UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
ESCOLA DE ENGENHARIA
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Digestão anaeróbia de resíduos sólidos da indústria coureira, co-digestão com resíduos agrícolas e utilização de digestato em cultivo agrícola

TESE DE DOUTORADO

Taysnara Simioni

Porto Alegre
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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Engenharia Química da Universidade Federal do Rio Grande do Sul como parte dos requisitos para obtenção do título de Doutora em Engenharia Química.

Área de concentração: Pesquisa e Desenvolvimento de Processos

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A Comissão Examinadora, abaixo assinada, aprova a Tese de Doutorado *Digestão anaeróbia de resíduos sólidos da indústria coureira, co-digestão com resíduos agrícolas e utilização de digestato em cultivo agrícola,* elaborada por Taysnara Simioni, como requisito parcial para obtenção do Grau de Doutora em Engenharia Química.

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Resumo

A crescente demanda por energia, devido ao crescimento da população humana e ao esgotamento dos recursos energéticos não renováveis, tem sido a principal causa da busca por recursos alternativos. O biogás, produzido a partir da degradação anaeróbia de compostos orgânicos, desempenha um papel importante no mercado em desenvolvimento de energia renovável. Resíduos de aterro sanitário, lodo de esgoto, esterco animal, resíduos agrícolas e industriais estão entre as principais fontes com potencial para geração de biogás. Além de fornecer energia renovável e reduzir a dependência por fontes de energia fósseis, o processo de digestão anaeróbia pode contribuir para uma prática eficaz de gerenciamento de resíduos, reduzindo a sua carga orgânica e gerando um subproduto rico em nitrogênio, fósforo e potássio, o digestato. A partir desses conceitos, o presente estudo propõe avaliar a produção de biogás e o balanço nutricional em ensaios controlados de co-digestão anaeróbia dos dois principais resíduos da indústria coureira, farelo de couro e lodo da estação de tratamento de efluentes. A pesquisa teve como principais objetivos (i) avaliar a influência dos componentes da solução de nutrientes empregada para garantir condições favoráveis ao crescimento dos microrganismos, (ii) aumentar o equilíbrio entre os macronutrientes presentes nos resíduos da indústria coureira através da sua co-digestão com resíduos agrícolas (palha de aveia e de trigo), onde também foi avaliado o efeito de pré-tratamentos (químico, térmico e a combinação entre esses) na biodegradabilidade das palhas, e (iii) avaliar o valor agronômico do digestato líquido obtido a partir da co-digestão anaeróbia de resíduos de couro. Os resultados mostraram que é possível reduzir 27% do custo agregado pela solução de nutrientes encontrada na literatura com a eliminação de um dos seus componentes, mantendo constante a produção de biogás e a mineralização dos resíduos. A adição de palha de aveia sem pré-tratamento e de palha de trigo pré-tratada com ácido aumentou a produção de biogás em 60% e 167%, respectivamente, quando comparado à co-digestão anaeróbia apenas dos resíduos de couro. Os demais pré-tratamentos aplicados alteraram a composição lignocelulósica das palhas; no entanto, a possível formação de compostos tóxicos ou esterilização de microrganismos não refletiu em uma maior produção de biogás. O desempenho do digestato em culturas agrícolas com relação ao crescimento de plantas de aveia foi equivalente ao de fertilizantes minerais comerciais. Os resultados obtidos representam avanços na busca por um processo mais equilibrado, eficiente e viável de produção de biogás.

Palavras-chave: resíduos; aveia; trigo; pré-tratamento; co-digestão anaeróbia; biogás; digestato.

Abstract

The growing demand for energy due to the growth of the human population and the depletion of non-renewable energy resources has been the leading cause of the search for alternative energy resources. Biogas, produced from organic compounds' anaerobic degradation, plays an essential role in the renewable energy development market. Landfill waste, sewage sludge, animal manure, and agricultural and industrial waste are among the primary sources of potential biogas generation. In addition to providing renewable energy and reducing dependence on fossil energy sources, the anaerobic digestion process can contribute to an effective waste management practice, reducing its organic load and generating a by-product rich in nitrogen, phosphorus, and potassium, the digestate. Based on these concepts, this study proposes to evaluate the biogas production and the nutritional balance in controlled anaerobic co-digestion tests of the two primary solid wastes of the tannery industry, leather shavings and sludge from wastewater treatment plant. The research aimed (i) to evaluate the influence of the components of the nutrient solution used to ensure favorable conditions for the growth of microorganisms, (ii) to improve the macronutrients balance through their co-digestion with agricultural wastes (oat and wheat straw), with the evaluation of the effect of pretreatments (chemical, thermal, and the combination thereof) on the biodegradability of straw, and (iii) to evaluate the agronomic value of the liquid digestate obtained from the anaerobic co-digestion of leather wastes. The results showed that it is possible to reduce 27% of the aggregate cost with the nutrient solution reported in literature by eliminating one of its components, maintaining biogas production and waste mineralization constants. The addition of untreated oat straw and acid pretreated wheat straw increased biogas production by 60% and 167%, respectively, compared to anaerobic co-digestion of leather wastes only. The other pretreatments applied changed the lignocellulosic composition of the straw; however, the possible formation of toxic compounds or sterilization of microorganisms did not reflect an increase in biogas production. The performance of digestate in agricultural crops concerning to the growth of oat plants was equivalent to commercial mineral fertilizers. The findings represent advances in the search for a more balanced, efficient, and viable process of biogas production.

Keywords: waste; oat; wheat; pretreatment; anaerobic co-digestion; biogas; digestate.

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Lista de Abreviações

ABR – *anaerobic baffled reactors*

AC – *activated carbon*

AcoD – *anaerobic co-digestion*

AD – *anaerobic digestion*

AGVs – ácidos graxos voláteis

AnBBR – *anaerobic sequencing batch biofilm reactor*

AOP – *advanced oxidation process*

APBR – *anaerobic packed-bed reactor*

ASTBR – *anaerobic structured-bed reactor*

BOD – *biochemical oxygen demand*

CI – carbono inorgânico

CNTP – condições normais de temperatura e pressão

co-DA – co-digestão anaeróbia

COT – carbono orgânico total

COVs – compostos orgânicos voláteis

CSTR – *continuous stirred-tank reactor*

CT – carbono total

DA – digestão anaeróbia

DIET – *direct interspecies electron transfer*

DRX – difração de raios-X

EGBR – *expanded granular bed reactor*

EGSB – *expanded granular sludge blanket*

ETEs – estação de tratamento de efluentes

FTIR – *Fourier transform infrared spectroscopy* (espectroscopia no infravermelho com transformada de Fourier)

GAC – *granular activated carbon*

GI – *germination index*

HPLC – *high performance liquid chromatography* (cromatografia líquida de alta eficiência)

HRT – *hydraulic retention time*

IC – *inorganic carbon*

ICP-OES – *inductively coupled plasma-optical emission spectrometry* (espectrometria de emissão ótica com plasma indutivamente acoplado)

IN – *inorganic nitrogen*

IRENA – *International Agency of Renewable Energy* (Agência Internacional de Energia Renovável)

LBR – *leachate bed reactor*

LD – *liquid digestate*

NI – nitrogênio inorgânico

NT – nitrogênio total

NTK – nitrogênio total Kjeldahl

OLR – *organic loading rate*

OM – *organic matter*

OS-HCl – *oat straw – acid pretreatment*

OS-HCl/THE – *oat straw – acid + thermal pretreatments*

OS-NaOH – *oat straw – alkaline pretreatment*

OS-NaOH/THE – *oat straw – alkaline + thermal pretreatments*

OS-THE – *oat straw – thermal pretreatment*

PCI – poder calorífico inferior

SBR – *sequential batch reactor*

SDV – sólidos dissolvidos voláteis

SHA – *shavings*

SLU – *sludge*

SRT – *solid retention time*

SSV – sólidos suspensos voláteis

SV – sólidos voláteis

TC – *total carbon*

TKN – *total Kjeldahl nitrogen*

TN – *total nitrogen*

TOC – *total organic carbon*

TOC-L – *total organic carbon of liquid*

TOC-S – *total organic carbon of solids*

TS – *total solids*

UASB – *upflow anaerobic sludge bioreactor*

UOS – *untreated oat straw*

UWS – *untreated wheat straw*

VDS – *volatile dissolved solids*

VFAs – *volatile fatty acids*

VS – *volatile solids*

VSS – *volatile suspended solids*

WS-HCl – *wheat straw – acid pretreatment*

WS-HCl/THE – *wheat straw – acid + thermal pretreatments*

WS-NaOH – *wheat straw – alkaline pretreatment*

WS-NaOH/THE – *wheat straw – alkaline + thermal pretreatments*

WS-THE – *wheat straw – thermal pretreatment*

WWTP – *wastewater treatment plant*

XRD – *X-ray diffraction*

Introdução

A busca por recursos alternativos de energias renováveis, aliada ao desafio que o gerenciamento dos resíduos causados pelo crescimento populacional e pelo desenvolvimento da indústria representa, tem motivado a pesquisa por tecnologias de geração de energia a partir da biodegradação de resíduos. A digestão anaeróbia (DA) destaca-se como uma tecnologia promissora nesta área, pois é capaz de converter diferentes tipos de resíduos em biogás com alto teor de metano.

Além do biogás, que pode ser utilizado para a produção de biocombustível, energia elétrica e calor, a DA também origina um subproduto. Trata-se do resíduo digerido ou degradado, o digestato, o qual por ser rico em nitrogênio e outros nutrientes mineralizados (principalmente fósforo e potássio) e de fácil absorção para as plantas, tem sido utilizado como fertilizante agrícola. A aplicação do digestato como fertilizante não só contribui para completar o ciclo biológico dos nutrientes, mas também reduz a demanda por fertilizantes minerais, promovendo o desenvolvimento de uma agricultura mais sustentável e contribuindo com a tão buscada economia circular.

O processo bioquímico de DA segue as etapas de hidrólise, acidogênese, acetogênese e metanogênese, que ocorrem de forma sequencial e paralela. Diferentes comunidades microbianas envolvidas em cada etapa trabalham em sinergia, decompondo a matéria orgânica na ausência de oxigênio, com os produtos de uma etapa servindo como substrato para a etapa seguinte. A DA é um processo complexo que depende de vários fatores, incluindo características do substrato e parâmetros de processo. O equilíbrio de nutrientes é um aspecto importante, e um processo de DA bem-sucedido requer uma relação entre carbono e nitrogênio

(C/N) equilibrada (20-30), o que é difícil de atingir na mono-digestão, visto que um único substrato pode não ter essa razão nutricional. Dessa forma, processos operando com a mistura de dois ou mais substratos tem sido cada vez mais estudados. A co-digestão anaeróbia (co-DA) fornece melhor equilíbrio de macro e micronutrientes, diluição de compostos tóxicos ou inibidores, adequação da umidade de acordo com a rota tecnológica escolhida e melhor capacidade de tamponamento. Além disso, apresenta vantagens econômicas por compartilhar o sistema e tratar mais de um resíduo ao mesmo tempo.

Os resíduos gerados representam um passivo ambiental e uma preocupação econômica recorrente. O setor coureiro apresenta alto potencial poluidor, visto que ao longo das operações empregadas para a transformação de pele em couro quantidades significativas de águas residuárias e resíduos sólidos são gerados. Os resíduos sólidos e o lodo gerado nas estações de tratamento de efluentes (ETEs) dos curtumes têm sido tradicionalmente destinados para aterros industriais de resíduos perigosos, devido ao elevado conteúdo de poluentes químicos e à presença de compostos recalcitrantes. No entanto, em resposta a novos regulamentos rigorosos e políticas ambientais que incentivam tratamentos alternativos, a DA tornou-se uma solução atraente na perspectiva do manejo sustentável e integrado de resíduos sólidos e de águas residuárias provenientes de curtumes.

Os trabalhos do grupo de pesquisa do Laboratório de Estudos em Couro e Meio Ambiente (LACOURO) têm se preocupado com as questões ambientais relacionadas aos curtumes, tendo como principais objetivos o desenvolvimento de tecnologias limpas, o tratamento de efluentes líquidos e a obtenção de subprodutos e energia a partir de resíduos sólidos. Na área de DA de resíduos sólidos de curtumes, estudos avaliaram principalmente: a produção de biogás por diferentes substratos contendo colágeno, incluindo resíduos de curtumes, através do teste de diferentes lodos provenientes de esgoto doméstico, curtumes e frigoríficos (PRIEBE et al., 2016); a geração de biogás e a variação da carga orgânica e inorgânica dos resíduos em ensaios controlados de co-DA de farelo de couro e lodo de ETEs de curtume (AGUSTINI et al., 2018a); a influência dos agentes curtentes (sal de cromo e tanino vegetal) e do pré-tratamento térmico do farelo na co-DA de farelo de couro e lodo de ETEs de curtume (AGUSTINI et al., 2018b); o escalonamento dos ensaios de co-DA de farelo de couro e lodo de ETEs para uma escala semi-piloto e a economia energética de um curtume onde os resíduos sólidos fossem destinados para a produção de biogás (AGUSTINI; DA COSTA; GUTTERRES, 2018); utilização do efluente bruto do processo de curtimento como suplementação nutricional na co-DA de farelo de couro e lodo de ETEs (AGUSTINI; DA

COSTA; GUTTERRES, 2020a); a influência da adição de pelo bovino, biomassa de microalga ou silicone comercial como terceiro co-substrato na co-DA de farelo de couro e lodo de ETEs (AGUSTINI et al., 2018c); e a composição microbiológica antes e após os ensaios de co-DA de farelo de couro e lodo de ETEs de curtume (AGUSTINI; DA COSTA; GUTTERRES, 2020b).

A atividade agrícola é um setor em crescente expansão para atender a demanda por alimentos da população mundial, mas que também gera uma quantidade substancial de resíduos, principalmente caracterizados pelas palhas de colheita. A grande disponibilidade e o alto teor de carbono tornam as palhas de cereais agrícolas biomassas atrativas para a geração de biogás. Apesar disso, a conversão de qualquer substrato lignocelulósico em biogás é prejudicada pela estrutura complexa desse material. Especificamente, a área de superfície acessível, a cristalinidade da celulose e o teor de lignina limitam a digestibilidade da matéria lignocelulósica. Como uma tentativa de remover essas barreiras, tornando o conteúdo orgânico do substrato mais acessível e, consequentemente, aumentando o rendimento do processo de produção de biogás, um grande número de técnicas tem sido investigadas para o pré-tratamento dessas biomassas, baseadas em abordagens físicas, químicas e biológicas.

Diante do exposto, buscou-se avaliar o gargalo tecnológico do processo de co-DA dos resíduos sólidos da indústria coureira e sugerir estratégias que viabilizem e melhorem a eficiência do processo de forma a aumentar a produção de biogás e metano.

O primeiro ponto de atenção concentrou-se na necessidade de adição de uma solução nutritiva para iniciar o processo de co-DA dos resíduos da indústria coureira. Embora a adição de nutrientes não seja um assunto abordado com clareza na literatura consultada, experimentos anteriores realizados pelo grupo de pesquisa indicavam a necessidade do fornecimento de nutrientes para garantir condições favoráveis ao crescimento e metabolismo dos microrganismos. No entanto, esta adição de nutrientes representa um custo adicional ao processo, e até o momento não se tinha conhecimento sobre a influência que cada componente da solução adicionada desempenhava.

Seguindo na linha do equilíbrio de nutrientes, por características intrínsecas da matéria-prima utilizada (pele animal), os resíduos da indústria coureira são ricos em proteína e de forma geral apresentam uma relação C/N abaixo do valor considerado ótimo para a estabilidade do processo de DA. Para superar esta limitação, buscou-se um resíduo de fácil acesso, ampla disponibilidade e rico em carboidrato que pudesse fornecer carbono de modo a aumentar a estabilidade do processo. A biomassa lignocelulósica, mais especificamente, palhas de aveia e de trigo, que são culturas de inverno bastante cultivadas pelo setor agrícola gaúcho, surgiram como opções interessantes de co-substrato, e o reporte do seu uso na co-DA com resíduos da indústria coureira não foi encontrado na literatura.

Como último ponto de atenção está a gestão do digestato obtido. Embora já seja de conhecimento que a DA dos resíduos de curtume gera um subproduto altamente estável e mineralizado, ele ainda precisa ser destinado de forma adequada. Neste sentido, buscou-se uma forma de aplicação sustentável para o digestato produzido a partir da co-DA dos resíduos sólidos da indústria coureira, através de sua utilização como fertilizante agrícola, assunto inexplorado até o momento, e que pode contribuir com a viabilização da cadeia produtiva do biogás.

1.1 Objetivos do trabalho

O objetivo deste trabalho é a obtenção de biogás a partir do tratamento de resíduos sólidos provenientes da indústria coureira e do setor agrícola através do processo de co-DA controlada, avaliando técnicas que viabilizem e melhorem a eficiência do processo.

Os objetivos específicos deste estudo são:

- Estudar a influência de nutrientes no processo de co-DA de farelo de couro *wet-blue* e lodo proveniente da ETEs de curtume;
- Verificar a eficiência do processo de co-DA no tratamento dos resíduos através da redução de carga orgânica e geração de biogás e metano;
- Analisar a viabilidade econômica do processo de co-DA de resíduos de curtume como uma alternativa à sua disposição em aterro sanitário;
- Avaliar a economia alcançada com o estudo da solução de nutrientes;
- Avaliar o processo de co-digestão de resíduos da indústria coureira (farelo *wet-blue* e lodo de ETEs) a partir da adição de um terceiro substrato, palha de aveia ou palha de trigo;
- Avaliar o efeito de diferentes técnicas de pré-tratamento aplicadas nas palhas de aveia e de trigo no que diz respeito a alterações da estrutura química das palhas e à produção de biogás e metano;

- Testar o digestato líquido obtido a partir da co-DA de resíduos de curtume em substituição aos fertilizantes minerais.

1.2 Estrutura do trabalho

O presente trabalho está estruturado em 9 capítulos.

Este capítulo 1 apresenta uma introdução sobre o tema abordado nesta tese, assim como os objetivos e a forma como o trabalho está estruturado.

No capítulo 2 estão apresentados os principais aspectos teóricos relevantes e estudos de revisão bibliográfica envolvendo a geração de biogás a partir da DA de resíduos. É feita uma contextualização sobre o panorama energético atual e a geração de biogás a partir de resíduos, além da apresentação das atividades (coureira e agrícola) que originam os resíduos utilizados no presente trabalho, bem como as principais características e etapas do processo de DA.

O capítulo 3 completa a revisão bibliográfica em um estudo submetido para contribuição a um capítulo de livro intitulado *Performance enhancement strategies of anaerobic digestion technology: a critical assessment*, para o livro *Anaerobic Biodigesters for Human Waste Treatment* da editora Springer Nature, e engloba as vantagens e limitações das principais técnicas que vem sido citadas na literatura como estratégias para melhorar o desempenho da DA e, consequentemente, aumentar a produção de biogás.

No capítulo 4 são apresentados os materiais e métodos utilizados na realização desta tese: os resíduos utilizados nos ensaios de DA e técnicas utilizadas na sua caracterização; detalhes acerca dos procedimentos experimentais e dos parâmetros avaliados nos ensaios de DA; e a apresentação das técnicas utilizadas para acompanhar a eficiência da biodegradação dos resíduos e aproveitamento do digestato.

No capítulo 5 é apresentado o artigo *Nutrient balance for anaerobic co-digestion of tannery wastes: energy efficiency, waste treatment and cost-saving*, publicado no periódico *Bioresource Technology*, onde foi determinada a influência dos componentes da solução nutritiva originalmente empregada em processos de co-DA de resíduos de curtume, com relação à eficiência energética (produção de biogás) e econômica (análise de economia de custos) e à biodegradação dos resíduos.

No capítulo 6 é apresentado o artigo *Anaerobic co-digestion of tannery wastes and untreated/pretreated oat straw*, publicado no periódico *BioEnergy Research*, onde se avaliou o efeito da aplicação de pré-tratamentos químicos e térmico na composição lignocelulósica da palha de aveia, e ainda, o potencial de geração de biogás quando estas palhas (tratadas ou não) foram adicionadas como substrato ao processo de co-DA com resíduos da indústria coureira (farelo de couro *wet-blue* e lodo de ETEs).

No capítulo 7 é apresentado o artigo *Enhancement of biogas production by anaerobic co-digestion of leather waste with raw and pretreated wheat straw*, submetido e em revisão em periódico, onde se avaliou a viabilidade do processo de co-DA entre farelo de couro *wet-blue*, lodo de ETEs de curtume e palha de trigo; além disso, neste trabalho também se estabeleceu uma comparação do efeito dos pré-tratamentos ácido, básico, térmico e da combinação destes, sobre a composição lignocelulósica da palha de trigo e no rendimento de metano obtido pela co-DA.

No capítulo 8 é apresentado o artigo *Use of tannery waste anaerobic digestate as agricultural fertilizer: an analysis of oat growth and soil fertility*, submetido e em revisão em periódico, onde se avaliou o valor agronômico e o efeito fitotóxico do digestato líquido obtido a partir da co-DA mesofílica de resíduos de curtume em substituição aos fertilizantes minerais no que diz respeito ao crescimento de plantas de aveia e ao conteúdo de macronutrientes presentes no solo após a fertilização.

No capítulo 9 são apresentadas as conclusões deste trabalho e sugestões para trabalhos futuros.

Capítulo 2

Fundamentos Teóricos e Revisão Bibliográfica

Neste capítulo estão colocados os principais aspectos teóricos relevantes ao estudo da geração de biogás a partir da DA de resíduos. É feita uma contextualização sobre o panorama energético atual e a geração de biogás, além da apresentação das atividades (coureira e agrícola) que originam os resíduos utilizados no presente trabalho, bem como as principais características e etapas do processo de DA.

2.1 O panorama energético atual

O consumo de energia tem aumentado em todo o mundo e deve continuar crescendo nos próximos anos, com crescimento estimado de quase 50% entre 2018 e 2050 (EIA, 2019). Os combustíveis fósseis são portadores de energia não renovável que compreendem carvão, petróleo e gás natural e que fornecem cerca de 80% da energia total consumida para a produção de eletricidade utilizada para fins industriais e domésticos em todo o mundo. No entanto, os combustíveis fósseis são finitos e consumidos por humanos mais rapidamente do que poderiam ser repostos (AZIZ; HANAFIAH; GHEEWALA, 2019). Além disso, a utilização de combustíveis fósseis é considerada como a principal razão para várias questões ambientais, como a poluição do ar e o aquecimento global (AZIZ; HANAFIAH; GHEEWALA, 2019; KHALIL et al., 2019), e a dependência mundial de uma única fonte de energia gera insegurança energética, podendo ocasionar crises econômicas e políticas (HAGOS et al., 2017). Atualmente, a conjuntura energética global encontra-se em um estágio de novas mudanças e ajustes, e a tendência é realizar a transição do sistema de energia fóssil para um sistema de energia de baixo carbono e, finalmente, entrar na era da energia sustentável baseada principalmente em energias renováveis (LI et al., 2020).

2.1.1 Energia renovável

É bem aceito que nenhuma única fonte de energia alternativa será capaz de cobrir a crescente demanda de energia da humanidade. Em vez disso, a tendência é que o sistema energético do futuro seja uma mistura de várias fontes de energia renováveis e, pelo menos nas próximas décadas, ainda complementadas pela energia fóssil (MILTNER; MAKARUK; HARASEK, 2017). A energia renovável é uma forma eficaz de lidar com o dilema de atender à crescente demanda de energia e reduzir as emissões de gases de efeito estufa. Também desempenha um papel importante na garantia da segurança energética, melhorando a proteção ambiental e contribuindo com o aumento da oferta de emprego em muitos países (LI et al., 2020). Atualmente, o desenvolvimento de recursos renováveis globais e sua escala de utilização têm se expandido continuamente e os custos de aplicação diminuíram (LU; GAO, 2021). O consumo mundial de energia renovável deve aumentar 3% ao ano entre 2018 e 2050 e se tornar a principal fonte de consumo de energia primária até 2050 (EIA, 2019).

As fontes de energia renováveis mais importantes são a energia eólica, energia solar fotovoltaica e energia de biomassa. Energia hidrelétrica, geotérmica e marinha também merecem destaque. Fontes cada vez mais amplas de energia eólica e fotovoltaica já fornecem energia razoavelmente barata (DESHMUKH et al., 2021), mas esses sistemas são caracterizados por um perfil de produção altamente flutuante e nem sempre previsível. Da mesma forma, a energia hidrelétrica depende do regime de chuvas, o que pode comprometer a segurança energética de um país. O Brasil, por exemplo, cuja matriz energética é altamente dependente de fontes hídricas, já experimentou grandes problemas de abastecimento de energia elétrica em decorrência de um grande período de estiagem em 2001, que comprometeu a capacidade dos reservatórios, reduzindo drasticamente a capacidade de geração hídrica (FREITAS et al., 2019). As técnicas de conversão de energia de biomassa, por outro lado, são capazes de produzir uma carga de base constante e até mesmo equilibrar as lacunas entre oferta e demanda no setor de energia (MILTNER; MAKARUK; HARASEK, 2017). A biomassa é um recurso neutro em carbono, bem como uma fonte de elementos C/H/O para gerar produtos orgânicos à base de carbono, como bioenergia (biocombustível e biogás) e produtos químicos (biorrefinaria) (JUNG et al., 2021). Assim, a valorização da matéria-prima de biomassa tem recebido considerável atenção nas últimas décadas e as fontes de energia baseadas em biomassa devem ter uma participação representativa no sistema energético do futuro (JUNG et al., 2021; MILTNER; MAKARUK; HARASEK, 2017). Do ponto de vista energético, biomassa pode ser compreendida como qualquer recurso renovável, oriundo de matéria orgânica (de origem animal ou vegetal), que possa ser utilizado para produção de energia (AZIZ; HANAFIAH; GHEEWALA, 2019).

As energias renováveis têm crescido fortemente e sua competitividade aumentou. De acordo com estatísticas da Agência Internacional de Energia Renovável (*International Renewable Energy Agency* - IRENA) (2020a), a capacidade instalada global de energia renovável mais que dobrou na última década, com o desenvolvimento e consolidação de novas fontes de energia renovável. Ao final de 2020, foi atingido o valor de 2.789.061 MW de capacidade instalada global de energia renovável, dos quais 43,41% correspondem à hidrelétrica, 25,37% à solar, 26,29% à eólica, 4,41% à bioenergia, 0,5% à geotérmica e 0,02% à energia marinha. A evolução da capacidade instalada global de energia renovável e o perfil de distribuição das fontes renováveis de energia na última década podem ser observados na Fig. 2.1.

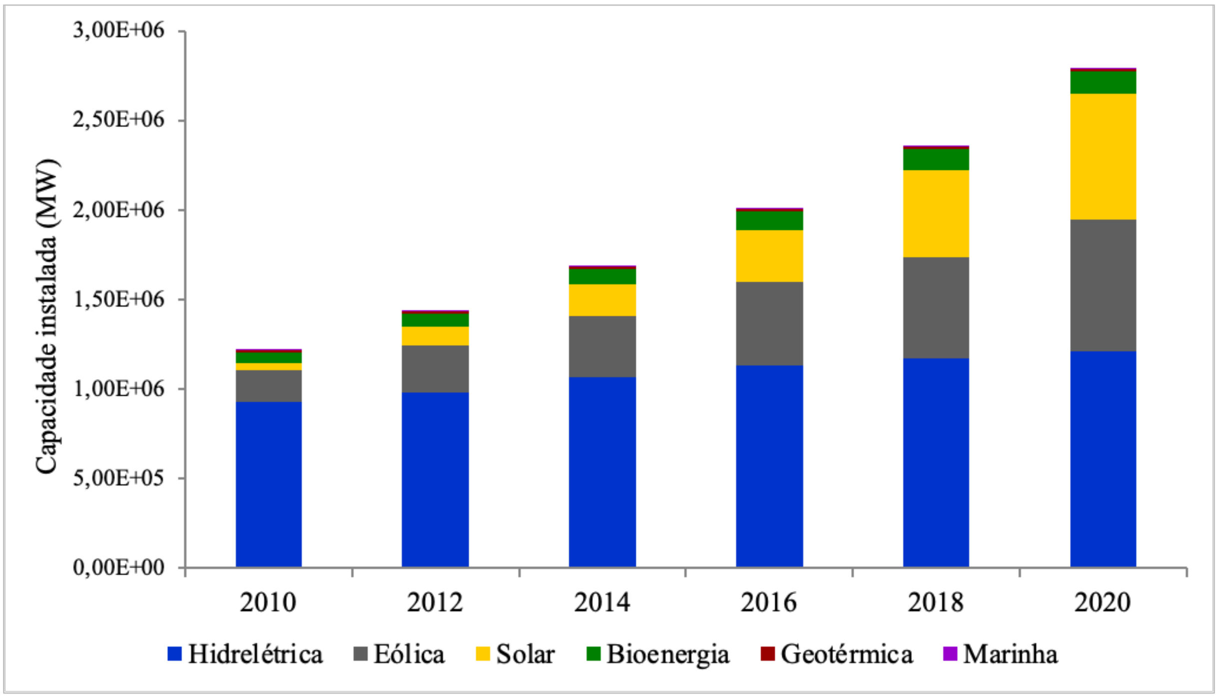

Fig. 2.1. Tendências em energia renovável. Adaptado de IRENA (2020a).

2.1.2 Resíduos para produção de energia

Os processos de transformação de resíduos em energia compreendem qualquer tecnologia de tratamento de resíduos que gere qualquer forma de energia, ou seja, calor, eletricidade ou combustíveis líquidos (por exemplo, diesel, petróleo ou querosene) a partir de uma matéria-prima de resíduos (RAFIEE et al., 2021). A chamada transformação de resíduos em energia tem múltiplas vantagens. Não só aborda o desafio do destino dos resíduos, mas também oferece uma boa oportunidade de segurança energética, pois tanto os processos de produção quanto de consumo de energia podem estar na mesma localização geográfica, ao contrário do que acontece com os combustíveis fósseis.

A utilização de resíduos como biomassa para produção de energia, como o biogás, vem se destacando como uma alternativa promissora para atender à alta demanda global por consumo de energia (KHALIL et al., 2019). Na literatura, estudos têm relatado que vários tipos de resíduos orgânicos, como resíduos animais (HOLLAS et al., 2021; PARRALEJO et al., 2019; RAMOS-SUÁREZ et al., 2019), resíduos alimentares (BOZYM et al., 2015; KUCZMAN et al., 2018), resíduos sólidos urbanos orgânicos (TYAGI et al., 2018), resíduos industriais, lodo de esgoto e resíduos agrícolas (MOMAYEZ; KARIMI; TAHERZADEH, 2019; ONTHONG; JUNTARACHAT, 2017) podem ser potencialmente utilizados como fontes para a produção de biogás através do processo de DA (KHALIL et al., 2019).

2.2 A indústria coureira e seus resíduos

A indústria do couro desempenha um papel significativo na economia global (TANG et al., 2021) devido ao couro ser uma das commodities mais comercializadas no mundo (MPOFU; OYEKOLA; WELZ, 2021). A confecção do couro envolve uma série de processos que transformam a pele, subproduto da indústria de carnes, em couros de alto valor agregado (TANG et al., 2021). Os países em desenvolvimento dominam este mercado e fornecem mais de 60% dos couros do mundo, o que pode ser atribuído à disponibilidade de mão de obra de baixo custo e regulamentações ambientais menos rigorosas (MPOFU; OYEKOLA; WELZ, 2021). O Brasil possui cerca de 200 curtumes em funcionamento, e exportou próximo a 1 bilhão de dólares no ano de 2020 para mais de 80 países, sendo China, Estados Unidos e Itália os principais destinos dos couros produzidos no Brasil (CICB, 2020).

O processamento do couro, realizado em curtume, consiste na transformação da pele (verde ou salgada) em couro, e segue várias etapas, com adições de água e produtos químicos às peles, intercaladas por processos de pesagem, lavagem e operações mecânicas, a depender do tipo de matéria-prima e do artigo final a ser fabricado. As etapas de processamento da pele em couro são agrupadas em três fases de operações: ribeira, curtimento e acabamento. Na fase de ribeira, são feitas operações de limpeza e preparação da pele para o curtimento (remolho, depilação, caleiro, descarne, divisão, desencalagem, purga e píquel). Após as operações de ribeira, a pele está preparada para receber o agente curtente. Durante o curtimento ocorre a verdadeira transformação da estrutura de colágeno da pele em couro. Essa modificação é obtida por ligações químicas das cadeias de colágeno com os agentes de curtimento, geralmente à base de sais de cromo ou taninos vegetais. O curtimento proporciona estabilidade da pele à putrefação (microbiana e enzimática), estabilidade química e hidrotérmica, além de conferir porosidade e flexibilidade ao material. A fase de acabamento do couro compreende as operações de acabamento molhado (desacidulação, recurtimento, tingimento e engraxe do couro), secagem, pré-acabamento e, finalmente, o couro é acabado com aplicações de camadas de produtos pigmentados e poliméricos formadores de filmes sobre sua superfície, com prensagens intermediárias e tratamentos finais. Essas operações conferem ao couro suas características finais, como firmeza, cor e maciez, dependendo exclusivamente da finalidade a que se destina o couro (GUTTERRES; MELLA, 2015).

A indústria do couro tem alto potencial poluidor devido à grande quantidade de resíduos gerados ao longo do processo (CHOJNACKA et al., 2021; DETTMER et al., 2010; POLIZZI; ALATRISTE-MONDRAGÓN; MUNZ, 2018; PRIEBE et al., 2016). Em média, 1 tonelada de pele é transformada em 250 kg de couro, gerando 15 a 50 toneladas de efluentes líquidos e 450 a 730 kg de resíduos sólidos (AGUSTINI; GUTTERRES, 2017a), além de odores, gases poluentes (CO_2, H_2S, NH_3) e compostos orgânicos voláteis (COVs), como aminas, aldeídos e hidrocarbonetos (CHOJNACKA et al., 2021).

A transformação de pele em couro requer grande quantidade de água, ocasionando a geração de um grande volume de água residuária. Estima-se que, em média, 30 a 35 m^3 de água residual são gerados por tonelada de pele processada, dependendo da matéria-prima, do produto de acabamento e dos processos empregados (LOFRANO et al., 2013). As águas residuais de curtumes normalmente contêm altas concentrações de compostos orgânicos, inorgânicos e nitrogenados (VAZIFEHKHORAN; SHIN; TRIOLO, 2018). Estão geralmente presentes cálcio, sulfeto, sulfato, cloreto, ácidos orgânicos e inorgânicos, taninos vegetais e sintéticos e cromo trivalente (AGUSTINI; GUTTERRES, 2017a). O tratamento dos efluentes líquidos dos curtumes é realizado na ETEs e geralmente inclui processos de tratamento preliminar, primário, secundário (biológico) e algumas vezes também terciário (MPOFU; OYEKOLA; WELZ, 2021), originando lodo como subproduto. O efluente líquido gerado no processamento de 1 tonelada de pele é transformado em cerca de 500 kg de lodo (teor de sólidos de 40%) (HU et al., 2011). O custo com a gestão do lodo gerado representa 50-55% dos custos de operação e manutenção da ETEs dos curtumes, o que reforça a necessidade de desenvolvimento de estratégias para gestão eficiente deste resíduo (AGUSTINI; GUTTERRES, 2017a).

O processo de fabricação de couro também gera quantidades substanciais de resíduos sólidos. Farelo de couro (serragem), aparas de couros curtidos, couros acabados e semi-acabados, assim como lodo proveniente da ETEs, estão entre os principais resíduos sólidos gerados (AGUSTINI; GUTTERRES, 2017a; PICCIN et al., 2016; PRIEBE et al., 2016). O farelo de couro está entre os principais resíduos sólidos gerados pelos curtumes, com cerca de 100-150 kg de farelo sendo gerados por tonelada de pele processada. O farelo é gerado na etapa de ajuste da espessura do couro, e é composto de matéria proteica orgânica pré-estabilizada termicamente pelo agente curtente (*wet-white*) ou finalmente estabilizadas (couro *wet-blue*)

(AGUSTINI; GUTTERRES, 2017a). A toxicidade destes resíduos é determinada principalmente pelo agente curtente utilizado no processo, que, em cerca de 80% dos couros produzidos no mundo, são sais de cromo (GUTTERRES; MELLA, 2015).

Os resíduos sólidos gerados pelos curtumes têm sido tradicionalmente destinados para aterros industriais perigosos, devido ao elevado conteúdo em poluentes químicos e à presença de compostos recalcitrantes (AGUSTINI; DA COSTA; GUTTERRES, 2018; DETTMER et al., 2010; ZUPANČIČ; JEMEC, 2010). No entanto, em resposta a novos regulamentos rigorosos e políticas ambientais que incentivam tratamentos alternativos, a DA tornou-se uma solução atraente na perspectiva do manejo sustentável e integrado de resíduos sólidos provenientes de curtumes, principalmente pela alta carga orgânica observada para estes resíduos (POLIZZI; ALATRISTE-MONDRAGÓN; MUNZ, 2018).

2.2.1 Digestão anaeróbia de resíduos de curtumes

O primeiro relato de tentativa de produzir energia através da DA de resíduos de curtume foi no ano de 1982 por Cenni, Dondo e Tombetti (1982). Desde então, vários autores têm investigado a viabilidade de processos anaeróbios para resíduos sólidos de curtumes, especialmente naqueles países onde a indústria do couro desempenha um papel proeminente na economia nacional.

Zupancic e Jemec (2010) investigaram o potencial de DA de diferentes tipos de resíduos de curtume (lodo de ETEs, farelo de couro e resíduo de descarne) e obtiveram potenciais específicos de metano estimados em 0,617 m^3/kg, 0,377 m^3/kg e 0,649 m^3/kg de sólidos suspensos voláveis (SSV), respectivamente, com o biorreator operando em temperatura termofílica. O teor de cromo e a salinidade dos resíduos não mostraram efeitos adversos; no entanto, uma redução da temperatura do reator de 4,4°C levou a uma queda na produção de biogás de 25%, indicando a necessidade de manter a temperatura constante em 55°C.

Kameswari *et al.* (2011) avaliaram a adição de uma lipase comercial para melhorar a hidrólise do processo de co-DA entre resíduos de descarne e uma mistura de lodo primário e secundário gerados na ETEs de curtume. Os resultados experimentais revelaram que quando a dosagem ótima de lipase era aplicada, um aumento de cerca de 15% na geração de biogás em comparação com o controle (sem adição de lipase) foi observado, além da redução de 30% no período de digestão.

Priebe *et al.* (2016) estudaram o mecanismo de biodegradação de substratos colagênicos contendo diferentes concentrações de cromo, inoculados com três lodos biológicos diferentes coletados em ETEs (lodo anaeróbio de esgoto, lodo anaeróbico de abatedouro e lodo aeróbio de curtume). Melhores resultados foram obtidos utilizando lodo de abatedouro e de curtume devido à sua pré-adaptação a substratos protéicos à base de colágeno.

Polizzi, Alatriste-Mondragõn e Munz (2018) avaliaram os efeitos da alta concentração de substrato em testes em batelada de DA de resíduos de pele do processo de curtimento. Os resultados indicaram que o processo é tecnicamente viável operando com baixa carga orgânica (5 gSV/L), mas que o sistema sofreu inibição por amônia em cargas orgânicas de 10 e 20 gSV/L. A co-DA com lodo primário de curtume também foi avaliada e mostrou-se viável, provavelmente devido ao efeito de diluição.

Agustini *et al.* (2018b) avaliaram a co-digestão de farelo de couro e lodo de ETEs de curtume antes e após o curtimento para dois agentes curtentes (cromo e tanino vegetal), tendo como variáveis de resposta a produção de biogás e de metano e a variação da carga orgânica e inorgânica. O lodo com cromo (7,6 mg/L) mostrou-se significativamente mais adequado para DA do que o lodo sem ele, produzindo 27,9 mL de biogás/gSSV com 60% de metano, redução de 62,8% do carbono orgânico total (COT) e aumento de 325,0% do carbono inorgânico (CI). O farelo com cromo também se mostrou significativamente mais adequado para DA e, embora com um valor inicial superior de cromo (16,4 mg/L), produziu 10,7 mL de metano/gSSV e uma redução de 55,2% de COT, confirmando que as concentrações de cromo nos resíduos não foram inibitórias. O farelo curtido com taninos vegetais foi desfavorável à mineralização dos resíduos por DA, apresentando resultados significativamente inferiores (4,1 mL de metano/gSSV).

Agustini *et al.* (2018a) avaliaram a geração de biogás e de metano e a variação da carga orgânica e inorgânica em ensaios controlados de co-DA de farelo de couro e lodo de ETEs, curtidos com os dois principais agentes curtentes utilizados na indústria, sal de cromo e tanino vegetal, analisando a interação entre os agentes curtentes e a influência do pré-tratamento térmico do farelo. Os ensaios com lodo contendo cromo produziram em média 19,6 mL de biogás/gSSV, 8,15 mL de metano/gSSV e apresentaram maior mineralização dos resíduos quando comparado aos ensaios com lodo contendo tanino vegetal, que se mostrou tóxico. O tipo de curtimento do farelo não apresentou influência significativa na produção de biogás e metano e o pré-tratamento térmico aplicado no farelo de couro mostrou-se benéfico somente para os ensaios com taninos vegetais.

Agustini, Da Costa e Gutterres (2018) avaliaram o escalonamento dos ensaios de co-DA de farelo de couro e lodo de ETEs para uma escala semi-piloto e os resultados mostraram uma consistência linear na produção total acumulada de biogás entre as duas escalas estudadas, com rendimentos de biogás entre 21 e 30 mL/kgSSV, teor máximo de metano de 59% v/v, e uma redução de COT entre 68 e 76% para ambas as escalas. A principal diferença entre os ensaios de biodegradação nas duas escalas estudadas foi na taxa de biodegradação, pois quando o volume reacional foi aumentado em cinco vezes, a velocidade de produção de biogás foi duas vezes mais rápida, mostrando que o processo em escala semi-piloto trataria os resíduos mais rapidamente.

Agustini, Da Costa e Gutterres (2020a) investigaram a possibilidade de utilização do efluente bruto do processo de curtimento como suplementação nutricional na co-DA de farelo de couro e lodo de ETEs, avaliando-se as eficiências energética, econômica e do tratamento dos resíduos. Os resultados mostraram que apesar de o processo ter sido fortemente inibido (produção de biogás de 1,9 ± 0,3 mL/gSSV e percentual de metano de 33%), a grande economia com a disposição dos resíduos sólidos e tratamento do efluente tornam o processo economicamente viável.

Agustini, Da Costa e Gutterres (2020b) caracterizaram a composição microbiológica antes e após os ensaios de co-DA de farelo de couro e lodo de ETEs de curtumes, e o filos *Bacteroidetes* e *Synergistetes* foram os que mais aumentaram em número ao longo do processo, mostrando que esses foram os principais responsáveis pela digestão dos resíduos. Os autores relacionaram a alta quantidade de metano observada com a família *Methanosaetaceae* e a ordem *Bacteroidales*.

Agustini *et al.* (2018c) avaliaram a influência da adição de pelo bovino, silicone comercial ou biomassa de microalga como terceiro co-substrato na co-DA de farelo de couro e lodo de ETEs. A adição de pelo bovino não influenciou a produção total de biogás cumulativa (19,4 mL/gSSV), mas adsorveu praticamente todo o cromo presente e aumentou em 16% a carga orgânica do meio, devido à hidrólise dos pelos. A adição de silicone comercial também não influenciou a produção cumulativa total de biogás, mas levou a uma alta capacidade de adsorção de cromo e de matéria orgânica (redução de 80% na matéria orgânica inicial). A adição de microalgas adsorveu três vezes mais cromo e dobrou a produção cumulativa de biogás (43,3 mL/gSSV) devido ao aumento da relação C/N inicial, mostrando o seu efeito benéfico ao processo.

Como conclusão geral, os estudos sobre DA de resíduos sólidos de couro concordam quanto à viabilidade do processo, alertando contra possíveis problemas operacionais relacionados ao desequilíbrio da relação C/N e às condições inibitórias de amônia e tanino vegetal.

2.3 A atividade agrícola e seus resíduos

A crescente demanda global por alimentos depende em grande parte da produção agrícola que vem se desenvolvendo de forma intensiva nas últimas décadas (ZHOU et al., 2021). Com o aumento da produção, aumenta também a quantidade de palha (MA; SHEN; LIU, 2020) ou resíduo de colheita (talo ou caule da cultura) que é deixado na lavoura (LI et al., 2018). Estima-se que cerca de 7 bilhões de toneladas de palha sejam geradas anualmente em escala global, das quais as palhas de arroz, de milho e de trigo respondem por cerca de 90% da quantidade total (MA; SHEN; LIU, 2020).

No Brasil, desde a década de 1970, é comum utilizar-se o sistema de plantio direto, técnica desenvolvida por agricultores da Inglaterra e dos Estados Unidos na década de 1950, e que hoje é utilizada por 80% das áreas de produção agrícola brasileira. Na prática, o plantio direto significa o cultivo de sementes sobre resíduos de cobertura vegetal, as palhas, sem a necessidade de revolver o solo (MOTTER; DE ALMEIDA, 2015). A palha contém matéria orgânica abundante, potássio, fósforo, nitrogênio e outros oligoelementos, que são considerados fertilizantes desejáveis para o crescimento das culturas, além de reduzir a erosão do solo (LI et al., 2018; ZHOU et al., 2021). A ideia do plantio direto é que a palha deixada nas lavouras de safras anteriores se decomponha para que os nutrientes sejam absorvidos pelo solo e pelas plantas na próxima safra (MOTTER; DE ALMEIDA, 2015). No entanto, em áreas agrícolas que utilizam o sistema de cultivo duplo, ou até mesmo triplo, não há tempo suficiente para a degradação da palha entre as safras, o que implica que a palha deve ser manuseada rapidamente após a colheita (LI et al., 2018).

Atualmente, o manejo da palha depende amplamente da queima em campo aberto (LI et al., 2018; MA; SHEN; LIU, 2020; SINGH; ARYA, 2021). A queima de palha gera vários gases de efeito estufa (CO_2, N_2O e CH_4), além de outros gases nocivos (CO, NO_X e SO_2), fumaça e poeira (hidrocarbonetos aromáticos policíclicos, dioxinas, etc.), causando a poluição do ar e representando uma ameaça à saúde humana (MA; SHEN; LIU, 2020). A utilização da palha varia significativamente nas diferentes regiões, dependendo da política nacional, do apoio financeiro, da conscientização dos produtores, bem como dos níveis econômico e industrial (MA; SHEN; LIU, 2020).

