an oxygen-free environment, AD is not only a path to reduce the environmental pollution caused by animal production, but also to generate energy as biogas and biofertilizer and soil conditioner from digestate (Zahedi, 2018).

Biogas can be used to produce heat, electricity or biomethane, a substitute for natural gas (Angelidaki et al., 2019), promoting a double-green transition, by combining an increase of renewables in the energy sector with an improvement with waste management (D'Adamo et al., 2021). It is also able to reach up to 240% and 202% GHG savings with electricity and biomethane generation from manure, respectively (EBA, 2019). Compared to other green energies, biogas is advantageous since, when updated to biomethane, it can be distributed by grid injection in the natural gas pipeline and/or stored (Pasini et al., 2019). Also, the storage of biomethane outweighs the seasonality of energy availability from other sources. Methane is easily stored in the natural gas grid, an underground reservoir, a compressed tank, liquefied storage, bottling, adsorbed storage (metal-organic framework, other porous materials) and physical and chemical conversion (hydrated clathrate, chemicals) (Budzianowski and Brodacka, 2017).

A circular economy model is an important way to control the pollution caused by animal production (Yunan et al., 2021). By converting organic wastes into resources such as biogas and biofertilizers, which can then be returned to the production chain, AD has an important role in the contribution for transitioning the livestock sector to a circular economy (Fagerström et al., 2018; Pirelli et al., 2021; Yunan et al., 2021). Besides the utilization of a cleaner energy source, the application of AD technology also generates GHG and non-GHG emissions savings related to the reduction of mineral fertilizer production and utilization, while allowing for nutrient recovery due to biofertilizer production (EBA, 2019; Pirelli et al., 2021), closing the production cycle.

Even though the AD of manure provides several valuable bioproducts, there are still some challenges to overcome. The high lignocellulosic content in cattle manure decreases AD efficiency, especially due its lignin concentration, that makes the substrate highly recalcitrant (Millati et al., 2020). The high concentration of nitrogenous compounds in chicken manure, due to its high protein content, is another challenge to AD processes (Sun et al., 2016). These compounds are detrimental for the anaerobic microbial community, since they are converted into ammonia, causing cell membrane injuries and pH imbalance (Ruiz-Sánchez et al., 2018).

To overcome these challenges, physical, chemical and biological pretreatments – as well as their combinations – have been applied prior to the anaerobic degradation of manure (Isikgor and Becer, 2015). Pretreatments improve AD efficiency by increasing the substrate accessibility, biomass de-crystallization and biodegradability of organic matter, besides removing pathogens present in the biomass and reducing the production and accumulation of inhibitory or toxic compounds in the system (Abraham et al., 2020; Hashemi et al., 2021; Meegoda et al., 2018; Orlando and Borja, 2020). Furthermore, it promotes the reduction of hydraulic retention time, which directly impacts the working volume required for reactors, reducing both capital and operating costs (Ivanova et al., 2016).

Different sources of manure (e.g., cattle, swine and chicken) are often grouped as farm animal waste (Bona et al., 2018), which implies subjecting them to the same pretreatment and AD configuration. However, differences in the chemical composition of manure from different sources make them distinct, resulting in different responses to AD and pretreatment strategies. The animal diet plays a crucial role in the composition of manure, as herbivores' diets are high in fiber and carbohydrates, while omnivorous diets consist predominantly of lipids, proteins and fibers (Ariunbaatar et al., 2018).

Many studies based on the pretreatment efficiency of AD have been reported in the literature, in which the large part of results was obtained from the mixture of manure, disregarding the intrinsic specificities of each animal group (Bona et al., 2018; Orlando and Borja, 2020; Qiao et al., 2011).The lack of targeted knowledge on manure source has led to the application of inappropriate pretreatments in the AD process (Qiao et al., 2011; Raju et al., 2013).

Based on a meta-analysis approach, this paper aimed to understand the effects of different pretreatments applied to different types of manure and their contribution to the increase of methane yield and energy potential from manure, as well as the interest of producing countries in the treatment of this environmental polluting source.

2. Materials and methods

2.1.Literature search and study selection

A peer-reviewed literature search was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Fig. 1), on the Web of Science and Scopus databases, with original articles published in English between 1945 and 2020. The following keywords were combined with the aid of Boolean operators (AND, OR) and wildcards (*): “hydrolysis”, “anaerobic digestion”, “manure”, “dung”, “livestock”, “cow”, “cattle”, “pig”, “hog”, “chicken”, “poultry”, “hen”, “slurry”, “methane” and “pretreatment”. The search resulted in 582 peer-reviewed studies.

The eligibility criteria to include articles in the review were: having manure as the only substrate for AD; manure chemical characterization; application of pretreatment for biogas production; determination of the methane yield with and without pretreatment (control). After screening, 54 articles were selected for this review (Table S1).

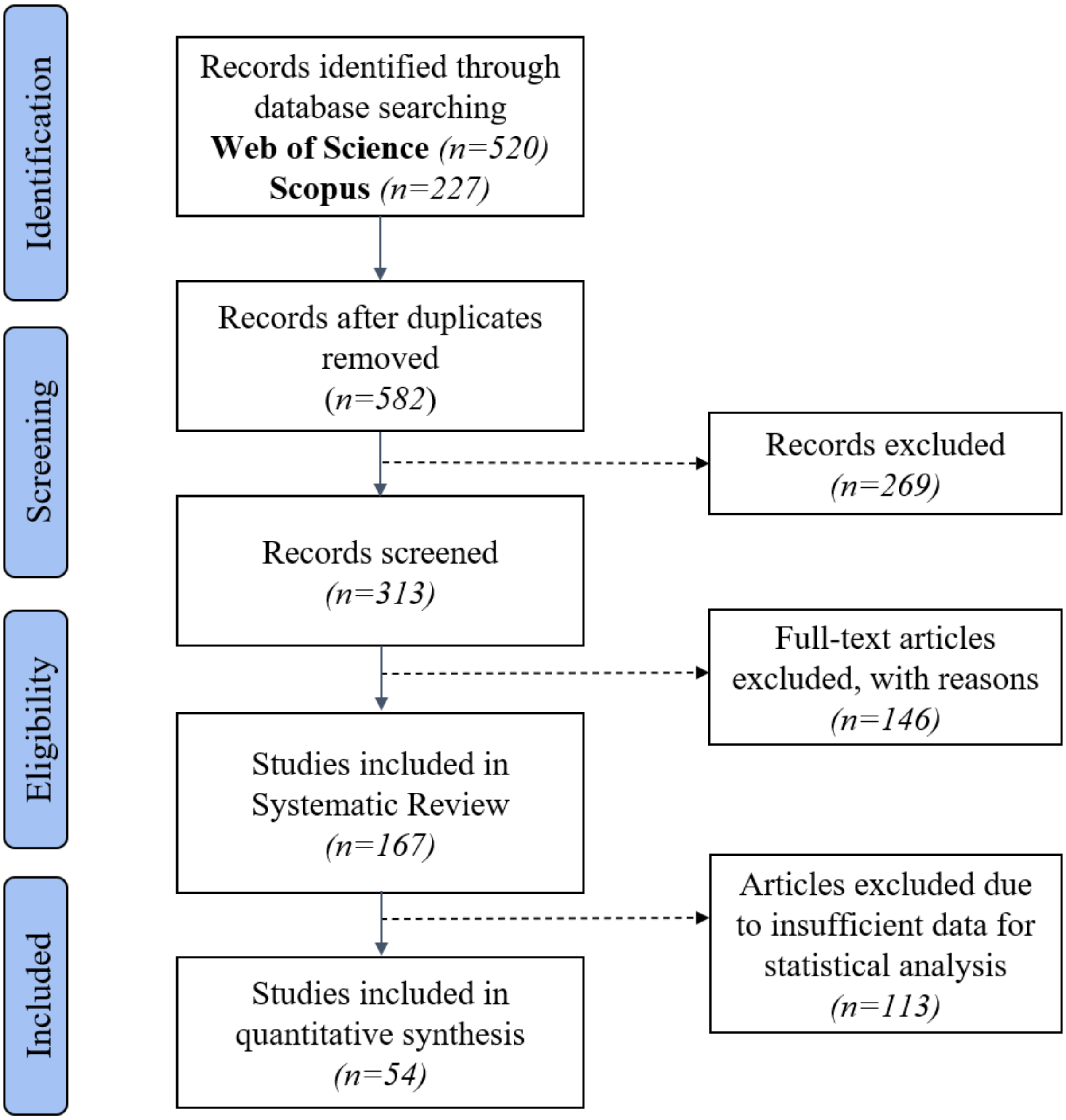

Fig. 1. PRISMA flow diagram showing the peer-reviewed literature search conducted in this study (adapted from http://www.prisma-statement.org/).

2.2.Pretreatment categories

The different pretreatments reported were classified into five categories, according to their characteristics (Table 1).

Table 1. Pretreatment categories of specific methods applied to improve the Anaerobic Digestion of manure.

Category	Methods	
Chemical (n=43)	Acid (n=2) Aerobic (n=2)	Chemical combinations (n=3) Iron-based (n=9)

	Alkaline (n=6) Aqueous ammonia soaking (n=8) Attapulgite (n=4)	Nano-bubble water (n=7) Trace Elements (n=2)
Biological (n=17)	Bioaugmentation (n=3) Biological Supplements (n=5)	Enzyme (n=4) Lignocellulolytic microbial consortium (n=5)
Physical (n=238)	Agitation (n=1) Autoclave (n=20) Electropolar (n=5) Hydrodynamic Cavitation (n=3) Hydrothermal (n=7) Mechanical (n=9)	Microwave (n=2) Pulsed Electric Field (n=3) Steam Explosion (n=8) Thermal (n=156) Ultrasound (n=24)
Chemical+Physical (n=88)	Acid+Steam Explosion (n=2) Alkaline+Autoclave (n=22) Alkaline+Microwave (n=1)	Alkaline+Steam Explosion (n=1) Alkaline+Thermal (n=61) Alkaline+Ultrasound (n=1)
Chemical+Biological (n=5)	Enzyme+Trace Elements (n=5)	

n= number of data extracted from the articles included in the meta-analysis.

2.3.Statistical analysis

Methane production was quantified using the natural log response ratio (RR) based on (Hedges et al., 1999), comparing the mean of the pretreatment ($\bar{X}_T$) with the control ($\bar{X}_C$) according to Equation 1:

$$RR = \ln(\bar{X}_T/\bar{X}_C) \qquad (1)$$

An unweighted meta-analysis was conducted in order to include the largest number of studies, even including the studies that did not report a measure of variance (Cao et al., 2019). Mean effect sizes were calculated in the R software using the "metafor" package and a confidence interval (CI) of 95% with bias correction. Pretreatments were considered significant ($p \leq 0.05$) when their CI and mean did not overlap the zero line. Mean and CI below the zero line indicate a negative response (pretreatment < control), while mean and CI above the zero line indicate a positive response (pretreatment > control).

2.4.Global Manure production and CH_4 potential

Data of the number of swine (breeding and beef), cattle (dairy and non-dairy) and chicken (broiler and layer) heads were collected from (FAOSTAT, 2018). Manure production and volatile solids (VS) content for each animal type was estimated according to Scarlat et al. (2018) (Equation 2; Table 2). The potential CH_4 production was estimated based on the average methane yield from untreated and pretreated manure, considering the pretreatments with the highest reported increase in this study and a lower calorific value of 9.97 kWh/m³ CH_4 (Ornelas-Ferreira et al., 2020) (Equation 3).

Table 2. Manure production and volatile solids content. Adapted from (Scarlat et al., 2018).

Animal type	Manure (kg/head.day)	Manure organic matter (%VS)
Cattle (dairy)	53	6.8
Cattle (non-dairy)	25	6.8
Chickens (broilers)	0.1	16
Chickens (layers)	0.2	16
Swine (breeding)	4.5	4.8
Swine (market)	4.5	4.8

$$MG = \frac{Heads \times SMG \times 365 \times \mathrm{OMManure}}{1000} \quad (2)$$

$$CH_4\ potential = \frac{MG \times \mathrm{CH4\ yield} \times 9.97}{1000000} \quad (3)$$

Where MG is manure generation (t VS/yr), SMG is the daily Specific Manure Generation (kg/head.day), 365 is the number of days in a year, OM_{Manure} is the organic matter content in manure (% VS) and 9.97 is the lower calorific value of CH_4. (kWh/m³ CH_4). Denominators were used for unit conversion.

3. Results and Discussion

3.1.Characterization of the different types of manure and their effect on CH_4 production

We evaluated three groups of manure: from animals with herbivore diets, omnivorous diets and a mixture of manure from both diet types. There was a similar contribution of animals with herbivore and omnivorous diets in the studies included in the meta-analysis (Fig. 2). Cattle manure prevailed in herbivore studies with 98%, against 2% for horse manure. The prevalence of cattle manure might be explained by the larger meat production and widespread distribution of cows in the world (Table S3). In manure from omnivorous animals, swine accounted for 71%, and chicken for 29% of the studies.

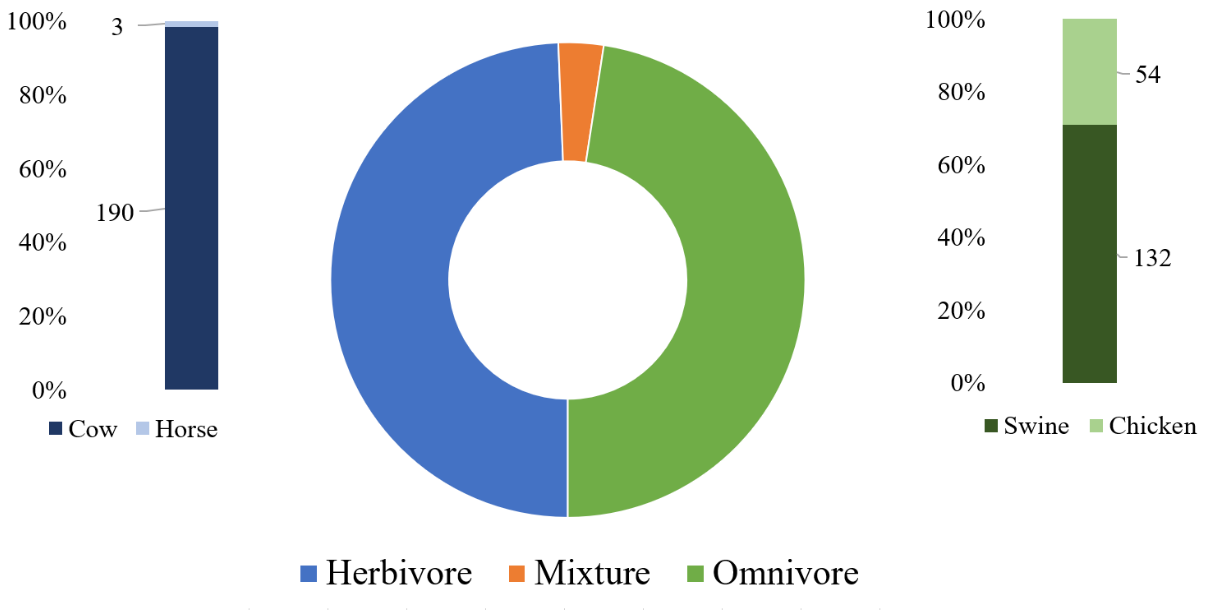

Fig. 2. Different sources of manure used in the AD studies evaluated in this meta-analysis.

Table 3 shows the chemical composition of the manure used in AD (swine, chicken, and cattle manure) reported in the literature. The lignocellulosic content in cattle manure is higher than in swine and chicken manure, mostly due to its lignin content, which reaches values on average two to four times higher than in manure from herbivores.

Lignin is a recalcitrant compound, due to its structure and chemical properties and is one of the main bottlenecks in the production of CH_4 from manure (Orlando and Borja, 2020). The lignocellulosic material in herbivorous manure is composed of polymeric structures of low degradability, what makes fermentation difficult, requiring pretreatments to weaken the intra and intermolecular hydrogen bonds (You et al., 2019). Some studies (Biswas et al., 2015; Millati et al., 2020; Zhao et al., 2018) have reported an increase in lignocellulosic material degradability after pretreatments, facilitating the enzymatic hydrolysis of these components into fermentable sugars.

Swine and chicken manure have similar compositions, with a protein content of 16.6 and 15.8% total solids (TS), respectively. Crude protein is usually included in animal food, being nitrogen (N) one of its main components; and while part of the N is used by the animals for growth, a large percentage is excreted in the urine and feces (Sajeev et al., 2018). The degradation of these nitrogenous compounds raises ammonium concentration (Yenigün and Demirel, 2013), which could explain the high content of Total Ammonia Nitrogen (TAN) in swine and chicken manure: an average of 6.3gN/kg for swine and 10.8gN/kg for chicken, ten times higher N content than N concentration in cattle manure (1.3gN/kg). High TAN content inhibits AD due to its cytotoxic effects, such as excessive energy waste and the decrease of metabolic and growth rates (Müller et al., 2006; Wang et al., 2016). Pretreatment is rated as an excellent strategy to overcome this loss, recovering the system's balance and increasing the performance of AD and biogas production.

Lipid content in manure from omnivorous animals has a higher lipid content than cattle manure (2.9%TS). Lipids are basically composed of long chain fatty acids and triglycerides, which are readily degraded into free fatty acids and glycerol and further degraded into carbon dioxide, alcohols, hydrogen gas and others (Rasit et al., 2015). Although lipids are highly biodegradable and their theoretical methane potential is higher compared to carbohydrates and proteins (Li et al., 2018; Rasit et al., 2015) high lipid concentrations can lead to the excessive formation of intermediate products during AD, such as long-chain fatty acids and volatile fatty acids, inhibiting several microbial pathways, leading to a reduction in biogas production (Sánchez-Bayo et al., 2020).

Table 3. Chemical characterization of different manure sources. Percentages are presented as average (minimum and maximum range) [a].

Substrate	Lignin (%TS)	Hemicellulose (%TS)	Cellulose (%TS)	Protein (%TS)	Lipid (%TS)	TAN (g/kg)
Swine manure	4.5 (1.8 – 8.4)	18.7 (14.6 – 21.7)	15.2 (6.6 – 23.6)	16.6 (14.8 – 17.9)	5.2 (2.8 – 8.8)	6.3 (3.1 – 12.5)
Chicken manure	3.3 (1.7 – 5.1)	14.7 (4.4 – 24.3)	16.4 (4 – 24.3)	15.8 (15.4 – 16.3)	6.4 (5.1 – 8.8)	10.8 (2.8 – 19.8)
Cattle manure	15.8 (10.2 – 25.1)	18.9 (14 – 30.4)	20.1 (15.3 – 29.3)	12.3 (11.1 – 13.8)	2.9 (1.9 – 4.4)	1.3 (0.9 – 1.7)

[a]References used were reported in Table S4.

3.2.Pretreatment as a strategy for overcoming AD bottlenecks

Five pretreatment categories were identified in this review. Physical pretreatments were the focus of 61% of the case studies, followed by 23% of combined chemical+physical pretreatments (Fig. 3A). Heat application (i.e., autoclave, hydrothermal, steam explosion and thermal) was the main strategy, present in more than 80% of the physical pretreatments (Fig. 3B), and in almost 99% of the chemical+physical combinations (Fig. 3E). This predominance can be explained by the need for thermal hygienization of animal products before AD to a safe use. According to Liu et al. (2021), the EU requires the pasteurization of animal products before AD, to reduce the risk of contamination by pathogens such as *Enterococcus faecalis* or *Salmonella senftenberg*, that are harmful to human health.

Chemical pretreatment methods Iron-based (21%) and Attapulgite (9%) additions have been explored in the attempt to improve AD (Fig. 3C). These additions play a key role as mineral sources for enzymatic activity, especially during methanogenesis (Liang et al., 2020). Additionally, they reduce the generation of toxic compounds and improve the AD process, favoring the production of biogas through a balanced system (Ugwu et al., 2020).

Aqueous ammonia soaking is a common chemical pretreatment (18%, Fig. 3C) as it promotes the degradation of the lignocellulosic components, exposing the substrate to the hydrolysis step. Furthermore, the process is conducted with low energy consumption and easy ammonia recovery, making it a good candidate for a large scale pretreatment (Lymperatou et al., 2020). The use of alkaline (14%) or acid compounds (5%) and their combination (7%) were also tested for lignin degradation (Fig. 3C). The alkaline pretreatments were more successful on substrates with lower lignin content, while the acid pretreatments and combinations performed better on high lignin content substrates (Kundu et al., 2021). However, acid additives can be extremely abrasive and toxic depending on their concentration and therefore, the inadequate configuration of these methods can lead to serious chemical imbalances and decrease in the AD system.

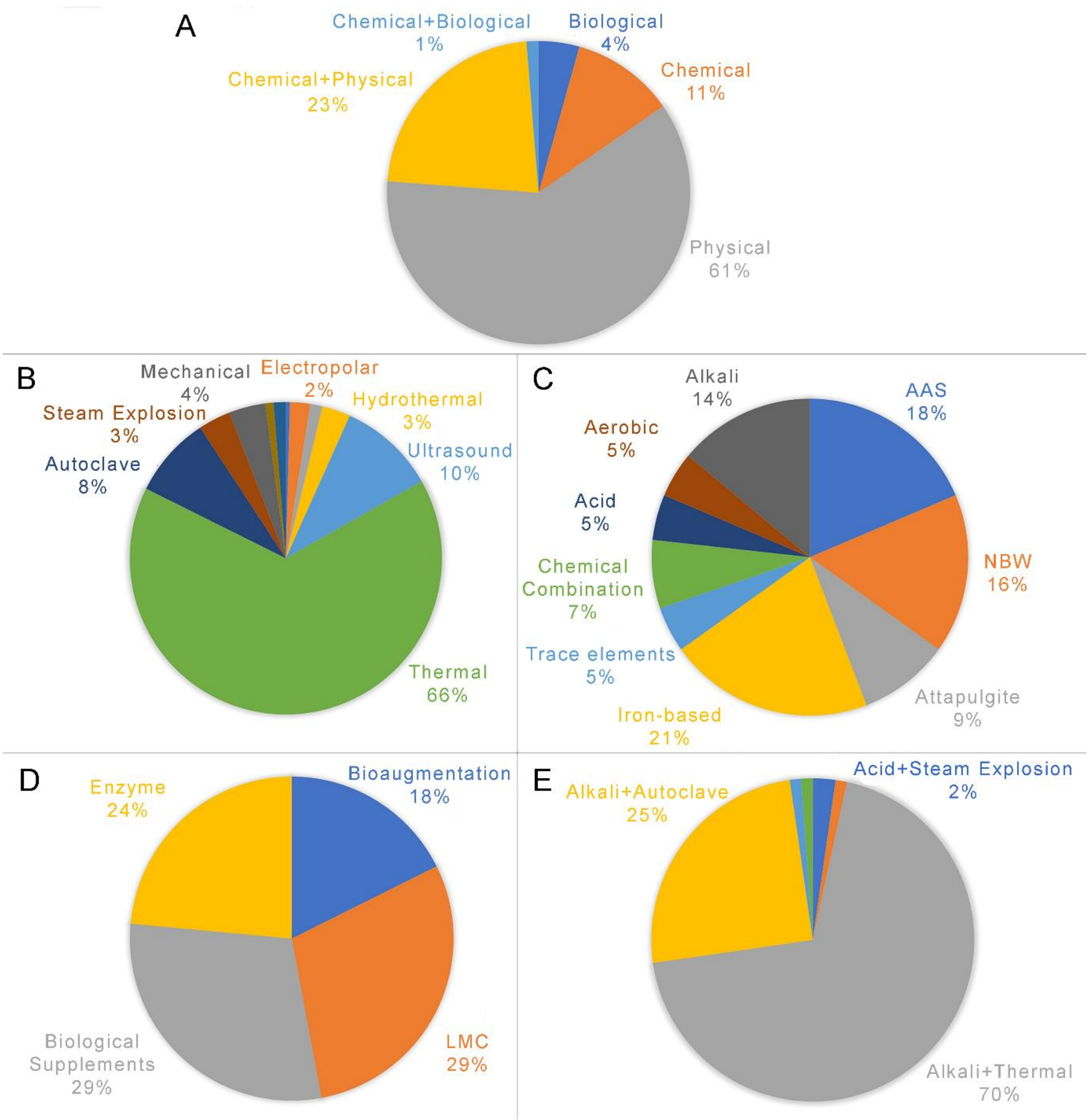

Fig. 3. Distribution (%) of pretreatment technologies applied to animal manure to increase biogas production. A: General pretreatments categories; B: Physical; C: Chemical; D: Biological; E: Chemical+Physical. LMC = Lignocellulolytic microbial consortium; NBW = Nano-bubble water; AAS = Aqueous ammonia soaking.

Biological pretreatments are the least applied to animal manure (4%) (Fig. 3A). Microbial consortium, fungus and enzymes are highly efficient and widely used to degrade lignocellulosic feedstock such as cattle manure, but their use in swine and chicken manure apparently has been poorly explored (Orlando and Borja, 2020). Almost 50% of the reported biological methods (bioaugmentation and LMC, Fig. 3D) consist on the addition of a complex mixture of microorganisms intended to repeat the biodegradation that occurs in natural environments, arising from the symbiotic relationship between microorganisms and the digestive system of ruminants (Dollhofer et al., 2015). However, this system does not support the physicochemical requirements for the degradation of non-ruminant manure (e.g., swine and chicken manure) due to differences in chemical composition.

Microorganisms are highly capable of converting high molecular weight compounds by breaking recalcitrant polymeric structures into smaller compounds, enabling the fermentation process (Ferdeş et al., 2020). Although the application of microorganisms as a pretreatment is highly efficient, it has drawbacks such as incubation time, which requires a time interval from weeks to months to reach the complete degradation of the biomass, making an industrial-scale application unfeasible (Kainthola et al., 2021). Even so, the non-addition of chemicals, low operating cost, prevention of the growth of unfavorable microorganisms and relatively simple equipment requirements make these pretreatments a promising option that should be further studied and developed (Tabatabaei et al., 2020).

The combination of pretreatments shows greater efficiency in breaking down highly recalcitrant substrates (Khan and Ahring, 2020), as the degradable content availability for hydrolysis increases. The combination of chemical and biological pretreatments has been explored in few studies (Fig. 3A). Trace elements have been applied to supplement enzyme activity, improving AD while reducing energy and costs (Mao et al., 2015). However, some studies reported that the use of trace elements as the only pretreatment resulted in a reduction of biogas production, compared to their addition associated with specific enzymes (Bhatnagar et al., 2020).

The simultaneous use of alkaline and thermal pretreatments is the most frequent combination applied to animal manure, with the most common methods being alkaline+thermal and alkaline+autoclave, in 70% and 25% of the studies, respectively (Fig. 3E). According to Khan and Ahring (2020), thermal pretreatments affect the biodegradability of cellulose, hemicellulose, and lignin fractions, while the addition of an alkaline pretreatment improves the degradation of lignin.

3.3. Impacts of animal diet on pretreatment efficiency

The composition of manure varies according to the animal's diet. Lignocellulosic content is higher in cattle manure, while protein is higher in swine and chicken manure (Table 3). Our results showed that the pretreatment efficiency on methane yield from animal manure is significantly depending on eating habits, indicating that a higher methane yield can be achieved using the appropriate pretreatment (Fig. 4). The application of the inappropriate pretreatment may lead to a decrease in biogas production (Fig. 4B).

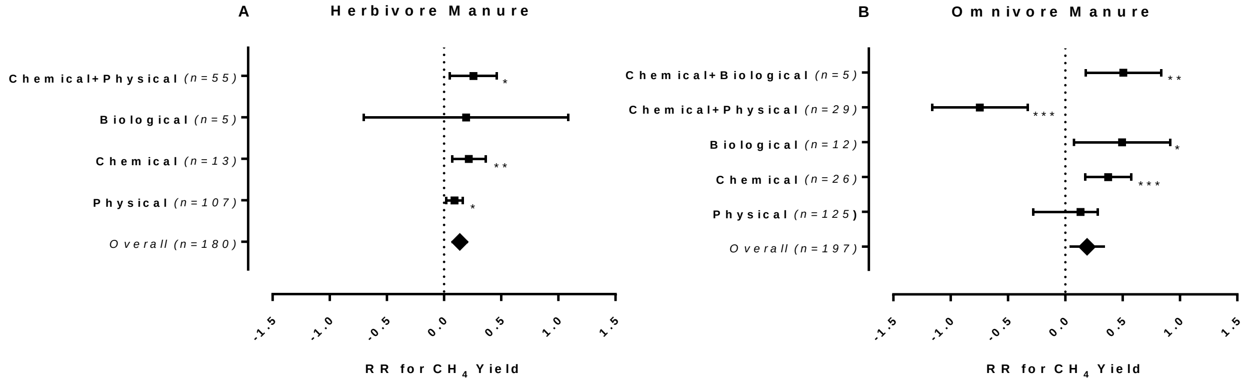

Fig. 4. Natural log response ratio (RR) of methane yield with different pretreatments for A: herbivore manure (cow and horse) and B: omnivore manure (chicken and swine) with 95% confidence intervals. Significance code: (***) p = 0.001; (**) p = 0.01; (*) p = 0.05 and ( ) p = 0.1. n = number of effect sizes per pretreatment type.

Herbivore manure had a positive response to chemical (RR= 0.217, 95% CI: 0.071 to 0.364), physical (RR= 0.092, 95% CI: 0.021 to 0.163) and chemical+physical pretreatments (RR= 0.258, 95% CI: 0.051 to 0.464), corresponding to a methane yield increase of 124%, 110% and 129%, respectively (Fig. 4A). The significant pretreatments ($p \leq 0.05$) coincide with those reported in literature as the most effective for crop residues (Hashemi et al., 2021; Khan and Ahring, 2021). Biological pretreatment had a marginal negative effect on methane yield; however, this result needs to be interpreted with caution since the number of observations is smaller compared to other pretreatments (Fig. 4A), suggesting a greater need for studies to determine its performance more accurately. Chemical and/or physical pretreatments are more effective if applied to substrates with higher lignocellulosic content, due to their physical (e.g., specific cellulose surface area, cellulose crystallinity, degree of polymerization, pore size and volume) and chemical constraints (e.g., lignin, hemicellulose and acetyl groups content and composition) (Zoghlami and Paës, 2019).

