UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA AGRÍCOLA

Felipe Vedovatto

PRODUÇÃO DE ETANOL 2G E BIOGÁS UTILIZANDO RESÍDUOS DA CULTURA DA SOJA

Santa Maria, RS
2021

Felipe Vedovatto

PRODUÇÃO DE ETANOL 2G E BIOGÁS UTILIZANDO RESÍDUOS DA CULTURA DA SOJA

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Engenharia Agrícola, da Universidade Federal de Santa Maria (UFSM), RS, como requisito parcial para obtenção do título de **Doutor em Engenharia Agrícola.**

Orientador: Prof. Dr. Marcus Vinícius Tres
Coorientador: Prof. Dr. Giovani Leone Zabot

Santa Maria, RS
2021

Vedovatto, Felipe
PRODUÇÃO DE ETANOL 2G E BIOGÁS UTILIZANDO RESÍDUOS DA CULTURA DA SOJA / Felipe Vedovatto.- 2021.
97 p.; 30 cm

Orientador: Marcus Vinícius Tres
Coorientador: Giovani Leone Zabot
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Engenharia Agrícola, RS, 2021

1. Palha 2. Casca 3. Hidrólise 4. Água Subcrítica 5. Biocombustíveis I. Tres, Marcus Vinícius II. Zabot, Giovani Leone III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Email: felipevedovatto@yahoo.com.br

Felipe Vedovatto

PRODUÇÃO DE ETANOL 2G E BIOGÁS UTILIZANDO RESÍDUOS DA CULTURA DA SOJA

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Engenharia Agrícola, da Universidade Federal de Santa Maria (UFSM), RS, como requisito parcial para obtenção do título de **Doutor em Engenharia Agrícola.**

Aprovado em: 08 de abril de 2021:

Marcus Vinícius Tres, Dr (UFSM)
(Presidente/Orientador) - Videoconferência

Giovani Leone Zabot, Dr (UFSM)
(Coorientador) - Videoconferência

Adriano Arrué Melo, Dr. (UFSM) - Videoconferência

Helen Treichel, Dra. (UFFS) - Videoconferência

Paulo Carteri Coradi, Dr. (UFSM) - Videoconferência

Rogério Marcos Dallago, Dr. (URI) - Videoconferência

Santa Maria, RS
2021

AGRADECIMENTOS

Agradeço a Deus, por me iluminar, me dar forças e me abençoar nessa caminhada.

Agradeço a minha família, meu pai, minha mãe e ao meu irmão pelo amor e pelo incentivo.

Agradeço a Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Engenharia Agrícola pela oportunidade de curso o doutorado.

Agradeço ao meu orientador Professor Dr. Marcus Vinícius Tres e ao meu coorientador Professor Dr. Giovani Leone Zabot pela orientação, pela confiança depositada em mim e pela ajuda nos momentos de dificuldade durante o Doutorado. Também agradeço a disponibilidade dos alunos de Iniciação Científica do Laboratório de Engenharia de Processos Agroindustriais (LAPE) para realização dos experimentos de hidrólise durante o Doutorado.

Agradeço ao Professor Dr. Marcio Antonio Mazutti pela ajuda e pela disponibilidade do Laboratório BiotecFactory para realização dos experimentos durante o Doutorado.

Agradeço a Professora Dra. Helen Treichel pela ajuda, pela confiança e pela disponibilidade do Laboratório de Microbiologia e Bioprocessos (UFFS/Erechim) para realização dos experimentos de produção de Etanol.

Agradeço ao pesquisador Ricardo L. R. Steinmetz e a Embrapa Suíno e Aves pela ajuda e confiança na realização dos experimentos de produção de biogás.

Agradeço a CAPES pelo apoio financeiro.

Enfim, agradeço a todas as pessoas que participaram de alguma forma no desenvolvimento deste trabalho. Que Deus abençoe cada um e ilumine suas vidas.

RESUMO

PRODUÇÃO DE ETANOL 2G E BIOGÁS UTILIZANDO RESÍDUOS DA CULTURA DA SOJA

AUTOR: Felipe Vedovatto
ORIENTADOR: Dr. Marcus Vinícius Tres
COORIENTADOR: Dr. Giovani Leone Zabot

Os resíduos de biomassas lignocelulósicas são uma alternativa para a produção de etanol de segunda geração (2G) e biogás, devido a sua composição química (celulose, hemicelulose e lignina), por serem recursos renováveis, de baixo custo e não competir com culturas alimentares. Estes resíduos são oriundos da agricultura e agroindústria. A hidrólise com água subcrítica de resíduos de biomassas lignocelulósicas é uma alternativa para a produção de açúcares fermentescíveis para produção de biocombustíveis. Diante disso, o presente trabalho teve como objetivo avaliar a hidrólise com água subcrítica dos resíduos da soja (palha e casca) para obtenção de açúcares fermentescíveis e a produção de biocombustíveis (etanol e biogás). O trabalho foi organizado em três etapas. Na etapa 1, foi realizada a obtenção, caracterização e preparo da palha e casca de soja. Na etapa 2, foram conduzidos os ensaios de hidrólise com água subcrítica. As condições experimentais investigadas foram a temperatura (180, 220 e 260 °C) e a razão massa líquido/sólido (9 g e 18 g de água/ g de palha e 7,5 e 15 g de água/ g de casca). O tempo de reação de hidrólise foi de 15 min com pressão fixada em 25 MPa. Foram avaliados o rendimento em açúcares redutores, eficiência, composição dos hidrolisados e os resíduos após a hidrólise. Na etapa 3, foi realizada a avaliação da produção de etanol utilizando a levedura *Wickerhamomyces* sp. e as condições de maior rendimento de açúcares redutores. Também nesta etapa, foram avaliados o potencial bioquímico de biogás e de metano dos resíduos *in natura*, hidrolisados e dos hidrolisados fermentados pela co-digestão anaeróbica. As condições de 220 °C/R-18 e 220 °C/R-15 proporcionaram o maior rendimento de açúcares redutores com 9,56 ± 0,53 g/100 g de palha e 10,15 ± 0,5 g/100 g de casca em 4 e 3 min de reação de hidrólise, respectivamente. A eficiência da hidrólise para os dois resíduos foi aproximadamente de 23 g/100 g de carboidratos. A produção de etanol foi de 5,57 ± 0,01 g/L e 6,11 ± 0,11 g/L de etanol para os hidrolisados da palha e casca diluídos e suplementado com glicose (10 g/L). A produção máxima de etanol em biorreator para os hidrolisados da palha e casca sem ajuste de pH foi de 48 h e com ajuste de pH foi de 24 h. O potencial bioquímico de biogás e metano foi possível para os resíduos *in natura*, hidrolisados e os hidrolisados fermentados. Destacam-se a produção de biogás de 739 ± 37 e 652 ± 34 NmL/g_{VSad} para o hidrolisado fermentado da palha com e sem ajuste de pH e 620 ± 26 NmL/g_{VSad} para o hidrolisado fermentado da casca sem ajuste de pH. Diante desses resultados, conclui-se que os resíduos da soja combinados com o processo de hidrólise com água subcrítica, com o processo de produção de etanol e com a co-digestão anaeróbica apresentam potencial para a produção de energias renováveis.

Palavras-chave: Palha. Casca. Hidrólise. Água Subcrítica. Biocombustíveis.

ABSTRACT

ETHANOL 2G AND BIOGAS PRODUCTION USING SOYBEAN CULTURE RESIDUES

AUTHOR: Felipe Vedovatto
ADVISOR: Dr. Marcus Vinícius Tres
COADVISOR: Dr. Giovani Leone Zabot

Lignocellulosic biomass residues are an alternative to the second-generation ethanol (2G) and biogas production, due to their chemical composition (cellulose, hemicelluloses, and lignin), because it is a renewable resource, low cost, and not compete with food crops. These residues come from agriculture and agroindustry. Subcritical water hydrolysis of lignocellulosic biomass is an alternative for obtaining fermentable sugars to produce biofuels. Therefore, the present paper aims to evaluate the subcritical water hydrolysis of soybean residues (straw and hull) for obtaining fermentable sugars and biofuels production (ethanol and biogas). The work was organized in three stages. In stage 1, the obtaining, characterized, and prepared soybean straw and the hull were performed. In stage 2, the assays of subcritical water hydrolysis were conducted. The experimental conditions investigated were the temperature (180, 220 e 260 °C) and liquid-solid mass ratio (9 g and 18 g water/g straw and 7.5 g and 15 g water/g hull). The time of hydrolysis reaction was 15 min with a set pressure at Mpa. The reducing sugars yield, efficiency, composition of hydrolysates, and the residues after hydrolysis were evaluated. In stage 3, was performed evaluating ethanol production using the yeast *Wickerhamomyces* sp. and the condition of higher reducing sugars yield. At this stage, the biochemical biogas and methane potential of new residues, hydrolysates, and fermented hydrolysates by anaerobic co-digestion were performed. Subcritical water hydrolysis provided the obtaining fermentable sugars. The conditions of 220 °C/R-18 e 220 °C/R-15 provided higher reducing sugars yield with 9.56 ± 0.53 g/100 g straw and 10.15 ± 0.50 g/100 g hull at 4 min and 3 min of the hydrolysis reaction, respectively. The efficiency of hydrolysis for both residues was approximately 23 g/100 g carbohydrates. The ethanol production was 5.57 ± 0.01 g/L e 6.11 ± 0,11 g/L ethanol for straw and hull hydrolysates diluted and supplemented with glucose (10 g/L). The maximum ethanol production in a bioreactor for straw and hull hydrolysates without changing the pH was 48 h and with changing the pH was 24 h. The biochemical biogas and methane potential were possible for new residues, hydrolysates, and fermented hydrolysates. Highlighting the biogas production of 739 ± 37 e 652 ± 34 NmL/g_{VSad} for straw hydrolysates fermented with and without changing the pH and 620 ± 26 NmL/g_{VSad} for hull hydrolysate fermented without changing the pH. Given these results, it is concluded that the soybean residues combined with the subcritical water hydrolysis process, ethanol production process, and anaerobic co-digestion have the potential for renewable energy production.

Keywords: Straw. Hull. Hydrolysis. Subcritical water. Biofuels

LISTA DE ILUSTRAÇÕES

LISTAS DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

2G	Produção de etanol de segunda geração
SWH	Hidrólise com água subcrítica
Y_{RS}	Rendimento de açúcares redutores
E	Eficiência
R	Razão massa líquido/ sólido
HPLC	Cromatografia líquida de alta performance
HMF	5-hidroximetilfurfural
FT-IR	Espectrofotometria de infravermelho com transformada de Fourier
MEV	Microscopia eletrônica de varredura

SUMÁRIO

CAPÍTULO 1 – APRESENTAÇÃO DA TESE

1.1 INTRODUÇÃO

A soja é umas das principais culturas no cenário mundial (ABDULKHANI et al., 2017). No Brasil, há muitos anos a soja é a principal cultura. Na safra 2020/2021 estima-se uma produção de grãos maior em relação à safra anterior (CONAB, 2021). Além, da utilização dos grãos para diferentes finalidades, os resíduos produzidos desta cultura quando processados podem gerar diferentes produtos (ABDULKHANI et al., 2017).

A produção dos resíduos agrícolas é resultante da colheita e do processamento de uma determinada cultura, podendo variar conforme a produtividade e a área cultivada. Os resíduos agrícolas, agroindustriais, florestais e de alimentos possuem na sua composição química, majoritariamente, celulose, hemicelulose e lignina. Quando a celulose e a hemicelulose são dissociadas e utilizadas, podem produzir diferentes produtos, tais como etanol, aditivos alimentares, enzimas, biogás, entre outros (MAITAN-ALFENAS; VISSER; GUIMARÃES, 2015).

A dissociação da celulose e hemicelulose pode ocorrer pelo processo de hidrólise. Existem alguns exemplos de processos de hidrólise sendo investigados na literatura: a hidrólise enzimática do bagaço de cana-de-açúcar (RABELO et al., 2011), a hidrólise ácida da casca de soja (CASSALES et al., 2011) e a hidrólise com água subcrítica da palha de arroz (ABAIDE et al., 2019b) e de resíduos de nogueira-pecã (SANTOS et al., 2020). O processo de hidrólise com água subcrítica é uma alternativa que tem sido amplamente investigada para a produção de açúcares fermentescíveis (CARDENAS-TORO et al., 2014; LACHOS-PEREZ et al., 2016). É um processo rápido e utiliza apenas água como solvente, apresentando a inofensividade do solvente, em comparação a outros processos (ZHU et al., 2011).

A produção de biocombustíveis surgiu como um potencial para produção de combustíveis renováveis. Esta produção tem sido usada para auxiliar na redução da utilização de combustíveis fósseis com o uso de biomassas lignocelulósicas sob a forma de resíduos (PITARELO et al., 2012). As biomassas lignocelulósicas têm grande potencial para produção de biocombustíveis, por serem fontes de carbono e são considerados resíduos vegetais não comestíveis. Logo, não competem com as culturas alimentares e tem baixo custo comparando-se com as matérias-primas agrícolas convencionais (ALVIRA et al., 2010).

Após o processo de hidrólise, os açúcares gerados podem ser destinados à produção de etanol e biogás. A produção de etanol a partir de resíduos lignocelulósicos é considerada

como sendo de etanol de segunda geração (2G) (NAIK et al., 2010). O processo de produção de etanol de segunda geração é um processo promissor para produção de energia renovável, pois utiliza resíduos não alimentares, de fácil obtenção e renováveis (SARKAR et al., 2012). A utilização de diversos resíduos proporcionou a produção de etanol de segunda geração, dentre eles casca de soja (ROJAS et al., 2014), a mistura de casca de soja e casca de aveia (DALL CORTIVO et al., 2020) e palha de trigo (SAHA et al., 2015).

A co-digestão anaeróbia é um processo biológico eficaz para a produção de biogás, que consiste na mistura de dois substratos: efluente animal e material lignocelulósico. A mistura é para obter uma relação C/N favorável para a digestão anaeróbica, melhorando a eficiência do processo (SHRESTHA et al., 2017). O pré-tratamento das biomassas lignocelulósicas é uma etapa importante no processo de digestão anaeróbica, pois é necessário expor os carboidratos à degradação microbiana, de modo a aumentar o rendimento em biogás (ZHENG et al., 2014). A co-digestão anaeróbica usando esterco de vaca leiteira e palha de milho, palha de soja e caule de girassol (KOVAČIĆ et al., 2019) e co-digestão anaeróbica com esterco suíno e talos de milhos (VENTURIN et al., 2018) proporcionaram a produção de biogás.

Diante do exposto, os resíduos lignocelulósicos apresentam grande potencial para a produção de biocombustíveis. Além disso, o processo de hidrólise com água subcrítica, a produção de etanol e a produção de biogás são promissores para obtenção de biocombustíveis, mas necessitam de novos estudos para melhorar e otimizar os seus processos (PRADO et al., 2016; SARKAR et al., 2012; ZHENG et al., 2014). Assim, este trabalho propôs avaliar a produção de biocombustíveis através dos resíduos da soja. Avaliou-se diversas condições experimentais da hidrólise com água subcrítica, a produção de etanol utilizando os hidrolisados da palha e casca de soja, o potencial bioquímico de biogás e metano utilizando os hidrolisados fermentados da produção de etanol e otimizou-se o processo de produção de biocombustíveis.

1.2 OBJETIVOS

1.2.1 Objetivo Geral

Avaliar a hidrólise dos resíduos da soja (palha e casca) com água subcrítica para obtenção de etanol e biogás.

1.2.2 Objetivos Específicos

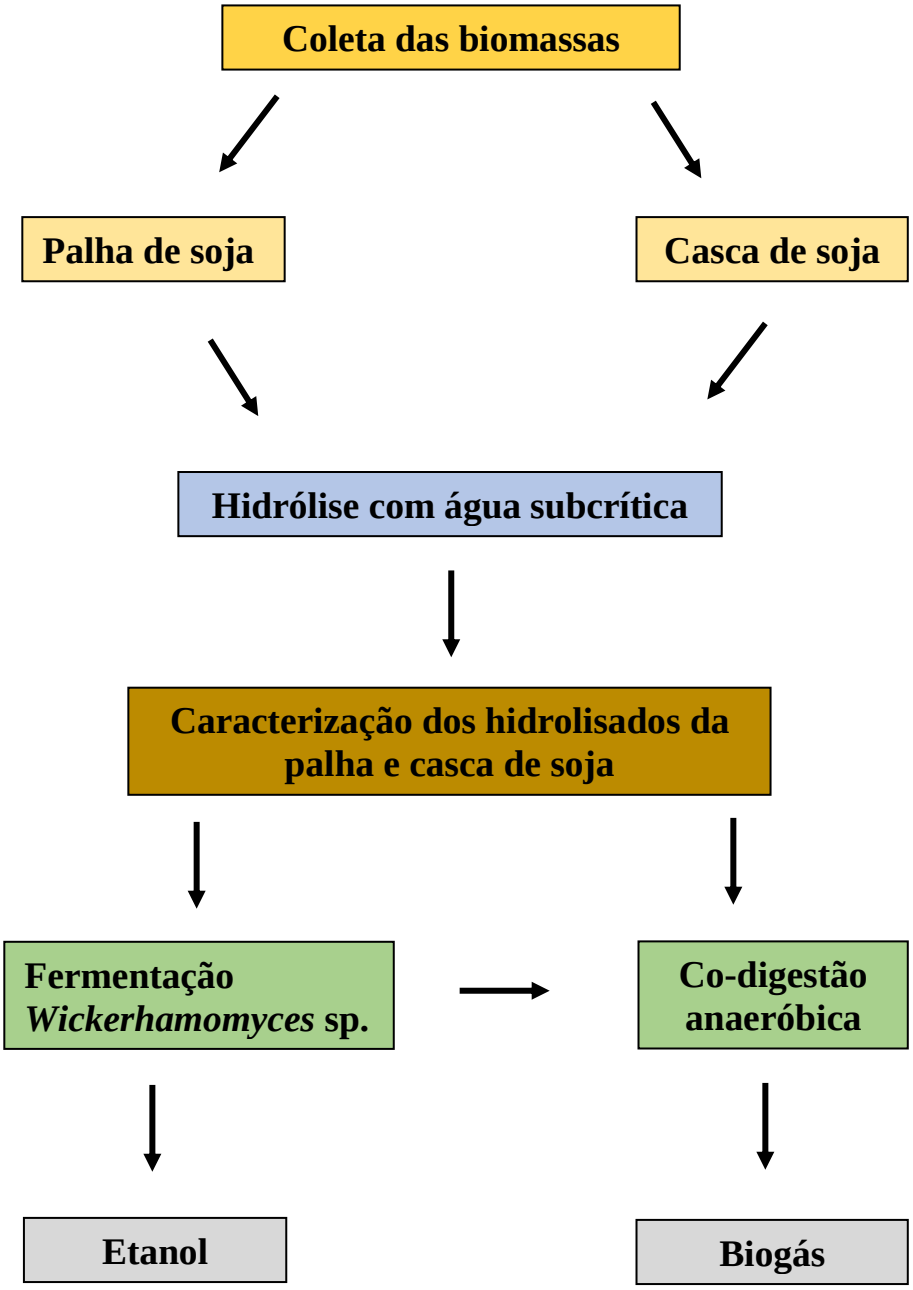
Os objetivos específicos investigados neste trabalho são abaixo apresentados:

a) Obtenção e caracterização de palha e de casca de soja em termos de celulose, hemicelulose e lignina;
b) Avaliação das variáveis temperatura e razão massa líquido/sólido na hidrólise dos resíduos da soja com água subcrítica;
c) Caracterização dos hidrolisados em termos de açúcares redutores totais, inibidores e ácidos orgânicos;
d) Produção de etanol dos hidrolisados usando a levedura *Wickerhamomyces* sp. UFFS-CE-3.1.2 via processo fermentativo;
e) Produção de biogás usando os resíduos da fermentação via co-digestão anaeróbica.