O descarte adequado ou reaproveitamento dos resíduos agrícolas tornou-se uma preocupação urgente não apenas pela questão ambiental, mas também para a utilização efetiva destes recursos, o que pode produzir enormes benefícios econômicos (HE; ZHANG; ZENG, 2019; LI et al., 2018). Materiais lignocelulósicos, como as palhas de colheita, são um vasto reservatório de energia inexplorada (NAIK; POONIA; CHAUDHARI, 2021), devido à sua abundância, alto teor de carbono e baixo custo (PAN et al., 2021).

Os materiais lignocelulósicos são compostos basicamente por celulose, hemicelulose e lignina. A celulose é o composto orgânico mais abundante da natureza e suas moléculas têm orientações diferentes ao longo da estrutura, levando a diferentes níveis de cristalinidade, de modo que quanto maior o índice de cristalinidade, mais difícil se torna a biodegradação da celulose. Enquanto a celulose possui forma rígida e cristalina, a hemicelulose possui propriedades amorfas e cadeias ramificadas, correspondendo a um polímero facilmente hidrolisável. A lignina, por sua vez, é um grande e complexo heteropolímero amorfo, aromático e hidrofóbico que desempenha o papel de reforço para a reticulação entre a celulose e a hemicelulose (FERREIRA et al., 2013; SABEEH; LIAQUAT; MARYAM, 2020; ZHENG et al., 2014). A principal função da lignina é manter as fibras unidas por uma forte força de ligação para aumentar a compactação e resistência da estrutura da planta. As propriedades da lignina a tornam o componente mais recalcitrante da parede celular da planta e, quanto maior o teor de lignina, maior a resistência da biomassa à degradação química e biológica. Ou seja, a lignina é a principal barreira para a utilização da biomassa lignocelulósica em processos de bioconversão (NESHAT et al., 2017; PATOWARY; BARUAH, 2018).

A estrutura da lignocelulose é muito complexa, resistente e recalcitrante. A separação da lignina do complexo celulose-hemicelulose-lignina é necessária para que a celulose e a hemicelulose possam ser acessadas e para que a hidrólise ocorra, uma vez que são estes os componentes com potencial de serem transformados em bioenergia. Devido à natureza complexa da ligação e interligação dos componentes da lignocelulose, é muito difícil hidrolisá-la em seus monômeros, por isso é necessário adotar uma estratégia de pré-tratamento antes do processo de hidrólise. O processo de pré-tratamento busca tornar as ligações mais fracas, diminuir a cristalinidade da celulose, remover a lignina do complexo lignocelulósico e aumentar a área de superfície interna, dentre outras características (NAIK; POONIA; CHAUDHARI, 2021).

2.3.1 Pré-tratamentos aplicados em resíduos lignocelulósicos

Na literatura, uma série de trabalhos envolvendo o uso de pré-tratamentos em resíduos com alto teor lignocelulósico pode ser encontrado. De forma geral, a aplicação dos pré-tratamentos objetiva a degradação dos compostos complexos presentes nesses resíduos com a formação de compostos solúveis simples que podem ser mais facilmente consumidos por microrganismos, aumentando a produção de metano através do processo de DA. Na sequência, são apresentados os resultados obtidos por alguns trabalhos que aplicaram diferentes pré-tratamentos nas palhas de aveia e de trigo.

Mancini *et al.* (2018) investigaram o efeito de três diferentes métodos de pré-tratamento químico na produção de biogás a partir da DA de palha de trigo. O material lignocelulósico foi pré-tratado separadamente utilizando: i) solvente orgânico N-óxido monohidratado de N-metilmorfolina (NMMO) a 120 °C por 3 h; ii) método organosolv, empregando etanol como solvente orgânico a 180 °C por 1 h e iii) pré-tratamento alcalino com NaOH a 30 °C por 24 h. Todos os pré-tratamentos foram eficazes no aumento da produção de biometano. Em particular, o rendimento cumulativo de produção de biometano de 274 mL de CH_4/gSSV, obtido com a matéria-prima não tratada, aumentou em 11% pelo pré-tratamento com NMMO e em 15% tanto pelo pré-tratamento organosolv como o alcalino. Os três métodos de pré-tratamentos tiveram um impacto diferente na composição química da palha. NMMO praticamente manteve a quantidade de carboidratos e lignina presentes na matéria-prima original. Organosolv teve um grande impacto na dissolução do componente hemicelulósico, enquanto o pré-tratamento alcalino foi o mais eficaz na remoção da fração de lignina. Além do aumento nos rendimentos de biogás, os pré-tratamentos aplicados aumentaram a cinética de produção de biometano.

Chandra *et al.* (2012) estudaram a produção de metano a partir da DA de palha de trigo submetida aos pré-tratamentos hidrotérmico e com NaOH. O substrato pré-tratado com NaOH produziu 87,5% mais biogás e 111,6% mais metano, quando comparado ao substrato de palha de trigo não tratada. O substrato submetido ao pré-tratamento hidrotérmico resultou em um aumento de 9,2% na produção de biogás e de 20,0% na produção de metano em comparação com o substrato de palha de trigo não tratado. Os ensaios de DA foram conduzidos a 37 °C (temperatura mesofílica) e a concentração de substratos foi mantida em 4,45% SV (44,5 g SV/L).

Rajput, Zeshan e Visvanathan (2018) conduziram um estudo sobre o efeito do pré-tratamento térmico na DA da palha de trigo, nas temperaturas de 120, 140, 160 e 180°C. Eles observaram diferenças significativas na produção de biogás para os casos com temperatura superior a 160°C, sendo que a palha de trigo pré-tratada a 180 °C apresentou um rendimento de biogás 53% maior em comparação com a palha não tratada. Além disso, mudanças nas ligações químicas da estrutura lignocelulósica da palha de trigo foram constatadas pela análise FTIR. De forma geral, os autores atribuem o aumento na produção de biogás à mudança na estrutura lignocelulósica e ao aumento no teor de celulose.

Moset *et al.* (2018) determinaram o efeito da combinação entre os pré-tratamentos mecânico e alcalino, utilizando álcali em baixa concentração e temperatura ambiente (19 °C por 24 h). Os autores testaram diferentes concentrações (0,5; 1; 2; 4 e 8%) de dois álcalis (NaOH e KOH) e encontraram uma correlação linear entre a concentração de álcali e a hidrólise do conteúdo de fibras, principalmente, hemicelulose. Um efeito positivo pela combinação da briquetagem (compactação da palha moída) da palha de trigo com adição de hidróxido de sódio foi encontrado, com uma redução de 66% no conteúdo de hemicelulose. De acordo com os resultados obtidos por esses autores, a melhor combinação dentre as testadas foi a palha de trigo briquetada, seguida de tratamento alcalino com NaOH 2%, que teve um aumento de 40% no rendimento do metano obtido após 30 dias. A briquetagem reduziu a concentração de álcali necessária para alcançar a máxima melhora no rendimento de metano em comparação com a palha moída.

Outros trabalhos relacionados ao tema foram encontrados na literatura. Alvarado-Morales *et al.* (2017) testaram a aplicação de um processo de oxidação avançada, combinando a irradiação UV na presença do fotocatalisador dióxido de titânio (TiO_2), visando aumentar a produção de biogás através da DA da palha de trigo. Ferreira *et al.* (2013) testaram o pré-tratamento de explosão a vapor sob diferentes combinações de temperatura e tempo, também para a palha de trigo. Gomez-Tovar *et al.* (2012) propuseram, para a hidrólise da palha da aveia, uma estratégia que consistiu na combinação de pré-tratamentos químicos e enzimáticos sequenciais que incluíram uma hidrólise ácida inicial (sacarificação da hemicelulose), seguida de hidrólise alcalina (remoção de lignina) e posterior hidrólise enzimática (sacarificação da celulose). Em todos os casos mencionados acima, um aumento na produção de metano como resultado do pré-tratamento aplicado na palha foi observado.

2.4 Digestão anaeróbia

A DA é um processo bioquímico de decomposição da matéria orgânica, realizado por um consórcio de microrganismos que vivem simbioticamente na ausência de oxigênio. Do ponto de vista tecnológico, a DA é uma alternativa promissora para o gerenciamento de materiais orgânicos, pois é capaz de converter praticamente todas as fontes de biomassa, incluindo diferentes tipos de resíduos, em biogás altamente energético. Este biogás pode ser usado para a produção de combustível, compostos químicos, energia elétrica e calor. A DA também origina um subproduto, o digestato, que é o resíduo do material degradado, um produto rico em nitrogênio e que tem potencial para ser utilizado como fertilizante agrícola (AGUSTINI; GUTTERRES, 2017b; SEADI et al., 2008; XU et al., 2019).

O processo de DA segue basicamente as etapas de hidrólise, acidogênese, acetogênese e metanogênese, de forma sequencial e paralela, conforme mostrado na Fig. 2.2. As etapas estão associadas porque as diferentes comunidades microbianas envolvidas em cada etapa trabalham em sequência, com os produtos de uma etapa servindo como substrato para a etapa seguinte. De forma geral, a matéria orgânica alimentada no biorreator é composta de diferentes porcentagens de carboidratos, proteínas e gorduras. Os microrganismos anaeróbios decompõem esses compostos complexos em compostos orgânicos mais simples e solúveis, como açúcares, aminoácidos e ácidos graxos, que são absorvidos e fermentados até que sejam transformados em compostos simples, como ácido acético, H_2 e CO_2. Na etapa final, esses compostos simples são absorvidos diretamente por microrganismos metanogênicos para produzir metano (HAGOS et al., 2017; KHALID et al., 2011; PARSAEE; KIANI; KARIMI, 2019; SEADI et al., 2008; XU et al., 2019).

A estequiometria da reação teórica de DA de diferentes matérias orgânicas pode ser escrita usando a equação de Buswell (Eq. 2.1), na qual C, H, O, N e S são convertidos em CH_4, CO_2, NH_3 e H_2S.

$$C_nH_aO_bN_cS_d + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{7}{4}c + \frac{d}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3}{8}c - \frac{d}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} - \frac{5}{8}c + \frac{d}{4}\right) CO_2 + cNH_4HCO_3 + dH_2S \qquad (2.1)$$

Teoricamente, o rendimento de metano a partir de diferentes substratos pode ser estimado a partir de sua composição elementar (Eq. 2.1). No entanto, na prática, os materiais orgânicos têm uma estrutura complexa e geralmente não se decompõem completamente durante a DA. Além disso, o rendimento real de metano depende de muitos outros fatores, tais como a bioacessibilidade e complexidade estrutural do material orgânico, composição nutricional dos substratos e parâmetros operacionais empregados na DA (XU et al., 2019).

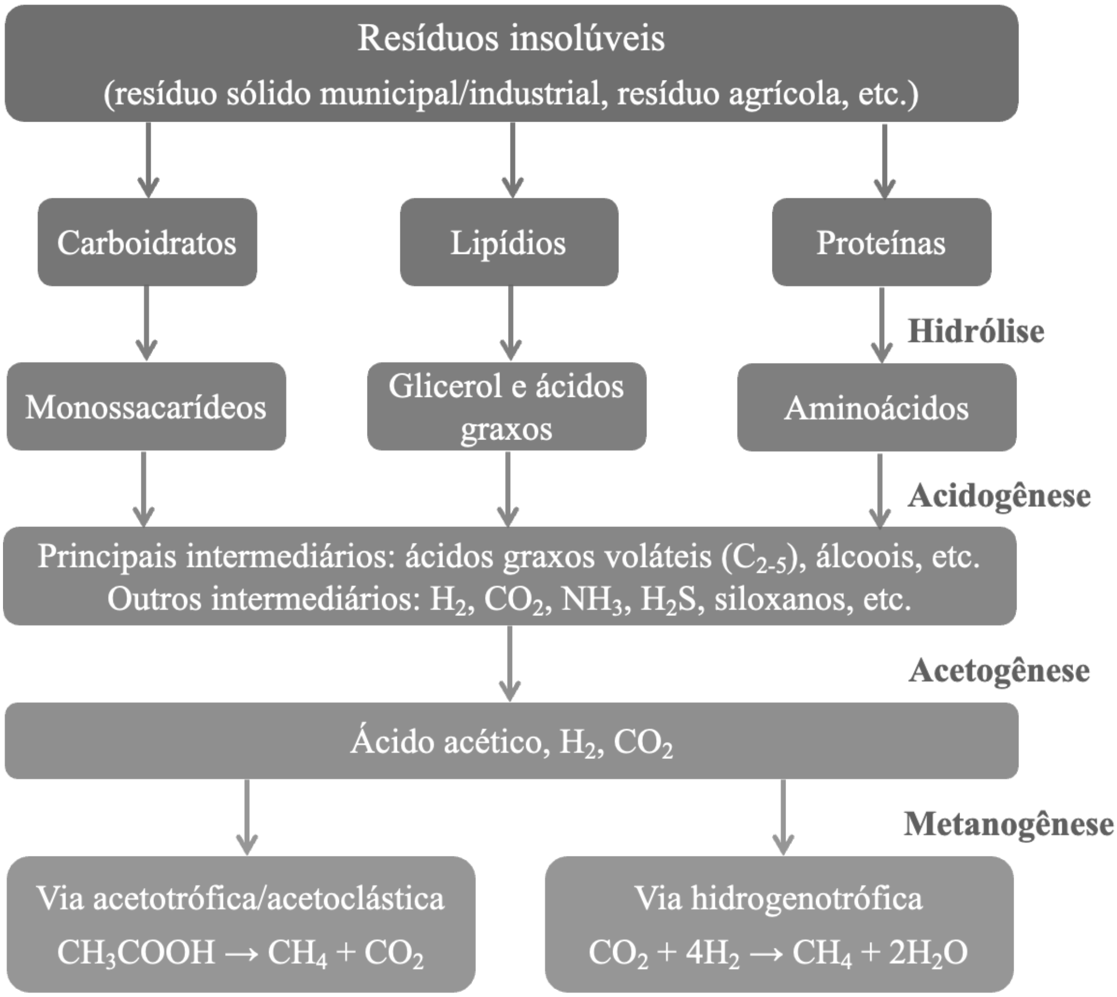

Fig. 2.2. Etapas envolvidas no processo de DA (JUNG et al., 2021).

A DA é um processo altamente complexo e muitos fatores interferentes ainda não são totalmente esclarecidos. Para alcançar o potencial máximo desta tecnologia, o controle de alguns parâmetros é crucial. Composição e estrutura química do substrato (relação C/N, biodegradabilidade, bioacessibilidade e biodisponibilidade), temperatura, pH e concentração de ácidos graxos voláteis (AGVs) estão entre os parâmetros mais importantes que afetam o desempenho de um sistema de DA. Além disso, existem alguns compostos e condições que podem causar efeito inibitório no processo e que precisam ser monitorados (HAGOS et al., 2017; NESHAT et al., 2017).

2.4.1 Etapas da digestão anaeróbia

A hidrólise é, teoricamente, o primeiro passo da DA, durante o qual macromoléculas orgânicas complexas (polímeros) são convertidas em compostos mais simples e solúveis (monômeros e oligômeros) (AGUSTINI; GUTTERRES, 2017b; LI; CHEN; WU, 2019; NESHAT et al., 2017). Lipídios, carboidratos e proteínas são despolimerizados através de enzimas extracelulares das bactérias hidrolíticas (lipase, celulase, amilase, protease) em ácidos graxos de cadeia longa, açúcares e aminoácidos (SEADI et al., 2008). Geralmente, a hidrólise é a etapa limitante do processo, determinando a velocidade e a eficiência de degradação (AGUSTINI; GUTTERRES, 2017b; MIRMOHAMADSADEGHI et al., 2019).

Os monômeros e oligômeros formados durante a hidrólise são então degradados por bactérias acidogênicas (fermentativas) em ácidos graxos de cadeia curta (propionato, acetato, butirato e lactato), álcoois, e subprodutos gasosos (NH_3, H_2, CO_2 e H_2S), etapa que é conhecida como acidogênese ou fermentação (APPELS et al., 2008; LI; CHEN; WU, 2019; MIRMOHAMADSADEGHI et al., 2019).

A terceira fase da DA é a acetogênese. Nesta etapa, bactérias acetogênicas convertem os ácidos e álcoois orgânicos de alto peso molecular produzidos na etapa anterior, em ácido acético, CO_2 e H_2, que são os substratos diretos para a etapa seguinte e final, a metanogênese (APPELS et al., 2008; MIRMOHAMADSADEGHI et al., 2019).

Na fase final da DA, metano é produzido por dois grupos de bactérias metanogênicas: as acetoclásticas, que são responsáveis pela decomposição do acetato em metano e dióxido de carbono, e as metanogênicas hidrogenotróficas, que produzem metano utilizando o hidrogênio como doador de elétron e o dióxido de carbono como receptor (APPELS et al., 2008; LI; CHEN; WU, 2019; NESHAT et al., 2017).

De acordo com Seadi *et al.* (2008), 70% do metano formado origina-se do acetato, enquanto os 30% restantes são produzidos a partir da conversão de hidrogênio (H_2) e dióxido de carbono (CO_2).

A metanogênese é um passo crítico em todo o processo de digestão anaeróbia, pois é a reação bioquímica mais lenta do processo, além de ser severamente influenciada pelas condições de operação. Composição do substrato, taxa de alimentação, temperatura e pH são exemplos de fatores que influenciam o processo de metanogênese. A sobrecarga do digestor, as mudanças de temperatura ou uma grande entrada de oxigênio podem resultar no cessamento da produção de metano (SEADI et al., 2008).

2.4.2 Biogás: características e panorama mundial

O biogás é uma mistura de gases produzido a partir da DA de compostos orgânicos (KHAN et al., 2017; WU et al., 2015). Resíduos de aterros, lodo de esgoto, esterco animal, resíduos agrícolas, entre outros, são as principais fontes de geração de biogás (WU et al., 2015). O biogás consiste principalmente de metano (CH_4), em uma faixa de 50 a 70%, e dióxido de carbono (CO_2), em uma concentração de 30 a 50%. O teor relativo de CH_4 e CO_2 no biogás é principalmente dependente da natureza do substrato e dos parâmetros empregados no processo de DA. Além desses dois gases, o biogás pode conter adicionalmente quantidades menores de outros compostos: nitrogênio (N_2), em concentrações de 0-3%, que podem ter origem no ar saturado do influente; vapor de água (H_2O), em concentrações de 5 a 10%, ou superior nos casos que operam com temperaturas termofílicas, derivada da evaporação do meio; oxigênio (O_2), em concentrações de 0–1%, que entra no processo a partir do substrato alimentado ou de vazamentos; sulfeto de hidrogênio (H_2S), em concentrações de 0–10.000 ppmv, que é produzido a partir da redução do sulfato contido em alguns resíduos; amônia (NH_3), proveniente da hidrólise de materiais proteicos; hidrocarbonetos em concentrações de 0-200 mg/m^3 e siloxanos em concentrações de 0-41 mg/m^3, originários por exemplo, de efluentes de indústrias cosméticas (ANGELIDAKI et al., 2018; GAO et al., 2018; KHAN et al., 2017; WU et al., 2015).

Além do CH_4, todos os outros gases contidos no biogás são considerados impureza. O conteúdo de energia do metano descrito pelo Poder Calorífico Inferior (PCI) é de 50,4 MJ/kg-CH_4 ou 36 MJ/m^3-CH_4 (nas condições CNTP). Quanto maior o teor de CO_2 ou N_2 no biogás, menor seu PCI. Por exemplo, para o biogás com teor de metano na faixa de 60 a 65%, o PCI é de aproximadamente 20 a 25 MJ/m^3 de biogás. Existem diversos tratamentos visando eliminar os compostos indesejáveis do biogás, ampliando sua gama de aplicações (ANGELIDAKI et al., 2018).

Em 2019, a capacidade máxima de geração de biogás instalada em usinas ao redor do mundo era de 19.381 MW, mais que o dobro do observado em 2010, 9.519 MW. A Alemanha lidera a lista de países com a maior capacidade instalada (mais de 7.061 MW), seguida por EUA (2.368 MW), Reino Unido (1.775 MW), Itália (1.575 MW), China (799 MW), Turquia (534 MW) e Tailândia (530 MW) (IRENA, 2020b).

Embora represente apenas 0,8% da energia renovável global instalada (IRENA 2020a), em comparação com outras fontes de energia renováveis, a produção de biogás é independente das flutuações sazonais, pode ocorrer de forma estável e, portanto, promete uma forma confiável de produzir energia (KOUPAIE et al., 2019).

2.4.2.1 Utilização e melhoramento do biogás

Existem quatro formas básicas de utilização do biogás: produção de calor e de vapor, geração/co-geração de eletricidade, uso como combustível em veículos e, mais recentemente, produção de químicos. No entanto, a utilização do biogás bruto é limitada pelos contaminantes presentes e, na maioria dos casos, tratamentos são necessários para viabilizar sua aplicação (APPELS et al., 2008). Atualmente, existem diferentes tratamentos visando remover os compostos indesejáveis do biogás, ampliando sua gama de aplicações.

O primeiro tratamento está relacionado à “limpeza do biogás” e inclui a remoção de compostos nocivos e/ou tóxicos (como H_2S, Si, COVs, siloxanos, CO e NH_3) (ANGELIDAKI et al., 2018). A adsorção com carvão ativado é utilizada para a remoção de siloxanos e de H_2S. Para este último contaminante, também são utilizados microrganismos que oxidam o H_2S a enxofre elementar e alguns sulfatos, membranas, adsorção líquida, dentre outras técnicas (APPELS et al., 2008; MILTNER; MAKARUK; HARASEK, 2017). Alguns autores recomendam também a secagem do biogás, uma vez que ele é saturado com vapor de água quando sai do digestor (APPELS et al., 2008).

A segunda forma de tratamento diz respeito à purificação do biogás e visa aumentar seu PCI e, assim, convertê-lo em um padrão próximo ao de combustível. Caso o biogás seja purificado de acordo com especificações semelhantes ao gás natural, o produto final é chamado biometano (ANGELIDAKI et al., 2018; SAHOTA et al., 2018). Atualmente, as especificações da composição do gás natural dependem das regulamentações nacionais e, em geral, é necessário um teor de metano superior a 95% (KHAN et al., 2021). No processo de purificação do biogás, o CO_2 presente pode ser removido ou convertido em CH_4 através da reação com H_2 (ANGELIDAKI et al., 2018; SAHOTA et al., 2018). Diversas tecnologias estão comercialmente disponíveis ou sob pesquisa, tais como adsorção por oscilação de pressão, purificação química, com água ou com solvente orgânico, e separação por membrana ou criogênica (KHAN et al., 2021). Além disso, o biogás também pode ser convertido por meio da reforma a seco do metano em produtos de maior valor agregado, como H_2, que é considerado uma energia limpa promissora e amplamente utilizada em células a combustível, ou ainda utilizada para a síntese de combustíveis líquidos e produtos químicos de valor agregado, como álcoois, plásticos e hidrocarbonetos (JUNG et al., 2021). A seleção da tecnologia apropriada para a purificação do biogás bruto depende do uso final do biogás, da economia envolvida e da eficiência do processo de melhoramento (KHAN et al., 2021).

2.4.3 Manejo do digestato anaeróbio

O digestato é o resíduo digerido resultante da produção de biogás (ALBURQUERQUE et al., 2012; BONETTA et al., 2014; KOSZEL; LORENCOWICZ, 2015). Muito mais que um resíduo, é um subproduto gerado durante o processo de DA que pode contribuir para a viabilidade técnica e econômica de toda a cadeia produtiva de biogás (DRAGICEVIC et al., 2018). A massa do digestato representa cerca de 87% da matéria-prima alimentada no biorreator (TAMPIO; MARTTINEN; RINTALA, 2016), e a medida que a DA se consolida no mercado de energia renovável, seu gerenciamento torna-se um desafio cada vez maior, de modo que sua reutilização tem sido bastante estudada nos últimos anos. Para facilitar o transporte, manuseio e gestão, o digestato é comumente separado nas frações líquida e sólida, resultando em um percentual de massa na proporção de 90 para 10%, respectivamente (DUTTA et al., 2021).

A composição do digestato depende do substrato utilizado, do inóculo, das condições de operação e da configuração do biorreator (LU; XU, 2021). De forma geral, o digestato contém resquício da matéria orgânica parcialmente degradada, biomassa microbiana (DUTTA et al., 2021), macro (N,P) e micronutrientes (K, Na, Ca e outros) (WANG; LEE, 2021). Ao promover a degradação da matéria orgânica, a DA produz um subproduto de elevada estabilidade biológica, alta maturidade e baixo risco de presença de patógenos (PIGOLI et al., 2021). Tanto o nitrogênio quanto o fósforo presentes no substrato são mineralizados durante o processo de DA, mas não são eliminados do sistema, o que gera um digestato rico nestes dois macronutrientes e com potencial de ser utilizado como corretivo de solo ou fertilizante agrícola (FERNÁNDEZ-RODRÍGUEZ et al., 2021; RIVA et al., 2016).

Por ser rico em nutrientes mineralizados essenciais (WANG; LEE, 2021) e de fácil absorção para as plantas (DUTTA et al., 2021), a aplicação mais comum do digestato tem sido como fertilizante agrícola. A adição ao solo do digestato obtido a partir da DA de diversos resíduos tem sido reportada na literatura dos últimos anos: digestato de esterco de gado misturado com palha agrícola (BARŁÓG; HLISNIKOVSKÝ; KUNZOVÁ, 2020; RIVA et al., 2016), de resíduos sólidos de moinho de oliva (FERNÁNDEZ-RODRÍGUEZ et al., 2021), de resíduos alimentares (CHEONG et al., 2020; REN et al., 2020), de esgoto (SAPP et al., 2015), co-DA de esterco de gado e de suíno, cama de aviário e resíduos de cebola (IOCOLI et al., 2019) e de esterco animal (aves, vacas e ovelhas), soro de leite, silagem de milho, resíduos de azeitona e polpa cítrica (MUSCOLO et al., 2017). Um exemplo prático de utilização do digestato como fertilizante acontece na Alemanha, onde a quantidade de N e P no digestato obtido em plantas de biogás espalhadas por todo o país fornece 26% da produção de fertilizante nitrogenado e 80% da produção de fertilizante fosfatado (LU; XU, 2021).

Além da aplicação direta no solo, o digestato líquido pode ser usado como fonte de nutrientes na produção de algas, e o digestato sólido pode ser usado para a produção de bioetanol ou como combustível em processos térmicos, ou ainda convertido em produtos de valor agregado por meio de um processo de pirólise. Mais recentemente, tem-se ainda estudado a recuperação de nutrientes do digestato (N, P e K) através da tecnologia de membranas (KAPOOR et al., 2020).

Capítulo 3

Performance enhancement strategies of anaerobic digestion technology: a critical assessment

Neste capítulo são destacadas as vantagens e limitações das principais técnicas que vem sendo citadas na literatura como estratégias para melhorar o desempenho da DA e, consequentemente, aumentar a produção de biogás. Este capítulo completa a revisão bibliográfica desta tese e foi submetido para publicação no livro *Anaerobic Biodigesters for Human Waste Treatment* da série *Environmental and Microbial Biotechnology*, editado pelo Dr. Mukesh Meghvansi e a ser publicado pela editora Springer Nature.

Abstract

Anaerobic digestion is one of the most promising conversion technologies for managing organic solid waste due to methane-rich biogas and the recycling of nutrients. However, some process limitations need to be mitigated for an efficient industrial application. This chapter consolidates and summarizes research associated with the advantages/limitations of various performance-enhancing strategies further to promote the development and industrial applications of AD technology. The proposed techniques are classified into six main areas: (i) parameter optimization, (ii) physical, chemical, and biological pretreatments, (iii) co-digestion, (iv) additives, (v) bioreactor design, and (vi) genetic engineering.

Keywords: parameter optimization; pretreatments; additives; co-digestion; bioreactor design; metagenomic.

3.1 Strategies to improve anaerobic digestion technology

Anaerobic digestion (AD) is a suitable and efficient technology for organic materials management, and it is also predicted to play a vital role in the future of renewable energy production. However, AD is a complex and susceptible process involving numerous microorganisms with ultimate operational environmental conditions (HAGOS et al., 2017), so knowing the main parameters that affect the process is essential for better decision making on the configuration of the reaction (CREMONEZ et al., 2021).

Some strategies have been reported in the literature for enhancing AD performance concerning biogas production and process stability. The proposed techniques can be classified into six primary areas: operating parameter optimization, pretreatments, additives, co-digestion, bioreactor design and optimization, and genetic technology. To analyze the relevance of each of these strategies and establish a trend for future research, a quantitative review of the literature published in the last decade was conducted, separated into two-time intervals, 2011-2015 and 2016-2020. The articles counted in this analysis were found in the Scopus database, including title, abstract and keyword. The terms entered in the search were "anaerobic" and "digestion", plus the term corresponding to the strategy being searched. The percentages corresponding to each strategy and for each time interval are shown in Fig. 3.1.

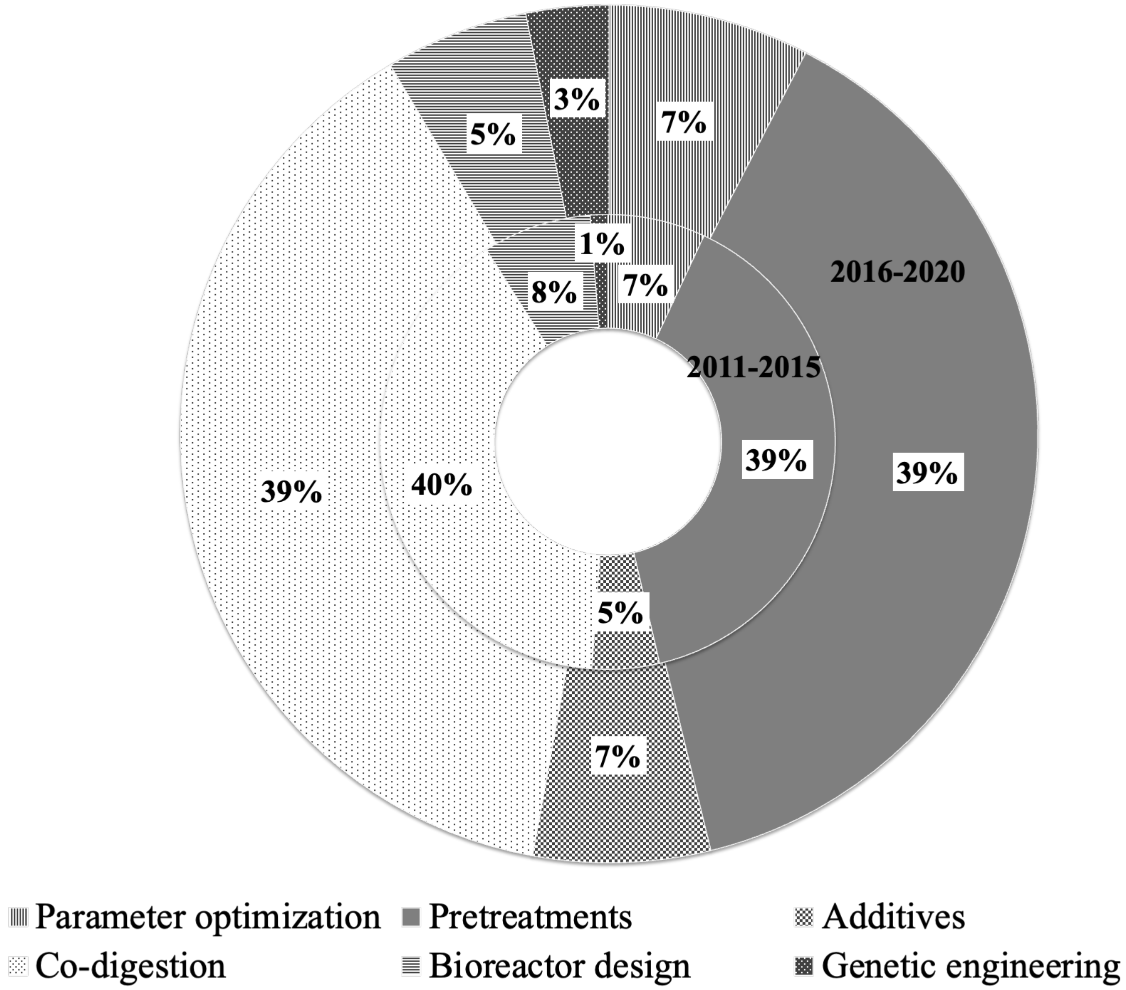

Fig. 3.1. Percentage of published literature on strategies for improving biogas production during 2011-2015 and 2016-2020.

Data presented in Fig. 3.1 provides a good representation of the research progress in the past decade, indicating popularity and future developmental potential. The main research focuses were almost equally placed on co-digestion and pretreatments during the first period. As characterization techniques and information on substrate influence on AD became available, the advantages of mixing different types of waste began to be studied, mainly as a strategy for nutrient balance. Furthermore, recognizing the abundance of lignocellulosic biomass as a possible substrate for AD gave rise to research associated with pretreatment strategies. During 2016–2020, while co-digestion methods and pretreatment techniques remain popular, genetic technology increased attention and the use of additives was highlighted. Concerning genetic technology, the increase is associated with the greater availability of highly efficient and easy-to-use biomolecular tools for genetic analysis and manipulation. The strategy of using additives to improve the performance of AD, in turn, has gained significance because of advancements in scientific knowledge and the application of new materials, such as nanoparticles.

It is also worth mentioning that considering the research as a whole, the number of published works more than doubled considering the time interval of 2016-2020 (4.419 articles) compared to 2011-2015 (2.173 articles), which confirms the interest of the scientific community in the AD of waste and consolidates biogas as renewable energy of extreme importance and potential for the near future.

3.1.1 Optimization of operating parameters

Operational parameters including pH, temperature, organic loading rate (OLR), solid/hydraulic retention time (SRT/HRT), moisture content, mixing/agitation condition, besides substrate characteristics (particle size, nutrients and C/N ratio), are key factors that determine the operational efficiency of biogas production and the process stability of AD. Optimal configuration and manipulation of the operating parameters of AD can enhance the activity and growth of crucial anaerobic microorganisms, potentially resulting in significantly improved process stability and biogas yield (WU et al., 2021).

Advanced monitoring methods can enable monitoring critical parameters in AD systems, allowing early detection of process disturbances. Ideal monitoring methods should be in-situ, online, automated, and continuous. The parameter detection process is directly carried out during plant operation, so that no delay or temporal dislocation is introduced in the process-monitoring scheme. However, monitoring AD in biogas plants has proven to be extremely difficult because of a lack of robust and feasible online-measurement systems and the high non-linearity of AD processes (WU et al., 2019).

3.1.1.1 pH

pH is a crucial parameter in the dynamic detection and regulation of the AD process, as operating stability and bacterial activity are significantly affected by changes in pH (ZHANG;

LOH; ZHANG, 2019). In addition, each group of microorganisms has a different range of optimum pH (APPELS et al., 2008). Methanogenic archaea are extremely sensitive to pH, with an optimal value in the range of 6.5 to 8.2 (KAINTHOLA; KALAMDHAD; GOUD, 2019; ZAMRI et al., 2021). For microorganisms acting in the steps of hydrolysis and acidogenesis, the optimum pH value is in the range of 5.5 and 6.5, respectively (KHALID et al., 2011; MAO et al., 2015). This is one of the fundamental reasons engineers prefer to disconnect the hydrolysis/acidification and acetogenesis/methanogenesis processes in two-stage AD reactors (PANIGRAHI; DUBEY, 2019).

Treating the process as a whole, the pH range from 6 to 8 seems to be the most suitable for AD (XU et al., 2018), with neutral pH being the ideal (KUMAR; SAMADDER, 2020; PANIGRAHI; DUBEY, 2019). It is noteworthy that methane formation is considerably suppressed at pH values below 6.0 or above 8.5 (KHALID et al., 2011; MIRMOHAMADSADEGHI et al., 2019).

Volatile fatty acids (VFAs) produced during the acidogenesis stage tend to reduce the pH. This reduction in pH is counterbalanced by the activity of methanogens, which produce alkalinity in the form of CO_2 (in the gas phase), ammonia, and bicarbonate (in the liquid phase) (APPELS et al., 2008; PANIGRAHI; DUBEY, 2019). Low buffering capacity can be mitigated by reducing OLR, adding salts for converting CO_2 to bicarbonate, or directly adding bicarbonate (PANIGRAHI; DUBEY, 2019).

3.1.1.2 Temperature

Temperature is one of the most critical operational parameters of AD because it directly affects the thermodynamic equilibrium of the biochemical reactions, the activity of various enzymes and co-enzymes, microbial growth rate, diversity of the microorganisms, the bioavailability of metals, sludge quality, stability and consequently, the production of biogas (PANIGRAHI; DUBEY, 2019; ZAMRI et al., 2021). Thermodynamic show that endergonic reactions, such as the decomposition of propionate into acetate, CO_2 and H_2, would become energetically more favorable at higher temperatures, while exergonic reactions (hydrogenotrophic methanogenesis) are less favored at higher temperatures (APPELS et al., 2008).

AD microorganisms can typically operate in three different temperature ranges: psychrophilic (below 20°C), mesophilic (35-40°C), and thermophilic (50-65°C). From the point of view of biochemical reactions, a higher performance of AD can be achieved at a higher temperature within the limit of the established range. A very low-temperature digester may not facilitate the enzyme catalytic efficiency, while a too high-temperature digester may denature sensitive enzymes and subsequently lead to process failure (ZHANG; LOH; ZHANG, 2019). Compared with the mesophilic AD process, thermophilic conditions typically exhibit many advantages, including greater capacity of biogas generation, higher specific growth rate of microbes, lower HRT, higher reduction of pathogens and degradation of cell walls in feedstock, and better separation of digestate into solids and liquid phases (PAN et al., 2021; PANIGRAHI; DUBEY, 2019). On the other hand, the high reaction rate of acidogenesis in the thermophilic process results in the accumulation of VFAs in the digester, which may inhibit the activities of the methanogens. Another concern in a thermophilic AD is the high energy requirement and process instability, which may negatively affect energy balance and the whole digestion process (PANIGRAHI; DUBEY, 2019). The ideal condition would be to operate at thermophilic temperature in the hydrolysis and acidogenesis steps and at mesophilic temperature in the methanogenesis step, consistent with a two-phase AD reactor (KAINTHOLA; KALAMDHAD; GOUD, 2019; MAO et al., 2015).

Generally speaking, selecting an operating temperature depends on multiple factors, including feedstock characteristics, energy demand, financial support, and sanitization requirements. However, the primary concern during the construction and operation of any AD system should be temperature management and control (WU et al., 2021). It is essential to maintain a stable operating temperature in the digester as frequent fluctuations in temperature affect bacteria, especially the methanogenics (APPELS et al., 2008).

3.1.1.3 Solid and hydraulic retention time

The retention time, usually expressed in days, is the time required for the complete degradation of organic matter by microorganisms (MAO et al., 2015; SIDDIQUE; WAHID, 2018; ZHANG; LOH; ZHANG, 2019). There are two types of retention time discussed: the SRT, which is defined as the average time that solids remain in the digester, and the HRT, which is defined as the average time interval that the sludge remains in the digester

(KHADAROO et al., 2019; PAN et al., 2021). Generally, a retention time of 15 to 30 days is required to treat organic solid waste under mesophilic AD conditions and 10 to 20 days for thermophilic conditions (WU et al., 2021). However, this parameter must be optimized according to process temperature, organic load, and substrate composition (PAN et al., 2021; PANIGRAHI; DUBEY, 2019).

A relatively long retention time can mitigate process instability to a certain degree, yield a higher cumulative methane production, and lead to a more significant reduction of the total volatile solids (VS). Still, the initial capital cost is significantly increased by the investment required to build high-volume digesters. In contrast, too low HRT may not provide sufficient time for the multiplication of anaerobic microorganisms. The discharging procedure may also cause "wash out" of microorganisms and smaller aggregates through the discharging operation, eventually resulting in VFAs accumulation like organic overload, which can cause bacterial inhibition and even process failure (PAN et al., 2021; WU et al., 2021; ZHANG; LOH; ZHANG, 2019).

3.1.1.4 Organic loading rate

The OLR can be considered the amount of organic matter fed to the AD process per unit of time and volume (SIDDIQUE; WAHID, 2018), generally expressed as $kg_{COD}m^{-3}d^{-1}$ or $kg_{VS}m^{-3}d^{-1}$ (WU et al., 2021). OLR varies depending on raw material characteristics, operating temperature, and HRT. Very low values can cause malnutrition of the fermentative microorganisms, resulting in low efficiency of the process (ZHANG; LOH; ZHANG, 2019). On the other hand, high values can lead to the accumulation of VFAs in the digester, imbalance of nutrients, and, consequently, the inhibition of bacteria, and even to the failure of the AD process (PANIGRAHI; DUBEY, 2019). Generally, biogas yield increases with increasing OLR (WU et al., 2021), but this ratio is limited. The maximum tolerable value of OLR is determined according to the type of organic solid waste and the operating conditions of the digester (ZHANG; LOH; ZHANG, 2019).

3.1.1.5 Moisture content

The metabolism and activities of microorganisms require moisture. AD can be operated with a total solids (TS) content ranging from 5% to 35%. Depending on the TS content, AD can be divided into three different categories: wet (<10% TS), semi-dry (10-20% TS), and dry (>20% TS) (KUMAR; SAMADDER, 2020). Both the processes (wet and dry) have their advantages and disadvantages. The wet process has the benefits of lower inoculum requirement, shorter retention time, higher methane production, and more significant reduction of VS. In contrast, the advantages of the dry (or solid-state) process include high OLR, smaller digester volume, less energy expenditure for system heating and agitation, and easier digestate handling (MIRMOHAMADSADEGHI et al., 2019). The literature indicates that the highest methane production rates occur for cases with moisture in the range of 60-80% (KHALID et al., 2011).

3.1.1.6 Mixing/agitation condition

Proper agitation of the digesters favors the supply of nutrients to the microorganisms, in addition to removing products of metabolism (especially the H_2 blocking layer), avoiding the formation of foam and temperature gradients, and homogenizing the solution, eliminating any division into layers (KAINTHOLA; KALAMDHAD; GOUD, 2019; MIRMOHAMADSADEGHI et al., 2019). There are currently three effective systems to accomplish agitation in the anaerobic digesters, mechanical, pneumatic, and hydraulic mixers, among which mechanical agitators are the most dominant systems being used. However, experimental results have demonstrated that aside from the type of substrate treated and agitation equipment applied in the AD process, the duration and intensity of mixing significantly affect the process stability and biogas yield. Vigorous mixing intensity may inhibit the process stability by disrupting microbial flocks and reducing the extracellular polymeric substances that affect the adhesion of microbial cells to each other and other surfaces. Regarding the mixing duration, intermittently or minimally mixed systems appear to have similar or even better biogas production and process stability than the continuous mixing regime while reducing energy requirements and maintenance costs of biogas-producing systems (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020).

3.1.1.7 Particle size

The particle size of the substrates directly influences the biodegradation rate and the stability of AD (MIRMOHAMADSADEGHI et al., 2019; SIDDIQUE; WAHID, 2018; ZHANG; LOH; ZHANG, 2019). Very large particles would lead to the clogging of the digester as well as difficulty in the digestion function for the responsible microbes, while reducing the particle size of the substrate could increase the specific surface area accessible to microbial attacks, thus allowing faster reaction rates and increased biogas yield (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020). However, the excessive reduction in particle size could over-quicken substrate hydrolysis, resulting in build-up of VFA and ammonia, which could destabilize the reactor (KUMAR; SAMADDER, 2020).

3.1.1.8 Nutrients and C/N ratio

Some micro and macronutrients are necessary for the survival and growth of microorganisms involved in the AD process (MIRMOHAMADSADEGHI et al., 2019). Carbon and nitrogen are essential sources for energy and development of new cell structures (ZAMRI et al., 2021). Also, phosphorus is a crucial element for capturing and transferring energy by energy carriers in the cellular activities of microorganisms and sulfur is needed as a nutrient for the growth of methanogens (MIRMOHAMADSADEGHI et al., 2019).

In mono-digestion, a single substrate is either carbon-rich or nitrogen-rich, such that it is difficult to maintain a balanced C/N ratio. The imbalance between nutrients has a negative effect on microbial activity, being a limiting factor in the AD of organic waste (NESHAT et al., 2017). At a high C/N ratio, excessive acidification occurs due to rapid substrate degradation during initial stage of the digestion, resulting in the process instability. The excess carbon content will slow down the degradation process, as the microorganisms will take more time to consume the available carbon. On the contrary, a low C/N ratio limits microbial growth due to lack of carbon, which can lead to the accumulation of ammoniacal nitrogen and VFAs in the digester (KUMAR; SAMADDER, 2020). Relatively high C/N ratios are found in oat straw (47-51), wheat straw (51-151), sugar cane waste (139-151), or sawdust (199-501). At the same time, relatively low C/N ratios are observed for pig manure (7-15) and food wastes (2-18) (SIDDIQUE; WAHID, 2018). For the success of the AD process, the literature recommends that the C/N ratio is in the range of 20:1 to 30:1 (PANIGRAHI; DUBEY, 2019). The C/N ratio can be modified by mixing different substrates (KHALID et al., 2011; XU et al., 2018; ZHANG; LOH; ZHANG, 2019).

Besides essential macronutrients, the anaerobic process requires microelements at a relatively lower concentration. Iron, cobalt, nickel, zinc, molybdenum, manganese, copper, selenium, and tungsten are the leading metal microelements with a recommended concentration between 1×10^{-6} and 1×10^{-15} M (RASAPOOR et al., 2020). Micronutrients are crucial cofactors in numerous enzymatic reactions involved in the biochemistry of methane formation (ROMERO-GÜIZA et al., 2016). Iron, as well as acting as a growth factor, plays an essential role as a stimulating agent in the formation of ferredoxins and cytochromes, vital components in cell metabolism. In addition, the iron may react with H_2S to precipitate sulfur and iron sulfide (II) and reduce the effects of corrosion of H_2S in the biogas. As a growth factor for acetogenic microorganisms, cobalt assists in the stability of the AD process. Nickel is necessary for the growth of methanogenic bacteria, especially methanogenic archaea. Zinc is required for the synthesis of carbonic anhydrase by methanogens. Depending on the methanogenic pathway (acetoclastic or hydrogenotrophic), several metalloenzymes are involved in the AD process and, consequently, different micronutrients are needed. Generally, micronutrient supplementation, or rather, the right combination of multinutrients, can improve AD's performance. On the other hand, high concentrations of micronutrients can inhibit the AD process (MIRMOHAMADSADEGHI et al., 2019).