In contrast, for omnivore manure, the chemical+physical pretreatment (RR= -0.745, 95% CI: -1.161 to -0.329) had a negative effect and decreased its methane yield when compared to untreated manure (Fig. 4B). Although the thermochemical pretreatment of swine manure is successful in the solubilization of lignocellulosic compounds during AD, high levels of solubilization can be toxic and, inhibitory to the system (Carrère et al., 2009). Physical pretreatments (RR= 0.133, 95% CI: -0.282 to 0.282; Fig. 4B) also showed a low performance when applied to omnivore manure. The continuous application of a physical pretreatment, even with negative results for biogas production, could be due to the mandatory sanitization of animal products, usually carried out at high temperatures. However, chemical additives such as calcium oxide (CaO), calcium hydroxide ($Ca(OH)_2$) and hydrogen peroxide (H_2O_2) have shown promise in eliminating pathogens (Manyi-Loh et al., 2016), and could replace some physical pretreatments. Furthermore, the use of a chemical pretreatment for omnivorous manure shows the largest significate increase in methane yield of up to 145% (RR= 0.374, 95% CI: 0.175 to 0.574; Fig. 4B) compared to all other pretreatments.

Biological pretreatments (RR= 0.496, 95% CI: 0.076 to 0.916) have effective results in increasing organic matter degradation, improving the chemical balance of the AD system (Wei et al., 2015) and contributed to the lignin degradation (Hashemi et al., 2021) increasing the CH_4 yield in up to 164% (Fig. 4B). However, the management of biological components requires constant monitoring of the environment to keep microbial populations controlled and balanced to carry out their activity. The inadequacy of these parameters can affect the AD performance, causing greater fluctuation in biogas yield when compared to chemical pretreatments (Fig. 4).

As expected, the combination of the pretreatments with the highest performance provided the best results, whether in the combination of chemical and physical pretreatments for herbivorous manure (Fig. 4A), or biological and chemical pretreatments for omnivorous manure (Fig. 4B). This indicates that the response to the pretreatment is strongly associated with manure composition, even when in different configurations. Additionally, chemical pretreatment ensured an increase in biogas production in all manure sources.

3.3.1. Best pretreatment responses for herbivore manure

Pretreatments involving heat application showed a high performance, reaching an improvement of up to 110% in methane yield when compared to untreated manure (Table 4). Optimum results were obtained at manure exposure to temperature ranged from 135°C to 170°C, for 30 to 60 minutes. High temperatures usually promote cell lysis, and consequently expose the intracellular content to degradation by microorganisms, enabling and accelerating the biogas production (Cano et al., 2014).

Table 4. Performance of the most efficient pretreatments for herbivore manure and maximum increase in CH_4 production under specific pretreatment configuration.

Pretreatment	**Average CH_4 Yield[a]**			**Maximum reported increase in methane production**	
	$\bar{X}$ **Control**	$\bar{X}$ **Treated**	$\bar{X}$ **Increase (%)**	**Specific pretreatment configuration [b]**	**Maximum increase in CH_4 yield (%)**
Physical					
Autoclave	66,7	100,1	50 $_{(n=2)}$	135 °C for 1 h [54]	53

Electropolar	60,3	103,8	72 (n=5)	Cathode electrode at 250 micro-voltages (mV) [37]	120
Hydrodynamic Cavitation	295,5	301,2	2 (n=3)	Inlet pressure of 7 bar for 60 min [18]	3
Hydrothermal	196,8	210,5	7 (n=2)	170 °C for 1 h [45]	14
Mechanical	128,1	139,8	9 (n=9)	Sandpaper against Sandpaper [33]	45
Steam Explosion	317	408	29 (n=1)	170 °C and 30 min [39]	29
Thermal	207,5	236,4	14 (n=83)	160 °C 60 min [17]	110
Ultrasonic	156,4	175	12 (n=2)	Ultrasound with 6000 kJ/kg TS [44]	19
Chemical					
Acid	111,8	140	25 (n=2)	1% H_2SO_4 at a pH 6 for 3 days [50]	128
Alkaline	146,4	200,2	37 (n=2)	8% NaOH for 0.5h [21]	50
Chemical combinations	191,3	241,6	26 (n=3)	7% NaOH + 2% Polyethylene glycol for 12h [21]	33
Iron-based	141	186,6	32 (n=6)	1000g/L of Waste Iron Powder [10]	57
Chemical+Physical					
Thermal+Alkaline	165,7	191,3	15 (n=49)	6% NaOH + 55 °C for 24 h [32]	314
Alkaline-Ultrasonic	102,8	122,44	19 (n=1)	8% KOH at 25 °C for 24 h + 20 kHz and power density of 2 W mL^{-1} for 20 min. [6]	19
Alkaline+Autoclave	66,7	147,7	121 (n=6)	3% NaOH + 100 °C for 6 h [54]	180

[a] mL/gVS or mL/gCOD.
[b] Reference number of the article in Table S1 shown in square brackets.
n= number of data samples reported.

Among physical pretreatments, electropolar had the best performance, with a 120% increase in methane yield (Table 4). This procedure changes and controls the microbial activity, while increasing pore size, leading to ruptures in the lignocellulosic substrate (Qu et al., 2014), and making it an efficient process for high fiber manure. Conversely, the efficiency of hydrodynamic cavitation was very low (Table 4), besides having a high energy cost due to the pressure demanded to collapse the substrate (Carpenter et al., 2017), thus indicating the unfeasibility of this pretreatment. Similarly, the ultrasonic pretreatment deals with pressures that change the physicochemical characteristics of the substrate, increasing digestibility (Kisielewska et al., 2020), but the methane yield only increased 19% (Table 4). Therefore, hydrodynamic cavitation and ultrasonic pretreatments should not be applied to manure.

Some physical pretreatments are based on mechanical principles, reducing particle size and increasing the contact area for enzymatic attack, leading to an increase of up to 45% in methane yield (Table 4). However, it is not as efficient as other physical pretreatments – such as thermal – due to its lower efficiency in changing the physical structure of organic matter. Furthermore, it is a procedure with a high energy demand (Hashemi et al., 2021).

Among the Chemical pretreatments, acid application acts disrupting van der Waals forces, hydrogen bonds and covalent bonds in lignocellulosic biomass without unbalancing the system (Amin et al., 2017). Although the addition of an optimized amount of acid improves methane yield in up to 128%, too high or too low concentrations can be toxic or ineffective, respectively, decreasing biogas production. A 57% methane yield increased were obtained for iron-based methods due to its efficiency as a nutrient supplement to AD, improving methanogenic activity (Farghali et al., 2020).

Alkaline pretreatments increased methane yield in up to 50% (table 4). A decrease in lignocellulosic biomass was reported at alkaline pretreatment, in response to the action of the hydroxyl ion (OH^-), promoting cellulose swelling and breaking the hydrogen bonds between cellulose and hemicellulose (Khan and Ahring, 2020).

Physical and chemical pretreatments are often combined in order to achieve the highest degradation rates of manure recalcitrant compounds. The alkaline method is considered the most promising in improving the enzymatic hydrolysis of agricultural waste (Salehian and Karimi, 2013), and its combination with a thermal method proved to be the most successful pretreatment, with a methane yield increase of up to 314% (Table 4).

3.3.2. Best pretreatment responses for omnivore manure

The largest increases in the methane yield for omnivore manure were observed after chemical pretreatments (Fig. 4; Table 5). The Nano-bubble water (NBW) was the method with highest increased methane yield (973%) when compared to untreated manure. Water plays a crucial role in the hydrolysis of organic matter, providing the components used by microorganisms in energy conversion (Fan et al., 2020).

Table 5. Performance of the most appropriate pretreatments for omnivore manure and maximum increase in CH_4 production under specific pretreatment configuration.

Pretreatment	**Average CH_4 Yield[a]**			**Maximum reported increase in methane production**	
	$\bar{X}$ **Control**	$\bar{X}$ **Treated**	$\bar{X}$ **Increase (%)**	**Specific pretreatment configuration [b]**	**Maximum increase in CH_4 yield (%)**
Chemical					
Aerobic	257,9	339,4	32 $_{(n=2)}$	Aerated 10 times a day for 15min at 1 L/min [7]	33
Ammonia	122,3	156,3	28 $_{(n=8)}$	Ammonia Stripping: Heated at 80 °C and aerated for 24 h	121

				[33]	
Attapulgite	153,4	187,2	22 (n=4)	10 g/L of attapulgite [2]	37
Iron-based	264,9	324,8	23 (n=3)	20 g/L Zero-Valence Iron + 10 g/L Fe_3O_4 [23]	26
Nano-bubble Water (NBW)	124,9	188,9	51 (n=7)	CO_2 was introduced into deionized water through NBW generator for 20 min at 20 °C [3]	973
Trace Elements	240,7	318,4	32 (n=2)	1 mL of Selenium Stock Solutions [12]	38
Biological					
Lignocellulose Microbial Consortium (LMC)	115	163	42 (n=5)	LMC/Swine manure ratio 3:1 [42]	61
Biological Supplements (BS)	270,3	312	15 (n=5)	BS includes *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus thuringiensis*, cellulase, corn, wheat bran and Yucca extract for 14 days [7]	34
Enzyme	288,3	415,9	44 (n=2)	8mL of fungal enzyme cocktail, commercially known as-Digest P3 [12]	91
Chemical+Biological					
Enzyme+Trace Elements	240,7	396,5	65 (n=5)	8 mL of fungal enzyme cocktail + 1 mL of trace elements [12]	72

[a] mL/gVS or mL/gCOD.
[b] Reference number of the article in Table S1 shown in square brackets.
n= number of data samples reported.

The use of ammonia as a pretreatment on manure with high fiber content promoted an increase in CH_4 yield in more than 120%. This pretreatment requires low energy input and is considered to be non-polluting and non-corrosive (Jurado et al., 2016; Lymperatou et al., 2017), suggesting the cost-effectiveness of this pretreatment.

Attapulgite, trace elements and iron-based additives act as a supplementary mineral additive that enables effective enzymatic action and, consequently, high methane yields (Liang et al., 2020). These methods showed a positive response compared to untreated manure, resulting in an increase in methane yield of 37, 38 and 26%, respectively.

Among the biological pretreatments, the lignocellulolytic microbial consortium (LMC) and the enzymatic methods had the best results, with a CH4 yield increase of up to 61 and 91%. LMC aims to replicate the natural activity of lignocellulose biodegradation, breaking the physical-chemical barriers by the action of selected microorganisms. Similarly, the enzymatic method reproduces the activity that occurs in natural environments, optimized by the possibility of selecting specific enzymes to degrade the organic content (Liu et al., 2016). The use of enzymes as a pretreatment is reported as effective, environmentally friendly and less costly (Baruah et al., 2018).

The combination of enzyme and trace elements was the only combination of chemical and biological pretreatments with an increased CH_4 yield in up to 72% (Table 5). The synergistic action between the two methods may have made it more powerful compared to other combinations, since trace elements play a key role in supplementing enzymatic activity.

3.4.Implications of the application of the adequate pretreatment

India, Brazil, China and USA produce almost 40% of all global manure (Fig. S1). The potential increase in energy production if manure was properly pretreated was estimated to increase twice for India, Brazil and USA, and ca. 72% in China due to the smaller amount of cattle manure produced (Table 6). Considering the current global manure production, an increase of 81% in methane production, corresponding to 2.7 million GWh/yr, could be achieved in case the appropriate pretreatment was applied to all produced manure (Table S3).

Table 6. Generation of waste and energy potential from manure pretreated by the largest producing countries.

Country	Type of manure	Manure generation (1000 t VS/yr)	CH_4 production[a]	
			Without pretreatment (GWh CH_4/yr)	With adequate pretreatment (GWh CH_4/yr)
India	Cattle	151,417	100,692	222,972
	Chicken	6,517	15,640	25,764
	Swine	669	1,605	2,645
	All	*158,604*	*117,938*	*251,381*
Brazil	Cattle	143,756	95,598	211,691
	Chicken	10,804	25,927	42,709
	Swine	3,267	7,841	12,916
	All	*157,828*	*129,366*	*267,317*
China	Cattle	43,225	28,745	63,652
	Chicken	48,647	116,743	192,307
	Swine	35,219	84,518	139,225
	All	*127,092*	*230,006*	*395,185*
USA	Cattle	65,067	43,269	95,815
	Chicken	13,794	33,103	54,529

	Swine	5,878	14,105	23,235
	All	*84,738*	*90,477*	*173,579*
Total	**-**	**528,261**	**567,787**	**1,087,462**

[a] Calculated with CH4 yields before and after the pretreatment with the highest increase of methane production for herbivore manure and omnivorous manure.

This extra amount of methane could potentially replace a large amount of fossil fuel, contributing to the transition to a more sustainable society from all these countries. For example, methane produced from pretreated manure in India could generate 251,381 GWh/yr (Table 6), enough to supply 42% of energy consumption from natural gas in the country (BP, 2021) – much more than the 20% that could be supplied with untreated manure (Fig. 5). In Brazil, the appropriate pretreatment application to all produced manure would generate enough CH_4 to offset 83% of the national consumption of natural gas. Without the pretreatment process, not even half of the demand would be supplied (Fig. 5).

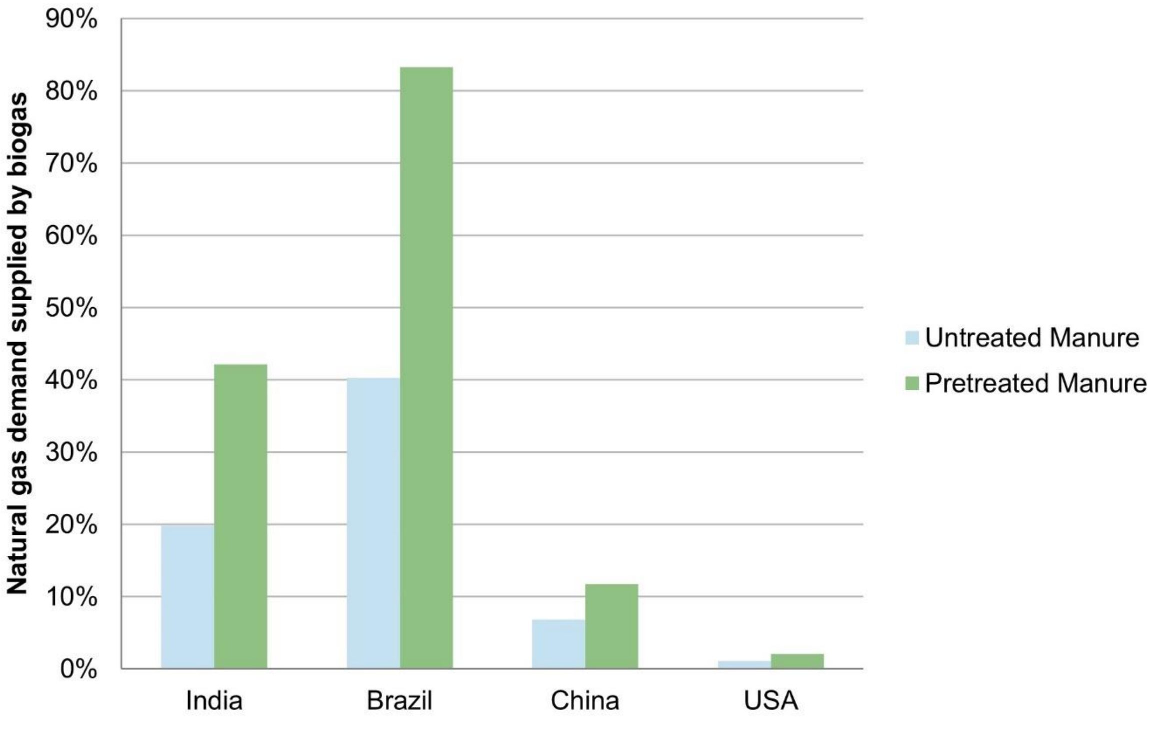

Fig. 5. Potential contribution to supply natural gas demand per year by biogas from untreated and pretreated manure.

It should be highlighted that the high installation costs for biogas facilities are among the main barriers for the implementation of anaerobic digesters (Nevzorova and Kutcherov, 2019) and are a fairly common complaint among farmers (Herrero et al., 2018). Thus, increasing the efficiency of AD is extremely important to make the process economically appealing. Although the adoption of a pretreatment method makes investment and operational costs rise, the increase in methane production may overcome these costs, making the overall process economically feasible (Cano et al., 2014; Shafiei et al., 2013; Zeynali et al., 2017).

It should be noted, however, that the application of incorrect pretreatment can potentially result in a decrease in methane production (González-Fernández et al., 2008; Ortega-Martinez et al., 2016) (Fig. 4), leading to less economic bonuses and jeopardizing the choice for biogas production from manure.

4. Conclusions

There is an acute disparity between the distribution of animal manure and the amount of studies on manure pretreatments worldwide, with less research coming from developing and underdeveloped countries – even though they are often great manure producers. This shows the importance of the implementation of public policies that encourage and enforce sustainable manure management and biogas use for the development of a greener economy, especially in countries with a high manure production.

Our results also show that not only the type of pretreatment (e.g., physical, chemical, biological) strongly influences methane production, but also that the animal diet is relevant for the maximum methane yield after manure pretreatment. Physical and chemical pretreatments are more efficient for manure from animals with herbivorous diets increasing the CH_4 yield in 110% and 124%, respectively, while the combination of these methods have the largest effect on CH_4 yield of 129%. On the other hand, chemical and biological pretreatments are more efficient for manure from omnivorous animals, reaching an increase of 145% and 164 % in CH_4 yield, respectively, and their combination promotes an increase of 166% in CH_4 yield. Choosing the appropriate pretreatment increases economic profit, reducing operating costs and potential risks to the environment, while increasing the potential energy generated from this waste source.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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4.3 Artigo III

Anacleto TM, Kozlowsky-Suzuki B, Wilson AE, Enrich-Prast A. Comprehensive Meta-Analysis of Pathways to Increase Biogas Production in the Textile Industry. Energies. 2022; 15(15):5574. https://doi.org/10.3390/en15155574

Comprehensive meta-analysis of pathways to increase the biogas production in textile industry

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Abstract: The textile industry is one of the most environmental polluters in the world. Although waste management via Anaerobic Digestion (AD) is a sustainable strategy to transform waste into clean energy and water recovery, the efficiency of the AD process is reduced by the presence of recalcitrant materials, chemicals, and toxic contents. This study aims to investigate the performance of several chemical, physical, and biological pretreatments applied to improve the biodegradability of textile waste. We performed a meta-analysis with 117 data extracted from 13 published articles that evaluated the efficiency of pretreatments prior to AD applied to textile waste to increase biogas production measured as methane (CH_4) yield. Even though the majority of the studies focused on the effect of chemical and physical pretreatments, our results showed that the application of biological pretreatments are more efficient and eco-friendlier. Biological pretreatments can increase CH_4 yield by up to 360%, with lower environmental risk and lower operating costs, while producing clean energy and a cleaner waste stream. The biological pretreatments also avoid the addition of chemicals and favor the reuse of textile wastewater, decreasing the current demand for clean water increasing the resource circularity in the textile industry.

Keywords: textile residues; biotechnology; methane; circular economy; fibers; cotton

1. Introduction

The textile industry is one of the most polluting sectors worldwide with an estimated waste production of 92 million tons per year [1] including pre- (i.e. agricultural production, fiber production, wastewater, solid waste) to post-consumer (i.e. manufacturing, logistics, retail and mixtures of discarded clothing or household items) from the supply chain [2]. Over 8000 chemicals (e.g. dyes, suspended solids, chlorinated aromatic hydrocarbons, surfactants, and heavy metals) are used in the textile supply chain [3,4]. As a result, effluents and solid waste with high loads of hazardous chemicals are discharged, thus increasing the toxicity of the produced waste, with a high pollution risk to the environment and human health [5].

Sustainable manufacturing is crucial to reduce the environmental impact of fashion and the textile industry. Projects and policies aiming at the sustainable development of the market, such as the [6], “Strategic Agenda on Textile Waste Management and Recycling”, Expert Network on Textile Recycling (ENTeR), Conference of the Parties (COP 21) as well as the 2030 Agenda for Sustainable Development Goals (SDGs), have been important players in reframing textile production. Incentive actions for reuse are also crucial to the implementation of the circular economy model as established by the European Union into this sector [7].

Approaches to minimize textile waste and increase its life span, such as clothing rental and repairing, second-hand market and reprocessing operations for the production of original or new products [7], are alternatives to manage the polluting potential. However, the advance of fast fashion follows a business model that produces large quantities of clothing and trends at low prices [1], and often of low quality. The low quality of textiles makes recycling unfeasible, restricting their use to the end of the waste management hierarchy, the energy recovery, as determined in the in the Directive 2008/98/EC (European Parliament, Council, 2008).

Anaerobic digestion (AD) is a widely applied biotechnology that has proven its effectiveness as a green solution for waste management, reducing the risk of contamination while producing energy as biogas. AD implementation in the textile industry can contribute to the waste use as a resource for the generation of clean energy and water reuse supply. However, the wide range of chemicals, organic pollutants and recalcitrant compounds of textile waste poses challenges, reducing the efficiency of degradation if AD is applied as the only strategy [9].

Several studies have suggested that the application of pretreatments is advantageous not only to improve organic matter degradability but also to increase the biogas yield [9–12], and further remove dyes and toxic compounds from wastewater and solid waste from aqueous solutions [4,9]. Pretreatments can be chemical, physical, biological or combinations of these, and their performance is influenced by the chemical composition of the waste [13]. Physical pretreatments (i.e., thermal, mechanical, irradiation, ultrasound) act disrupting cells through physical force [14]. As a result, the contact surface of the organic matter is increased by reducing the particle size, facilitating microbial attack [15]. Although physical pretreatments are advantageous as no toxic compounds are generated, some techniques (e.g. thermal) can increase energy costs becoming unfeasible at a large-scale [15].

Chemical pretreatments (i.e., acid, alkali, organic solvent) act breaking chemical bonds from complex structures causing an internal increase in the surface by swelling of the cell [16]. Chemical techniques are highly efficient in degrading complex materials and are more often applied than biological and physical pretreatments [16]. However, they require extra care since depending on the chemical reagents applied toxic compounds can be formed. Some chemicals can further damage operational equipment by corrosion of digesters [17,18].

Biological pretreatments (i.e., fungi, bacteria, microbial consortia, enzymes) act in synergy with the microbial metabolism promoting the acceleration of the degradation of organic matter [15]. Avoiding the addition and generation of harmful chemicals promotes an environmentally healthier AD system. In addition, the lower capital and energy costs compared to physical and chemical pretreatments turn the application of biological techniques extremely attractive even at full-scale[18,19].

In this context, we performed a systematic review followed by a meta-analysis of the available data in the literature to evaluate the effect of pretreatments applied to the textile industry waste. The aim of this study was to assess the efficiency of several pretreatments applied to textile waste prior to AD. Based on the peer-reviewed literature, we compared several physical, chemical, biological and combinations of pretreatments to identify the best choice in terms of the highest organic waste reduction through biogas production and with the highest cost-benefit ratio.

2. Materials and Methods

The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) was used as a guide to conduct the execution of the sampling design, determining the search strategy, keywords, and exclusion/inclusion criteria for evaluating the quality of articles [20]. A systematic review followed by a meta-analysis was carried out using the Web of Science and Scopus database and Google Scholar search engine. We filtered our search using the keywords combined with the aid of Boolean operators (AND) and wildcards (*, $): *anaerobic digestion, textil* and *pretreatment*, from 1945 to 2021, English language, and article as the type of document. The eligibility criteria to include articles in the meta-analysis were: i) Textile waste as a unique substrate for AD; ii) Application of pretreatment for biogas production; iii) Measurement of methane yield from the organic fraction; iv) Experiments with specific description and measurements for control and treatment samples.

Based on Hedges et al. [21], methane production data were quantified using the natural log response ratio (RR). Thus, the mean of the pretreatment performance ($\overline{X}_{T}$) was compared to the mean of the control ($\overline{X}_{C}$). Following the equation:

$$RR = \ln(\overline{X}_{T}/\overline{X}_{C}) \quad (1)$$

An unweighted meta-analysis was conducted to include the largest number of studies, even those that did not report a measure of variance for the response variable [22]. Mean effect sizes and 95% confidence intervals (CI) with bias correction were conducted using R software (R Core Team, 2021) using the "metafor" package (version 3.0-2). Pretreatments were considered significant ($p < 0.05$) when their mean effect and CI did not overlap the zero line. Mean and upper CI below the zero line indicates a negative response (i.e., treatment < control, or treatment less efficient than control) while mean and lower CI above the zero line represent a positive response (i.e., treatment > control, or treatment more efficient than control).

We assessed the potential production of CH_4 that could be generated from cotton and polyester waste if the most appropriate pretreatment is applied. Cotton and polyester waste data from the textile industry were collected, respectively, from [23,24]. The volatile solids (VS) and total solids (TS) content for each textile waste was based on data reported in the literature [25,26] (Equation 2, 3; Table S1). The potential production of CH_4 was estimated based on the average methane yield of untreated and pretreated cotton and polyester, considering the pretreatments with the highest increase reported in this study (Table S2) and a lower calorific value of 9.97 kWh/m³ CH_4 [27] (Equation 4).

$$GCW = (CW \times TS\%) \times OM_{cotton} \quad (2)$$

$$GPW = (PW \times TS\%) \times OM_{polyester} \quad (3)$$

$$CH_4 \text{ potential} = GPW \times CH_4 yield \times 9.97 \quad (4)$$

Where GCW is global cotton waste (million t VS/y), CW is the cotton waste (million t/ y), GPW is global polyester waste (million t VS/y), PW is the polyester waste (million t/ y), OM is the organic matter content in the waste (e.g., cotton, polyester) (VS%) and 9.97 is the lower calorific value of CH_4 (kWh/m³ CH_4).

3. Results and Discussion

A total of 117 data were extracted from 13 articles (Table S3) that applied pretreatments to improve the AD of textile waste (Figure S1). The solid fraction of textile waste prevailed (81%) over the liquid fraction, as the type of waste that undergoes pretreatment prior to AD. Wool and pure cotton were the waste sources with the largest contribution ca. 38% each (Figure 1).

Cotton represents a robust fraction of textile waste [28] with its chemical composition consisting mostly of cellulose (<88%), a polymer structure with high crystallinity, that requires to be pretreated for successful AD [29]. Wool is a natural protein fiber highly exploited as a raw material in the textile industry due to its high quality such as high heat retention, high stain and static resistance and good flexibility [30]. As the highest methane yield is usually observed in protein-rich substrates [31], the high protein content in wool indicates its potentially high energy value. In addition, wool and cotton come originally from the agricultural sector, where AD is largely applied as a "Waste-to-energy" technology promoting waste recovery and energy generation [32]. Thus, both residues are strong candidates to be explored for energy recovery, as well as to strengthen the textile supply chain since its pre-consumer phase, in agricultural production.

The high consumer demand upon the textile industry has driven several waste management strategies. Incineration and landfill are the main destinations of most textile waste at their end of life [1]. Although waste volume decrease by 90% is achieved via incineration, and this process is viewed as an effective energy recovery [2,33], contaminated, damp, ripped or stained textile waste does not contribute to energy recovery [34]. In addition, incineration can produce flue gases [e.g., sulphur dioxide (SO_2), hydrogen chloride (HCI), hydrogen fluoride (HF), nitrogen dioxide (NO_2)] that are harmful to the environment [33]. AD is, thus, the most promising technology when compared in terms of both environmental performance and energy recovery [35]. Even though it is a great challenge to stabilize the AD process since its stages are driven by microorganisms [36], the generation of clean energy and biofertilizer makes it advantageous from an environmental perspective [37].

Blue jeans waste mainly consists of cotton and polyester fiber [29] and had a large contribution to the solid fraction, representing 23% of all solid waste (Figure 1). Although the conversion of cotton to biogas through the breakdown of cellulose into simple sugars has been extensively described in the literature, its blending with polyester is poorly described and requires further research [28,29,38]. Furthermore, as polyester represents a robust fraction of the textile sector and an increase in its production is projected due to cost-effectiveness in line with the consumption pattern of emerging countries, AD of this fraction requires attention [1]. The main challenge and bottleneck is the hydrolysis of polyester as its large molecules hinder the enzymatic attack by microorganisms limiting the process to the surface of the material and, consequently, extending the duration of the process by months [7].