1.3 FLUXOGRAMA DA TESE

Na Figura 1 é apresentado o fluxograma com as atividades realizadas nesta Tese de Doutorado.

Figura 1 - Fluxograma de atividades do trabalho.



Fonte: Autor.

CAPÍTULO 2 - REVISÃO BIBLIOGRÁFICA

2.1 CULTURA DA SOJA

A expansão da soja no Brasil possibilitou o aumento da produção de grãos em novas fronteiras agrícolas. A soja é rica em diversos nutrientes e seus grãos são utilizados para diversos fins, e tem como destaque a produção de farelo para alimentação animal e óleo para alimentação humana (EPE, 2014).

A produção mundial de soja no ano de 2019 foi de 333,67 milhões de toneladas (FAO, 2019). No Brasil, estima-se uma produção de 135,54 milhões de toneladas para a safra de 2020/2021, com um aumento esperado de 8,6%, comparado à safra 2019/2020 e uma estimativa de produtividade de 3.523 kg/ha (CONAB, 2021). Em relação à área plantada, estima-se um aumento de 4,1% comparado com a safra anterior, com aproximadamente 38.473,0 mil ha para safra 2020/2021 (CONAB, 2021).

A cultura da soja tem grande potencial como matéria-prima para as biorrefinarias. Os seus resíduos são usados para produzir bioetanol, biogás, biodiesel e outros subprodutos (proteína, enzimas, açúcares fermentescíveis e inibidores). No entanto, ainda há a carência de estudos sobre o uso da cultura em biorrefinarias. A biorrefinaria de matérias lignocelulósicos inclui a conversão de celulose, hemicelulose e lignina em diversos produtos para serem destinados a bioenergia e outros produtos (ABDULKHANI et al., 2017).

2.2 BIOMASSAS LIGNOCELULÓSICAS

As biomassas lignocelulósicas incluem os resíduos agrícolas e agroindustriais, como bagaço de cana-de-açúcar, palha de arroz e trigo, casca de arroz, dentre outros, e também inclui os materiais lenhosos e as culturas energéticas (MUSSATTO; DRAGONE, 2016). As biomassas lignocelulósicas são compostas por celulose, hemicelulose e lignina, com maiores porcentagens. Os compostos com menores porcentagens são as proteínas, as frações de extrativos e os minerais inorgânicos (KNEZ et al., 2018; MUSSATTO; DRAGONE, 2016; VAN DYK; PLETSCHKE, 2012). As biomassas possuem compostos inorgânicos (potássio, sódio, cálcio dentre outros) (YU; LOU; WU, 2008). Os extrativos das biomassas lignocelulósicas são constituídos por compostos orgânicos como gorduras, ceras, alcaloides, proteínas, fenólicos, açúcares simples, pectinas, mucilagens, gomas, resinas, terpenos, amidos, glicosídeos, saponinas e óleos essenciais. Os extrativos são considerados reserva de

energia e funcionam como defesa contra a ataques de patógenos e de insetos (MOHAN; PITTMAN; STEELE, 2006).

A celulose é uma componente de rigidez e sustentador da parede celular. A hemicelulose e a lignina conferem a rigidez da microfibra celulósica (OGEDA; PETRI, 2010). Os materiais lignocelulósicos são compostos por cadeia de celulose, sendo recoberta por hemicelulose e lignina.

A celulose é o maior composto encontrado nos materiais lignocelulósicos. É um homopolímero linear de glicose ($C_6H_{12}O_6$) com unidades ligadas entre si, sob a forma de unidades de D-anidroglucopiranose através de ligação β-(1,4)-glicosídica. A celulose é encontrada em plantas em duas formas: estrutura cristalina e estrutura amorfa. A celulose cristalina é fortemente agrupada e ligada entre si por ligações de hidrogênio, sendo mais difíceis de serem hidrolisadas do que a celulose amorfa (MUSSATTO; DRAGONE, 2016; VAN DYK; PLETSCHKE, 2012). O segundo composto mais encontrado nos materiais lignocelulósicos é a hemicelulose. A hemicelulose é formada de açúcares 5-carbono ou pentoses (D-xilose e L-arabinose), açúcares 6-carbono ou hexoses (D-glicose, D-galactose e D-manose) e alguns ácidos (LIMAYEM; RICKE, 2012; MUSSATTO; DRAGONE, 2016).

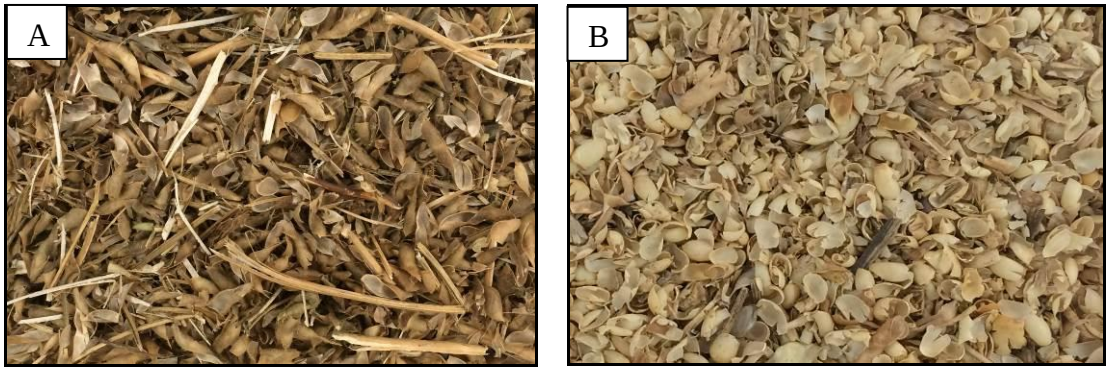
A lignina é o terceiro composto mais encontrado nos materiais lignocelulósicos e a sua composição varia conforme a biomassa. A lignina é um polímero tridimensional amorfo de unidades fenilpropanoides (MUSSATTO; DRAGONE, 2016) exibindo as estruturas p-coumaril, coniferil e sinapil (YU; LOU; WU, 2008). Na sua estrutura amorfa tem muitas ligações de éter, ao contrário das funções acetais encontradas na celulose e na hemicelulose. Também possui ligações covalentes entre a lignina e os polissacarídeos, aumentando fortemente a força adesiva entre as fibras de celulose e sua matriz de lignina (YU; LOU; WU, 2008).

A porcentagem da composição química dos resíduos depende do tipo e da característica da biomassa lignocelulósica. Por exemplo: a palha de arroz apresenta 32,70% de celulose, 15,59% de hemicelulose e 21,24% de lignina (SINDHU et al., 2016). Já a palha de milho possui 37% de celulose, 29% de hemicelulose e 18% de lignina (CAO et al., 2015). A palha de cana-de-açúcar, coletada em cinco estados brasileiros (São Paulo, Minas Gerais, Paraná, Alagoas e Mato Grosso) apresentou composição química diferente entre os estados, apresentando média de 44,26% de celulose, 31,10% de hemicelulose e 19,01% de lignina (SANTOS et al., 2014).

A palha e a casca de soja (Figura 2) são resíduos gerados durante a colheita e o processamento da cultura. A produção de palha de soja é de 2,5 t de biomassa para cada de

tonelada de grãos, com um umidade de 15% (KOOPMANS; KOPPEJAN, 1997). A casca da soja representa, aproximadamente, 8% do grão e são obtidas por meio do processamento da soja (GNANASAMBANDAM; PROCTOR, 1999). A palha da soja é composta por folha, caules e vagens e é uma fonte abundante, renovável anualmente pelo cultivo da soja e de baixo custo (CABRERA et al., 2015; REDDY; YANG, 2009). A casca de soja geralmente é descartada no meio ambiente, tem baixo uso na alimentação animal e é pouco aproveitada como matéria-prima (CASSALES et al., 2011; QING et al., 2017).

Figura 2 - Palha (A) e casca (B) de soja.



Fonte: Autor.

Os resíduos da soja são considerados resíduos lignocelulósicos ou biomassas lignocelulósicas. Os resíduos são compostos principalmente por celulose, hemicelulose e lignina (Tabela 1). A celulose e a hemicelulose quando dissociadas geram diversos açúcares e bioprodutos, que posteriormente podem ser utilizadas para a produção de biocombustíveis (YU; LOU; WU, 2008).

Tabela 1 - Composição química da palha e da casca de soja.

Biomassa	**Componentes (%, em base mássica)**				**Ref.**
	Celulose	Hemicelulose	Lignina	Lignina ácida solúvel	
Casca de soja	31,19	2,28	1,54	-	(MERCI et al., 2015)
	35,80	23,10	-	4,30	(ROJAS et al., 2014)
Palha de soja	24,99	11,91	17,64	-	(XU et al., 2007)
	35,30	16,90	-	0,90	(MUÑOZ et al., 2015)

Na literatura científica, diversos trabalhos usando os resíduos da soja e diversos processos de hidrólise proporcionaram a produção de açúcares fermentescíveis. Cassales et al. (2011) destacam o potencial da casca de soja como fonte de bioprodutos, usando a hidrólise ácida e obtendo uma eficiência de 87% e com baixa produção de inibidores. Cabrera et al. (2015) investigaram a hidrólise enzimática combinada com o pré-tratamento da palha de soja com ácido sulfúrico e hidróxido de sódio e obtiveram uma conversão de açúcares de 93% e 86,5%, respectivamente.

Além disso, os resíduos da soja proporcionam a produção de biocombustíveis. Mielenz; Bardsley; Wyman (2009) investigaram o processo de sacarificação e fermentação por *Saccharomyces cerevisiae*, sem nenhum pré-tratamento da casca de soja. Tais autores obtiveram produção de etanol, preservando a proteínas das cascas e a conversão de carboidratos em etanol. Rojas et al. (2014) produziram etanol da fração lignocelulósica remanescente da recuperação de proteínas da casca de soja, com uma produção de etanol de 13,4 g/L e um rendimento de 93,7% pré-tratada com proteólises, hidrólise ácida e hidrólise celulolíticas e produziram 12,8 g/L de etanol e um rendimento de 89,2% para a casca pré-tratada com hidrólise ácida e hidrólise celulítica. Onthong; Juntarachat (2017) utilizaram cinco resíduos (soja, casca de mamão, bagaço de cana-de-açúcar, palha de arroz e gengibre) para avaliar o potencial de produção de biogás usando os processos de digestão em batelada e contínuo. Os autores obtiveram a maior taxa de produção de biogás em relação as outras biomassas, com 63,01 L/dia para o resíduo da soja.

2.3 HIDRÓLISE COM ÁGUA SUBCRÍTICA

A hidrólise com água subcrítica é uma alternativa para obtenção de açúcares fermentescíveis. Degrada menos os açúcares em relação a outros métodos, não gera resíduo e é extremamente rápida, podendo evitar o pré-tratamento da biomassa (PRADO et al., 2016).

A água subcrítica é um fluido com temperatura entre 150°C e 373°C (temperatura crítica) e com uma pressão superior que sua pressão de saturação de vapor (CARDENAS-TORO et al., 2014). Nos estados supercrítico e subcrítico, a água é considerada um solvente não polar, ideal para formação de ligações C-C ou reações catalisadas por compostos organometálicos. Mesmo assim, a estrutura de uma única molécula de água continua sendo polar, podendo interagir com íons. Com isso, a água subcrítica comparada com a água em condições ambientais tem maior compressibilidade, suportando reações iônicas ou radicais livres (YU; LOU; WU, 2008).

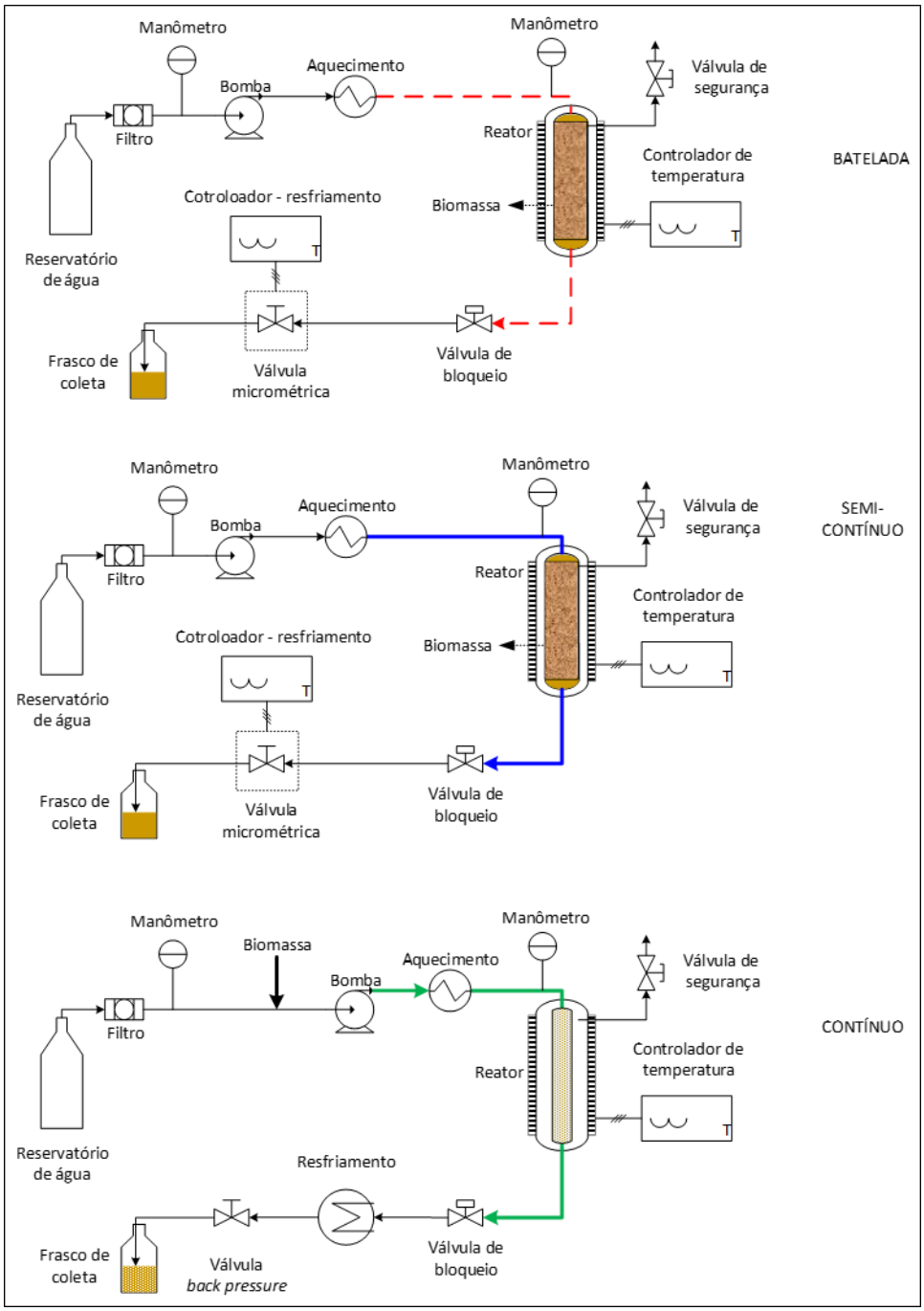
As propriedades físicas da água subcrítica (densidade, produto iônico e constante dielétrica) são dependentes da temperatura e da pressão. Altas temperaturas e pressões, aumentam o produto iônico, diminuem a viscosidade, aumentam a difusividade e afetam a constante dielétrica, que é proporcional à densidade e inversamente proporcional à temperatura. Portanto, o produto iônico da água a 300 °C, possui maior concentração de íons H^+ e OH^- favorecendo as reações iônicas (COCERO et al., 2018; PRADO et al., 2016).

Água subcrítica apresenta baixa viscosidade e alta difusividade, o que facilita a penetração da água na complexa estrutura da matriz lignocelulósica. No entanto, a constante dielétrica apresenta características de solvente apolar, aumentando a solubilidade de compostos orgânicos, apresentando rápida hidrólise e intensificando o processo. Porém, a velocidade da reação apresenta uma desvantagem na seletividade em períodos maiores, levando à degradação dos produtos da hidrólise e resultando em misturas complexas (COCERO et al., 2018).

A hidrólise com água subcrítica é um método promissor para a recuperação de açúcares, mas necessita de novos estudos com o objetivo de otimizar o processo. Há necessidade de estudos de cinética e desenvolvimento de modelos matemáticos, adição de catalisadores, otimização dos parâmetros operacionais (temperatura e pressão) e redução de etapas. Consequentemente, ocorre redução da demanda de energia, quantidade de água e redução de resíduos (COCERO et al., 2018; KNEZ et al., 2018; PRADO et al., 2016; YU; LOU; WU, 2008).

O processo de hidrólise com água sub/supercrítica é baseado em três modos de operação batelada, semi-contínuo e contínuo (Figura 3). No modo em batelada, a matéria-prima e o reagente (água) reagem no processo sem adicionar e/ou retirar os produtos. No modo semi-contínuo a água subcrítica flui através da biomassa e os líquidos são eliminados rapidamente pelo sistema. No modo contínuo, a biomassa e a água passam pelo reator em conjunto, sendo a biomassa e a água continuamente bombeados através de um aquecedor e mantidos sob reação constate e/ou pré-aquecendo a água e colocando em contato com a corrente de biomassa, na qual a reação é finalizada por resfriamento (PRADO et al., 2016; YU; LOU; WU, 2008). Os produtos obtidos nos modos semi-contínuo e contínuo apresentam menor degradação em comparação ao modo batelada, pois o tempo de permanência dos produtos no reator são curtos (YU; LOU; WU, 2008).

Figura 3 - Modos de operação (batelada, semi-contínuo e contínuo) do processo de hidrólise com água sub/supercrítica.



Fonte: Autor.