3.1.1.9 Inhibitor compounds

Inhibitory compounds can either be present in the substrate or intermediate compounds generated during the AD process that inhibit the process at high concentrations. The main inhibitory compounds are VFAs, ammonia, metals and toxic compounds (KUMAR; SAMADDER, 2020; PANIGRAHI; DUBEY, 2019).

VFAs (acetic, propionic, butyric, and valeric acid) are intermediate products of the initial stage of the AD process and act as indicators of the correct balance between hydrolysis, acidogenesis, and methanogenesis since the produced VFAs should get converted into CH_4 and CO_2 by the active microorganisms. In a stable anaerobic digester, the concentration of VFAs is about 50 to 250 mg/L. On the other hand, high concentrations of VFAs decrease the pH of the medium, which can inhibit methanogenesis (KUMAR; SAMADDER, 2020). The inhibitory concentration of VFAs has been reported to be about 1500 mg/L (NESHAT et al., 2017).

Ammonia has advantages and disadvantages for AD. It can act as a pH neutralizer and be a valuable nitrogen source for methanogenic bacteria (NESHAT et al., 2017). On the other hand, high concentrations of ammonia can poison microorganisms and inhibit AD. Ammonia inhibition occurs mainly during the AD of protein-rich solid waste. This behavior occurs because the digestion of these residues results in the hydrolysis and solubilization of the protein into amino acids through proteolytic bacteria. Amino acids, in turn, are further hydrolyzed by hydrolytic/hydrogenic bacteria to release ammonia, H_2, CO_2, and fatty acids (AGUSTINI; GUTTERRES, 2017). Ammonia inhibiting concentration in the anaerobic digester mainly depends on the pH, temperature, C/N ratio, type of substrate, and inoculum (KUMAR; SAMADDER, 2020).

Metallic elements, including light metals (sodium, potassium, magnesium, calcium, and aluminum) and heavy metals (chromium, cobalt, copper, zinc, and nickel), are among the micronutrients necessary for the survival of microorganisms. However, at high concentrations, they can cause inhibition of AD by interrupting the function of enzymes (MIRMOHAMADSADEGHI et al., 2019). Phenolic compounds, furans, cyanides, and sulfur oxides are also toxic to microorganisms and inhibit the AD process when in high concentrations (NESHAT et al., 2017).

3.1.1.10 Chemical composition of substrates

Substrates contain the full range of complex and straightforward organic materials that can be used in the AD process, being carbohydrates, proteins, and lipids the main ones. Depending on their sources (agricultural, urban, food, industrial waste), specific organic compounds may predominate, although in most cases, the exact composition of substrates is complex to obtain (RASAPOOR et al., 2020). A comprehensive understanding of feedstock's types and biochemical characteristics plays a critical role in preventing process instability and optimizing AD systems. Commonly used parameters for monitoring feeding substrates are TS, VS, C/N ratio, macro and micro elements, particle size, pH, quickly degradable compounds (e.g., soluble sugar, protein, carbohydrate, lipids), low degradable or undigested compounds (e.g., hemicelluloses, cellulose, and lignin) and impurities and inhibitors (e.g., ammonia, heavy metals). Unfortunately, it remains difficult for most commercial biogas plants to achieve online/in-situ monitoring and analysis of feedstock with equipment and methods currently available at an acceptable cost (WU et al., 2021).

3.1.1.10.1 Carbohydrate-rich biomass waste

Carbohydrates are considered an essential organic component in solid waste for biogas production (KHALID et al., 2011). Carbohydrates (commonly sugars) are present in all substrates in different proportions. AD from organic wastes with high levels of simple sugars can result in rapid accumulation of VFAs in the reactor, a decrease in pH, and consequent suppression of methanogenesis. For the balanced operation of anaerobic reactors, mixing a substrate containing large amounts of simple carbohydrates with residues with a lower content of easily biodegradable organic components (HAGOS et al., 2017; SIDDIQUE; WAHID, 2018). Residues composed of lignocellulosic carbohydrates, by contrast, show slow degradability, considerably increasing the HRT of digesters and consequently reducing biogas production rates (CREMONEZ et al., 2021). A pretreatment step may be essential to improve the digestibility of these residues.

3.1.1.10.2 Protein-rich biomass waste

Like carbohydrates, proteins are also present in the majority of organic substrates. Protein-rich substrates can produce biogas with high methane content. The microbial degradation of proteins results in the release of the ammonium ion, which can be a potent inhibitor of methanogenic bacteria and ammonia when in high concentration. Ammonia and ammonium are in balance to maintain the process stability, which depends heavily on operating factors (pH, temperature). Choosing suitable co-substrates and adjusting the C/N ratio can minimize this problem (HAGOS et al., 2017; SIDDIQUE; WAHID, 2018).

3.1.1.10.3 Lipid-rich biomass waste

Organic materials with high-fat content are readily degradable and therefore have high biogas production. However, lipids cause different problems in anaerobic digesters in high concentrations, including blocking, adsorption to biomass (causing mass transfer problems), and microbial inhibition. Mixing carbohydrate-rich with fat-rich materials (slowly and rapidly degradable, respectively) is advantageous in nutritional balance, enriches microorganisms, reduces inhibitor accumulation, and increases stability, resulting in high efficiency in biogas production and methane yield (HAGOS et al., 2017; SIDDIQUE; WAHID, 2018).

3.1.1.11 Inoculum

The choice of the inoculum is a fundamental step on the excellent performance of the AD process, as it not only provides trace elements, moisture content, and nutrients (macro and micro) but also provides the buffering capacity in the system (CREMONEZ et al., 2021; KAINTHOLA; KALAMDHAD; GOUD, 2019). The use of sludge from digesters or treatment ponds to degrade residues of similar characteristics to the substrates of interest makes the systems more efficient and adapted. It may reduce lag phase time considerably, especially in more complex systems (CREMONEZ et al., 2021).

Substrate and inoculum concentrations or substrate-to-inoculum (S/I) ratio are among the most important factors influencing AD performance and stability. Very high or low inoculum concentrations may disrupt the AD process by affecting the bacterial lag phase time, reactions rate, nutrient consumption, biomass growth behavior, and so on. Therefore, establishing a balance between substrate and inoculum concentrations, or in other words, optimizing the S/I ratio, seems to be a good strategy for the successful operation of AD processes (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020). Results showed that lower S/I ratios favored biogas production due to the rapid degradation of VFAs. Conversely, bioreactors operated at higher S/I ratios (50:50 and above) experienced excessive VFA concentrations, a sharp drop in pH, and consequently biogas production levels lower than theoretical values. In general, the accumulation of toxic intermediate products such as VFAs at higher S/I ratios indicates a kinetic imbalance between the microorganisms responsible for producing and consumping acids inside the anaerobic reactor (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020).

3.1.2 Pretreatment

Substrate pretreatment is a common step in raw material processing, especially for those with high lignocellulosic content (VENTURIN et al., 2018). Characteristics such as the presence of lignin and cellulose, crystallinity and degree of polymerization of cellulose, accessible surface area, and degree of acetylation of hemicellulose impact the biodegradability of biomass (ZHENG et al., 2014). During pretreatment, difficult-to-degrade compounds present in the substrate, such as hemicellulose, cellulose, and lignin, are transformed into soluble compounds, which are more easily hydrolyzed by bacterial enzymes (NESHAT et al., 2017;

ZHANG; LOH; ZHANG, 2019). A successful pretreatment should preserve the organic materials in biomass, develop the progress of beneficial to hydrolysis, avoid the formation of any toxic and/or inhibitory compounds, and be environmental friendly and economically feasible (PANIGRAHI; DUBEY, 2019).

In the literature, pretreatments are classified as physical, chemical or biological. The choice of which pretreatment to apply depends on several factors, such as the crystallinity of the lignocellulose, the degree of polymerization, the accessible surface area, and the relative amount of acetyl groups (ZHANG; LOH; ZHANG, 2019; ZHENG et al., 2014). A combination of pretreatments can also be used and is often found in the literature.

3.1.2.1 Physical pretreatment

Physical pretreatment refers to methods that do not use chemicals or microorganisms during the process. The physical pretreatment methods most commonly used in substrate preparation for AD include mechanical and thermal pretreatment (NESHAT et al., 2017).

Mechanical pretreatment (AKBAY; DIZGE; KUMBUR, 2021; MOSET et al., 2018) of biomass reduces particle size and is typically applied before other pretreatment methods (MILLATI et al., 2020). The reduction in particle size can breakdown the lignin-hemicellulose complex, changing the morphology of the lignocellulosic biomass and reducing the degree of crystallinity and polymerization of cellulose, which increases the available cellulosic content. Furthermore, it increases the accessible surface area, providing better contact between the substrate and anaerobic bacteria, resulting in improved digestibility of solid waste (ARIUNBAATAR et al., 2014; KAINTHOLA; KALAMDHAD; GOUD, 2019; TIAN; ZHAO; CHEN, 2018). However, if the mechanical pretreatment is excessive (obtained particle size is less than the optimal particle size), digestion performance will deteriorate, such as the accumulation of VFAs, and net energy output will not be positive (CAI et al., 2021). The advantages of mechanical pretreatment include no odor generation, an easy implementation, better dewaterability of the final anaerobic residue and moderate energy consumption. Disadvantages include no significant effect on pathogen removal and the possibility of equipment clogging or scaling (ARIUNBAATAR et al., 2014).

Thermal treatment technique (ENNOURI et al., 2016; RAJPUT; ZESHAN; VISVANATHAN, 2018) can be of two types, thermal pretreatment (only temperature is controlled, like hot air oven, microwave, hot water bath) and hydrothermal pretreatment (both temperature and pressure are controlled, like autoclave and steam explosion) (PANIGRAHI; DUBEY, 2019). The optimal temperature is a wide range for the different substrates and pretreatment time. In general, for the hot air oven, microwave, autoclave, and hot water bath pretreatment, the optimal temperature ranges are 90-170 °C, 140-200 °C, 90-175 °C, and 90-100 °C, respectively (CAI et al., 2021). Although high temperature can achieve a better pretreatment effect, it is worth noting that temperatures above 150 °C could cause the formation of some inhibitory compounds (such as phenolic, furfural, and hydroxymethylfurfural), which are toxic to anaerobic microorganisms (HASHEMI et al., 2021). The main effect of thermal pretreatment is the disintegration of cell membranes, thus resulting in solubilization of organic compounds. Thermal pretreatment also leads to pathogen removal and reduction of sludge viscosity (ARIUNBAATAR et al., 2014). The disadvantages are the formation of odorous compounds and high operation and maintenance costs (KHADAROO et al., 2019).

3.1.2.2 Chemical pretreatment

Chemical pretreatment of solid organic waste is carried out using strong acids, alkalis, ionic liquids, and oxidizing agents. Compared to physical pretreatment techniques, chemical pretreatments have received more attention in recent years from researchers due to their better performance in increasing the yield of biogas production (ZHANG; LOH; ZHANG, 2019).

The effect of chemical pretreatment depends on the type of method applied and the characteristics of the substrates. It is not suitable for easily biodegradable substrates containing high amounts of carbohydrates due to their accelerated degradation and subsequent accumulation of VFAs, which leads to failure of the methanogenesis step. In contrast, it can have an apparent positive effect on substrates rich in lignin (ARIUNBAATAR et al., 2014). The significant impact of chemicals on lignocellulosic substrates is the removal of lignin and hemicellulose, which increases the accessible surface area for enzymatic hydrolysis and the reduction in cellulose crystallinity. Although chemical pretreatment methods are efficient for pretreatment with a short substrate retention time, some concerns such as the high cost, effectiveness of chemical recovery, and chemical discharge into the environment are the most critical barriers in large-scale applications (HASHEMI et al., 2021).

Acid pretreatment (DAI; DONG, 2018; SYAICHURROZI et al., 2019) commonly involves the usage of H_2SO_4, H_2O_2, HCl, HNO_3, CH_3COOH, etc. (CAI et al., 2021; KAINTHOLA; KALAMDHAD; GOUD, 2019). The main objective of acid pretreatment is to solubilize hemicellulose, reduce cellulose and hydrolyze hemicellulose into respective monosaccharides by disrupting the covalent hydrogen bonds and Van Der Waals forces (PANIGRAHI; DUBEY, 2019). Strong acidic pretreatment may produce inhibitory by-products, such as furfural and hydroxymethylfurfural (HMF). Hence, strong acidic pretreatment is avoided, and pretreatment with dilute acids is commonly coupled with thermal methods. Other disadvantages associated with the acid pretreatment include the loss of fermentable sugar due to the increased degradation of complex substrates, a high cost of acids, and the additional cost for neutralizing the acidic conditions before the AD process (ARIUNBAATAR et al., 2014).

Alkaline pretreatment (RAHMAN et al., 2018; SABEEH; LIAQUAT; MARYAM, 2020) uses NaOH, $Ca(OH)_2$, KOH, and $NH_3•H_2O$ to modify the structure of lignocellulosic substrate components and make them more degradable to microorganisms and enzymes. By removing cross-links, alkaline pretreatment increases porosity and internal surface area, decreases the degree of polymerization and crystallinity, disrupts the lignin structure, and breaks bonds between lignin and other polymers. The effectiveness of alkaline pretreatment is associated with the lignin content of the substrates. The main disadvantage of this technique is the high cost of the base (PATINVOH et al., 2017; TIAN; ZHAO; CHEN, 2018; ZHENG et al., 2014).

Ionic liquid (MANCINI et al., 2018; PÉREZ-PIMIENTA et al., 2021) pretreatment uses molten salts (like 1-ethyl-3-methylimidazolium acetate), and organic components such as N-methyl morpholine N-oxide (NMMO) in moderated temperatures to cause the dissolution of the biomass components (HASHEMI et al., 2021). The cellulose dissolution mechanism in these reagents involves the oxygen and hydrogen atoms of the hydroxyl groups of the cellulose molecule, which form electron donor-receptor complexes and interact with ionic liquids. After this interaction, hydrogen bonds are broken, leading to the opening of the hydrogen bonds between the cellulose molecular chains, resulting in the dissolution of the molecule. The solubilized cellulose can be precipitated with anti-solvents such as ethanol, methanol, acetone, or water. It was found that the recovered cellulose has the same degree of polymerization and polydispersity as the initial cellulose, but significantly different macro and microstructures, especially concerning decreasing crystallinity and increasing porosity (ZHENG et al., 2014). Along with structural changes, ionic liquids can partially remove lignin (HASHEMI et al., 2021). Despite the high cost of ionic liquids (ZHENG et al., 2014), the main advantage of this method is the easy recycling of the pretreatment solvent by distillation (MANCINI et al., 2018).

The oxidative pretreatment (CESARO; BELGIORNO, 2013; HODAEI et al., 2021) accelerates the reaction rates by applying oxygen or air at high temperature (above 260°C) and pressure (10 MPa) to the feedstock before the AD process. The oxidative methods are Fenton, peroxymonosulfate, dimethyldioxirane, and advanced oxidation process (AOP). Ozone treatment enhances hydrolysis by solubilizing and/or breaking lignin (PANIGRAHI; DUBEY, 2019). Ozonation leaves no chemical residues compared to other chemical pretreatment methods (ARIUNBAATAR et al., 2014). The disadvantage of this process is the high operating cost (KHADAROO et al., 2019).

3.1.2.3 Biological pretreatment

The fundamental concept of biological pretreatment is to improve biodegradability through applying fungi, microbial consortium, and enzymes ensiling the biomass or by adding a biological treatment step before the AD (KOUPAIE et al., 2019). Mild operating conditions and no chemical added make biological pretreatment a less energy-demanding and more environmentally friendly process (PANIGRAHI; DUBEY, 2019). However, the slow reaction rate of biological pretreatment remains a problem (MILLATI et al., 2020).

Fungal pretreatment (TISMA et al., 2018; ZANELLATI et al., 2021) is a standard microbial pretreatment method. Fungi can secrete cellulases, hemicellulases and ligninase. Specifically, those functions of enzymes are involved in the structure change of lignocellulosic biomass, mainly including modifying lignin structure (the ratio of guaiacyl/sinapyl), decreasing cellulose crystalline, increasing substrate porosity, and changing hemicellulose structure (the ratio of xylose/arabinose) (CAI et al., 2021). White-rot fungi are very popular to be used in this kind of pretreatment due to their effectiveness in degrading lignin among other fungi such as brown-, white-, and soft-rot fungi (MILLATI et al., 2020).

Besides pure fungi, the microbial consortium (RAUT; PANDHAL; WRIGHT, 2021; WANG et al., 2021) was also developed and used for the pretreatment of lignocellulosic biomass. The microbial consortium can be obtained through restrictive culture technology, which can select an ideal microbial consortium; artificially, combining a variety of pure bacteria with decomposing lignocellulosic biomass; or directly from natural sources, such as biogas slurry. Among the three types of microbial consortia, biogas slurry is the most likely to be applied due to its economic viability, environmental friendliness, and strong operability. The optimal pretreatment condition depends on the source of microorganisms. Pretreatment time is the most crucial factor that needs to be optimized to reduce organic matter loss and obtain the best results. In addition to the incubation time, pH range, temperature, and oxygen concentration are also vital parameters. In general, microbial pretreatment results in the lab-scale study due to controllable conditions (CAI et al., 2021).

Enzymatic pretreatment (ÇAKMAK; UGURLU, 2020; DOMINGUES et al., 2015) involves oxidative and hydrolytic enzymes often produced by the bacteria and fungi. This pretreatment method is gaining more interest due to the relatively short reaction time, the lower nutrition requirement for the enzymatic reactions, and the fact that most enzymes are not affected by the presence of inhibitors and other microbial metabolisms. However, although enzymatic pretreatment does not require expensive processing equipment, the high cost of the enzyme remains an obstacle for its large-scale application (KOUPAIE et al., 2019). Several strategies have been studied to achieve an effective and cost-efficient enzymatic pretreatment, such as optimizing enzyme activity, enzyme recycle, development of genetically modified organisms that can produce high-quality enzymes, and improving enzyme quality by genetic engineering (MILLATI et al., 2020).

3.1.2.4 Hybrid pretreatments technologies

Combined pretreatment (AMBROSE et al., 2020; PATOWARY; BARUAH, 2018) incorporates two or more pretreatment techniques from the same or different categories. The coupling of different pretreatments may add the isolated advantages of each, leading to better preparing the substrate for the AD process (HASHEMI et al., 2021; ZHENG et al., 2014). Physical pretreatments would increase the accessible surface area and decrease cellulose’s degree of polymerization and crystallinity, while chemical and/or biological pretreatments would facilitate the accessibility of enzymes to cellulose (ZHANG; LOH; ZHANG, 2019). However, combining two or more individual pretreatment methods would require more energy input and higher costs of chemical or biological reagents, which could be a significant obstacle to industrial applications. Thus, deciding on an appropriate combined pretreatment method requires a balanced consideration of chemical and enzyme costs, energy requirements, and potential gain in biogas production (ZHANG; LOH; ZHANG, 2019; ZHENG et al., 2014).

3.1.3 Co-digestion

Some factors can hamper the use of a single substrate in the AD process. The nutritional imbalance of the substrate used stands out as the main one (HAGOS et al., 2017). Anaerobic co-digestion (AcoD), the AD from a mixture of two or more different substrates, appears as a promising option to overcome the drawbacks of mono-digestion and enhance the economic feasibility of AD process (KARKI et al., 2021).

The main advantage of the AcoD-based process is the improvement in biogas production and methane yield. Co-digestion can increase biogas production from 25% to 400% over mono-digestion, considering the same substrates acting separately (HAGOS et al., 2017; KARKI et al., 2021).

AcoD of two or more substrates (GHOSH et al., 2020; SIMIONI et al., 2021; ZAHAN et al., 2018) provides better availability and balance of macro and micronutrients (for good microbial growth), dilution of toxic or inhibitory compounds, moisture balance and better-buffering capacity. AcoD also allows positive synergistic effects on process efficiency, increase in biodegradable organic load, expansion of the microbial community involved in the process, and higher concentrations of active biomass. This leads to better process stability and greater biogas generation (HAGOS et al., 2017; KHALID et al., 2011; PANIGRAHI; DUBEY, 2019; TYAGI et al., 2018). In addition, the economic advantages of sharing the AD system and treating more waste simultaneously deserve to be highlighted (HAGOS et al., 2017; SIDDIQUE; WAHID, 2018).

Recalling that to optimize the AcoD process, the adjustment of operational parameters (temperature, pH, OLR, etc.) of the reactor and the characterization of the substrates involved as to the C/N ratio, biodegradability, bioaccessibility, and bioavailability, remain crucial (HAGOS et al., 2017; SIDDIQUE; WAHID, 2018).

3.1.4 Additives

AD depends on a set of enzymatic reactions facilitated by complex microorganisms. Many researchers argue that applying some additives mixed with the substrate could intensify waste biodegradation and increase methane production (ZHANG; LOH; ZHANG, 2019).

Additives have been successfully used to improve methane production in anaerobic digesters by different approaches, such as (i) supplying nutrients at low concentrations; (ii) adsorbing inhibitory elements at high concentrations; (iii) improving digester buffer capacity; and (iv) enhancing substrate biodegradability (ROMERO-GÜIZA et al., 2016). The performance of additives may not be directly compared because many factors of different AD systems are not the same, such as substrate type, digester configuration, and anaerobic microbial community composition. An excess of additives may deteriorate the performance of the AD process (LIU; WEI; LENG, 2021).

Some studies have reported that various metal-based additives (HASSANEIN; LANSING; TIKEKAR, 2019; SCHMIDT et al., 2014) can enhance AD performance and improve anaerobic bioconversion processes by stimulating methanogens to increase CH_4 production during AD. The addition of trace metals is advantageous for the growth of methanogens during enzymatic synthesis. Many enzymes include transition metals as catalytic centers at active sites or as cofactors for electron transport. Trace metals can be added to the AD process in various forms, e.g., chloride salts, metal oxides, and metal nanoparticles. The presence of trace metals in AD systems is beneficial for the hydrolysis and acidogenesis stages and it also augments the microbial species to improve biogas production. The optimal concentration of trace elements in an AD system depends on substrate type, especially for micronutrient-deficient substrates. The different species of methanogens have additional requirements for trace element concentrations. The combined addition of the different kinds of metals resulted in a high biogas yield in AD. In contrast, a high concentration of metals may inhibit the methanogens, thereby causing low methane production (LIU; WEI; LENG, 2021).

Carbon-based additives (CHEN et al., 2020; TIAN et al., 2017), such as activated carbon (AC), graphene, biochar, carbon cloth, graphite, granular activated carbon (GAC), and carbon nanotubes, have been widely employed to enhance methanogenesis through direct interspecies electron transfer (DIET) (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020; ROMERO-GÜIZA et al., 2016). Carbon-based materials, with its favorable physicochemical properties (fine pore structure, good electrical conductivity, large porosity, and surface area), promote microbial activity, electron transfer among anaerobes, and biogas production because these materials provide a good immobilization matrix for microorganisms. Furthermore, carbon-based accelerants with conductive capacity can enhance AD performance by building bioelectrical relations between the methanogens and acetogens, which is beneficial for relieving VFAs accumulation (LIU; WEI; LENG, 2021).

Most of the goals achieved by inorganic additives can also be reached through biological additives (ROMERO-GÜIZA et al., 2016). Bioaugmentation technique (AKYOL et al., 2019; MLAIK et al., 2019; PESSUTO et al., 2016) consists of adding specifically selected microorganisms into biogas digesters to improve the performance of the AD process (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020). The addition of bacterial consortia or cellulolytic bacteria can promote the hydrolysis rate and increase the CH_4 yield (LIU; WEI; LENG, 2021). The addition of anaerobic fungi increased VFAs degradation, which would be favorable to alleviate the accumulation of these acids in anaerobic digesters and, consequently, increase biogas production (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020). Enzymes can also be directly dosed into AD systems as they are capable of acting in the presence of various toxic and recalcitrant substrates and under a wide range of environmental conditions (e.g., pH, temperature, and salinity), remaining active even if these conditions quickly change. They can work in the presence of microorganisms and inhibitors of microbial metabolism, and, due to their smaller size, higher solubility and mobility, have easier access to the substrates than microbes do (ROMERO-GÜIZA et al., 2016). Bioaugmentation with a mixture of different species of anaerobic fungi or with different enzymatic compositions is more efficient since each enzyme degrades only a few specific substrates (LIU; WEI; LENG, 2021).

Supplementation of mineral-based additives (KOTSOPOULOS et al., 2008) has been a cost-effective approach to control the accumulation of undesired VFAs and enhance the biogas production from the AD process. Among various minerals existing in nature, silicate minerals like wollastonite ($CaSiO_3$) appear to react effectively with H^+ ions provided by VFAs dissociation, thus neutralizing the pH inside the AD reactors. Aluminosilicate minerals like zeolite have also been widely used as the ion exchanger and adsorbent for the removal of organic molecules, as well as supplying the trace elements in AD (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020).

In recent years, numerous research efforts have been made to use inorganic wastes as cost-effective supplements for dealing with VFAs inhibition in AD processes. Among them, ashes (LO et al., 2010) from the thermochemical processing of biomass have shown promising results mainly via providing alkalis and trace metals required for balancing the AD process (ALAVI-BORAZJANI; CAPELA; TARELHO, 2020).

3.1.5 Bioreactor design and optimization

The design and configuration of the bioreactor used for the AD of organic solid waste have a strong influence on the performance of the process. Several types of bioreactors can be used to perform AD processes, continuous stirred-tank reactor (CSTR), upflow anaerobic sludge bioreactor (UASB), anaerobic baffled reactors (ABR), anaerobic sequencing batch biofilm reactor (AnBBR), packed-bed reactor (APBR), anaerobic structured-bed reactor (ASTBR), expanded granular bed reactor (EGBR), expanded granular sludge blanket (EGSB), sequential batch reactor (SBR), and leachate bed reactor (LBR) (CREMONEZ et al., 2021). About the configuration mode, the three main groups include batch reactors, one-stage continuously fed reactors, and two- or multi-stage continuously fed systems.

The operation of reactors in batch mode is quite simple: the reactor is fed with raw material, which is degraded for a certain period (HRT). After this time, the bioreactors are emptied, and a new batch is fed. Although they are equipment simple to build and operate and lower cost, batch reactors have some limitations. Low quality, fluctuations in biogas production, and loss of biogas during the depletion of bioreactors are among them (KHALID et al., 2011).

Regarding the other two configurations of bioreactors mentioned, systems fed continuously from one or more stages, the only difference is that in the first, all biochemical reactions take place in one bioreactor, and in the second, the stages of AD (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) occur separately. The system with two or more stages is considered a promising method for treating organic waste with high efficiency in terms of waste degradation and biogas production yield. This type of system allows the selection and enrichment of different bacteria in each stage and increases the process's stability by optimizing the HRT to avoid overloading and toxic material accumulation (HAGOS et al., 2017; KHALID et al., 2011). Temperature, pH, organic load, and other conditions can be independently optimized for each stage to favor the AD step's specific microbial reactions taking place there (CREMONEZ et al., 2021).

3.1.6 Genetic technology

The efficiency of methane production from the AD of waste is related to the number of species and the physiological behavior of the microbial consortium involved. Therefore, the description of these two aspects can be used to characterize an AD process and improve its efficiency. The development of genetic engineering techniques, such as gene sequencing technologies, metagenome technology, and synthetic biology, allows not only to change a DNA sequence but to build entirely new sequences and put them into operation in microbial cells to make AD faster and more efficient (ZHANG; LOH; ZHANG, 2019).

Over the past decade, the development of high-throughput sequencing technology and the decrease in its cost facilitated the application of bioinformatics tools in the studies of metagenomics data of microbial communities in anaerobic digesters. For instance, it is now known that there is a clear correlation between taxonomic and functional genes patterns of anaerobic microorganisms in biogas-producing digesters. Additionally, based on the analysis of functional genes by metagenomics studies and network-based approaches, corresponding metabolic pathways can be estimated, consequently pointing to identifying the actual dominant metabolic pathways and mechanisms in the biogas digesters (ZHANG et al., 2019). Moreover, it has been established that both taxonomic and functional patterns can be influenced by environmental variables such as digester configuration, operational parameters, and feedstock characteristics (LUO; FOTIDIS; ANGELIDAKI, 2016).

The ongoing development of high-throughput molecular tools and bioinformatics allows sequencing of the bulk DNA instead of only 16S rRNA genes and thereby provides both taxonomic and functional information of microbiomes to the extent that was unimaginable even a few years ago (LUO; FOTIDIS; ANGELIDAKI, 2016). For instance, metagenomics analysis and functional characterization of the biogas microbiome using high throughput shotgun sequencing and a novel binning strategy were performed to disclose nearly one million genes and extract 106 microbial genomes. As a result, several key microbial genomes encoding enzymes involved in metabolic pathways including amino acids fermentation, fatty acids degradation, carbohydrates utilization and syntrophic acetate oxidation were identified

(ZHANG et al., 2019). It is noteworthy that traditional microbiological methodologies (e.g., isolation and cultivation of pure strains) continue to be used in order to study the physiology and metabolism of new isolates derived from biogas reactors, which could not be accomplished by metagenomic sequencing. Therefore, the combination of the new molecular technologies with traditional microbiological methodologies is necessary for future studies (LUO; FOTIDIS; ANGELIDAKI, 2016).

The application of genetic engineering to improve biogas production is done through the manipulation of genes in specific pathways and/or incorporation of specific DNA fragments into target species. Current and future research trends are directed towards the development and applications of genetically modified organisms to address the challenges encountered from naturally occurring conventional strains (CHRISTY; GOPINATH; DIVYA, 2014). For example, a super microorganism could be created to degrade recalcitrant waste such as lignocellulosic structures, converting cellulose and hemicellulose into biogas and making the AD of these wastes more efficient. Of course, cost-effectiveness will be an important factor to consider even if the genetic engineering approach proves effective in the biogas industry (ZHANG; LOH; ZHANG, 2019).

Knowledge obtained from the modeling of microbial communities provides the possibility of optimizing the AD process by regulating the microorganisms. Feedstock composition, digester configuration, operating parameters, and environmental conditions are the leading driving factors for community structure changes (ZHANG et al., 2019).

3.2 Conclusions

AcoD is a promising technology for effective waste management and resource recovery while promoting economic and environmental sustainability. However, AD is a complex process and depends on several factors, so some strategies to improve AD performance concerning biogas production and process stability have been studied in this chapter. Some of the key findings related to these strategies include: (i) Optimal configuration and manipulation of the operational parameters of AD (pH range of 6–8, stable operating temperature, appropriate moisture content and proper agitation, C/N ratio between 20−30) can enhance the activity and growth of key anaerobic microorganisms, resulting in significantly improved process stability and biogas yield; (ii) Different pretreatment methods (physical, chemical, biological or hybrid) have been applied to eliminate physical and chemical barriers, by transforming recalcitrant compounds into soluble ones, which are more easily hydrolyzed by bacterial enzymes. The selection of the optimum pretreatment technique depends on multiple factors such as the characteristics of the biomass, the capital and operating cost of the pretreatment and the ease of the operation; (iii) Co-digestion of two or more substrates appears as a promising option to overcome the drawbacks of mono-digestion (mainly with regard to nutritional imbalance) and enhance the economic feasibility of AD process; (iv) Supplementation of metal-, carbon- and mineral-based or biological additives has shown to be a practical approach to enhance AD performance and improve anaerobic bioconversion processes by stimulating methanogens to increase CH_4 production during AD; (v) Two- or more stages AD bioreactors could be used to optimize and synchronize the rates of reaction of the multi-step AD process, with the selection and enrichment of different bacteria in each stage; (vi) The development of genetic engineering techniques allows the description of species and physiological behavior of the microbial consortium involved in AD, which can be used not only to characterize the process, but also to make it faster and more efficient. As a general conclusion, it is clear that the challenge regarding this topic is not only the optimization of each strategy separately but to combine and synergize the various enhancement strategies, seeking to optimize the AD process and the sustained conversion of waste into sustainable bioenergy.

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Capítulo 4

Material e Métodos

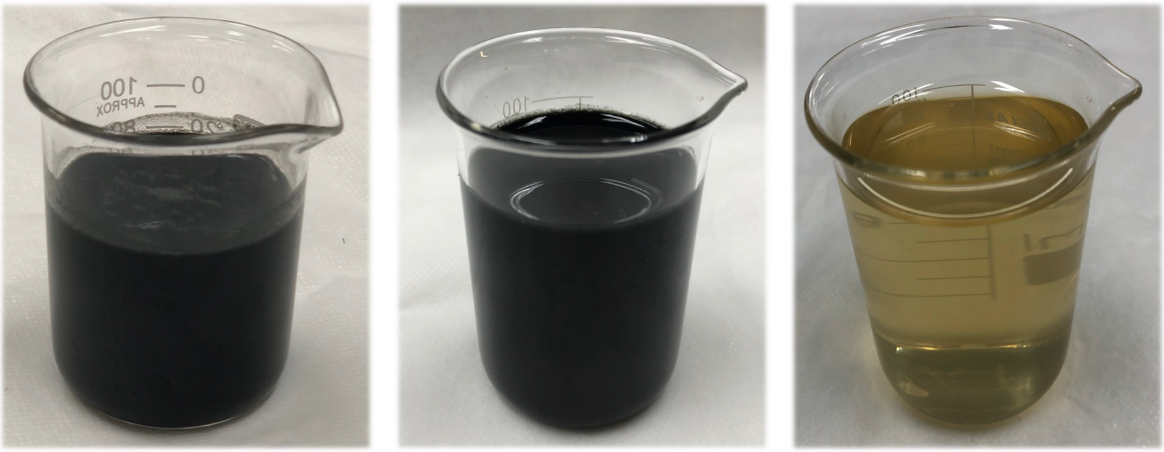
Neste capítulo é apresentado o detalhamento dos materiais e métodos utilizados na realização desta tese: os resíduos utilizados nos ensaios de DA e técnicas utilizadas na sua caracterização; detalhes acerca dos procedimentos experimentais e dos parâmetros testados nos ensaios de DA; e a apresentação das técnicas utilizadas para acompanhar a eficiência da biodegradação dos resíduos e aproveitamento do digestato.

4.1 Resíduos

Os resíduos utilizados no desenvolvimento deste trabalho foram:

- Farelo de couro *wet-blue* (Fig. 4.1d), obtido a partir da etapa de rebaixamento da pele curtida com sais de cromo, coletado no curtume Nimo (Montenegro - RS), que realiza o processamento completo do couro;
- Lodo da estação de tratamento de efluente (ETE) do curtume Bender (Estância Velha - RS), que realiza o processamento completo do couro com sais de cromo, após o processamento do efluente do processo completo (Fig. 4.1a); e lodo da ETEs proveniente do curtume Minuano (Lindolfo Collor - RS), coletado após o processamento do efluente do processo completo, onde foram utilizados sais de cromo na etapa de curtimento e taninos vegetais na etapa de recurtimento (Fig. 4.1b);
- Palhas de aveia (Fig. 4.1e) e de trigo (Fig. 4.1f), coletadas em lavouras agrícolas localizadas na região norte do Rio Grande do Sul;
- Outros materiais: i) digestato líquido (Fig. 4.1c) obtido a partir da co-DA de resíduos de curtume (lodo de ETEs + farelo de couro *wet-blue*) incubados em temperatura mesofílica; ii) fertilizante comercial NPK 3-21-21 (3% N, 21% P_2O_5 e 21% K_2O, além de Ca, S, B, Mn e Zn) (Fig. 4.1h); iii) ureia comercial 27-0-0 (27% N, além de Ca e Mg) (Fig. 4.1i); e iv) solo agrícola (Fig. 4.1g) coletado em lavoura no norte do Rio Grande do Sul a uma profundidade de aproximadamente 10 cm.

Os resíduos foram mantidos em temperatura ambiente antes da sua utilização, com exceção do digestato líquido, que foi armazenado em geladeira (4°C).



a. Lodo de ETE de um curtume que utiliza sais de cromo na etapa de curtimento

b. Lodo de ETE de um curtume que utiliza sais de cromo na etapa de curtimento e taninos vegetais no recurtimento

c. Digestato líquido obtido pela DA de resíduos de couro curtido com sais de cromo

d. Farelo de couro *wet-blue*

e. Palha de aveia

f. Palha de trigo

g. Solo agrícola

h. Adubo comercial

i. Ureia comercial

Fig. 4.1. Resíduos e materiais utilizados ao longo deste trabalho.

4.1.1 Pré-tratamentos aplicados nas palhas de aveia e de trigo

As palhas de aveia e de trigo foram previamente moídas e peneiradas (passante na peneira de 6 *mesh*), e, buscando aumentar a sua biodisponibilidade, submetidas às metodologias de pré-tratamentos apresentadas no Quadro 4.1 (GOMEZ-TOVAR et al., 2012; MANCINI et al., 2018; TAHERDANAK; ZILOUEI; KARIMI, 2016).

Quadro 4.1. Pré-tratamentos aplicados nas palhas de aveia e de trigo.

Pré-tratamento	Metodologia
Ácido	10 g de palha imersa em 100 mL de HCl (2%) e incubada por 2 h a uma temperatura de 90°C. Após o pré-tratamento, a palha foi filtrada, lavada até pH neutro, seca em estufa (60°C) e armazenada para posterior utilização.
Alcalino	10 g de palha imersa em 100 mL de NaOH (1,6%) e incubada por 24 h a uma temperatura de 30°C. Após o pré-tratamento, a palha foi filtrada, lavada até pH neutro, seca em estufa (60°C) e armazenada para posterior utilização.
Térmico	Autoclavagem da palha a 121 °C (1 atm) por 15 minutos.
Ácido + térmico	10 g de palha imersa em 100 mL de HCl (2%) e autoclavada a 121 °C (1 atm) por 15 minutos. Após o pré-tratamento, a palha foi filtrada, lavada até pH neutro, seca em estufa (60°C) e armazenada para posterior utilização.
Alcalino + térmico	10 g de palha imersa em 100 mL de NaOH (1,6%) e autoclavada a 121 °C (1 atm) por 15 minutos. Após o pré-tratamento, a palha foi filtrada, lavada até pH neutro, seca em estufa (60°C) e armazenada para posterior utilização.

4.1.2 Caracterização dos resíduos

A caracterização dos resíduos foi realizada de acordo com as seguintes técnicas: sólidos voláteis (SV), carbono orgânico total (COT), carbono total (CT), carbono inorgânico (CI), nitrogênio total Kjeldahl (NTK), teor de fenóis, de taninos vegetais e de cromo, e pH. O efeito dos pré-tratamentos aplicados às palhas de aveia e de trigo foi avaliado em relação às mudanças na composição química (teor de lignina, celulose e hemicelulose), cristalinidade, através de difração de raios-X (DRX), e grupos funcionais, pela técnica de espectroscopia no infravermelho com transformada de Fourier (FTIR).

A descrição da metodologia empregada em cada técnica de caracterização é apresentada na seção 4.4.

4.2 Ensaios de biodegradação

Os ensaios de biodegradação foram realizados em duas configurações de biorreatores de bancada de 300 mL, confeccionados em vidro, hermeticamente fechados, ambos contendo dois pontos para coleta de amostra gasosa: uma válvula superior, para medição do volume de biogás produzido e concomitante alívio da pressão interna, e uma válvula rosca lateral com septo, para coleta de amostras para análise cromatográfica da composição do biogás. As duas configurações de biorreator utilizadas diferem pela ausência (Fig. 4.2a) ou presença (Fig. 4.2b) de um terceiro ponto de coleta: uma válvula rosca inferior com septo, para coleta de amostra líquida. Os biorreatores foram incubados em uma estufa para cultura bacteriológica modelo DL-CBE18L da marca DeLeo a 35 °C (temperatura mesofílica).

A relação entre as quantidades de lodo (25 mL) e farelo de couro *wet-blue* (1 g) utilizada em todos os experimentos é equivalente, em proporção, à quantidade desses resíduos gerada pelos curtumes da região (AGUSTINI et al., 2018a). Para os demais resíduos analisados (palhas de aveia e de trigo), se estipulou a quantidade de 1 g. Por conter uma alta carga de microrganismos, o lodo também atuou como inóculo dos ensaios. Para garantir condições favoráveis ao crescimento e metabolismo dos microrganismos, 200 mL de uma solução de nutrientes foi adicionada aos ensaios.

Após a incubação, os biorreatores foram abertos e as suas amostras precipitadas e dissolvidas foram coletadas, separadas e armazenadas a 4°C.

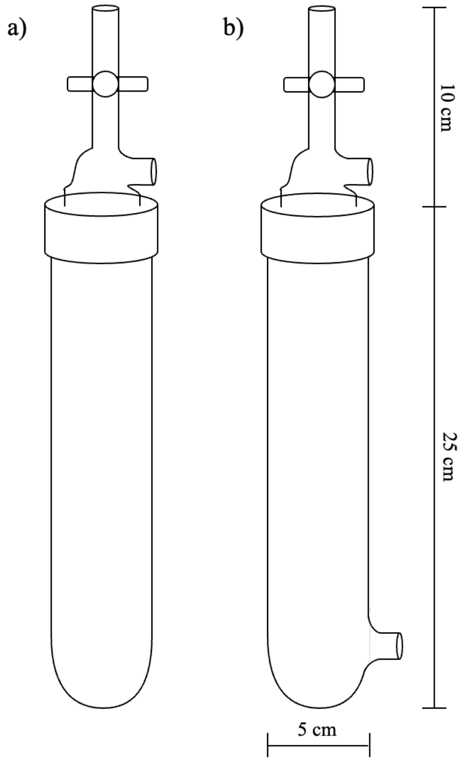


Fig. 4.2. Biorreatores de bancada utilizados nos experimentos.

4.2.1 Análise das misturas antes da biodegradação

Ensaios análogos aos realizados nos biorreatores foram montados em frascos graduados de 500 mL para a realização das análises nos resíduos antes da biodegradação. Os frascos foram agitados por 1 h em temperatura ambiente em um agitador de Wagner da marca ACB Labor e armazenados por 24 h a 4 °C para sedimentação. Decorrido esse tempo, os frascos foram abertos e as suas amostras precipitadas e dissolvidas foram coletadas separadamente e armazenadas a 4°C.

4.2.2 Monitoramento do volume de biogás

O monitoramento da produção volumétrica de biogás nos biorreatores foi realizado com o auxílio de um dispositivo que utilizava o princípio de Mariotte (Fig. 4.3). A medida do volume produzido era realizada da seguinte forma: uma mangueira era conectada entre o dispositivo e o biorreator, a válvula do dispositivo era aberta e, na sequência, a válvula do biorreator era aberta, resultando na saída de uma quantidade de água presente no interior do dispositivo equivalente ao volume de biogás gerado no biorreator acima da pressão atmosférica. Essa quantidade de água era pesada e convertida em volume através da massa específica na temperatura ambiente no momento da medida. O pH da solução aquosa presente no interior do dispositivo era mantido abaixo de 3,0 para evitar a solubilização de componentes do biogás (CO_2 e H_2S). A medida era realizada três vezes por semana, para a pressão dentro do biorreator manter-se próxima da pressão atmosférica e, assim, evitar a inibição por pressão. A condição de parada adotada foi produção de biogás menor de 1 mL/dia.

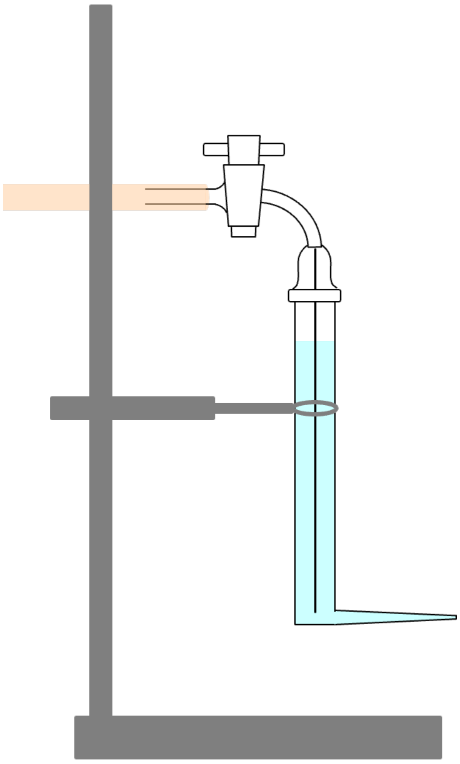


Fig. 4.3. Dispositivo para medição volumétrica de biogás.

4.2.3 Composição do biogás

A determinação das frações de ar (O_2 e N_2), metano (CH_4) e dióxido de carbono (CO_2) do biogás produzido nos biorreatores foi realizada em um cromatógrafo gasoso modelo GC-2014 da marca Shimadzu equipado com um detector de condutividade térmica (TCD) e uma coluna empacotada ShinCarbon (ST 100/120 2 m 1 mmID 1/16” OD Silco). O gás de arraste utilizado foi Hélio 5.0 Analítico (White Martins) a uma vazão de 10 mL/min. As temperaturas do injetor, do pré-detector e do detector foram mantidas a 200, 240 e 250°C, respectivamente. O programa de temperaturas do forno foi: 40 °C por 3 min, rampa a 15°C/min até 150°C, e 150 °C por 0,67 min, totalizando 11 min de análise. Os resultados foram expressos em percentuais de O_2, N_2, CH_4 e CO_2.

4.2.4 Análise da biodegradação dos resíduos

A biodegradação dos resíduos foi avaliada através de análises de sólidos totais, fixos e voláteis, COT, CT, CI, NTK e pH. As análises foram realizadas antes e após a biodegradação. Para alguns casos, foram também analisados os AGVs formados, através da cromatografia líquida de alta eficiência (HPLC). A descrição da metodologia empregada em cada técnica de caracterização é apresentada na seção 4.4.

4.3 Ensaio de fitotoxicidade do digestato líquido

O nível de fitotoxicidade do digestato líquido foi estabelecido por meio de testes de germinação e desenvolvimento de plantas de aveia.