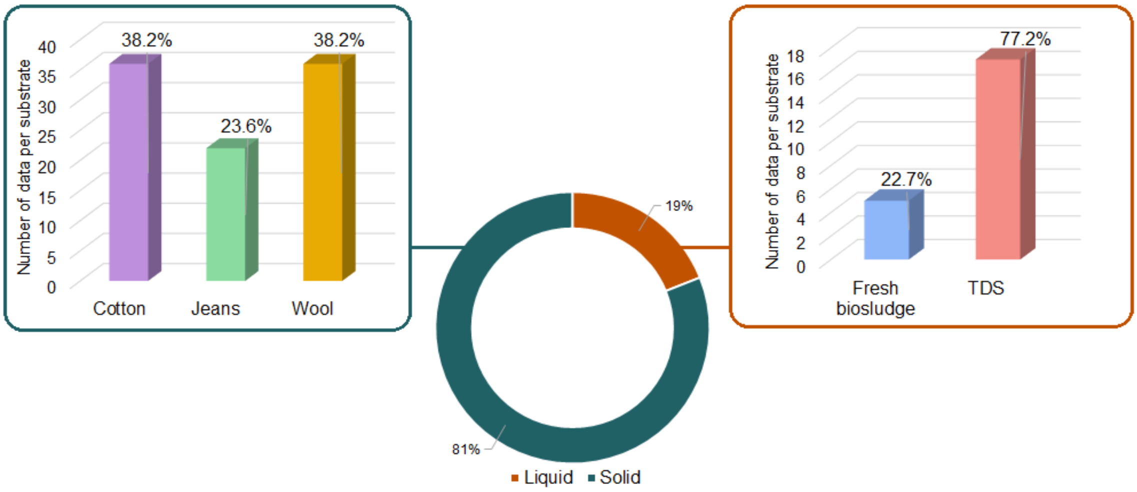

Figure 1. Different sources of textile waste used in AD reported in the literature included in the meta-analysis. TDS= Textile dyeing sludge

The liquid fraction of the residual organic matter represents 19% of all organic waste (Figure 1) and is mainly composed of textile dye sludge (TDS) (77.2%) and fresh sludge (22.7%) (Figure 1). Even though the liquid waste volume is much lower than the solid waste, the liquid fractions are highly toxic, and its pollutant potential is very high [4], losing only to tanneries and the pulp and paper industry [3]. The amount and chemical content of textile effluents are of huge concern due to the presence of highly water-soluble chemicals. The conventional activated sludge system or municipal sewage treatment systems are inefficient for the treatment of this residue [39] indicating a clear need for investments to water reuse and energy recovery from this waste stream. Successful pretreatment application to textile effluents has led to dye removal, decolorization of wastewater and reduction of toxicity [4,9]. Moreover, the proper selection of the AD pretreatment results in a cost-effective and secondary treatment to alleviate environmental damage [4]. Pretreatments promote the reduction of pollutant loading, mitigating impacts on environmental and human health. Furthermore, water reuse would supply the high-water demand of the textile industry itself.

Several pretreatments, such as physical, chemical, biological and their combinations (Figure 2, Table S2) have been applied to improve AD. Pretreatments applied alone, especially physical and chemical, are the most explored while their combinations are less observed (Figure 2, Table S2). This could be explained by the high capital cost investment required to integrate pretreatments into the AD process and also by the difficulty of large-scale implementation [38].

Heat application (i.e., autoclave and thermal) is the most reported among physical pretreatments both as unique and in combination (Figure 2). High temperatures are successful in lysing cells and increasing the solubilization of organic materials such as polysaccharide, protein and soluble chemical oxygen demand in liquid and solid fraction of the textile waste [40,41], favoring biogas generation.

Sonication and Liquid Nitrogen (LN_2) are mechanical pretreatments highly efficient in the disruption and disintegration of complex bonds in the substrate chemical structure [42,43]. Although the application of these pretreatments have a positive effect, the increase in methane yield is limited, which may be a result of the reduction and/or inhibition of AD caused by the presence of some chemicals released from the substrate. As a result, the implementation of this pretreatment may become economically unfeasible [43].

The diversity in chemical pretreatments was the largest (Figure 2), with microaeration (n=11) being the most applied. Oxygen addition impacts the biological microbial process and microbial electrolysis in addition to inhibiting the production of hydrogen sulfide (H_2S), promoting a more stable AD process [25]. Other chemical pretreatments such as alkaline, acids and organic solvents additions have shown to be highly efficient in breaking down complex structures and increasing the availability of fermentative sugars for enzymatic hydrolysis [44]. However, when applied at high doses, they can generate methanogenesis-inhibiting bioproducts such as furfural and vanillin [45].

Heat combined with sodium hydroxide (NaOH, n=1) and sodium carbonate (Na_2CO_3, n=24) were the thermochemical pretreatments reported (Figure 2). The predominance of Na_2CO_3+thermal can be a consequence given the negative effects of sodium hydroxide such as corrosion, need for neutralization and generation of hazardous content to the environment [29].

In contrast, sodium carbonate is successful in reducing cellulose crystallinity and consequently achieves high bioconversion of lignocellulosic content in addition to low cost [29].

As the use of enzymes provides a very selective and specific action on the organic matter [46], the predictability of its response could suggest a lower need for extensive testing, as required in other pretreatments. Therefore, the commercial enzyme alkaline endopeptidase (n=16) was the only biological pretreatment applied to wastes from the textile industry (Figure 2, Table S2).

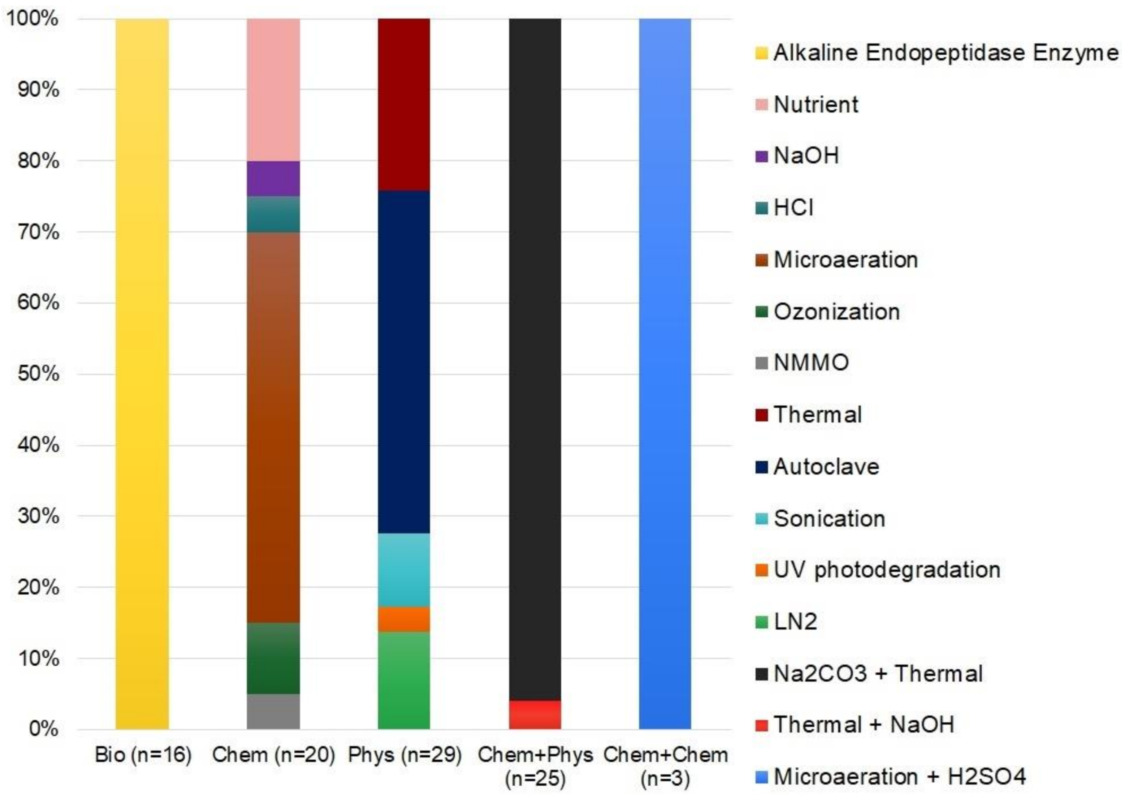

Figure 2. Diversity of methods composing the pretreatment categories applied to improve AD of textile waste reported in articles included in the meta-analysis. *Biological:* Alkaline Endopeptidase (n=16); *Chemical:* Nutrient (n=4), NaOH (n=1), HCl (n=1), Microaeration (n=11), Ozonization (n=2), N-Methylmorpholine N-oxide (NMMO) (n=1); *Physical*: Thermal (n=7), Autoclave (n=14), Sonication (n=3), UV photodegradation (n=1), Liquid Nitrogen (LN_2) (n=4); *Chemical+Physical:* Na_2CO_3+Thermal (n=24), Thermal+NaOH (n=1); *Chemical+Chemical:* Microaeration+H_2SO_4 (n=3).

Biological pretreatments are the most eco-friendly, as they have a relatively low energy cost and require no addition of chemicals/inhibitory compounds. Thus, they generate less pollution and high methane yields [47]. The application of alkaline endopeptidase led to an increase in methane yield in up to 360% (RR= 1.28, 95% CI: 0.15 to 2.41, $p<0.05$) indicating a high efficiency as a pretreatment for the textile industry (Figure 3, Table S2). The use of commercial enzymes has become more attractive due to their pure nature of speeding reactions turning biodegradation faster [48]. Addition of the alkaline endopeptidase enzyme shows stable performance with high protein solubilization, which improves methane production [40,48]. In fact, the success of enzyme application has led to an expressive growth in the last decade in the textile sector [46]. However, the few enzymes and so far investigated highlights the crucial need for more research in the area for the development of new enzymes with higher efficiencies targeting textile wastes.

Despite a better performance of biological pretreatments (Figure 3), physical and chemical pretreatments remain the most applied on textile waste (Figure 2). This is probably due to their high performance when applied on other waste sources. However, our result (Figure 3) clearly showed that their application may not necessarily payoff and can even lead to decreases in the methane yield in comparison to untreated controls, especially when applied to cotton waste (Table S2). Therefore, pretreatments should be carefully chosen before large-scale application.

The use of inappropriate pretreatments can in fact reduce the methane yield [49]. For instance, formation of inhibitory/toxic compounds following pretreatments, such as volatile fatty acids (VFAs) [50,51], can be pointed out as causing the negative effect on the biogas production due to a low capacity to degrade specific organic compounds and to deal with usual chemical loads in textiles such as dyes.

Chemical pretreatments used both individually and/or in combination showed a large variability over methane yield (Figure 3). This can be explained by the choice of additive (i.e. H_2SO_4, NaOH), and the concentration added, which requires care since high doses can inhibit methanogenesis [37,45].

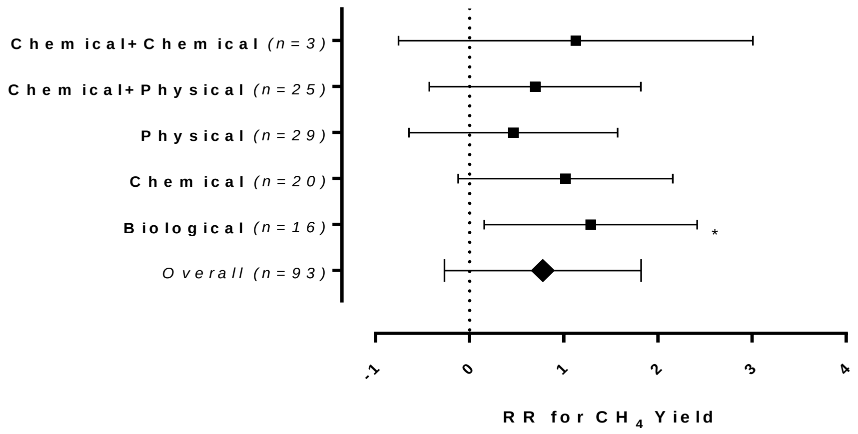

Figure 3. Effect size by natural log response ratio (RR) of methane yield with 95% confidence interval (CI) (p-value=0.05), comparing the performance of the Biological, Chemical, Physical, Chemical+Physical and Chemical+Chemical pretreatments. Significant code p≤0.05 (*); n = number of effect sizes per treatment type.

Even though significant results were obtained in this study, our analysis must be viewed with caution as the application of pretreatments in textile waste is limited. Despite the highest performance of the biological pretreatment (Figure 3), it cannot be assumed the same efficiency on all textile wastes as the prevailing chemical composition of the substrate is likely to have an effect on its efficiency. Therefore, careful attention is needed to the individual performance of the different substrates subjected to pretreatments case-by-case (Table S2), especially if we consider textile residues with strong environmental impacts such as cotton and polyester [29].

In fact, some textile wastes e.g., textile dyeing sludge (TDS), have high loads of dyes, auxiliary chemicals, surfactants and heavy metals [52]. Such chemicals can severely impair the AD microbial community, especially during hydrolysis and acidogenesis [51]. AD alone has shown CH_4 yield of 0.1 mL/gVS which is dramatically increased, after its combination with pretreatment, to 56.1 mL/gVS, corresponding to an increase of 56000% (Table S2).

Cotton and polyester represent 37% and 63% of the total fibers that is produced within the textile industry, respectively [53]. High energy demand is required from the production of those fibers to their transport to the final destination. Energy consumption is estimated at 48 kWh and 101 kWh just to produce one kilogram of cotton and polyester fibers, respectively (Figure 4A).

Replacing virgin cotton and polyester with second-hand clothes can save up to 65kWh and 90kWh of energy per kilogram of fiber reused, respectively [7]. However, the lifespan of textile products is continuously decreasing [7] and, therefore, the reuse of clothing as a strategy to reduce energy consumption is inefficient.

The destination of textile waste for AD can be a sustainable alternative for more efficient energy recovery and supply to the production chain. Considering the global annual waste production of cotton (11.7 million tons) and polyester (42 million tons) exclusively generated by the textile industry [23,24], we estimated the potential energy production of 155.835 GWh and 298.805 GWh from polyester and cotton, respectively (Figure 4B), which can be increased by applying the pretreatment with the best performance for those types of residues (Table S2).

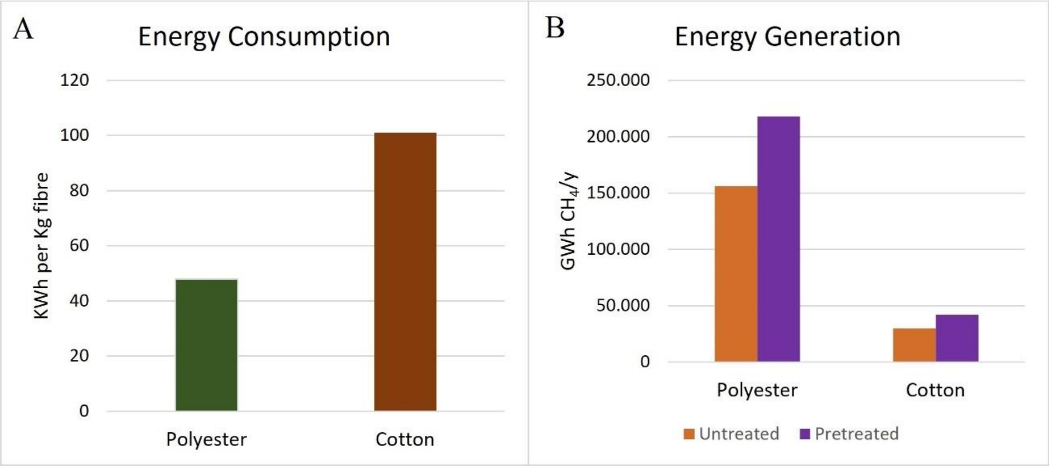

Figure 4. Consumption and generation of energy from the main textile fibers (cotton and polyester). (A) Energy consumption per kilogram of textile fiber produced. Adapted from [1]. (B) Global energy generation potential from polyester and cotton untreated (orange) and pretreated (purple).

4. Conclusions

Our results showed that biological pretreatments, among those commonly applied in the textile industry, promote a larger biogas production via AD. In comparison with chemical and physical pretreatments, enzyme pretreatment may lead to an increase in methane yield up to 360%, on average. Biological pretreatments also require no addition of chemicals and favor the reuse of textile wastewater, decreasing the current demand for clean water while increasing the resource circularity in the textile industry. Moreover, biological pretreatments are very efficient in removing highly soluble chemicals that cause damage to human and environmental health and given their lower energy demand, the cost-benefit is expected to be low. The implementation of AD improved by pretreatment in the textile industry promotes adequate management of the huge amount of waste generated, converting textile residues into economic value for the industry in line with the circular economy. However, more research is crucial on the quest for new enzymes with higher efficiencies and, especially extensive screening on largely neglected residues e.g., textile wastewater, boosting this sector in a more sustainable model.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, **Figure S1.** Flow diagram summarizing quantitatively the selection of studies from the systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA, http://www.prisma-statement.org/). n= number of articles; Table S1. Global production of fiber waste from the textile industry and volatile solids (VS) and total solids (TS) content; Table S2. Summary of pretreatments applied to different sources of textile waste reported in the systematic review; Table S3: Studies included in the meta-analysis.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, T.M.A. and A.E-P.; methodology, T.M.A.; software, T.M.A. and A.E.W.; validation, A.E.W., B.K.S. and A.E-P.; formal analysis, T.M.A.; investigation, T.M.A.; resources, A.E-P.; data curation, T.M.A. A.E.W. and B.K.S.; writing—original draft preparation, T.M.A., B.K.S., A.E.W. and A.E-P.; writing—review and editing, B.K.S., A.E.W. and A.E-P.; visualization, T.M.A., B.K.S., A.E.W. and A.E-P.; supervision A.E-P.; project administration, A.E-P.; funding acquisition, A.E-P..

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4.4 Artigo IV

Maximizing biogas production from algal biomass

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Abstract

Energy security is a serious concern on the global agenda for sustainable development. Addressing this challenge, anaerobic digestion (AD) is a successful technology able to alleviate the energy crisis while mitigates environmental impacts. Although algae species have shown great potential to produce bioenergy, the chemical composition of algae poses challenges that may hinder the efficiency of AD. To overcome this limitation, several pretreatments have been applied on algae biomass prior to AD as a strategy to break down their physicochemical properties and increase biogas production. We carried out a quantitative synthesis and showed that the performance of pretreatments is determined by the chemical composition of the algal biomass. Notably, micro- and macroalgae achieve higher methane (CH_4) yields after being submitted to different pretreatments. Biological, physical, and chemical+physical pretreatments were the most efficient for microalgae, increasing CH_4 yields by up to 141%, 125% and 151%, respectively, while the physical pretreatments led to the highest performance on macroalgae, with an increase of 129% in CH_4 yield. Moreover, our conservative estimate suggests that using 10% of the current global algal biomass production (3.6 Mt) could yield over 5.5 TWh y^{-1}.

Appropriate pretreatment could double this potential energy production. These findings should be considered in future clean energy projections, emphasizing their potential for societal decarbonization.

Keywords

anaerobic digestion, microalgae, macroalgae, biomass, biogas, pretreatment

1. Introduction

Global energy consumption is projected to increase by 45–60% by 2030 (Tvaronavičienė et al., 2019), and currently, 85% of the world's energy system is fossil dependent, which poses a significant challenge to decarbonization (Ediger, 2019). The European Union (EU) predicts that by 2050, energy from biomass could increase to 250 million tons of oil equivalent (Mtoe), with over 70 Mtoe by biogas as a decarbonization strategy (Iglesias et al., 2021). Furthermore, prediction models show that the net-zero carbon emission target expected to be achieved between 2050 and 2070 could be reached earlier if biomass is applied as energy (van Soest et al., 2021). Therefore, renewable energies play a crucial role in global decarbonization.

Energy based on biomass has not only environmental advantages, such as clean energy generation, nutrient recovery, "carbon neutral", and reduced dependence on fossil fuels, but also socioeconomic benefits by increasing employment in rural areas and promoting a circular economy (Angouria-Tsorochidou et al., 2022; Awosusi et al., 2022). Additionally, biomass offers a decentralized energy source that increases energy security, especially in light of recent geopolitical instabilities. The energy crisis in Europe caused by dependence on natural gas from Russia, which supplies approximately 50% of European demand, highlights the urgent need for advancements in the energy sector (Carfora et al., 2022). Biomass can help mitigate such risks by reducing reliance on centralized and vulnerable energy sources.

First-generation (e.g., corn, soy, sugarcane) and second-generation (e.g., lignocellulosic) biofuels provide direct pressure on the increase in food prices and present a high cost to lignin removal (Chen et al., 2015; Montingelli et al., 2015), limiting its conversion to energy. The third generation (e.g., algae) overcomes the limitations in biomass from previous generations, such as carbohydrate richness, high carbon fixation rates and low lignin content, and their energy potential is over 100 EJ yr^{-1}, which is greater than that of terrestrial crops at 22 EJ yr^{-1} (Dave et al., 2019; Montingelli et al., 2015).

Micro- and macroalgae are classified according to their size and morphology, with chemical composition directly related to their physiological features (Pourkarimi et al., 2019). While microalgae are richer in protein and lipids, macroalgae have relatively higher carbohydrate contents (Dave et al., 2019; Niccolai et al., 2019). The use of algae biomass is highly advantageous due to its short growth cycle (Montingelli et al., 2015), ability to occupy restricted and nonfarmable areas, and low impact on water and soil resources (Adeniyi et al., 2018). Currently, 36 million tons (Mt) of algae are produced worldwide, with the highest percentage cultivated (Cai et al., 2021). It has been reported to be successful for industrial applications in animal health and nutrition, pharmaceuticals, cosmetics, wastewater bioremediation, and biofuel/bioenergy production (Khoo et al., 2021; Kumar et al., 2021a; Niccolai et al., 2019). Within the energy context, algae are a promising biomass source owing to their advantages over other feedstocks, such as biomass productivity, renewability, and sustainability (Kumar et al., 2021a).

Anaerobic digestion (AD) is one of the most effective waste treatment technologies that converts biomass into biogas while mitigating greenhouse gas (GHG) emissions (Iglesias et al., 2021). AD is a process driven by microorganisms in the absence of oxygen that convert organic matter into biogas, mainly composed of CH_4 (60–70%) and CO_2 (20–40%) with traces of H_2S, N_2, and NH_3 (Kendir and Ugurlu, 2018; Uddin et al., 2021). AD is the most tolerable to biomass with high moisture content compared with other energy conversion processes (Sarwer et al., 2022). The high moisture content of algae facilitates the access of AD microorganisms to the substrate, accelerating microbial growth and consequently improving bioconversion (Thompson et al., 2019).

Although algae are a promising source for biogas production due to their high carbohydrate, protein and lipid contents, the diversity of structural chemical components among several species is a challenge to AD stabilization and microbial community performance (Ganesh Saratale et al., 2018; Kumar et al., 2021a). Additionally, the presence of polyphenols, halogen content, accumulation of volatile fatty acids (VFAs), low C/N ratio, ammonia (NH_4^+ and NH^3) and production of H_2S act as AD inhibitors (Chen et al., 2015; Kumar et al., 2021a; Montingelli et al., 2015).

Pretreatment application prior to AD can significantly improve its efficiency by weakening the cell wall, breaking down complex sugars into monomers, and increasing the accessibility of other cellular components such as proteins and lipids (Yukesh Kannah et al., 2021). Moreover, the correct choice of pretreatment can enhance the solubilization of organic matter, remove pathogens present in the biomass, and reduce the production and accumulation of inhibitory or toxic compounds (Anacleto et al., 2022; Kendir and Ugurlu, 2018). It is also crucial to consider the specific group of algae being used, whether macroalgae or microalgae, as their distinct morphologies may result in varying responses in terms of severity and type of pretreatment approaches.

Several methods (e.g., chemical, physical, biological and combination methods) have been tested for macro- and microalgae to select the most cost-effective method in terms of biogas yield performance, energy consumption, and operational cost.

In this study, we conducted a comprehensive meta-analysis to evaluate the efficiency of several pretreatments applied to both macro- and microalgae prior to AD to increase biogas production, as well as the potential for this AD optimization to meet the global energy demand and contribute to energy security.

2. Methods

The systematic review was performed on the Web of Science and Scopus databases following the preferred reporting items guide for systematic reviews and meta-analyses (PRISMA, http://www.prisma-statement.org/) (Fig. S1). The search included keywords, the Boolean operator (AND) and wildcards (*): “anaerobic digestion” “pretreatment” “methane” and “algae”. The selected studies were filtered to articles in English that were published between 1945 and 2020 (Table S1).

The eligibility criteria to include the articles in the meta-analysis were microalgae/macroalgae as substrate for AD, algae chemical characterization, application of pretreatment, methane yield of untreated (control) and pretreated algae, and satisfactory data for statistical analysis, such as the mean, standard deviation (SD) and number of replicates.

The natural log response ratio (RR) is a standard way to quantify the effect size in meta-analysis. The RR was used to compare the methane yield of pretreated algae to untreated (control) algae (Hedges et al., 1999). Thus, the mean of the pretreatment performance ($\bar{X}_T$) was compared to the mean of the control ($\bar{X}_C$) based on the based on the equation:

$$RR = ln\left(\frac{\bar{X}_T}{\bar{X}_C}\right) \qquad (1)$$

The mean effect sizes and 95% confidence intervals (CI) with bias correction were calculated using R software and the "metafor" package. Pretreatments were considered significant if their CI and mean effect did not overlap the zero line. Mean and upper CI below the zero line indicate a negative response (treatment < control), while a mean and lower CI above the zero line represent a positive response (treatment > control). The p<0.05 significance level was considered.

The chemical characterization data of both micro- and macroalgae were analyzed through one-way analysis of variance (ANOVA) with Tukey’s post hoc comparison tests using GraphPad Prism version 7.04.

We assessed the potential CH_4 production from algal biomass equivalent to 10% of the world’s production (Cai et al., 2021), specifically when the most appropriate pretreatment is applied. The content of volatile solids (VS) for both micro- and macroalgae was obtained from studies included in the systematic review (Equation 2; Table S2). The CH_4 potential was estimated by considering the average methane yield for untreated and pretreated micro- and macroalgae, with a focus on the pretreatment methods that showed the highest increase in yield (Equation 3):

$$AG = (AB \times TS\%) \times OM_{algae} \quad (2)$$

$$CH_4 \text{ potential} = AG \times CH_4\ yield \times 9.97 \quad (3)$$

where AG is algae generation (Mt VS/y), AB is the algae biomass (Mt wet weight/y), OM_{algae} is the organic matter content in the algae biomass (VS%), and 9.97 is the lower calorific value of CH_4 (kWh/m^3 CH_4) (Ornelas-Ferreira et al., 2020).

3. Results and Discussion

3.1. World algae production

Algae production, including wild collection and cultivation, accounts for the second largest fraction of the global aquaculture sector, representing a production of 36 Mt (wet weight) (Cai et al., 2021; Naylor et al., 2021). World cultivation increased by 97% from 1969 to 2019, while wild collection remained at 1.1 Mt (Cai et al., 2021), indicating a growing interest in algae uses. Macroalgae are the most produced type of algae and show large geographic decentralization (Fig. 1A), with higher production in eastern and southeastern Asia (Cai et al., 2021). In contrast, microalgae cultivation only comprises 16% of total algal production and is less widespread globally (Fig. 1B). This imbalance may be related to the cultivation system since microalgae cultivation requires nutritional supplementation (i.e., glucose, glycerol, sodium acetate, sucrose, CO_2, and bicarbonate), specific conditions (i.e., light intensity, temperature and pH) and operational energy input to the culture system, which is not the case for macroalgae cultivation (Kumar et al., 2021b).

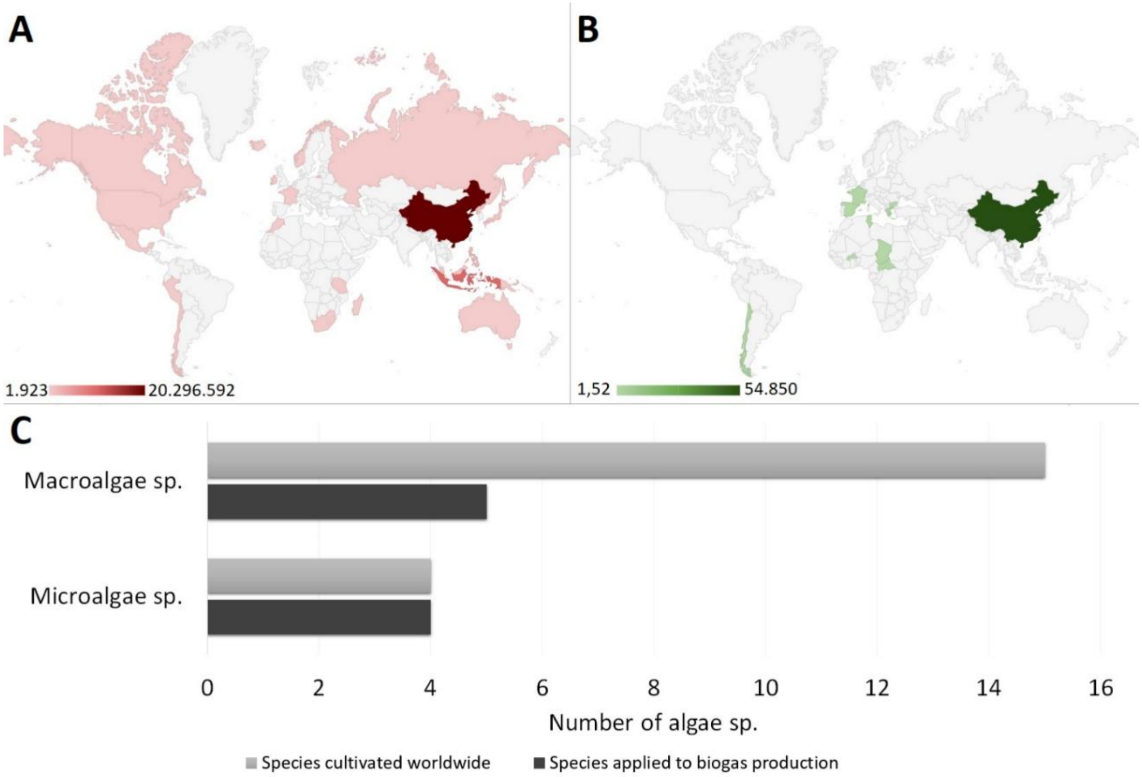

Fig. 1. World algal biomass production (tons wet weight) and species applied to biogas production. A) Geographical distribution of macroalgae biomass (tons). B) Geographic distribution of microalgae biomass (tons). C) Comparison of the number of species for both micro- and macroalgae worldwide applied to biogas production based on a systematic review. Adapted from Cai et al (2021).