A celulose passa por um processo de despolimerização por duas vias de reação: (1) desidratação da glicose da extremidade redutora via clivagem pirolítica da ligação glicosídica da celulose, que ocorre com o aumento da temperatura e a diminuição da pressão e (2) hidrólise da ligação glicosídica via intumescimento e dissolução da celulose, localizada em regiões de alta densidade em água sub e supercrítica (MATSUMURA et al., 2006). No

processo de decomposição, a celulose produz gases, óleo e carvão com aumento da temperatura (MATSUMURA et al., 2006).

Durante o processo de hidrólise pode ocorrer a decomposição da glicose e formação de outros compostos (5-hidroximetilfurfural, frutose, eritose, glicolaldeído, dihidroxyacetotana, glicealdeído,1,6 anidroglicose e piruvaldeído) pela influência da temperatura, pressão ou aditivos (ácidos e bases) (YU; LOU; WU, 2008). A decomposição da glicose ocorre por dois mecanismos: quebra de ligação ou desidratação. A desidratação da glicose gera furfurais e fenóis. A quebra de ligação da glicose produz ácidos, aldeídos e gases. Porém com a quebra de ligação dos furfurais e fenóis, também há produção de ácidos e aldeídos (SINAG; KRUSE; SCHWARZKOPF, 2003).

O processo de decomposição da hemicelulose na hidrólise é muito parecido com o da celulose. A decomposição ocorre de duas formas: desidratação e condensação retro-aldólica. Esta decomposição ocorre nos monômeros de xilose produzindo compostos aldeídos e acetonas. Portanto, a decomposição da celulose, hemicelulose e lignina das biomassas lignocelulósicas ocorrem conforme a temperatura do processo. A hemicelulose é hidrolisada a uma temperatura mais baixa e a celulose precisa de uma temperatura mais elevada (YU; LOU; WU, 2008).

2.4 PRODUÇÃO DE BIOCOMBUSTÍVEIS

As biomassas lignocelulósicas após processadas, apresentam várias aplicações em diferentes bioprocessos. Os processos de conversão em energia das biomassas são: combustão direta, gaseificação, pirólise, fermentação, digestão anaeróbica, liquefação, hidrólise, transesterificação e craqueamento (ABAIDE et al., 2019a; FARDIN; DE BARROS; DIAS, 2018).

A produção de biocombustíveis como etanol, biogás e biodiesel podem ser de diferentes gerações. A produção de biocombustíveis de 1ª geração utiliza cultura alimentares, sementes oleaginosas e gordura animal. Biocombustíveis de 2ª geração utilizam resíduos de materiais lignocelulósicos. Biocombustíveis de 3ª geração utilizam como fonte de matéria-prima as algas. Já a produção de biocombustíveis de 4ª geração utilizam algas e microrganismos como fonte de matéria-prima e são uma produção emergente pois as matérias que absorvem CO_2 são convertidas em combustíveis (PRASAD et al., 2019).

2.4.1 Produção de Etanol

A produção total de etanol (milho e cana-de-açúcar) no Brasil para safra 2019/2020 foi de 35,67 bilhões de litros. Já a estimativa para a safra 2020/2021 é de 32,85 bilhões de litros, apresentando uma redução de 7,9% em relação à safra passada. Esta redução na estimativa da produção de etanol é devido às indústrias da cana-de-açúcar terem sido afetadas pela pandemia do COVID-19, clima mais seco observado na safra e uma redução significativa na demanda por combustíveis, contrapondo as condições favoráveis de mercado para produção do açúcar (CONAB, 2020).

No Brasil, a produção de etanol é predominantemente realizada à base de açúcares. A produção de etanol de primeira geração é a mais clássica, utilizando açúcares e amido. Já a produção de etanol de segunda geração (2G) utiliza diferentes biomassas lignocelulósicas, mas este processo está em desenvolvimento e novas pesquisas devem ser realizadas para melhorar as etapas do processo (COOPER et al., 2020; SU et al., 2020).

A produção de etanol de segunda geração não é tão desenvolvida quanto a produção de primeira geração, mas apresenta um grande potencial pela disponibilidade de matéria-prima (ADITIYA et al., 2016). A produção de etanol depende de diversas rotas de produção, dentre elas: o pré-tratamento, hidrólise, fermentação e destilação. Cada rota de produção é muito importante, vai influenciar na eficiência, qualidade e nos custos de produção (ADITIYA et al., 2016).

O pré-tratamento das biomassas lignocelulósicas consiste na redução do tamanho das partículas das biomassas, melhorar a exposição dos componentes, melhorar a eficiência da hidrólise, evitar a degradação dos açúcares e formação de inibidores, redução do grau de cristalinidade da matriz de celulose e consequentemente minimizar os custos operacionais (ADITIYA et al., 2016; SARKAR et al., 2012). Os pré-tratamentos são divididos em biológicos, químicos, físicos e físico-químicos, tecnologia de fluido supercrítico, pré-tratamento ácido, alcalino, uso de líquidos iônicos, explosão de vapor, entre outros (ADITIYA et al., 2016).

A hidrólise é um processo de dissociação de carboidratos com adição de molécula de água, geralmente catalisada por enzima e ácido. A etapa da hidrólise é um processo de grande importância na produção de etanol, pois influenciará na qualidade do hidrolisado e disponibilizará os açúcares para os microrganismos utilizarem como substrato (ADITIYA et al., 2016).

Os açúcares obtidos no processo de hidrólise são destinados a etapa da fermentação. A fermentação é um processo importante na produção de etanol, pois ele é produzido diretamente pela atividade metabólica do agente de fermentação (ADITIYA et al., 2016). O agente de fermentação pode ser a levedura ou a bactéria (ADITIYA et al., 2016). A levedura *Saccharomyces* spp. é a principal escolha para a produção de etanol porque apresenta uma ótima capacidade fermentativa, tolerância a inibidores e crescimento rápido sob condições anaeróbicas (MUSSATTO et al., 2010). Os processos de fermentação normalmente empregados são a sacarificação e a fermentação simultânea (SSF) e a hidrólise e a fermentação separadas (SHF) (SARKAR et al., 2012), mas há outros processos como a sacarificação simultânea e a fermentação combinada (SSCF), hidrólise separada e co-fermentação (SHCF) e bioprocessamento consolidado (CBP) (PRASAD et al., 2019).

A destilação é a última etapa na produção de etanol. A destilação é necessária para a retirada do teor de água que é feita pela diferença dos pontos de ebulição da água e do etanol, originando o etanol de alta qualidade. O processo consiste no aquecimento do fermentado a 78,2 °C (ponto de ebulição do etanol), consequentemente o etanol é vaporizado e separado da água. Além disso, existem outros processos de destilação, tais como: processo de adsorção, destilação azeotrópica, desidratação química, destilação por difusão, destilação extrativa, processos de separação com membranas e destilação a vácuo (ADITIYA et al., 2016).

A produção de etanol de segunda geração apresentou resultados positivos utilizando diferentes tipos de biomassas lignocelulósicas: bagaço de cana de açúcar, alga (*Ulva prolifera*), palha de arroz e losna-branca (*Parthenium hysterophorus* (LI et al., 2016; SINDHU et al., 2016; TAVVA et al., 2016). Canabarro et al. (2017) avaliaram a otimização e a ampliação da produção de etanol utilizando o resíduo de farelo de arroz por sacarificação em estado sólido e fermentação. Primeiramente as fermentações foram realizadas em frascos Erlenmeyer para definir as melhores variáveis e depois realizou-se a fermentação em biorreator de leito fixo. A produção de etanol obtida pelo biorreator de leito fixo foi semelhante ao obtido pelos frascos Erlenmeyer, atingindo produção de 138,7 g/kg. Artifon et al. (2018) demostraram um aumento na produção de etanol de 1,1 g/L para 7,4 g/L de etanol, após a desintoxicação do hidrolisado de cana-de-açúcar via adsorção com carvão ativado e decapagem com ar seco para remoção de inibidores.

A casca de soja pré-tratada com líquido iônico, com hidrólise enzimática de um complexo enzimático de *Penicillium echinulatum* S1M29 (DSM 18942) e fermentação dos hidrolisados utilizando a levedura *Candida shehatae* HM 52.2 proporcionou a produção de etanol com uma produção de 6 g/L de etanol e um rendimento de 0,31 $g_{etanol}/ g_{açúcares}$ (DA

CUNHA-PEREIRA et al., 2016). DALL CORTIVO et al. (2020) avaliaram a produção de etanol utilizando a levedura *Spathaspora hagerdaliae* UFMG-CM-Y303 e os hidrolisados da mistura 1:1 de casca de soja e casca de aveia obtidos por hidrólises ácida, por hidrólise enzimática e a combinação da hidrólise ácida-enzimática. A levedura *Spathaspora hagerdaliae* UFMG-CM-Y303 proporcionou a produção de etanol e xilitol em todas as condições testadas.

2.4.2 Produção de Biogás

A produção de biogás ocorre pelo processo de digestão anaeróbica. A digestão anaeróbica é um processo metabólico em condições anaeróbicas e para a produção de biogás depende da atividade de vários microrganismos (KUNZ; STEINMETZ; AMARAL, 2019). O biogás é composto principalmente por metano e dióxido de carbono (CO_2) e também é composto, mas em pequenas quantidades por nitrogênio, hidrogênio, sulfeto de hidrogênio, amônia e vapor de água (NESHAT et al., 2017). O biogás produzido contém metano (50-75%) e dióxido de carbono (FARDIN; DE BARROS; DIAS, 2018).

O processo de digestão anaeróbica pode ser dividido em quatro etapas: hidrólise, acidogênese, acetogênese e metanogênese (KUNZ; STEINMETZ; AMARAL, 2019; NESHAT et al., 2017). O processo de hidrólise degrada os compostos complexos como lipídios, polissacarídeos, proteínas e ácidos nucléicos em substâncias orgânicas mais simples e solúveis, como os ácidos graxos, monossacarídeos, aminoácidos, purinas e pirimidinas (KUNZ; STEINMETZ; AMARAL, 2019; NESHAT et al., 2017). O processo ocorre pela ação das bactérias hidrolíticas e a velocidade e tempo de hidrólise é dependente da característica do substrato (KUNZ; STEINMETZ; AMARAL, 2019).

No processo de acidogênese, as substâncias geradas na hidrólise são degradadas em ácido orgânicos de cadeia curta, ácido acético, ácido propiônico, ácido butírico, álcoois, óxidos de nitrogênio, sulfeto de hidrogênio, hidrogênio e dióxido de carbono por diferentes bactérias anaeróbias e facultativas (KOTHARI et al., 2014; KUNZ; STEINMETZ; AMARAL, 2019). No processo de acetogênese, os ácidos de cadeia longa (ácido propiônico, ácido butírico, ácido lático) são degradados em ácidos com um ou dois átomos de carbono (ácido fórmico e ácido acético), e em conjunto produzem o hidrogênio e o dióxido de carbono pelas bactérias acetogênicas (KUNZ; STEINMETZ; AMARAL, 2019).

A etapa da metanogênese é a responsável pela produção do metano. A produção de metano ocorre a partir do ácido acético, etanol, metanol ou dióxido de carbono e hidrogênio

(KOTHARI et al., 2014). A produção do biogás é através das arqueas metanogênicas em duas vias metabólicas (acetoclásticas e hidrogenotróficas) (KUNZ; STEINMETZ; AMARAL, 2019). As metanogênicas acetoclásticas usam o acetato para a produção de metano e as metanogênicas hidrogênotroficas usam o hidrogênio e dióxido de carbono para produção de metano (KUNZ; STEINMETZ; AMARAL, 2019).

A co-digestão anaeróbica é um processo que consiste na mistura de dois materiais biodegradáveis (efluente de animal e material lignocelulósico) para melhorar a digestão anaeróbica. O objetivo do processo é compensar o baixo teor de carbono no efluente animal, com a mistura de um substrato rico em carbono, oferecendo uma relação C/N ideal para a digestão anaeróbica (NESHAT et al., 2017). A co-digestão anaeróbica de resíduos animais e lignocelulósicos é promissora para a produção de biogás, mas se tem a necessidade de buscar novos conhecimentos para melhorar o desempenho e a estabilidade do processo (NESHAT et al., 2017). Alguns trabalhos na literatura, mostram que o processo de co-digestão anaeróbica foi eficiente na produção de biogás utilizando diferentes efluentes de animais e resíduos lignocelulósicos (LI et al., 2015; RISBERG et al., 2013).

A co-digestão anaeróbica utilizando resíduos do processamento da soja (palha de soja, resíduos da extração de óleo de soja e terra de diatomácea, usada no branqueamento do óleo de soja), feno e efluente de um digestor anaeróbio foi investigada para a produção de biogás. Os resultados mostram que a mistura dos resíduos da soja e do feno melhoraram o rendimento de biogás, obtendo a maior produção em 258 L/kg de sólido volátil (ZHU et al., 2014). Kovačić et al. (2019) avaliaram a aplicação de eletroporação (técnica de dano seletivo da membrana biológica, através da exposição a um campo elétrico) nos resíduos da colheita de milho, soja e girassol, como pré-tratamento, para produção de biogás por co-digestão com esterco de vaca-leiteira. Os autores demonstram que o pré-tratamento dos resíduos pela eletroporoção melhora o processo de co-digestão para todos os resíduos. Venturin et al. (2018) avaliaram a produção de biogás dos caules de milho em co-digestão anaeróbica e obtiveram uma produção de biogás de 346,4 ± 5,6 e 644,2 ± 16,4 $L_{Nbiogas}/Kg_{VSad}$ para caules de milhos pré-tratados com ácido sulfúrico (H_2SO_4) e peróxido de hidrogênio (H_2O_2), respectivamente.

2.5 CONSIDERAÇÕES FINAIS

Diante do exposto nesta revisão da literatura, as biomassas lignocelulósicas são uma fonte alternativa de matéria-prima para produção de biocombustíveis, devido a sua

composição conter celulose, hemicelulose e lignina. A hidrólise com água subcrítica é um processo promissor na dissociação da celulose e hemicelulose para a produção de açúcares fermentescíveis. A produção de biocombustíveis é uma alternativa como fonte de energia renovável, pois utiliza matérias-primas renováveis que são, na maioria das vezes descartadas.

A busca por novas alternativas para os processos de hidrólise com água subcrítica e de produção de biocombustíveis é uma realidade para melhorar e otimizar os processos. Este trabalho vai ao encontro com a busca destas novas alternativas. O trabalho irá avaliar a hidrólise da palha e casca de soja com água subcrítica e a avaliar a produção de biocombustíveis dos hidrolisados obtidos. O diferencial deste estudo se dá na utilização dos resíduos da fermentação (hidrolisados fermentados) para avaliar o potencial bioquímico de biogás e metano. Portanto, esta pesquisa é relevante porque visa melhorar e otimizar as etapas de produção dos biocombustíveis.

CAPÍTULO 3 – ARTIGO 1

Neste capítulo estão apresentados os resultados relacionados ao cumprimento dos três primeiros objetivos desta Tese, que foram: obtenção e caracterização de palha e de casca de soja, avaliação das variáveis temperatura e razão massa líquido/sólido na hidrólise dos resíduos da soja com água subcrítica e a caracterização dos hidrolisados em termos de açúcares redutores totais, inibidores e ácidos orgânicos.

Subcritical water hydrolysis of soybean residues for obtaining fermentable sugars

Artigo publicado: The Journal of Supercritical Fluids
Vol. 167, p.105043, 2021
https://doi.org/10.1016/j.supflu.2020.105043

Felipe Vedovatto[a,d], Gustavo Ugalde[a], Charline Bonatto[b,e], Suzana F. Bazoti[b], Helen Treichel[b], Marcio A. Mazutti[a,c], Giovani L. Zabot[d], Marcus V. Tres[d]

[a] Department of Agricultural Engineering, Federal University of Santa Maria, 1000, Roraima av., Santa Maria, 97105-900, Brazil
[b] Laboratory of Microbiology and Bioprocess, Federal University of Fronteira Sul, 200, ERS 135 – Km 72, Erechim, 99700-970, Brazil
[c] Department of Chemical Engineering, Federal University of Santa Maria, 1000, Roraima av., Santa Maria, 97105-900, Brazil
[d] Laboratory of Agroindustrial Processes Engineering (LAPE), Federal University of Santa Maria, 1040, Sete de Setembro av., Cachoeira do Sul, 96506-322, Brazil
[e] Department of Chemical and Food Engineering, Federal University of Santa Catarina, Trindade, Florianópolis, 88040-900, Brazil

Abstract

The objective of this study was to evaluate subcritical water hydrolysis (SWH) of soybean straw and hull for obtaining fermentable sugars in a semi-continuous mode. The experimental conditions investigated were temperature (180, 220 and 260 °C) and liquid/solid mass (R) ratio (9 g and 18 g water/g straw and 7.5 g and 15 g water/g hull). The hydrolysis was performed for 15 min at 25 MPa. The characterization of residues after hydrolysis and reducing sugars yield (Y_{RS}), efficiency (E) and composition of hydrolysates were evaluated. The condition of 220 °C/R-18 and R-15 provided the highest Y_{RS} of 9.56 ± 0.53 g/100 g straw at 4 min and 10.15 ± 0.50 g/100 g hull at 3 min of hydrolysis. The efficiencies were 23.65 ± 1.32 g/100 g carbohydrates and 23.04 ± 1.14 g/100 g carbohydrates for soybean straw and hull, respectively. SWH modified the structure of the residues and allowed the production of fermentable sugars.

Keywords: bioproducts, fermentable sugars, hydrolysis, soybean residues, subcritical water.

1. Introduction

Soybean is an important source of food [1], which is rich in proteins, lipids, fiber, minerals and vitamins. Currently, the soybean culture is the most cultivated worldwide, with a global production of 352.64 million tons in 2017 [2]. In Brazil, the production was 115.03 million tons for the 2018/2019 harvest [3]. During soybean harvesting and grain processing residues, straw and hull are generated [1]. Generally, soybean residues are disposed of in the environment because the use as animal feed is limited [1,4].

Soybean straw and hull are considered lignocellulosic biomass because their chemical composition contains cellulose, hemicelluloses and lignin as major components [5]. Soybean straw and hull composition can vary according to the extraction process, ranging from 25 to 35% cellulose, 2.28 to 23% hemicelluloses and 1 to 17% lignin [1,6–8]. Lignocellulosic residues present the potential to obtain fermentable sugars [9–14], including soybean residues [4,7,15].

The disassociation of cellulose and hemicelluloses into sugars and secondary products can be performed by acid or enzymatic hydrolysis [16,17] or sub/supercritical hydrolysis [18]. The success of enzymatic hydrolysis depends on the enzymes, substrate

characteristics, and pretreatment. The biomass pretreatment has a high cost and influences on efficiency [19,20]. The disadvantage of acid hydrolysis is the need for acid recovery before fermentation, thus increasing the operational cost [16,21].