4.3.1 Índice de germinação das sementes

O teste de germinação das sementes foi avaliado em placas de Petri (90 mm de diâmetro), contendo 2 papéis filtro (base e tampa) (peso de 80 g/cm^2 e espessura de 205 μm), que foram umedecidos com 1 mL de líquido. Digestato, fertilizante comercial NPK e ureia comercial foram testados nas concentrações de 100% (puro), 10% e 1% (fração volumétrica), diluídos em água destilada. Um teste controle também foi realizado apenas com água destilada. Em cada placa, foram adicionadas 10 sementes e o experimento foi realizado em duplicata. As placas foram então transferidas para uma câmara de germinação sob condições controladas de temperatura (20°C) e escuridão por 5 dias (ALBURQUERQUE et al., 2012). Após este período, o número de sementes germinadas foi contado, os comprimentos das radículas dessas sementes medidos e o índice de germinação (IG) calculado como uma porcentagem do controle (água destilada) conforme especificado pelas Eq. 4.1, 4.2 e 4.3, de acordo com a metodologia descrita por Zucconi *et al.* (1981) *apud* Selim, Zayed e Houssam (2012).

$$Germinação\ da\ semente\ (\%) = \frac{N°\ de\ sementes\ germinadas\ no\ tratamento}{N°\ de\ sementes\ germinadas\ no\ controle} \times 100 \tag{4.1}$$

$$Comprimento\ da\ radícula\ (\%) = \frac{Média\ do\ comprimento\ das\ radículas\ no\ tratamento}{Média\ do\ comprimento\ das\ radículas\ no\ controle} \times 100 \tag{4.2}$$

$$Índice\ de\ germinação\ (\%) = \frac{Germinação\ da\ semente\ (\%) \times Comprimento\ da\ radícula\ (\%)}{100} \tag{4.3}$$

4.3.2 Bioensaios de crescimento de plantas

O valor agronômico do digestato líquido foi avaliado através do acompanhamento da fase de crescimento inicial de plantas de aveia. Os experimentos foram realizados em vasos plásticos (36 x 15 cm) incubados em estufa cedida pelo Departamento de Agronomia da Universidade Federal do Rio Grande do Sul, de acordo com adaptações da Norma OECD 208 (2006). Em cada vaso, foi adicionado 1,5 kg de solo e quatro condições foram testadas, sendo uma controle (apenas solo) e 3 tratamentos (digestato líquido, fertilizante comercial NPK e ureia comercial).

O digestato líquido, o fertilizante comercial NPK e a ureia comercial foram adicionados e misturados manualmente com o solo, em quantidades equivalentes de nitrogênio. Além disso, o fertilizante NPK e a ureia, originalmente sólidos, foram dissolvidos em água destilada para que o aspecto físico do tratamento não fosse uma variável no processo de absorção de nutrientes. Em cada vaso foram plantadas oito sementes de aveia, separadas por pelo menos 2 cm e a uma profundidade de aproximadamente 3 cm. Após a germinação das sementes, o desenvolvimento das plantas foi monitorado diariamente por um período de 21 dias, contado após 50% da emergência das plantas no grupo controle. Cada vaso foi manualmente irrigado a cada 48 horas com aproximadamente 200 mL de água. Ao final do experimento, cada planta foi colhida e o material da raiz foi removido. As plantas foram secas a 60 °C por 24 h e resfriadas à temperatura ambiente em um dessecador antes do peso seco ser medido.

4.3.3 Caracterização do solo

A caracterização do solo (controle e tratamentos) foi realizada através das técnicas de SV, COT e CI, nitrogênio total (NT), nitrogênio inorgânico (NI), teor de Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn, P e K e pH. A descrição da metodologia empregada em cada técnica de caracterização é apresentada na seção 4.4.

4.4 Técnicas analíticas

Nesta seção estão descritas as metodologias e equipamentos utilizados em análises realizadas no decorrer de todo o trabalho.

4.4.1 Sólidos totais, fixos e voláteis

A determinação do teor de sólidos totais, fixos e voláteis das amostras foi realizada segundo o método gravimétrico (NBR ISO 4684:2014), com o auxílio de uma balança analítica digital modelo EEQ9003F-B da marca Edutec, uma estufa de secagem da marca De Leo, um forno mufla modelo Q318M da marca Quimis e cadinhos de porcelana modelo B-47 marca Chiarotti. O procedimento consistiu em calcinar o cadinho durante 1 h a 600 °C no forno mufla (M_1), adicionar a amostra ao cadinho e secar o conjunto a 100 °C na estufa de secagem até peso constante (M_2), e calcinar o cadinho com a amostra seca a 600 °C no forno mufla por 1 h (M_3). Os cálculos para sólidos totais, fixos e voláteis são mostrados nas Eq. 4.4, 4.5 e 4.6:

$$Sólidos\ totais\ (g) = M_2 - M_1 \tag{4.4}$$

$$Sólidos\ fixos\ (\%) = \frac{M_3 - M_1}{M_2 - M_1} \times 100 \tag{4.5}$$

$$Sólidos\ voláteis\ (\%) = \left(1 - \frac{M_3 - M_1}{M_2 - M_1}\right) \times 100 \tag{4.6}$$

4.4.2 Carbono orgânico total, carbono total e carbono inorgânico

As determinações dos teores de COT, CT e CI das amostras líquidas foram realizadas no equipamento analisador de carbono orgânico total (TOC-L Shimadzu). Todo o carbono presente na amostra é oxidado a CO_2 por combustão catalítica a 680 °C e a detecção é realizada por infravermelho não dispersivo (NDIR). Ar sintético 4.7 (marca White Martins) a 150 mL/min foi utilizado como gás de arraste.

Para as amostras sólidas, as análises foram realizadas no equipamento SSM-500A, também da marca Shimadzu, que utiliza o método de combustão catalítica na temperatura de 900 °C e detector NDIR.

Os dois equipamentos analisam CT e CI, e determinam o COT pela diferença entre os dois primeiros.

4.4.3 Nitrogênio total e nitrogênio inorgânico

O NT das amostras pôde ser determinado através de duas técnicas: NTK ou combustão catalítica. NI foi determinado através de extração com KCl.

4.4.3.1 Nitrogênio total Kjeldahl

A análise de NTK das amostras líquidas foi realizada segundo a norma ASTM D3590-11. A digestão das amostras foi feita com uma solução digestora, composta por H_2SO_4, K_2SO_4 e HgO, em um equipamento digestor Velp Scientifica, modelo DK20 (1 hora em 230 °C + 2 horas em 350°C). A amostra digerida foi destilada em um equipamento destilador de nitrogênio Velp Scientifica, modelo UDK 129. A determinação da amônia destilada foi realizada por titulação com H_2SO_4 0,02 N.

Para as amostras sólidas, a determinação do NTK foi feita de acordo com uma adaptação da norma D2868-10, com adição de H_2SO_4 e de uma mistura catalítica (K_2SO_4 + $CuSO_4$) para a digestão. No caso das palhas de aveia e trigo, também foi inicialmente adicionado H_2O_2, conforme recomendação de (TEDESCO et al., 1995). Após a destilação das amostras digeridas, a determinação da amônia foi realizada por titulação com H_2SO_4 0,03 N.

4.4.3.2 Nitrogênio total

A determinação do NT das amostras de solo foi realizada em um equipamento analisador de carbono, modelo TOC-L da marca Shimadzu equipado com um acessório para determinação de nitrogênio total (TNM-L) e um amostrador automático (OCT-L). A oxidação da amostra ocorre através da sua combustão catalítica a 680 °C e a detecção é realizada por NDIR, utilizando ar sintético 4.7 (marca White Martins) a 150 mL/min como gás de arraste.

As amostras foram previamente digeridas com a adição de H_2O_2, H_2SO_4 e de uma mistura catalítica (K_2SO_4 e $CuSO_4.5H_2O$), e aquecidas em um bloco digestor DK20, Velp Scientifica, nas temperaturas subsequentes de 70 °C por 2 horas, 150 °C por 1,5 horas e 330 °C até que a solução clareasse, atingindo coloração amarelo-esverdeada (aproximadamente 4 horas) (BREMNER; MULVANEY, 1982; TEDESCO et al., 1995; TEIXEIRA et al., 2017).

4.4.3.3 Nitrogênio inorgânico

A determinação da parcela inorgânica de nitrogênio (NH_4^+, NO_3^- e NO_2^-) nas amostras de solo foi realizada através de extração com KCl 1M, na proporção de 1:10 (massa:volume) (VALENTINUZZI et al., 2020). As amostras foram agitadas por 30 minutos, decantadas por 30 minutos e a quantidade de NI extraído foi lida no acessório TNM-L acoplado ao equipamento TOC-L, da marca Shimadzu, de forma similar ao apresentado no item anterior.

4.4.4 pH

O pH foi determinado pelo método potenciométrico com um pHmetro modelo DM-22 da marca Digimed equipado com sensor de temperatura. Para a medição do pH dos resíduos sólidos, 2,5 g de amostra foi adicionada à 50 mL de água destilada e a solução foi mantida em agitação por 24 h (AGUSTINI; DA COSTA; GUTTERRES, 2018). Para a determinação do pH do solo, a relação solo/água destilada utilizada foi de 1:2,5 (DRAGICEVIC et al., 2018).

4.4.5 Cromo

A concentração de óxido de cromo (III) foi determinada pelo método ABNT NBR 13341 (2010) para banho residual de curtimento e recurtimento. Para o farelo de couro, 1 g de amostra foi previamente dissolvida em HCL 50% até a desintegração total. As amostras foram submetidas à digestão em etapas, utilizando os seguintes reagentes: NaOH 4%, H_2O_2 20%, $NiSO_4$ 5%, HCl, 50% e KI 10%. As amostras digeridas foram tituladas com tiossulfato de sódio ($Na_2S_2O_3$) 0,01 N e a concentração de óxido de cromo (III) foi calculada através da Eq. 4.7.

$$Cr_2O_3(g/L) = \frac{V_{Na_2S_2O_3}(mL) \times F_C \times 25{,}33}{V_{alíquota}\,(mL)} \tag{4.7}$$

Onde F_C é o fator de correção da solução de $Na_2S_2O_3$.

4.4.6 Taninos vegetais

A concentração de taninos vegetais foi determinada a partir da equação da reta obtida da curva de calibração preparada com soluções padrão de tanino de acácia (Tanfood/Tanac, 0,01-0,1 g/L). A absorbância das amostras foi lida em um espectrômetro modelo T80 + UV/Vis (PG Instruments) a 278 nm, comprimento característico para taninos (AGUSTINI et al., 2018b).

4.4.7 Fenóis

A concentração de fenóis totais foi estimada como equivalentes de ácido tânico, de acordo com o ensaio de Folin-Ciocalteau (MIRON et al., 2011): 0,5 mL de amostra + 2,5 mL da solução de Folin-Ciocalteau 10% (v/v) + 2,0 mL da solução de carbonato de sódio 4% (m/v). Após 30 min de repouso no escuro, a absorbância das amostras era lida em 760 nm (Visible Spectrophotometer Genesys 30, Thermo Scientific) e comparada com a curva de calibração do ácido tânico (0,01-0,1 g/L, diluições em etanol) elaborada da mesma maneira.

4.4.8 Cromatografia líquida de alta eficiência

A determinação dos AGVs formados foi realizada em um equipamento de cromatografia líquida de alta eficiência (HPLC), marca Agilent, modelo 1200 Infinity, com injetor automático e detector de arranjo de diodos (DAD). A coluna Rezex RHM - Monosaccharide H^+ (Phenomenex, EUA), aquecida a 40°C, com ácido sulfúrico 5 mM como fase móvel a uma taxa de fluxo de 0,6 mL/min foi utilizada. Os ácidos testados foram: fórmico, acético, propiônico e butírico.

4.4.9 Espectroscopia no Infravermelho com Transformada de Fourier

Espectroscopia no infravermelho com transformada de Fourier (FTIR) (Frontier, Perkin Elmer) foi utilizada para analisar alterações nos grupos funcionais das palhas de aveia e de trigo antes e após as mesmas serem submetidas aos pré-tratamentos. Os espectros foram formados de 4000 a 500 cm^{-1} com varredura de 32 vezes.

4.4.10 Difração de Raios-X

As análises de DRX das palhas antes e após os pré-tratamentos foram realizadas em um difratômetro da marca Bruker, modelo D2 Phaser, em um ângulo de $2\theta = 10° - 40°$. A determinação do índice de cristalinidade (CrI) foi baseada nos dados de DRX e calculada através da Eq. 4.8, fornecida por Segal *et al.* (1959).

$$CrI(\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100 \tag{4.8}$$

Em que, I_{002} (fração cristalina) é a intensidade do pico 002 (2θ = 22) e I_{am} (fração amorfa) é a intensidade do pico em 2θ = 18.

4.4.11 Composição lignocelulólisa

Os teores de lignina, hemicelulose e celulose das palhas antes e depois de cada pré-tratamento foram determinados com base no método químico (LI et al., 2004; RAJPUT; ZESHAN; VISVANATHAN, 2018). Inicialmente, a quantidade de extrativos na biomassa foi determinada usando extração por solvente, para a qual, 60 mL de acetona foram usados para 1 g de amostra de palha seca (G_0, g) a uma temperatura constante de 90 °C por 2 h. Em seguida, a amostra foi seca em estufa a 105 °C até peso constante ser atingido (G_1, g). Para determinar o teor de hemicelulose, 150 mL de solução de NaOH 20 g/L foi adicionada a 1g de biomassa seca livre de extrativos (G_1, g). A mistura foi fervida por 3,5 h com reciclo de água destilada, filtrada e lavada até que o pH se aproximasse de 7. A amostra foi então seca até peso constante, resfriada à temperatura ambiente em um dessecador e pesada (G_2, g). O teor de lignina foi determinado usando 30 mL de ácido sulfúrico 72% para cada grama de amostra de biomassa seca livre de extrativos ($G_1 = G_3$, g). Depois disso, a amostra foi mantida à temperatura de 8 - 15 °C por 24 h. Decorrido esse tempo, adicionou-se 300 mL de água destilada e ferveu-se a amostra por 1 h com reciclo de água destilada. A mistura foi então resfriada, filtrada, e a palha foi lavada até que íons sulfato não fossem mais detectados pela titulação com solução de cloreto de bário 10%. A amostra foi então seca até peso constante, resfriada em dessecador e pesada (G_4, g). Os teores de extrativo (W_1, % em peso seco), hemicelulose (W_2, % em peso seco) e lignina (W_3, % em peso seco) foram calculados de acordo com as Eq. 4.9, 4.10 e 4.11, respectivamente. O teor de celulose (W_4, % em peso seco) foi calculado pelo método da diferença, assumindo que extrativos, lignina, hemicelulose e celulose são os únicos componentes da biomassa (Eq. 4.12).

$$W_1(\%) = \frac{G_0 - G_1}{G_0} \times 100\% \tag{4.9}$$

$$W_2(\%) = \frac{G_1 - G_2}{G_0} \times 100\% \tag{4.10}$$

$$W_3(\%) = \frac{G_4(1 - W_1)}{G_3} \times 100\% \tag{4.11}$$

$$W_4(\%) = 100 - (W_1 + W_2 + W_3) \tag{4.12}$$

4.4.12 Espectrometria de emissão ótica com plasma indutivamente acoplado

A concentração dos elementos Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb e Zn nas amostras de solo foi analisada através da técnica de espectrometria de emissão ótima com plasma indutivamente acoplado (ICP-OES), em um equipamento Agilent Technologies 5110. As amostras de solo foram previamente digeridas de acordo com o Método EPA 3050B, com adições repetidas de ácido nítrico (HNO_3) e peróxido de hidrogênio (H_2O_2).

4.4.13 Teor de fósforo e de potássio

A extração do fósforo (P) e do potássio (K) “disponível” no solo foi efetuada através da adição de 30 mL da solução contendo ácido clorídrico (0,05 mol/L) e ácido sulfúrico (0,0125 mol/L), conhecida como solução Mehlich-1 (MEHLICH, 1953) à 3 g de solo, seguido de agitação por 5 min e repouso para decantação do solo por 10-16 h.

Decorrido este tempo, para a determinação do P, 3 mL do sobrenadante foi pipetado, e a este extrato foi adicionado 3 mL de uma solução contendo HCl 0,87 mol/L e $(NH_4)_6Mo_7O_{24}.4H_2O$ 0,038%) e 0,1 mL de ácido ascórbico 20% (m/m). Após 10-15 min da adição do ácido ascórbico, determinou-se a absorbância da solução utilizando espectrofotômetro UV-Vis (colorímetro) (marca Thermo Scientific, modelo Genesys 30), em comprimento de onda de 660 nm. Para a determinação do K, 3 mL do sobrenadante foi pipetado, após o período de decantação do solo, e a este extrato foi adicionado 3 mL de água destilada. A intensidade da emissão de K da solução foi medida utilizando fotômetro de chama (marca Micronal, modelo B462), ajustado em curva padrão (TEDESCO et al., 1995). A curva de calibração foi preparada a partir de uma solução multielementar (KCl, NaCl e KH_2PO_4), em concentrações que variaram de 0 a 10 mg/L.

4.5 Análise estatística

A análise estatística para os ensaios de biodegradação e de fitotoxicidade foi realizada no software Microsoft Excel (versão 2010) para Windows. A significância estatística foi avaliada através da análise de variância ANOVA fator único, com teste *post hoc* de Tukey. Foram consideradas diferenças significativas os casos que apresentassem valor-p < 0,05.

Capítulo 5

Nutrient balance for anaerobic co-digestion of tannery wastes: energy efficiency, waste treatment and cost-saving

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Neste artigo, avaliou-se a solução de nutrientes empregada na co-DA de farelo de couro *wet-blue* e lodo de ETEs de curtumes, com relação à eficiência energética (produção de biogás) e econômica (análise de economia de custos) e à biodegradação dos resíduos.

Abstract

The macronutrients ratio present in tannery wastes is normally not ideal for anaerobic digestion (AD). In most cases, it is necessary to add nutrients to obtain a more balanced AD process and to ensure favorable conditions for the growth and metabolism of microorganisms. The aim of this study was to verify the influence of the components of nutrient solution added to AD of tannery wastes with regard to the energy and waste treatment efficiency and to the cost-saving analysis. The findings provide better understanding of the nutritional requirement of co-AD of tannery wastes, with the highest value of biogas production of 30.14 mL/g of added VSS, besides representing a step in the search for a more balanced, efficient and viable process. The results obtained proving the competitiveness of co-AD of tannery wastes instead of disposing it in landfills (saving off about 71% in terms of electric consumption).

Keywords: biogas, nutrient solution, waste, anaerobic co-digestion.

5.1 Introduction

Besides providing renewable energy and reducing dependence on fossil energy sources, biogas production from industrial wastes can contribute to effective waste management (AZIZ; HANAFIAH; GHEEWALA, 2019; LĂZĂROIU et al., 2017a). The growing demand for energy, due to the growth of the human population and the depletion of nonrenewable energy resources, has been the main cause of the search for alternative sustainable energy resources (KHALIL et al., 2019; LI; CHEN; WU, 2019). Biogas plays an important role in the developing renewable energy market and it is estimated that its use worldwide will double in next years, ranging from 14.5 GW in 2012 to 29.5 GW in 2022 (KHAN et al., 2017). Biogas is a mixture of gases produced from the anaerobic digestion of organic compounds (KHAN et al., 2017; WU et al., 2015). Waste from landfill, sewage sludge, animal manure, agricultural waste, among others, are the main sources of biogas generation (WU et al., 2015). Biogas consists mainly of CH_4 (50-70%) and CO_2 (30-50%). The relative content of CH_4 and CO_2 in biogas is dependent on the nature of the substrate and the parameters used in the AD process (ANGELIDAKI et al., 2018; GAO et al., 2018; KHAN et al., 2017; MIHAESCU et al., 2018; WU et al., 2015). This biogas can be used for the production of fuel, electricity, chemicals and heat (AGUSTINI; GUTTERRES, 2017a; SEADI et al., 2008; XU et al., 2019).

The amount of waste generated is increasing, due to daily human activities and the progress of industry (LI; CHEN; WU, 2019). The tanning industry belongs to the polluting activities regarding to the high production of wastewater and solid waste (LOFRANO et al., 2013; POLIZZI; ALATRISTE-MONDRAGÓN; MUNZ, 2018; PRIEBE et al., 2016). It is estimated that for the processing of 1 ton of rawhide, which produces 250 kg of finished leather, between 15 and 50 tons of liquid effluents and 450-730 kg of solid waste are generated (AGUSTINI; GUTTERRES, 2017b). Solid waste have traditionally been disposed of in hazardous industrial landfills due to the high content of chemical pollutants and the presence of recalcitrant compounds (AGUSTINI; DA COSTA; GUTTERRES, 2018; ORTIZ-MONSALVE et al., 2019; SPIER; GUTTERRES, 2018).

In response to new strict regulations and environmental policies that encourage alternative treatments, AD has become an attractive solution in the perspective of sustainable and integrated management of solid waste and wastewater from tanneries (LAZAROIU et al., 2018; POLIZZI; ALATRISTE-MONDRAGÓN; MUNZ, 2018). AD is a biochemical process of decomposition of organic matter, carried out by a consortium of microorganisms that live symbiotically in the absence of oxygen. From the technological point of view, AD is a promising alternative for the management of organic materials, since it is capable of converting practically all sources of biomass, including different types of waste, into a highly energetic biogas. However, the biological process of AD is complex and depends on several factors, including substrate characteristics (chemical composition, nutrient balance, particle size) and process parameters (pH, temperature, organic loading rate, retention time) (APPELS et al., 2008; MIRMOHAMADSADEGHI et al., 2019; SIDDIQUE; WAHID, 2018; ZHANG; LOH; ZHANG, 2019). Some micro and macronutrients are necessary for the survival and growth of the microorganisms involved in the AD process. As macronutrients, carbon, nitrogen, phosphorus and sulfur are the most important. Micronutrients include iron, cobalt, nickel, zinc, selenium, tungsten, magnesium, chromium and molybdenum and are required at low concentrations for the survival of microorganisms (MIRMOHAMADSADEGHI et al., 2019).

The macronutrients ratio present in tannery wastes is normally not ideal for anaerobic digestion, mainly due to excessive nitrogen abundance (MPOFU; OYEKOLA; WELZ, 2019). Therefore, this study investigates nutrients added in a co-anaerobic digestion (co-AD) process of leather shavings and sludge from tannery. The nutrient balance is an important aspect and a successful AD process needs balanced C/N ratios (20-30) (XU et al., 2018). In mono-digestion, a single substrate is either carbon-rich or nitrogen-rich, so it is difficult to maintain a balanced C/N ratio, which has a negative effect on the microbial activity (NESHAT et al., 2017). Co-AD of two or more substrates provides better macro and micronutrient balance, dilution of toxic or inhibitory compounds, moisture balance and better buffering capacity (KHALID et al., 2011; TYAGI et al., 2018). Moreover, it presents economic advantages by sharing the system and by treating more than one waste at the same time (HAGOS et al., 2017).

Previously studies that performed co-AD between leather shavings and sludge from wastewater treatment plants (WWTPs) are reported (AGUSTINI et al., 2018b; AGUSTINI; DA COSTA; GUTTERRES, 2018; PRIEBE et al., 2016). In these cases, the authors observed C/N ratios of shavings (2.8) and of sludge (1.8) very far from the optimum range for AD, which may limit microbial growth due to lack of carbon, leading to accumulation of ammonia nitrogen and volatile fatty acids (VFAs) in the digester. In most cases, it is necessary to add nutrients to obtain a more balanced AD process and to ensure favorable conditions for the growth and metabolism of microorganisms (AGUSTINI et al., 2018b; PRIEBE et al., 2016). Based on that, Agustini, Da Costa and Gutterres (2020) investigated the influence of a nutrient solution of known composition specific compared with the absence of nutrient supply (water only) for co-AD establishment of two tannery solid waste and found that the addition of the known nutrient solution was very beneficial for the establishment of AD. Besides that, the absence of nutrient supply produces virtually no biogas; reinforcing the need and importance of this subject. Therefore, the objective of this study was to optimize the nutrient solution originally used in previous works. For that, the statistical importance of each component of this solution was determined in relation to the energy (biogas production) and economic (cost-saving analysis) efficiency and to the biodegradation of the waste from the tannery industry submitted to the co-AD process.

5.2 Materials and Methods

5.2.1 Waste characterization

The leather shavings and the sludge were obtained from tannery companies and their respective WWTP located close to Porto Alegre, Brazil. Wet-blue leather shaving and sludge from tannery WWTPs were both collected in tanneries that perform the complete processing of leather, from salted hide to finished leather (GUTTERRES; MELLA, 2015), and that use chromium salts as tanning agent.

The characterization of the wastes used in this work was previously performed by Agustini *et al.* (2018a) and is presented in Table 5.1.

Table 5.1. Characterization of wastes (AGUSTINI et al., 2018a).

Waste	Tannin agent	TOC	IC	TN	C/N ratio	Water amount (%)	Density (g/mL)	VS (%)
		(ppm) - sludge (%(g/g)) - shaving						
Leather shaving	7.79% (g/g) of chromium	38.1	0.2	13.6	2.8	26.2	-	94.0
Sludge	7.35 mg/L of chromium	2,889	903	972	3.9	96.6	1.07	97.6

5.2.2 Biodigestion experiments

The biodegradation assays were performed in 300 mL bioreactors, built in glass, hermetically sealed, containing an upper valve, to measure the volume of biogas produced and a lateral screw valve with septum, for the collection of samples for chromatographic analysis of the composition of biogas. The components and its respective amounts added to the bioreactors are shown in Table 5.2. The ratio between the amounts of sludge and leather shavings is equivalent in proportion to the amount of such waste generated by the tanneries of the region. Because it contained a high load of microorganisms, the sludge also acted as inoculum of the tests. After 120 days of incubation, the bioreactors were opened and the precipitated and dissolved samples were collected. To analyze the organic and inorganic loads of the biomass mixture before biodegradation, the same tests were carried out in closed bottles, shaken for 1 h at room temperature on an ACB Labor Wagner shaker and stored for 24 h at 4 °C for sedimentation before opening and the collection of liquid and solid samples. All tests were performed in duplicate. The bioreactors were incubated in a DL-CBE18L (DeLeo) bacterial culture oven at 35°C.

Table 5.2. Components and their amounts added in each condition evaluated.

Condition	Sludge (mL)	Leather shaving (g)	Components of the nutrient solution	Concentration (g/L)
1	25	1	Peptone	1
			K_2HPO_4	7
			KH_2PO_4	3
2	25	1	Yeast extract	2
			K_2HPO_4	7
			KH_2PO_4	3
3	25	1	Yeast extract	2
			Peptone	1
			KH_2PO_4	3
4	25	1	Yeast extract	2
			Peptone	1
			K_2HPO_4	7
5	25	1	Yeast extract	2
			Peptone	1
			K_2HPO_4	7
			KH_2PO_4	3

5.2.2.1 Nutrient Solution

To find out the optimal nutrient solution for the AD, variations were tested in a previously employed nutrient solution consisted of: 2 g/L of yeast extract, 1 g/L of peptone, 7 g/L of K_2HPO_4 and 3 g/L of KH_2PO_4 (AGUSTINI; DA COSTA; GUTTERRES, 2018). In order to evaluate the effect of each component on the AD performance, one component was restricted in each condition. The four conditions tested are presented in Table 5.2. In order to compare, Table 5.2 also presents the assay performed by Agustini *et al.* (2017) (Cond. 5), which used the complete nutrient solution in AD tests of equivalent wastes to those employed in this work.

5.2.3 Biogas measurements

The monitoring of biogas volumetric production in the bioreactors was carried out with a device based on the principle of Mariotte (AGUSTINI; DA COSTA; GUTTERRES, 2018; PRIEBE et al., 2016). To perform the measurement, a hose was connected between the device and the bioreactor. First, the device was depressurized. Then, the bioreactor valve was opened, which resulted in the release of a volume of water equivalent to the volume of biogas generated in the bioreactor. This amount of water was weighed and converted to volume by the density of water at room temperature. The pH of the aqueous solution was kept at less than 3.0 to avoid the solubilization of some of the biogas components (CO_2 and H_2S). The measurements were performed three times a week, attempting to maintain local atmospheric pressure (avoiding pressure inhibition).

5.2.4 Analytical methods

The determination of the air (O_2 and N_2), methane (CH_4) and carbon dioxide (CO_2) fractions of the biogas produced in the bioreactors was carried out weekly in a gas chromatograph (GC-2014 Shimadzu) equipped with a thermal conductivity detector (TCD) and a ShinCarbon packed column (ST 100/120 2 M 1 MMID 1/16 "OD Silco) using Helium 5.0 Analytical (White Martins) as carrier gas. The biodegradation of the residues was evaluated through analysis of volatile suspended solids (VSS) and volatile dissolved solids (VDS), total organic carbon (TOC), inorganic carbon (IC), total Kjeldahl nitrogen (TKN) and pH. All analyzes were performed before and after AD. The determination of VSS and VDS was performed according to the gravimetric method (NBR ISO 4684: 2014). The determination of the TOC and IC of the liquid samples was performed in a total organic carbon analyzer (Shimadzu TOC-L). TKN analysis was performed according to ASTM D3590-11. The pH was determined with a Digimed model DM-22 pH meter, equipped with a temperature sensor. The nutrients were also individually analyzed by TOC, IC and TKN. VFAs were determined in a High-Performance Liquid Chromatography (HPLC), model 1200 Infinity (Agilent), with automatic injector and diode array detector (DAD). Products were using a Rezex RHM - Monosaccharide H^+ column (Phenomenex, USA), heated at 40°C, using 5 mM sulfuric acid as the mobile phase at a flow rate of 0.6 mL/min. Acetic, propionic and butyric acid were tested.

5.2.5 Statistical and economic analysis

Statistical analysis was realized using Microsoft Excel (version 2010) software for Windows. Statistical significance was evaluated using one-way ANOVA analysis of variance, with a Tukey *post hoc* test. Differences were accepted as significant when p-value < 0.05.

Economic analysis calculated the cost saving from the production of biogas from the co-digestion of all shavings and sludge generated by a midsize tannery (on lab-scale) and the cost of disposal of such waste. The values were estimated (AGUSTINI; DA COSTA; GUTTERRES, 2018) through local research carried out with midsize tanneries operating in Brazil to obtain data of average production of residues. The cost involved with nutrient solution was estimated by research with suppliers. The reduction in the consumption and in the cost of electric and thermal energy if all shavings and sludge generated be co-digested for biogas production was estimated according to equations presented in Table 5.3.

Table 5.3. Equations to estimate the reduction in the consumption and in the cost of electric and thermal energy.

	Equation
Reduction in electric consumption	$\frac{\text{Gross electric energy potential production}}{\text{Electric consumption of an average tannery}}$
Reduction in thermal consumption	$\frac{\text{Gross thermal energy potential production}}{\text{Thermal consumption of an average tannery}}$
Reduction in cost in terms of electric consumption	$\frac{\text{Cost of landfill disposal} + \text{transport of total waste produced}}{\text{Cost of electric energy consumption}} + \frac{\text{Gross electric energy potential production} \times \text{Cost of electric energ}}{\text{Cost of electric energy consumption}}$
Reduction in cost in terms of thermal consumption	$\frac{\text{Cost of landfill disposal} + \text{transport of total waste produced}}{\text{Cost of thermal energy consumption}} + \frac{\text{Gross thermal energy potential production} \times \text{Cost of thermal energy}}{\text{Cost of thermal energy consumption}}$

5.3 Results and discussion

5.3.1 Biogas production and composition

The volume of biogas produced daily and its composition are presented in Fig. 5.1. The cumulative biogas production per gram of VSS added for all tests is shown in Fig. 5.2.

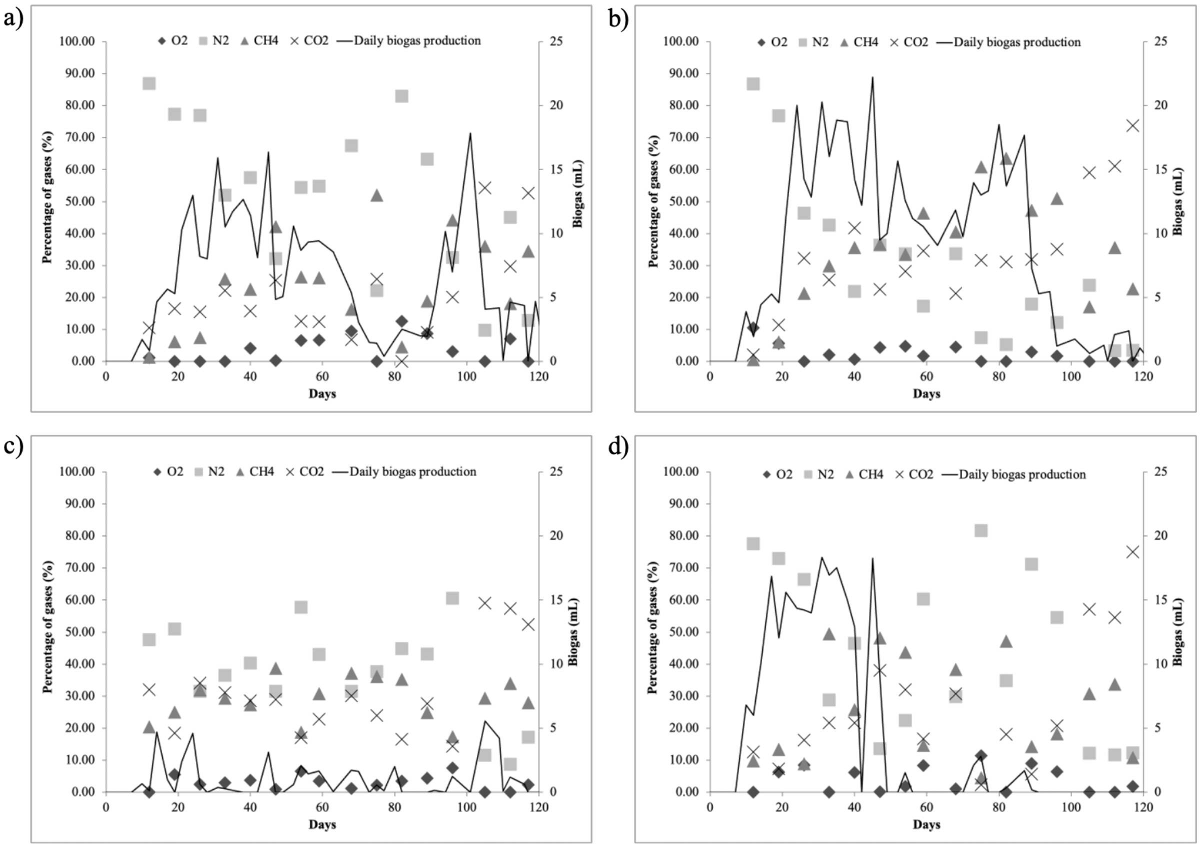

Fig. 5.1. Daily volume and composition of the biogas produced for (a) condition 1; (b) condition 2; (c) condition 3 and (d) condition 4.

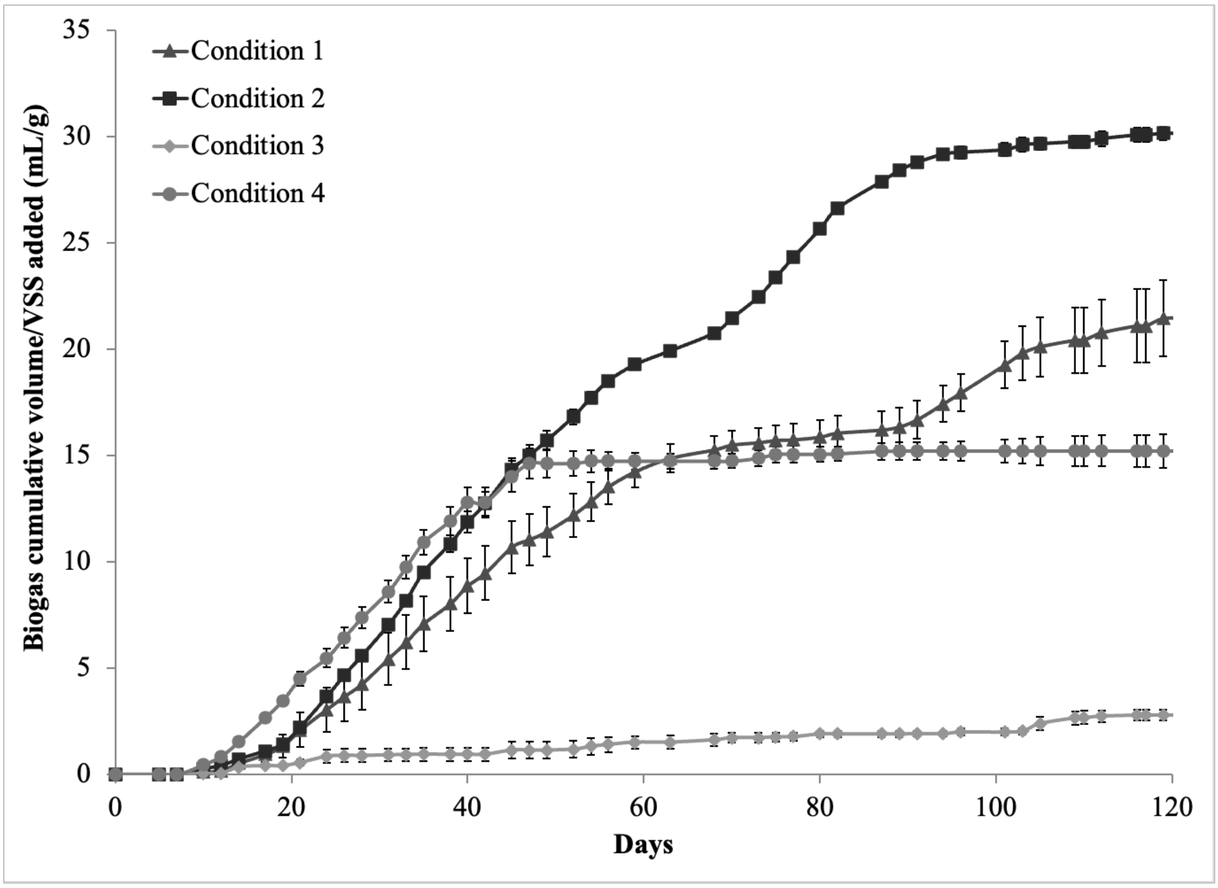

Fig. 5.2. Cumulative biogas production per gram of VSS added for biodegradation tests of tannery wastes.

Regarding the evolution of the gas composition throughout the AD process, the results found for condition 2 agree with that expected for a well-established process (AGUSTINI; DA COSTA; GUTTERRES, 2018). In general, the O_2 concentration is expected to be eliminated at the beginning of the AD process and is maintained close to zero during the sequence. However, even with small inlets of oxygen in the system, the CH_4 concentration was not affected, since oxygen was only in the gas phase and its diffusion in dense media, such as the waste, is very low, not affecting the anaerobiosis of the medium. The concentration of N_2 tends to be slowly eliminated as the pressure is relieved (although being a gas inert to AD, its presence has no effect on methane production). The concentrations of CO_2 and CH_4, as expected, increased throughout the experiment. The concentration of CO_2 increases first, indicating that the acidogenesis and acetogenesis stages are stable before the methanogenic phase. Methane concentration increased along with biogas production, as expected for a well-established AD process, its percentage reached a maximum of 63.63%, during the transition from the log to the stationary phase (Table 5.4). The daily production of biogas showed a characteristic behavior; the highest daily production was between days 20 and 85, within the log phase.

The final volume of methane produced per gram of added VSS and the maximum yield of methane throughout the biodegradation tests are shown in Table 5.4 for all cases.

Table 5.4. Volume and maximum yield of methane throughout the biodegradation tests of tannery wastes.

Condition	Final methane cumulative volume/VSS added (mL/g)	Maximum yield of methane (%)	Day of maximum yield of methane
1	5.28	52.01	75
2	10.93	63.56	82
3	0.85	35.22	82
4	2.94	38.58	33

The AD was established in all tests, with a maximum percentage of methane ranging from 35 to 63%, depending on the studied case, confirming that the concentration of chromium (Cr^{+3}) in the residues is not inhibitory of the AD process (AGUSTINI et al., 2018b; LĂZĂROIU et al., 2017b). The biogas cumulative volume (Fig. 5.2) was significantly the same ($p > 0.05$) for conditions 1 and 2 and for conditions 3 and 4, as can be seen in Table 5.7. Conditions 1 and 2 are highlighted by the highest values of biogas production: 21.45 and 30.14 mL/g of added VSS, respectively. Regarding the volume of methane accumulated per gram of VSS added, condition 2 is significantly different ($p < 0.05$) and higher than the others conditions, with production of 10.93 mL/g VSS (Table 5.7).

5.3.2 Volatile fatty acids production

The VFAs are important intermediates in the metabolic pathway of CH_4 production. Acetic, propionic and butyric acid are the main VFAs present during AD and can be used to evaluate the process performance (KARTHIKEYAN; VISVANATHAN, 2013; NESHAT et al., 2017). Table 5.5 shows the concentration of acetic, propionic and butyric acid in the initial stage of AD, for the four conditions studied. The concentrations of VFAs under the conditions in which one of the buffering agents (K_2HPO_4 and KH_2PO_4) was restricted (conditions 3 and 4), indicate that AD was established previously in the waste. For condition 3, the three acids tested were detected at the beginning of AD, indicating that the degradation of residues and the reduction of organic matter were already occurring in the sludge, which may be related to the fact that this condition had the lowest production of biogas. The concentrations of VFAs found are far below the inhibitory concentration (> 1500 ppm) (NESHAT et al., 2017). At the end of AD, none of the acids was detected for any of the conditions, indicating that acetogenesis was not the limiting step, since all acids produced during the acidogenesis were consumed by acetogenesis and transformed into CH_4 and CO_2 in methanogenesis.

Table 5.5. VFAs concentration in the initial stage of AD.

Condition	Acetic acid (ppm)	Propionic acid (ppm)	Butyric acid (ppm)
1	0	0	0
2	0	0	0
3	474.11	62.71	194.93
4	0	263.92	268.79

5.3.3 Efficiency of AD in waste treatment

The TOC, IC, TKN and pH parameters, analyzed in the mixtures before and after biodegradation, are presented in Fig. 5.3 and Fig. 5.4, respectively.

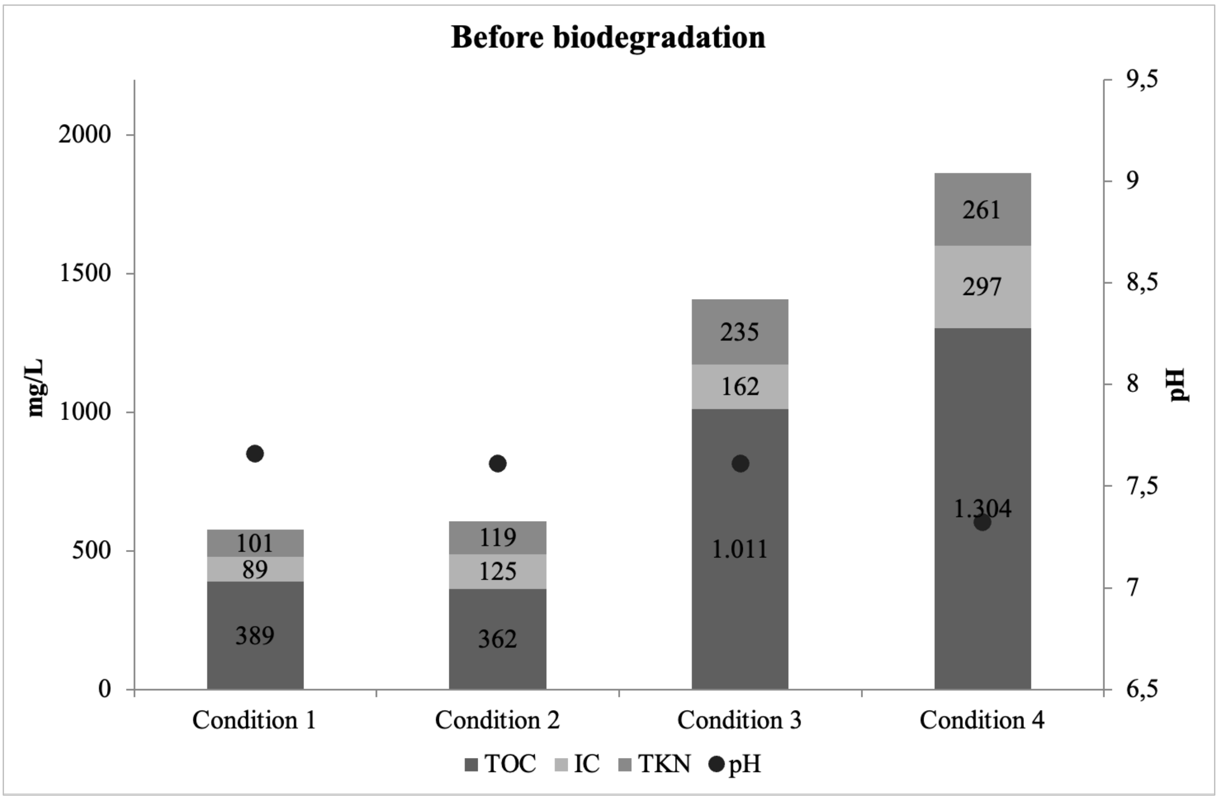

Fig. 5.3. Characterization of the tannery wastes before the biodegradation.