Both micro- and macroalgae production are prevalent in Asia, especially China, with a production of 54,850 and 20,296,592 tons of wet weight, respectively, in 2019 (Fig. 1A and B). Eastern Asian countries have a long history of commercial cultivation of algae mainly for human food and medicinal use, in addition to encouraging research to improve cultivation techniques since 1950 (Hwang et al., 2019). Algae cultivation has historically been associated with regions facing water scarcity and less fertile land (Kumar et al., 2021b), as they can increase food security (Naylor et al., 2021). Furthermore, occurrences of large floating algae blooms have been frequently reported on the Asia coast in recent decades (Chen et al., 2022; Marquez et al., 2020). From an energy perspective, this environmental issue presents an opportunity to transform it into socioeconomic benefits in the affected regions (Chen et al., 2022).

European countries, Canada, Australia and the USA have shown a huge interest in the commercial cultivation of algal biomass to produce various bioproducts as an economically viable and sustainable industry (Pankratz et al., 2017). The conversion of algal biomass into bioproducts is highly environmentally advantageous, as it relieves pressure on natural resources, such as water and land, for food production (Naylor et al., 2021). Moreover, it has been identified as a promising resource to replace fossil energy sources while mitigating the levels of GHG emitted into the atmosphere (Adeniyi et al., 2018).

Although the European Union is one of the global leaders in biogas energy generation (Grando et al., 2017), the use of algal biomass on an industrial scale is rarely implemented (Murphy et al., 2016). Policies such as Renewable Energy Directive 2018/844/EC (REDII) are game changers that boost the conversion of algal biomass into biomethane, meeting the growing demand for energy in the EU (Prussi et al., 2021). Moreover, these policies actively contribute to achieving the target of a 32% share of biogas in the EU's renewable energy share by 2030 (Prussi et al., 2021).

The United States, Canada, and Australia have dedicated significant research efforts to the development of strategies aimed at enhancing algae cultivation technologies for industrial applications (Pankratz et al., 2017). These strategies aim to minimize both capital and operating costs while improving the competitiveness of algae-based energy compared to other renewable sources. Furthermore, there is a strong focus on achieving the lowest market prices to drive the growth of biorefineries that utilize algae as a feedstock. This approach seeks to create a favorable economic environment for industry, encouraging investment and scaling up production (Kumar et al., 2021a; Pankratz et al., 2017).

Even at a disadvantage in terms of the amount of cultivated biomass and geographical distribution, microalgae stand out in the energy sector compared to macroalgae (Fig. 1C). Microalgae and macroalgae had 18 and 15 species, respectively (Fig. S2 and S3), tested for AD. Regarding the algal species cultivated in the world according to (Cai et al., 2021), 100% of the listed microalgal species and 33% of the macroalgae have been tested for biogas production (Fig. 1C). Indeed, the low number of algal species tested for AD remains a limiting factor in expanding the knowledge about biogas production potential, especially considering that there are approximately 170,000 described species of algae (De Clerck et al., 2013). Moreover, there is a scarcity of both biomass production and biogas production in countries located in tropical zones, which possess favorable light and temperature conditions for AD. These countries have the potential to encourage the generation of energy through biogas from algae. By harnessing their advantageous environmental conditions.

Microalgal physiology plays a decisive role in further biotechnological exploration (Adeniyi et al., 2018). Its high production and accumulation of biopolymers, such as proteins, lipids and carbohydrates, make it extremely attractive as feedstock for AD applications (Kendir and Ugurlu, 2018). On the other hand, macroalgae contain high levels of polysaccharides and have a low lignin content, which makes them more effective in AD.

3.2. Characterization of algal biomass

Microalgae and macroalgae represent 79% and 21% of the total algal biomass applied to biogas production, respectively, as reported in the searched articles (Fig. S4). The use of microalgae biomass in the energy sector prevails due to its higher yield per hectare compared to macroalgae (158 t vs. 60–100 t) (Chen et al., 2015), which can be attributed to its successful large-scale cultivation, rapid growth rate, and vigorous vitality (Øverland et al., 2019a). In addition, microalgae are excellent candidates for biogas production since protein is their major cellular biopolymer, resulting in a high CH_4 yield.

Organic biopolymers (e.g., protein, carbohydrate, and lipid) have different biodegradation rates and theoretical CH_4 yields (Xue et al., 2020). Highlighting the distinct dominance of biopolymers in algal biomass, our study grouped them into microalgae and macroalgae (Fig. 2). The chemical composition of macroalgae biomass is dominated by carbohydrates (31.5% of DW), followed by lipid (13% of DW) and protein (8.2% of DW) contents (Fig. 2A). Macroalgae contain a wide range of carbohydrates and complex polysaccharides that mainly serve structural functions, such as alginate, laminarin, ulvan, agar, and carrageenan (Dave et al., 2019; Øverland et al., 2019b). The efficiency of carbohydrate hydrolysis in AD is determined by its chain complexity. Simple sugars are rapidly hydrolyzed, resulting in a high CH_4 yield, while polysaccharides are not freely available, leading to a decline in the CH_4 yield by ca. 55% (Chen et al., 2015). Additionally, macroalgae contain high levels of alkali metals, halogens and sulfur that can inhibit the growth of anaerobic microorganisms (Chen et al., 2015). In particular, secondary halogenated metabolites found in red algae (90%) exhibit strong antimicrobial properties that block essential binding sites in the methanogenic pathway, with green and brown algae containing only 7% and 1%, respectively (Nielsen et al., 2020).

Microalgal biomass is predominantly composed of protein (45.1% of DW), followed by carbohydrates (23.2% of DW) and lipids (15% of DW) (Fig. 2B). Although proteins are highly successful for energy applications, the breakdown of nitrogen (N) bonds in their molecules leads to the production of ammonium (NH_4^+) and free ammonia (NH_3) (Khedim et al., 2018), which at high levels can be harmful to the microbial community driving AD.

Lipids have a complex and larger structure, and due to their complex polymeric substances, they are the main energy storage substances of microalgae and the basis for biomass energy utilization (Xue et al., 2020). Microalgae contain a variety of lipids, such as sterols, lipoproteins, phospholipids and triacylglycerols, with the amount varying with the type of species, growth conditions, and environmental factors (Liu et al., 2017). The lipid structure must be broken down to produce biogas, a process that occurs slowly and can generate inhibitory compounds that may possibly inhibit some phase of the AD process, producing long-chain fatty acids and ammonia (Anacleto et al., 2022; Xue et al., 2020). Microalgae, when exposed to stress conditions, accumulate lipids in the form of triacylglycerols, and lipids are found in the cell membranes in the form of phospholipids, which facilitates the oil extraction process (Li et al., 2019).

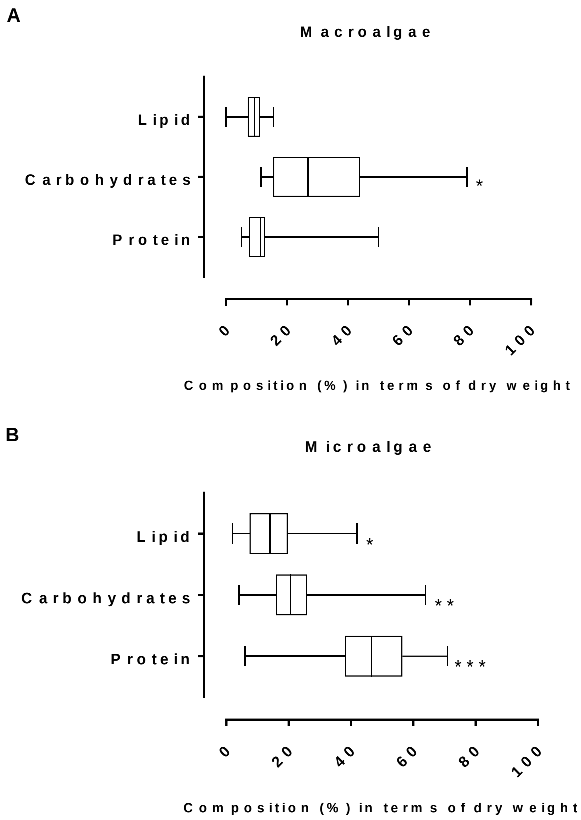

Fig. 2. Chemical composition of A) macroalgae (n= 37) and B) microalgae (n=65) based on dry matter (% DW). *p<0.01, **p<0.001, ***p<0.0001 (one-way ANOVA with Tukey's *post hoc* test). All data are presented as the mean±SD. References are provided in Table S3.

3.3. Effect of pretreatment on the CH_4 yield of macro- and microalgae

Microalgal biomass showed high responses in terms of CH_4 yield after pretreatment application (Fig. 3A). The application of chemical+physical (RR= 0,410; CI: 0,212 to 0,608), biological (RR= 0,373; CI: 0,173 to 0,572) and physical (RR= 0,192; CI: 0,084 to 0,301) pretreatments resulted in significant increases in CH_4 yield when compared to untreated microalgae.

High protein and lipid contents can hinder AD and reduce CH_4 yield (Abdallah et al., 2018), which could require interventions, such as pretreatments to mitigate inhibitory effects for the achievement of higher CH_4 yields. The most tested pretreatments in microalgae are physical (n= 325) and biological (n= 100) (Fig. 3A).

The high incidence of physical pretreatment applications may be related to their success in weakening the cell wall (Yukesh Kannah et al., 2021). The use of ultrasound, mechanical and heat methods is highly effective in disintegrating cells and breaking hydrogen bonds between polymer complexes while increasing the surface area for enzymatic attack in AD (Abraham et al., 2020; Di Capua et al., 2020). However, the optimum operational condition range of the physical pretreatments should be carefully considered, as heating applications greater than 70 °C reduce the organic fraction convertible to biogas (Yukesh Kannah et al., 2021), leading to a decline in biogas production.

Biological pretreatments are successful in microalgae compared to other approaches (e.g., alkaline, irradiation, heating, maceration) due to their high biodegradability rate, no formation of inhibitory compounds, little or no addition of chemicals/toxic compounds, and low energy consumption and capital cost (Zabed et al., 2019). Furthermore, avoiding pretreatments that form inhibitory compounds such as chemical pretreatments is crucial since substrates rich in proteins and lipids are more unstable due to the self-formation of various compounds, such as NH_4 (Anacleto et al., 2022). The high concentration of these toxic compounds in AD can decrease the CH_4 yield to lower than the untreated microalgae (Fig. 3).

Combining different pretreatment methods is an efficient strategy to increase the severity of the biodegradation of organic matter. Our results show that the combination of chemical+ physical pretreatments led to the highest CH_4 yield in microalgae, reaching an increase of 151% (Fig. 3). This synergistic effect of combined pretreatments not only improves sugar yield and enzymatic digestibility but also reduces energy consumption compared to individual pretreatments (Kim et al., 2016). Moreover, the integrated effect of chemical+physical pretreatment (i.e., alkaline and mechanical) can reduce energy consumption and waste generation, proving advantageous for industrial-scale implementation (Areepak et al., 2022).

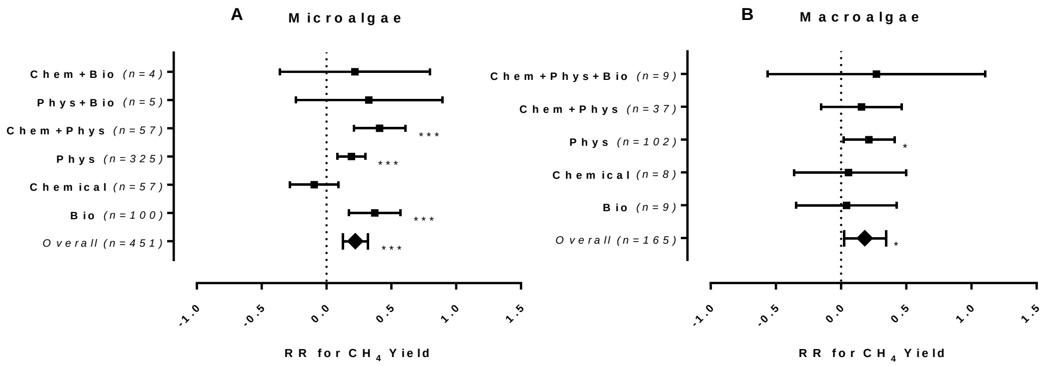

Fig. 3. Mean effect size (RR) and 95% confidence intervals of methane yield from algae biomass submitted to different pretreatments. Phys= Physical, Chem= Chemical and Bio= Biological and their combinations. A: Microalgae consists of using a single species or a mixture of them. B: Macroalgae consists of using a single species or a mixture of them. Significance level: $p \leq 0.001$ (***); $p \leq 0.01$ (**); $p \leq 0.05$ (*); $p \geq 0.1$ (). n= number of effect sizes per pretreatment type.

Macroalgal biomass is converted to biogas more easily than microalgae biomass due to its high carbohydrate content. Since carbohydrates have quicker degradation rates, rapid hydrolyses can promote VFA accumulation, leading to an imbalance during the acidogenesis and methanogenesis steps (Potdukhe et al., 2021). Physical pretreatments (RR=0.214; CI: 0.018 to 0.411) were significantly the most efficient when applied to macroalgae, with an average increase in CH4 yield of 124% (Fig. 2B). Although macroalgae have a more flexible cell wall than terrestrial plants, they contain several complex carbohydrates, polysaccharides and 'lignin-like' compounds (Øverland et al., 2019b), which create a protective structure preventing enzymatic attack and, consequently, requiring their breakdown.

3.4. Comparison of physical pretreatments applied to algal biomass

Among all evaluated pretreatments, physical pretreatments (RR= 0.222; CI: 0.147 to 0.298) showed a significant increase of 125% in CH4 yield for both micro- and macroalgae (Fig. 4). This finding is particularly valuable since it identifies a pretreatment that can be applied for both groups, boosting the use of algae biomass in industrial-scale AD applications, where precise species screening may not always be feasible. Additionally, the mixing of micro- and macroalgae has benefits in terms of cost savings and logistical efficiency, as a large volume of waste can be processed in a single reactor.

Homogenizer (RR= 0.635; CI: 0.361 to 0.909), thermal (RR= 0.391; CI: 0.251 to 0.530) and ultrasound (RR= 0.241; CI: 0.122 to 0.359) pretreatments promoted the highest increase in CH_4 yield (Fig. 4). Homogenizing pretreatment achieved an 189% increase in CH_4 yield compared to untreated algae. Its mechanical function efficiently disrupts cells through a highspeed and high-pressure rotor, increasing the availability of intracellular material for enzymatic attack (Carpenter et al., 2017; Sarwer et al., 2022). Despite a significant increase in biogas production and being safer for the AD system compared to chemical pretreatment, the high energy cost of operating this pretreatment on a large scale is a barrier to its implementation (Carpenter et al., 2017).

The highest performance in CH_4 yield under heat application was obtained in the range of 100–120 °C for 30–120 minutes (Table S4; S5). Thermal pretreatment is widely investigated since it is highly efficient in the solubilization of organic compounds, reduction of pollutant contents, and destruction of pathogens (Li et al., 2016). This favors not only the hydrolysis step, increasing biogas production but also improving digestate quality.

Ultrasonic pretreatment promoted an increase of 128% in CH_4 yield. As with other mechanical pretreatments, it disrupts cells, increasing the digestibility of the organic material. It stands out due to its low cost required for large-scale application (Kisielewska et al., 2020).

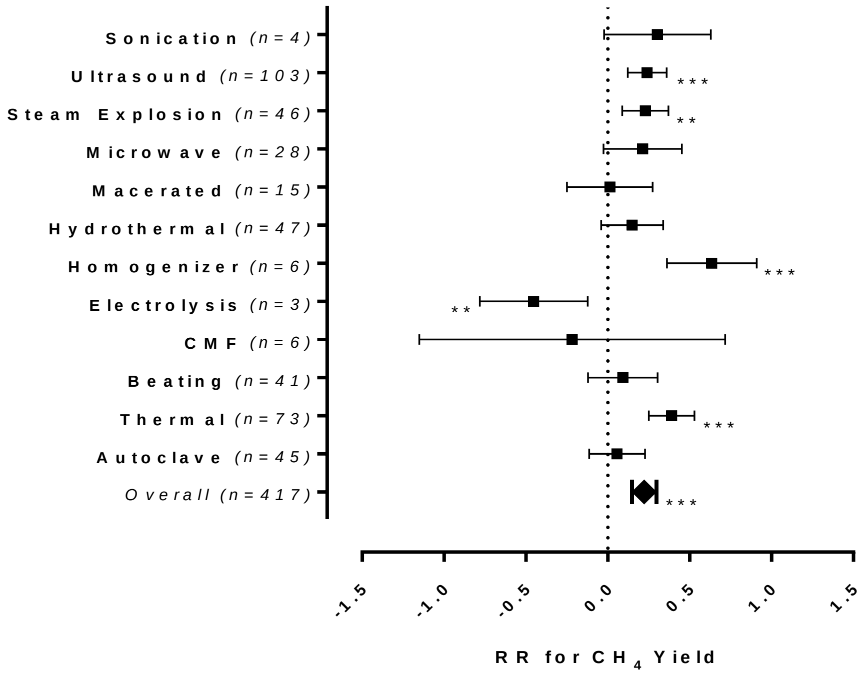

Fig. 4. Mean effect size (RR) and 95% confidence intervals of methane yield from micro- and macroalgae applied to physical pretreatments. CMF: constant magnetic field. Significance level: $p \leq 0.001$ (***); $p \leq 0.01$ (**); $p \leq 0.05$ (*); $p \geq 0.1$ (). n= number of effect sizes per pretreatment type. Methods with n<2 were removed from the statistical analysis.

3.5. Global potential for energy production from algal biomass

Currently, less than 1% of macroalgae biomass is utilized for energy production, and the percentage of microalgae utilization is unknown (Chen et al., 2015). A conservative estimate suggests that if 10% of the global algal biomass produced was applied in the energy sector, it would lead to an energy production of 5.58 TWh per year. Considering that the appropriate pretreatment is applied to micro- or macroalgae, this energy output could potentially reach 12.55 TWh (Fig. 5). Furthermore, when considering the conversion of biogas into electrical and thermal energy through combined heat and power (CHP) processes, which typically exhibit average efficiencies ranging from 30% to 45% (Koç et al., 2019), the findings of this study could correspond to approximately 16% of the global electricity consumption, which amounted to 22.848 TWh in 2019 (IEA, 2021).

The impressive energy generation values of AD make it a game-changer for renewable energy technologies. While 24,281 m² of land area is required to produce just 1 MW solar power plant, AD can produce over 12 TWh without competing for land and without efficiency being dependent on climate and seasonality (Chew et al., 2021). Additionally, AD is superior to other renewable energy technologies in terms of environmental costs to society. For instance, according to a life-cycle assessment (LCA), 2 MW wind power turbines produce 13.4 g CO2e/kW/h, accompanied by high levels of noise pollution and negative impacts on flying fauna (Chew et al., 2021).

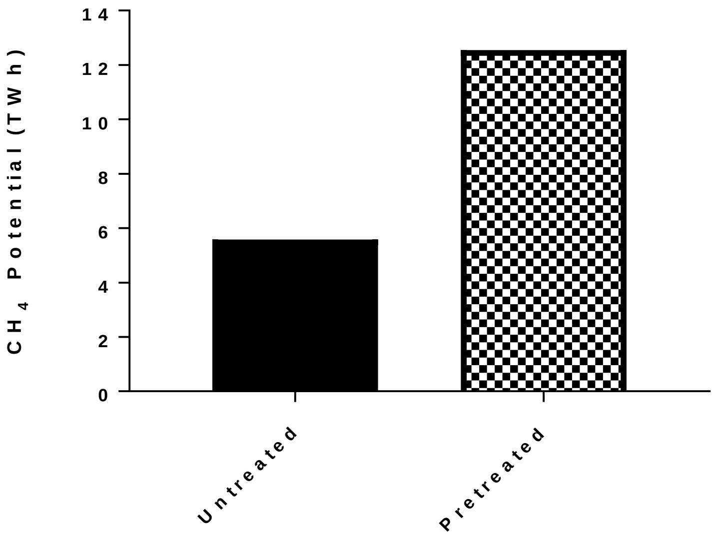

Fig. 5. Estimate of global energy generation potential from 10% of the total cultivated algal biomass under untreated and pretreated conditions.

Conclusion

The use of algal biomass as a feedstock for energy generation can provide a powerful tool to decarbonize and increase societal sustainable energy sources. With its decentralized energy generation and ability to meet over 16% of global electricity consumption, algal biomass ensures energy security without competing with food production. Notably, pretreatments, especially physical ones, have substantially enhanced anaerobic digestion, yielding resulting in a 125% increase in CH_4 yield for both micro- and macroalgae. These findings pave the way for efficient and cost-effective large-scale implementation of algal biomass, making a significant contribution to meeting our energy needs while reducing production costs.

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4.5 Artigo V

Assessing phytotoxicity of anaerobic digestate: Effect of feedstock composition and liquid-solid separation

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Abstract

Anaerobic digestate is widely recognized as a potential biofertilizer and soil amendment, offering promising prospects for reducing reliance on mineral fertilizers and facilitating nutrient recycling within the circular economy model. This study investigates the phytotoxicity of various digestate types (e.g., sewage sludge, food waste, agricultural biomass, and manure) from industrial-scale biogas digesters, assessing their influence on germination through the germination index (GI). Our findings revealed that the improvement of digestate GI is highly dependent on the digestate fraction, i.e., solid (SD), liquid (LD), and whole (WD) digestate. Liquid-solid separation was found to reduce phytotoxicity in the liquid fraction while increasing it in the solid fraction. Notably, sewage sludge and agricultural biomass-derived digestates exhibited non-phytotoxic characteristics in both WD and LD forms. The results indicate that LD from sewage sludge demonstrated the highest GI (99.31 ± 32.67%), signifying non-phytotoxicity, whereas manure-derived digestate showed the lowest GI. These results underscore the importance of feedstock composition and solid-liquid separation in determining the phytotoxicity and suitability of digestate as a potential biofertilizer.

Keywords

Anaerobic digestion, biofertilizer, nutrient recovery, germination, manure, agricultural biomass

1. Introduction

Anaerobic digestion (AD) is increasingly recognized as a key technology for renewable energy production, with biogas as its main product. The global expansion of AD is notable, with an annual growth rate of 12.8% and approximately 132,000 digesters in operation, alongside 50 million micro digesters (Borges et al., 2021; Jain et al., 2019). This growth is driven by AD's ability to enhance energy security, provide socioeconomic benefits, and maintain ecological balance (Boluk et al., 2019). The European Union's REPowerEU plan exemplifies this momentum, aiming to reach 35 billion cubic meters (bcm) of biomethane by 2030 and 151 bcm by 2050 to meet emission reduction goals and net-zero targets (Alberici et al., 2022).

A significant challenge associated with the widespread adoption of AD technology is digestate management, a nutrient-rich by-product. An approximate estimation indicates that a biogas plant producing 500 kW of power, produces over 10,000 tons of digestate annually, containing about 10% dry matter (Kratzeisen et al., 2010). Effective digestate management is essential for the sustainability of the biogas supply chain, consistent with circular economy principles that emphasize recycling and resource efficiency (Pecorini et al., 2020).

Digestate contain essential nutrients such as Nitrogen (N), Phosphorus (P) and Potassium (K) as well as micronutrients, making it a valuable organic fertilizer and soil conditioner (Peng et al., 2020; Surendra et al., 2014). Although digestate is considered waste due to handling and disposal cost, its use as a biofertilizer can enhance the economic viability of biogas production (IEA, 2020). According to Surendra et al. (2014), digestate application improves soil physical, chemical, and biological properties, thereby enhancing soil security and agricultural productivity.

The agronomic value of digestate is influenced by its chemical composition and origin. Digestates from different sources may introduce various contaminants, such as agrochemicals residues from manure and agricultural biomass, and heavy metals, pharmaceuticals, antibiotics, and antibiotic-resistant bacteria from sewage sludge (Czekała, 2022; Sica et al., 2023; Soukupová and Koudela, 2023; Świechowski et al., 2020). Additionally, the solid-liquid separation process affects nutrient content distribution, with the liquid fraction being rich in nitrogen and the solid fraction higher in phosphorus (Logan and Visvanathan, 2019). Given the need to reduce reliance on mineral fertilizers and support global food security, assessing the suitability of various digestate sources is crucial.

Despite advancements in technologies capable of detecting contaminants such as metals, pesticides, and mycotoxins, assessing anaerobic digestate toxicity directly in living organisms remains essential (Lencioni et al., 2016). The germination index (GI) is a phytotoxicity test proposed by environmental and regulatory agencies such as the US Environmental Protection Agency (US EPA), the Organization for Economic Co- operation and Development (OECD), and the International Organization for Standardization (ISO) using crops species guidelines such as tomato (*Solanum lycopersicum L.*) (Lencioni et al., 2016).

Tomato plants are highly sensitive to herbicides, making them suitable for phytotoxicity assessments (Fast et al., 2011). Even herbicides deemed low-risk by the European Food Safety Authority (EFSA), such as aminopyralid (4-amino-3,6-dichloro-2-pyridinecarboxylic acid), can cause significant damage at low soil concentrations (Abdourahime et al., 2019; Soukupová and Koudela, 2023). For instance, annual applications rate of aminopyralid in permanent pasture and non-agricultural areas range from 0.05 to 0.12 kg ae ha (kilograms of active ingredient per hectare), with concentration as low as 0.2 μg kg^{-1} in soil leading to severe agricultural biomass damage and up to 95% yield loss in tomatoes (Fast et al., 2011).

This study aims to evaluate the potential use of the whole digestate and its fractions from various sources as a biofertilizer, by assessing its nutrient composition and potential phytotoxicity. The results from this study could support the broader adoption of AD technology in agricultural practices.

2. Material and methods

2.1. Digestate sampling

Digestate samples were collected from 23 industrial-scale biogas digesters across Sweden, Norway, and Denmark (Table S1). The sampled digesters had agricultural biomass, food waste, manure or sewage sludge as their main feedstock source. Samples were collected in triplicate using 10 L containers and promptly transported to the laboratory. They were kept in a water bath to maintain their original operating temperatures before chemical analyses and phytotoxicity evaluation.

2.2. Digestate characterization

The pH was determined using a pH meter (InoLab 7310, WTW, Germany). Total ammonia nitrogen (TAN) was analyzed after centrifugation and filtration, with filtrates stored at −20 °C. The samples were thawed, diluted up to 7900 times and analyzed in an AutoAnalyzer (SEAL Analytical, USA). The free ammonia concentration was calculated according to Equation 1 (Sarker et al., 2019).

$$NH_3 - N = \frac{TAN}{1+\frac{10^{-pH}}{10^{-\left(0,09018+\frac{2729,92}{T}\right)}}} \qquad (1)$$

Total organic carbon (TOC) and total nitrogen (TN) were measured using a CHN elemental analyzer (Thermo Fischer, Flash 2000). Inorganic elements were measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 8900), reaction gases varied with the element analyzed, using helium for most metals, oxygen for arsenic and selenium, and no specific gas for lithium, beryllium, and boron.

2.3. Phytotoxicity test

Phytotoxicity was evaluated in vitro using commercial tomato seeds (*Solanum lycopersicum*). Digestate was separated into solid digestate (SD), liquid digestate (LD), and whole digestate (WD), through centrifugation at 10,000 rpm for 10 minutes at 20 °C. SD was dried at 70 °C for 20h in a muffle. A 1 g of digestate was mixed with 9 mL deionized water, and seeds were incubated with this solution in Petri dishes at room temperature (23.9±0.7 °C) for 72 h. As a control, seeds were incubated with 10 mL deionized water. The experiment was performed in triplicates, with 10 seeds per Petri dishes.

The germination index (GI) was determined by Equation 2 (Quina et al., 2015):

$$\text{GI } (\%) = \frac{RSG\ (\%) x\ RRG\ (\%)}{100} \qquad (2)$$

Where RSG is the relative percentage of seed germinated and RRG is the relative percentage of root length, calculated using the Eqs. (3) and (4), respectively:

$$\text{RSG } (\%) = \frac{N_{SG,T}}{N_{SG,B}} \times 100 \qquad (3)$$

$$\text{RRG } (\%) = \frac{L_{R,T}}{L_{R,B}} \times 100 \qquad (4)$$

Where $N_{SG,T}$ and $N_{SG,B}$ are the mean number of germinated seeds in the extract (treatment) and in control (deionized water), respectively, and $L_{R,T}$ and $L_{R,B}$ are the mean length of roots in the extract and in the control, respectively.

2.4. Statistical analysis

Normal distribution was analyzed using D'Agostino–Pearson and Shapiro–Wilk normality tests. Two-way analysis of variance (ANOVA) and post hoc Tukey multiple comparisons ($p \leq 0.05$) were conducted for GI analysis. Statistical analyses were performed using GraphPad Prism 6.01 software.

Principal component analysis (PCA) with 95% confidence ellipses was performed using with the FactoMineR, factoextra, and ggplot2 packages to compare chemical properties in each feedstock. The analysis was executed using R software version 4.2.3.

3. Results and discussion

3.1.Chemical characterization of different digestate sources

Digestate derived from food waste and agricultural biomass were rich in macronutrients, especially N (3.7 and 3.4%, respectively) and TOC (36.5 and 33.1%, respectively) (Table 1).

This alternative nitrogen source is promising given that, in 2019, the European Union (EU) had to import 30% of its nitrogen consumption (IEA, 2020). However, careful management is necessary as digestate application significantly affects soil nitrogen levels; excessive rates can lead to nitrogen leaching into deeper soil layers, emphasizing the importance of tailored application based on specific soil properties and plant nutritional demands (Pranckietienė et al., 2023).

The elevated TOC content in agricultural biomass digestate is probably due to the presence of recalcitrant organic compounds such as lignin and cellulose, which remain after anaerobic digestion (García-López et al., 2023). All tested digestates exhibited a C:N ratio of around 9, which is considered satisfactory since it maintains the C:N ratio below 20, indicating a quality compound stabilized and suitable for soil amendment purposes (Islam et al., 2021).

Table 1. Chemical characterization of the digestate from industrial-scale biogas digesters. Data are presented as mean ± standard deviation.