The subcritical water hydrolysis (SWH) process is an alternative for dissociation of cellulose and hemicelluloses into sugars and secondary products. The process has been investigated because it is a fast process, can avoid biomass pretreatment, it uses only water as a solvent, it does not generate solvent residues and can degrade less amount of sugars when compared to other methods [22,23]. The process is based on the critical temperature of water and pressure higher than its vapor saturation pressure [24]. The SWH has been efficiently reported for obtaining reducing sugars [25,26].

Even though, the SWH needs further studies to optimize the process, as kinetics studies, mathematical models, operational parameters, and step reduction (temperature and pressure) [23,27,28]. Likewise, soybean residues have potential as lignocellulosic biomass for fermentable sugars production, but there is still a lack of studies on the use of these residues in biorefineries [29]. The composition of residues in terms of hemicellulose, cellulose and lignin tends to influence the dissociation of polysaccharides into fermentable sugars. Therefore, the present work aims to evaluate the SWH of soybean residues (straw and hull) to obtain fermentable sugars for biofuels production in a semi-continuous mode. Characterization of soybean residues, evaluation of experimental conditions of hydrolysis (temperature and liquid/solid ratio), and characterization of hydrolysates medium (sugars, inhibitors and organic acids) by high-performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM) are presented.

2. Materials and methods

2.1 Soybean straw and hull

The residues were obtained in the northwest region of the state of Rio Grande do Sul/Brazil. The soybean straw was collected directly from the farm after harvested. The soybean hull was obtained from a grain-processing unit (Olfar, Erechim, Brazil). The residues were oven-dried (Lucadema, model LUCA 80/27, Brazil) at 60 °C until constant weight and ground in a Willey Knife Mills equipment (Solab, model SL 30, Brazil) with a 20 mesh separation grid. Afterward, they were sieved and the particles

higher than 0.25 mm were used to avoid clogging of pipes. The residues were packed in plastic bags and stored in a refrigerator (Fricon, model HCED 503-C, Brazil) at -5 °C until the experimental assays.

2.2 Reagents and solvents

The reagents and solvents of analytical grade were acquired at local commerce. Sulfuric acid (72%), 3,5- dinitrosalicylic acid, sodium hydroxide 2N, phenol, potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$), acetonitrile (99.9%), sulfuric acid (99.9%), D-glucose, D-xylose, D-arabinose, D-cellobiose, acetic acid, formic acid, HMF, and furfural were used. Distilled water was obtained from a laboratory distiller (Solab, SL-71, Brazil). Ultrapure water was obtained from a purifier system (Merck, Direct-Q, Germany).

2.3 Soybean straw and hull characterization

The methodology of Association of Official Analytical Chemists was used for the determination of moisture and ash [30]. For moisture determination content (%), 5 g of residue was placed in an oven dryer (Lucadema, model LUCA 80/27, Brazil). For ash determination, 5 g of residue was weighed and placed in melting pots. The samples were calcined in an oven (Fornitec, model MDS 15X15X30, Brazil) at 400°C for 1 h and 800 °C for 2 h. The ash was determined by mass difference. The moisture content and ash determinations were performed in triplicate for each residue (straw and hull soybean).

The methodology of National Renewable Energy Laboratory was also used for determination of cellulose, hemicelluloses, and lignin [31]. 0.3 g of fresh residue was weighed individually. An aliquot of the hydrolysis solution was used to measure the absorbance in a UV-Visible spectrophotometer (BEL, model UV-M51, Italy) to determine soluble lignin. The remaining hydrolysis solution was used to determine cellulose and hemicelluloses. After filtering, the filter paper + solid was rinsed with ultra-pure water. Subsequently, it was oven-dried (Lucadema, model LUCA 80/27, Brazil) at 105 °C until constant weight for the determination of insoluble lignin. The determinations of cellulose, hemicellulose, and lignin from straw and hull were performed in quadruplicate.

2.4 Subcritical water hydrolysis

The SWH procedure was based on scientific papers [26,32] and performed individually for each biomass. The experimental unit used in the hydrolysis process consisted of a high-pressure pump (Jasco, model PU4087, Japan), a thermostatic bath (Solab, model SL-152, Brazil), a flow non-return valve, a reactor (Citua, Brazil) manufactured with 316L stainless steel with an internal volume of 50 mL and height to internal diameter ratio of 6 (height of 13.2 cm and diameter of 2.2 cm) capable of supporting 60 MPa, a ceramic band heater, and a micrometering valve.

In each hydrolysis assay, 25 g of straw or 30 g of hull was used. After loading the reactor with straw or hull, distilled water was pumped until reaching a pressure of 25 MPa. Thereafter, the reactor heating was started until reaching the desired temperature. The micrometering valve was partially opened to maintain the pressure at 25 MPa during the heating. For hydrolysis in semi-continuous mode, the experimental conditions tested in the work were temperatures of 180, 220 and 260 °C and flow rates of 15 and 30 mL/min, corresponding to final liquid/solid ratios (R) of 9 g and 18 g water/g straw and 7.5 g and 15 g water/g hull, respectively. A total of 4 samples were collected at intervals of 0.5 min until reaching 2 min. The other 8 samples were collected at intervals of 1 min from 2 to 10 min. One final sample was collected in 15 min. The hydrolyzed solutions obtained in each interval were evaluated for reducing sugars, efficiency of carbohydrates conversion, compositions of sugars, HMF, furfural and organic acids, and pH. The solid residues after SWH were oven-dried (Lucadema, model LUCA 80/27, Brazil) at 105 °C until reaching constant weight and subsequently analyzed by Fourier-transform infrared spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). All the assays were performed in duplicate and completely randomized, totalizing 12 assays.

2.5 Hydrolyzed solution analysis

2.5.1 Determination of reducing sugars

The reducing sugars were analyzed by dinitrosalicylic acid (DNS) colorimetric method [33]. The samples obtained in each assay were analyzed once. The calibration

curve was prepared with glucose using a concentration range from 0.10 to 1.00 g/L of glucose. For analysis, 1 mL of sample and 1 mL of DNS reagent were mixed and were heated in a water bath with boiling water at 100 °C for 5 min. After, 8 mL of potassium sodium tartrate solution (15.1 g/L) was added and the solution was cooled in a water bath (25 °C). The absorbance was taken in a spectrophotometer (Shimadzu, model UV-2700, Japan) at 540 nm. The reducing sugars yield (Y_{RS}); g sugar equivalents in glucose /100 g residue) was calculated according to Equation 1 and the efficiency E (g sugar /100 g carbohydrates (hemicellulose and cellulose)) was calculated according to Equation 2.

$$Y_{RS} = \frac{m_{RS}}{m_{SA}} x\ 100 \quad (1)$$

$$E = \frac{m_{RS}}{m_{CA}} x\ 100 \quad (2)$$

Where: m_{RS} is the mass (g) of reducing sugars in the hydrolyzed solution, m_{SA} is the initial mass (g) of residue loaded in the reactor vessels at the beginning of the hydrolysis process, and m_{CA} is the mass (g) of carbohydrates (hemicellulose and cellulose).

The results of Y_{RS} and E were evaluated by Analysis of Variance and Tukey's test for comparison of means ($p < 0.05$) using the Statistica 7.0® (StatSoft, Inc) software.

2.5.2 pH

The pH was determined for each sample of using a digital pHmeter (Lucadema, model Luca-210, Brazil) and the samples reading was done at 20 °C and the pHmeter was calibrated before the readings with the standards of pH 4, 7 and 10. The determination of pH was performed once for each sample of each assay.

2.5.3 Analysis of sugars

For the determination of sugars (glucose, xylose, arabinose, and cellobiose), an HPLC (Shimadzu, model Proeminence UFLC- Nexera XR, Japan) equipped with a refractive index detector (RID 10A, Shimadzu, Japan) was used. An amino column Asahipak NH2P-50 (250 mm x 4.6 mm i.d.) (Asahi Kasei, Tokyo, Japan) was used at

50 °C. The mobile phase was prepared with a solution of acetonitrile:water (69:31) 1% H_3PO_4 prepared with ultrapure water and it was filtered under vacuum using 0.45 μm and 47 mm diameter porosity cellulose esters (Millipore, USA). After, it was degassed in an ultrasonic bath (USC-1400, Unique, São Paulo, Brazil). The volumetric flow rate of the mobile phase was 0.5 mL/min and the injection volume of samples was 15 μL. The compounds concentration was obtained by the correlation between the areas of the chromatograms and calibration curves previously determined by standards of components (D-glucose, D-xylose, D-arabinose, and D-cellobiose). The calibration curves were prepared in concentrations ranging from 0.05 to 20.00 g/L for all sugars. The samples were analyzed once for collected samples of each experimental condition.

2.5.4 Analysis of organic acids

The analytical methodology for the determination of organic acids (acetic acid and formic acid) was based on Bazoti et al [34]. The samples were analyzed by HPLC (Shimadzu, model LCMS-2020, Japan) equipped with a refractive index detector RID 10-A and an AMINEX® BIORAD HPX87H column. Samples of 20 μL were analyzed by chromatography at 45 °C and the flow rate of the mobile phase was 0.6 mL/min. The compounds concentration was obtained by the correlation between the areas of the chromatograms and calibration curves previously determined by standards of components (acetic acid and formic acid). The calibration curves were prepared in concentrations ranging from 0.06 to 0.4 g/L of acetic acid and from 0.07 to 0.5 g/L of formic acid. The samples were analyzed once for collected samples of each experimental condition.

2.5.5 Analysis of inhibitors

The determination of inhibitors (hidroximetilfurfural (HMF) and furfural) was based on Fleig et al. [35]. The samples were analyzed by HPLC (Shimadzu, model Proeminence UFLC- Nexera XR, Japan) equipped with a photodiode array detector (PDA 20-A, Shimadzu, Japan) and a Shim-Pak ODS C18 column (Shimadzu, Japan). The conditions for the analysis were 10 μL of injection volume, flow rate of 0.8 mL/min, column temperature of 30 °C, wavelength of 280 nm, detector temperature of 30 °C, and run time of 10 min. The compounds concentration was obtained by the

correlation between the areas of the chromatograms and calibration curves previously determined by standards of components (HMF and furfural). The calibration curves were prepared in concentrations ranging from 0.0005 to 0.05 g/L of HMF and from 0.005 to 0.1 g/L of furfural. The samples were analyzed once for collected samples of each experimental condition.

2.6 Characterization of solid residues

2.6.1 Analysis of Fourier-transform infrared spectroscopy

The solid residues obtained after SWH were analyzed by FT-IR spectroscopy (Shimadzu, model IRPrestige-21, Japan) with a nominal resolution of 4 cm^{-1} and within the spectral range between 4000-100 cm^{-1}. The disks were prepared with 100 mg of KBr and 1 mg of the sample, macerated and mixed into smooth agate gravel and pressing under 8 tons until the disks were thin with a thickness of approximately 1 mm and 13 mm in diameter. For the analysis of solid residues, one sample of each experimental condition was analyzed.

2.6.2 Scanning electron microscopy

The morphology of samples was taken by a scanning electron microscope (Tescan, model VEGA-3G, Czech Republic). The fresh residues (soybean straw and hull) and a sample of the condition of 220 °C/R-15 (higher Y_{RS} and E) processed by SWH were analyzed by scanning electron microscopy.

3. Result and discussion

3.1 Residues characterization

The soybean straw and hull compositions are shown in Table 1. The values obtained for cellulose, hemicellulose and lignin were close to those found in the literature [1,6–8]. The values of lignin for both residues were high, with 24.12 ± 2.60% for soybean straw and 30.60 ± 1.94% for soybean hull. These relatively high values are a consequence of the fraction used in the hydrolysis. After milled, soybean residues

were sieved and the particles higher than 0.25 mm were used to avoid clogging of pipes. Consequently, the particles were concentrated in lignin since other more fragile substances than lignin could be separated with the fraction of fine particles (< 0.25 mm). The ash contents in soybean straw and hull were 4.29 ± 0.03% and 4.65 ± 0.29%, respectively, which were close to the values reported by Wan, Zhou, Li [36] and Rojas et al. [7]. Overall, it is difficult to make a straight comparison of the chemical compositions of lignocellulosic biomasses because they can vary depending on the cultivar type and climatic conditions of cultivation [37].

Table 1. Composition of fresh soybean straw and hull.

Residues	Components (%, dry basis)				
	Cellulose	**Hemicellulose**	**Lignin**	**Ash**	**Moisture (%)**
Soybean straw	22.69 ± 0.17	17.73 ± 0.13	24.12 ± 2.60	4.30 ± 0.10	6.94 ± 0.40
Soybean hull	26.46 ± 0.18	17.59 ± 0.13	30.60 ± 1.94	4.65 ± 0.29	6.34 ± 0.22

3.2 Reducing sugars and efficiencies

The Y_{RS} and E of SWH of soybean straw and hull (Table 2 and 3) presented a significant difference ($p < 0.05$) between the conditions studied. The conditions of 220 °C/R-18 and 220 °C/R-15 presented the highest Y_{RS} and E for soybean straw and hull, respectively. In the SWH of soybean straw for 4 min, it was obtained Y_{RS} of 9.56 ± 0.53 g/100 g. In the SWH of soybean hull for 3 min, it was obtained Y_{RS} of 10.15 ± 0.50 g/100 g. At 15 min, no significant differences ($p < 0.05$) for reducing sugars and efficiencies of SWH of soybean straw and hull were observed for the conditions of 220 °C/R-9 and R-7.5 and 220 °C/R-18 and R-15. This behavior is most likely associated with the recovery of dissociated compounds at the beginning of hydrolysis applying such temperature. Consequently, no matter the changes are done in the operational conditions near the end of reaction time (15 min).

Table 2. Y_{RS} and efficiency of SWH of soybean straw at 25 MPa in the different experimental conditions.

Assay*	Conditions	Y_{RS} (g /100 g soybean straw)		E (g /100 g carbohydrates)	
		4 min	15 min	4 min	15 min
1	180 °C/R-9	2.35 ± 0.83^{c}	3.97 ± 0.04^{b}	5.80 ± 2.06^{c}	9.83 ± 0.11^{b}
2	180 °C/R-18	2.00 ± 0.23^{c}	2.40 ± 0.03^{b}	4.96 ± 0.57^{c}	5.94 ± 0.08^{b}
3	220 °C/R-9	6.72 ± 0.94^{b}	8.63 ± 0.99^{a}	16.64 ± 2.34^{b}	21.35 ± 2.46^{a}
4	220 °C/R-18	9.56 ± 0.53^{a}	10.27 ± 0.58^{a}	23.65 ± 1.32^{a}	25.40 ± 1.42^{a}
5	260 °C/R-9	2.32 ± 0.26^{c}	4.17 ± 0.49^{b}	5.75 ± 0.64^{c}	10.38 ± 1.21^{b}
6	260 °C/R-18	3.14 ± 0.62^{c}	4.90 ± 1.19^{b}	7.77 ± 1.54^{c}	12.12 ± 2.87^{b}

*Averages followed by the same letter, in the same column, do not differ by the Tukey's test at 5 % of significance.

Y_{RS}: reducing sugars yield

E: efficiency

R: liquid/solid mass ratio

Table 3. Y_{RS} and efficiency of SWH of soybean hull at 25 MPa in the different experimental conditions.

Assay*	Conditions	Y_{RS} (g /100 g soybean hull)		E (g /100 g carbohydrates)	
		3 min	15 min	3 min	15 min
1	180 °C/R-7.5	0.69 ± 0.07^{d}	1.66 ± 0.30^{cd}	1.56 ± 0.17^{d}	3.77 ± 0.79^{cd}
2	180 °C/R-15	0.69 ± 0.04^{d}	0.97 ± 0.06^{d}	1.57 ± 0.08^{d}	2.20 ± 0.14^{d}
3	220 °C/R-7.5	7.18 ± 0.59^{b}	9.21 ± 0.02^{a}	16.31 ± 1.34^{b}	20.94 ± 0.04^{a}
4	220 °C/R-15	10.15 ± 0.50^{a}	10.52 ± 0.41^{a}	23.04 ± 1.14^{a}	23.88 ± 0.94^{a}
5	260 °C/R-7.5	2.41 ± 0.27^{c}	4.24 ± 0.97^{b}	5.47 ± 0.62^{c}	9.62 ± 2.21^{b}
6	260 °C/R-15	2.94 ± 0.59^{c}	3.57 ± 0.86^{bc}	6.68 ± 1.33^{c}	8.11 ± 1.95^{bc}

*Averages followed by the same letter, in the same column, do not differ by the Tukey's test at 5 % of significance.

Y_{RS}: reducing sugars yield

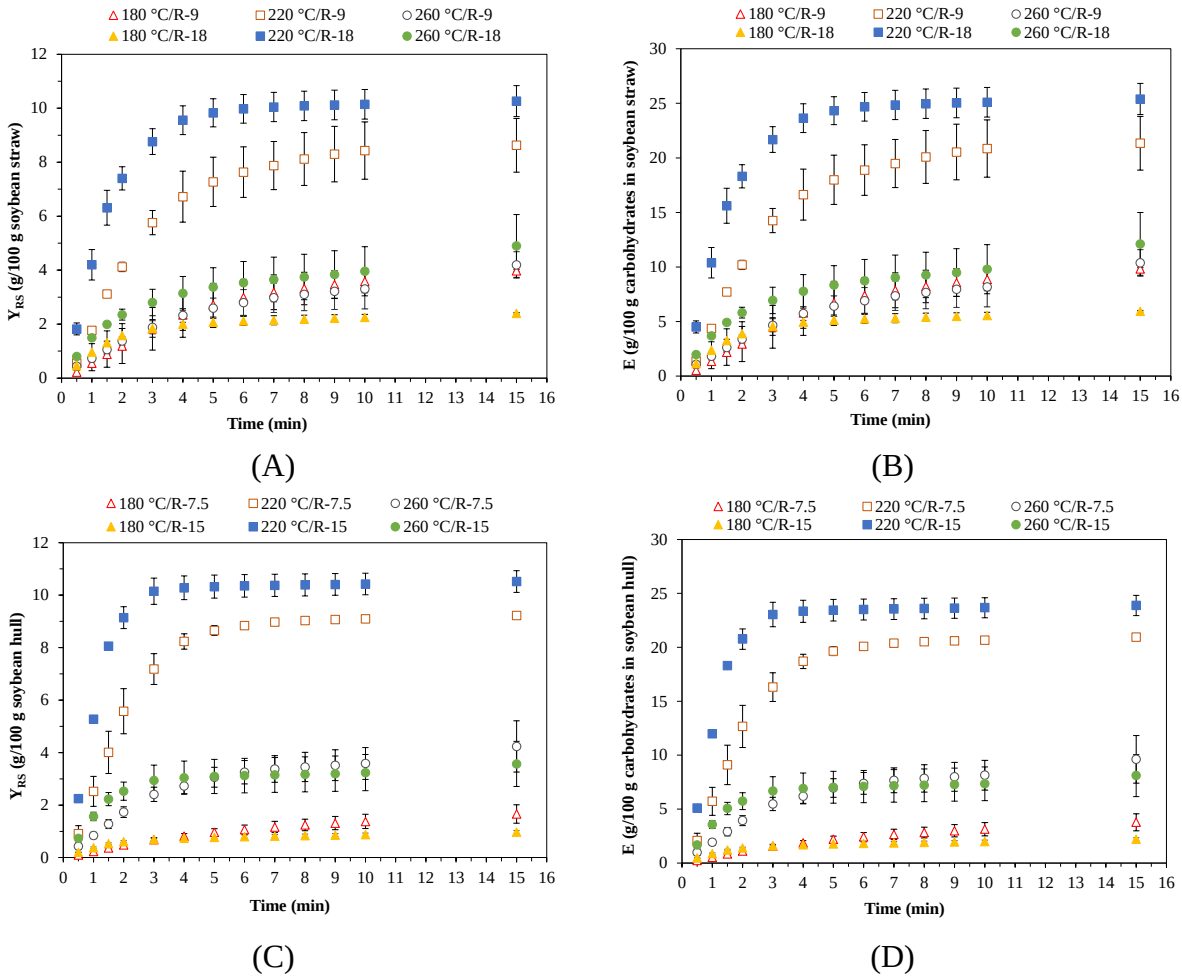
E: efficiency

R: liquid/solid mass ratio

The E (Table 2 and 3) presented a behavior similar to Y_{RS}, presenting a significant difference ($p < 0.05$) between the conditions. In the SWH of soybean straw and hull, the conditions 220 °C/R-18 and 220 °C/R-15 presented the highest E, with 23.65 ± 1.32 g/100 g carbohydrates at 4 min and 23.04 ± 1.14 g/100 g de carbohydrates at 3 min, respectively. In the evaluation of E at 15 min, the conditions 220 °C/R-9 and R-7.5 and 220 °C/R-18 and R-15 did not present a significant difference ($p < 0.05$) for SWH of residues. Based on these results, it is suggested that an intermediary temperature is sufficient to act in the hydrolysis of hemicellulose and cellulose with only water as reactant, thus no needing the use of a larger temperature, such as 260 °C.