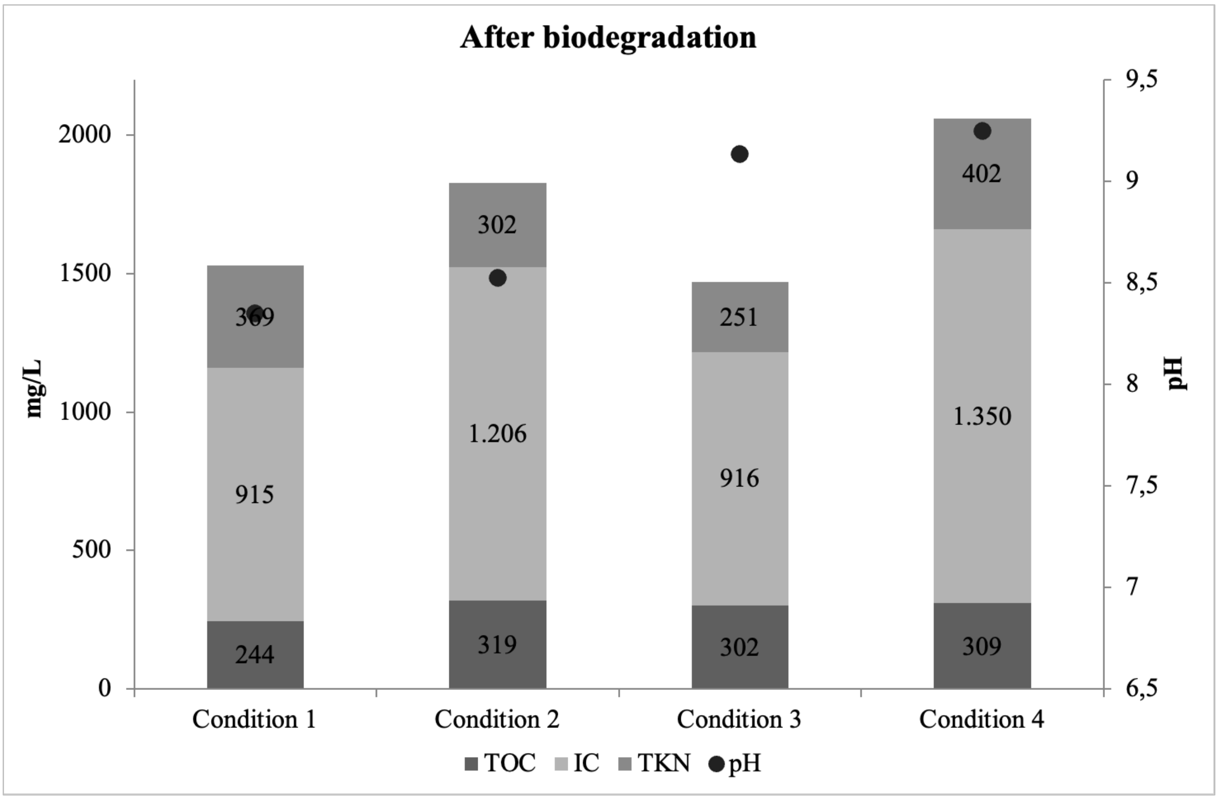

Fig. 5.4. Characterization of the tannery wastes after the biodegradation.

The efficiency of waste treatment (reduction) during the AD process is as important as the production of biogas, since the remaining waste still requires destination. A more mineralized and less organic waste is environmentally more inert for disposal, and can be used in a variety of applications, such as incorporation into fertilizers and cement (KHALIL et al., 2019; XU et al., 2019). The analysis of the results of TOC and IC refers to the efficiency of the mineralization of the residues. In a well-established and efficient AD process, TOC, which is related to organic carbon content, is expected to decrease, and IC, related to inorganic carbon content, is expected to increase (AGUSTINI et al., 2018b; TAYYAB et al., 2019).

The initial TOC of 1010.70 ± 29.4 and 1304 ± 27 mg/L (average value of two replicates ± standard deviation) of conditions 3 and 4, respectively, is higher than the other cases, which is attributed to the higher content of organic matter of the nutrient solutions used in these assays (Table 5.6). For all cases, reduction of TOC was observed. Especially for conditions 1 and 2, this reduction was already expected, as a result of the higher biogas production, where organic matter is metabolized by the microorganisms and transformed into CH_4 and CO_2.

Table 5.6. TOC, IC and TKN of the components of nutrient solution.

Component of nutrient solution	TOC (mg/L)	IC (mg/L)	TKN (mg/L)
Yeast extract (2 g/L)	670.10	38.90	153.72
Peptone (1 g/L)	352.80	23.13	87.08
K_2HPO_4 (7 g/L)	18.34	2.79	0
KH_2PO_4 (3 g/L)	13.05	1.23	0

The initial IC concentration of 162.30 ± 1.52 and 297.20 ± 5 mg/L (average value of two replicates ± standard deviation) was also higher for conditions 3 and 4, respectively, showing that nutrient solutions employed in these cases also added inorganic matter to the mixture (Table 5.6). The increase of IC for all the studied conditions shows a mineralization of the residues.

The TKN values of 234.5 ± 3.5 and 261.1 ± 34.65 mg/L (average value of two replicates ± standard deviation) for conditions 3 and 4, respectively, are higher than the other cases studied; which is attributed to the higher organic nitrogen content of the nutrient solutions employed in these cases (Table 5.6). The increase of TKN, observed in all the cases studied, is characteristic of the microbial activity of the AD process (MA et al., 2018; MOMAYEZ; KARIMI; TAHERZADEH, 2019).

Reduction in TOC values and increases in IC and TKN values are common for cases where the AD process was well established, with satisfactory production of biogas and methane. In this study, even conditions 3 and 4, which presented low production of biogas and methane, had a considerable reduction in TOC and increases in IC and TKN. This behavior can be attributed to the activity of microorganisms not involved in AD, so that the mineralization of the residues is not only linked to the biogas production.

For all conditions studied, the initial pH was in the range of 7.3-7.6. Also for all cases, an increase in pH after AD was observed, especially for the test with conditions 3 and 4, which obtained the highest values of final pH (9.135 ± 0.055 and 9.25 ± 0.17, respectively). The increase in pH is expected due to the probable formation of ammonia, since the residues used in the tests present large amounts of organic nitrogen. Furthermore, for conditions 3 and 4, the absence of the components K_2HPO_4 and KH_2PO_4 in the nutrient solution, acting as buffering agents, may have contributed to the higher observed pH values. Although statistically, the increase in pH for all cases was significantly the same ($p > 0.05$), the activity of the bacteria is affected by the variation in pH (ZHANG; LOH; ZHANG, 2019) and the formation of methane is considerably suppressed at pH values below 6.0 or at values higher than 8.5 (KHALID et al., 2011; MIRMOHAMADSADEGHI et al., 2019).

The reduction of VSS was observed for all cases (Table 5.7). The observed reductions for conditions 1 and 2, corroborate what has been presented so far, reinforcing the fact that the reduction of these parameters is directly related to the great amount of organic matter that left the system in the form of biogas. For conditions 3 and 4, this behavior can be attributed to the activity of microorganisms not involved in AD, since the biogas production was small for these cases. The analysis of the results of the obtained variation of VDS for all cases by means of the statistic inference of variance (test F with 0.05 significance level), showed no significant differences (Table 5.7). VDS is related to the total mass of volatile organic matter, not only carbon (AGUSTINI; DA COSTA; GUTTERRES, 2018). So even though dissolved TOC was reduced, organic nitrogen compounds increased in concentration and, thus, VDS can slight increased, as observed for condition 4.

5.3.4 Statistical analysis

Statistical comparison of biogas and methane volumes produced per gram of VSS added and the parameters related to waste treatment obtained from tests before and after AD are presented in Table 5.7. The data obtained by this study were also compared to the results obtained by Agustini *et al.* (2017) (Cond. 5 in Table 5.2 and Table 5.7), which used the complete nutrient solution in AD tests of equivalent residues to those employed in this work.

Table 5.7. Statistical comparison of the volumes of biogas and methane produced per gram of VSS added and changes in the parameters related to the treatment of residues before and after AD. Tukey's test indicates significant differences in each line.

	Cond. 1	Cond. 2	Cond. 3	Cond. 4	Cond.5
Biogas cumulative volume/VSS added (mL/g)	21.45^a	30.14^b	2.80^c	13.05^d	35.6^b
Methane cumulative volume/VSS added (mL/g)	5.28^a	10.93^b	0.85^a	2.93^a	14.9^c
TOC reduction (mL/g) (%)	144.4^a (37.33%)	43.52^a (12.08%)	708.80^b (65.35%)	994.95^b (76.12%)	296.55^a (64%)
IC increase (mL/g) (%)	826.02^a (951.94%)	1080.9^b (862.16%)	753.6^a (463.32%)	1058.3^b (356.43%)	391.1^a (103%)
TKN increase (mL/g) (%)	268.1^a (276%)	182.7^b (150%)	16.1^c (58%)	140.7^d (74%)	n.d.
pH increase	0.69^a	0.91^a	1.52^a	1.92^a	0.42^a
VSS reduction (%)	23.77^a	23.55^a	15.11^b	24.78^a	3.39^c
VDS reduction (%)	5.81^a	12.15^a	15.28^a	-5.02^a	10.64^a

n.d.: not determined

As can be seen in Table 5.7, regarding the volume of cumulative biogas per g of added VSS, conditions 2 and 5 are significantly the same ($p > 0.05$). Although the volume of methane produced by condition 2 is lower compared to condition 5 ($p < 0.05$), a reduction of 27% in the cost added by the nutrient solution would still justify the choice of the optimized solution. With respect to the parameters used to evaluate the biodegradability of the residues during the AD process, it is observed that condition 2 is statistically the same (reduction of TOC, pH, reduction of VDS) or better (increase of IC, reduction of VSS) than condition 5, with a significance level of 95%.

5.3.5 Energy valuation

The amount of remained waste (shavings and sludge), the cost of disposal of such waste and the energy consumption of a midsize tannery operating in Brazil are shown in Table 5.8.

Taking into account the biogas cumulative production per initial mass of VSS added and extrapolating this values to a real tannery, an evaluation of the potential energetic valuation of the biogas generated by the tannery waste was calculated (Table 5.9), assuming an average calorific value for biogas of 6 kWh/m^3 (ANGELIDAKI et al., 2018), a 35% conversion from primary to electric energy production and 42% conversion from primary to thermal energy conversion (RUFFINO et al., 2015).

Table 5.10 presents the reduction in the consumption of electric and thermal energy if all shavings and sludge generated be co-digested for biogas production. A reduction of 6.5% of electric or 1.5% of thermal energy consumption can be achieved in condition 2. Besides this energy saving, there is a great reduction with the cost of disposal of this waste, since all waste previously sent to landfill is now turned into biogas. Counting up cost-savings with waste disposal and with the generated energy, a saving off about 71% in terms of electric consumption and 35% in terms of thermal consumption can be achieve, proving the competitiveness of AD of tannery solid waste rather than disposing it in landfills.

Table 5.8. Evaluation of the cost of waste disposal and energy consumption of a midsize tannery (AGUSTINI; DA COSTA; GUTTERRES, 2018).

Waste produced*, **	
Hides processed	10,000
Weight of shavings produced (kg)	5,000
Weight of volatile matter in shavings produced (kgVSS)	4,700
Volume of shavings produced (m^3)	30
Weight of sludge produced (kg)	100,000
Weight of volatile matter in sludge produced (kgVSS)	98,000
Volume of sludge produced (m^3)	90
Weight of volatile matter of total waste produced (kgVSS)	102,700
Volume of total waste produced (m^3)	120
Cost of landfill disposal (US$/$m^3$)	50
Cost of landfill disposal of total waste produced (US$)	6,000
Cost of transport (US$/$m^3$)	20
Cost of transport of total waste produced (US$)	2,400
Cost of landfill disposal + transport of total waste produced (US$)	8,400
Energy consumption	
Electric consumption of an average tannery (MWh)	100
Cost of electric energy (US$/MWh)	130
Cost of electric energy consumption (US$)	13,000
Thermal consumption of an average tannery (MWh)	500
Cost of thermal energy (US$/MWh)	50
Cost of thermal energy consumption (US$)	25,000
Total cost of energy consumption (US$)	38,000

*The values were estimated by local research carried out with the tanneries of the region.

**Monthly average values of a midsize tannery operating in Brazil.

Table 5.9. Evaluation of the potential energetic valorization of the biogas generated by the tannery wastes.

Biogas/energy potential production from waste	
Condition 1	
Biogas cumulative volume (mL/gVSS)	21.45
Biogas generated (m^3)	2,202.9
Primary energy potential production (kWh)	13,217.49
Gross electric energy potential production (kWh)	4,626.12
Gross thermal energy potential production (kWh)	5,551.34
Condition 2	
Biogas cumulative volume (mL/gVSS)	30.14
Biogas generated (m^3)	3,095.38
Primary energy potential production (kWh)	18,572.27
Gross electric energy potential production (kWh)	6,500.29
Gross thermal energy potential production (kWh)	7,800.35
Condition 3	
Biogas cumulative volume (mL/gVSS)	2.80
Biogas generated (m^3)	287.56
Primary energy potential production (kWh)	1,725.36
Gross electric energy potential production (kWh)	603.88
Gross thermal energy potential production (kWh)	724.65
Condition 4	
Biogas cumulative volume (mL/gVSS)	13.05
Biogas generated (m^3)	1,340.23
Primary energy potential production (kWh)	8,041.41
Gross electric energy potential production (kWh)	2,814.49
Gross thermal energy potential production (kWh)	3,377.39

Table 5.10. Evaluation of the potential energy saving by exploitation of tannery solid wastes through biogas.

Consumption reduction	
Condition 1	
Reduction in electric consumption (%)	4.63
Reduction in thermal consumption (%)	1.11
Reduction in cost in terms of electric consumption (waste disposal + energy generated) (%)	69.24
Reduction in cost in terms of thermal consumption (waste disposal + energy generated) (%)	34.71
Condition 2	
Reduction in electric consumption (%)	6.50
Reduction in thermal consumption (%)	1.56
Reduction in cost in terms of electric consumption (waste disposal + energy generated) (%)	71.12
Reduction in cost in terms of thermal consumption (waste disposal + energy generated) (%)	35.16
Condition 3	
Reduction in electric consumption (%)	0.60
Reduction in thermal consumption (%)	0.14
Reduction in cost in terms of electric consumption (waste disposal + energy generated) (%)	65.22
Reduction in cost in terms of thermal consumption (waste disposal + energy generated) (%)	33.74
Condition 4	
Reduction in electric consumption (%)	2.81
Reduction in thermal consumption (%)	0.67
Reduction in cost in terms of electric consumption (waste disposal + energy generated) (%)	67.43
Reduction in cost in terms of thermal consumption (waste disposal + energy generated) (%)	34.28

5.4 Conclusions

The results obtained to energy and waste treatment efficiency and to cost-saving analysis showed that a reduction of 71% of electric or 35% of thermal energy consumption can be achieved if all the shavings and sludge produced be co-digested using the nutrient solution tested in condition 2, proving the competitiveness of co-AD of tannery wastes instead of disposing it in landfills. The findings provide better understanding of the nutritional requirement of co-AD of tannery wastes, besides representing a step in the search for a more balanced, efficient and viable process and suggest the possibility of replacing nutrients with other residues.

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Capítulo 6

Anaerobic co-digestion of tannery wastes and untreated/pretreated oat straw

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Neste artigo, avaliou-se o efeito da aplicação de pré-tratamentos químicos e térmico na composição lignocelulósica da palha de aveia, e ainda, o potencial de geração de biogás quando estas palhas (tratadas ou não) foram adicionadas como substrato ao processo de co-DA com resíduos da indústria coureira (farelo de couro *wet-blue* e lodo de ETE).

Abstract

The combination of carbon and nitrogen-rich co-substrates results in a better balance and increase the stability of the anaerobic co-digestion (AcoD) process. In this work, the AcoD process of waste from the leather industry (shavings and sludge) with waste from agriculture (oat straw) was assessed with regard to the energy (biogas production) and waste treatment efficiency (reduction of organic matter). The results indicate that the addition of untreated oat straw improved the AcoD process, increasing biogas production (25.44 mL of cumulative biogas/g of VSS added) by almost 60% when compared to the AcoD of only leather waste (16.17 mL/gVSS). Also, the effect of acid, alkaline, thermal and the combination of these pretreatments techniques was evaluated on the lignocellulosic composition of oat straw and on methane yields. Pretreatments improved the characteristics and bioavailability of oat straw, particularly in methodologies that use alkali, with a significant increase in cellulose content accompanied by a decrease in hemicellulose and lignin content. However the possible formation of secondary products or sterilization of important microorganisms did not reflect in a greater production of biogas: 21.06 mL/gVSS for oat straw pretreated only with HCl and 21.91 mL/gVSS for oat straw pretreated with HCl in autoclave; 5.20 mL/gVSS for oat straw pretreated with NaOH and 3.43 mL/gVSS for oat straw pretreated with NaOH in autoclave; thermal pretreatment probably have generated toxic compounds from hemicellulose and cellulose degradation, which inhibited the AcoD process and, as consequence, virtually no biogas was produced.

Keywords: renewable energy, anaerobic digestion, lignocellulosic biomass, leather waste, pretreatment, methane.

6.1 Introduction

Renewable energy production methods have been examined to provide accessible and sustainable energy sources which are essential for economic growth, increasing energy security, and reducing the risk of climate change (KOUPAIE et al., 2019). Worldwide renewable energy consumption is expected to increase by 3% per year between 2018 and 2050 and become the leading source of primary energy consumption by 2050 (EIA, 2019). Among renewable forms of energy, the use of waste energy is one of the most attractive for treating waste while producing energy from it.

Anaerobic digestion (AD) is a viable alternative to renewable energy, which can be described as a process in which biomass resources are submitted to a microbial process under anaerobic conditions to produce two major products: energy-rich biogas and nutrient-rich digested residues (ABRAHAM et al., 2020). Biogas production is independent of seasonal fluctuations, can be stably produced and, therefore, promises a reliable way to produce energy (KOUPAIE et al., 2019). Despite producing alternative fuels and high-quality fertilizer, the biogas production technology offers multiple benefits like complete waste recycling, greenhouse gas reduction and environmental protection from pollutants (EDWIGES et al., 2019).

The production of biogas from lignocellulosic biomass creates a good opportunity to convert vast biomass resources into renewable energy (ABRAHAM et al., 2020). Unlike purpose-grown energy crops, the residue of lignocellulosic material (straw) does not compete with food production. The grain is seen as the main product and much of the straw generated world-wide is left in the field after harvesting. Straw is therefore often available in large quantities at a low price (FJØRTOFT et al., 2019).

Lignocellulosic material is mainly composed of three different types of polymers: cellulose, hemicellulose and lignin. While cellulose has a rigid and crystalline form, hemicellulose has a lower molecular weight and short lateral chains, which corresponds to an easy hydrolysable polymer. The third polymer, lignin, is composed mainly of phenolic compounds which binds together hemicellulose and cellulose and runs throughout the three-dimensional structure providing a protective sheath over hemicellulose and cellulose, giving resistance to microbial attack. Hemicellulose and cellulose, collectively called holocellulose, are polymers of sugars which are biodegradable and can be effectively utilized to produce biogas (FERREIRA et al., 2013; SABEEH; LIAQUAT; MARYAM, 2020). The presence of lignin is a hindrance to the enzymatic hydrolysis of cellulose during the anaerobic digestion, what makes the hydrolysis of lignocellulose often becomes the rate-limiting step during process (NESHAT et al., 2017; PATOWARY; BARUAH, 2018). In fact, the major difficulty with the lignocellulosic substrate is the proper digestion of biomass during the AD due to the complex and recalcitrant nature of feedstock, which makes absolutely necessary a pretreatment step (GOMEZ-TOVAR et al., 2012; PATINVOH et al., 2017).

Different pretreatment methods have been applied to eliminate physical and chemical barriers, making cellulose accessible for enzymatic hydrolysis (EDWIGES et al., 2019; WYMAN et al., 2018). The selection of the optimum pretreatment technique depends on multiple factors such as the characteristics of the lignocellulosic material (crystallinity of the lignocellulose, the degree of polymerization, accessible surface area), the capital and operating cost of the pretreatment and the ease of the operation (KOUPAIE et al., 2019; ZHANG; LOH; ZHANG, 2019). There are several studies reported in the literature with methodologies for chemical and thermal pretreatments of lignocellulosic materials, resulting in changes in the lignocellulosic composition of these materials and influence on the performance of subsequent anaerobic biological processes.

Gomez-Tovar *et al.* (2012) reported the acid pretreatment (2% HCl, 90°C) of oat straw as part of a sequential pretreatment (acid/alkaline/enzymatic) to convert the lignocellulosic material in soluble sugars and observed that 85.5% of the hemicellulose and 30.3% of the cellulose were hydrolyzed in the acid step. Mancini *et al.* (2018) investigated the effect of alkaline pretreatment (NaOH at 30 °C for 24 h) on the biogas production from the anaerobic digestion of wheat straw and observed that the cumulative biomethane production yield of the untreated feedstock was enhanced by 15% by alkaline pretreatment. Similarly, rice straw was pretreated with NaOH (0.5%, 1%, 1.5%, and 2% w/v for 3 h at 37°C) and, as compared to raw rice straw, maximum biogas and methane enhancement of 50 and 71%, respectively, was observed for 1.5% w/v NaOH pretreated rice straw (SABEEH; LIAQUAT; MARYAM, 2020). Rajput, Zeshan and Visvanathan (2018) studied the effect of thermal pretreatment (120, 140, 160 and 180 °C for 60 min) on anaerobic digestion of wheat straw and found a 53% increase in biogas yield for wheat straw pretreated at 180 °C as compared to untreated. Ferreira *et al.* (2013) evaluated the biochemical methane potential of steam exploded wheat straw under different temperature-time combinations (150-220 °C and 1-15 min) and reported that the optimum was obtained for 1 min and 220°C, resulting in a 20% increase in methane production respect non-treated straw. They also observed that for more severe pretreatment conditions the biodegradability of wheat straw decreased due to a possible formation of inhibitory compounds. Chandra *et al.* (2012) presented the results of an experimental methane fermentation study on untreated, NaOH (4% at 37 °C for 120 h) and hydrothermal (200 °C for 10 min) pretreated substrates of wheat straw which produced, respectively, 111.6% and 20% higher methane compared to the untreated wheat straw substrate.

The combination of chemical and thermal pretreatments has also been investigated. Taherdanak, Zilouei and Karimi (2016) evaluated the improvement of biomethane production from wheat submitted to pretreatment with H_2SO_4 1% (v/v) at room temperature for 10 min followed by autoclave at 121 °C for four different time (10, 30, 60, and 120 min) and achieved an increase in methane yield of 8.9% after pretreatment for 60 min and 15.5% after 120 min, compared to untreated wheat. Patowary and Baruah (2018) investigated the combination of chemical (banana peel ash and calcium hydroxide) and thermal treatments (60-90 °C for time intervals of 2, 6 and 10 h) of rice straw and corn stalk and reported that biogas production of rice straw and corn stalk pretreated at 90 °C for 6 h was enhanced by 62% and 66%, respectively as compared to untreated rice straw and corn stalk. Kim *et al.* (2018) assessed the effects of autoclaving (121 °C, 1.45 atm, 60 min) of rice straw after addition of 2% H_2SO_4 and the results showed a reduction of about 20% in methane yield, indicating the inhibitory effect of the acid in the biogas production process.

In addition to structural features, lignocellulosic wastes have a high carbon/nitrogen (C/N) ratio which limits its efficient use in AD processes for methane production (MAO et al., 2015). For example, oat straw, the biomass used in this study, has a C/N ratio around 50, higher than the optimal range for AD (20-30) (SIDDIQUE; WAHID, 2018). To overcome this limitation, lignocellulosic biomass is often co-digested with nitrogen-rich feedstock, in order to maintain the optimum C/N ratio and increase the stability of the process (HAGOS et al., 2017). The leather industry generates substantial quantities of nitrogen-rich solid waste (AGUSTINI; DA COSTA; GUTTERRES, 2018) and could be a good option to balance the C/N ratio of AcoD with oat straw. Leather shavings and sludge from wastewater treatment plants (WWTP) are the main solid wastes generates by tanneries (AGUSTINI et al., 2018b) and AD has become an attractive solution in the perspective of sustainable and integrated management of these wastes (PESSUTO et al., 2016; SIMIONI et al., 2020). Additionally, the combination of co-substrates results in other positive effects to the system, such as a better balance (pH, moisture), dilution of potentially toxic compounds and supplementation of trace elements. Thus, AcoD of lignocellulosic and tanneries wastes is a possible way to solve the limitations of mono-digestion, increase biogas production, improve waste recycling and contribute to environmental protection (XU et al., 2018). Furthermore to the economic advantage of sharing the AD system and treating more than one waste at the same time (HAGOS et al., 2017; SIDDIQUE; WAHID, 2018).

The novelty of this work is the possibility of co-digesting waste from agricultural cultivation and from leather industry, in order to maintain a balanced C/N ratio during the AcoD process. There are no reported studies involving AcoD of leather shavings, sludge from WWTP of tanneries and oat straw. This works also aimed to compare the effects of acid, alkaline, thermal and a combination of these pretreatments techniques on the lignocellulosic composition of oat straw and on the methane yields from its subsequent AcoD. The AcoD process was assessed with regard to the energy (biogas production) and waste treatment efficiency (reduction of organic matter).

6.2 Materials and methods

6.2.1 Substrates and inoculum

The leather shavings from tannery factories that use chromium as tanning agent and vegetable tannin as retanning agent and the sludge (inoculum) of WWTP were collected in tanneries, located close to Porto Alegre, Rio Grande do Sul (Brazil). Oat (*Avena sativa*) straw was harvested from agricultural fields in the north of Rio Grande do Sul (Brazil).

6.2.1.1 Pretreatments of oat straw

The whole oat straw sample that was used in this work was previously oven dried at 60°C, ground in a knife mill and passed through a 6-mesh sieve. This initial preparation was not a variable evaluated in this study, so that “untreated oat straw” corresponds to dry and ground oat straw, but not subjected to any of the pretreatments evaluated in this study. The strategy proposed for the previously hydrolysis of the oat straw consisted of acid, alkaline and thermal pretreatments and the combination of those. Fig. 6.1 shows the experimental flow adopted.

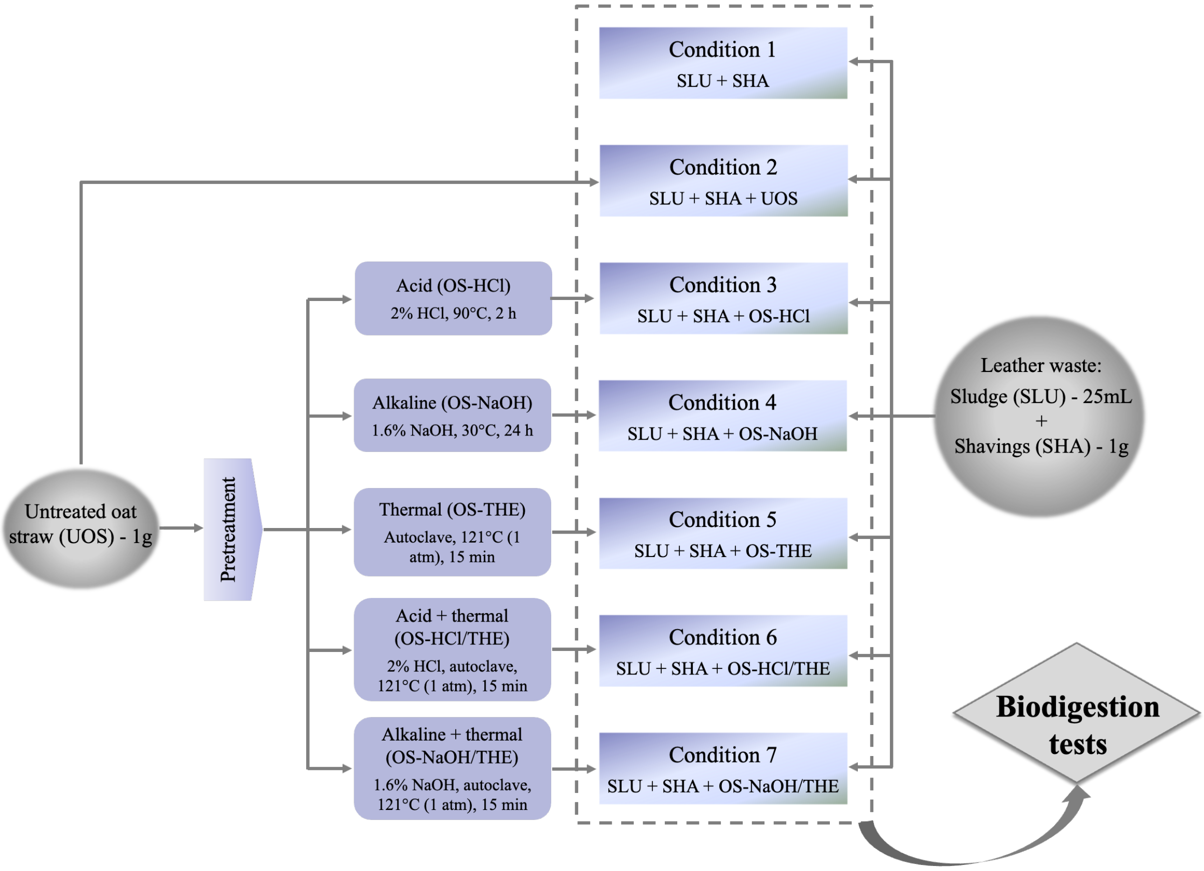

Fig. 6.1. Schematic of the experimental flow used in this study and composition of each reactor. SLU: sludge; SHA: shavings; UOS: untreated oat straw; OS-HCl: oat straw submitted to acid pretreatment; OS-NaOH: oat straw submitted to alkaline pretreatment; OS-THE: oat straw submitted to thermal pretreatment; OS-HCl/THE: oat straw submitted to acid + thermal pretreatments; OS-NaOH/THE: oat straw submitted to alkaline + thermal pretreatments.

The chosen pretreatment conditions were based on previous studies (GOMEZ-TOVAR et al., 2012; MANCINI et al., 2018; TAHERDANAK; ZILOUEI; KARIMI, 2016) performed on lignocellulosic materials and which helped to increase the bioavailability of these materials. Acid hydrolysis was carried out in 10 g of oat straw that was suspended in 0.1 L of HCl (2%) at 90 °C for 2 h (GOMEZ-TOVAR et al., 2012). Alkaline pretreatment was carried out in 10 g of oat straw that was suspended in 0.1 L of NaOH (1.6%) at 30 °C for 24 h (MANCINI et al., 2018). After each pretreatment, the suspension was filtered and the liquid fraction was discarded, whereas the remaining fiber was washed with distilled water until neutral pH (7.0) was reached. Finally, the fiber was dried at 60 °C and stored. Thermal pretreatment was carried out by autoclaving (vertical autoclave, Phoenix Luferco) the untreated oat straw at 121 °C (1 atm) for 15 min (TAHERDANAK; ZILOUEI; KARIMI, 2016).

The combinations of thermal and chemical pretreatments were also studied (TAHERDANAK; ZILOUEI; KARIMI, 2016). Acid + thermal pretreatment was carried in 10 g of untreated oat straw immersed in 0.1 L of HCl (2%) and autoclaved at 121 °C (1 atm) for 15 minutes. Alkaline + thermal pretreatment was carried in 10 g of untreated oat straw immersed in 0.1 L of NaOH (1.6%) and autoclaved at 121 °C (1 atm) for 15 minutes. After each pretreatment, the straw was filtered, washed to neutral pH and oven dried (60°C).

6.2.2 Biodigestion tests

Biodigestion tests were performed in 300 mL hermetically sealed bench scale bioreactors, built in cylindrical glasses, containing an upper valve, to measure the volume of biogas produced, a lateral screw valve with septum, for the collection of samples for chromatographic analysis of the composition of biogas and a lower thread septum valve for liquid sample collection. The composition of the seven conditions studied is presented in Fig. 6.1. The ratio of sludge/shavings of 25 mL/1g is equivalent, in proportion, to the amount of such waste generated by the tanneries of the region south of Brazil, according to local research (AGUSTINI; DA COSTA; GUTTERRES, 2018). 200 mL of nutrient solution (2 g/L of yeast extract, 7 g/L of K_2HPO_4 and 3 g/L of KH_2PO_4) was employed to ensure favorable conditions for the growth and metabolism of the microorganisms (SIMIONI et al., 2020). The bioreactors were maintained at 35 °C (mesophilic temperature range) in a DL-CBE18L (DeLeo) bacterial culture oven. After 274 days of incubation, the bioreactors were opened and the precipitated and dissolved samples were collected. To analyze the composition of the biomass before biodegradation, the same tests were carried out in closed bottles, shaken for 1 h at room temperature on an ACB Labor Wagner shaker and stored for 24 h at 4 °C for sedimentation before opening and the collection of liquid and solid samples (AGUSTINI; DA COSTA; GUTTERRES, 2018). All tests were performed in duplicate.

Statistical analysis was performed using Microsoft Excel (version 2010) software for Windows. Statistical significance was evaluated using one-way ANOVA analysis of variance, with a Tukey *post hoc* test. Differences were accepted as significant when *p-value* < 0.05.

6.2.3 Analytical parameters

6.2.3.1 Waste characterization

The wastes were characterized according to the following techniques. Gravimetric method (NBR ISO 4684:2014) was used to determine volatile solids (VS), using an analytical balance model EEQ9003F-B (Edutec), a drying oven (DeLeo), a muffle furnace (model Q318M/Quimis) and porcelain crucibles B-47 (Chiarotti). The determination of total organic carbon (TOC) and inorganic carbon (IC) were performed in a total organic carbon analyzer: TOC-VCSH Shimadzu for the liquid samples and SSM-500A Shimadzu, for the solids. Total Kjeldahl nitrogen (TKN) analysis was performed according to ASTM D3590-11, for liquid samples, and according to adapted D2868-10, for solid samples. The digestion of the samples was performed in a Velp Scientifica digester equipment, model DK20. The digested sample was distilled in a nitrogen distiller equipment (Velp Scientifica) and the determination of the distilled ammonia was performed by titration.

Vegetable tannin concentration was measured in a T80+ UV/Vis Spectrometer (PG Instruments) at 278 nm with a calibration curve prepared with standard solutions of tannin of acacia (0.01-0.1 g/L). Total phenols were estimated as tannic acid equivalents, according to the Folin-Ciocalteau assay (MIRON et al., 2011). The absorbance of the samples was measured at 760 nm (T80+ UV/Vis Spectrometer by PG Instruments) and compare to the tannic acid calibration curve (0.01-0.1 g/L) elaborated in the same manner. For both analyzes, vegetable tannin and total phenols, the sludge samples were diluted 100 and 10 times, respectively. Chromium (III) oxide concentrations were determined with ABNT NBR 13341 (2010) method for residual bath. For the shavings, 1 g of the waste was dissolved in HCL 50% until total disintegration. pH was determined with a Digimed pH-meter (DM-22). For pH measurement of solid wastes, 2.5 g of shavings/oat straw were stirred for 24 h in 50 mL of distilled water.

6.2.3.1.1 Analysis of the pretreatment efficiency in the composition of oat straw

Fourier transform infrared (FTIR) spectrophotometer (Frontier, Perkin Elmer) was used to analyze changes in the functional groups of oat straw before and after pretreatments. The spectra were formed from 4000 to 500 cm^{-1} with 32 times scanning.

Lignin, hemicellulose and cellulose contents of oat straw before and after each pretreatment were measured based on chemical method (LI et al., 2004; RAJPUT; ZESHAN; VISVANATHAN, 2018). First, the amount of extractives in the biomass was determined by using solvent extraction, for which, 60 mL of acetone was used for 1 g of dried oat straw sample (G_0, g) at a constant temperature of 90 °C for 2 h. After that, biomass sample was oven dried at 105 °C until constant weight was achieved (G_1, g). For determining hemicellulose content, 150 mL of NaOH solution 20 g/L was added to 1 g of extractive-free dried biomass (G_1, g). The mixture was boiled for 3.5 h with recycled distilled water, filtered and washed until the value of pH approached 7. The sample was then dried to constant weight, cooled to room temperature in a desiccator and weighted (G_2, g). Lignin was determined by using 30 mL of sulfuric acid 72% for each gram of extractive-free dried biomass sample (G_3, g). The mixture was kept at 8-15 °C for 24 h and transferred into a flask, diluted with 300 mL of distilled water and boiled for 1 h with recycled distilled water. The mixture was then filtered, and the residues were washed until there was no more sulfate ion in the filtrate (detected by 10% barium chloride solution). The residue was then dried to a constant weight, cooled to room temperature in a desiccator and weighted (G_4, g). The extractive W_1 (wt.%, db), hemicellulose W_2 (wt.%, db) and lignin W_3 (wt.%, db) were calculated according to Eq. 6.1, 6.2 and 6.3, respectively. Cellulose content W_4 (wt.%, db) was calculated by difference method as it was assumed that extractives, lignin, hemicellulose and cellulose are the only components in biomass (Eq. 6.4).

$$W_1(wt.\%, db) = \frac{G_0 - G_1}{G_0} \times 100\% \quad (6.1)$$

$$W_2(wt.\%, db) = \frac{G_1 - G_2}{G_0} \times 100\% \quad (6.2)$$

$$W_3(wt.\%, db) = \frac{G_4(1 - W_1)}{G_3} \times 100\% \quad (6.3)$$

$$W_4(wt.\%, db) = 100 - (W_1 + W_2 + W_3) \quad (6.4)$$

6.2.3.2 Waste biodegradation analysis

The biodegradation of the residues was evaluated through analysis of volatile suspended solids (VSS), total organic carbon of the suspended solids (TOC-S), total organic carbon of liquid portion (TOC-L), IC, TKN and pH. The details of the methodologies used in these analyzes have already been presented in section 2.3.1. All analyzes were performed before and after AD. A carbon-based mass balance was conducted to calculate the percentage of carbon conversion throughout the experiment, taking into account the initial total carbon (TC) (converted to mol) and the carbon that left the system in the biogas (CH_4 and CO_2, in mol) (AGUSTINI; DA COSTA; GUTTERRES, 2020).

6.2.3.3 Biogas monitoring

Biogas volumetric production was measured every 2 days by water displacement with a device based on the Mariotte principle (PRIEBE et al., 2016). To perform the measurement, a hose was connected between the device and the bioreactor. First, the device was depressurized. Then, the bioreactor valve was opened, which resulted in the release of a volume of water equivalent to the volume of biogas generated in the bioreactor. This amount of water was weighed and converted to volume by the density of water at room temperature. The pH of the aqueous solution was kept acid (< 3.0) to avoid the solubilization of some of the biogas components (CO_2 and H_2S). The composition of biogas was accessed weekly through a gas chromatograph (GC-2014 Shimadzu) equipped with a ShinCarbon column (ST 100/120 2m 1 mmID 1/16” OD Silco) and TCD detector. Helium was used as the carrier gas at a flow rate of 10 mL/min. The injector and detector temperatures were held at 200 and 250°C, respectively. The oven program was: 40 °C (3 min), ramp at 15°C/min to 150°C, and hold for 0.67 min. The results were expressed as percentages of O_2, N_2, CH_4 and CO_2.

6.3 Results and discussion

6.3.1 Waste characterization

The initial characteristics of feedstock strongly affect startup, process stability and biogas production during anaerobic digestion. Table 6.1 presents the characteristics of the wastes used in this work.

Table 6.1. Characterization of waste used for biogas production.

	Chromium	TKN	TOC	IC	C/N ratio	VS (%)	pH
	(g/L) - sludge (% (g/g)) - shaving and oat straw						
Sludge	0.31	1.21	11.27	1.89	10.88	34.42	7.45
Leather shavings	1.14%	2.95%	32.29%	0	10.95	90.24	4.09
Oat straw		0.54%	44.29%	0	82.02	94.10	8.15

TKN: total Kjeldahl nitrogen; TOC: total organic carbon; IC: inorganic carbon; C/N ratio: carbon/nitrogen ratio; VS: volatile solids

The sludge exhibited high organic carbon content (TOC/TC around 85%), C/N ratio close to 11, pH close to neutral and a concentration of chromium well below the reported tolerance for the microorganisms involved in the AD process, around 500-5,000 ppm (AGUSTINI; DA COSTA; GUTTERRES, 2020) and close to the value reported by Zupancic and Jemec (2010). In addition to the presence of chromium, the sludge also had a high concentration of vegetable tannins and phenols, probably from the retanning operation, since the tannery from which the sludge was obtained uses chromium as tanning agent. The concentration of vegetable tannins and phenols in sludge were of 7.83 and 0.96 g/L, respectively. This high concentration is due to an excess of vegetable tannin extracts used during vegetable tanning or retanning process to ensure full penetration and reaction of tannins with hide collagen. Consequently, many tannin components inevitably remain in the wastewater and in the sludge. Thus, added to the toxic phenolic and recalcitrant nature of vegetable tannins, tannin sludge have a high concentration of vegetable tannin and phenols in their composition (AGUSTINI et al., 2018a; KALYANARAMAN; KAMESWARI; RAO, 2015). Agustini *et al.* (2018a) evaluated the AD of sludge obtained from a tannery that uses vegetable tannin as the tanning agent, which has proven to be toxic to AD. The concentration of vegetable tannin present in the sludge used by Agustini *et al.* (16.52 g/L) was twice as high as the value found in the present work.

Shavings exhibited high organic load, C/N ratio close to observed to sludge (10.95), acidic pH values and a concentration of chromium of 1.14%. The concentration of chromium in the shavings is due to absorption (fixation) of chromium in the hide during the tanning process (AGUSTINI; DA COSTA; GUTTERRES, 2018) and did not impart any toxicity for its

AcoD, both in this study and in previous ones (AGUSTINI et al., 2018b; PRIEBE et al., 2016; ZUPANČIČ; JEMEC, 2010).

The oat straw exhibited high organic carbon load and thus a high C/N ratio, of 82. This could limit its efficient use in mono-AD processes for methane production. Also, oat straw (raw and pretreated) was characterized in relation to its chemical composition and functional groups and the results will be presented and discussed in the next section.

Despite the complexity of the sludge, the recalcitrant characteristics of all the wastes and the low C/N ratio of the mixture used (around 13), the AD was established, proven by the production of methane.

6.3.2 Effect of the pretreatments on oat straw

In this study, the effect of different pretreatments applied to oat straw was evaluated with respect to changes in chemical composition and functional groups.

6.3.2.1 Changes in main chemical composition

The cellulose, hemicellulose, and lignin contents of untreated and pretreated oat straw are shown in Table 6.2. No correction for ash content was done. Extractives (or soluble matter) include pectins, proteins, fats, glucoronic and galacturonic acids, among others (GOMEZ-TOVAR et al., 2012).

Table 6.2. Effects of pretreatment on the chemical composition of oat straw. For each column, similar letters a, b, c represent values that are not significantly different by the Tukey's test ($p > 0.05$).

	Extractives (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Untreated	4.14 ± 0.32^{a}	51.92 ± 0.83^{a}	24.41 ± 1.63^{b}	19.53 ± 0.74^{b}
Acid pretreatment	1.23 ± 0.16^{a}	45.59 ± 0.09^{c}	30.90 ± 1.00^{b}	22.29 ± 1.00^{b}
Alkaline pretreatment	3.83 ± 0.28^{a}	31.26 ± 0.29^{d}	56.37 ± 1.68^{a}	8.54 ± 0.33^{c}
Thermal pretreatment	3.26 ± 0.03^{a}	50.90 ± 0.04^{a,b}	24.19 ± 0.46^{b}	21.65 ± 0.30^{b}
Acid + thermal pretreatment	2.22 ± 0.26^{a}	46.46 ± 0.08^{b,c}	19.89 ± 1.55^{b}	31.44 ± 0.15^{a}
Alkaline + thermal pretreatment	2.52 ± 0.26^{a}	21.07 ± 0.64^{e}	67.47 ± 1.44^{a}	8.94 ± 0.55^{c}

The untreated oat straw presented 51.92% of hemicellulose, 24.41% of cellulose and 19.53% of lignin. This composition is different from that presented by Gomez-Tovar *et al.* (2012) (28.2, 35.0, and 4.1%, respectively), which can be attributed to climatic and geographic conditions, as well as the sample preparation and extraction method (DE OLIVEIRA et al., 2017).

The influence of the pretreatments on lignocellulosic composition of oat straw was also investigated. The extractives content did not show significant difference among the tested cases. After acid, alkaline and alkaline + thermal pretreatment, increase in cellulose content was accomplished by the decrease in hemicellulose, which is in accordance with the findings of Krishania, Vijay and Chandra (2013), Rajput, Zeshan and Visvanathan (2018) and Shen *et al.* (2019). Cellulose content increased by about 176% due to alkaline + thermal pretreatment as compared to untreated oat straw. Ran *et al.* (2018) reported the same increase in cellulose contents due to hydrothermal pretreatment of lignocellulosic biomass at 160°C. Lignin content decreased by about 55% due to alkaline and alkaline + thermal pretreatments as compared to untreated oat straw. The structural changes caused by these pretreatments can be attributed to the action of the alkali on the ether and ester linkages between hemicellulose and lignin. Due to the breaking of these internal bonds, both hemicellulose dissolution and lignin removal can be achieved, which can contribute to improve the digestibility of the substrate (MANCINI et al., 2018; SHEN et al., 2019).

6.3.2.2 Changes in functional groups

The change in the chemical bonds of oat straw due to pretreatments was analyzed by the FTIR technique. The spectra of pretreated and untreated straw in the 4000-500 cm^{-1} region are shown in Fig. S6.1.

The spectra of untreated and pretreated oat straw have similar profiles but varied intensities. The infrared spectra showed characteristic bands of the functional groups of the components of the lignocellulosic fibers (cellulose, hemicelluloses and lignin) (LIU et al., 2019). These components showed mainly alkanes, aromatic groups and different functional groups, such as ester, ketone and alcohol in their structures (DE OLIVEIRA et al., 2017; MORÁN et al., 2008). Similar to that reported in the literature (KUMAR et al., 2019; RAJPUT; ZESHAN; VISVANATHAN, 2018; YAO; DAVID; DAVARITOUCHAEE, 2018), all samples presented two main absorbance regions. The first one, in wavelengths in the range of 700-1800 cm^{-1}, and the second one at higher wavelengths corresponding to the range 2800-3500 cm^{-1}.

As can be seen in Fig. S6.1, the absorption band at 3330 cm^{-1} attributed to the O-H stretching in the hydrogen bond of cellulose, hemicellulose and lignin (LIU et al., 2019; RAJPUT; ZESHAN; VISVANATHAN, 2018; YANG et al., 2007) decreased to oat straw submitted to thermal (d), alkaline (c) and acid pretreatments (b). This implies that crystalline cellulose of oat straw was disrupted by these pretreatments.

The peak of 2920 cm^{-1} correspondent to $C–H_n$ and related to asymmetric methylene stretching of cellulose, hemicellulose and lignin becomes weaker with acid pretreatment (b) (KUMAR et al., 2019). For oat straw submitted to thermal (d), acid + thermal (e), alkaline + thermal (f) and alkaline pretreatments (c), a peak formation was observed in 2850 cm^{-1}, also related to the $C–H_n$ stretching and characteristic of hemicellulose compounds (YANG et al., 2007).

The absorption band at 1640 and 1600 cm^{-1} is originated from lignin, which includes C=C stretching of aromatic ring and aromatic skeleton vibration of C=C, respectively (KUMAR et al., 2019). The intensity of these peaks decreased for the oat straw submitted to acid + thermal (e), alkaline (c), acid (b) and alkaline + thermal (f) pretreatments and are related to the removal of lignin.

Changes in the intensity of the peaks 1370 and 1318 cm^{-1} are detected to acid + thermal (e), alkaline + thermal (f), alkaline (c) and acid (b) pretreated oat straw. These bands are related to aliphatic C–H deformation vibration and $C–H_2$ wagging of cellulose, respectively (KUMAR et al., 2019).