	Agricultural biomass	Food waste	Manure	Sewage sludge
N (% of DM)	3.4 ± 0.9	3.7 ± 1.1	2.9 ± 0.4	3.3 ± 1
TOC (% of DM)	33.1 ± 8.8	36.5 ± 11.1	28.8 ± 3.9	28.9 ± 2.7
C:N ratio	9.73	9.86	9.93	8.75
TS (%)	9.7 ± 0.5	5.4 ± 0.9	7.0 ± 0.4	3.8 ± 0.1
TS/VS (%)	82.5 ± 1.5	67.6 ± 1	74.6 ± 0.9	62.0 ± 1.1
NH_4-N (mgN/L)	1012 ± 19.6	1097.6 ± 71.2	1690.7 ± 584.8	487.5 ± 57
NH_3-N (mgN/L)	31.9 ± 0.7	186.3 ± 23.7	164.4 ± 21.7	13.2 ± 2
pH	7.3 ± 0	7.8 ± 0	7.8 ± 0	7.3 ± 0
K (g/Kg)	38.0 A± 17.2	35.2 ± 20.4	25.3 ± 16.2	23.1 ± 23.4
Ca (g/Kg)	35.8 ± 7.6	26.0 a± 12	22.9 ± 11.8	24.5 ± 4
Mg (g/Kg)	4.5 ± 1.3	4.7 ± 1.9	4.4 ± 1.7	4.7 ± 2.8
Fe (g/Kg)	16.4 ± 17.6	12.9 ± 14.8	26.4 ± 23.4	31.7 ± 32.3
Mn (mg/Kg)	221.8 ± 62.8	229.6 ± 67.9	224.2 ± 83.3	235.3 ± 55.8
Zn (mg/Kg)	278.7 ± 111.6	254.2 ± 132.8	260.4 ± 150	378.2 ± 120.5
Cu (mg/Kg)	90.5 ± 94	83.6 ± 72.2	129.2 ± 128.4	230.9 ± 124.7
Mo (mg/Kg)	3.7 ± 1.3	3.9 ± 1.7	4.1 ± 3.3	5.2 ± 2.4

B (mg/Kg)	24.3 ± 6.8	20.4 ± 10.1	16.9 ± 8.6	23.6 ± 8.1
Ni (mg/Kg)	10.6 ± 3.2	8.1 ± 3.2	10.1 ± 5.9	10.9 ± 4.4

DM: dry matter.

The pH values of all tested digestates were within the neutral range, close to the optimal pH for plant growth (Table 1). This range falls within the recommended range of 5.5–9.0 for compost material (Ezemagu et al., 2021). A pH of around 8 is considered ideal as it supports microbial activity necessary for the decomposition of OM during the composting process (Ezemagu et al., 2021).

Manure-derived digestate showed the highest levels of potentially inhibitory nitrogen compounds, such as TAN and FAN. This nitrogen forms significantly influence plant growth, with high concentrations of NH_4^+ (> 0.1 mmol/ L) inducing stress and toxicity in plants (Britto and Kronzucker, 2002; Coleto et al., 2023). This compound can inhibit growth due to interactions with other nutrients and cause acidification of the cell external medium, impacting nutrient availability (Coleto et al., 2023). For instance, in tomato plants, a balanced NO_3:NH_4 ratio of 75:25 with adequate calcium (Ca) levels improved yield and prevented disorders such as blossom-end rot, whereas lower ratios (higher NH_4^+) negatively affected growth and nutrient partitioning (Gholamnejad et al., 2023).

On the other hand, sewage sludge digestate presented the highest micronutrients content (Table 1). Essential elements such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu), have been reported to have higher concentrations in digestate from human waste digestate compared to livestock waste and abattoir waste digestates (Kirawa et al., 2020). These micronutrients such as Zn, Fe, Cu, boron (B), molybdenum (Mo), manganese (Mn), and chlorine (Cl) are vital for various physiological and biochemical processes, including photosynthesis and enzymatic activities (Panchal and Maitreya, 2023). However, their effects on plants can be beneficial or harmful, depending on the concentration. While adequate levels are essential for plant health, excessive accumulation can pose risks. For example, mining activities have been linked to elevated levels of Cu, Zn, and other metals in soils, which subsequently accumulate in crops like wheat and rice, sometimes exceeding safe consumption levels (Xu et al., 2022). Cu and Cd, which are absorbed via the same transporters as essential minerals, complicate the development of crop cultivars that can differentiate between essential and toxic elements (Hussain, 2022).

3.2.Digestate phytotoxicity across different feedstocks

Among the different digestate sources analyzed, sewage sludge digestate exhibited the highest GI, at 99.31 ± 32.67% (Figure 1). This can be attributed to the low levels of NH_4-N and NH_3, indicating low phytotoxicity, as well as the highest micronutrients content of sewage sludge digestate (Table 1). The balanced nutrient profile and lower concentration of phytotoxic compounds contribute to its high suitability as a biofertilizer. Additionally, the lower C:N ratio in sewage sludge facilitates faster nutrient mineralization, enhancing availability for seeds germination compared to other digestates. Although there are concerns about the presence of pathogens in sewage sludge digestate, sanitization can eliminate most pathogens, such as *Salmonella spp*. and SARS-CoV-2 (Carraturo et al., 2022). This sanitization is mandatory for the use of sewage sludge in agriculture and is regulated by the Sewage Sludge Directive (86/278/EEC).

EU regulations have historically supported the use of sewage sludge in agriculture as a means of recycling nutrients and promoting sustainability (Bauer et al., 2020; Çapan Mustafaoğlu et al., 2023; Grecu and Mâşu, 2018). However, recent regulations, such as EU Regulation 1009/2019, have excluded sewage sludge from the list of possible constituents of organic fertilizers based on their origin rather than their quality, which has raised concerns about the circular bioeconomy principles (Cucina et al., 2021).

It was previously reported that crop yields were about 8% higher with sewage sludge fertilization compared to mineral fertilizers (Dubis et al., 2022). Long-term field trials in Sweden, initiated in 1981, have demonstrated that applying sewage sludge to agricultural land increases crop yields without negative effects on heavy metal uptake by plants (Sugurbekova et al., 2023). The results from this study corroborate that sewage sludge have characteristics that improve soil quality, while not inhibiting plant growth.

Manure digestate showed the lowest performance, with GI < 30% (Figure 1), likely due to high NH_4-N and NH_3 levels. Additionally, the immaturity of the applied digestate could further reduce GI performance. Adding unstable residues or immature compost into agricultural soil can lead to phytotoxicity, negatively affecting root quality and plant growth due to high salt concentrations, OM biodegradation, and nitrogen immobilization (Rigane et al., 2011). GI levels below 50% are indicative of phytotoxicity (Logan and Visvanathan, 2019).

Food waste digestate also had a GI below 50%, showing signs of phytotoxicity (Figure 1). The low GI may be due to potential inhibitory factors such as volatile fatty acids accumulation (Zhang et al., 2015). Additionally, the chemical composition of food waste is highly influential on its performance, as it constitutes a complex organic matrix whose final composition depends on eating habits and varies between countries, regions and periods of the year (Anacleto et al., 2024).

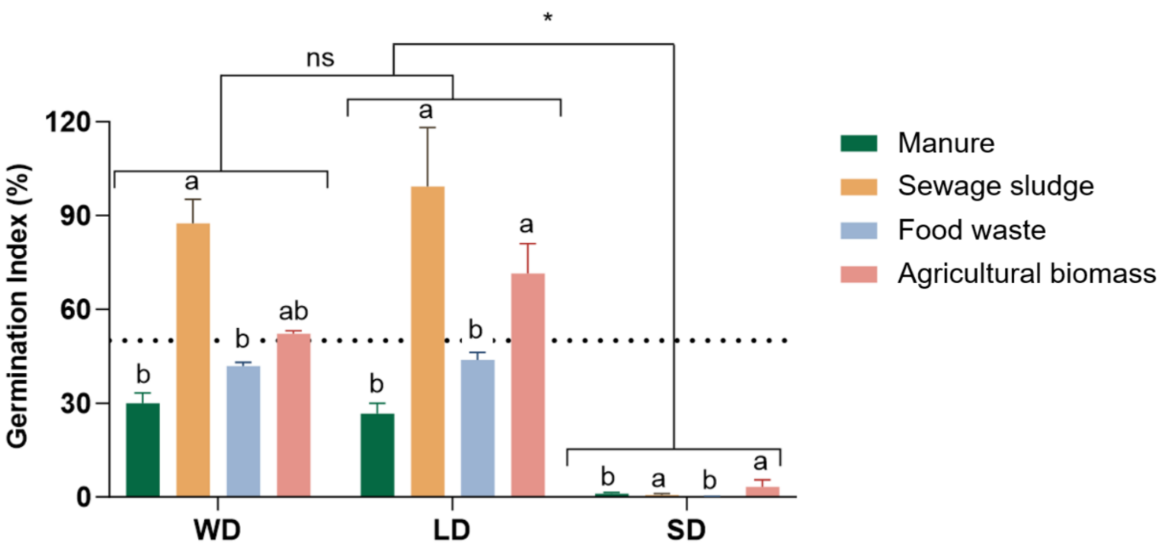

Figure 1. Germination index of digestates from industrial-scale biogas digesters with different feedstocks. WD: Whole digestate; LD: Liquid digestate; SD: Solid digestate. Statistical analysis used Two-way ANOVA with

Tukey's post hoc test. Different letters indicate significant differences between feedstocks, and * denotes significant differences between digestate fractions (WD, LD, and SD). ns: non-significant. Dashed line represents the phytotoxic effect limit.

Agricultural biomass-derived digestate showed non-phytotoxic results, with a GI of 52.16 ± 1.35% (Figure 1). This high GI value is linked to the elevated macronutrient concentration of this digestate, which enhances soil organic carbon, total nitrogen, plant-available phosphorus, potassium, and mineral nitrogen content, thereby improving soil fertility and nutrient availability (Barłóg et al., 2020). The liquid fraction of agricultural biomass digestate, with its high nitrogen content, performed similarly to mineral fertilizers in agronomic tests, suggesting its potential as a sustainable substitute in intensive cropping systems (Grillo et al., 2021; Tambone et al., 2017).

3.3.Effect of solid-liquid separation on digestate phytotoxicity

The effect of solid-liquid separation on digestate phytotoxicity was significant across all tested samples, except for those derived from manure, for which there was no difference between WD, LD and SD. LD consistently showed higher GI values, while SD exhibited severe phytotoxicity, with GI values lower than 7% for all sources (Figure 1). This suggests that the separation process enhances the agronomic value of the digestate, effectively reducing the phytotoxicity of the liquid fraction while concentrating harmful compounds in the solid fraction.

Interestingly, when solid-liquid separation was performed, the GI of the solid fraction was lower, which could be attributed to the accumulation of heavy metals in this organic fraction. Solid-liquid separation has been reported to significantly affect heavy metal content, nearly eliminating phosphorus and heavy metals from the liquid fraction while concentrating them in the solid fraction (Beggio et al., 2022). Chemically enhanced separation significantly reduces the heavy metal content in the liquid fractions, ensuring compliance with EU regulations for agricultural reuse of organic soil amendments (Beggio et al., 2022).

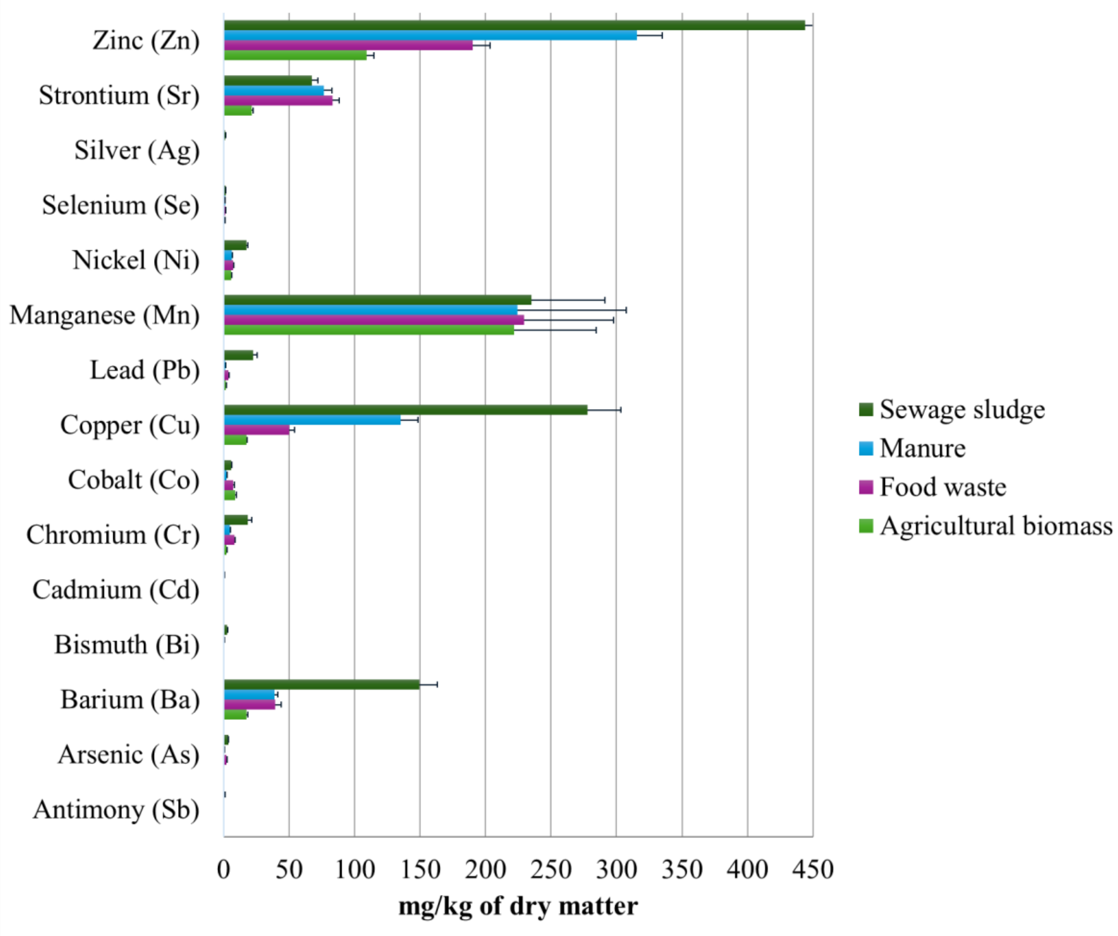

Figure 2. Mean *and standard deviation of* heavy metal content in digestates from full-scale digesters with different feedstocks.

Solid-liquid separation of digestate can also increase its biofertilizer potential by OM stabilization, aiding soil fertility, carbon sequestration, and reducing the risk of N leaching (Egene et al., 2021). Studies have shown that LD can provide agronomic performances comparable to mineral fertilizers, leading to satisfying agro-environmental sustainability indices (García-López et al., 2023; Grillo et al., 2021). The application of SD has been found to increase soil organic carbon content, while the LD enhances carbon and nitrogen levels in deeper soil layers, impacting the mobile forms of carbon and nitrogen in the soil (Slepetiene et al., 2023).

All digestates evaluated in this study meet the limits for Zn, Cu, and Ni set by the EEC Directive 86/278. The directive allows these limits for heavy metals concentrations in soil to be exceeded by up to 50% if the pH is above 7. At low pH, heavy metals become more mobile due to increased release, especially during oxidation processes (Sintorini et al., 2021). As pH increases, the mobility of heavy metals decreases in the order of Cd > Zn > Ni > Cu > Pb. At pH 7, Zn and Cd ions start to dissociate from their compounds, with Cd becoming 80% dissolved at this pH, while Pb dissolution reaches 80-90% at pH 5-6 (Sintorini et al., 2021).

3.4. Correlation between digestate chemical properties and germination index

PCA of whole digestate chemical composition from various sources, such as manure, agricultural biomass, sewage sludge and food waste, has revealed significant insights into which factors influence digestate performance. The analysis identified five variables with eigenvalues ≥ 1, explaining 59% of the variation (Figure 3), indicating that the feedstock used in anaerobic digestion plays a crucial role in the resulting digestate's characteristics. A strong similarity between digestates from the same origin was revealed, regardless of operational/chemical variations among digesters, suggesting that the digester's feedstock is a determinant of the digestate's performance.

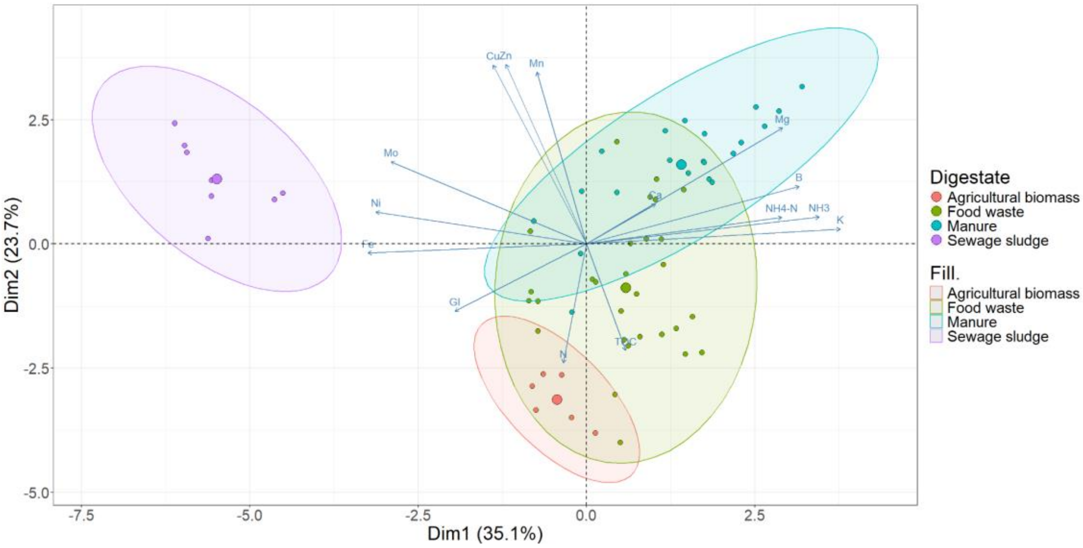

Figure 3. Principal component analysis (PCA) for chemical parameters of digestates. Ellipses show 95% confidence interval of clusters aggregation, highlighting the clustering of digestates according to their origins: agricultural biomass (red), food waste (green), manure (blue), and sewage sludge (purple). NH_4-N: ammonium nitrogen; NH_3: ammonia; GI: germination index; TOC: total organic carbon.

Food waste and agricultural biomass digestates were clustered closely, reflecting their similar chemical compositions rich in N content. In contrast, sewage sludge formed a distinct cluster, characterized by higher concentrations of trace metals (Zn, Cu, Mo, and Fe) and a higher GI.

Manure digestate, however, was dominated by higher levels of inhibitory compounds such as NH_4-N and NH_3, contributing to their lower GI and potential phytotoxicity. The overlapping chemical characteristics of agricultural biomass, food waste and manure digestate were reflected in their GI performance, while the distinct chemical profile of sewage sludge was associated with high GI performance.

Conclusion

Our study highlights that the phytotoxicity of anaerobic digestate is critically dependent on feedstock composition and the liquid-solid separation process. The liquid fraction, particularly from sewage sludge, demonstrated superior performance with the highest germination index. These findings challenge current EU regulations that prohibit the use of sewage-derived digestate as a biofertilizer, suggesting a reevaluation is warranted. Conversely, the solid fraction of all digestates exhibited high toxicity, likely due to the presence of toxic compounds such as heavy metals. This underscores the importance of solid-liquid separation in enhancing the agronomic potential of digestate, thereby significantly contributing to global soil and food security.

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5 CONCLUSÃO

A seleção adequada de métodos de pré-tratamento, levando em consideração a composição química dos resíduos orgânicos, resulta em aumentos substanciais na produção de biogás. Essa abordagem tem um grande potencial para contribuir significativamente para a transição energética em níveis nacional e global, garantindo segurança energética de forma descentralizada.

Além disso, esta pesquisa identificou importantes lacunas, especialmente relacionadas à gestão de esterco animal, resíduos da indústria têxtil, biomassa algal e à aplicação de pré-tratamentos. Os resultados destacam a variabilidade na resposta dos pré-tratamentos de acordo com a composição química de cada substrato, demonstrando o potencial promissor desses resíduos quando adequadamente manuseados na digestão anaeróbica.

Finalmente, este estudo evidencia o potencial do digestato como biofertilizante e/ou emenda do solo, ressaltando a importância da separação líquido-sólido para maximizar seu potencial agronômico. Essa descoberta tem implicações significativas para a promoção da agricultura sustentável e para a redução da dependência de fertilizantes minerais. A aplicação de digestato devidamente tratado pode melhorar a qualidade do solo, promover a reciclagem de nutrientes e contribuir para um modelo de economia circular, alinhando-se aos objetivos de sustentabilidade e descarbonização globais

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APÊNDICE A – Material suplementar do artigo I

Supplementary information for

Methane yield response to pretreatment is dependent on substrate chemical composition: A meta-analysis on anaerobic digestion systems

Thuane Mendes Anacleto, Betina Kozlowsky-Suzuki, Annika Björn, Sepehr Shakeri Yekta, Laura Shizue Moriga Masuda, Vinícius Peruzzi de Oliveira, Alex Enrich-Prast

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Supplementary Figures

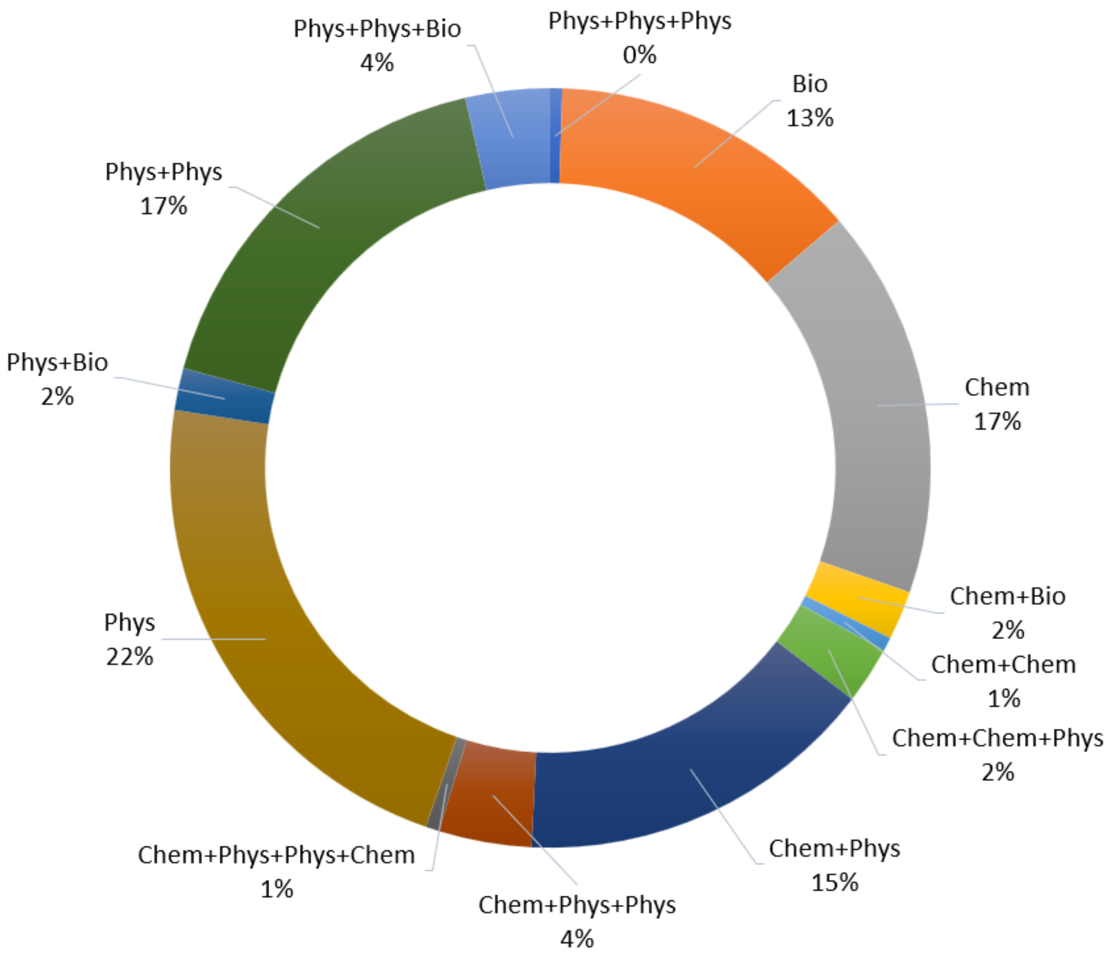

Figure S1. Quantification in percent of single and combined pretreatments of different nature (i.e., Biological= Bio, Chemical= Chem and Physical= Phys) applied prior to AD from the studies (n= 415) included in this systematic review and listed in Table S5. Detailed information about the different pretreatments can be found in Fig. S2-S4 and Tables S1-S4.

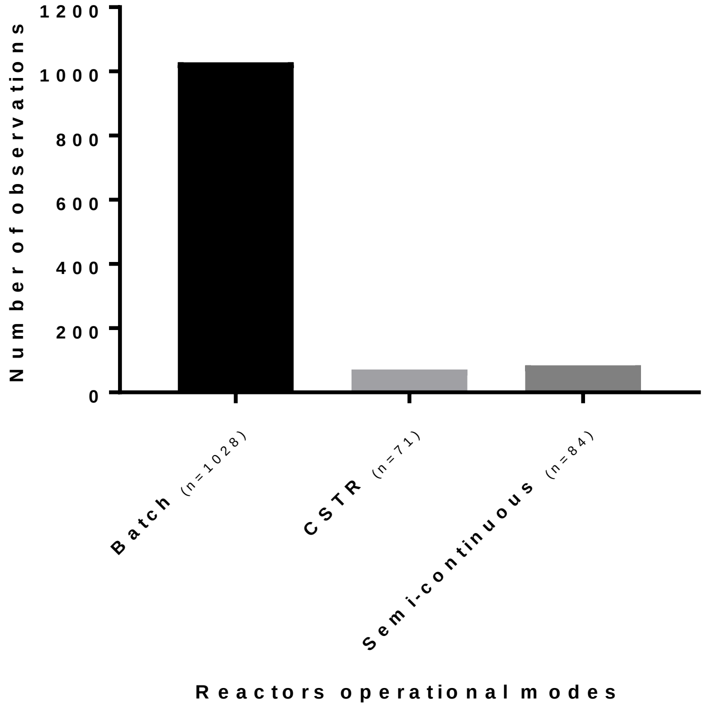

Figure S2. Summary of reactor operational model used in the studies included in this meta-analysis. The majority of observations were retrieved from studies applying batch incubations, followed by semi continuous feeding and by CSTR: Continuous stirred-tank reactor model.

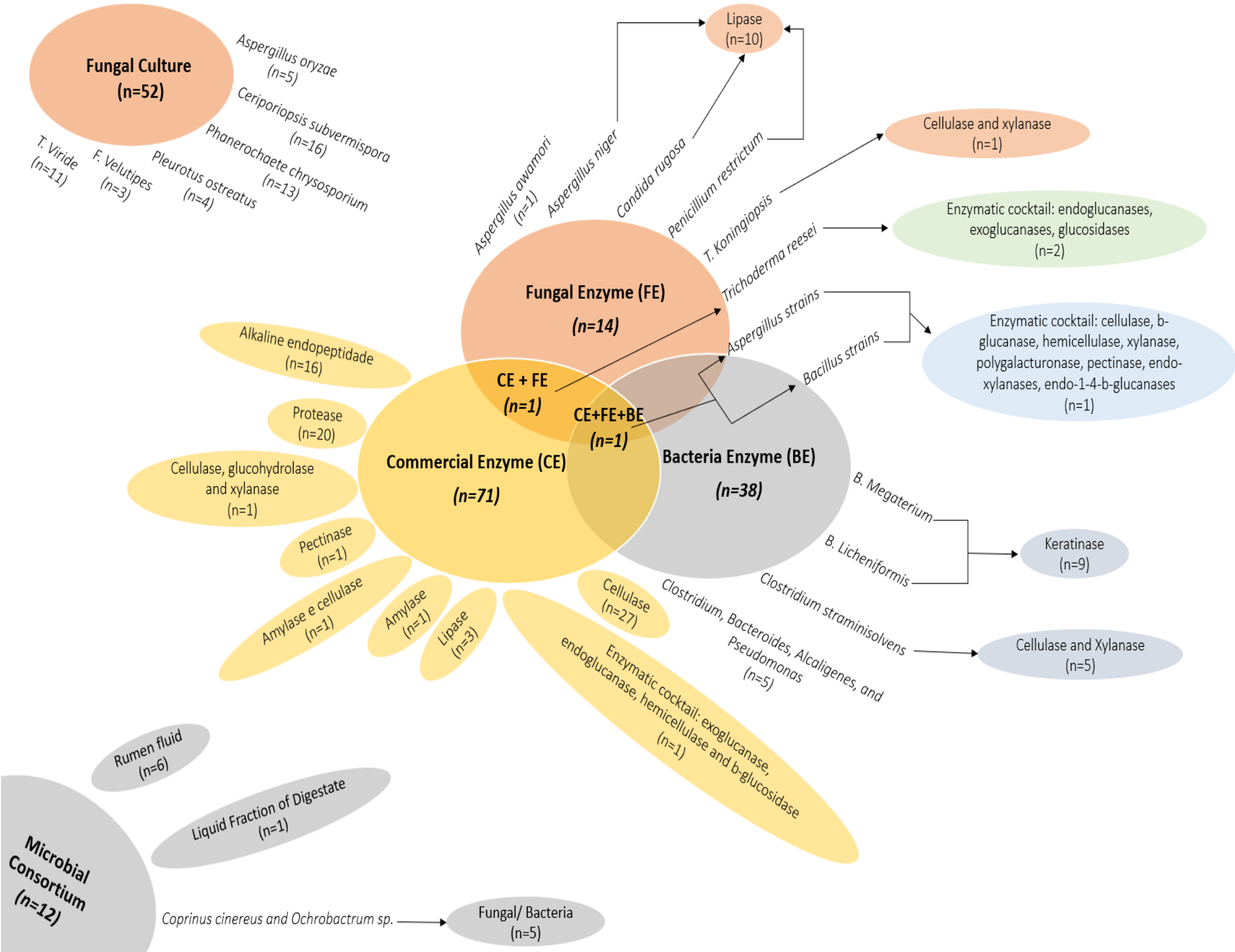

Fig. S3. Overview of the different biological pretreatments reported from the studies included in this meta-analysis. The largest share of cases applied Commercial Enzyme (n= 71), followed by Fungal Culture (n= 52) and Bacterial Enzyme (n= 38). The application of Fungal Enzymes (n= 14) and Microbial Consortium (n= 12) were the least used pretreatments.