In the kinetic profiles of soybean straw and hull (Fig. 1), the 220 °C/R-18 and 220 °C/R-15 conditions presented the highest Y_{RS} and E. The profile shown for 220 °C/R-18 condition (Fig. 1A) for straw demonstrates an almost constant rate of hydrolysis reaction in the beginning. It kept increasing the reducing sugars yield until 4 min, obtaining Y_{RS} of 9.56 ± 0.53 g/100 g soybean straw. For the other conditions, the hydrolysis reaction continued increasing with a small rate until stabilization at 15 min. For 220 °C/R-18, Y_{RS} of 10.27 ± 0.58 g/100 g soybean straw was reached at 15 min, presenting only an increase of reducing sugars of 0.71 g/100 g soybean straw from 4 to 15 min. The increase is not pronounced in this period because most of the reducing sugars have been recovered until 4 min.

Figure 1. Kinetic profile of Y_{RS} and E of hydrolyzed solution of soybean straw (A and B) and hull (C and D) at 25 MPa in the different experimental conditions.



The kinetic behavior of 220 °C/R-15 for SWH of soybean hull (Fig. 1C) shows an increase of hydrolysis reaction until 3 min, with Y_{RS} of 10.15 ± 0.50 g/100 g soybean hull. The hydrolysis reaction rate reduced from 3 to 15 min, which only presented an increase of reducing sugars of 0.37 g /100 g soybean hull. The other experimental conditions presented Y_{RS} values below 5 g/100 g soybean straw and 4 g/100 g soybean hull. Indeed, the behaviors of kinetics shown for both types of soybean residues were similar. Only small differences are observed in the initial rates of the hydrolysis reaction.

The kinetic profiles (Fig. 1B and 1D) of efficiency were similar to Y_{RS}. The 220 °C/R-18 and 220 °C/R-15 conditions, corresponding to a flow rate of 30 g/min, showed higher efficiencies in the hydrolysis process than the other conditions. Such flow rate was the highest level tested in this work, which suggests that lower residence times are suitable to dissociate the polymers and to avoid the degradation of sugars into other compounds. The influence of

liquid/solid ratios mainly occurred in the first minutes of the hydrolysis reaction, like 3-4 min. For hydrolysis reactions longer than 4 min, the increase in efficiency seems to be independent of the amount of water in contact with soybean residues.

The condition 260 °C obtained higher E and Y_{RS} compared to the condition of 180 °C (Fig. 1). Overall, although the absolute values of these responses were higher for 260 °C, no significant differences ($p < 0.05$) were achieved (Table 2 and 3). The increase of temperature from 180 °C to 220 °C favored the production of fermentable sugars in the SWH of soybean straw and hull (Fig. 1). Optimized temperatures and pressures can modify the physicochemical properties of subcritical water, increasing the ionic product (H^+ and OH^-), decreasing viscosity, and increasing diffusivity, consequently facilitating the penetration into the complex lignocellulosic matrix. Subcritical water presents non-polar solvent characteristics, increasing the solubility of organic compounds, and presenting a process rapid for converting cellulose and hemicellulose into sugars in short reaction times. However, in longer hydrolysis reactions, degradation of products can occur, resulting in complex mixtures [23,27,38,39].

In the study reported by Watchararuji et al. [40], SWH of rice bran and soybean meal (raw and defatted) presented the potential for obtaining reducing sugars, proteins, and amino acids. The condition of 220 °C, extraction time of 20 min and liquid/solid ratio of 1:5 showed higher Y_{RS} for all biomasses. The amount of reducing sugars from soybean residues was below rice residues, with a maximum of 47 mg/g soybean meal.

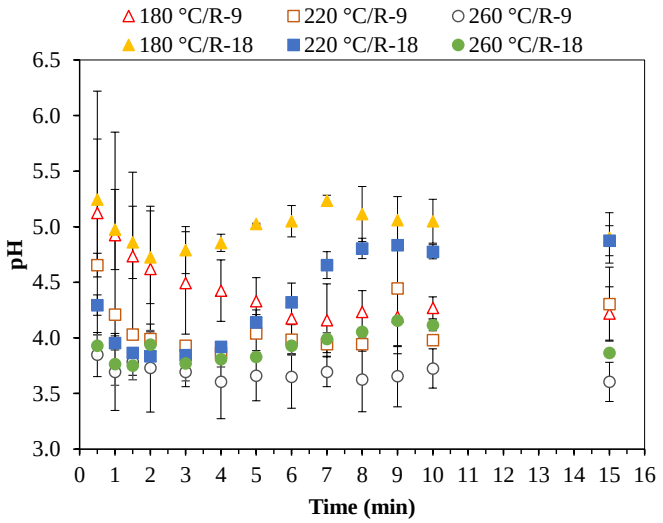
In the same trend, SWH of rice straw and husks was efficient for obtaining fermentable sugars [26,32]. The condition that obtained the highest Y_{RS} was 220 °C/R-7.5 with 33.6 ± 4.3 g/100 g rice straw [26] and 18.3 ± 2.1 g/100 g rice hull [32]. In both studies, the kinetic profiles were similar to this work (Fig. 1), with a reaction rate higher at the beginning of hydrolysis (up to 7 min). This hydrolysis reaction behavior was also observed in SWH of sugarcane bagasse [39,41]. Furthermore, other studies with different lignocellulosic biomass presented a production of reducing sugars using temperatures close to 200 °C. Mayanga-Torres et al. [42] using coffee power and defatted cake observed high values of Y_{RS} after hydrolysis at 175 °C and 22.5 MPa. In the study reported by Cardenas-Toro et al. [24], the temperature of 250 °C and pressure of 15 MPa provided high production of reducing sugars in SWH of palm fiber, with 22.9 g glucose/100 g carbohydrates.

In the SWH process of soybean residues, the increase of temperature from 220 °C to 260 °C showed a lower final yield (Fig. 1), which could be the consequence of degradation of reducing sugars. For example for soybean straw, the condition of 260 °C/R-18 yielded

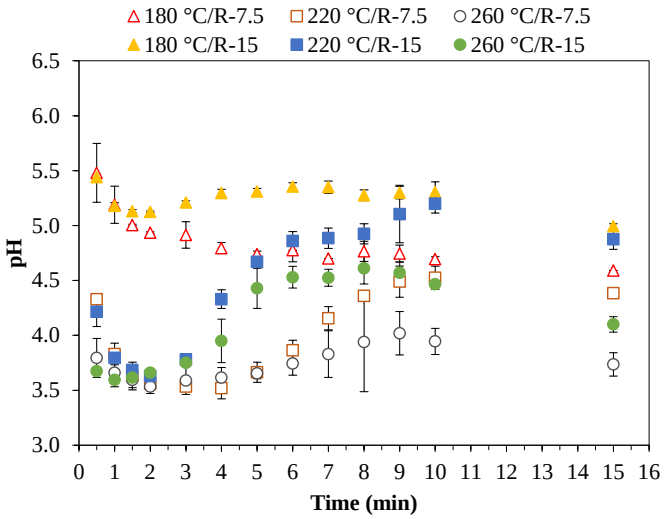
approximately 52% lower reducing sugars than the condition of 220 °C/R-18. The same comparison can be done for soybean hull, where the reducing sugars were approximately 66% lower in the highest temperature. In terms of energetic evaluation, these findings are interesting because it is possible to process soybean residues by SWH expending lower energy.

The results for the pH of hydrolyzed soybean straw and hull (Fig. 2) were influenced by temperature. The conditions processed at 180 °C resulted in higher values of pH if compared to 220 °C and 260 °C. In the condition of 220 °C/R-18 (highest Y_{RS} and E) for soybean straw (Fig. 2A), the pH reduced from 4.29 ± 0.09 to 3.92 ± < 0.01 for the reaction until 4 min. Thereafter, the pH increased from 4 min to 15 min. This behavior was also observed for the condition of 220 °C/R-15 (highest Y_{RS} and E) for soybean hull (Fig. 2B). The pH reduced from 4.21 ± 0.13 to 3.78 ± 0.03 for the reaction until 3 min and increased to 4.87 ± 0.09 at 15 min. The reduction of pH at the beginning of hydrolysis is related to the reaction rates of dissociation of cellulose and hemicellulose and degradation of reducing sugars, where most of the organic acids were also produced. These behaviors and explanations of pH were also reported for SWH of sugarcane bagasse [39], rice straw [26,43], and rice hull [32].

Figure 2. Kinetic profile of pH of hydrolyzed solution of soybean straw (A) and hull (B) at 25 MPa in the different experimental conditions.



(A)



(B)

3.3 Composition of hydrolyzed solution

The composition of the hydrolyzed solution of soybean residues (Figs. 3, 4 and 5) was influenced by temperature and the liquid/solid ratio. The conditions of 180 °C/R-9 and 220 °C/R-7.5 presented higher sugars yield for both hydrolysates at 4 and 3 min, respectively. The yield of sugars in short reaction times was similar to those reported in the literature [26,32]. The SWH has the advantage of obtaining high sugar yield in short reaction times and avoids the degradation in other products [44].

Figure 3. Production of sugars and inhibitors obtained of subcritical water hydrolysis of soybean straw at 25 MPa in the different experimental conditions

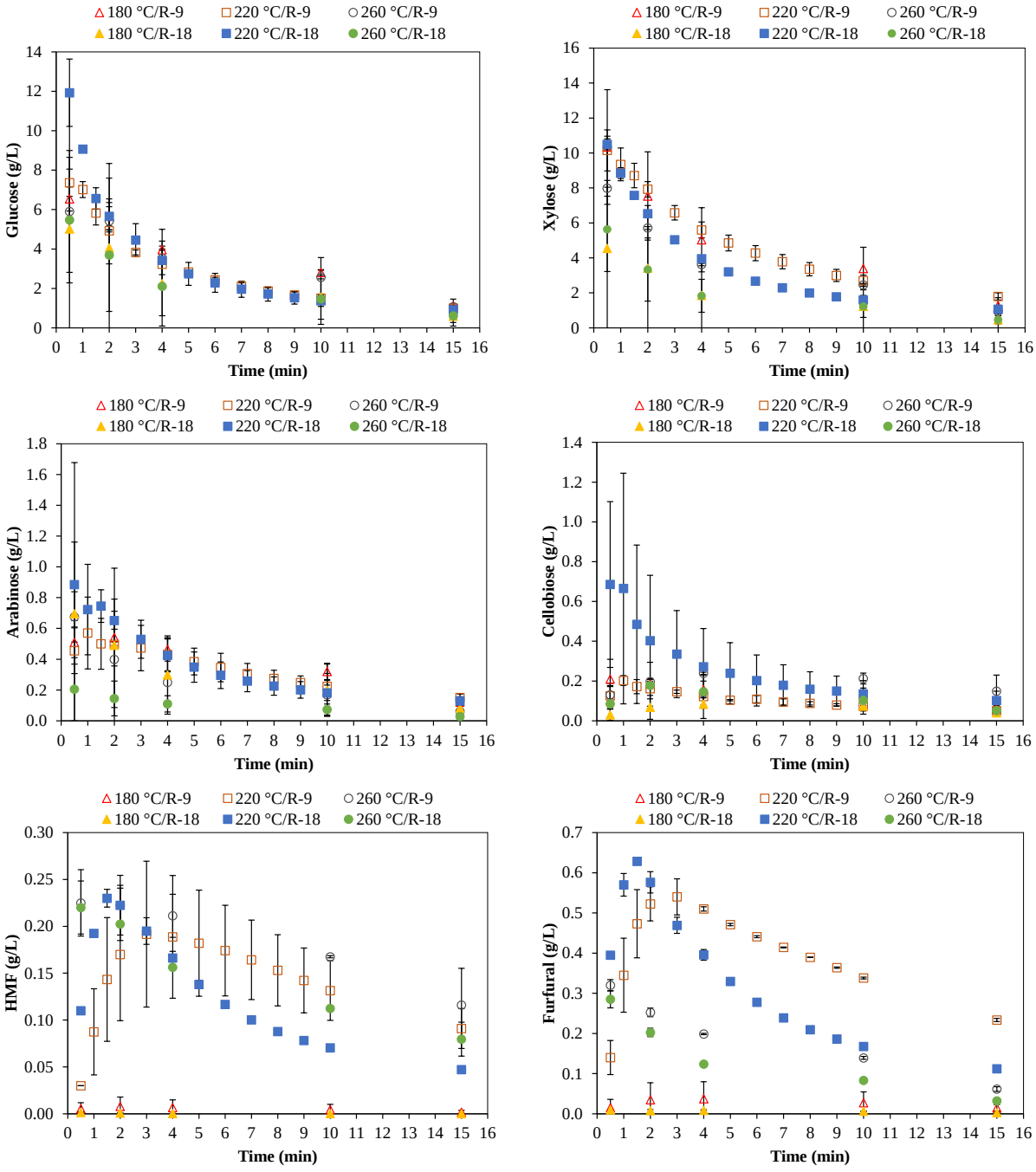


Figure 4. Production of sugars and inhibitors obtained of subcritical water hydrolysis of soybean hull at 25 MPa in the different experimental conditions.