The stretching vibration of C–O–C for cellulose and hemicellulose is described at the approximate wavenumber of 1158 cm^{-1} (COLOM et al., 2003; KUMAR et al., 2019). Compared to the untreated oat straw (a), the decrease in wavenumber intensity observed in acid (b) and alkaline pretreatment (b) might be because of the removal of hemicellulose (SHEN et al., 2019).

The absorption band at 1100 cm^{-1}, also related to the vibration of C–O–C glycosidic linkages of the cellulose (DE OLIVEIRA et al., 2017), and 1031 cm^{-1}, related to C–O stretching of cellulose and hemicellulose (KUMAR et al., 2019; YANG et al., 2007) becomes weaker for alkaline + thermal (f), alkaline (c) and acid pretreatments (b).

The intensity at 899 cm^{-1} band corresponding to β-D-cellulose linkages had a significant decrease in acid pretreatment (b), which indicated the removal of amorphous components (YAO; DAVID; DAVARITOUCHAEE, 2018; ZHANG; LOH; ZHANG, 2019).

FTIR spectra indicate that pretreatments result in some level of delignification of the fiber. However, some peaks corresponding to lignin components (1740, 1510, 1455 and 1234 cm^{-1}) increased or even was only detected in pretreated oat straw (DE OLIVEIRA et al., 2017; KUMAR et al., 2019; LIU et al., 2019). This contradiction can be attributed to the formation of pseudo-lignin droplets, spherical structures containing a lignin-like compound that can be found on the surface of pretreated biomass (YAO; DAVID; DAVARITOUCHAEE, 2018). This phenomenon is possible when some pretreatments generate products such as furfural repolymerize and/or lignin and form a lignin-like material named pseudo-lignin (HU; JUNG; RAGAUSKAS, 2012).

Taken together these FTIR results indicate that specially the oat straw pretreated with acid, alkaline and by the combination of alkaline + thermal, presented changes in the lignocellulosic structure of the fiber related to solubilization of carbohydrate and lignin, breakage of association between lignin with carbohydrate and changes in aromatic rings. These changes might be beneficial for AD, because the solubilization of lignin can made cellulose fibers become more available for enzymes attack to convert it into fermentable sugars.

6.3.3 Effect of pretreatment on biogas production and waste treatment

The cumulative biogas production per gram of VSS added for each experimental condition tested is shown in Fig. 6.2. The daily biogas production and biogas composition for all the tests are shown in Fig. S6.2. Waste analyses, expressed by the parameters TOC-L, TOC-S, VSS, IC, TKN, and pH, before and after biodegradation assays, are shown in Table 6.3 and Table 6.4.

The definition of the acronyms that represent the composition of the reactors is: sludge + shavings (SLU + SHA), sludge + shavings + untreated oat straw (SLU + SHA + UOS), sludge + shavings + oat straw submitted to acid pretreatment (SLU + SHA + OS-HCl), sludge + shavings + oat straw submitted to alkaline pretreatment (SLU + SHA + OS-NaOH), sludge +

shavings + oat straw submitted to thermal pretreatment (SLU + SHA + OS-THE), sludge + shavings + oat straw submitted to acid + thermal pretreatments (SLU + SHA + OS-HCl/THE), sludge + shavings + oat straw submitted to alkaline + thermal pretreatments (SLU + SHA + OS-NaOH/THE).

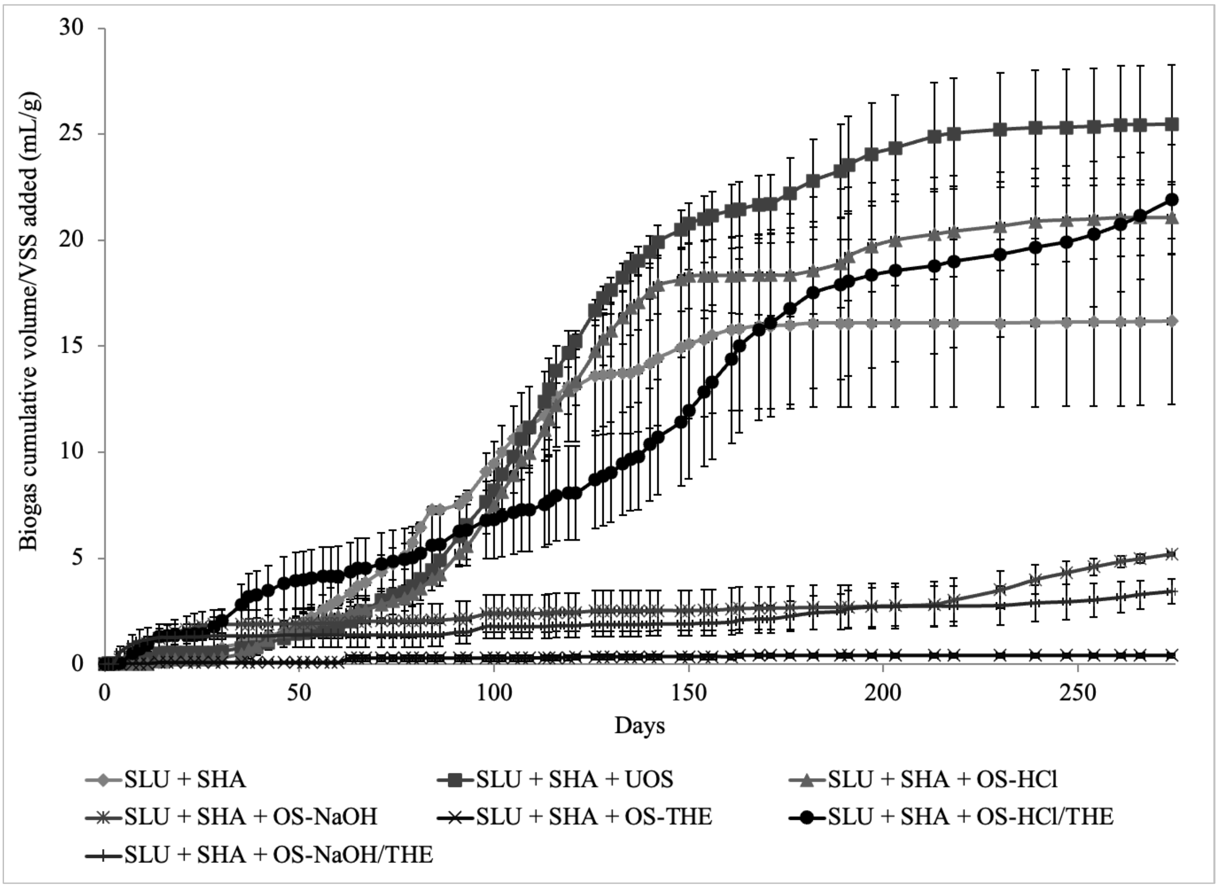

Fig. 6.2. Biogas cumulative production per gram of VSS added in the biodegradation reactors.

Table 6.3. Mean values of organic matter (TOC and VSS) of bioreactors before and after biodegradation and variation due to biodegradation. For each column, similar letters a, b represent values that are not significantly different by the Tukey's test ($p > 0.05$).

(continue)

Reactor	TOC-L (mg/L)			TOC-S (%)			VSS (%)		
	Before	After	Variation	Before	After	Variation	Before	After	Variation
SLU + SHA	289.80 ± 4.40	399.82 ± 51.09	+39.16 ± 19.74%^{b}	23.88 ± 0.74	14.43 ± 0.01	-39.33 ± 1.90%^{b}	48.33 ± 0.86	31.29 ± 0.29	-35.13 ± 1.75%^{a}
SLU + SHA + UOS	428.80 ± 40.30	562.60 ± 29.10	+38.65 ± 19.82%^{b}	28.86 ± 0.52	22.6 ± 0.20	-21.62 ± 0.73%^{b}	70.36 ± 2.30	60.92 ± 2.59	-13.52 ± 0.85%^{a}

(conclusion)

Reactor	TOC-L (mg/L)			TOC-S (%)			VSS (%)		
	Before	After	Variation	Before	After	Variation	Before	After	Variation
SLU + SHA + OS-HCl	323.60 ± 4.20	449.90 ± 9.25	+38.97 ± 1.05%^{b}	27.53 ± 0.27	33.77 ± 0.32	+22.76 ± 2.36%^{a}	67.34 ± 1.21	61.25 ± 0.89	-8.83 ± 2.96%^{a}
SLU + SHA + OS-NaOH	314.10 ± 13.35	2509.82 ± 51.09	+702.08 ± 17.82%^{a}	29.97 ± 1.86	28.21 ± 1.19	-5.39 ± 1.89%^{a,b}	72.00 ± 2.06	49.32 ± 3.15	-31.78 ± 2.43%^{a}
SLU + SHA + OS-THE	285.80 ± 5.50	501.00 ± 45.20	+76.78 ± 19.22%^{b}	29.74 ± 1.35	21.33 ± 0.52	-28.02 ± 1.52%^{b}	65.90 ± 0.04	62.28 ± 2.92	-5.49 ± 4.50%^{a}
SLU + SHA + OS-HCl/THE	302.50 ± 6.25	732.70 ± 119.15	+145.89 ± 44.47%^{b}	32.20 ± 1.28	27.46 ± 0.67	-13.83 ± 5.50%^{b}	67.35 ± 1.04	54.44 ± 1.49	-18.95 ± 4.50%^{a}
SLU + SHA + OS-NaOH/THE	356.40 ± 20.50	1970.50 ± 52.75	+463.79 ± 47.23%^{a}	29.88 ± 0.10	23.55 ± 0.72	-21.13 ± 2.66%^{b}	68.31 ± 0.38	46.30 ± 0.99	-32.19 ± 1.83%^{a}

Table 6.4. Mean values of IC, TKN and pH of bioreactors before and after biodegradation and variation due to biodegradation. For each column, similar letters a, b represent values that are not significantly different by the Tukey's test ($p > 0.05$).

Reactor	IC (mg/L)			TKN (mg/L)			pH		
	Before	After	Variation	Before	After	Variation	Before	After	Variation
SLU + SHA	198.36 ± 9.32	872.90 ± 8.55	+344.80 ± 25.21%^{a}	207.2 ±14.00	644.00 ± 19.60	+213.99 ± 11.76%^{a}	8.30 ± 0.06	7.79 ± 0.04	-6.08 ± 0.11%^{a}
SLU + SHA + UOS	336.30 ± 20.75	933.30 ± 13.25	+182.80 ± 21.39%^{a}	201.60 ± 0.00	663.60 ± 15.40	+229.17 ± 7.64%^{a}	7.86 ± 0.33	7.87 ± 0.06	+0.68 ± 3.34%^{a}
SLU + SHA + OS-HCl	274.00 ± 21.10	857.03 ± 25.68	+217.43 ± 15.07%^{a}	207.20 ± 2.80	556.73 ± 22.63	+168.30 ± 7.30%^{a}	7.67 ± 0.15	7.80 ± 0.09	+1.94 ± 3.10%^{a}
SLU + SHA + OS-NaOH	222.87 ± 13.66	695.90 ± 84.85	+226.49 ± 58.09%^{a}	176.40 ± 1.40	613.20 ± 14.00	+247.45 ± 5.18%^{a}	7.68 ± 0.13	6.93 ± 0.06	-9.61 ± 2.31%^{a}
SLU + SHA + OS-THE	305.70 ± 3.25	862.20 ± 23.20	+182.49 ± 10.59%^{a}	198.80 ± 1.40	473.20 ± 64.40	+137.16 ± 30.72%^{a}	7.53 ± 0.02	7.75 ± 0.02	+2.92 ± 0.01%^{a}
SLU + SHA + OS-HCl/THE	274.70 ± 1.55	957.30 ± 16.45	+248.40 ± 4.02%^{a}	182.00 ± 9.80	618.80 ± 5.60	+243.32 ± 15.41%^{a}	7.65 ± 0.01	8.06 ± 0.06	+5.36 ± 0.71%^{a}
SLU + SHA + OS-NaOH/THE	255.97 ± 43.61	654.80 ± 7.80	+187.07 ± 45.87%^{a}	190.40 ± 5.60	641.20 ± 28.00	+236.20 ± 4.82%^{a}	8.00 ± 0.26	7.02 ± 0.02	-11.86 ± 2.59%^{a}

6.3.3.1 No oat straw - SLU + SHA

The reactors without the addition of oat straw produced on average 16.17 ± 1.80 mL of cumulative biogas/g of VSS added (12.57 g) with a maximum percentage of 38% of methane (Fig. 6.2 and Fig. S6.2). The lag-phase of the cumulative biogas production curve was approximately 30 days, showing that the recalcitrance and complexity of the residues caused the hydrolysis phase to take longer to complete. Similarly, the log-phase was also long, beginning at day 30 of incubation and ending around day 150, and also not having a large slope, again due to the complexity of the residues, so that the microorganisms could not quickly metabolize the wastes (Fig. 6.2). A plateau phase was verified in the cumulative biogas production curve, phenomenon that is also known as diauxie and that could be related to the accumulation of volatile fatty acids (VFAs), indicating an imbalance between the early stages of AD and methanogenesis. The methanogenic archaea are not able to process all the intermediate products so fast and the high concentration of the acids that starts to accumulate reduces the pH of the medium which causes the inhibition of methane production. Biogas production continues when the VFAs concentration drops (GOMES; REPKE; MEYER, 2019; KIM; KIM, 2017). The methane concentration rose along with the biogas production (Fig. S6.2) as expected for an established AD process. Its percentage reached a maximum of 38% at the end of the log-phase.

The biogas production and methane yield obtained in this work were lower than that reported in other studies carried out with equivalent wastes, which produced up to 30 mL of biogas/g of VSS, with a methane percentage of 60% (AGUSTINI; DA COSTA; GUTTERRES, 2018; SIMIONI et al., 2020). Comparing the wastes used in the studies cited above with that used in the present work, the main difference is in the composition of the sludge. In addition to a higher concentration of chromium, the sludge used in this study contains phenols and vegetable tannin, which have been reported as toxic to the final stages of AD in previous studies (AGUSTINI et al., 2018a, 2018b) and which may have partially inhibited the AD of the residues.

In our study, the efficiency of waste treatment (reducing its organic load) is as important as the production of biogas, since the remaining waste (after the AD process) still needs to be managed and a more mineralized waste is environmentally inert for disposal. It is worth remembering that, in addition to producing biogas, the AD process also generates a nitrogen-rich digestate that can be used as fertilizer in the field, which can be particularly interesting in this case, where one of the wastes comes from agriculture.

In relation to the reduction of the organic load of the wastes, a reduction of 35.13% was observed for VSS (Table 6.3), which represents the suspended organic matter. Similar to what occurs with VSS, the balance between TOC-S and TOC-L is related to the migration of organic carbon, initially precipitated in the waste and which, due to AD, undergoes hydrolysis and is solubilized in the medium to be metabolized by microorganisms. The variation of TOC-S and TOC-L over the tests is shown in Table 6.3. For the SLU + SHA test, a reduction in TOC-S (-39.33%) was observed, which is in accordance with the discussion above, and an increase in TOC-L (+39.16%), which shows that the suspended carbon was hydrolyzed and dissolved in the medium, but also indicates that the following steps (acidogenesis, acetogenesis and methanogenesis) were partially inhibited.

The concentrations of TKN and IC increased throughout the process (Table 6.4), as expected (AGUSTINI; DA COSTA; GUTTERRES, 2020a; SIMIONI et al., 2020). The increase in TKN concentration (+213.99%) is expected due to the natural characteristic of the waste, which, because of the animal hide origin, is a nitrogen-rich source. As there was no large outflow of gaseous nitrogen, it remained in the residues and increased its concentration due to the volumetric reduction of the system. The IC concentration also increased (+344.80%), as expected due to the mineralization of the waste.

6.3.3.2 Untreated oat straw - SLU + SHA + UOS

The AcoD between tannery waste (shavings + sludge) and untreated oat straw (SLU + SHA + UOS) produced on average 25.44 ± 0.69 mL of cumulative biogas/g of VSS added (19 g), which represents an increase of almost 60% in relation to the case discussed above, without the addition of oat straw. The periods comprising the lag and log-phases were practically the same as those observed for the SLU + SHA. However, a greater slope of the curve was observed for SLU + SHA + UOS, indicating that the wastes were more easily metabolized in this case, probably due to the greater nutritional balance provided by the addition of oat straw (C/N ratio of 13, closest to optimal for AD) (Fig. 6.2). As in the case without the addition of oat straw, discussed above, the cumulative production of biogas also showed diauxic growth curves. The concentration of CH_4 increased with the increase in biogas production, reaching the maximum percentage of 40% at the end of the log-phase (Fig. S6.2).

Regarding the treatment of waste (Table 6.3 and Table 6.4), the parameters analyzed follow the same trend as observed for SLU + SHA. Reduction of VSS (-13.52%) and TOC-S (-21.62%) and increase in TOC-L (+38.65%), indicate a decrease in organic matter and the migration of organic carbon from the suspended to the dissolved phase, with partial inhibition of the steps subsequent to hydrolysis. Also, the concentrations of TKN and IC increased (+229.17% and +182.80%, respectively) throughout the process, as expected.

6.3.3.3 Pretreated oat straw - SLU + SHA + OS-HCl; SLU + SHA + OS-NaOH; SLU + SHA + OS-THE; SLU + SHA + OS-HCl/THE; SLU + SHA + OS-NaOH/THE

The cumulative production of biogas per gram of added VSS for the cases in which pretreated oat straw was added to the bioreactors was: 21.06 ± 0.99 mL/g VSS (18.18 g) for SLU + SHA + OS-HCl; 5.20 ± 0.74 mL/g VSS (19.44 g) for SLU + SHA + OS-NaOH; 21.91 ± 2.06 mL/g VSS (18.19 g) for SLU + SHA + OS-HCl/THE; 3.43 ± 0.58 mL/g VSS (18.44 g) for SLU + SHA + OS-NaOH/THE. The test in which the oat straw was submitted only to the thermal pretreatment (SLU + SHA + OS-THE) produced virtually no biogas (0.42 ± 0.09 mL/g VSS), being statistically not different ($p > 0.05$) from SLU + SHA + OS-NaOH and SLU + SHA + OS-NaOH/THE.

The curve profile (lag and log phases and slope of the curve) of the SLU + SHA + OS-HCl case is very similar to that observed for SLU + SHA + UOS. For the SLU + SHA + OS-HCl/THE case, although biogas production started earlier (lag-phase started around the 10th day), the slope of the curve is much lower (Fig. 6.2), indicating that the combination of acid and thermal pretreatments was an aggressive process, which sterilized the wastes and caused an excessive degradation of the organic matter that would later be digested (AGUSTINI et al., 2018a). For both cases, diauxie behavior was observed, which, as mentioned previously, could be related to the VFAs concentration (GOMES; REPKE; MEYER, 2019; KIM; KIM, 2017).

The percentage of methane showed the same growth as the biogas production growth for SLU + SHA + OS-HCl and SLU + SHA + OS-HCl/THE, reaching a maximum of 40.85% and 30.31%, respectively, both at the end of the process (Fig. S6.2), showing that anaerobic digestion took longer to establish and did not establish so well. For SLU + SHA + OS-NaOH and SLU + SHA + OS-NaOH/THE, the percentage of methane reached was 17.46% and 10%, respectively. For thermal pretreatment (SLU + SHA + OS-THE), the percentage of methane reached 18% in the process (Fig. S6.2), showing that even though AD was not fully established, methanogenic activity did occur. The high concentration of CO_2 (up to 70%) observed for all cases corroborates the hypothesis that mainly the final stage of AD was inhibited. It means, the methanogenic bacteria were not able to metabolize the CO_2 formed during the initial stages and convert it into methane, however, acetogenesis was established (APPELS et al., 2008; MIRMOHAMADSADEGHI et al., 2019).

The amount of biogas produced for these cases is lower than that observed for the case in which the oat straw was not pretreated (SLU + SHA + UOS) (25.44 mL of cumulative biogas/g of VSS added), suggesting that pretreatment probably generated inhibitory compounds. This inhibition can be attributed to the presence of toxic compounds from dehydration of pentoses (furfural) and hexoses (hydroxymethyl furfural) and/or to phenolic compounds from the rupture of lignin, which inhibit anaerobic fermentation because it is difficult for anaerobic microorganisms to metabolize them (FERREIRA et al., 2013; GOMEZ-TOVAR et al., 2012; RAN et al., 2018). Particularly for cases involving thermal pretreatment, the observed inhibition can also be due to autoclaving being a very aggressive process, inducing much sterilization and excessive degradation of the organic matter that would be further digested (AGUSTINI et al., 2018a).

Lower biogas production and methane yields observed for cases where the oat straw was pretreated with NaOH can also be attributed to the presence of Na^+ ions. Sodium is a known process inhibitor in anaerobic systems and impacts on methanogens through an increase of osmotic pressure or complete dehydration of microorganisms (FJØRTOFT et al., 2019; SHETTY et al., 2017). Although pretreatment with NaOH has a positive effect on a series of studies reported involving AD of lignocellulosic materials (CHANDRA et al., 2012; MANCINI et al., 2018; SABEEH; LIAQUAT; MARYAM, 2020), and even in the lignin removal observed for this study (Table 6.2), it is believed that the possible presence of ammonia due to the nature of the leather wastes, along with Na^+ ions originating from the pretreatment of the straw may have caused the observed inhibition. This hypothesis is supported since Hierholtzer and Akunna (2012) found that relatively low sodium levels (0.2 mol/L) can bring about significant levels of process inhibition in the presence of high levels of ammoniacal nitrogen. In addition, the pH values observed after biodegradation, 6.93 for SLU + SHA + OS-NaOH and 7.02 for SLU + SHA + OS-NaOH/THE (Table 6.4), well below the average observed for the other cases, are an indicative that the acidogenesis was established, but the products were not converted into CH_4 and CO_2 by inhibition of methanogenesis, corroborating the assumption made above.

Regarding the efficiency in the treatment of the wastes, a reduction in VSS (Table 6.3) was observed in all cases containing pretreated oat straw as a co-substrate. The migration of organic carbon (TOC-S to TOC-L), which was initially precipitated in the residue and, after hydrolysis, is solubilized in the medium, was also observed for the cases discussed in this section. The values of TOC-L for SLU + SHA + OS-NaOH and SLU + SHA + OS-NaOH/THE increased more than 400% (Table 6.3). These results indicate the toxicity of the wastes to the final stages of AD, since only the hydrolysis, which releases organic matter in the medium, was able to establish (AGUSTINI et al., 2018b). The concentrations of TKN and IC increased after AD for all cases, proving the mineralization of the waste, even though AD has not been fully established in some cases.

Reduction in TOC and VSS values and increases in IC and TKN values are common for cases where the AD process was well established, with satisfactory production of biogas and methane. For SLU + SHA + OS-THE, even with practically no biogas production, there was a reduction in VSS (-5.49%) and TOC-S (-28.02%) and an increase in TKN (+137.16%) and IC (+182.49%). This behavior can be attributed to the activity of microorganisms not involved in AD, so that the mineralization of the wastes is not only linked to the biogas production.

6.3.3.4 Carbon-based mass balance

A balance that involves TC (TOC + IC) was carried out for the mixtures in bioreactors in order to compare the amount of initial carbon with the amount of carbon that was converted to CH_4 and CO_2 throughout the experiment. The results are detailed in Table 6.5, where it is shown that the AcoD between tannery waste (shavings + sludge) and untreated oat straw was the most efficient, converting almost 27% of carbon from the waste to CH_4 and CO_2. The AcoD of tannery wastes (shavings + sludge) converted 17%, the same carbon conversion observed by Agustini, Da Costa and Gutterres (2020a) in their study involving the AcoD of leather wastes similar to those used in this work. Among the pretreatments tested on oat straw, acid pretreatment stood out as the most efficient option, converting 21% of carbon from the waste into CH_4 and CO_2.

Table 6.5. Carbon-based mass balance. For each column, similar letters a, b represent values that are not significantly different by the Tukey's test ($p > 0.05$).

Reactor	Initial C amount (×10^{-2} g)	Initial C (×10^{-2} mol)	C (×10^{-2} mol) CH_4	C (×10^{-2} mol) CO_2	C conversion
SLU + SHA	34.86 ± 0.43	2.90 ± 0.04	0.17 ± 0.05	0.34 ± 0.05	17.25 ± 3.37%^{a}
SLU + SHA + UOS	74.93 ± 0.33	6.24 ± 0.03	0.51 ± 0.06	1.17 ± 0.09	26.80 ± 2.46%^{a}
SLU + SHA + OS-HCl	68.50 ± 0.04	5.71 ± 0.003	0.29 ± 0.03	0.94 ± 0.04	21.54 ± 1.31%^{a}
SLU + SHA + OS-NaOH	72.01 ± 03.11	6.00 ± 0.26	0.02 ± 0.008	0.22 ± 0.004	4.17 ± 0.11%^{b}
SLU + SHA + OS-THE	72.79 ± 2.49	6.07 ± 0.21	0.003 ± 0.0002	0.02 ± 0.004	0.36 ± 0.07%^{b}
SLU + SHA + OS-HCl/THE	77.37 ± 2.45	6.45 ± 0.20	0.30 ± 0.05	0.98 ± 0.10	20.24 ± 3.13%^{a}
SLU + SHA + OS-NaOH/THE	73.53 ± 1.24	6.13 ± 0.10	0.009 ± 0.002	0.11 ± 0.009	2.00 ± 0.21%^{b}

The greater efficiency in carbon conversion observed for SLU + SHA + UOS highlights the importance of the AcoD process. In fact, the balance of nutrients provided by AcoD has resulted in a more efficient process with greater biogas production, in addition to the economic advantage of sharing the AD system and treating three different wastes at the same time. Although the pretreatments studied here have improved the characteristics and bioavailability of oat straw, there is still the possible formation of secondary products or sterilization of important microorganisms that did not reflect in a greater production of biogas.

6.4 Conclusion

The experiments of AcoD of sludge, shavings and oat straw (untreated and pretreated) indicate that: (1) the addition of untreated oat straw improved the AcoD process, increasing biogas production by almost 60% when compared to the AcoD of only leather waste; (2) acid and acid + thermal pretreatments of oat straw did not enhanced biogas and methane production compared to untreated oat straw; (3) lignin content decreased by about 55% due to alkaline and alkaline + thermal pretreatments as compared to untreated oat straw, but the presence of inhibitory compounds remaining of NaOH pretreatment along with the presence of ammoniacal nitrogen due to the nature of the leather wastes, may have partially inhibited methanogenesis, limiting the production of biogas for these cases; (4) thermal pretreatment of oat straw probably have generated toxic compounds from hemicellulose and cellulose degradation, which inhibited the AcoD process and, as consequence, virtually no biogas was produced.

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Supplementary material

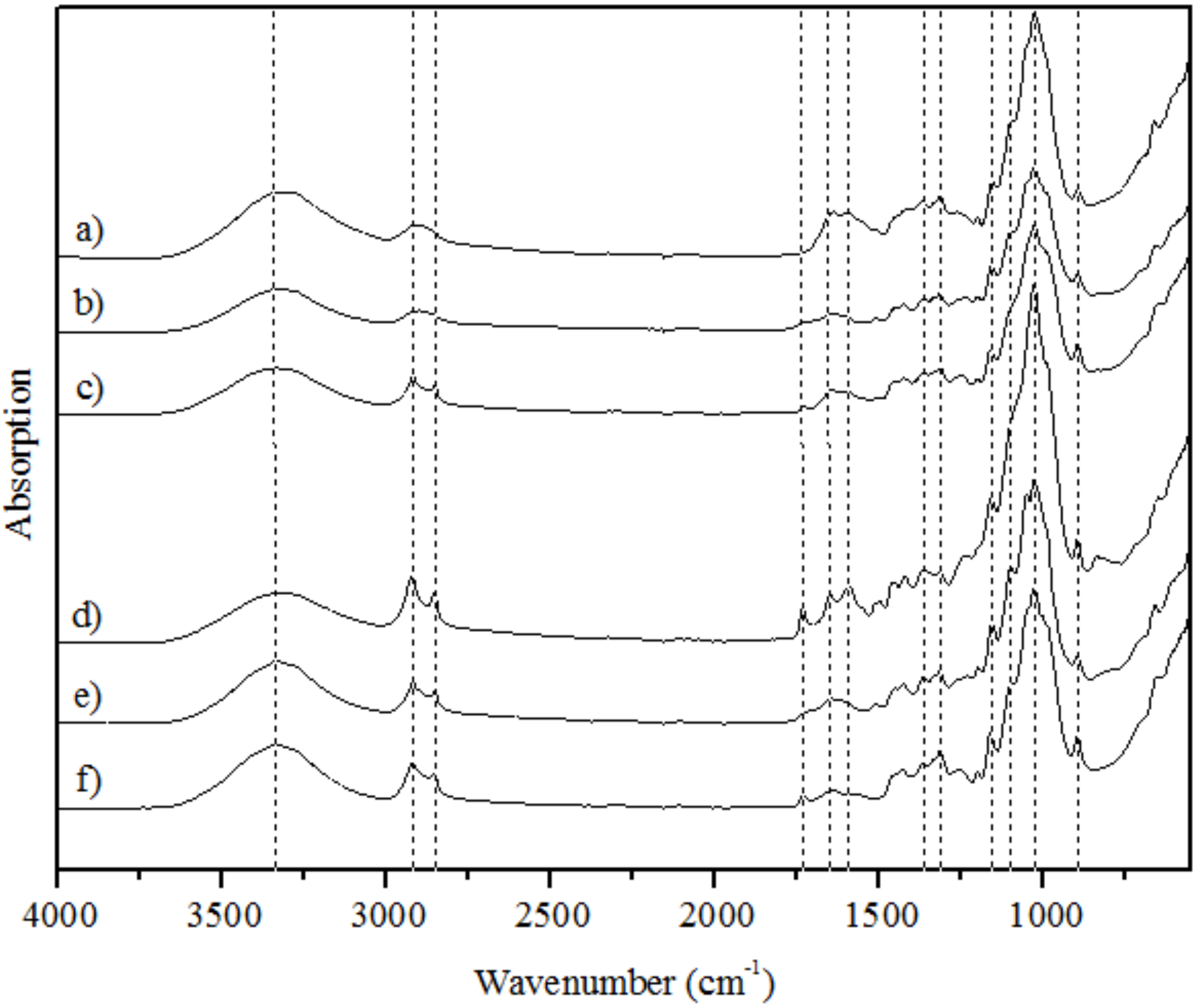

Fig. S6.1. FTIR spectra for oat straw (a) without pretreatment and submitted to (b) acid pretreatment, (c) alkaline pretreatment, (d) thermal pretreatment, (e) acid + thermal pretreatment and (f) alkaline + thermal pretreatment.

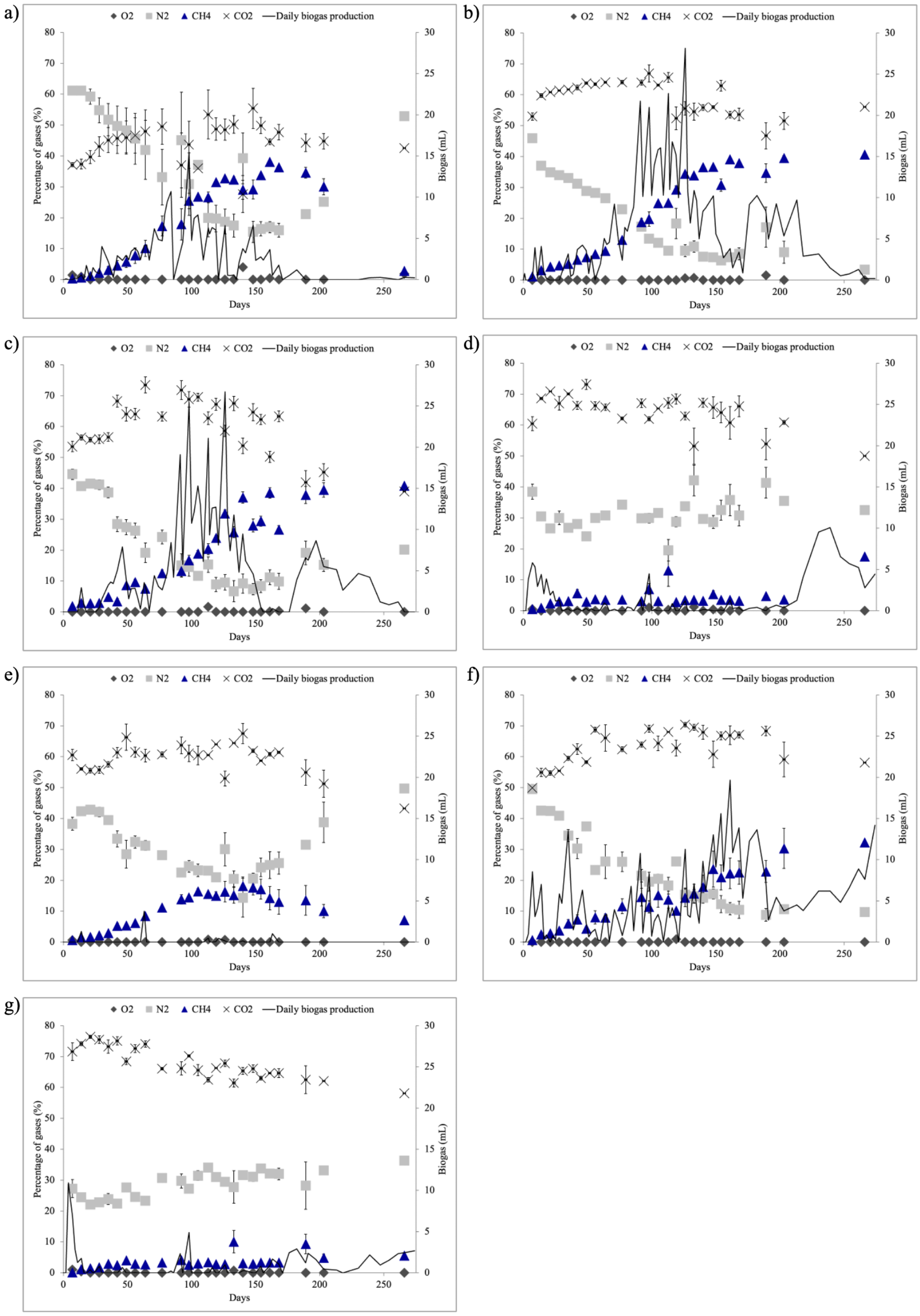

Fig. S6.2. Daily production and composition of the biogas produced in the biodegradation reactors: a) SLU + SHA, b) SLU + SHA + UOS, c) SLU + SHA + OS-HCl, d) SLU + SHA + OS-NaOH, e) SLU + SHA + OS-THE, f) SLU + SHA + OS-HCl/THE, g) SLU + SHA + OS-NaOH/THE.

Capítulo 7

Enhancement of biogas production by anaerobic co-digestion of leather waste with raw and pretreated wheat straw

Artigo submetido e em revisão em periódico.

Neste artigo, avaliou-se a viabilidade do processo de co-DA entre farelo de couro *wet-blue*, lodo de ETEs de curtume e palha de trigo; além de estabelecer uma comparação do efeito dos pré-tratamentos ácido, básico, térmico e da combinação destes, sobre a composição lignocelulósica da palha de trigo e no rendimento de biogás obtido pela co-digestão anaeróbia.

Abstract

This work investigated the effect of the addition of wheat straw on the anaerobic co-digestion of leather waste regarding biogas production. In an attempt to improve biodegradability, wheat straw was pretreated using (i) acid pretreatment with HCl 2% at 90 °C for 2 h, (ii) alkaline pretreatment with NaOH at 30 °C for 24 h and (iii) thermal pretreatment by autoclaving the straw at 121 °C (1 atm) for 15 min. In addition, the combination between the pretreatments (iv) acid + thermal and (v) alkaline + thermal were tested. Pretreatment with alkali seems to affect the wheat straw more with a significant increase in cellulose content and a decrease in hemicellulose and lignin content. However, methanogenesis appears to have been inhibited in these cases, resulting in the lowest biogas production among all the tested issues (8.84 mL/gVSS for alkaline pretreatment and 4.01 mL/gVSS for the combination between alkaline and thermal pretreatments). The highest cumulative biogas yield of 43.15 mL/gVSS was obtained for wheat straw pretreated with HCl, 59% higher than untreated wheat straw (27.12 mL/gVSS) and almost 167% higher than that without wheat straw (16.17 mL/gVSS), indicating that the codigestion studied in this condition is a promising alternative.

Keywords: waste-to-energy; lignocellulosic biomass; tannery waste; pretreatment.

7.1 Introduction

Energy consumption has increased worldwide and should keep rising in the upcoming years, with an estimated growth of almost 50% between 2018 and 2050 (EIA, 2019). Therefore, the search for solutions that increase energy supply is an urgent need, and these solutions must be renewable and sustainable. Renewable energy offers the opportunity to restrain the increasing depletion of fossil resources and the environmental impacts resulting from the indiscriminate use of non-renewable resources (FREITAS et al., 2019).

Waste-to-energy technologies have enormous potential to increase the share of renewable energy in the Brazilian electricity matrix, given the amount of waste generated in the agricultural, industrial, and urban sectors (FERREIRA et al., 2018). Studies have shown that anaerobic digestion (AD) is one of the most widespread technologies used to convert waste into energy worldwide (KHALIL et al., 2019). AD is a complex biochemical process that includes hydrolysis, acidogenesis, acetogenesis, and methanogenesis steps, during which organic materials are degraded by a consortium of microorganisms in the absence of oxygen, resulting in the production of methane-rich biogas (XU et al., 2018; ZHANG; LOH; ZHANG, 2019). Biogas can be used in various applications such as the production of electricity, heat and steam generation in households and industry, injection into the natural gas grid, and vehicular fuel (KHAN et al., 2017).

The leather industry is considered one of the major engines of the Brazilian economy, exporting about 1.15 billion dollars to 80 countries in 2019 (CICB, 2019). However, the economic importance of this industry has as a counterpoint the environmental issues inherent to the leather process and the waste generated. In this context, the search for alternative treatments of the waste generated by the leather industry is becoming increasingly essential, and AD stands out as an attractive solution from the perspective of sustainable and integrated management of solid waste and tannery wastewater (AGUSTINI; DA COSTA; GUTTERRES, 2020, 2018; LAZAROIU et al., 2018; POLIZZI; ALATRISTE-MONDRAGÓN; MUNZ, 2018). Mono-digestion of the recalcitrant feedstocks, highly protein-rich substrates or feedstocks with harmful compounds often results in a slow process and a low biogas yield (PATINVOH et al., 2017). These restrictions can be overcome by the co-digestion of different feedstocks with an appropriate mixing ratio, seeking better availability and balance of macro and micronutrients (for good microbial growth), dilution of toxic or inhibitory compounds, moisture balance, and better-buffering capacity. Anaerobic co-digestion (AcoD) also allows positive synergistic effects in the efficiency of the process, increase in the biodegradable organic load, expansion of the microbial community involved in the process, and higher concentrations of active biomass (HAGOS et al., 2017; KHALID et al., 2011; TYAGI et al., 2018). Another great advantage of AcoD system is that it can treat more than one waste simultaneously (HAGOS et al., 2017; SIDDIQUE; WAHID, 2018).

The main points that must be considered to evaluate the co-digestion with leather waste are: (i) they are not as bioavailable to micro-organisms due to the processing of leather (AGUSTINI et al., 2018a); (ii) recycle techniques, such as incorporation in other materials or their direct use as fertilizer, are restrictive because of the large number of inhibiting compounds such as tanning agents and chlorides in the wastes (ZUPANČIČ; JEMEC, 2010); (iii) the main management technique of these wastes is their disposal in landfills (POLIZZI; ALATRISTE-MONDRAGÓN; MUNZ, 2018), which is highly detrimental to groundwater, soil and atmosphere due to the uncontrolled release of methane (AGUSTINI et al., 2018a). Therefore, besides producing biogas, the biodegradation of these wastes also aims to reduce their organic load and volume since the remaining waste still needs to be managed, and a more mineralized waste is environmentally inert for disposal (SIMIONI et al., 2020).

The inhibition occasioned by a low C/N ratio can be potentially alleviated by adding carbon-rich co-substrates to raise the C/N ratio and improve methane yield (RAHMAN et al., 2018; XU et al., 2018). Wheat straw is the second abundant agricultural waste globally, second only to rice straw (KIM; DALE, 2004). According to the National Supply Company (CONAB) (CONAB, 2020), the annual average of wheat production in Brazil in the last five years (2015-2019) was 5,427.22 thousand tons. With an organic carbon load of around 50% (HASSAN et al., 2017) and a C/N ratio of around 100 (SIDDIQUE; WAHID, 2018), it can be an excellent option co-digestion with nitrogen-rich substrates, like a leather waste (C/N ratio of around 10) (SIMIONI et al., 2021).

However, the biodegradability of wheat straw still needs to be improved effectively. The main reason for the low utilization efficiency of wheat straw is that cellulose, hemicellulose, and indigestible lignin components combine together tightly and form a complex structure, which provides chemical and physical recalcitrance to microorganisms and then limits the initial hydrolysis step of AD (SHEN et al., 2019). Various pretreatment strategies have been introduced to modify the recalcitrant structure and enhance the digestibility of lignocellulosic biomass for AD. In recent years many studies have evaluated the feasibility of these methods to increase the bioavailable carbon load by reducing lignin and cellulosic crystallinity to enhance the fermentation stability and improve biogas production (ABRAHAM et al., 2020; HASSAN et al., 2017). The state-of-the-art pretreatment strategies applied to wheat straw to improve the AD process are shown in Table 7.1.

Table 7.1. Overview of studies of AD of pretreated wheat straw.

(continue)

Pretreatment	Co-substrate	Inoculum	Findings	Reference
Thermoalkaline - 10% CaO at 75 °C for 24 h.	Microalgal biomass	Sludge from a sugar factory.	15% increase in methane yield after pretreatment.	(SOLÉ-BUNDÓ et al., 2017)

(continuation)

Pretreatment	Co-substrate	Inoculum	Findings	Reference
Alkaline - KOH 2% (w/w).	Poultry droppings	Cattle manure with wheat straw and grass.	13.07% and 16.96% increase in methane production at mesophilic and thermophilic temperatures, respectively, compared to no KOH addition.	(RAHMAN et al., 2018)
Steam-exploded - 210 °C for 10 min.	Cattle manure	From a biogas plant.	Pretreatment did not increase methane yield.	(RISBERG et al., 2013)
Mechanical - cylindrical briquettes (68 mm diameter). + Alkaline - NaOH and KOH (0.5, 1, 2, 4 or 8% w/w at ambient temperature for 24 h).	-	From a biogas plant.	The addition of alkali improved methane yield in all cases and mechanical pretreatment produced higher yields at all alkali concentrations tested. The best combination was briquetted wheat straw + NaOH 2%, which increased methane yield by 40%.	(MOSET et al., 2018)
i. Alkaline - KOH and $Ca(OH)_2$ (1, 2, 3, 4, and 5%, w/v); ii. Co-pretreatment (CP) - KOH (1, 2, and 3% w/v) were mixed with $Ca(OH)_2$ (1, 2, and 3% w/v) at 25 °C for 24 h.	-	From a biogas plant.	KOH 2% + $Ca(OH)_2$ 1% was the best tested condition, with methane yield of 239.8 mL/gVS, 17% higher than observed for untreated WS.	(SHEN et al., 2019)
Thermal - 120, 140, 160 and 180 °C for 60 min.	-	Digested manure.	Highest biogas yield of 615 NmL/gVS (53% higher than untreated) for wheat straw (WS) pretreated at 180°C.	(RAJPUT; ZESHAN; VISVANATHAN, 2018)
Alkaline - NaOH 4% (w/w) at 37 °C for 120 h;	-	95.70% moisture + 4.30% of total solids content on weight basis of wet biomass.	87.5% increase in biogas (353.2 L/kg VS_a) and 111.6% in methane production (165.9 L/kg VS_a) to WS pretreated with NaOH.	(CHANDRA et al., 2012)

(conclusion)

Pretreatment	Co-substrate	Inoculum	Findings	Reference
i. Organic solvent N-methylmorpholine N-oxide (NMMO) at 120 °C for 3 h; ii. Organosolv method - ethanol at 180 °C for 1 h; iii. Alkaline pretreatment - NaOH at 30 °C for 24 h.	-	Digestate from a full-scale AD plant.	Methane production increased by 11% for NMMO pretreatment and by 15% for both organosolv and alkaline pretreatment.	(MANCINI et al., 2018)
i. Dilute acid pretreatment - H_2SO_4 2% w/v; steam temperature of 121 °C for 30 min, with steam gauge pressure of 100 kPa. ii. Alkali pretreatment - NaOH 2% for 72 h at ambient temperature; iii. Physical pretreatment - 2 and 3 mm of particle size; iv. $Ca(OH)_2$ 3% w/v + Na_2CO_3 3% w/v for 48 h at ambient temperature; v. H_2SO_4 2% w/v + NaOH 2% w/v with steam temperature of 121 °C and working gauge pressure of 100 kPa for 30 min.	Cattle manure	Digested slurry from a cattle manure biogas plant.	Pretreatments ii and iv improved biogas and methane yields by 94% and 99%, respectively, in comparison to untreated WS. Cumulative methane yields of 0.191 ±0.004, 0.125±0.002, 0.370± 0.02, 0.003±0.005, 0.380±0.017 and 0.241±0.005 m^3/kg VS, respectively, were found for untreated and for i, ii, iii, iv and v pretreatments of WS.	(KRISHANIA; VIJAY; CHANDRA, 2013)
Alkaline - $Ca(OH)_2$ 0%, 20%, 40%, and 60% (w/w) for 8 h at ambient temperature.	-	From a biogas plant (cow manure at mesophilic temperatures).	The biogas yield for WS pretreated with 0%, 20%, 40% and 60% $Ca(OH)_2$ was 412, 450, 491 and 518 mL/g VS, respectively, which represents an increase ranging from 14 to 42%, compared to untreated WS.	(KUMAR et al., 2019)
Alkaline - KOH 1%, 3%, 6% and 9% at ambient temperature.	-	-	Biogas production of WS pretreated with KOH 6% was 45% higher than untreated one.	(JAFFAR et al., 2016)

The literature shows that chemical (especially with the addition of alkali) and thermal pretreatments are among the most efficient for increasing the bioavailability of wheat straw, with cases in which the increase in biogas production reached close to 100% (KRISHANIA; VIJAY; CHANDRA, 2013). This research attempts to evaluate the feasibility of the AcoD process between protein-rich wastes - shavings and sludge from the leather industry - and a carbohydrate-rich waste - straw from wheat cultivation, to maintain a more balanced C/N ratio and increase the stability of the process. Until this moment, studies involving AcoD between these residues were not found. This work also aimed to compare the effects of different pretreatment techniques (chemical and thermal) on the lignocellulosic composition of wheat straw and the methane yields from its subsequent AcoD.