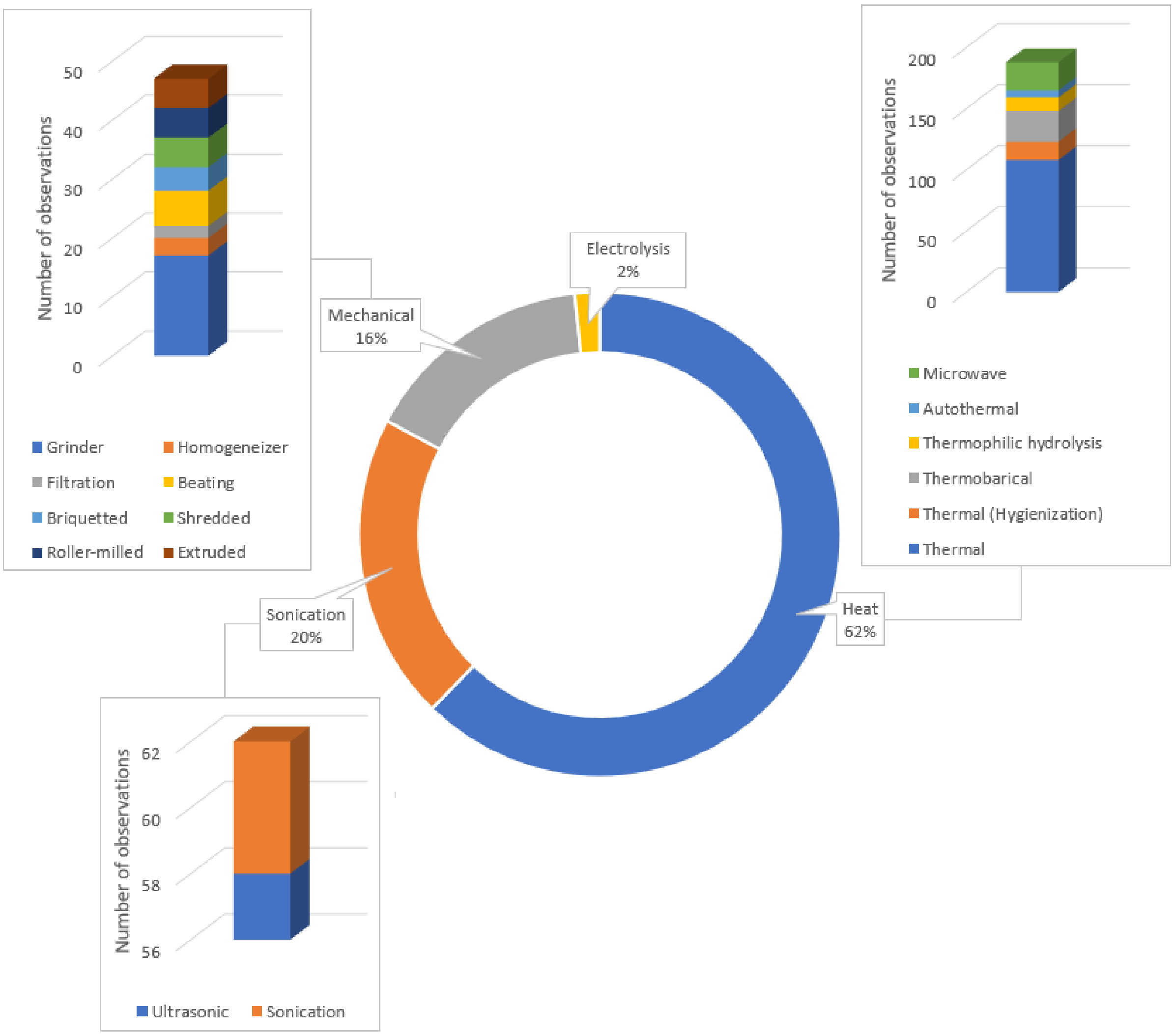

Figure S4. Overview of the different physical pretreatments reported from the studies included in this meta-analysis. Heat is by far the most common physical pretreatment, followed by mechanical methods where grinder prevails and by sonication. Electrolysis was applied in only 2% of the reported cases.

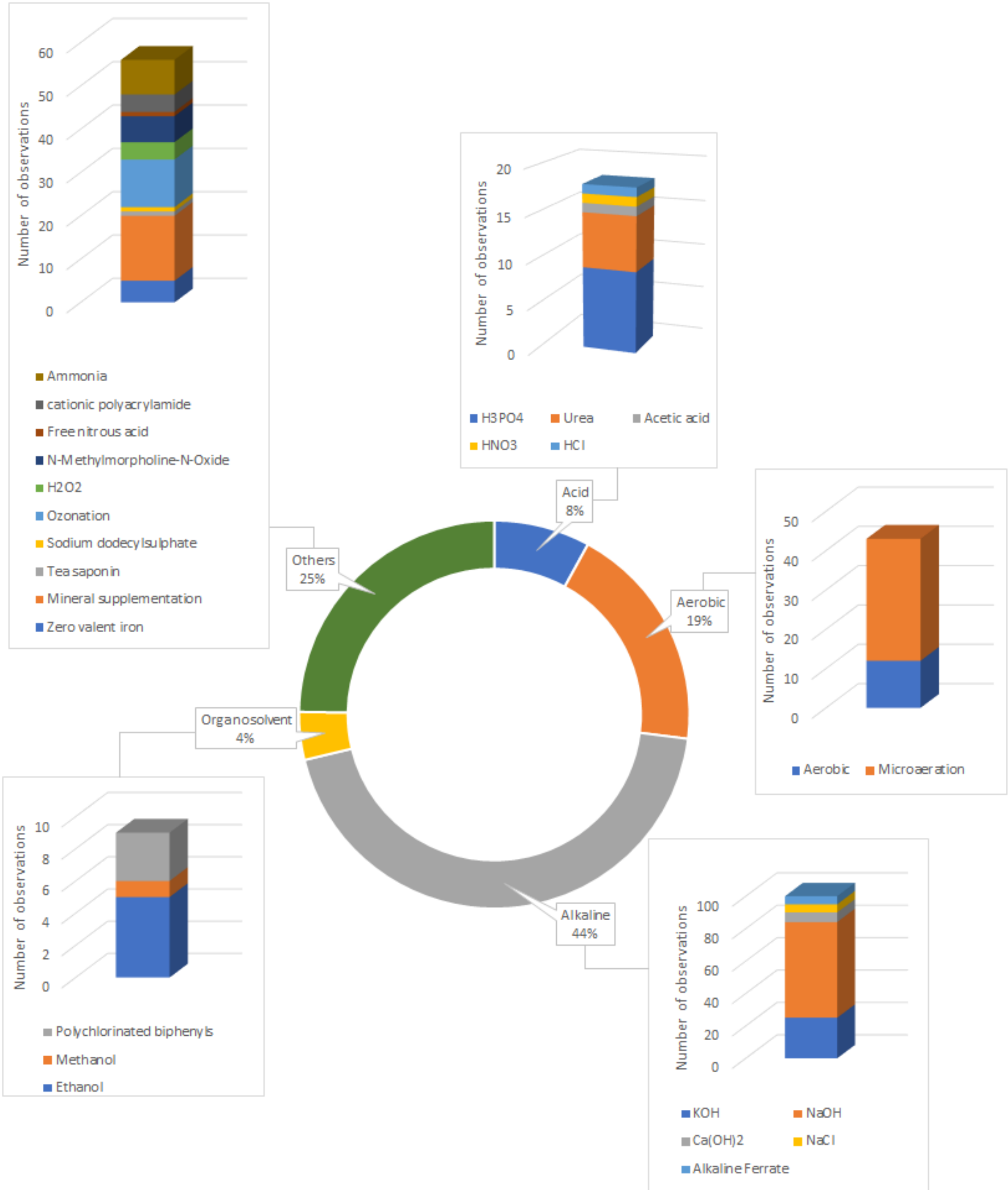

Figure S5. Overview of the different chemical pretreatments reported from the studies included in this meta-analysis. Alkaline methods responded for 44% of the reported cases. Mineral supplementation, ozonation and ammonia removal were among the most applied methods within the category Others with 25% of the studied cases. Among aerobic methods microaeration prevails, while ethanol is the most applied organosolvent.

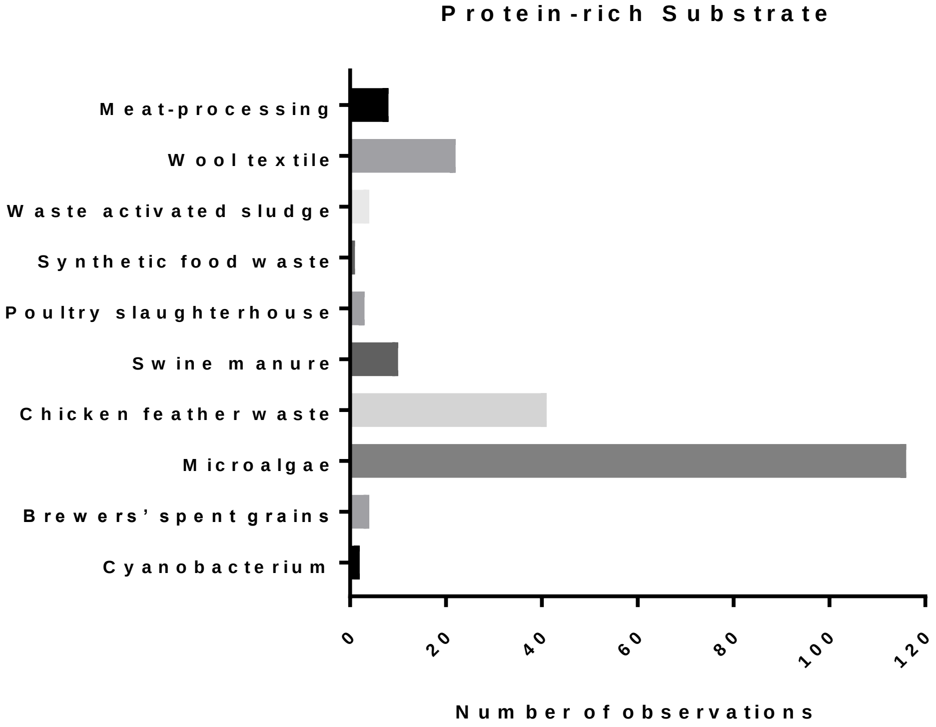

Figure S6. Biomass sources of the protein-rich substrate. Protein-rich substrate is the substrate of any source with majority in protein (>40% dry matter) such as microalgae and different sorts of animal waste.

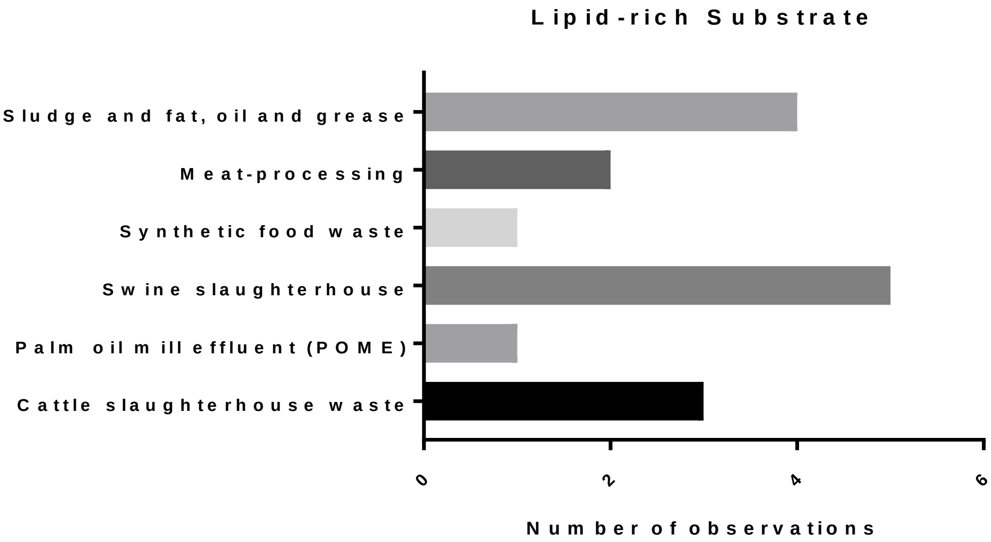

Figure S7. Biomass sources of the lipid-rich substrate. Lipid-rich substrate is predominantly composed of agricultural oil residues and swine slaughterhouse wastewater. This category of substrate had the lowest number of cases (n= 13) reported.

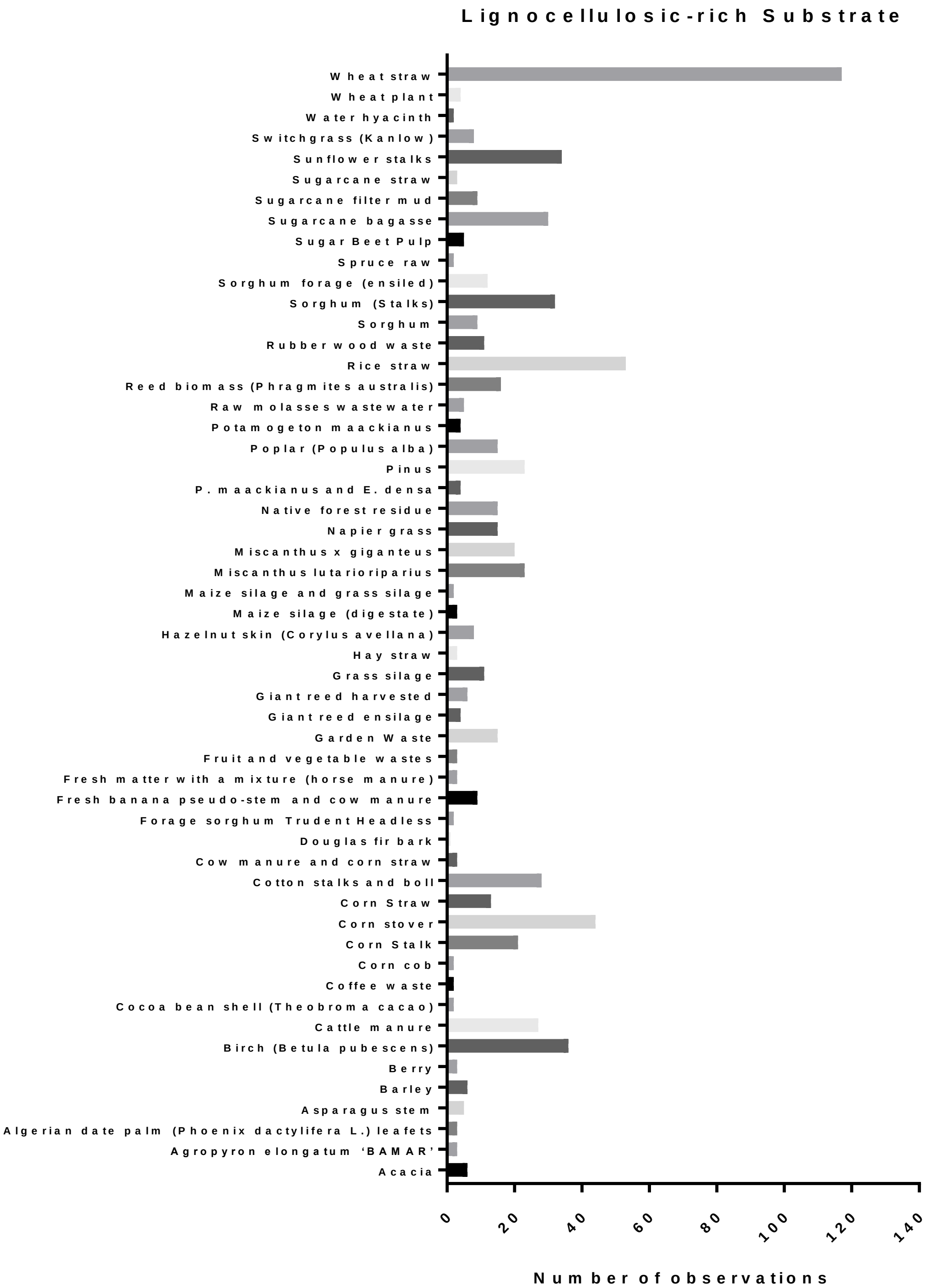

Figure S8. Biomass sources of the lignocellulosic-rich substrate. This category, predominantly composed of crop residues and cattle manure, was by far the one with the highest number of observations (n= 745).

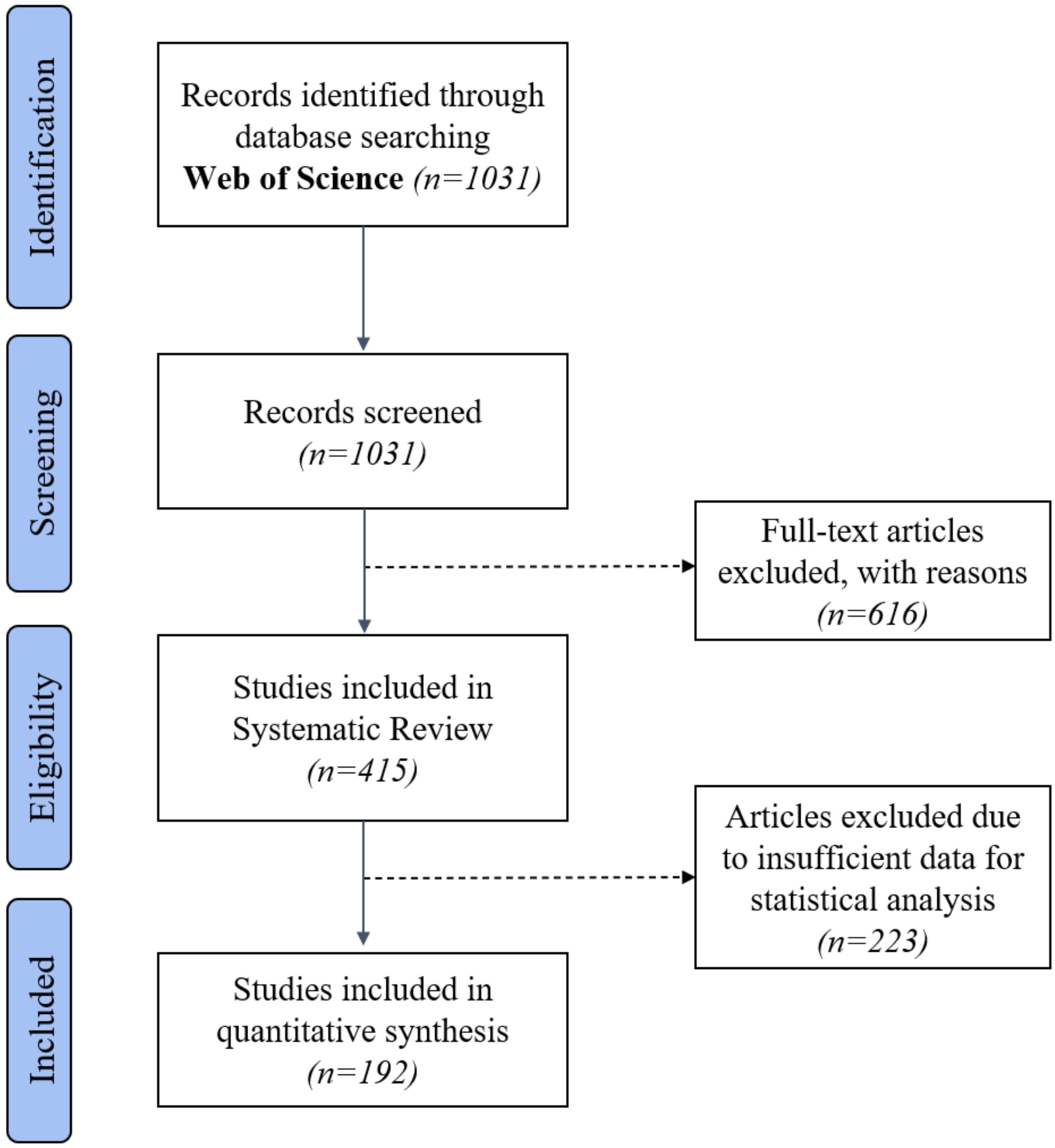

Figure S9. Flow diagram summarizing quantitatively the selection of studies from the systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA, http://www.prisma-statement.org/). n= number of articles

Supplementary Tables

Table S1. Studies included in the meta-analysis (attached in excel).

Table S2. Performance of the most efficient pretreatments for protein-rich substrate and maximum increase in CH_4 production under specific pretreatment configurations. ($\overline{X} = average$)

	CH_4 Yield [a]			
	$\overline{X}$ Untreated	$\overline{X}$ Pretreated	Change (%)	Specific pretreatment configuration with the maximum increase in CH_4 yield (%)[b]
Biological				
Bacterial Keratinase (n=9)	180	270	**50**	↑ 128% From *B. megaterium*, 8 days of degradation [148]
Cellulase (n=2)	253.13	374.34	**47.8**	↑ 72% Concentrated enzyme-substrate, pH 7, for 24 h [58], [158]
Endopeptidase	23.8	244.5	**927.3**	↑ 2163% Alkaline endopeptidase 2 h [88], [122]

(n=16)				
Enzyme Mix (n=1)	188.6	217.3	**15.2**	↑ 15% Enzyme mix (cellulase, glucohydrolase and xylanase) was added (0.5 and 1% w/w) [58]
Lipase (n=2)	410	624.5	**52.3**	↑ 106% From fungus *Penicillium restrictum* [130]
Protease (n=12)	131.9	260.9	**97.8**	↑ 129% Enzyme Alkaline Serine Protease (Savinase 16 L, Type EX, Novozymes, Denmark) [132]
Saccharified (n=1)	255.35	373.03	**46**	↑ 46% Buffer solution (50 mM sodium citrate and pH 4.8 + Celluclast 1.5 L and Novozyme® 188 were added at 35 filter-paper unit (FPU) and 61.5 cellobiose activity units (CBU) in each reactor, respectively at 50 °C [96]
Physical+Physical				
Autoclave (n=13)	196.2	232.9	**18.7**	↑ 1005% 120 °C for 10 min [12], [109], [119], [122], [132]
Hydrothermal (n=6)	102	182.3	**78.8**	↑ 309% 0.82 MPa 200 °C for 0 min [89], [160]
Steam explosion (n=26)	263.6	314.8	**19.4**	↑ 62% 110 ± 5 °C (1.0 ± 0.2 bar) [104], [120], [121], [153]
Thermal+Pressure (Sterilized) (n=1)	580	960	**65.5**	↑ 65% 133 °C and 3 bars for 20 min [131]
Ultrasonication + Electrolysis (n=1)	138	257	**86.2**	↑ 86% Ultrasonication 30 W + Electrolysis 30 V 10 min [49]
Physical				
Electrolysis (n=3)	138	123.6	**10.4**	↑ 31% Electrolysis 30 V 10 min [49]
Homogeneizer (n=3)	317.66	407	**28.1**	↑ 39% Homogeneizer: 220 W and 30 min [153]
Microwave (n=6)	307.6	360.6	**17.3**	↑ 59% 900 W of output power and 3 min [85], [108]
Sonication (n=40)	242.7	282.2	**16.2**	↑ 113% Ultrasonication 30 W 10 min [49], [98], [120], [121], [153]
Thermal (n=12)	276	285.9	**3.5**	↑ 50% Frozen 20 °C [8], [119], [131], [136], [153]
Physical+Physical+Biological				
Autoclave + Alkaline serine protease (n=12)	110	234.1	**112.8**	↑ 475% 0.53 mL/g VS enzyme concentration + 120 °C for 10 min [132]
Autoclave+Lipase from *C. rugosa* (n=2)	731.5	786	**7.4**	↑ 20% 121 °C for 20 min prior to addition of the enzyme [119]

[a] mL/gVS or mL/gCOD.
[b] Reference number of the article in Table S1 shown in square brackets.
n= number of data samples reported.

Table S3. Performance of the most appropriate pretreatments for lignocellulosic-rich substrates (lignin<10% DW) and maximum increase in CH_4 production under specific pretreatment configurations. ($\bar{X} = average$)

	CH_4 Yield [a]			
	$\bar{X}$ **Untreated**	$\bar{X}$ **Pretreated**	**Change (%)**	**Specific pretreatment configuration with the maximum increase in CH_4 yield (%)[b]**
Biological				
Mix Bacteria (n=5)	185	214.8	**16.1**	↑ 33% Bacteria (*Clostridium, Bacteroides, Alcaligenes, and Pseudomonas*) 13 days [95]
Cellulolytic bacteria	185	212.4	**14.8**	↑ 27% Cellulolytic bacteria (*Clostridium*

(n=5)				*straminisolvens*) 13 days [95]
Mix Fungal (n=1)	277.3	351.4	**26.7**	↑ 27% Enzyme (Mixture of Celustar XL and Agropect pomace (3:1) from *Trichoderma longibrachiatum* (fungi) [10]
Fungal (n=3)	125.75	150.69	**19.8**	↑ 34% Fungal (*F. velutipes*) [63]
Fungi and bacteria mixing (n=5)	185	253.4	**36.9**	↑ 50% Fungi and bacteria mixing (*Coprinus cinereus* and *Ochrobactrum sp.*, respectively) [95]
Rumen fluid (n=6)	156.1	238.5	**52.7**	↑ 83% 24 h of pretreatment rumen fluid from the fresh stomach of cattles [30]
Chemical+Physical				
Briquetted + Alkali (n=8)	309.15	335	**8.3**	↑ 14% Briquetted + Injected concentration KOH 6,27% (w/w) [31]
Alkali+Thermal (n=7)	152	171.4	**12.7**	↑ 43% 40 °C at 1 h 10 (% w/w) NaOH dosage [116], [117]
Ultrasonic+Alkaline (n=2)	187	275.5	**47.3**	↑ 71% Dual-frequency (20 KHz and 57 KHz) ultrasonic for 30 min + (2% NaOH) for 36 h [169]
Physical+Physical +Biological				
Autoclave + Enzyme (n=1)	277.3	534.3	**92.6**	↑ 93% 120 °C, 4 bars for 15 min + Mixture of Celustar XL and Agropect pomace (3:1) [10]

[a] mL/gVS or mL/gCOD.
[b] Reference number of the article in Table S1 shown in square brackets.
n= number of data samples reported.