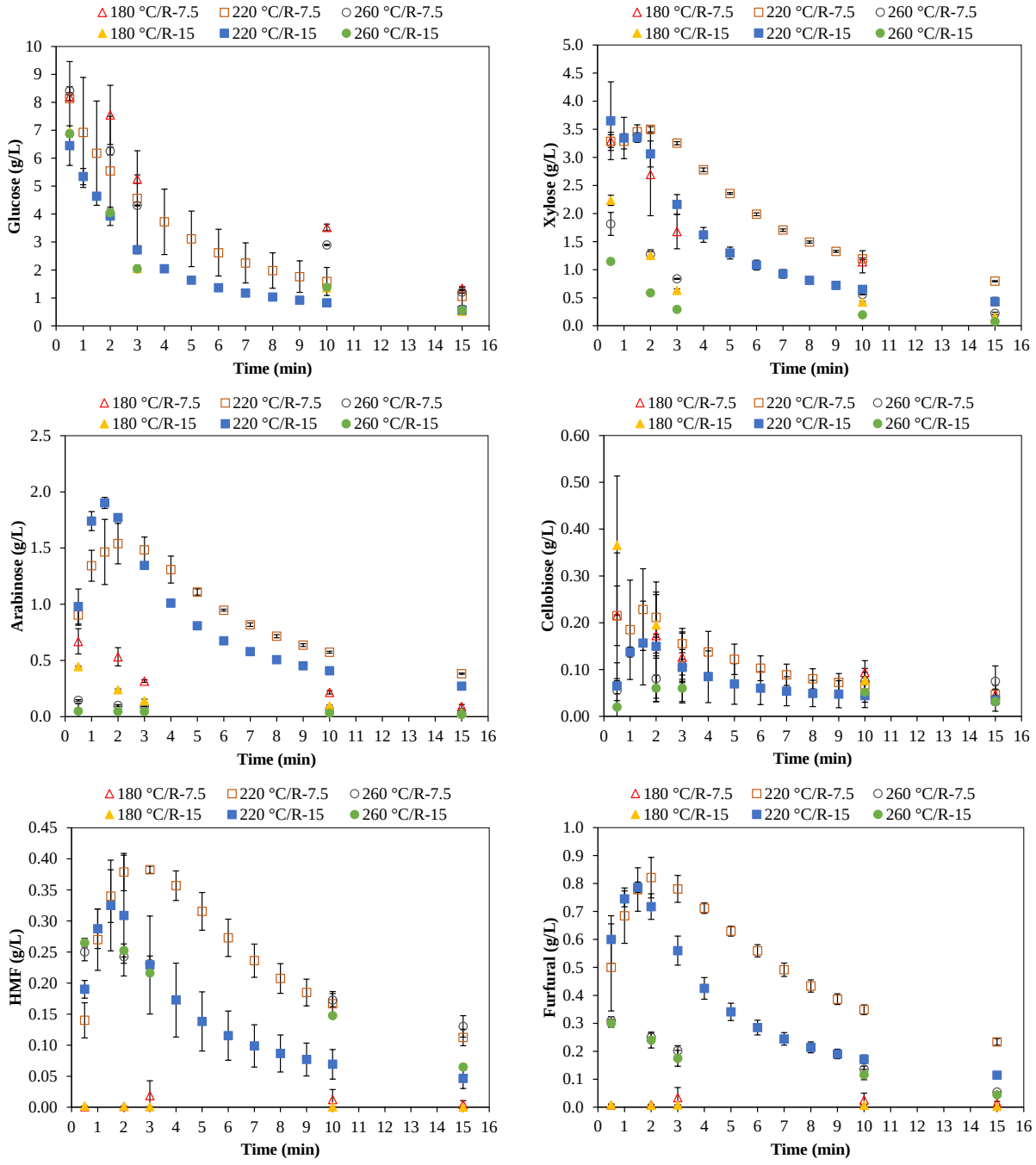
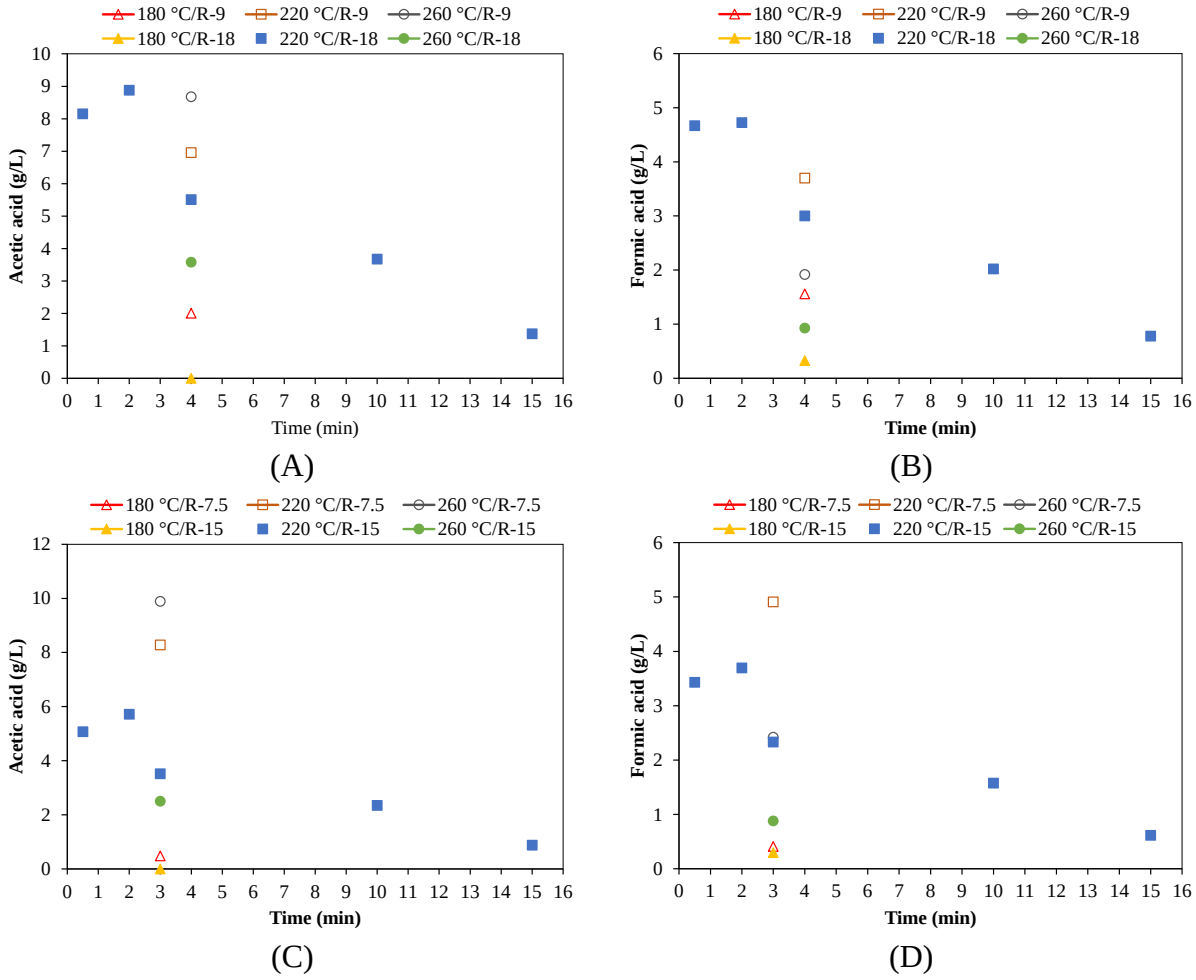


Figure 5. Production of organic acids obtained of subcritical water hydrolysis of soybean straw (A and B) and hull (C and D) at 25 MPa in the different experimental conditions.



The ratios of 9 g water/g soybean straw and 7.5 g water/g soybean hull produced a higher yield of specific sugars and bioproducts when compared to the condition of 18 g water/g soybean straw and 15 g water/g soybean hull (Figs. 3, 4 and 5). Lower ratios have lower water flows when compared to higher ratios, thus resulting in longer residence times of reactant in the reactor vessel. Overall, this longer contact of water with polysaccharides resulted in yields of xylose, furfural, and organic acids slightly higher than the yields of other compounds in 3-4 min of reaction times.

The temperatures of 180 °C and 220 °C increased the levels of sugars, inhibitors and organic acids for all hydrolyzed solutions (Figs. 3, 4 and 5). As stated elsewhere, the increase in temperature provides higher efficiency in the hydrolysis process [45,46]. In the condition of 220 °C, xylose and arabinose were produced with higher yields than 180 °C and 260 °C (Fig. 3 and 4). The hemicellulose is responsible for the production of C5 (xylose and arabinose) and C6 (galactose, glucose, and mannose) sugars [44]. The hydrolysis of hemicellulose occurs

between 150 °C to 200 °C and the decomposition of sugars in byproducts commonly occurs when the temperature is increased from 175 °C to 200 °C for SWH of industrial coffee residues [42].

According to the results of sugars content (Figure 3), there is a similarity between the amount of xylose and glucose in the hydrolysate. It most likely was obtained because the hydrolysis of cellulose was partial in the current work. If the hydrolysis of all polymers would be complete, the content of glucose could be higher than that one reported herein. However, a partial degradation into other compounds also occurred during the process, thus resulting in approximately 2.6 g sugars (mainly glucose and xylose) recovered from 25 g soybean straw and 3.2 g sugars recovered from 30 g soybean hull for the condition 220 °C/R-18. Considering all conditions, the extent of polymers that were not converted into monomers or that were degraded accounted for 74-94% for straw and 76-97% for hull at 15 min of reaction. For example, in the condition of 220 °C/R-18 at 15 min, inhibitors (0.05 g/L HMF and 0.11 g/L furfural) and organic acids (1.38 g/L acetic acid and 0.78 g/L formic acid) were produced by the degradation of sugars (Figures 3, 5A and 5B).

In the process of SWH, the condition of 220 °C/R-7.5 resulted in higher production of organic acids for both hydrolysates (Fig. 5). The yields of acetic acid and formic acid were 6.96 g/L and 3.70 g/L for the hydrolysate of soybean straw at 4 min. The hydrolysate of soybean hull resulted in 8.28 g/L of acetic acid and 4.91 g/L formic acid at 3 min. The production of acetic acid and formic acid is influenced by the temperature and is directly associated with the reduction of the pH of the hydrolysates [44].

The conditions of 220 °C/R-9 and 220 °C/R-18 presented a total yield of 0.70 ± 0.08 g/L and 0.57 ± 0.02 g/L (HMF + furfural) for the hydrolysate of soybean straw at 4 min (Fig. 3), respectively. The conditions of 220 °C/R-7.5 and 220 °C/R-15 presented a total of 1.16 ± 0.06 g/L and 0.79 ± 0.13 g/L (HMF + furfural) for the hydrolysate of soybean hull at 3 min (Fig. 4), respectively. The conditions 180 °C/R-9, 180 °C/R-7.5, 180 °C/R-18 and 180 °C/R-15 showed a production (HMF + furfural) less than 0.05 g/L at 4 min and 3 min for hydrolysates of soybean straw and hull, respectively. Temperatures above 200 °C can result in the production of HMF and furfural, which can cause the decomposition of sugars in other products [38].

The increase of temperature from 220 °C to 260 °C caused a decrease in sugars and an increase of inhibitors and organic acids (Figs. 3, 4 and 5). The use of very high temperatures provides hydrolysis of cellulose and hemicelluloses, but it degrades the sugars [41]. In the SWH of rice straw, the increase from 250 °C to 260 °C provided an increase in sugar yield,

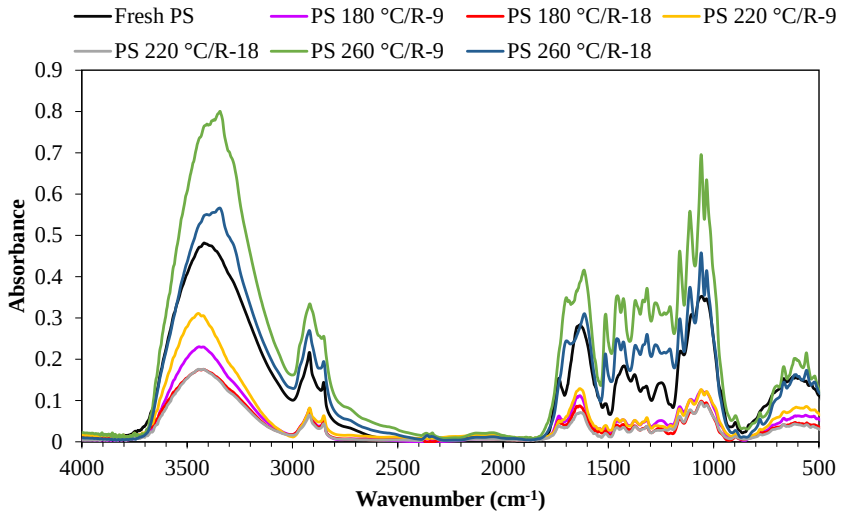
but when used 300 °C, it presented degradation of glucose and xylose into 2-ethyl tetra-hydrofuran and acetic acid [44]. Also, the high concentration of products of degradation can form insoluble products, consequently causing the clogging of pipes, valves and filters [41].

3.4 Analysis of solids after SWH

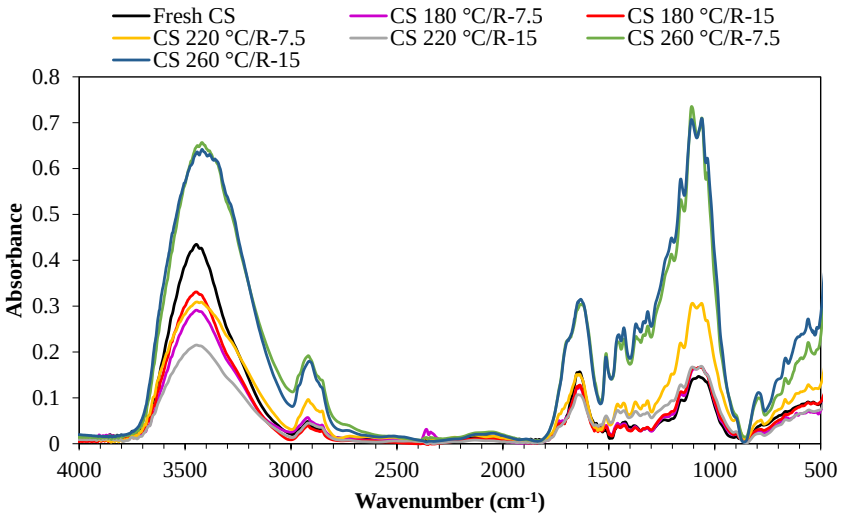
3.4.1 Analysis of spectrophotometry

The evaluation of FT-IR was performed for soybean straw and hull solid residues before and after SWH. The FT-IR spectra of soybean straw and hull (Fig. 6) presented different band intensities for fresh residues and the solids residues after SWH. The different intensities indicate the removal of different components. For example, the samples from the condition of 260 °C presented the highest intensity between the peaks from 2900 to 3500 cm^{-1} and from 800 to 1700 cm^{-1} compared to the samples from the conditions of 220 °C and 180 °C. The samples processed at 180 °C resulted in an opposite behavior to the condition of 260 °C, showing lower intensity between the peaks from 2900 to 3500 cm^{-1} and from 800 to 1700 cm^{-1} and indicating lower compound degradation during the hydrolysis. The removal compound in the condition of 220 °C was intermediate.

Figure 6. FT-IR spectroscopy of fresh and processed soybean straw (A) and hull (B) in the different experimental conditions; PS: soybean straw; CS: soybean hull.



(A)



(B)

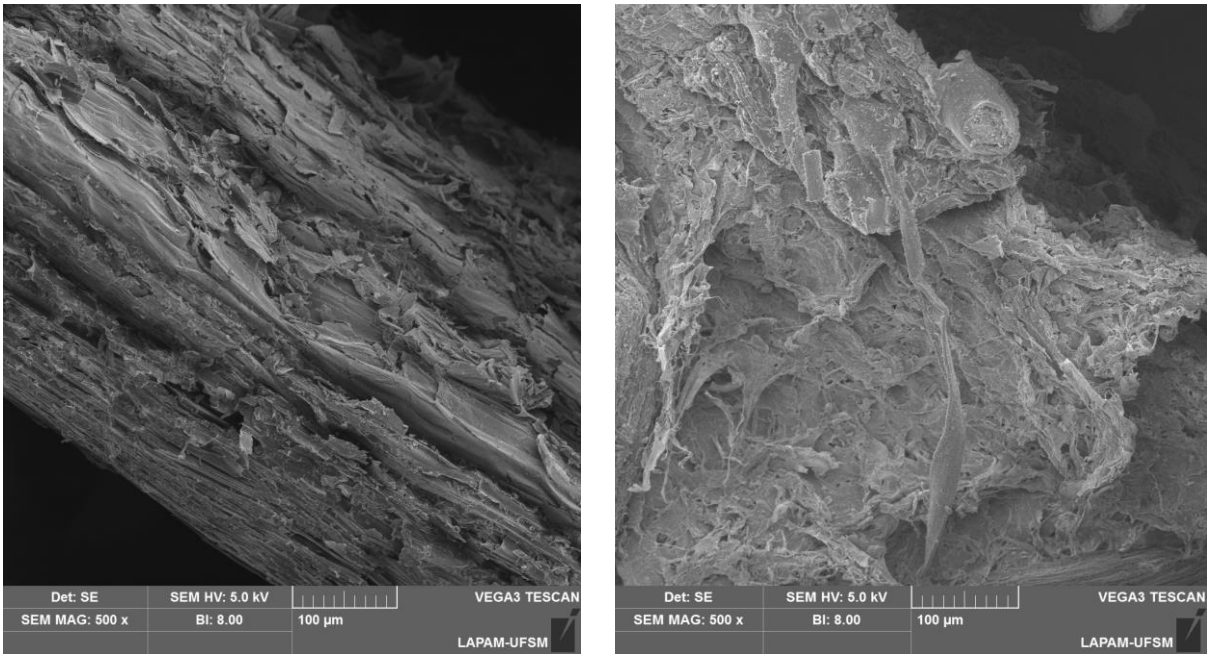
The soybean straw and hull spectra (Fig. 6) observed in the region of approximately 3400 cm^{-1} are attributed to the vibration of the elongation O-H, corresponding to lignin [47,48]. The highest intensity for samples processed at 260 °C can be attributed to a higher dissociation of hemicellulose [41,49], consequently leaving the lignin more exposed in the solids residues, thus corroborating the findings discussed in the previous sections. The region of 1100 cm^{-1} is attributed to the vibration of C-O, C=C and C-C-O corresponding to the polymer of cellulose. According to scientific literature, the increase of temperature also provided higher dissociation of hemicellulose and cellulose found in sugarcane bagasse [41]. The region of 1600 cm^{-1} is attributed to the C=O functional group [47]. We can observe that

the region around 1600 cm^{-1} (1500-1800 cm^{-1}) was also influenced by SWH conditions, especially temperature. The carbonyl stretch in the solid residues was influenced by the hydrolysis. Hydrolytic stripping of acetyl side chains present on hemicellulose could have occurred. In such case, ketones and aldehyde groups associated with lignin remained in the solid material, mainly in lower temperatures. These inferences are correlated with the decrease of pH for the hydrolyzed solutions processed at 180 °C, in which the acetyl side chains tend to produce acetic acid, thus decreasing the pH.

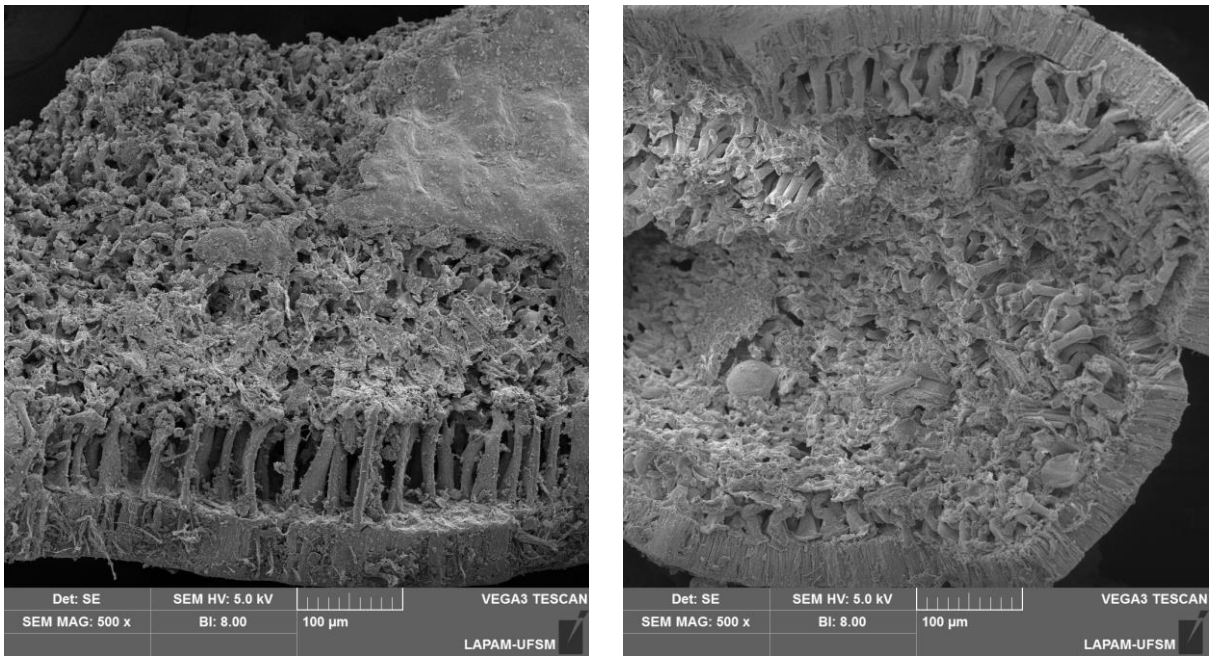
3.4.2 Scanning electron microscope

The morphologic structure of residues before and after SWH in the conditions 220 °C/R-18 and 220 °C/R-15 were observed using SEM (Fig. 7). The samples (Fig. 7A and 7C) showed a consistent morphology with the cell wall before hydrolysis. The images indicate that the microstructures of samples before SWH were not modified with drying and grinding. The untreated hull and straw evidenced rigid and compact surface structures and an intact morphology. After SWH, the samples (Fig. 7B and 7D) showed a morphology with ruptured and twisted vessels. The structural modification of residues is directly linked to the production of higher Y_{RS} and efficiency for both residues in the condition of 220 °C/R-18 and 220 °C/R-15. The structural modification also indicated that the temperature used in SWH could cause the breakdown of polymers. Hemicellulose and cellulose were dissociated into sugars, inhibitors, and organic acids.