7.2 Materials and methods

7.2.1 Substrates and inoculum

The leather shavings and the sludge were obtained from tannery factories that use chromium salts as tanning agent and vegetable tannins in the retanning step located in Rio Grande do Sul (Brazil). Sludge was collected after the primary treatment stage (mainly anaerobic) of a wastewater treatment plant (WWTP) of a tannery that perform the entire final leather processing, and also acted as inoculum. Wheat (*Triticum aestivum*) straw (WS) was harvested from agricultural fields in the north of Rio Grande do Sul (Brazil).

7.2.1.1 Pretreatments of wheat straw

Before use, the whole wheat straw sample was subjected to a constant mechanical pretreatment by being cut into pieces smaller than 5 mm.

Five different pretreatment methods were conducted in this study: (i) acid, (ii) alkaline, (iii) thermal, (iv) acid + thermal, and (v) alkaline + thermal. The chosen pretreatment conditions were based on previous studies performed on lignocellulosic materials (GOMEZ-TOVAR et al., 2012; MANCINI et al., 2018). Table 7.2 describes experiments.

Table 7.2. Design of anaerobic digestion bioreactors.

Bioreactor	**Substrate 1 (25 mL)**	**Substrate 2 (1 g, db)**	**Substrate 3 (1 g, db)**
SLU + SHA	Chromium tanning sludge (*inoculum*) (SLU)	Chromium tanned leather shavings (SHA)	---
SLU + SHA + UWS	Chromium tanning sludge (*inoculum*) (SLU)	Chromium tanned leather shavings (SHA)	Untreated wheat straw (UWS)
SLU + SHA + WS-HCl	Chromium tanning sludge (*inoculum*) (SLU)	Chromium tanned leather shavings (SHA)	Wheat straw – acid pretreatment (WS-HCl)
SLU + SHA + WS-NaOH	Chromium tanning sludge (*inoculum*) (SLU)	Chromium tanned leather shavings (SHA)	Wheat straw – alkaline pretreatment (WS-NaOH)
SLU + SHA + WS-THE	Chromium tanning sludge (*inoculum*) (SLU)	Chromium tanned leather shavings (SHA)	Wheat straw – thermal pretreatment (WS-THE)
SLU + SHA + WS-HCl/THE	Chromium tanning sludge (*inoculum*) (SLU)	Chromium tanned leather shavings (SHA)	Wheat straw – acid + thermal pretreatment (WS-HCl/THE)
SLU + SHA + WS-NaOH/THE	Chromium tanning sludge (*inoculum*) (SLU)	Chromium tanned leather shavings (SHA)	Wheat straw – alkaline + thermal pretreatment (WS-NaOH/THE)

Acid hydrolysis was carried out in 10 g of wheat straw that was suspended in 0.1 L of 2% HCl at 90 °C for 2 h. Alkaline pretreatment was carried in 10 g of wheat straw that was suspended in 0.1 L of 1.6% NaOH at 30 °C for 24 h. Thermal pretreatment was carried out by autoclaving the straw at 121 °C (1 atm) for 15 min. Acid + thermal pretreatment was carried in 10 g of straw immersed in 0.1 L of HCl (2%) and autoclaved at 121 °C (1 atm) for 15 minutes. Alkaline + thermal pretreatment was carried in 10 g of straw immersed in 0.1 L of NaOH (1.6%) and autoclaved at 121 °C (1 atm) for 15 minutes. In cases involving acid or alkaline solution, the liquid fraction was filtered after pretreatment, and the straw was washed with distilled water until neutral pH (7.0). Afterwards it was dried at 60 °C and stored.

7.2.2 Biodigestion assays

Biodigestion assays were performed in 300 mL hermetically sealed bench scale bioreactors, built in cylindrical glasses, containing an upper valve, to measure the volume of biogas produced, a lateral screw valve with septum, for the collection of samples for chromatographic analysis of the composition of biogas and a lower thread septum valve for waste sample collection. The composition in each bioreactor is presented in Table 7.2. The ratio of the quantity of sludge/shavings of 25 mL/1 g was stipulated by local research carried out with the tanneries of the region, based on the proportion of generation of such wastes (AGUSTINI et al., 2018a). 200 ml of nutrient solution (2 g/L of yeast extract, 7 g/L of K_2HPO_4, 3 g/L of KH_2PO_4) was employed to ensure favorable conditions for the growth and metabolism of the microorganisms (SIMIONI et al., 2020). The bioreactors were maintained at 35 °C (mesophilic temperature) in a bacteriological incubator (DL-CBE18L, DeLeo, Brazil), and they were not shaken during the experiment to prevent the transport of oxygen into the medium and prevent the biomass flake from breaking. The bioreactor was operated for 274 days and, after this period, the precipitated and dissolved samples were collected separately and stored (4 °C) for further analysis. To analyze the composition of the biomass before biodegradation, the same tests were carried out in closed bottles, shaken for 1 h at room temperature (Wagner shaker, ACB Labor, Brazil), and stored for 24 h at 4 °C for sedimentation before opening and the collection of liquid and solid samples. All tests were performed in duplicate.

7.2.3 Biogas measurements and modeling

Biogas volumetric production was measured every 2 days by water displacement with a device based on the Mariotte principle (PRIEBE et al., 2016; SIMIONI et al., 2020). The proportion of biogas components was accessed weekly through a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a ShinCarbon column (ST 100/120 2m 1 mmID 1/16” OD Silco) and TCD detector, with helium as the carrier gas at a flow rate of 10 mL/min.

The biogas production data determined by the biodigestion assays were adjusted according to the modified Gompertz model, shown in Eq. 7.1 (ZWIETERING et al., 1990).

$$P_t = P_m \times exp\left\{-exp\left[\frac{\mu_m \times e}{P_m}(\lambda - t) + 1\right]\right\} \tag{7.1}$$

Where P_t is the cumulative biogas production (mL/gVSS) at digestion time t, P_m is the maximum biogas potential (mL/gVSS), μ_m is the maximum biogas production rate (mL/gVSS day), λ is the lag-phase time (day), and e is the Euler´s number taken as 2.71828. μ_m was obtained from the slope of the line during the exponential phase of biogas production, and λ is the x-axis intercept of this slope (RAUT; PANDHAL; WRIGHT, 2021). The coefficient of correlation (R^2) between observed and predicted biogas production was determined to evaluate the model fit.

7.2.4 Analytical parameters

7.2.4.1 Analysis of the efficiency of pretreatment of wheat straw

Fourier transform infrared (FTIR) spectrophotometer (FT-IR Frontier, PerkinElmer, USA) was used to analyze changes in the functional groups of wheat straw before and after pretreatments. The spectra were formed from 4000 to 500 cm^{-1} with 32 times scanning. X-ray diffraction (XRD) analyses of pretreated and untreated wheat straw were performed in a diffractometer (D2 Phaser, Bruker, USA), at an angle of $2\theta = 10° - 40°$. The determination of crystallinity index (CrI) was based on the XRD data and computed with the help of Eq. 7.2, provided by Segal *et al.* (1959).

$$CrI(\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100 \tag{7.2}$$

In which, I_{002} (the crystallinity fraction) is the intensity of the 002 peak (at about $2\theta = 22$) and I_{am} (the amorphous fraction) is the intensity of peak at $2\theta = 18$.

Lignin, hemicellulose and cellulose contents of wheat straw before and after each pretreatment were measured, in duplicate, based on chemical method (LI et al., 2004; RAJPUT; ZESHAN; VISVANATHAN, 2018). First, the amount of extractives (W_1) was determined by solvent extraction, using 60 mL of acetone for each gram of dried wheat straw sample (G_0) at a constant temperature of 90 °C for 2 h. After that, biomass sample was oven dried at 105 °C until constant weight was achieved (G_1). For determining hemicellulose content (W_2), 150 mL of NaOH 20 g/L was added to 1 g of extractive-free dried biomass (G_1). The mixture was boiled for 3.5 h with recycled distilled water, filtered and washed until the value of pH approached 7. The sample was then dried to constant weight, cooled to room temperature in a desiccator and weighted (G_2). Lignin (W_3) was determined by using 30 mL of H_2SO_4 72% for each gram of extractive-free dried biomass sample (G_3). The mixture was kept at 8 – 15 °C for 24 h, diluted with 300 mL of distilled water and boiled for 1 h with recycled distilled water. The mixture was then filtered, and wheat straw sample was washed until there was no more sulfate ion in the filtrate (detected by 10% barium chloride solution), dried to constant weight, cooled to room temperature in a desiccator and weighted (G_4). W_1 (%, db), W_2 (%, db) and W_3 (%, db) were calculated according to Eq. 7.3, 7.4 and 7.5, respectively. Cellulose content W_4 (%, db) was calculated by difference, considering that extractives, lignin, hemicellulose and cellulose are the only components of biomass (Eq. 7.6). All analyzes were performed in duplicate.

$$W_1(\%, db) = \frac{G_0 - G_1}{G_0} \times 100\% \tag{7.3}$$

$$W_2(\%, db) = \frac{G_1 - G_2}{G_0} \times 100\% \tag{7.4}$$

$$W_3(\%, db) = \frac{G_4(1 - W_1)}{G_3} \times 100\% \tag{7.5}$$

$$W_4(\%, db) = 100 - (W_1 + W_2 + W_3) \tag{7.6}$$

7.2.4.2 Waste biodegradation analysis

The wastes were characterized according to the following techniques. Gravimetric method was used to determine volatile solids (VS), using analytical balance and a muffle furnace. The determination of total organic carbon (TOC) and inorganic carbon (IC) were performed in a total organic carbon analyzer: TOC-VCSH (Shimadzu, Japan) for the liquid samples and SSM-500A (Shimadzu, Japan), for the solids. Total Kjeldahl nitrogen (TKN) analysis was performed according to ASTM D3590-11, for liquid samples, and according to adapted D2868-10, for the solid samples. Vegetable tannin concentrations were measured in a UV/VIS Spectrometer (PG Instruments Ltd, United Kingdom) at 278 nm with a calibration curve prepared with standard solutions of tannin of acacia. Total phenols were estimated as tannic acid equivalents, according to the Folin–Ciocalteau assay (MIRON et al., 2011). Chromium (III) oxide concentrations were determined with ABNT NBR13341 method for residual bath; for the shavings, 1 g of the waste was previously dissolved in HCL 50% until total disintegration. pH was determined with a DM-22 pH-meter (Digimed, Brazil). For pH measurement of solid wastes, 2.5 g of shavings or wheat straw were stirred for 24 h in 50 mL of distilled water.

The biodegradation of the wastes was evaluated through analysis of volatile suspended solids (VSS) and volatile dissolved solids (VDS), TOC-S (TOC of the suspended solids), TOC-

L (TOC of liquid portion), IC and TKN. All analyzes were performed before and after AD. Volatile fatty acids (VFAs) were determined in a High-Performance Liquid Chromatography (HPLC) (model 1200 Infinity, Agilent, USA), with automatic injector and diode array detector (DAD). Rezex RHM - Monosaccharide H^+ column (Phenomenex, USA), heated at 40 °C, using 5 mM sulfuric acid as the mobile phase at a flow rate of 0.6 mL/min was used. Formic, acetic, propionic and butyric acids were tested.

The percentage of carbon conversion was calculated through a mass balance, taking into account the initial total carbon (TC) and the carbon that left the system in the biogas (CH_4 and CO_2).

7.2.5 Statistical analytical

Statistical analysis was performed using Microsoft Excel (version 2010) software for Windows. Statistical significance was evaluated using one-way ANOVA analysis of variance with a Tukey *post hoc* test. Differences were accepted as significant when *p-value* < 0.05.

7.3 Results and discussion

7.3.1 Waste characterization

The initial characteristics of feedstock affect startup, process stability and biogas production during anaerobic digestion. Table 7.3 presents chromium, TKN, TOC, IC, C/N ratio, VS and pH of the sludge, leather shavings and raw wheat straw.

Table 7.3. Characterization of waste used in anaerobic digestion bioreactors.

	Chromium	TKN	TOC	IC	C/N ratio	VS (%)	pH
	(g/L) - sludge (% (g/g)) - shaving and wheat straw						
Sludge	0.31	1.21	11.27	1.89	10.88	34.42	7.45
Leather shavings	1.14%	2.95%	32.29%	0	10.95	90.24	4.09
Wheat straw		0.60%	41.32%	0	68.87	92.37	5.84

C/N ratios of individual substrates used in this study were either lesser or greater than that of optimum range of C/N ratio for AD (20-30) (KHALID et al., 2011). C/N ratio of wheat straw was around 69, which was comparatively higher than that of sludge (10.88) and leather shavings (10.95). The combination of this co-substrates according to the configuration presented in Table 7.2 provides a C/N ratio around 12.

Despite the recalcitrant characteristics of all the wastes and the C/N ratio outside the optimum range, the AD was established, proven by the production of methane for all cases tested (Table 7.6).

7.3.2 Effect of the pretreatments on the composition of wheat straw

This study evaluated the effect of different pretreatments applied to raw wheat straw concerning changes in chemical composition, crystallinity and functional groups.

7.3.2.1 Changes in main chemical composition

The cellulose, hemicellulose, and lignin contents of untreated and pretreated wheat straw are shown in Table 7.4. No correction for ash content was done.

Table 7.4. Effects of pretreatment on the chemical composition and on crystallinity index (CrI) of wheat straw. Tukey's *post hoc* test indicates significant differences ($p < 0.05$) in each column, represented by different letters.

	Extractives (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	CrI (%)
UWS	3.17 ± 0.17^{a}	49.32 ± 0.85^{a}	19.29 ± 0.09^{c}	28.22 ± 0.70^{a}	64.93
WS-HCl	0.99 ± 0.25^{b}	51.44 ± 0.13^{a}	16.43 ± 0.49^{c}	31.14 ± 0.34^{a}	58.42
WS-NaOH	2.11 ± 0.24^{b}	35.96 ± 1.09^{b}	43.67 ± 0.23^{b}	18.26 ± 1.35^{b}	72.49
WS-THE	0.90 ± 0.15^{b}	52.13 ± 0.02^{a}	19.71 ± 0.18^{c}	27.26 ± 0.04^{a}	64.83
WS-HCl/THE	1.57 ± 0.17^{b}	48.94 ± 0.11^{a}	17.78 ± 0.11^{c}	31.71 ± 0.29^{a}	73.08
WS-NaOH/THE	1.38 ± 0.12^{b}	30.54 ± 1.54^{b}	60.85 ± 0.85^{a}	7.23 ± 0.51^{c}	77.97

Both WS-NaOH and WS-NaOH/THE pretreatments seem to affect more the chemical composition of the wheat straw with a significant ($p < 0.05$) increase in cellulose content accomplished by the decrease in hemicellulose and in lignin content. This finding is in accordance with Rajput, Zeshan and Visvanathan (2018) and Shen *et al.* (2019). The alkali acts breaking the internal bonds between hemicellulose and lignin causing both hemicellulose dissolution and lignin removal (MANCINI et al., 2018; SHEN et al., 2019). Since lignin acts as a protective layer of cellulose, its removal makes the cellulose more accessible, which can improve the biodegradability of wheat straw (YAO; DAVID; DAVARITOUCHAEE, 2018).

The acid and thermal pretreatments, isolated and combined, did not show significant influence on the composition of wheat straw, in comparison with the untreated one.

7.3.2.2 Changes in cellulose crystallinity

X-ray diffraction spectra of untreated and pretreated WS were carried out to examine the change occurring in the crystalline nature of the WS. Crystallinity index (CrI) was also calculated to measure the relative amount of crystalline cellulose and it is shown in Table 7.4.

As can be seen, CrI decreased to WS-HCl due to the degradation of crystalline cellulose caused by the acid pretreatment. For other cases (WS-NaOH, WS-HCl/THE and WS-NaOH/THE), an increase of CrI was observed, which, according to literature (KUMAR et al., 2019), might be attributed to the dissolution of partial lignin and the removal of the hemicellulose and amorphous cellulose. The damage observed in lignin and hemicellulose structure might also be supported with the results of change in lignocellulosic composition, also shown in Table 7.4.

As crystalline material is relatively more resistant to chemical and biological biodegradation compared to amorphous material, in theory, the higher the CrI, the more difficult the biodegradation of cellulose becomes (RAJPUT; ZESHAN; VISVANATHAN, 2018; ZHENG et al., 2014). However, there are a number of studies in literature reporting an increase in CrI of lignocellulosic biomass after pretreatment and relating this to the increase in biogas production (RAJPUT; ZESHAN; VISVANATHAN, 2018; RAN et al., 2018; SHEN et al., 2019; YAO; DAVID; DAVARITOUCHAEE, 2018).

7.3.2.3 Changes in functional groups

Changes in chemical bonds of wheat straw due to pretreatment was accessed through FTIR spectra, as shown in Fig. 7.1.

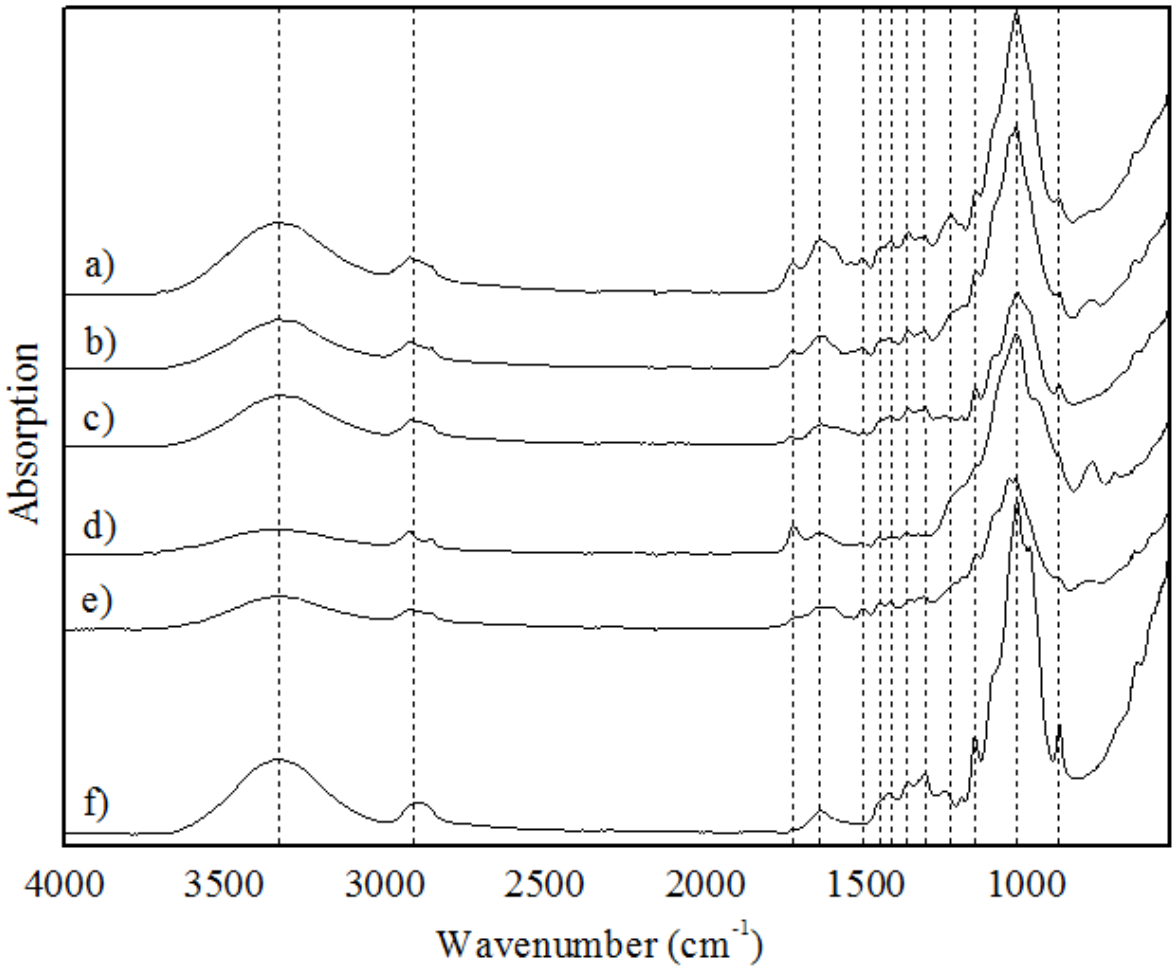

Fig. 7.1. FTIR spectra for (a) UWS, (b) WS-HCl, (c) WS-NaOH, (d) WS-THE, (e) WS-HCl/THE and (f) WS-NaOH/THE.

The infrared spectra showed characteristic bands of the functional groups of the lignocellulosic fibers components (cellulose, hemicelluloses and lignin) and are in agreement with that reported in the literature (KUMAR et al., 2019; RAJPUT; ZESHAN; VISVANATHAN, 2018; YAO; DAVID; DAVARITOUCHAEE, 2018). Table 7.5 shows the description of the functional groups corresponding to each band (COLOM et al., 2003; KUMAR et al., 2019; MORÁN et al., 2008; TAHERDANAK; ZILOUEI, 2014; YANG et al., 2007; YAO; DAVID; DAVARITOUCHAEE, 2018), as well as the pretreatments applied to the wheat straw in an increasing order of band weakness.

Table 7.5. Assignments of peaks in FTIR spectra of untreated and pretreated wheat straw (COLOM et al., 2003; KUMAR et al., 2019; MORÁN et al., 2008; TAHERDANAK; ZILOUEI, 2014; YANG et al., 2007; YAO; DAVID; DAVARITOUCHAEE, 2018).

(continue)

Wavenumber (cm^{-1})	Functional group	Compound	Fiber component	Pretreatment
3330	O–H	Acid; methanol	Cellulose; hemicellulose; lignin	WS-NaOH; WS-HCl; WS-HCl/THE; WS-THE
2920	C–H_n stretching	Alkyl; aliphatic; aromatic	Cellulose; hemicellulose; lignin	WS-NaOH; WS-HCl; WS-HCl/THE; WS-THE
1730	C=O	Acetyl; carboxylic acid; ester between lignin and hemicellulose	Hemicellulose; lignin	WS-THE; WS-HCl; WS-NaOH; WS-HCl/THE; WS-NaOH/THE
1640	C=C	Stretching of aromatic ring	Lignin	WS-NaOH/THE; WS-NaOH; WS-HCl/THE; WS-THE; WS-HCl
1510	C=C	Stretching of aromatic ring	Lignin	WS-HCl/THE; WS-NaOH; WS-NaOH/THE; WS-THE; WS-HCl
1455	C–H	Asymmetric bending in CH_3	Lignin	WS-NaOH/THE; WS-NaOH; WS-HCl/THE; WS-THE; WS-HCl
1425	C–H_2	C–H_2 symmetric banding	Cellulose	WS-NaOH/THE; WS-NaOH; WS-HCl/THE; WS-THE; WS-HCl
1370	Aliphatic C–H deformation vibration or C–H bending		Cellulose	WS-NaOH/THE; WS-NaOH; WS-HCl/THE; WS-THE; WS-HCl

(conclusion)

Wavenumber (cm^{-1})	Functional group	Compound	Fiber component	Pretreatment
1318	C–H_2 wagging		Cellulose	WS-NaOH; WS-HCl/THE; WS-HCl; WS-HCl/THE
1234	Syringyl ring breathing and C-O stretching out of lignin and xylene		Lignin	Not identified in pretreated wheat straw
1158	C–O–C asymmetric stretching		Cellulose	WS-THE; WS-HCl; WS-HCl/THE; WS-NaOH
1031	C–O stretching	C–OH (ethanol)	Cellulose and Hemicellulose	WS-THE; WS-HCl; WS-NaOH; WS-HCl/THE
899	Asymmetric out of phase ring stretching	Acetyl groups	Cellulose	WS-HCl; WS-NaOH; WS-HCl/THE

FTIR spectra (Fig. 7.1) in the present study shows that the absorption peaks in all the above-mentioned regions reduced in some level for all of the pretreatments applied to wheat straw, with emphasis on WS-NaOH and WS-HCl/THE pretreatments, which implies that the lignocellulosic structure of wheat straw got disrupted. Thus, protective layer (hemicellulose-lignin) of cellulose was broken by these pretreatments which changed the chemical structure of wheat straw as compared to raw material.

7.3.3 Effect of pretreatment on biogas production and waste treatment

7.3.3.1 Biogas production

The cumulative biogas generation per gram of VSS added is shown in Fig. 7.2, for all the tests. To validate the modified Gompertz model, the predicted cumulative biogas production curve for SLU + SHA + WS-HCl was plotted against the experimental data obtained in the biodegradation assays (Fig. 7.2). The cumulative biogas and methane generation per gram of VSS added, the maximum yield of methane and the respective day of biodegradation on which it occurred for each experimental condition tested are shown in Table 7.6. Statistical comparison of biogas and methane volumes produced per gram of VSS added is also presented in Table 7.6.

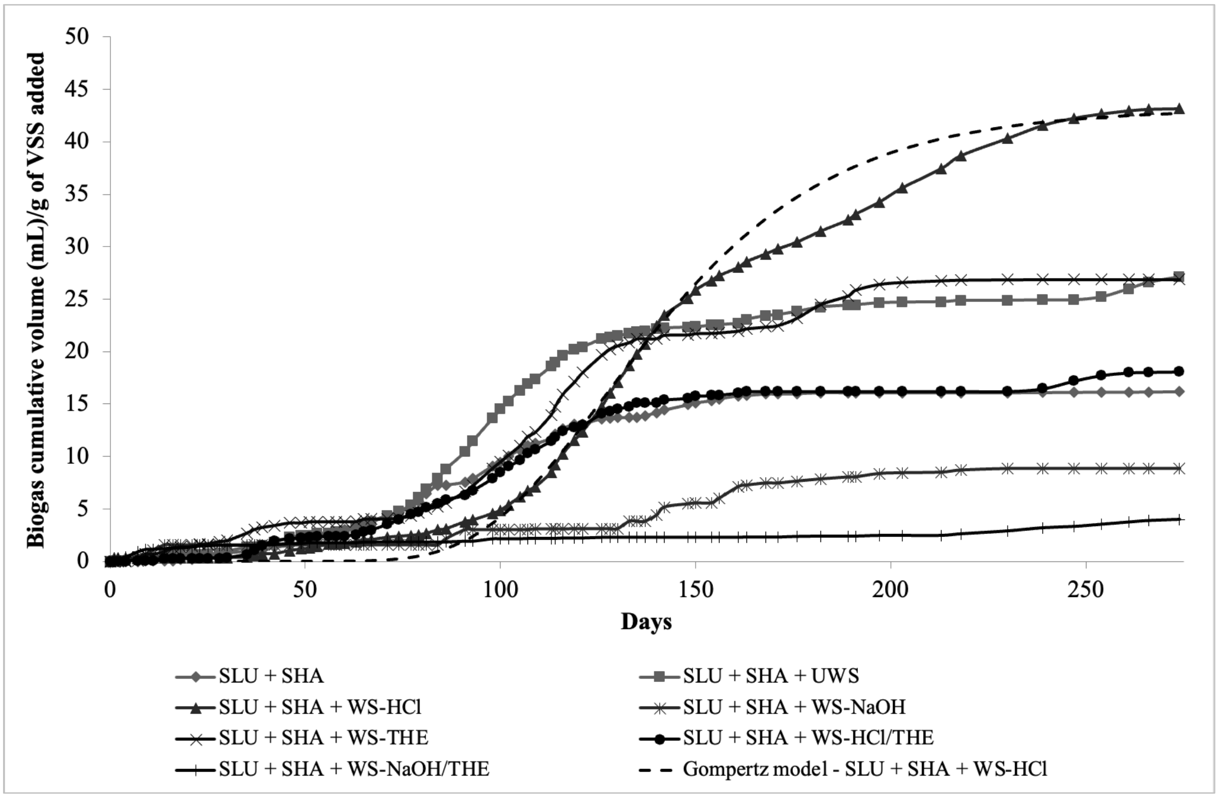

Fig. 7.2. Biogas cumulative production per gram of VSS added in the bioreactors.

Table 7.6. Average biogas and methane cumulative production per gram of VSS added and maximum methane yield during biodegradation assays. For each column, different letters a,b, and c represent significantly different values by Tukey's test ($p < 0.05$).

Bioreactor	Biogas cumulative volume/VSS added (mL/g)	Methane cumulative volume/VSS added (mL/g)	Maximum yield of methane (%)	Day of maximum yield of methane
SLU + SHA	$16.17^{b,c}$	$3.12^{b,c}$	38.05	161
SLU + SHA + UWS	$27.12^{a,b}$	$5.04^{a,b}$	40.61	191
SLU + SHA + WS-HCl	43.15^{a}	10.06^{a}	34.45	269
SLU + SHA + WS-NaOH	8.84^{c}	$1.56^{b,c}$	35.71	203
SLU + SHA + WS-THE	$26.87^{a,b}$	$4.71^{a,b,c}$	39.84	269
SLU + SHA + WS-HCl/THE	$18.08^{b,c}$	$4.10^{b,c}$	38.89	269
SLU + SHA + WS-NaOH/THE	4.01^{c}	0.14^{c}	7.71	266

As can be seen in Fig. 7.2 and confirmed by ANOVA results presented in Table 7.6, the addition of wheat straw in the co-digestion of residues of the leather industry affected significantly ($p < 0.05$) the production of biogas and methane, as well as the pretreatments applied to straw in all analyzed conditions. The highest cumulative biogas yield of 43.15 mL/gVSS was obtained for the wheat straw pretreated with HCl (SLU + SHA + WS-HCl), which was 59% higher than that of untreated wheat straw (SLU + SHA + UWS) and almost 167% higher than that without wheat straw (SLU + SHA). The increase in biogas production is related to the decrease in cellulose crystallinity - represented by CrI - caused by acid pretreatment (Table 7.4 and section 7.3.2.2), which made hemicellulose and amorphous cellulose, naturally more easily degradable, accessible to microbial attack (ABRAHAM et al., 2020; HASSAN et al., 2017; MONLAU et al., 2013; RAJPUT; ZESHAN; VISVANATHAN, 2018). The cumulative yield of biogas obtained from the modified Gompertz model was 42.71 mL/gVSS. An adequate fit was observed between the modeled and experimental data, with coefficient of determination (R^2) equal to 0.997, implying that the Gompertz model could simulate the biogas production for SLU + SHA + WS-HCl.

The lag-phase of the cumulative biogas production curve was approximately 60 days for SLU + SHA, SLU + SHA + UWS, SLU + SHA + WS-THE and SLU + SHA + WS-

HCl/THE, and even longer, approximately 80 days, for SLU + SHA + WS-HCl, showing that the recalcitrance and complexity of the residues caused the hydrolysis phase to take longer to complete and that even with pretreatments, the biodegradable organic matter in the substrate was not readily available for microbial decomposition, since the residues of the leather industry are also recalcitrant. Similarly, the log-phase was also long, beginning at day 60 of incubation and ending around day 130 for SLU + SHA, SLU + SHA + UWS and SLU + SHA + WS-HCl/THE. Among these cases, SLU + SHA + UWS had the highest slope of the curve, followed by SLU + SHA + WS-HCl/THE and SLU + SHA, again due to the complexity of the residues, so that the microorganisms could not quickly metabolize the waste. A plateau during biogas production, also known as diauxie, was verified for SLU + SHA + WS-HCl and for SLU + SHA + WS-THE. This behavior could be related to the accumulation of VFAs, indicating an imbalance between the early stages of AD and methanogenesis, as can be seen in Fig. S7.1 (Supplementary material). The methanogenic archaea are not able to process all the intermediate products so fast which then start to accumulate. The high concentration of these acids reduces the pH of the medium which causes the inhibition of methane production and so, the production of biogas falls. Biogas production continues when the volatile fatty acid concentration drops (GOMES; REPKE; MEYER, 2019; KIM; KIM, 2017).

For SLU + SHA + WS-NaOH, the log phase started on the 130th day of incubation and ended on the 160th day, having a little slope and culminating in a small production of biogas. SLU + SHA + WS-NaOH/THE did not present the characteristic curve as observed for the other cases and had the lowest biogas production (4.01 mL/gVSS).

The bioreactors without the addition of wheat straw produced on average 16.17 mL of cumulative biogas/g of VSS added (12.57 g) with a maximum percentage of 38% of methane at the end of the log-phase. The biogas production and methane yield obtained in this work were lower than that reported in other studies carried out with equivalent wastes, which produced up to 30 mL of biogas/g of VSS, with a methane percentage of 60% (AGUSTINI; DA COSTA; GUTTERRES, 2018; SIMIONI et al., 2020). The sludge used here seems to have a different composition of those used in the reported works. In addition to a higher concentration of chromium, the sludge used in this study also contains vegetable tannins (7.83 g/L) and phenols (0.96 g/L), which have been reported as toxic to the final stages of AD in previous studies (AGUSTINI et al., 2018a, 2018b) and which may have partially inhibited the AD of the residues.

Unlike what is reported in the literature and presented in Table 7.1, the addition of alkaline pretreated wheat straw (SLU + SHA + WS-HCl/THE and SLU + SHA + WS-NaOH/THE) resulted in the lowest biogas yields, 8.84 and 4.01 mL of cumulative biogas/g of VSS added with a maximum methane yield of 35.71 and 7.71%, respectively. This goes against to the expectancy, since the lignin removal was higher for the cases in which pretreatment involved NaOH and, as mentioned in the literature (CHANDRA et al., 2012; MANCINI et al., 2018), lignin hampers the accessibility of carbohydrates for enzymatic hydrolysis, leading to a low cellulose and hemicellulose degradation and thus a lower biogas production yield. Since the lignin content in wheat straw pretreated with alkali was significantly lower ($p < 0.05$) (Table 7.4) than that of untreated wheat straw, hydrolysis may not be a limiting step for the degradation of this case, and the lower biogas production and methane yield observed can be attributed to the aromatic compounds released from lignin degradation, and to the presence of sodium ions remaining from the straw pretreatment (AKBAY; DIZGE; KUMBUR, 2021; FANG; BOE; ANGELIDAKI, 2011; HIERHOLTZER; AKUNNA, 2012). Sodium is a known process inhibitor in anaerobic systems (FJØRTOFT et al., 2019; SHETTY et al., 2017), whose effect can be potentiated by the presence of ammonia (in this case due to the nature of leather wastes), impacting on methanogens through an increase of osmotic pressure or complete dehydration of microorganisms. This hypothesis is supported since Hierholtzer and Akunna (2012) found that relatively low sodium levels (0.2 mol/L) can bring about significant levels of process inhibition in the presence of high levels of ammoniacal nitrogen.

7.3.3.2 Waste treatment

Waste analyses, expressed by the parameters VSS, VDS, TOC-L, TOC-S, IC and TKN, before and after biodegradation assays, are shown in Fig. 7.3. The horizontal bars mean no significant difference between ($p > 0.05$) the beginning and the end of biodegradation for each individual assay.

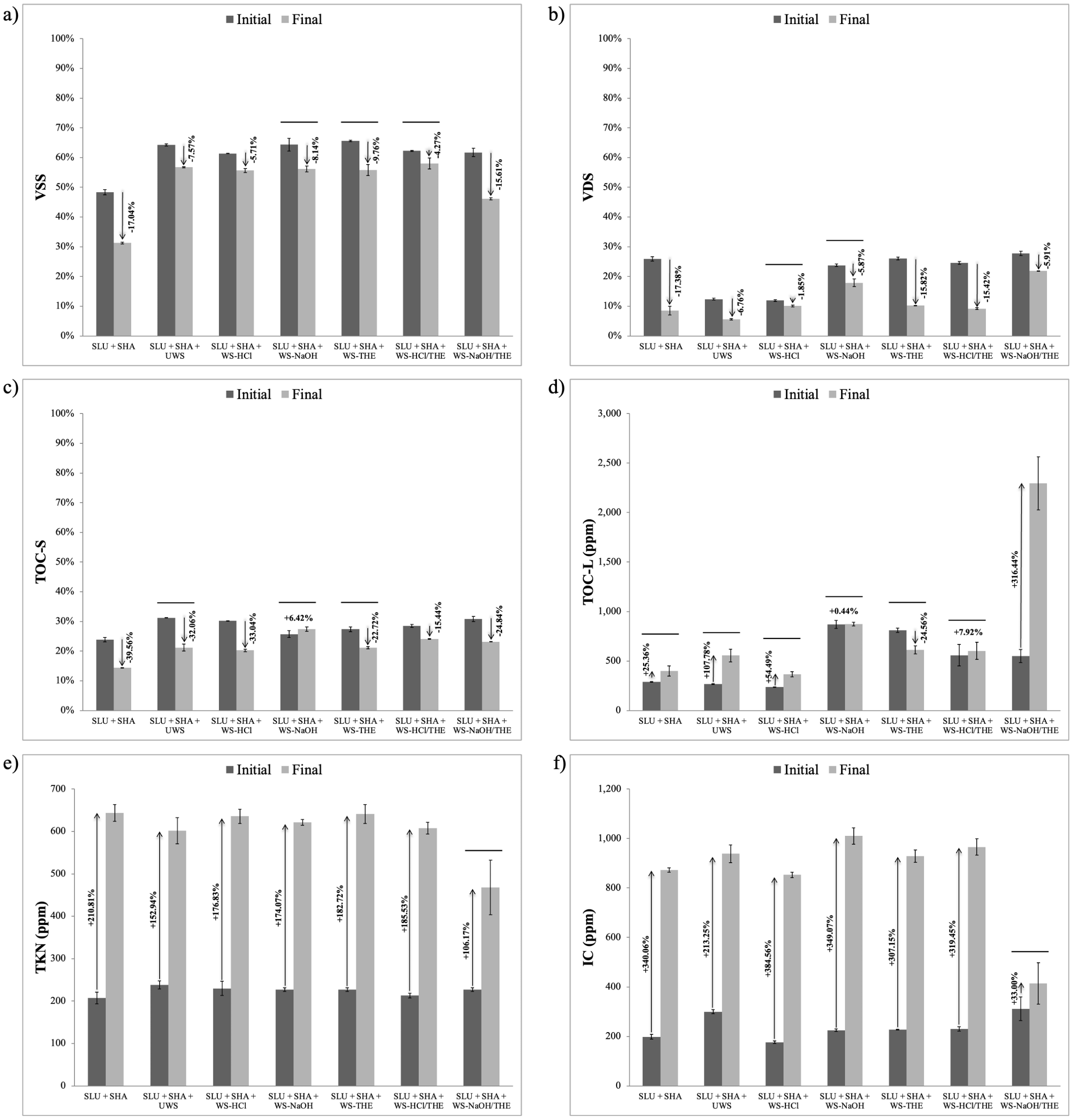

Fig. 7.3. Waste analyses before and after biodegradation assays. The horizontal bars mean no significant difference ($p > 0.05$) between the beginning and the end of biodegradation for each assay.

The volatile solids represent the organic matter dissolved (VDS) and suspended (VSS) in the system, giving an idea of the migration of the organic matter initially complex and precipitated in the residues, which underwent hydrolysis to the point of lowering the molar mass and being able to solubilize in the medium, to be metabolized by microorganisms. The variation of VSS and VDS throughout the assays is shown in Fig. 7.3 (a-b). Significant reduction ($p < 0.05$) in VSS was observed for SLU + SHA, SLU + SHA + UWS, SLU + SHA + WS-HCl, and

SLU + SHA + WS-NaOH/THE. For VDS, only SLU + SHA + WS-HCl and SLU + SHA + WS-NaOH did not show significant reductions ($p > 0.05$).

Similar to what occurs for volatile solids, the balance between TOC-S and TOC-L is related to the migration of organic carbon, initially precipitated in the waste and which, through AD, undergoes hydrolysis and solubilizes in the medium to be metabolized by microorganisms. The variation of TOC-S and TOC-L over the tests is shown in Fig. 7.3 (c-d). SLU + SHA, SLU + SHA + WS-HCl, SLU + SHA + WS-HCl/THE and SLU + SHA + WS-NaOH/THE showed significant reductions ($p < 0.05$) in TOC-S, which is in accordance with the theory previously presented. For TOC-L, only SLU + SHA + WS-NaOH/THE showed a significant increase ($p < 0.05$) which proves that the suspended carbon was hydrolyzed and dissolved in the medium, but also indicates that the following steps (acidogenesis, acetogenesis and methanogenesis) were partially inhibited.

The concentrations of TKN (Fig. 7.3e) and IC (Fig. 7.3f) increased significantly for all tests, excepting SLU + SHA + WS-NaOH/THE. This behavior is expected in cases witch AD was established, since it is characteristic of the metabolization and mineralization of the waste (AGUSTINI; DA COSTA; GUTTERRES, 2020; SIMIONI et al., 2020).

Table 7.7 shows the C/N ratio before and after biodegradation assays for each combination of wastes.

Table 7.7. C/N ratios before and after biodegradation assays.

Assay	Initial C/N ratio	Final C/N ratio	Variation (%)
SLU + SHA	7,59 ± 0,84	2,91 ± 0,06	-61,64%
SLU + SHA + UWS	8,28 ± 0,81	4,11 ± 0,58	-50,38%
SLU + SHA + WS-HCl	7,82 ± 1,18	4,05 ± 0,84	-48,17%
SLU + SHA + WS-NaOH	9,84 ± 0,40	4,71 ± 0,22	-52,19%
SLU + SHA + WS-THE	9,95 ± 0,51	3,87 ± 0,11	-61,09%
SLU + SHA + WS-HCl/THE	9,75 ± 1,65	4,11 ± 0,16	-57,84%
SLU + SHA + WS-NaOH/THE	9,86 ± 0,54	8,41 ± 1,50	-14,74%

Although the C/N ratios observed for all cases are below the optimal ratio recommended for the process, the AD was established. The reduction in the C/N ratio observed after AD is characteristic of the process, since the C initially present in the wastes is converted into CH_4

and CO_2, and the N concentration increases as a result of the reduction in the volume of the system.

7.3.3.3 Carbon balance

A balance that involves TC (TOC + IC) was carried out for the mixtures in bioreactors in order to compare the amount of initial carbon with the amount of carbon that was converted to methane or carbon dioxide. The results are detailed in Table 7.8.

Table 7.8. Carbon-based mass balance.

Bioreactor	Initial C amount (g)	Initial C (moles)	C (moles) CH_4	C (moles) CO_2	C conversion
SLU + SHA	0.3486	0.0290	0.0017	0.0034	17.42%
SLU + SHA + UWS	0.7517	0.0626	0.0036	0.0123	25.43%
SLU + SHA + WS-HCl	0.6975	0.0581	0.0069	0.0208	47.70%
SLU + SHA + WS-NaOH	0.7599	0.0633	0.0012	0.0042	8.60%
SLU + SHA + WS-THE	0.7803	0.0650	0.0040	0.0124	25.26%
SLU + SHA + WS-HCl/THE	0.7475	0.0623	0.0028	0.0075	16.61%
SLU + SHA + WS-NaOH/THE	0.8103	0.0675	0.0001	0.0015	2.33%

The carbon balance confirmed that the AcoD process between the leather wastes and the wheat straw pretreated with HCl was more efficient, converting almost 48% of carbon from the waste to CH_4 and CO_2. Of all the converted carbon, 23% became CH_4 and 70% CO_2. Although it is a promising result, it is also an indication that some problems related to inhibition of methanogenesis need to be overcome to obtain a higher CH_4 yield.

7.4 Conclusion

This research evaluated the efficiency of the AcoD process of wastes from the leather industry and wheat cultivation, in addition to assessing the effects of different pretreatment techniques on the composition of wheat straw and in the biogas yields. Although the pretreatment involving NaOH seems to affect more the chemical composition of wheat straw, the biogas production for these cases was the lowest among all the conditions, which may be linked to the dehydration of microbial cells, caused by the release of aromatics compounds from lignin degradation and the presence of residual sodium ions. The biodigestion between leather waste and wheat straw pretreated with HCl was the most efficient, converting almost 48% of carbon from the waste into 43.15 mL/g of VSS with 23% of CH_4 and 70% of CO_2. The accumulated biogas yield for this case was 59% higher than that observed for untreated wheat straw and almost 167% higher than that observed without the addition of wheat straw. The waste treatment efficiency (reducing its organic load) was verified in all tested conditions, which in this study is as important as the production of biogas since the remaining residue (after the AD process) still needs to be managed and a more mineralized waste is environmentally inert for disposal or even liable to be used as a fertilizer in the field since it is rich in nitrogen.

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Supplementary material

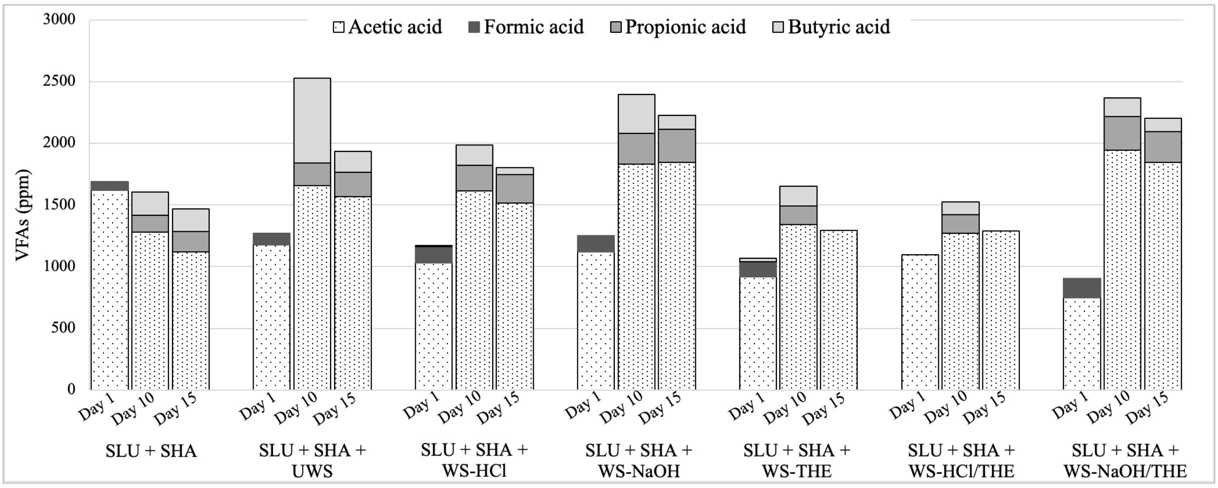

Fig. S7.1. VFAs concentration for the first 15 days of AD.