Table S4. Performance of the most appropriate pretreatments for lignocellulosic-rich substrates (lignin10 – 25% DW) and maximum increase in CH_4 production under specific pretreatment configuration. ($\overline{X} = average$

	CH_4 Yield [a]			
	$\overline{X}$ **Untreated**	$\overline{X}$ **Pretreated**	**Change (%)**	**Specific pretreatment configuration with the maximum increase in CH_4 yield (%)[b]**
Biological				
Fungal (n=16)	136.2	230.8	**69.4**	↑ 231% Fungal strain (*Phanerochaete chrysosposrium*) [4], [13], [187]
Cellulolytic enzyme (n=4)	146.5	166.2	**13.4**	↑ 36% 1% Cellulolytic enzyme [88]
Microbes (n=1)	174.3	233.3	**33.8**	↑ 34% 2% of liquid fraction of digestate [26]
Saccharified (n=2)	271.9	366	**34.6**	↑ 38% 30 mL citratre buffer solution (50 mM sodium citrate and pH 4.8 at 50°C) [96]
Chemical				
Alkali (n=28)	27.6	22	**20.2**	↑ 273% 7% NaOH 50g/L [59], [76], [83], [102]
Acid (n=3)	100.7	128.9	**28**	↓ 11%8 mL of 85% phosphoric acid at 60 °C for 45 min in 50 mL plastic centrifuge tube [40]
Urea (n=2)	210.4	289.4	**7.5**	↑ 45%1% Urea [52]
Ammonia (n=1)	174.3	214.5	**23**	↑ 23% 2% ammonia solution [26]
Microaeration (n=4)	152	234.2	**54**	↑ 82% Aeration time: 48 h; equivalent aerated O_2 intensity (mL O_2/gVS): 431 [29]
Nutrient (n=4)	146.5	160	**9.2**	↑ 20% Nutrient concentrations for the basal medium (1 g/L substrate, containing inorganic macronutrients) were (mg/L): NH_4Cl (76.4), KH_2PO_4 (5.18), $MgSO_4 \cdot 7H_2O$ (0.27), $CaCl_2 \cdot 2H_2O$, (10.00), and trace nutrients, 1 mL/L [88]
Chemical+Biological				
Fungal+Moisture (n=18)	120	179.9	**49.9**	↑ 119% *P. ostreatus* 75% at 20 days [20]
Fungal+Acid (n=4)	120	257.5	**14.5**	↑ 150% *N. intermedia* CBS 131.92 (inoculum) + Phosphoric Acid concentration 1.2% (w/v), residence

				time 7 min, and temperature 195 ± 2 °C [141]
Bacterium+Microaeration (n=5)	230.2	256	**11.2**	↑ 17% 0.10 U/mL min after 24 h bacteria microaerobic pretreatment 1 (v/v, biogas liquid/bacteria solution (*Bacillus Subtilis*) [54]
CaO + Liquid Fraction of Digestate (microbes) (n=1)	174.3	274.6	**57.4**	↑ 57% 6% CaO + Liquid Fraction of Digestate (LFD) [26]
Chemical+Physical				
Acid + Mold Size (n=2)	213	223	**4.7**	↑ 3% Acetic acid (HAc) 1% + 68 mm [64]
Size reduction + Alkali (n=16)	274.5	261.7	**-4.6**	↑ 9% KOH 1% + 68 mm [64], [27]
Alkaline + Thermal (n=1)	122.3	173.3	**41.7**	↑ 41% NaOH (0.25 N, temp. 50 ºC, incubation time 30 min) [72]
Acid + thermal (n=9)	160.8	176.1	**9.5**	↑ 22% Furfural acid + 6 days 35 °C [186]
Thermal + Organosolv (n=18)	92.9	172.7	**85.9**	↑ 270% 160 ºC + 50% ethanol [81], [156]
Chemical+Physical+ Physical				
Lewis acids organosolv catalysed FeCl2 + Autoclave (n=6)	189	294.6	**55.8**	↑ 61% H_2SO_4 (40 g of WS was mixed with 1.25 L of aqueous ethanol (EtOH 65%, H_2O 35%) with 8 mmol L1 Lewis acid (or 4.4 mmol L1 H_2SO_4) in a 2 L autoclave during 2 h at 160 ºC [45]
Physical				
Mechanical (Size reduction) (n=28)	154.8	175.6	**13.4**	↑ 55% The extruder barrel is 2.84 m long and the retention time for the biomass varied from 37 to 82 s. Feeding was done at 528 rpm and the extrusion screws ran at 600 rpm [13], [27], [42], [64]
Thermal (n=12)	316.4	411.1	**29.9**	↑ 70% 35 °C for 6 days [9], [23], [178]
Physical+Biological				
Milling + Fungal (n=6)	97	217.6	**124.3**	↑ 166% Fungus *Pleurotus ostreatus* (DSM 11191) 20 days + Milling (<2 mm) [13]
Physical+Physical				
Autoclave (n=7)	219.1	231.5	**5.6**	↑ 18% 121 °C for 30 min [4], [13]
Hydrothermal (n=18)	173	178	**2.9**	↑ 69% 175 °C at 30 min [1], [191]
Steam explosion (n=44)	239.3	264.4	**10.5**	↑ 89% 200 °C for 15 min [15], [114]
Filtration + Temperature (n=2)	261	228.	**-12.6**	↓ 5% Sieved fresh incoculum stored at 4 °C in cold room prior to batch tests [23]

[a] mL/gVS or mL/gCOD.
[b] Reference number of the article in Table S1 shown in square brackets.
n= number of data samples reported.

Table S5. Performance of the most appropriate pretreatments for lignocellulosic-rich substrates (lignin content >25% DW) and maximum increase in CH_4 production under specific pretreatment configuration. ($\overline{X} = average$

	CH_4 Yield [a]			
	$\overline{X}$ **Untreated**	$\overline{X}$ **Pretreated**	**Change (%)**	**Specific pretreatment configuration with the maximum increase in CH_4 yield (%)[b]**
Chemical				
Acid (n=5)	166.32	254.9	**53.2**	↑ 560% Acetic acid (without catalyst) [90], [192]
Alkali (n=10)	235.7	250.8	**6.4**	↑ 132% 12% NaOH [19], [92], [93], [189]
Micro-aeration (n=15)	257	261.3	**1.6**	↑ 7% 5 mL O_2/g VS, Pulse: 1 for 3 days [17]

Organosolv (n=2)	50	265	**430**	↑ 500% Ethanol (without catalyst) [90]
Urea (n=4)	157.5	164.2	**4.2**	↑ 7% Urea 2% 60 day [75]
Chemical+Chemical				
Organosolv+ Acid (n=4)	50	325	**550**	↑ 580% Methanol + Sulfuric Acid/ Ethanol and Sulfuric Acid [90]
Acid + Acid (n=2)	50	295	**490**	↑ 520% Acetic acid and Sulfuric Acid [90]
Chemical+Chemical+Physical+Physical				
Hydrothermal + Organosolv + Acid (n=8)	124	239.1	**92.8**	↑ 123% 160 °C for 30 min and H_2SO_4 with 1% w/w and isopropanol [56]

[a] mL/gVS or mL/gCOD.
[b] Reference number of the article in Table S1 shown in square brackets.
n= number of data samples reported.

APÊNDICE B – Material suplementar do artigo II

1. Unequal geographic distribution of research on biogas from manure

Most studies included in the systematic review (Table S2) were conducted in Asia and Europe: 85% (Fig. S1A). China, the third largest manure global producer, was the country with the highest number of studies (25%), followed by Denmark, Spain, and Germany – surprisingly, not leading countries in manure production (Fig. S1B). Although India and Brazil rank among the largest global manure producers, these countries did not represent a proportional share in the number of studies on manure pretreatment. Their low engagement can be attributed to the lack of public policies towards manure management, renewable energies and biogas production. AD is broadly applied in countries with financial incentives that encourage the involvement of farmers (Loyon et al., 2016; Wissman et al., 2013).

In the USA - the fourth largest manure producer - inconsistent incentives for renewable energy, financial hurdles, difficulties in energy transition, and the lack of a federal climate policy have hindered the further development of AD technologies in the country (Edwards et al., 2015; Gloy and Dressler, 2010). However, the USA made expressive investments in technologies for the reduction of GHG emissions, and is a global leader in research and development, which could explain the relevant number of studies (IEA, 2007).

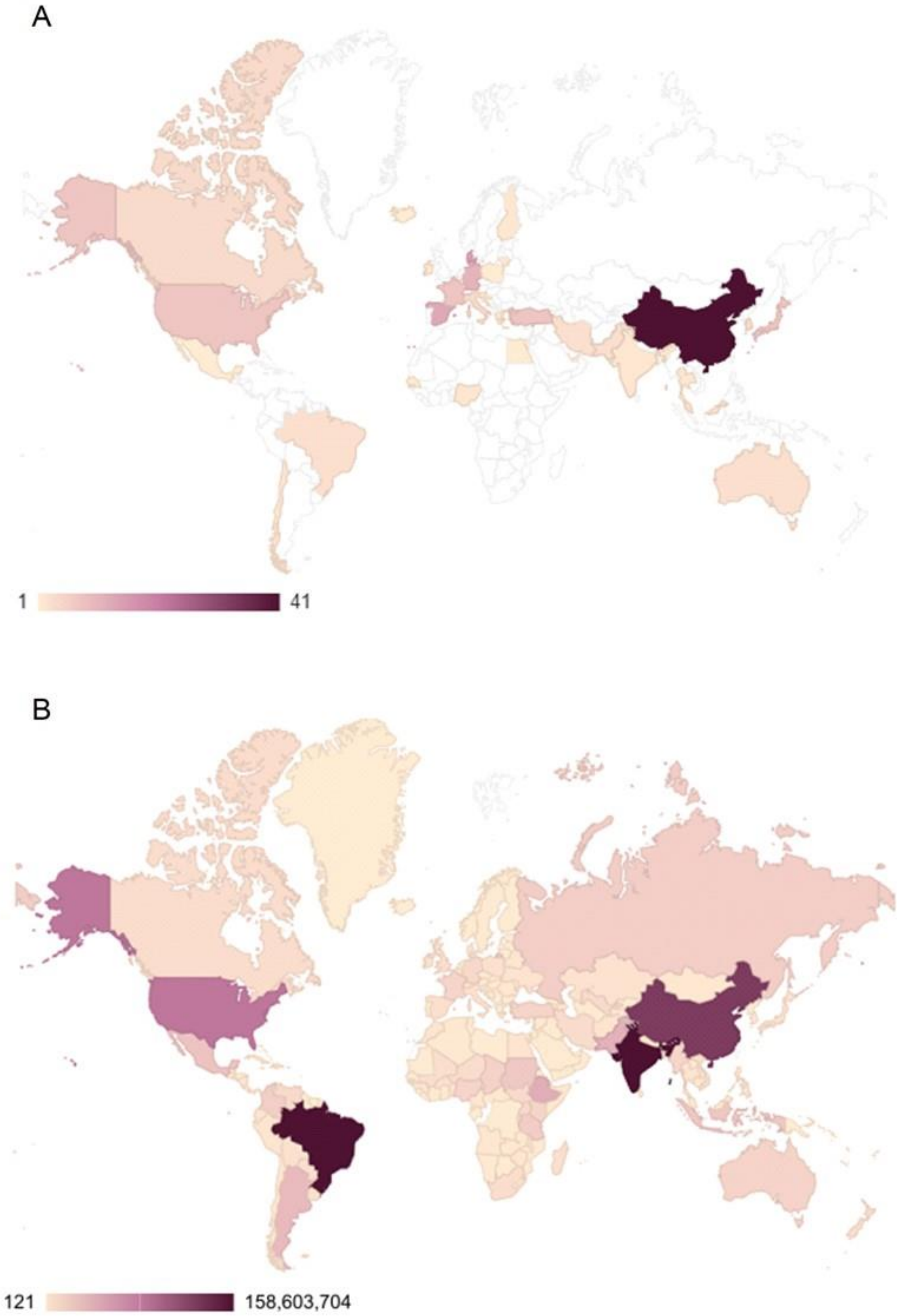

Fig. S1. Geographic distribution of A: number of studies on manure pretreatment and B: manure production (tVS/year). Adapted from (FAOSTAT, 2018).

The high number of studies in Europe could be explained by the EU regulation, that poses limits to manure application on land (Council Directive 91/676/EEC) and describes AD as a suitable treatment for animal manure (Regulation EU 1069/2009). Furthermore, directives such as the Common Agricultural Policy and the Directive on promoting the use of energy from renewable sources (Directive (EU) 2018/2001) established bases for a more sustainable development and provided supporting actions for the implementation of biogas plants. Germany, Spain and Denmark, the three most productive European countries in studies of manure pretreatment (Fig. S1A), have particularly strong policies supporting biogas production (Capodaglio et al., 2016; Zhu et al., 2019).

Among Asian countries, China stands out for having several policies, establishing stronger agricultural control and programs including the production of organic fertilizers, electricity and biogas, such as "Recycling of Livestock Manure policy", "Zero Fertilizer Increase Input Policy" and the "Agricultural Green Development Program in China" (Khoshnevisan et al., 2021; Xu et al., 2018). This reflects on the dominance of this country in studies on pretreatment for AD.

Though India is the largest manure producer in the world (Fig. S1B), the absence of contributions about manure pretreatment for AD in the country is remarkable, with only two studies. Although there is a National Biogas and Manure Management Program, it was designed for small biogas plants, and mostly located in rural and semi-urban households (India, n.d.). In such cases, the use of pretreatment on the manure is unlikely.

In Africa and South America, high amounts of livestock are produced, and animal farming corresponds to 40% and 46% of the agricultural Gross Domestic Product, respectively – reaching up to 80% in some countries (FAO, n.d.; Malabo Montpellier Panel, 2020). However, there are few policies on manure management and AD, and they are often poorly implemented.

In South America, Argentina and Brazil have regional regulations concerning manure and dairy effluent management, while Chile has agreements between the government and farmer federations such as "Cleaner Production Agreements" (Herrero et al., 2018). Even though there are policies to promote the use of renewable energy sources, often enforcement and compliance to their implementation has been low (Venier and Yabar, 2017). Lack of knowledge and legislation have been identified as the main barriers for manure management development in South America (Herrero et al., 2018). An improvement in the regulatory framework could boost the research and development of management methods, helping to solve these issues.

In most of the African continent, energy and nutrient recovery potential in manure is disregarded and management policies are often a part of waste management, shared by multiple ministries, leading to incoherent policies (Ndambi et al., 2019). Even when policies do exist, their enforcement is weak (Teenstra et al., 2014). Ethiopia, Kenya and Rwanda have national biogas programs or strategies to implement biogas and biofertilizer production (EREDPC and SNV/Ethiopia, 2007; Ngigi, 2010; Rwanda, 2011). Furthermore, Burkina Faso, Ethiopia, Kenya, Uganda and Tanzania are part of the Africa Biogas Partnership Program, aiming to provide sustainable and accessible energy through biogas generation and use (ABPP, 2021).

The collection and appropriate treatment of manure can have a high positive economic, social and environmental impact in the manure largest producing countries. Brazil, India and the USA are the largest net importers of fertilizers in the world (OEC, 2019) and the digestate produced from manure AD could allow for an improvement in their autonomy and security of food production, besides increasing sustainability in agriculture and contributing to the construction of a circular economy model (Manyi-Loh et al., 2019).

Currently, large discrepancies in manure treatment are observed among continents. According to the Food and Agriculture Organization (2018), all of the generated swine manure is treated worldwide. In Europe and North America, 99% of chicken manure is also treated, while in Africa, it is only 19%. In Africa and South America, less than 2% of non-dairy cattle manure is treated, as a consequence of specific policies for adequate manure disposal. The regions with the highest amount of manure treatment also concentrate the highest number of studies of pretreatment, indicating that AD is an important manure management technology.

Energy generation from biogas has no geographical limitations and allows for a decentralized energy production, close to the waste source (Santos et al., 2016). Until 2017, the gas pipeline system had a global total length of almost 3 million km (Lu et al., 2020b), distributed over all continents with the largest fraction in North America with 43% of the world total (Lu et al., 2020a). This existing infrastructure can facilitate the biomethane transport, increasing its contribution to the national energy matrix and, consequently, increase the proportion participation of green energy sources.

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APÊNDICE C – Material suplementar do artigo III

Supplementary Materials

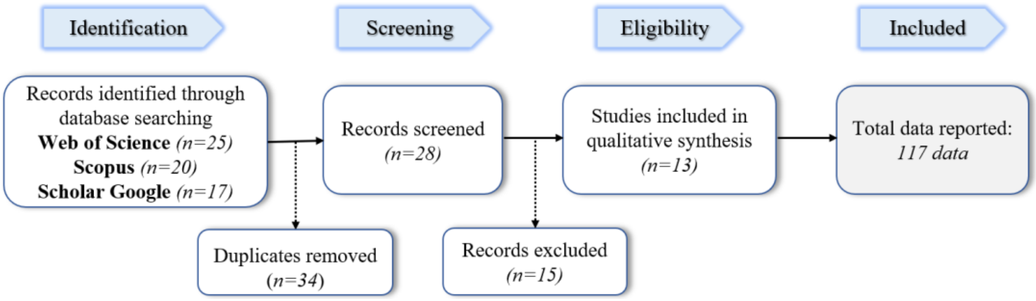

Figure S1. Flow diagram summarizing quantitatively the selection of studies from the systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA, http://www.prisma-statement.org/). n= number of articles

Table S1. Global production of fiber waste from the textile industry and volatile solids (VS) and total solids (TS) content.

Parameters	Fiber waste		unit
	Polyester	Cotton	
World waste production	42	11,7	million tonnes per year
VS%	99,4	79	%TS
TS%	97,5	98,7	%

Table S2. Summary of raw data from studies used to perform meta-analysis of evaluation of pre-treatments applied to different sources of textile waste.

	Textile Waste	Reactor conditions	Pretreatment conditions	CH_4 Yield (mL/g)[3]			Reference
				Average Control	Average Pretrated	Change (%)	
Biological							
	Wool textile[1]	Batch; 37 °C; 50 days;	Alkaline Endopeptidase (Kilo Novo Protease Unit)	43,5	78	79	[1]
	Wool textile[1]	Batch; 55 °C; 46 days;	Alkaline Endopeptidase (Kilo Novo Protease Unit)	19,5	300	1438	[2]
Chemical							
	Wool textile[1]	Batch; 37 °C; 50 days;	Inorganic macronutrients + trace nutrients	43,5	62	42	[1]
	Blue jeans[1]	CSTR – UASB; 55 °C – 24 °C; 30 days;	N-methyl- morpholine-N-oxide (NMMO) soluion concentrated to 85%	179,1	250,5	40	[8]
	Cotton Waste[1]	Batch; 38 °C;	Microaeration [Oxygen Flow Rates (OFR): 0.5 – 4.6 mL/h]	653,3	197,3	-70	[6]
	Cotton Waste[1]	Batch; 38 °C; 30 days	Microaeration [OFR: 0.5 – 4.6 mL/h]	653,3	160,4	-75	[10]
	Textile dyeing sludge[2]	35 °C; 15 days;	NaOH [pH 10]	0,1	33,3	33200	[4]
	Textile dyeing sludge[2]	35 °C; 15 days;	HCl [pH 2]	0,1	21,1	21000	[4]
	Fresh biosludge	Batch; 35 °C; 30 days;	Ozonization [0.005 – 0.01 g O_3/g COD]	246	286,5	16	[7]
Physical							
	Wool textile[1]	Batch; 55 °C; 46 days	Autoclave [120 °C for 10 min]	19,5	130	567	[2]
	Wool textile[1]		Liquid Nitrogen (LN_2)	34	129,7	281	[9]
	Cotton Waste[1]	Semicontinuous anaerobic digesters; 37 °C; 40 days;	Autoclave [120 °C for 10 min]	157,5	203,9	29	[3]
	Textile dyeing sludge[2]	35 °C; 15 days;	Thermal [water bathing at 70 °C for 10 h]	0,1	56,1	56000	[4]
	Textile dyeing sludge[2]	Batch; 35 °C; 23 days;	Thermal [60 °C – 100 °C]	82,1	169,1	106	[5]
	Fresh biosludge[2]	Batch; 35 °C; 30 days;	Sonication [51 kH±6% frequency, 120 watts 30 – 60min]	246	278,5	13	[7]

Textile dyeing sludge[2]		Sonication [4 kHz, 255 W, 0.73 W/mL and 15 min]	113	125	10	[11]
Textile dyeing wastewater[2]	Batch; 37 °C;	UV photodegradation	8,9	15,5	74	[12]
Textile dyeing sludge[2]	Batch; 35 °C; 25 days;	90 °C for 1 h	288,9	347,7	20	[13]
Chemical+Physical						
Cotton Waste[1]	Semicontinuous anaerobic digesters; 37 °C; 40 days;	[Na_2CO_3 + 150 °C 120 min]	157,5	253,8	61	[3]
Textile dyeing sludge[2]	35 °C; 15 days;	Thermal [water bathing at 90 °C for 10 h + NaOH]	0,1	23,6	23500	[4]
Cotton Waste[1]	Batch; 38 °C; 30 days;	Microaeration [OFR: 1.0 – 4.6 mL/h+ H_2SO_4]	653,3	246,6	-62	[10]

[1] Solid Fraction

[2] Liquid Fraction

[3] mL/gVS or mL/gCOD

Reference number of the article in Table S3 shown in square brackets.

Table S3. Studies included in the meta-analysis.

n	Article Title	Authors	Year	DOI
1	Dry anaerobic digestion of lignocellulosic and protein residues	Kabir, M.M., Taherzadeh, M.J., Sárvári Horváth, I.	2015	10.18331/BRJ2015.2.4.5
2	Enhanced methane production from wool textile residues by thermal and enzymatic pretreatment	Kabir, MM; Forgacs, G; Horvath, IS	2013	10.1016/j.procbio.2013.02.029
3	Enhancing energy production from waste textile by hydrolysis of synthetic parts	Hasanzadeh, E; Mirmohamadsadeghi, S; Karimi, K	2018	10.1016/j.fuel.2018.01.035
4	Anaerobic digestion of recalcitrant textile dyeing sludge with alternative pretreatment strategies	Xiang, X., Chen, X., Dai, R., Luo, Y., Ma, P., Ni, S., Ma, C.	2016	10.1016/j.biortech.2016.09.098
5	Effect of low temperature of thermal pretreatment on anaerobic digestion of textile dyeing sludge	Chen, X., Xiang, X., Dai, R., Wang, Y., Ma, P	2017	10.1016/j.biortech.2017.06.138
6	Rapid hydrogen generation from cotton wastes by mean of dark fermentation	Solowski, G; Konkol, I; Shalaby, M; Cenian, A	2020	10.1007/s42452-020-03247-3
7	Effect of Ozonation and Sonication on Biochemical Methane Potential of Biosludge from Textile Mill Effluent	Desiana, D., Setiadi, T.	2009	10.1007/s11267-009-9239-5
8	High-rate biogas production from waste textiles using a two-stage process	Jeihanipour, A; Aslanzadeh, S; Rajendran, K; Balasubramanian, G; Taherzadeh, MJ	2013	10.1016/j.renene.2012.10.042
9	Effect of liquid nitrogen pre-treatment on various types of wool waste fibres for biogas production	Kuzmanova, E., Zhelev, N., & Akunna, J. C.	2018	10.1016/j.heliyon.2018.e00619
10	Methane and hydrogen production from cotton waste by dark fermentation under anaerobic and micro -aerobic conditions	Solowski, G; Konkol, I; Cenian, A	2020	10.1007/978-3-030-13068-8_71
11	Co-digestion Potential of Industrial Sludges with Municipal Sludge	Aksu Bahçeci, H., Sanin, S.L., Sanin, F.D.	2021	10.1007/s12649-021-01409-x
12	Integrated UV photodegradation and anaerobic digestion of textile dye for efficient biogas production using zeolite	Apollo, S., Onyango, M.S., Ochieng, A.	2014	10.1016/j.cej.2014.02.027
13	Optimization and system energy balance analysis of anaerobic co-digestion process of pretreated textile dyeing sludge and food waste	Zhou, WZ; Tuersun, N; Zhang, YZ; Wang, Y; Cheng, C; Chen, XG	2021	10.1016/j.jece.2021.106855

APÊNDICE D – Material suplementar do artigo IV

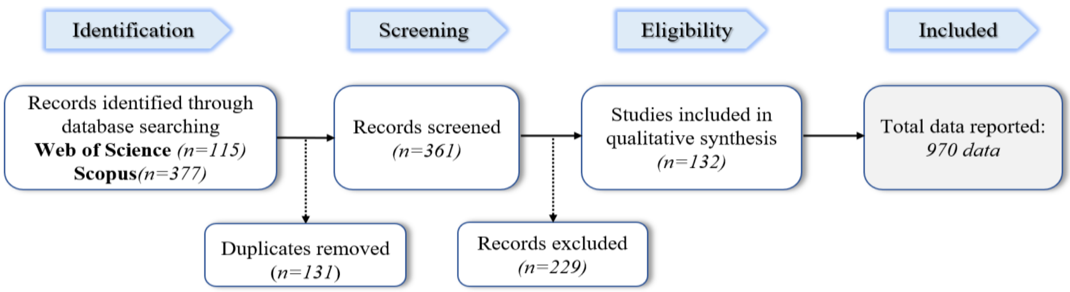

Fig. S1. Flow diagram summarizing quantitatively the selection of studies from the systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA, http://www.prisma-statement.org/). n= number of articles.

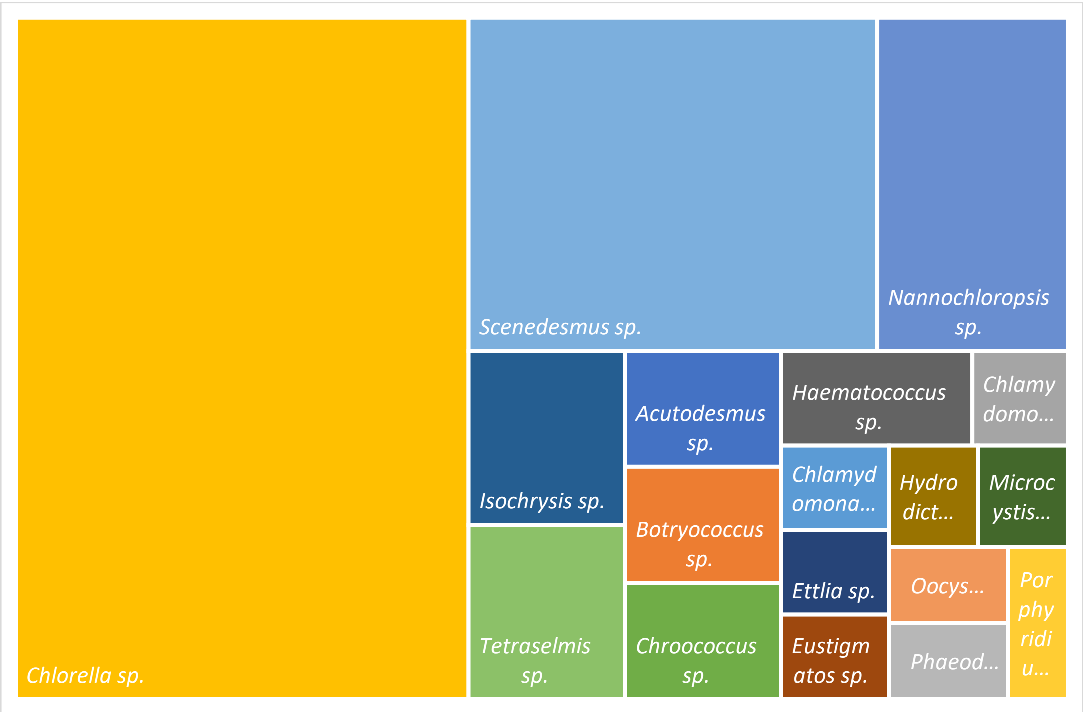

Fig. S2. Diversity of microalgae species reported in the systematic review applied to biogas production.

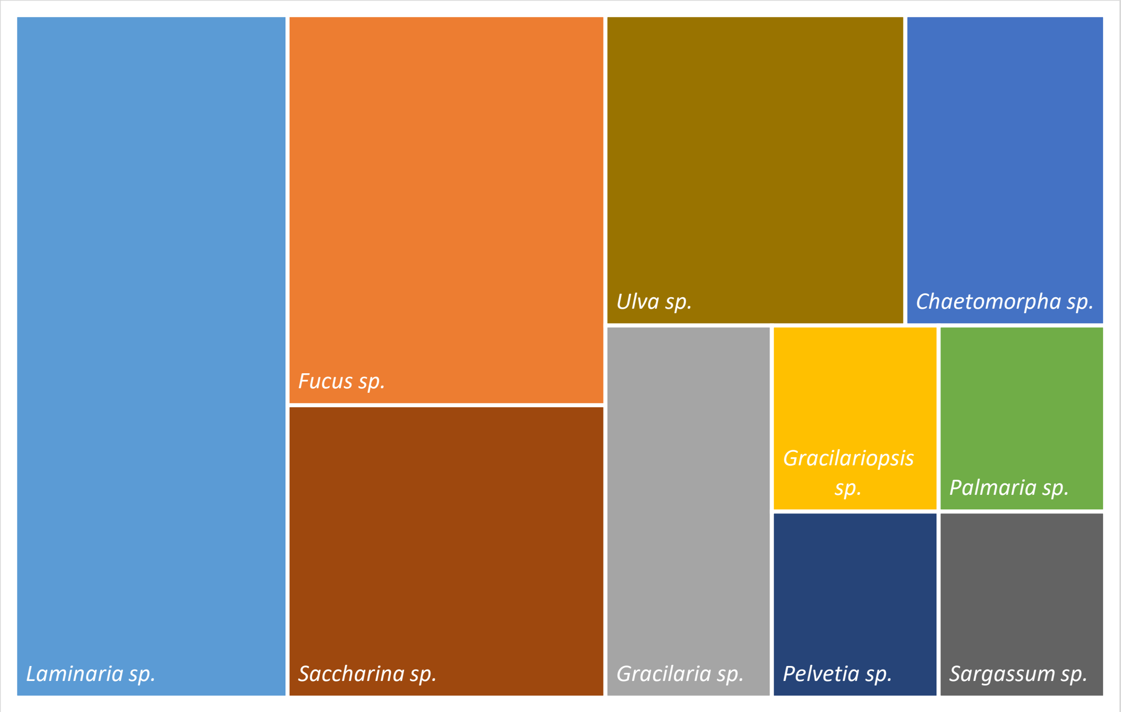

Fig. S3. Diversity of macroalgae species reported in the systematic review applied to biogas production.

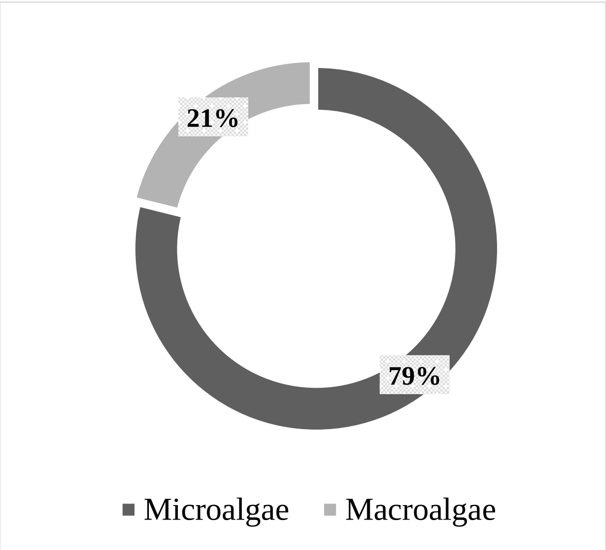

Fig. S4. Quantification (%) of micro and macroalgae reported in the studies included in the meta-analysis.

Table S1. Studies included in the meta-analysis. (Attached in excel file).

Table S2. Global production of algal biomass and volatile solids (VS) and total solids (TS) content.

Parameters	Algae	Unit	Reference
World production	36	million tonnes per year	(Cai et al., 2021)
World production (10%)	3,6	million tonnes per year	
VS%	87,65	%TS	[2,3]
TS%	91,9	%	

Reference number of the article in Table S1 shown in square brackets.

Table S3. Chemical composition in terms of dry matter (%DW) from species of algal applied in biotechnology (minimum–maximum). References are provided in Table S1 and adapted from (Suganya et al., 2016).