Figure 7. SEM images of solid samples of fresh (A) and processed soybean straw (B), and fresh (C) and processed soybean hull (D); SWH at 25 MPa in the condition of 220 °C/R-18 for soybean straw and 220 °C/R-15 for soybean hull.



(A) (B)



(C) (D)

The analysis by SEM demonstrated differences in the morphologic structure before and after SWH of residues. This observation agrees with previous results, mainly related to FT-IR. In Fig 7B, hairy like structures were seen, which might be lignin, a structure that is not targeted during hydrolysis reaction. The hydrolysis resulted in the exposure of internal structure, with visibly separated microfibers. Likewise, the modification of morphology after

SWH of coffee wastes was reported [42]. SWH also changed the morphology of sugarcane bagasse, disrupted, and twisted the lignocellulosic structure with the increase of temperature [41]. Hydrothermal fractionation-hydrolysis processes of grape seeds influenced in morphology, with the formation of carbon spheres in the solid residue at 340 °C [50].

4. Conclusion

The SWH of soybean straw and hull showed to be a potential technique for the production of reducing sugars. The conditions of 220 °C/R-18 and 220 °C/R-15 provided higher Y_{RS} and efficiencies for both residues. The highest Y_{RS} and efficiency were obtained at 4 and 3 min for soybean straw and hull, respectively. The 220 °C provided a higher yield of specific sugars (glucose, xylose, arabinose, cellobiose) and inhibitors (HMF and furfural) evaluated by HPLC when compared to 180 °C and 260 °C. With FT-IR and MEV analyses, modifications of solid biomasses structure were seen after SWH. Therefore, SWH seems to be a clean and promising technology for obtaining fermentable sugars and bioproducts from soybean culture residues.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The authors thank National Council of Technological and Scientific Development (CNPq) and Support Foundation of the State of Rio Grande do Sul (FAPERGS) for the support and financial support. The authors also thank Coordination for the Improvement of Higher Education Personnel (CAPES) for scholarships. H. Treichel, M. A. Mazutti (303482/2015-0), M. V. Tres (308936/2017-5) and G. L. Zabot (304882/2018-6) thank CNPq for the productivity grants.

REFERENCES

[1] E. Cabrera, M.J. Muñoz, I. Caro, A.B. Díaz, C. Curbelo, R. Martín, Comparison of industrially viable pretreatments to enhance soybean straw biodegradability, Bioresour. Technol. 194 (2015) 1–6. https://doi.org/10.1016/j.biortech.2015.06.090.

[2] FAOSTAT-Food and Agriculture Organization of the United Nations, Production of Crops, (2017). http://www.fao.org/faostat/en/#data/QC (accessed January 6, 2020).

[3] CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO., Acompanhamento da safra brasileira de grãos, v. 7 Safra 2019/2020 - Terceiro levantamento, Brasília, 2019.

[4] A. Cassales, P.B. de Souza-Cruz, R. Rech, M.A. Záchia Ayub, Optimization of soybean hull acid hydrolysis and its characterization as a potential substrate for bioprocessing, Biomass and Bioenergy. 35 (2011) 4675–4683. https://doi.org/10.1016/j.biombioe.2011.09.021.

[5] Q. Qing, Q. Guo, L. Zhou, X. Gao, X. Lu, Y. Zhang, Comparison of alkaline and acid pretreatments for enzymatic hydrolysis of soybean hull and soybean straw to produce fermentable sugars, Ind. Crops Prod. 109 (2017) 391–397. https://doi.org/10.1016/j.indcrop.2017.08.051.

[6] Z. Xu, Q. Wang, Z.H. Jiang, X. xin Yang, Y. Ji, Enzymatic hydrolysis of pretreated soybean straw, Biomass and Bioenergy. 31 (2007) 162–167. https://doi.org/10.1016/j.biombioe.2006.06.015.

[7] M.J. Rojas, P.F. Siqueira, L.C. Miranda, P.W. Tardioli, R.L.C. Giordano, Sequential proteolysis and cellulolytic hydrolysis of soybean hulls for oligopeptides and ethanol production, Ind. Crops Prod. 61 (2014) 202–210. https://doi.org/10.1016/j.indcrop.2014.07.002.

[8] A. Merci, A. Urbano, M.V.E. Grossmann, S. Mali, C.A. Tischer, Properties of microcrystalline cellulose extracted from soybean hulls by reactive extrusion, Food Res. Int. 73 (2015) 38–43. https://doi.org/10.1016/j.foodres.2015.03.020.

[9] F.C. Lunelli, P. Sfalcin, M. Souza, E. Zimmermann, V. Dal Prá, E.L. Foletto, S.L. Jahn, R.C. Kuhn, M.A. Mazutti, Ultrasound-assisted enzymatic hydrolysis of sugarcane bagasse for the production of fermentable sugars, Biosyst. Eng. 124 (2014) 24–28. https://doi.org/10.1016/j.biosystemseng.2014.06.004.

[10] J.M. Moscon, W.L. Priamo, D. Bilibio, J.R.F. Silva, M. Souza, F. Lunelli, E.L. Foletto, R.C. Kuhn, A. Cancelier, M.A. Mazutti, Comparison of conventional and alternative technologies for the enzymatic hydrolysis of rice hulls to obtain fermentable sugars, Biocatal. Agric. Biotechnol. 3 (2014) 149–154. https://doi.org/10.1016/j.bcab.2014.07.008.

[11] R. Sindhu, M. Kuttiraja, T.P. Prabisha, P. Binod, R.K. Sukumaran, A. Pandey, Development of a combined pretreatment and hydrolysis strategy of rice straw for the production of bioethanol and biopolymer, Bioresour. Technol. 215 (2016) 110–116. https://doi.org/10.1016/j.biortech.2016.02.080.

[12] S.S.M.D. Tavva, A. Deshpande, S.R. Durbha, V.A.R. Palakollu, A.U. Goparaju, V.R. Yechuri, V.R. Bandaru, V.S.R. Muktinutalapati, Bioethanol production through separate hydrolysis and fermentation of Parthenium hysterophorus biomass, Renew. Energy. 86 (2016) 1317–1323. https://doi.org/10.1016/j.renene.2015.09.074.

[13] A. Procentese, F. Raganati, G. Olivieri, M. Elena Russo, A. Marzocchella, Pre-treatment and enzymatic hydrolysis of lettuce residues as feedstock for bio-butanol

production, Biomass and Bioenergy. 96 (2017) 172–179. https://doi.org/10.1016/j.biombioe.2016.11.015.

[14] E.R. Abaide, M. V. Tres, G.L. Zabot, M.A. Mazutti, Reasons for processing of rice coproducts: Reality and expectations, Biomass and Bioenergy. 120 (2019) 240–256. https://doi.org/10.1016/j.biombioe.2018.11.032.

[15] H. Han, L. Wei, B. Liu, H. Yang, J. Shen, Optimization of biohydrogen production from soybean straw using anaerobic mixed bacteria, Int. J. Hydrogen Energy. 37 (2012) 13200–13208. https://doi.org/10.1016/j.ijhydene.2012.03.073.

[16] S. Brethauer, C.E. Wyman, Review: Continuous hydrolysis and fermentation for cellulosic ethanol production, Bioresour. Technol. 101 (2010) 4862–4874. https://doi.org/10.1016/j.biortech.2009.11.009.

[17] A. Limayem, S.C. Ricke, Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects, Prog. Energy Combust. Sci. 38 (2012) 449–467. https://doi.org/10.1016/j.pecs.2012.03.002.

[18] L. Luft, T.C. Confortin, I. Todero, G. Ugalde, G.L. Zabot, M.A. Mazutti, Transformation of residual starch from brewer's spent grain into fermentable sugars using supercritical technology, J. Supercrit. Fluids. 140 (2018) 85–90. https://doi.org/10.1016/j.supflu.2018.06.006.

[19] P. Alvira, E. Tomás-Pejó, M. Ballesteros, M.J. Negro, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review, Bioresour. Technol. 101 (2010) 4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093.

[20] S. Sun, S. Sun, X. Cao, R. Sun, The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials, Bioresour. Technol. 199 (2016) 49–58. https://doi.org/10.1016/j.biortech.2015.08.061.

[21] D. Chiaramonti, M. Prussi, S. Ferrero, L. Oriani, P. Ottonello, P. Torre, F. Cherchi, Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method, Biomass and Bioenergy. 46 (2012) 25–35. https://doi.org/10.1016/j.biombioe.2012.04.020.

[22] G. Zhu, X. Zhu, Q. Fan, X. Wan, Recovery of biomass wastes by hydrolysis in sub-critical water, Resour. Conserv. Recycl. 55 (2011) 409–416. https://doi.org/10.1016/j.resconrec.2010.12.012.

[23] J.M. Prado, D. Lachos-Perez, T. Forster-Carneiro, M.A. Rostagno, Sub- A nd supercritical water hydrolysis of agricultural and food industry residues for the production of fermentable sugars: A review, Food Bioprod. Process. 98 (2016) 95–123. https://doi.org/10.1016/j.fbp.2015.11.004.

[24] F.P. Cardenas-Toro, T. Forster-Carneiro, M.A. Rostagno, A.J. Petenate, F. Maugeri Filho, M.A.A. Meireles, Integrated supercritical fluid extraction and subcritical water hydrolysis for the recovery of bioactive compounds from pressed palm fiber, J. Supercrit. Fluids. 93 (2014) 42–48. https://doi.org/10.1016/j.supflu.2014.02.009.

[25] J. Liang, X. Chen, L. Wang, X. Wei, H. Wang, S. Lu, Y. Li, Subcritical carbon dioxide-water hydrolysis of sugarcane bagasse pith for reducing sugars production, Bioresour. Technol. 228 (2017) 147–155. https://doi.org/10.1016/j.biortech.2016.12.080.

[26] E.R. Abaide, S.R. Mortari, G. Ugalde, A. Valério, S.M. Amorim, M. Di Luccio, R. de F.P.M. Moreira, R.C. Kuhn, W.L. Priamo, M. V. Tres, G.L. Zabot, M.A. Mazutti, Subcritical water hydrolysis of rice straw in a semi-continuous mode, J. Clean. Prod. 209 (2019) 386–397. https://doi.org/10.1016/j.jclepro.2018.10.259.

[27] M.J. Cocero, Á. Cabeza, N. Abad, T. Adamovic, L. Vaquerizo, C.M. Martínez, M.V. Pazo-Cepeda, Understanding biomass fractionation in subcritical & supercritical water, J. Supercrit. Fluids. 133 (2018) 550–565. https://doi.org/10.1016/j.supflu.2017.08.012.

[28] Ž. Knez, M.K. Hrnčič, M. Čolnik, M. Škerget, Chemicals and value added compounds from biomass using sub- and supercritical water, J. Supercrit. Fluids. 133 (2018) 591–602. https://doi.org/10.1016/j.supflu.2017.08.011.

[29] A. Abdulkhani, P. Alizadeh, S. Hedjazi, Y. Hamzeh, Potential of Soya as a raw material for a whole crop biorefinery, Renew. Sustain. Energy Rev. 75 (2017) 1269–1280. https://doi.org/10.1016/j.rser.2016.10.082.

[30] AOAC - Association of Official Analytical Chemists, Official methods of analysis, 16th ed., Washington, USA, 1997.

[31] NREL, Biomass Compositional Analysis Laboratory Procedures, (n.d.). https://www.nrel.gov/bioenergy/biomass-compositional-analysis.html (accessed March 10, 2018).

[32] E.R. Abaide, G. Ugalde, M. Di Luccio, R. de F.P.M. Moreira, M. V. Tres, G.L. Zabot, M.A. Mazutti, Obtaining fermentable sugars and bioproducts from rice husks by subcritical water hydrolysis in a semi-continuous mode, Bioresour. Technol. 272 (2019) 510–520. https://doi.org/10.1016/j.biortech.2018.10.075.

[33] G. L. Miller, Use of DinitrosaIicyIic Acid Reagent for Determination of Reducing Sugar, Anal. Chem. 31 (1959) 426–428.

[34] S.F. Bazoti, S. Golunski, D. Pereira Siqueira, T. Scapini, É.T. Barrilli, D. Alex Mayer, K.O. Barros, C.A. Rosa, B.U. Stambuk, S.L. Alves, A. Valério, D. de Oliveira, H. Treichel, Second-generation ethanol from non-detoxified sugarcane hydrolysate by a rotting wood isolated yeast strain, Bioresour. Technol. 244 (2017) 582–587. https://doi.org/10.1016/j.biortech.2017.08.007.

[35] L.P.T. Olivia Paniz Fleig, Emília Savioli Lopes, Elmer Ccopa Rivera, Rubens Maciel Filho, Concept of rice husk biorefining for levulinic acid production integrating three steps: Multi-response optimization, new perceptions and limitations, Process Biochem. 65 (2018) 146–156. https://doi.org/https://doi.org/10.1016/j.procbio.2017.11.015.

[36] C. Wan, Y. Zhou, Y. Li, Liquid hot water and alkaline pretreatment of soybean straw for improving cellulose digestibility, Bioresour. Technol. 102 (2011) 6254–6259. https://doi.org/10.1016/j.biortech.2011.02.075.

[37] P.R.D. Cortivo, L.R. Hickert, R. Hector, M.A.Z. Ayub, Fermentation of oat and soybean hull hydrolysates into ethanol and xylitol by recombinant industrial strains of Saccharomyces cerevisiae under diverse oxygen environments, Ind. Crops Prod. 113 (2018) 10–18. https://doi.org/10.1016/j.indcrop.2018.01.010.

[38] D. Lachos-Perez, G.A. Tompsett, P. Guerra, M.T. Timko, M.A. Rostagno, J. Martínez, T. Forster-Carneiro, Sugars and char formation on subcritical water hydrolysis of sugarcane straw, Bioresour. Technol. 243 (2017) 1069–1077. https://doi.org/10.1016/j.biortech.2017.07.080.

[39] J.M. Prado, L.A. Follegatti-Romero, T. Forster-Carneiro, M.A. Rostagno, F. Maugeri Filho, M.A.A. Meireles, Hydrolysis of sugarcane bagasse in subcritical water, J. Supercrit. Fluids. 86 (2014) 15–22. https://doi.org/10.1016/j.supflu.2013.11.018.

[40] K. Watchararuji, M. Goto, M. Sasaki, A. Shotipruk, Value-added subcritical water hydrolysate from rice bran and soybean meal, Bioresour. Technol. 99 (2008) 6207–6213. https://doi.org/10.1016/j.biortech.2007.12.021.

[41] D. Lachos-Perez, F. Martinez-Jimenez, C.A. Rezende, G. Tompsett, M. Timko, T. Forster-Carneiro, Subcritical water hydrolysis of sugarcane bagasse: An approach on solid residues characterization, Elsevier B.V., 2016. https://doi.org/10.1016/j.supflu.2015.10.019.

[42] P.C. Mayanga-Torres, D. Lachos-Perez, C.A. Rezende, J.M. Prado, Z. Ma, G.T. Tompsett, M.T. Timko, T. Forster-Carneiro, Valorization of coffee industry residues by subcritical water hydrolysis: Recovery of sugars and phenolic compounds, J. Supercrit. Fluids. 120 (2017) 75–85. https://doi.org/10.1016/j.supflu.2016.10.015.

[43] R. Lin, J. Cheng, L. Ding, W. Song, F. Qi, J. Zhou, K. Cen, Subcritical water hydrolysis of rice straw for reducing sugar production with focus on degradation by-products and kinetic analysis, Bioresour. Technol. 186 (2015) 8–14. https://doi.org/10.1016/j.biortech.2015.03.047.

[44] Y. Yu, X. Lou, H. Wu, Some Recent Advances in Hydrolysis of Biomass in Hot-Compressed Water and Its Comparisons with Other Hydrolysis Methods, Energy and Fuels. 22 (2008) 46–60. https://doi.org/10.1021/ef700292p.

[45] P.C. Torres-Mayanga, S.P.H. Azambuja, M. Tyufekchiev, G.A. Tompsett, M.T. Timko, R. Goldbeck, M.A. Rostagno, T. Forster-Carneiro, Subcritical water hydrolysis of brewer's spent grains: Selective production of hemicellulosic sugars (C-5 sugars), J. Supercrit. Fluids. 145 (2019) 19–30. https://doi.org/10.1016/j.supflu.2018.11.019.

[46] T.F.-C. Francisco W. Maciel-Silva, Solange L. Mussato, Integration of subcritical water pretreatment and anaerobic digestion technologies for valorization of açai processing industries residues, J. Clean. Prod. 228 (2019) 1131–1142. https://doi.org/https://doi.org/10.1016/j.jclepro.2019.04.362.

[47] F. Xu, J. Yu, T. Tesso, F. Dowell, D. Wang, Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: A mini-review, Appl. Energy. 104 (2013) 801–809. https://doi.org/10.1016/j.apenergy.2012.12.019.

[48] Z. Liu, L. Meng, J. Chen, Y. Cao, Z. Wang, H. Ren, The utilization of soybean straw III: Isolation and characterization of lignin from soybean straw, Biomass and Bioenergy. 94 (2016) 12–20. https://doi.org/10.1016/j.biombioe.2016.07.021.

[49] M.S.N. dos Santos, G.L. Zabot, M.A. Mazutti, G.A. Ugalde, K. Rezzadori, M.V. Tres, Optimization of subcritical water hydrolysis of pecan wastes biomasses in a semi-continuous mode, Bioresource Technology. 306 (2020) 123129. https://doi.org/10.1016/j.biortech.2020.123129.

[50] F.M. Yedro, J. García-Serna, D.A. Cantero, F. Sobrón, M.J. Cocero, Hydrothermal fractionation of grape seeds in subcritical water to produce oil extract, sugars and lignin, Catal. Today. 257 (2015) 160–168. https://doi.org/10.1016/j.cattod.2014.07.053.

CAPÍTULO 4 – ARTIGO 2

Neste capítulo estão apresentados os resultados relacionados ao cumprimento do quarto e quinto objetivos desta Tese, que foram: avaliar a produção de etanol dos hidrolisados da palha e casca de soja usando a levedura *Wickerhamomyces* sp. UFFS-CE-3.1.2 via processo fermentativo e avaliar a produção de biogás usando os resíduos da fermentação via co-digestão anaeróbica.