Capítulo 8

Use of tannery waste anaerobic digestate as agricultural fertilizer: an analysis of oat growth and soil fertility

Artigo submetido e em revisão em periódico.

Neste artigo, avaliou-se a o valor agronômico e o efeito fitotóxico do digestato líquido obtido a partir da co-DA mesofílica de resíduos de curtume em substituição aos fertilizantes minerais no que diz respeito ao crescimento de plantas de aveia e ao conteúdo de macronutrientes presentes no solo após a fertilização.

Abstract

Anaerobic digestion (AD) process produces a biologically stable and potential high-value fertilizer product, the digestate, which can be used as an alternative to mineral fertilizers on crops, completing biological nutrient cycles and contributing to a circular economy. This work evaluated the agronomic value and phytotoxic effect of the tannery waste liquid digestate (LD) by seed germination and oat growth bioassays. Its performance was compared to mineral fertilizers. Germination index (GI) close to 120% (compared to the control) was obtained for germination tests with 1% LD, indicating that this digestion solution contains nutrients and may have bio stimulating effects on plant development. The results of the oat growth bioassays confirmed these findings since plants grown in soil treated with LD reached statistically similar final mean height ($p > 0.05$) to plants grown in soil treated with NPK fertilizer or urea, and final dry weight significantly higher than the condition treated with NPK fertilizer ($p < 0.05$). The analysis of the soil before and after application of the treatments showed that the LD provided the soil with the same nutrients – nitrogen (N), phosphorus (P) and potassium (K) – as the other fertilizers tested and that the N added by all the treatments is essentially in inorganic form. The application of LD does not seem to have affected the pool of soil organic matter, nor did it cause the accumulation of metals in the soil. The digestate used in this work appeared to have a high degree of stability and can be used directly in soils as mineral fertilizer.

Keywords: anaerobic digestion; tannery waste; liquid digestate; phytotoxicity; inorganic fertilizer.

8.1 Introduction

The anaerobic digestion process successfully converts biowastes into two economically valuable by-products: a renewable energy source (biogas) and a potential fertilizer and soil amendment: the anaerobic digestate (NKOA, 2014). Agronomical use of anaerobic digestates is nowadays seen as an additional advantage of the biogas industry since considerable amounts of digestate are made available as fertilizer, thus facilitating organic waste disposal with other benefits to both companies and farmers (DRAGICEVIC et al., 2018). From an agronomic and economic point of view, digestate use as fertilizer can be considered not only as a supplement for traditional organic fertilizers but also as an alternative fertilizer, which under certain soil conditions, is more effective than using handled NPK fertilizer (BARŁÓG; HLISNIKOVSKÝ; KUNZOVÁ, 2019). AD is reported as a suitable treatment to quickly recover P and N fertilizers from digestate due to the large amount of carbon that leaves the system as biogas (CH_4 and CO_2) (AGUSTINI; DA COSTA; GUTTERRES, 2020a) and to organic matter and free organic nutrients that are biodegraded into mineral forms (JIMENEZ et al., 2020). Tannery waste digestate is a viable fertilizer to be investigated, as previous work has shown that it is a highly stable and mineralized product (AGUSTINI et al., 2018b; AGUSTINI; DA COSTA; GUTTERRES, 2018; SIMIONI et al., 2021), in addition to being a sustainable form of final destination for the digestate and contributing to the viability of the production chain of biogas.

The application of digestate as fertilizer does not only contributes to completing the biological nutrient cycle (ANGOURIA-TSOROCHIDOU; THOMSEN, 2021) by returning nutrients to agriculture (TAMPIO; ERVASTI; RINTALA, 2015) but also reduces the demand for mineral fertilizer, which are energetically intensives and economically expensive (CHEONG et al., 2020), promoting the development of more sustainable agriculture (PARRA-OROBIO et al., 2021) and contributing to a circular economy (CÂNDIDO et al., 2022). Nutrients from digested feedstock remain in the digestate after the digestion process and serve as the basis for plant growth. N, P, K, Ca, Mg, Na, and S are among the macronutrients usually present in the digestate (STÜRMER et al., 2020).

Digestate consists of water, mineral components, and not fully degraded organic compounds (STÜRMER et al., 2020). During AD, a high proportion of organic carbon is converted to methane, bringing about considerable changes in physical and chemical properties of digestate compared to the substrate fed into the bioreactor, which may affect the C and N flows in the soil-plant system. Generally, a reduction of the C/N ratio and an increase in the ratio of NH_4-N to total N and of pH value can be assumed, which may result in a higher short-term N fertilizer value (HERRMANN et al., 2017). On the other hand, by promoting organic matter degradation of easily decomposable organic components, AD leads to relatively high biological stability of the digestate, reducing or even nullifying the content of pathogens, ensuring hygienic products and avoiding pathogens ending up in the soil. Moreover, promoting organic matter mineralization increases nutrient availability for plants, reducing potential odor production (PIGOLI et al., 2021). Digestate biochemical composition and stability degree can vary greatly depending on the type of biomass inputs (feedstock) used for digestion/co-digestion and the configuration of the digester (ALBURQUERQUE; DE LA FUENTE; BERNAL, 2012; NKOA, 2014).

The use in agriculture avoids the need for treatment of the digestate (CHEONG et al., 2020), besides having a positive impact on soil microbial community composition and diversity, microbial respiration, and enzyme activities. Additionally, the application of anaerobic digestate in soils can have positive effects on their physical properties, such as reducing the apparent density, increasing the saturated hydraulic conductivity and improving the moisture retention capacity (PARRA-OROBIO et al., 2021). Digestate contains other organic elements which can also result in positive influences on plant growth and development (REN et al., 2020). In terms of rapidity of action (absorption of elements by plants), it resembles mineral fertilizers since N, P, and K elements are easily available for plants (KOSZEL; LORENCOWICZ, 2015) besides providing other essential plant macronutrients required for growth, health, and wellbeing of crop plants without detrimental effect on the soil (DAHUNSI et al., 2021).

Anaerobic digestate is usually separated into solid and liquid fractions (CHINI et al., 2021; TAYIBI et al., 2021). The solid fraction is rich in P and fiber (TAYIBI et al., 2021). It has more significant potential as a soil amendment (NKOA, 2014), being generally used as an organic additive (TAMBONE et al., 2017). The liquid fraction of the digestate has been indicated to substitute N-mineral fertilizers due to its high ammonia content (RIVA et al., 2016). Moreover, because of the repartition of N and P in solid and liquid fractions, the N/P ratio tends to increase in the liquid fraction, offering a more balanced nutrient content for crops and reducing P accumulation in the soil (TAMBONE et al., 2017).

Even though digestates are a relatively new type of fertilizer, many research publications highlight its benefits for soil and plants (FERNÁNDEZ-RODRÍGUEZ et al., 2021; KOSZEL et al., 2018; MUSCOLO et al., 2017; ZILIO et al., 2021) and compare its effectiveness with that of mineral fertilizers (BARŁÓG; HLISNIKOVSKÝ; KUNZOVÁ, 2019; RIVA et al., 2016; TSACHIDOU et al., 2019; WALSH et al., 2012). No works were found in the literature using digestate obtained from the AD of tannery waste as fertilizer. In this light, this study aimed to examine the possibility of agricultural utilization of LD obtained from the mesophilic anaerobic co-digestion (AcoD) of tannery wastes (sludge and leather shavings) in replacement of mineral fertilizers, concerning oat growth and to the content of macroelements present in the soil after fertilization.

8.2 Materials and methods

8.2.1 Biogas digestate, commercial fertilizer, and soil

LD was obtained from assays of batch AcoD of tannery waste incubated at 35 °C in a semi-pilot scale with chromium tanned leather shavings and sludge from the wastewater treatment plant (WWTP) of a tannery (beamhouse + chromium tanning processing). The AcoD assays were performed by Agustini, Da Costa and Gutterres (2018) and the characterization of the digestate, performed by the same author, is shown in Table S8.1 (supplementary material). LD was sampled directly after AcoD (without post-treatments) and stored at a temperature < 4 °C to prevent any chemical or biological alteration.

NPK commercial fertilizer 3-21-21 (3% N, 21% P_2O_5 and 21% K_2O, besides 7% Ca, 5% S, 0.03% B, 0.05% Mn and 0.1% Zn) and commercial urea 27-0-0 (27% N, besides 4% Ca and 2% Mg) were also used as comparative treatments.

The soil used in the plant growth assays was collected in a depth of 10 cm in an agricultural farm at the north of Rio Grande do Sul (geo-coordinates 27°58’48”S, 52°15’36”O), Brazil, sieved (5 mesh) and mixed before use.

8.2.2 Potential phytotoxicity

To determine digestate maturity, the level of phytotoxicity was established through germination tests, using oat seeds. These sensitive, fast-growing, and easy-to-handle indicator species exposes phytotoxic substances through their harmful effects on seed germination and growth.

8.2.2.1 Seed germination bioassays

The seed germination tests were assessed in duplicate using Petri dishes (90 mm diameter), containing 2 filter papers (base and cover) (80 g/cm^2 weight and 205 μm thickness), which were moistened with 1 mL of liquid. LD, NPK fertilizer, and urea were tested at concentrations of 100% (pure), 10% and 1% (volume fraction), diluted in distilled water. A control test was also performed with distilled water only. Ten seeds per dish were sown and each experimental treatment was carried out in duplicate. The dishes were then transferred to a germination chamber under controlled conditions of temperature (20°C) and darkness for 5 days (ALBURQUERQUE et al., 2012). After this period, the number of germinated seeds was counted, the radicle lengths of these seeds measured and the germination index (GI) calculated as a percentage of the control (distilled water) as specified by Eq. 8.1, 8.2 and 8.3, according to methodology described by Zucconi *et al. apud* Selim, Zayed and Houssam (2012).

$$Seed\ germination\ (\%) = \frac{N^{\circ}\ of\ seeds\ germinated\ in\ treatment}{N^{\circ}\ of\ seeds\ germinated\ in\ control} \times 100 \tag{8.1}$$

$$Root\ elongation\ (\%) = \frac{Mean\ root\ length\ in\ treatment}{Mean\ root\ length\ in\ control} \times 100 \tag{8.2}$$

$$Germination\ index\ (\%) = \frac{Seed\ germination\ (\%) \times Root\ elongation\ (\%)}{100} \tag{8.3}$$

To assess the phytotoxicity levels, the results obtained were analyzed considering that: (i) GI ≤ 50% indicate a strong presence of phytotoxic substances, (ii) GI values between 50 and 80% indicate moderate presence of phytotoxic substances, (iii) GI ≥ 80% indicate a positive effect, and (iv) GI ≥ 120% evidence biostimulation effects (PARRA-OROBIO et al., 2021).

8.2.2.2 Plant growth bioassays

The agronomic value of tannery LD was evaluated by determining the growth parameters during an initial vegetative stage, using oat as the plant model. Plant experiments with oat seed were performed in plastic pots (36 x 15 cm) placed in a greenhouse (Department of Agronomy, Federal University of Rio Grande do Sul), according to OECD Standard 208 (OECD, 2006). Ambient temperature and relative humidity were recorded with a data logger placed out inside the greenhouse. The sensors were programmed to record readings every hour. Four configurations were tested, in duplicate:

- control: 1.5 kg of soil;
- treatment 1: 1.5 kg of soil + 150 mL of LD;
- treatment 2: 1.5 kg of soil + 7.36 g of NPK fertilizer dissolved in 150 mL of distilled water;
- treatment 3: 1.5 kg of soil + 0.82 g of urea dissolved in 150 mL of distilled water.

LD, NPK fertilizer, and urea were added manually and mixed thoroughly with the soil. 150 mL of LD used in the "treatment 1" experiments is equivalent to 0.22 g of N. From this value, the necessary amounts of NPK fertilizer and urea were determined (according to the concentrations informed by the manufacturers) so that the same amount of N was added in all cases. Also, the amount of commercial urea added is equivalent to 150 kg/ha, which corresponds to the technical indicators of the product. NPK fertilizer and urea, which are originally solids, were dissolved in distilled water so that the physical aspect of the treatment would not be a variable in the nutrient absorption process. Eight oat seeds were planted in each pot, separated by at least 2 cm and at a depth of approximately 3 cm, 5 days after adding the treatments to the soil. After seed germination, plant development was monitored daily for 21 days, counted after 50% of seedling emergence in the control group. Each pot was manually sub-irrigated every 48 h with approximately 200 mL of water. At the end of the experiment, each plant was harvested, and root material was removed. Plants were dried at 60 °C for 24 h and cooled to ambient temperature in a desiccator before the dry weight was measured.

The results were expressed as the mean height of the plants and the mean dry weight of the aerial plants considering each condition tested. The comparison of the impact of different forms of treatment applied concerning the control was statistically evaluated using analysis of variance ANOVA and Tukey's post-hoc test, with a 95% confidence level ($p < 0.05$).

8.2.3 Analytical methods for soil characterization

The parameters were determined in the soil (control and treatments 1, 2, and 3) before seed sowing and at the end of the experiment. The gravimetric method was used to determine volatile solids (VS), using an analytical balance model EEQ9003F-B (Edutec), a drying oven (DeLeo), a muffle furnace (model Q318M/Quimis), and porcelain crucibles B-47 (Chiarotti). The determination of total organic carbon (TOC) and inorganic carbon (IC) were performed in a total organic carbon analyzer SSM-500A Shimadzu. Total nitrogen (TN) analysis was measured in a total organic carbon analyzer (Shimadzu TOC-L) equipped with a total nitrogen measuring unit (Shimadzu TNM-L) and 8-port sampler (Shimadzu TOC-L). The samples were previously digested with H_2O_2, H_2SO_4, and a catalytic mixture (K_2SO_4 and $CuSO_4.5H_2O$), and heated in a Velp Scientifica DK20 digester block (2 hours at 70 °C + 1.5 hours at 150 °C + 4 hours at 330°C) (BREMNER; MULVANEY, 1982). The inorganic nitrogen (IN) was extracted with a 1M KCl (1:10, w:v) solution (VALENTINUZZI et al., 2020) and determined in the same equipment used for TN. The following elements were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent Technologies 5110): Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, and Zn, after digestion of the samples according to EPA Method 3050B. The extraction of P and K available in the soil was carried out with the Mehlich-1 solution (hydrochloric acid 0.05 mol/L and sulfuric acid 0.0125 mol/L) (MEHLICH, 1953) and its concentration determined, respectively, in 760 nm (Visible Spectrophotometer Genesys 30, Thermo Scientific) and flame photometer Micronal B462. The soil pH was determined with a Digimed pH-meter (DM-22) using a dry soil to water ratio of 1:2.5 (DRAGICEVIC et al., 2018).

8.3 Results and discussion

8.3.1 Liquid digestate stability

The biochemical oxygen demand (BOD), TOC, and TOC/TN ratio can be reliable parameters describing digestate biodegradability. A digestate suitable for use as a fertilizer was defined based on TOC < 1.5 g/L, BOD < 2.5 g/L and TOC/TN < 1 (ALBURQUERQUE et al., 2012). According to the values of these parameters obtained for the digestate produced by Agustini, Da Costa and Gutterres (2018) (Supplementary material Table S8.1) from the AcoD of leather waste, the digestate used in this work has a high degree of stability and can be directly used in the soil as a fertilizer, indicating that AcoD, in this case, produced highly stable materials. Similar values for TOC, TN, and BOD were reported by other authors (AGUSTINI et al., 2018a; AGUSTINI; DA COSTA; GUTTERRES, 2020b; SIMIONI et al., 2020) who studied the AcoD of tannery waste, confirming that this process, in addition to producing a highly energetic biogas, can also generate a stable digestate with potential use in agriculture.

The proportion of these parameters is related to the dynamics of C and N in the soil after the addition of the digestate. In cases where the digestate has microbial stability, as the used in this work, can lead to a lower production of CO_2-C and to an improvement in the N balance of the soil, representing a source of N available to the plants (ALBURQUERQUE; DE LA FUENTE; BERNAL, 2012).

8.3.2 Evaluation of the potential phytotoxicity of liquid digestate

The potential phytotoxicity of LD was first evaluated through seed germination. The effects of three concentrations (100%, 10% and 1%) of LD, NPK fertilizer and urea on the GI of oat seeds, expressed as percentage of the control (distilled water), are shown in Fig. 8.1. Daily temperature and relative humidity records during this experiment are summarized and presented in Fig. S8.1 in Supplementary material.

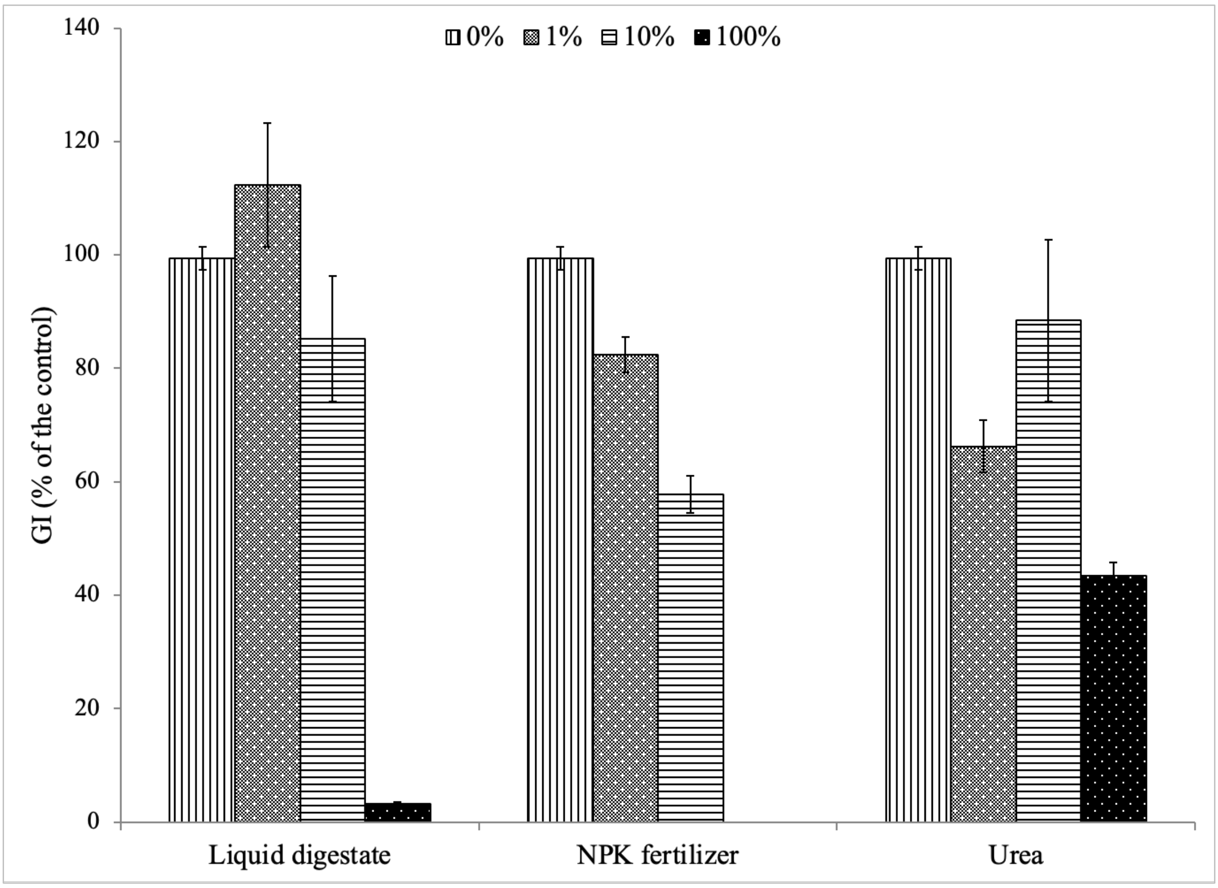

Fig. 8.1. The effects of treatments and concentrations on oat seeds' germination index (GI, expressed as percentage of control).

Statistically significant effects were found comparing the different concentrations and treatments tested. The GI values reported an inverse relationship with the doses assessed for LD and NPK fertilizer. In this sense, the assay with 1% dose produced better results for the variable associated with phytotoxicity. From a statistical point of view, no significant differences after the exposure from 0% to 10% of LD ($p > 0.05$) were evidenced, while inhibition was detected for pure treatment (100% concentration). At the assay with 1% digestate concentration, the GI value was close to 120% (compared to the control); indicating that this digestion solution contains enough nutrients and could have biostimulated positive effects on plant development (ALBURQUERQUE et al., 2012). Analysis of variance indicated that the results for GI with NPK fertilizer were statistically similar ($p > 0.05$) after exposure to 0 and 1%, and significantly different ($p < 0.05$) for higher concentrations (10% and 100%). For urea, no significant differences ($p > 0.05$) were found among the applied doses, and the highest GI was observed at the concentration of 10%, indicating a positive effect on seed germination (GI > 80%).

The results obtained are consistent with the findings reported by Parra-Orobio *et al.* (2021), Alburquerque *et al.* (2012b) and Da Ros *et al.* (2018), who achieved better GI with doses under 10%, as these conditions allow nutrients to be assimilated more easily by the plant, in addition to diluting substances potentially inhibiting the root system.

The agronomic value and phytotoxic effect of the tannery LD used in this work was also evaluated by determining the growth parameters during oat plants' first vegetative growth stage. In plant growth bioassays, LD was applied pure to the soil. However, to avoid phytotoxicity, the application of digestate to the soil was done 5 days before sowing, avoiding direct contact with young plants or germinating seeds (ALBURQUERQUE et al., 2012). In addition, the irrigation water added to the soil during the bioassays acted as a diluting agent, seeking to provide the maximum benefit of the nutrients present in the digestate, and minimizing, or even nullifying, any possible inhibitory effect.

Fig. 8.2a shows the average growth of oat plants for all conditions studied (control and treatments). The final average height reached by the plants, in addition to the final average dry weight, also for the control condition and the three types of treatments studied, are presented in Table 8.1.

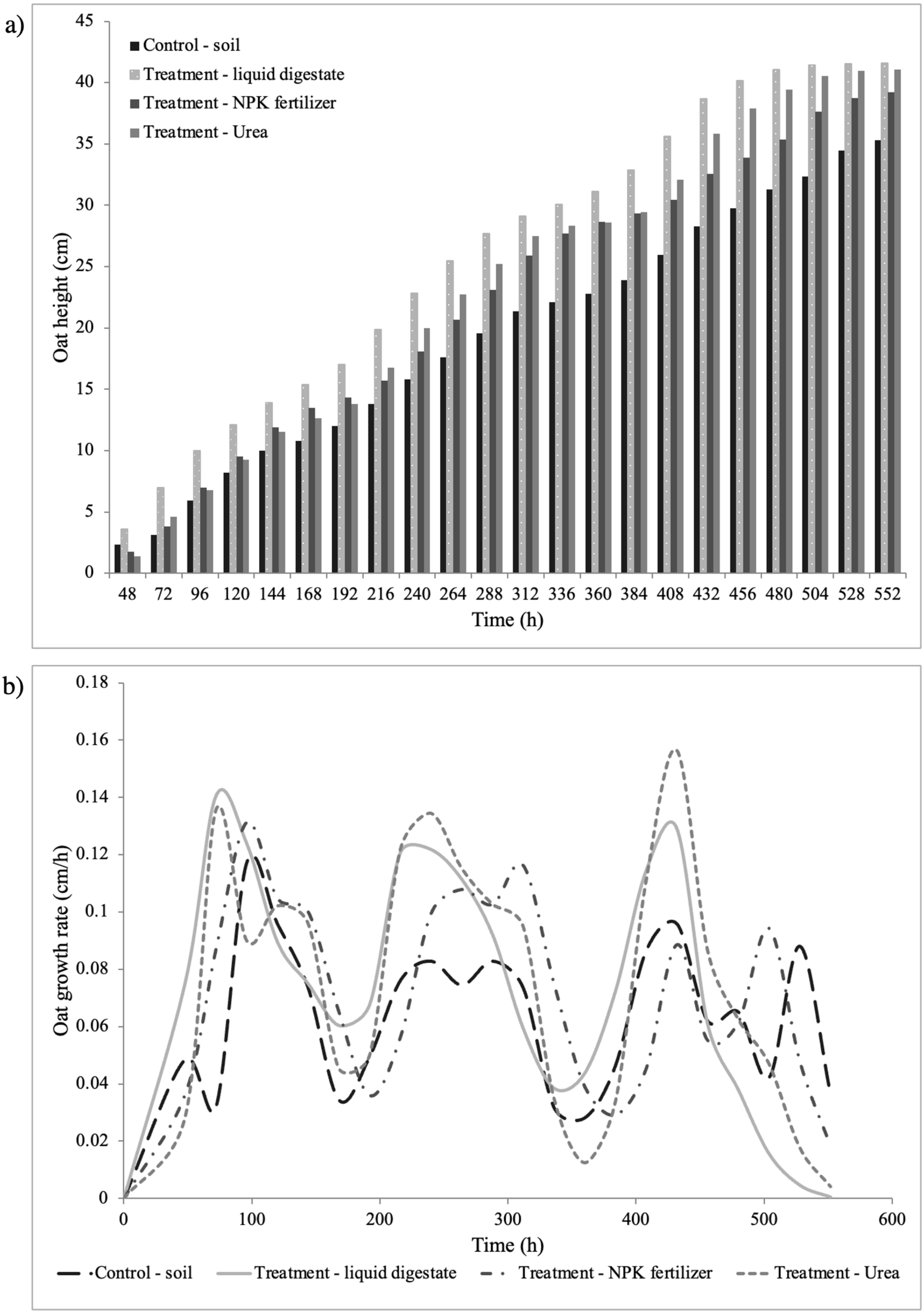

Fig. 8.2. (a) Oat growth column chart; (b) Oat growth rate graph.

Table 8.1. Final oat height and final oat dry weight for all conditions studied (control and treatments). For each column, different letters represent statistically different values by Tukey's test ($p < 0.05$).

	Oat final height (cm)	Oat final dry weight (mg)
Control - soil	35.31 ± 1.44^{b}	80.81 ± 0.76^{C}
Treatment 1 - LD	41.57 ± 0.52^{a}	144.04 ± 11.16^{A}
Treatment 2 - NPK fertilizer	$39.20 \pm 0.19^{a,b}$	$99.10 \pm 0.90^{B,C}$
Treatment 3 - urea	41.06 ± 0.56^{a}	$117.85 \pm 2.13^{A,B}$

Statistical analysis showed that the final average height of the plants obtained under the conditions in which the soil was subjected to any treatment (LD, NPK fertilizer, or urea) are statistically similar, with a 95% confidence level ($p > 0.05$). When analyzing the final average dry weight of the plants, this result is even more interesting, since the value obtained for plants grown in soil treated with LD is statistically similar only to the condition that uses urea as a treatment ($p > 0.05$), and significantly superior to the treatment with NPK fertilizer ($p < 0.05$).

These results confirm the ability of LD to improve plant growth. Similar results were previously demonstrated for digestates obtained from the AD of cattle slurry mixed with energy crops (BARŁÓG; HLISNIKOVSKÝ; KUNZOVÁ, 2020; RIVA et al., 2016), olive mill solid waste (FERNÁNDEZ-RODRÍGUEZ et al., 2021), food waste (CHEONG et al., 2020; REN et al., 2020), sewage sludge (SAPP et al., 2015), AcoD of cattle manure, poultry litter, pig slurry and onion waste (IOCOLI et al., 2019) and animal manures (poultry, cow and sheep), milk serum, maize silage, olive waste and citrus pulp (MUSCOLO et al., 2017). However, no studies were found that used the digestate obtained from the AcoD of leather waste as fertilizer and that could be compared.

Growth rate curves of oat plants were obtained from the differentiation of growth curves and are shown in Fig. 8.2b. In general, plants grown in soil treated with LD grew faster than plants in the control condition and with a profile and speed similar to that observed for plants treated with urea.

8.3.3 Soil analysis

The soil conditions used in this work (control and treatments) were analyzed before planting and after harvesting the oats, concerning their organic matter content (TOC and VS), pH, macronutrients (N, P, and K), and metals (Pb, Ni, Cd, Cr, Cu, Zn, Mn, Mg, Ca, Al, Fe). The results are shown in Table 8.2.

Table 8.2. Soil analysis for the studied conditions (control and treatments) before planting and after oat harvest.

(continue)

	Control - soil		Treatment - liquid digestate		Treatment - NPK fertilizer		Treatment - urea	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
pH	6.3250 ± 0.0575	6.5200 ± 0.0250	6.6350 ± 0.0175	6.5750 ± 0.0150	6.1650 ± 0.0025	6.3250 ± 0.0400	6.4150 ± 0.0075	6.2750 ± 0.0025
VS (%)	17.6247 ± 0.2611	15.7974 ± 0.0476	17.9581 ± 0.2165	16.4074 ± 0.0376	17.7516 ± 0.1815	16.6826 ± 0.0313	16.9249 ± 0.2075	14.8665 ± 0.6504
TOC (%)	4.9748 ± 0.1196	4.3346 ± 0.0659	4.4629 ± 0.0028	4.8733 ± 0.0687	4.3924 ± 0.0413	4.8177 ± 0.0205	4.6729 ± 0.0796	4.4507 ± 0.1062
IC (%)	0.0322 ± 0.0001	0.0279 ± 0.0021	0.0122 ± 0.0028	0.0347 ± 0.0043	0.0191 ± 0.0034	0.0318 ± 0.0002	0.0321 ± 0.0044	0.0388 ± 0.0070
TN (mg/g)	7.8113 ± 0.1138	7.1534 ± 0.0312	6.8916 ± 0.2736	7.5074 ± 0.1362	7.4706 ± 0.1042	7.7686 ± 0.0173	7.2195 ± 0.0332	7.0970 ± 0.0750
IN (mg/g)	0.3167 ± 0.0198	0.3975 ± 0.0165	0.5614 ± 0.0150	0.5966 ± 0.0126	0.6229 ± 0.0095	0.7008 ± 0.0340	0.7048 ± 0.0083	0.6178 ± 0.0304
P (mg/g)	3.1433 ± 0.0324	2.8976 ± 0.0007	4.7014 ± 0.3450	4.2792 ± 0.0147	7.9486 ± 0.0074	7.5587 ± 0.1332	3.4273 ± 0.0478	3.1227 ± 0.0368
K (mg/g)	7.4925 ± 0.0763	7.2700 ± 0.0350	9.0900 ± 0.0850	9.9100 ± 0.0550	13.8200 ± 0.5900	14.0300 ± 0.3350	6.8600 ± 0.2400	6.9700 ± 0.0350
Al (mg/g)	56.3118 ± 1.3500	53.1991 ± 2.2172	54.8360 ± 4.0863	49.0127 ± 2.2014	56.2037 ± 5.3199	59.0086 ± 1.9260	55.6432 ± 7.3089	56.6975 ± 0.0917

(conclusion)

	Control - soil		Treatment - liquid digestate		Treatment - NPK fertilizer		Treatment - urea	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Ca (mg/g)	5.6055 ± 0.0638	5.6195 ± 0.2072	5.1619 ± 0.0418	6.3088 ± 0.1253	5.5448 ± 0.1027	6.5419 ± 0.1353	6.5882 ± 0.1114	5.4369 ± 0.0980
Cd (mg/g)	0.0813 ± 0.0018	0.0848 ± 0.0026	0.0811 ± 0.0047	0.0895 ± 0.0008	0.0828 ± 0.0011	0.0799 ± 0.0023	0.0896 ± 0.0006	0.0924 ± 0.0010
Cr (mg/g)	0.1419 ± 0.0075	0.1240 ± 0.0011	0.1185 ± 0.0101	0.1256 ± 0.0026	0.1220 ± 0.0001	0.1445 ± 0.0038	0.1374 ± 0.0056	0.1107 ± 0.0062
Cu (mg/g)	0.1493 ± 0.0002	0.1463 ± 0.0006	0.1608 ± 0.0185	0.1280 ± 0.0058	0.1298 ± 0.0103	0.1549 ± 0.0031	0.1358 ± 0.0115	0.1418 ± 0.0108
Fe (mg/g)	112.9719 ± 1.4351	112.2431 ± 0.1114	110.9235 ± 3.3159	111.9000 ± 1.5451	105.2986 ± 0.8253	111.7633 ± 0.4592	114.0276 ± 0.9102	107.2943 ± 0.2377
Mg (mg/g)	2.6869 ± 0.0249	2.4853 ± 0.0683	2.4471 ± 0.0347	2.9037 ± 0.0363	2.2751 ± 0.0802	2.8406 ± 0.0721	3.2253 ± 0.1914	2.5304 ± 0.0138
Mn (mg/g)	1.2668 ± 0.0202	1.2622 ± 0.0070	1.2297 ± 0.0229	1.3173 ± 0.0077	1.1605 ± 0.0009	1.2743 ± 0.0169	1.3011 ± 0.0012	1.6629 ± 0.2232
Ni (mg/g)	0.0266 ± 0.0054	0.0540 ± 0.0061	0.0359 ± 0.0032	0.0040 ± 0.0232	0.0485 ± 0.0109	0.0581 ± 0.0052	0.0044 ± 0.0147	0.0257 ± 0.0025
Pb (mg/g)	ND	ND	ND	ND	ND	ND	ND	ND
Zn (mg/g)	0.3281 ± 0.0166	0.3019 ± 0.0346	0.1947 ± 0.0034	0.3513 ± 0.0465	0.4111 ± 0.0749	0.3756 ± 0.0675	0.2413 ± 0.0025	0.2828 ± 0.0041

ND: not determined

Literature indicates that LD contains all the nutrients necessary for crops, and the most abundant macronutrients in LD are N, P and K (VALENTINUZZI et al., 2020). In general, N is the most abundant macronutrient in LD, with concentrations that commonly vary between 200 and 900 mg/L, reaching values above 1,000 mg/L (WANG et al., 2021b), as the case of the LD used in this work (TN > 1,400 mg/L). Regarding the nutrient content for the plant and, therefore, its value as fertilizer, the most significant property of digestates is that generally a large proportion of TN occurs in inorganic form. As can be seen in Table 8.2, all treatments applied to the soil presented a large part of their TN in inorganic forms, indicated by higher IN values in these cases than in the control. It is reported that IN is present in LD mainly as ammoniacal nitrogen (NH_4^+-N), this form being highly available to the crops, which allows for rapid plant growth. Although this form of N can be easily lost by ammonia volatilization during storage and spreading in the soil, the digestate's neutral pH (7.3) and slightly acidified soil (around 6.5) probably prevent this phenomenon from happening (ALBURQUERQUE et al., 2012). As the surface of most soil particles have fixed negative charges, NH_4^+ is strongly adsorbed and shows seasonal and spatial homogeneity, providing plants with the long-term N they need. On the other hand, the anion NO_3^-, which is the main source of N in most chemical fertilizers, does not bind to soil particles and, consequently, can move freely through the soil, with the potential to invade reserves of groundwater and deprive plants of available N. Furthermore, there is a difference between the energy required for plants to absorb NH_4^+ and NO_3^-, which can impact their growth (TSACHIDOU et al., 2019). Long-term field experiments would be interesting mainly to assess the behavior of N in the soil and its absorption by plants over time.

As can be seen in Table 8.2, the soil treated with the LD presented higher concentrations of P and K than the control soil, indicating that the LD used in this work actually contains significant levels of these two macronutrients, as expected. P is a vital macronutrient for plant development and maturity as it is involved in photosynthesis, nutrient transport, energy storage and transfer and cell division (FERNÁNDEZ-RODRÍGUEZ et al., 2021). High K content in the digestate is also beneficial to the soil, as it increases the water retention capacity (JAFFAR et al., 2016), in addition to being an essential macro element for plant nutrition (KOSZEL; LORENCOWICZ, 2015). It plays a key role for several metabolic processes through the activation of enzymes, adenosine triphosphate (ATP) biosynthesis, transport assimilates, participation in the photosynthesis process and in the plant water balance (BARŁÓG; HLISNIKOVSKÝ; KUNZOVÁ, 2019; KOSZEL; LORENCOWICZ, 2015).

The addition of LD does not seem to have affected the pool of soil organic matter since neither the soil organic carbon nor the VS content showed significant changes after the treatment application. This is linked to the low TOC/TN ratio of the digestate (around 0.20), proving that the mineralization of organic matter was efficient during the AcoD process. Furthermore, it is possible that the small amount of organic matter remaining in the digestate and supplied to the soil by its addition, was easily degraded and transformed by the microbial activity of the soil in short term, not having contributed to the increase in the total C content of the treated soil (REN et al., 2020).

With respect to heavy metals and other elements analyzed, the total concentration in soil samples increased in the following order: Pb < Ni < Cd < Cr < Cu < Zn < Mn < Mg < Ca < Al < Fe, for all conditions tested (control and treatments). Analyzes indicated that the application of LD did not cause significant accumulation of these elements in the soil; the same was observed for the soil treated with NPK fertilizer and urea.

8.4 Conclusion

The agronomic value of LD obtained from the AcoD of tannery wastes was accessed. Its performance on oat growth was compared to that of commercial mineral fertilizers (NPK and urea). Tannery LD can be used directly in soils as fertilizer, achieving similar efficiency values as inorganic fertilizer. The results showed that LD has a high degree of stability and contains all essential macronutrients for plant development without the risk of contaminating the soil with metals. The use of digestate as the sole source of N can maintain nutrient supply and crop yield at similar levels to mineral fertilizers. The application of LD to the soil did not increase its organic matter content, and analyzes indicated that the added N was essentially inorganic, corroborating the hypothesis that the LD studied in this work has agronomic properties equivalent to mineral-based fertilizers.

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Supplementary material

Table S8.1. Characterization of liquid digestate (AGUSTINI; DA COSTA; GUTTERRES, 2018).

Parameter	Value
Chromium (mg/L)	< 2.5
pH	7.3 ± 0.11
BOD (mg/L)	1,010 ± 10
TOC (mg/L)	279 ± 2
TC (mg/L)	1,266 ± 6
TN (mg/L)	1,473 ± 13
VS (%)	26 ± 3
C/N ratio	0.86

BOD: biochemical oxygen demand; TOC: total organic carbon; TC: total carbon; TN: total nitrogen; VS: volatile solids

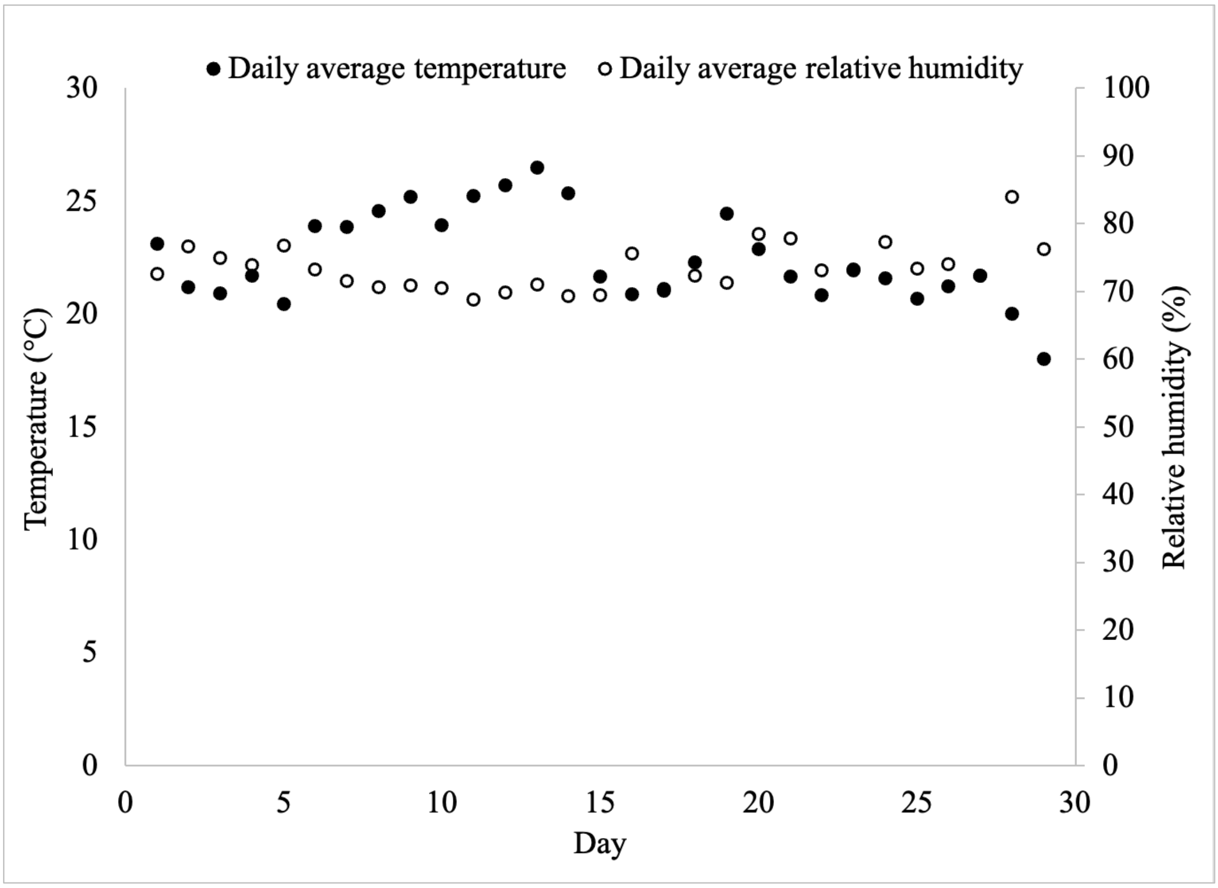

Fig. S8.1. Average daily temperature and relative humidity recorded during the experiment.

Capítulo 9

Conclusões

O tratamento dos resíduos de curtume e agrícola através das diferentes configurações de co-DA avaliadas ao longo deste trabalho mostrou-se eficaz, atingindo altas remoções de matéria orgânica e produção de biogás com alto teor de metano.

A influência dos componentes da solução de nutrientes avaliada no capítulo 5 mostrou que a solução nutritiva completa, composta por peptona, extrato de levedura e sais tamponantes (K_2HPO_4 e KH_2PO_4) pode ser substituída pela solução com restrição do componente peptona sem prejuízo algum ao processo de DA de resíduos da indústria coureira, com uma redução de 27% no custo agregado. O volume de biogás e metano acumulado por grama de SSV adicionado produzidos por essa condição foi estatisticamente igual ao produzido pela solução completa. Da mesma forma, os parâmetros que avaliam a biodegradabilidade dos resíduos durante o processo de DA foram estatisticamente iguais (redução de COT, pH, redução de SDV) ou superiores (aumento de CI, redução de SSV) para a condição com restrição de peptona em comparação com a solução de nutrientes completa. A análise econômica mostrou que uma redução de 71% do consumo de energia elétrica ou 35% do consumo de energia térmica pode ser alcançada se todo o farelo e lodo gerados em um curtume de médio porte fossem co-digeridos.

Os ensaios de co-DA entre farelo de couro, lodo de ETEs e palha de aveia (não tratada e pré-tratada) avaliados no capítulo 6 indicaram que a adição da palha de aveia não tratada melhorou o processo de co-DA, aumentando a produção de biogás em quase 60% quando comparado à co-DA apenas dos resíduos de couro. Os pré-tratamentos aplicados melhoraram as características e a biodisponibilidade da palha de aveia, principalmente nas metodologias que utilizaram álcalis, com aumento significativo do teor de celulose acompanhado de diminuição dos teores de hemicelulose e lignina. Porém, a possível formação de produtos secundários ou esterilização de microrganismos importantes não refletiu em uma maior produção de biogás. No caso em que apenas o pré-tratamento térmico foi aplicado à palha de aveia, compostos tóxicos provenientes da degradação da hemicelulose e da celulose provavelmente tenham sido gerados, inibindo o processo de co-DA e, como consequência, praticamente não foi observada produção de biogás.

A eficiência do processo de co-DA entre resíduos da indústria coureira e do cultivo de trigo, além dos efeitos de diferentes técnicas de pré-tratamento na composição da palha do trigo e no rendimento do biogás, foram avaliadas no capítulo 7. Embora o pré-tratamento com NaOH pareça ter afetado mais a composição química da palha de trigo, a produção de biogás nesse caso foi a menor dentre todas as condições testadas, o que pode estar relacionado à desidratação de células microbianas, causada pela liberação de compostos aromáticos da degradação da lignina e pela presença de íons sódio residuais. A co-DA entre os resíduos de couro e a palha de trigo pré-tratada com HCl foi a mais eficiente, convertendo cerca de 48% do carbono presente nos resíduos em biogás, o que representa um rendimento de biogás acumulado 59% superior ao observado para a palha de trigo não tratada, e aproximadamente 167% superior ao observado para o caso sem a adição de palha de trigo.

A eficiência no tratamento dos resíduos (redução de sua carga orgânica) foi verificada em todas as condições testadas, o que neste estudo é tão importante quanto a produção de biogás, uma vez que o resíduo remanescente (após o processo de DA) ainda precisa ser gerenciado, e resíduos mineralizados são ambientalmente inertes para disposição ou ainda, por apresentarem alta concentração de nitrogênio, passíveis de serem utilizados como fertilizantes na agricultura.

O valor agronômico do digestato líquido obtido a partir da co-DA de resíduos de couro foi avaliado no capítulo 8. Os resultados mostraram que o digestato líquido possui alto grau de estabilidade e contém os macronutrientes (N, P e K) essenciais para o desenvolvimento da planta, sem risco de contaminar o solo com metais. O seu desempenho com relação ao crescimento de plantas de aveia foi equivalente ao de fertilizantes minerais (NPK comercial e uréia), corroborando a ideia de que o digestato líquido obtido a partir da co-DA de resíduos de couro possui propriedades agronômicas equivalentes às de fertilizantes de base mineral e pode ser diretamente aplicado no solo como tal, fechando o ciclo biológico dos nutrientes, promovendo o desenvolvimento de uma agricultura mais sustentável e contribuindo para uma economia circular.

Algumas sugestões para trabalhos futuros são:

- substituição do extrato de levedura, componente remanescente da solução de nutrientes, por lodo proveniente da indústria cervejeira;
- purificação do biogás gerado a partir da co-DA de resíduos da indústria coureira a partir da adsorção de CO_2;
- escalonamento do processo para escala piloto e industrial, em parceria com curtumes e produtores rurais da região;
- avaliação da parcela sólida do digestato obtido a partir da co-DA de resíduos de couro como fertilizante agrícola.

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