	Protein (%DW)	**Carbohydrates (%DW)**	**Lipid (%DW)**
MICROALGAE			
Acutodesmus platenses	38,2	26,5	26,2
Anabaena cylindrica	43–56	25–30	4–7
Botryococcus braunii	9,1	21,5	19,2
Chlamydomonas reinhardtii	48–64,7	17–22,6	18–21
Chlorella pyrenoidosa	57	26	2
Chlorella sorokiniana	45,5	23,7	26,2
Chlorella sp.	42,9–65,8	16–24,6	4–17
Chlorella vulgaris	25,2–66,9	12–37	3,5–24,5
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Ettlia sp.	35	45,5	5,5
Euglena gracilis	39–61	14–18	14–20
Nannochloropsis gaditana	41,9–46,8	20,6–23	6,5–16,3
Nannochloropsis oculate	46,8–54,2	14,9–19,3	15,7–19,5
Nannochloropsis salina	16,3	18	42
Oocystis sp.	58	22	20
Phaeodactylum tricornutum	45,6	14,4	27,4
Porphyridium cruentum	28–39	40–57	9–14
Prymnesium parvum	28–45	25–33	22–39
Scenedesmus	26,9–53	13,5–25,9	8,1–13
Scenedesmus dimorphus	8–1 8	21–52	16–40
Scenedesmus obliquus	50–56	10–17	12–14
*Spirogyra*sp.	6–20	33–64	11–21
Spirulina máxima	60–71	13–16	6–7
Spirulina platenses	46–63	8–14	4–9
Synechoccus sp.	63	15	11
Tetraselmis maculate	52	15	3
Tetraselmis striata	49,4	16	6,4
MACROALGAE			
Acanthophora spicifera	12–13,2	11,6–13,2	10–12
Boergesenia forbesii	7,4	21,4	11,4
Caulerpa cupressoides	7,4	51,8	11
Caulerpa fergusonii	7,8	23,6	7,2
Caulerpa laetevirens	8,8	56,3	8,8
Caulerpa peltate	6,4	45	11,4
Caulerpa racemose	8,8–12,5	16–33,8	9–10,6
Caulerpa sertularioides	9,1	49,5	7
Chaetomorpha aérea	10,1	31,5	8,5
Chaetomorpha antennina	10,1–50	15–27	2,1–11,5
Chaetomorpha linoides	9,5	27	12

Cladophora fascicularis	15,5	49,5	15,7
Codium adhaerens	7,3	40,5	7,4
Codium decorticatum	6,1	50,6	9
Codium tomentosum	5,1	29,3	7,2
Dictyosphaeria cavernosa	6	42,8	10,5
Enteromorpha compressa	7,3	24,8	11,5
Gracilaria manilaensis	14,2	75,7	0,4
Gracilaria vermiculophylla	35,3–42,9	26,5–34,5	0–0,2
Gracilariopsis pérsica	10,5	79	0,2
Halimeda macroloba	5,4	32,6	9,9
Hypnea valentiae	11,8–12,6	11,8–13	9,6–11,6
Laurencia papillosa	11,8–12,9	12–13,3	8,9–10,8
Microdictyon agardhianum	20,9	27	9,4
Palmaria palmata	14,3	47,3	1,1
Ulva lactuca	11,4–12,6	11,6–13,2	9,6–11,4
Ulva reticulate	12,8	16,9	8,5
Ulva sp.	11,4	33,2	1,8
Valoniopsis pachynema	8,8	31,5	9,1

Table S4. Performance of the most efficient pretreatments for macroalgae substrate and maximum increase in CH_4 production under specific pretreatment configuration.

	CH_4 Yield (mL/g of VS)				
	Untreated	**Pretreated**	**Change (%)**	**Specific pretreatment configuration with the maximum increase in CH_4 yield (%)**	**References**
Physical					
Autoclave (n=4)	308	261	**-15,3**	120 °C for 30 min (-3%)	[77]
Dispenser (n=2)	33	124,5	**277,3**	Thermo disperser liquefaction -80 °C for 30 min (351,5%)	[92]
Hydrothermal (n=24)	208,8	203,5	**-2,5**	140 °C for 30 min SF 2.65 (180,8%)	[54], [90], [105], [114]
Macerated (n=43)	181,2	197,2	**8,8**	Beating for 5 min (147,8%)	[49], [60], [66], [67], [71], [89], [112], [124]
Microwave (n=8)	107,2	144,9	**35,2**	600 W, 2 min (1465%)	[33], [49], [66], [71]
Microwave + Ultrasonic (n=1)	13,7	260	**1797,8**	600 W, 2 min; 110 V for 15 min (797,8%)	[49]
Steam Explosion (n=2)	223	264	**18,4**	130 °C for 10 min (20%)	[108]
Thermal (n=4)	130,8	165,4	**26,5**	100 °C for 120 min (58,8%)	[33], [84], [77]
Ultrasonic (n=1)	13,7	230	**1578,8**	110 V, 15 min (1578,8%)	[49]
Washed (n=1)	295	430	**45,8**	Rinsed with water to remove any salt (45,8%)	[67]
Washed + Macerated (n=1)	295	481	**63,1**	Rinsed with water to remove any salt + cutted less than 0.5 cm and crushed with a motar (63,1%)	[67]
Washed + Thermal + Macerated (n=1)	295	349	**18,3**	Rinsed with water to remove any salt + dried at 37 °C + cutted less than 0.5 cm and crushed with a motar (18,3%)	[67]

Whased + Thermal (n=2)	363	334	**-8**	Rinsed with water to remove any salt + dried at 37 °C (9,8%)	[67], [86]

Reference number of the article in Table S1 shown in square brackets.

Table S5. Performance of the most efficient pretreatments for microalgae substrate and maximum increase in CH_4 production under specific pretreatment configuration.

	CH_4 Yield (mL/g of VS or COD)				
	Untreated	**Pretreated**	**Change (%)**	**Specific pretreatment configuration with the maximum increase in CH_4 yield (%)**	**References**
Biological					
Bacteria (n=16)	264	337,6	**27,9**	Ruminal fluid (Fibrobacter and Ruminococcus) (176%)	[43], [45], [74], [96] [113], [121]
Enzyme (n=70)	204,1	348,5	**70,7**	Enzymatic (Cellulase, Endogalactouronase, Esterase and Protease) (561,3%)	[5], [13], [14], [30], [38], [44], [47], [50], [63], [65], [83], [106], [116], [120], [123]
Fungal (n=15)	319,9	369,7	**15,6**	Mix of Anaeromyces, Neocallimastix, Orpinomyces and Piromyces (85,7%)	[46], [115], [120], [128]
Physical					
Autoclave (n=37)	176,7	236,9	**34,1**	121 °C for 10 min (461%)	[1], [2], [14], [18], [28], [32], [37], [39], [68], [74], [75], [106], [111], [127]
Constant Magnetic Field (CMF) (n=6)	285	222,02	**-22,1**	6 pumping cycles through the CMF area (times/h) / 24 dm³/h (11%)	[129]
Electrolysis (n=3)	138	123,6	**-10,4**	Electrolysis 30 V 10 min (31%)	[4]
Homogenizer (n=6)	317,6	407	**28,1**	Disperser (IKA T25 ultra Turrax Disinter homogenizer) (203%)	[30], [56], [92]
Hydrothermal (n=23)	222,1	217,5	**-2,1**	180 °C for 30 min with hydrothermal treatment severity 4,06 (209%)	[8], [31], [40], [48], [93], [94], [102], [107]
Microwave (n=20)	155,7	164,2	**5,4**	Microwave (Samsung M1914, 2450 MHz frequency) 900W for 3 min at 98 °C (78%)	[16], [79], [98], [126]
Milling (n=11)	191,1	196,5	**2,8**	Glass beads (1 mm diameter) was agitated at 900 RPM using a 20-mm diameter Rushton turbine for 4 h (79%)	[59], [69], [116]
Steam Explosion (n=29)	234,6	280,1	**19,4**	165 °C 7 bar for 30 min (141%)	[14], [22], [23], [27], [18], [34]
Thermal (n=80)	156,4	218,2	**39,5**	100 °C for 8 h (180%)	[6], [14], [15], [26], [28], [35], [38], [41], [45],

					[63], [70], [73], [78], [91], [95], [97], [98], [109], [116], [117]
Thermal + Pressure (n=9)	193,1	391,4	**102,7**	160 °C 6 bar for 10 min (65%)	[6], [10], [19]
Ultrasonic (n=107)	211,8	253,6	**19,7**	Constant frequency of 20 kHz and an ultrasonic power of 150 W (164%)	[4], [7], [12], [14], [22], [23], [18], [23], [28], [34], [51], [57], [59], [61], [63], [68], [79], [82], [98], [116], [122], [125]
Ultrasonication + Electrolysis (n=1)	138	257	**86,2**	Ultrasonication (Tomy UD 201) 30 W for 30 min + Electrolysis 30 V 10 min (86%)	[4]
Chemical + Physical					
Alkaline + Thermal (n=22)	185,6	226,6	**22,1**	2% (w/v) NaOH + 95 °C for 24 h (108%)	[1], [35], [70], [80], [107]
Acid + Autoclave (n=4)	134,9	232,1	**72,1**	H_2SO_4 (0.1% v/v) + 150 °C for 60 min relative pressure of 101.3 kPa (93%)	[18], [29]
Acid + Hydrothermal (n=2)	213,4	277,8	**30,2**	H_2SO_4 (2% v/v) + 135 °C for 15 min (45%)	[102], [110]
Acid + Steam Explosion (n=1)	206,82	246,13	**19**	H_2SO_4 (2% v/v) + 135 °C for 15 min (19%)	[110]
Acid + Thermal (n=2)	138,3	168,4	**21,8**	HCl (3% (w/w)) + 105 °C for 1.7 h (26,8%)	[15], [36]
Acid + Ultrasonic (n=9)	104	209,7	**101,6**	3 N HCl (pH 1) + 20 kHz frequency and a power of 150 W at 60 min (129%)	[51]
Alkaline + Microwave (n=1)	124	161	**29,8**	1% NaOH + Microwave at 250 W (29%)	[17]
Alkaline + Autoclave (n=3)	133,9	217,8	**62,7**	4 M NaOH + 120 °C for 40 min (73%)	[17], [18]
Alkaline + Ultrasonic (n=5)	240,4	250,2	**4,1**	0.2 M NaOH + 130 W (20 kHz) for 5 min (50%)	[17], [35], [82]
Organic Solvent + Thermal (n=4)	319	296,3	**-7,1**	20 mL of organic solvents (mixture of 80 % vol n-hexane and 20 % vol. of diethyl ether) + 110 °C for 1 h (15%)	[80]
Ultrasonic + Zero-Valent Iron (ZVI) (n=5)	33,1	48,46	**46,4**	Ultrasound 40 kHz for 30 min + 20 g ZVI/g TS for 30 min (84,8%)	[57]

Reference number of the article in Table S1 shown in square brackets.

Alzate, M.E., Muñoz, R., Rogalla, F., Fdz-Polanco, F., Pérez-Elvira, S.I., 2012. Biochemical methane potential of microalgae: Influence of substrate to inoculum ratio, biomass concentration and pretreatment. Bioresour Technol 123, 488–494. https://doi.org/10.1016/j.biortech.2012.06.113

Cai, Y., Zheng, Z., Schäfer, F., Stinner, W., Yuan, X., Wang, H., Cui, Z., Wang, X., 2021. A review about pretreatment of lignocellulosic biomass in anaerobic digestion: Achievement and challenge in Germany and China. J Clean Prod. https://doi.org/10.1016/j.jclepro.2021.126885

Suganya, T., Varman, M., Masjuki, H.H., Renganathan, S., 2016. Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach. Renewable and Sustainable Energy Reviews. https://doi.org/10.1016/j.rser.2015.11.026

Zou, Y., Xu, X., Li, L., Yang, F., Zhang, S., 2018. Enhancing methane production from U. lactuca using combined anaerobically digested sludge (ADS) and rumen fluid pre-treatment and the effect on the solubilization of microbial community structures. Bioresour Technol 254, 83–90. https://doi.org/10.1016/j.biortech.2017.12.054

APÊNDICE E – Material suplementar do artigo V

Table S1. Characteristics of digestate from the original collection in the industrial-scale biogas digesters.

Code	Temperature (°C)	Dominant feedstock	HRT (d)	OLR (kg VS/m^3 per day)	Treatments	Country
2A	40	Manure		N.A.	N.A.	Sweden
2B	37	Sewage sludge	22	N.A.	No	Sweden
2C	36	Sewage sludge	20	N.A.	No	Sweden
2D	41	Agricultural biomas		N.A.	N.A.	Sweden
3A	41	Food waste	40	N.A.	Hygienisation (Post AD)	Sweden
3B	38	Manure	41	3,7	Hygienisation (Post AD)	Sweden
3C	52	Food waste	41,5	2,56	Hygienization (Pre-AD)	Sweden
3D	38	Food waste	30	4	Hygienization (Pre-AD)	Sweden

4A	40	Food waste	32,5	2,4	Hygienization (Pre-AD)	Norway
4B	53	Food waste			N.A.	Sweden
4C	53	Food waste	23	5,4	No	Sweden
5A	44	Food waste		N.A.	N.A.	Sweden
5B	40	Agricultural biomas		N.A.	N.A.	Sweden
5C	56	Food waste	43	2,6	No	Sweden
5D	53	Food waste		N.A.	N.A.	Sweden
6A	52,5	Manure		N.A.	N.A.	Denmark
6B	51	Manure		N.A.	N.A.	Sweden
6C	51	Manure		N.A.	N.A.	Denmark
6D	50	Manure		N.A.	N.A.	Denmark
7A	41	Food waste	25	3,3	No	Sweden
7B	40	Manure	30		N.A.	Sweden
7C	42	Food waste	35	4,5	Hygienization (Pre-AD)	Sweden
7D	38	Sewage sludge	20	2	No	Sweden

HTR: hydraulic retention time; OLR: organic loading rate; N.A.: Not available; AD: anaerobic digestion.

APÊNDICE F – Produção científica

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Methane yield response to pretreatment is dependent on substrate chemical composition: a meta-analysis on anaerobic digestion systems

Thuane Mendes Anacleto[1,2], Betina Kozlowsky-Suzuki[3,4,5], Annika Björn[6,7], Sepehr Shakeri Yekta[6,7], Laura Shizue Moriga Masuda[8], Vinícius Peruzzi de Oliveira[2] & Alex Enrich-Prast[2,6,7,9]

Proper pretreatment of organic residues prior to anaerobic digestion (AD) can maximize global biogas production from varying sources without increasing the amount of digestate, contributing to global decarbonization goals. However, the efficiency of pretreatments applied on varying organic streams is poorly assessed. Thus, we performed a meta-analysis on AD studies to evaluate the efficiencies of pretreatments with respect to biogas production measured as methane yield. Based on 1374 observations our analysis shows that pretreatment efficiency is dependent on substrate chemical dominance. Grouping substrates by chemical composition e.g., lignocellulosic-, protein- and lipid-rich dominance helps to highlight the appropriate choice of pretreatment that supports maximum substrate degradation and more efficient conversion to biogas. Methane yield can undergo an impactful increase compared to untreated controls if proper pretreatment of substrates of a given chemical dominance is applied. Non-significant or even adverse effects on AD are, however, observed when the substrate chemical dominance is disregarded.

Anaerobic digestion (AD) is a successful and robust waste treatment biotechnology converting organic waste into clean energy in the form of biogas[1] and recovering nutrients as fertilizers and soil conditioners[2]. AD plays a crucial role in achieving the ambitious goal of the European Climate Law, aiming for climate neutrality by 2050[2]. An estimated increase from 0.3 EJ to 8.3 EJ by 2050 from biogas upgraded to biomethane (90% methane) makes it the non-fossil source with the greatest potential to be carbon neutral[2]. AD systems mitigate the emission of greenhouse gases (GHG), by recovering methane (CH_4) from organic wastes, and, when used as a combustion fuel, release carbon–neutral carbon dioxide (CO_2)[3]. About 60 to 80% of GHG emissions from transportation can be reduced by replacing gasoline with biomethane produced from AD[4]. Currently, the global potential for energy generation from biogas is estimated to be 10,000 to 14,000 TWh, with the potential to replace up to 10% of the world's primary energy consumption[5] of electric power, heat and automotive fuel. Unlike other sources of non-fossil energy, organic residues are the raw primary source for biogas production, which is relatively less sensitive to seasonality or scarcity.

Due to integrated socioenvironmental benefits[1] e.g., the replacement of energy resources such as firewood by biogas can improve quality of life, and promote gender equality, and higher educational levels[6]. AD surpasses several other renewable energy sources[7] representing the major technological pathway for the implementation of the

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Boosting manure biogas production with the application of pretreatments: A meta-analysis

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ABSTRACT

Anaerobic digestion (AD) is a versatile manure management approach that can combine waste treatment, energy generation and nutrient recovery, thus playing a central role in circular economy. The AD process is highly influenced by manure composition which, depending on the source, may contain high loads recalcitrant materials (e.g., lignocellulosic and fibers) or lead to the formation of toxic compounds (e.g., NH_3), decreasing the energetic potential of the waste and requiring specific pretreatments to increase its degradability and biogas production. Although there are distinctions in the chemical composition of manure according to animal diets, different manure sources are usually grouped together, leading to a suboptimal performance of both the pretreatment and the AD process. Here, we performed a meta-analysis of 54 studies to evaluate the effects of different pretreatments on different manure types and their effect on methane (CH_4) yield and we estimated the energy potential if the appropriate pretreatment is applied to largest manure producing countries. The results showed that chemical and/or biological pretreatments were more effective for omnivore manure (e.g., swine, chicken), while physical and a combination of chemical and physical pretreatments negatively affected CH_4 production. Physical and/or chemical pretreatments had a positive effect on CH_4 yield from herbivore manure (e.g., cattle, horses), while biological pretreatments had a negative effect. The application of the adequate pretreatment can more than double the energy recovered from manure, allowing for an important substitution of fossil fuels, while decreasing operational costs and environmental risks and ultimately improving profitability. The development of pretreatment technologies and their application are strongly related to public policies for sustainable manure management and biogas use and production.

1. Introduction

The global consumption of animal products per capita has doubled over the last 40 years, boosting the growth of the livestock sector especially in developing countries (Shober et al., 2018; Zhang et al., 2019). Consequently, huge amounts of waste and manure are produced, which lead to a growing concern over environmental issues. Inadequate manure disposal leads to several environmental problems, such as greenhouse gas (GHG) emissions, boosting climate change; acidification; particulate matter formation caused by NH_3 e NO_x; and eutrophication of soils, waterbodies and groundwaters (De Vries et al., 2012; Petersen et al., 2013).

Anaerobic digestion (AD) is one of the most versatile strategies for manure management. Based on the biochemical degradation of organic

Abbreviations: AAS, Aqueous Ammonia Soaking; AD, Anaerobic Digestion; BS, Biological Supplements; CI, Confidence Intervals; COD, Chemical Oxygen Demand; EU, European Union; GHG, Greenhouse Gas; LMC, Lignocellulolytic Microbial Consortium; MG, Manure Generation; NBW, Nano-bubble Water; OM, Organic Matter; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis; RR, Response Ratio; SMG, Specific Manure Generation; TAN, Total Ammonia Nitrogen; TS, Total Solids; USA, United States of America; VS, Volatile Solids.

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Article

Comprehensive Meta-Analysis of Pathways to Increase Biogas Production in the Textile Industry

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Abstract: The textile industry is one of the largest environmental polluters in the world. Although waste management via anaerobic digestion (AD) is a sustainable strategy to transform waste into clean energy and water recovery, the efficiency of the AD process is reduced by the presence of recalcitrant materials, chemicals, and toxic contents. This study aims to investigate the performance of several chemical, physical, and biological pretreatments applied to improve the biodegradability of textile waste. We performed a meta-analysis with 117 data extracted from 13 published articles that evaluated the efficiency of pretreatments applied to textile waste prior to AD to increase biogas production measured as methane (CH_4) yield. Even though the majority of the studies have focused on the effect of chemical and physical pretreatments, our results showed that the application of biological pretreatments are more efficient and eco-friendlier. Biological pretreatments can increase CH_4 yield by up to 360% with lower environmental risk and lower operating costs, while producing clean energy and a cleaner waste stream. Biological pretreatments also avoid the addition of chemicals and favor the reuse of textile wastewater, decreasing the current demand for clean water and increasing resource circularity in the textile industry.

Keywords: textile residues; biotechnology; methane; circular economy; fibers; cotton

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1. Introduction

The textile industry is one of the largest polluting sectors worldwide, with an estimated waste production of 92 million tons per year [1], including pre- (i.e., agricultural production, fiber production, wastewater, solid waste) to post-consumer (i.e., manufacturing, logistics, retail and mixtures of discarded clothing or household items) waste in the supply chain [2]. Over 8000 chemicals (e.g., dyes, suspended solids, chlorinated aromatic hydrocarbons, surfactants, and heavy metals) are used in the textile supply chain [3,4]. As a result, effluents and solid waste with high loads of hazardous chemicals are discharged, thus increasing the toxicity of the produced waste, with a high pollution risk to the environment and human health [5].

Sustainable manufacturing is crucial to reducing the environmental impact of fashion and the textile industry. Projects and policies aiming at the sustainable development of the market, such as the [6], "Strategic Agenda on Textile Waste Management and Recycling", Expert Network on Textile Recycling (ENTeR), and Conference of the Parties (COP 21), as well as the 2030 Agenda for Sustainable Development Goals (SDGs), have been important players in reframing textile production. Incentive actions for reuse are also crucial to the

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RESEARCH Open Access

Effluent solids recirculation to municipal sludge digesters enhances long-chain fatty acids degradation capacity

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Abstract

Background: Slow degradation kinetics of long-chain fatty acids (LCFA) and their accumulation in anaerobic digesters disrupt methanogenic activity and biogas production at high loads of waste lipids. In this study, we evaluated the effect of effluent solids recirculation on microbial LCFA (oleate) degradation capacity in continuous stirred-tank sludge digesters, with the overall aim of providing operating conditions for efficient co-digestion of waste lipids. Furthermore, the impacts of LCFA feeding frequency and sulfide on process performance and microbial community dynamics were investigated, as parameters that were previously shown to be influential on LCFA conversion to biogas.

Results: Effluent solids recirculation to municipal sludge digesters enabled biogas production of up to 78% of the theoretical potential from 1.0 g oleate l^{-1} day^{-1}. In digesters without effluent recirculation, comparable conversion efficiency could only be reached at oleate loading rates up to 0.5 g l^{-1} day^{-1}. Pulse feeding of oleate (supplementation of 2.0 g oleate l^{-1} every second day instead of 1.0 g oleate l^{-1} every day) did not have a substantial impact on the degree of oleate conversion to biogas in the digesters that operated with effluent recirculation, while it marginally enhanced oleate conversion to biogas in the digesters without effluent recirculation. Next-generation sequencing of 16S rRNA gene amplicons of bacteria and archaea revealed that pulse feeding resulted in prevalence of fatty acid-degrading *Smithella* when effluent recirculation was applied, whereas *Candidatus Cloacimonas* prevailed after pulse feeding of oleate in the digesters without effluent recirculation. Combined oleate pulse feeding and elevated sulfide level contributed to increased relative abundance of LCFA-degrading *Syntrophomonas* and enhanced conversion efficiency of oleate, but only in the digesters without effluent recirculation.

Conclusions: Effluent solids recirculation improves microbial LCFA degradation capacity, providing possibilities for co-digestion of larger amounts of waste lipids with municipal sludge.

Keywords: Anaerobic digestion, Primary and activated sewage sludge, Microbial community, Oleate, Feeding frequency, Sulfide

Background

Increasing biogas production is of strategic importance for achieving Sweden's goal of zero net greenhouse gas emissions by 2045 [1]. The national biogas strategy has set the goal of annual biogas energy use of 15 TWh by 2030, which requires a substantial increase in Sweden's biogas production capacity [2]. Anaerobic digester units at wastewater treatment plants (WWTP) account for

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ANAEROBIC DIGESTION AS A TOOL TO REDUCE ANTHROPOGENIC IMPACTS ON AQUATIC ECOSYSTEMS

Thuane Mendes Anacleto[1,3]*, *Helena Rodrigues Oliveira*[1,3], *Cristiane Fonseca Caetano da Silva*[1,3], *Rubens Perez Calegari*[5], *Mariana Erthal Rocha*[6], *Tiphane Andrade Figueira*[3], *Marcelo Côrtes Silva*[8], *Laura Shizue Moriga Masuda*[7], *Renato Paquet*[4], *Vinicius Peruzzi de Oliveira*[3] & *Alex Enrich-Prast*[2]

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Abstract: The large global generation and improper management of waste lead to the pollution of the environment and efforts toward reducing the impacts of anthropogenic activities on aquatic environments should be prioritized. The United Nations (UN) declared 2018-2028 as the international decade for action on "Water for Sustainable Development" and integrated management of water resources. Several international initiatives, such as the UN 2030 Agenda, the Sendai Framework for Disaster Risk Reduction and the Paris Agreement, have highlighted and strongly recommended the development of new technologies to reverse the current environmental scenario of global water bodies. The use of anaerobic digestion (AD) for treating organic wastes can minimize and avoid several adverse effects on aquatic environments while promoting nutrient cycling and the production of biogas, a renewable energy source that can replace fossil fuels and therefore decrease the emission of greenhouse gases. We performed a systematic review to evaluate the contribution of AD in preventing and reducing human impacts on aquatic ecosystems. China (15.1%), Spain (7.3%) and Italy (7.3%) are countries with a pronounced research focus on this topic, indicating their awareness on the importance of managing and preserving their water resources. The integration of co-digestion and pretreatment methods into AD improved the production of byproducts (especially

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A novel approach to estimate methanogenic pathways in biogas reactors via stable carbon isotope analysis

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ABSTRACT

Two microbial pathways are responsible for most of the methane produced during anaerobic digestion: acetoclastic methanogenesis (AM) and hydrogenotrophic methanogenesis (HM) coupled with syntrophic acetate oxidation (SAO). Identifying the dominant methanogenic pathway active in a system provides the information necessary to manage and optimize productivity, stability, process control, and gas quality in biogas reactors. In this study, a modified method is proposed to estimate methanogenic pathways in different biogas systems via short-term parallel incubations with methyl-labeled acetate (2-^{13}C-acetate). Cavity ring-down spectroscopy was applied to measure the $\delta^{13}C$–CH_4 and $\delta^{13}C$–CO_2 isotopic signatures of produced biogas. Preliminary experiments demonstrated that longer incubation times led to significant variations in $\delta^{13}C$–CH_4 and $\delta^{13}C$–CO_2 and consequently interfered with the calculated fraction of CH_4 produced from HM (f_{HM}). This variability is likely caused by the dilution of $^{13}CH_4$ and $^{13}CO_2$ as 2-^{13}C-acetate is consumed, along with potential changes in organic matter quality and quantity, microbial community composition, and environmental factors such as pH, volatile fatty acid content, and ammonia levels, during longer incubations. We applied this new approach to sludge from six full-scale reactors (three mesophilic and three thermophilic) and validated its potential with consistent estimates of f_{HM} with minimal variation. Mesophilic reactors exhibited AM dominance, while HM was the dominant pathway in thermophilic reactors, aligning with reports in the literature.

1. Introduction

Two major pathways are related to methane production in anaerobic reactors: acetoclastic methanogenesis (AM), during which acetate is converted to CH_4 and CO_2, and hydrogenotrophic methanogenesis (HM), during which CO_2 and H_2 are converted to CH_4 [1]. HM is often coupled with syntrophic acetate oxidation (SAO), which generates substrates for HM from acetate. AM should theoretically contribute to 67% of produced methane [2]. However, HM is often dominant, especially in environments with high temperatures and ammonia concentrations [1,3,4].

Establishing conditions suitable for a specific methanogenic pathway can lead to an increase in productivity and an improvement in process control and gas quality [5], in addition to preventing instability and low performance resulting from sudden pathway shifts [6,7]. A comprehensive understanding of AM and HM is crucial to the identification of factors that optimize biomethanation rate and reliability, increasing its competitivity [5]. Identifying the methanogenic pathways in biogas digesters also allows the detection of early imbalances, enabling the implementation of corrective actions to maintain operational efficiency [8]. However, acquiring an accurate estimate of the dominant methanogenic pathway active in biogas sludge can be challenging.

Methods such as microbial community analysis and incubation with ^{14}C-labeled compounds have been employed to determine

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Bioremediation Potential of the Macroalga *Ulva lactuca* (Chlorophyta) for Ammonium Removal in Elastomer Industry Wastewater

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Abstract

During the production of nitrile rubber, significant amounts of nitrogen in the form of ammonium are generated in the wastewater. The discharge of this high-nitrogen wastewater can lead to serious environmental issues, including eutrophication, disruption of aquatic ecosystems, and groundwater contamination. To mitigate these impacts, this research explored the bioremediation capabilities of the macroalgae *Ulva lactuca* (Chlorophyta) for removing nitrogen from nitrile rubber production wastewater. The study employed single-phase and Michaelis-Menten decay models based on ammonium consumption, using various dilutions of wastewater to identify the optimal concentration for treatment. The physiological state of the macroalgae was monitored by measuring the photosynthetic capacity and specific growth rate during the experiments. In the presence of *U. lactuca*, ammonium concentrations decreased in all treatment groups, confirming that the ammonium kinetics conformed to both applied models. Our results show that *U. lactuca* effectively reduces ammonium concentrations, with an approximate removal rate of 0.020 $\mu M \cdot g^{-1} \cdot min^{-1}$ across different wastewater concentrations (70%, 80%, 90%, and 100%). Notably, the treatments with 70%, 80%, and 90% wastewater strength achieved about 67% reduction in ammonium, demonstrating the alga's capacity to treat high-nitrogen wastewater. The photosynthetic performance of *U. lactuca* initially declined in control conditions but stabilized across all treatments, highlighting its adaptability. The kinetic analysis