Production of biofuels from soybean straw and hull hydrolysates obtained by subcritical water hydrolysis

Artigo publicado: Bioresource Technology
Vol. 328, p.124837, 2021.
https://doi.org/10.1016/j.biortech.2021.124837

Felipe Vedovatto[a,b], Charline Bonatto[c,d], Suzana F. Bazoti[c], Bruno Venturin[e], Sérgio L. Alves Jr.[f], Airton Kunz[c,e,g], Ricardo L. R. Steinmetz[g], Helen Treichel[c], Marcio A. Mazutti[a,h], Giovani L. Zabot[b], Marcus V. Tres[b]

[a] Department of Agricultural Engineering, Federal University of Santa Maria, 1000, Roraima av., Santa Maria, 97105-900, Brazil

[b] Laboratory of Agroindustrial Processes Engineering (LAPE), Federal University of Santa Maria, 1040, Sete de Setembro av., Cachoeira do Sul, 96506-322, Brazil

[c] Laboratory of Microbiology and Bioprocess, Federal University of Fronteira Sul, 200, ERS 135 – km 72, Erechim, 99700-970, Brazil

[d] Department of Chemical and Food Engineering, Federal University of Santa Catarina, Trindade, Florianópolis, 88040-900, Brazil

[e] Western Paraná State University, R. Universitária, Cascavel, 85819-110, Brazil

[f] Laboratory of Biochemistry and Genetics, Federal University of Fronteira Sul, Rodovia SC 484 – Km 02, Chapecó, 89815-899, Brazil

[g] Embrapa Suínos e Aves, BR 153 – Km 110, Concórdia, 89710-028, Brazil

[h] Department of Chemical Engineering, Federal University of Santa Maria, 1000, Roraima av., Santa Maria, 97105-900, Brazil

Abstract

The objective of this study was to evaluate the ethanol production by *Wickerhamomyces* sp. using soybean straw and hull hydrolysates obtained by subcritical water hydrolysis and, afterward, the biogas production using the fermented hydrolysates. Ethanol was produced using the straw and hull hydrolysates diluted and supplement with glucose, reaching 5.57 ± 0.01 g/L and 6.11 ± 0.11 g/L, respectively. The fermentation in a bioreactor with changing the pH to 7.0 allowed achieving maximum ethanol production of 4.03 and 3.60 g/L for straw and hull hydrolysates at 24 h, respectively. The biogas productions obtained for the fermented hydrolysates of straw with and without changing the pH were 739 ± 37 and 652 ± 34 NmL/g_{VSad}, respectively. The fermented hydrolysate of hull without changing the pH presented 620 ± 26 NmL/g_{VSad}. The soybean residues produced biofuels, indicating these residues show potential as raw material for renewable energy production.

Keywords: bioethanol, biogas, hull, straw, subcritical fluid.

1. Introduction

Soybean is one of the primary cultures in the world. Latina America, North America, and Asia are considered the principal producers of soybean, highlighting the United States, Brazil, and Argentina as the top countries producers (Abdulkhani et al., 2017). The global soybean production was 348.71 million tons in 2018 (FAO, 2018). Soybean is the main culture cultivated in Brazil, with an average production of 124.8 million tons for the 2019/2020 harvest (CONAB, 2020). The soybean residues (straw and hull) are obtained after the harvest and are considered lignocellulosic residues (Qing et al., 2017). Furthermore, soybean residues present potential for biofuel production (Cortivo et al., 2018; Salakkam et al., 2017; Xiong et al., 2020; Zhu et al., 2014).

The lignocellulosic residues are promising biomasses to obtain biofuels because they are composed of cellulose, hemicelluloses, and lignin (Kucharska et al., 2018). The cellulose and hemicelluloses are dissociated in carbohydrates to be converted into biofuels by microorganisms (Aditiya et al., 2016; Kucharska et al., 2018). Hydrolysis is

the responsible process for dissociating cellulose and hemicelluloses in sugars (Aditiya et al., 2016; Zabed et al., 2017). Cellulose is responsible for the provision of glucose and cellobiose, and the hemicelluloses are responsible for the provision of sugars with five carbons (xylose and arabinose) and six carbons (galactose, glucose, and mannose) (Yu et al., 2008).

Subcritical water hydrolysis (SWH) is a process able to dissociate cellulose and hemicelluloses in various fermentable sugars. In SWH, the residue is submitted to water condition that involves the critical temperature and pressure higher than its vapor saturation pressure (Cardenas-Toro et al., 2014). Based on the recent literature, the SWH of different agricultural residues demonstrated fermentable sugars production (Abaide et al., 2019b, 2019a; Lachos-Perez et al., 2017).

Based on the sugars available in the hydrolytic stage, different biofuels can be produced. The bioethanol produced using lignocellulosic residues is called second-generation ethanol, which has the purpose of using renewable, non-food, low-cost and abundant raw material (Aditiya et al., 2016; Ayodele et al., 2020; Sarkar et al., 2012). The bioethanol production steps are the pretreatment, hydrolysis, fermentation, and distillation (Aditiya et al., 2016). The lignocellulosic residues, different from the agricultural origin, produced second-generation ethanol (Canabarro et al., 2017; Saha et al., 2015). Although second-generation ethanol production is an efficient and promising technology, more studies are necessary to improve and optimize the ethanol production process (Sarkar et al., 2012; Zabed et al., 2017).

According to the biorefinery concepts, fermentation residues can be an alternative for biogas production (Elsayed et al., 2018; Rempel et al., 2019). The biogas is produced through anaerobic digestion, divided into four main stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Kothari et al., 2014; Zheng et al., 2014). One method to improve the biogas production is co-digestion (mixing a lignocellulosic residue with an animal residue); it improved and optimized the production process (Paul and Dutta, 2018; Shrestha et al., 2017). The mixture of both substrates is efficient on biogas production (Rempel et al., 2019; Venturin et al., 2018; Xiong et al., 2020). Also, biogas production requires new studies to improve the production process related to pretreatment, biomass degradability, effects of inhibitors, and production combined with other biofuel production processes to improve energy efficiency (Zheng et al., 2014).

Therefore, this work aims to evaluate biofuels (ethanol and biogas) production using the hydrolysates of soybean straw and hull obtained by subcritical water hydrolysis in a semi-continuous mode. Characterization of hydrolysates (sugars, inhibitors, and organics acids) by high-performance liquid chromatography (HPLC), ethanol production by fermentation using the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2, and biogas production using the fermentation residues by anaerobic co-digestion were performed to optimize the biofuels production process. The yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 was used because it was recently isolated and identified (Bazoti et al., 2017) and it presented a potential for ethanol production in the presence of acetic acid (Bazoti et al., 2017; Bonatto et al., 2020), but little information in the literature about this yeast is seen. This work is a continuation of a subcritical water hydrolysis study using soybean residues, based on Vedovatto et al. (2021).

2. Materials and methods

2.1 Soybean residues

The soybean straw was collected from the farm after harvested in the northwest region of the state of Rio Grande do Sul/Brazil. The soybean hull was donated by a grain-processing unit (Olfar, Erechim, Brazil). After the reception, the residues were oven-dried at 60 °C and ground in a Wily knife Mills equipment (Solab, model SL 30, Brazil) with a 20 mesh separation grid. The residues were sieved and the particles higher than 0.25 mm were used to avoid clogging of pipes. The residues were packed and stored in a refrigerator at -5 °C until the experimental assays.

2.2 Subcritical water hydrolysis

The SWH procedure was based on Vedovatto et al. (2021) and Abaide et al. (2019a). The experimental condition of higher sugar production for straw and hull was selected for this work: temperature of 220 °C and a liquid/solid mass (R) ratio of 18 g water/g straw and 15 g water/g hull.

The experimental unit consisted of a high-pressure pump (Jasco, model PU4087, Japan), a thermostatic bath (Solab, model SL-152, Brazil), a non-return flow valve, a reactor (Citua, Brazil) manufactured with 316 L stainless steel with an internal volume

of 50 mL and height to internal diameter ratio of 6 (height of 13.2 cm and diameter of 2.2 cm) capable of supporting 60 MPa, a ceramic and heater, and a micrometering valve.

The hydrolysis was performed individually for each residue. The hydrolysis process started with loading the reactor with 25 g of straw or 30 g of a hull. After that, distilled water was pumped until reaching a pressure of 25 MPa, and the reactor was heated. The temperature was 220 °C, and the flow rate was 30 mL/min, corresponding to a liquid/solid mass (R) ratio of 18 g water/g straw and 15 g water/g hull. The reaction time was 4 min for straw and 3 min for the hull. The hydrolysates obtained in the process were evaluated in terms of sugars, hydroxymethylfurfural (HMF) and furfural, and acetic acid and formic acid. Both hydrolysates were used for producing bioethanol and biogas.

2.3 The fermentation process for bioethanol production

Wickerhamomyces sp. UFFS-CE-3.1.2 (access numbers on GenBank MF538579 e MF538580) identified and described by Bazoti et al. (2017) was the yeast used in this work. The fermentation was performed in an Erlenmeyer flask containing 10 mL of inoculum and 90 mL of hydrolysates. The yeast was stored in test tubes of YPD (yeast extract, peptone, and dextrose) medium composed of 1% of yeast extract, 2% of peptone and 2% of glucose, and 2% of agar-agar in the refrigerator. The test tubes were transferred to a bacteriological oven at 30 °C for 72 h to activate the yeast. The inoculum was prepared with the aid of a platinum handle in test tubes containing 10 mL of liquid YPD medium composed of 1% yeast extract, 2% peptone, and 2% glucose on a mass/volume basis, and kept for 24 h at 30 °C in a bacteriological oven. Afterward, the inoculum was transferred to the Erlenmeyer flask with the hydrolysate sterilized and supplemented. The fermentation was fulfilled initially in an orbital shaker at 30 °C and 50 rpm (Bazoti et al., 2017). The straw and hull hydrolysates, the straw and hull hydrolysates supplemented with glucose (10 g/L), and the hydrolysates diluted (1:4 for straw hydrolysates and 1:2 for hull hydrolysates) and supplemented with glucose (10 g/L) were used. The dilution of the hydrolysates was performed to obtain a concentration of acetic acid of 2 g/L in the hydrolysates. The dilution and supplementation were based on Bazoti et al. (2017). The assays were performed in duplicate.

The experimental conditions of higher ethanol yield in the fermentation in orbital shaker were selected for fermentation in a bioreactor. The experimental conditions were: straw hydrolysate diluted by 1:4 and supplemented with glucose (10 g/L) and hull hydrolysate diluted by 1:2 and supplemented with glucose (10 g/L). The fermentation without changing the pH and changing the pH (7.0) was performed. The pH change was done using the pH control system of the bioreactor, with NaOH 3M and HCl 1 M. The fermentation was performed in a bioreactor (BIO-TEC, Tecnal, Brazil), and the fermentation process was performed to both hydrolysates individually at 30 °C and 80 rpm (Bonatto et al., 2020). The samples were collected in intervals of 24 h to complete 96 h of fermentation. The cell concentration in the bioreactor at the beginning of fermentation was 2.74×10^8 cells/mL for all assays. Sugars, inhibitors (HMF and furfural), organics acids (acetic acid and formic acid), and ethanol were determined.

2.4 Analyses of hydrolysates before and after fermentation

2.4.1 Sugars, organic acids, inhibitors, and ethanol

The samples were analyzed by High-Performance Liquid Chromatography (HPLC) (Shimadzu, model LCMS-2020, Japan) equipped with RID 10-A detector and an AMINEX® BIORAD HPX87H column for determination of glucose, xylose, arabinose, cellobiose, acetic acid, formic acid, and ethanol. The samples (20 µL) were analyzed at 45 °C with 5 mM H_2SO_4 as a mobile phase and a flow rate of 0.6 mL/min. For the determination of inhibitors (furfural and HMF), an HPLC (Shimadzu, model LCMS-2020, Japan) equipped with a PDA 10-A detector and a C18 column was used. The samples were analyzed at 30 °C with a mobile phase of 1:8 acetonitrile/water and 1% acetic acid and a flow rate of 0.8 mL/min (Bazoti et al., 2017).

2.5 Biochemical biogas and methane potential

For biogas production, straw and hull hydrolysates and the fermented hydrolysates in a bioreactor were used. The mesophilic anaerobic inoculum was obtained from a laboratory-scale bioreactor as described by Steinmetz et al. (2016). The inoculum was enriched and acclimated in 40 L reactors, feed with starters inoculants (anaerobic sludge treated with swine manure, fresh dairy cattle manure, and anaerobic

mesophilic granular sludge from a gelatin manufactory), and acclimated with daily feed with a standard mixture of nutrients and acclimated at 37 °C (Steinmetz et al., 2016).

The biochemical biogas potential (BBP) and methane potential (BMP) assays were performed at batch and individual experiments, in 250 mL glass reactors connected to 500 mL glass eudiometers. In each reactor, 2 g of straw or hull and 30 g for the samples of hydrolysates or fermented hydrolysates were mixed. The amount of inoculum added in each reactor was 170 g to 200 g, respecting the ratio of $VS_{inoculum}/VS_{substrate}$ (RIS) of 2. The headspace was purged with N_2 and the batch reactor was stored at 37 °C (VDI 4630, 2016). The volatiles solids and the total of the solids were analyzed by APHA (2012). The biogas production was measured daily by sealing liquid level displacement (sealing solution according to DIN 38414-8, 1985) until stability ($dV/dt \leq 1\%$ of cumulative volume). The gas volumes were normalized to atmospheric pressure (1013 hPa), 273 K, and zero moisture content (VDI 4630, 2016). The accumulated biogas production was determined by the mass of volatile solids added in each test. In parallel, tests were conducted with the inoculum alone to allow the endogenous respiration of the inoculum subtracted. Microcrystalline cellulose (20 l size, Sigma-Aldrich) was used as an internal standard for the evaluation of digestion efficiency according to Holliger et al. (2016) and Hafner et al. (2020) recommendations. The pH of the inoculum and substrate mixture was evaluated before and after the batch assay. The analysis of biogas composition (CH_4 and CO_2) was done by collecting a minimum of 60 mL of accumulated on the eudiometer tube and analyzed by infrared sensors (Landtec, BIOGAS5000, EUA). All assays were performed in triplicate.

3. Results and discussion

3.1 Characterization of soybean straw and hull hydrolysates

The characterization of the hydrolysates obtained by SWH is shown in Table 1. The glucose values were 2.16 g/L and 0.96 g/L for straw and hull hydrolysates, respectively. In the composition of both hydrolysates, inhibitors (HMF and furfural) and organic acids (acetic acid and formic acid) were produced. The degradation of sugars during the hydrolysis with a temperature of 220 °C produced HMF and furfural. The formation of HMF and furfural results from the degradation of sugars (Cardenas-Toro et al., 2014; Lachos-Perez et al., 2017; Yu et al., 2008). The concentration of acetic acid

for straw and hull hydrolysates was 8.22 g/L and 3.14 g/L, respectively. The acetic acid production is associated with the degradation of hemicelluloses with temperature increase during SWH (Abaide et al., 2019a; Lachos-Perez et al., 2016; Vedovatto et al., 2021).

Table 1. Composition of soybean straw and hull hydrolysates obtained by SWH.

	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Cellobiose (g/L)	Formic acid (g/L)	Acetic acid (g/L)	HMF (g/L)	Furfural (g/L)
Soybean straw	2.16	1.33	0.08	-	4.76	8.22	0.28	0.48
Soybean hull	0.96	1.11	0.43	0.09	3.24	3.14	0.16	0.31

In a concentration above 1.4 g/L, the acetic acid can inhibit the fermentation depending on the yeast strain type (Olsson and Hahn-Hägerdal, 1996). Due to the high concentration of acetic acid in both hydrolysates, the dilution of the hydrolysates was performed to obtain a concentration of approximately 2 g/L for the fermentation process. In the assays where the hydrolysates were diluted, supplementation with 10 g/L of glucose was also performed, based on Bazoti et al. (2017).

3.2 Ethanol production by Erlenmeyer flasks

The conditions that yielded the highest ethanol production were diluted and supplemented with glucose (Table 2). The straw hydrolysate diluted by 1:4 and supplemented with glucose (10 g/L) yielded an ethanol production of 5.57 ± 0.01 g/L at 72 h of fermentation. The hull hydrolysate diluted by 1:2 and supplemented with glucose (10 g/L) resulted in ethanol production of 6.11 ± 0.11 g/L after 96 h of fermentation. The results of this work were lower than the obtained by Bazoti et al. (2017) in the study of fermentation of sugarcane bagasse hydrolysate without changing the pH (4.88, which the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 yielded the highest ethanol production of 9.8 g/L at 72 h of fermentation. The results were also lower than those obtained by Artifon et al. (2018) after the detoxification process of sugarcane bagasse hydrolysate. In such study, ethanol productions of 7.4 g/L and 8.3 g/L were achieved at 48 h and 72 h of fermentation, respectively, using the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2.

Table 2. Composition of assays at 72 h and 96 h of fermentation of soybean straw and hull hydrolysates using *Wickerhamomyces* sp. UFFS-CE-3.1.2 in Erlenmeyer flasks.

Assays	Time (h)	Ethanol (g/L)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Cellobiose (g/L)	Acetic acid (g/L)	Formic acid (g/L)	HMF (g/L)	Furfural (g/L)
FPS/ B*	72	0.69 ± 0.06	2.04 ± 0.17	1.08 ± 0.02	-	-	7.35 ± 0.65	4.17 ± 0.19	0.30 ± <0.01	0.30 ± <0.01
FPS/ G*	72	0.74 ± 0.01	8.00 ± 0.09	0.95 ± 0.03	-	-	7.83 ± 0.14	4.51 ± 0.06	0.31 ± <0.01	0.31 ± <0.01
FPS/ G/ D 1:4	72	5.57 ± 0.01	0.57 ± 0.02	0.24 ± 0.01	-	-	1.74 ± 0.01	1.03 ± 0.04	0.02 ± <0.01	0.02 ± <0.01
FCS/ B*	96	0.72 ± 0.01	1.02 ± 0.25	0.90 ± 0.04	0.40 ± <0.01	0.09 ± <0.01	2.85 ± 0.05	2.96 ± 0.02	0.18 ± <0.01	0.18 ± <0.01
FCS/ G*	96	0.79 ± 0.01	7.96 ± 0.43	0.72 ± 0.01	0.39 ± 0.01	0.09 ± <0.01	2.83 ± 0.02	3.03 ± 0.02	0.19 ± 0.02	0.19 ± 0.04
FCS/ G/ D 1:2	96	6.11 ± 0.11	0.39 ± 0.03	0.40 ± 0.01	0.20 ± <0.01	-	1.48 ± 0.02	1.31 ± 0.01	0.09 ± <0.01	0.09 ± 0.02

*The assays did not present ethanol production during the fermentation.

FPS/ B: Fermentation of straw hydrolysate.

FPS/ G: Fermentation of straw hydrolysate supplemented with glucose (10 g/L).

FPS/ G/ D 1:4: Fermentation of straw hydrolysate diluted by 1:4 and supplemented with glucose (10 g/L).

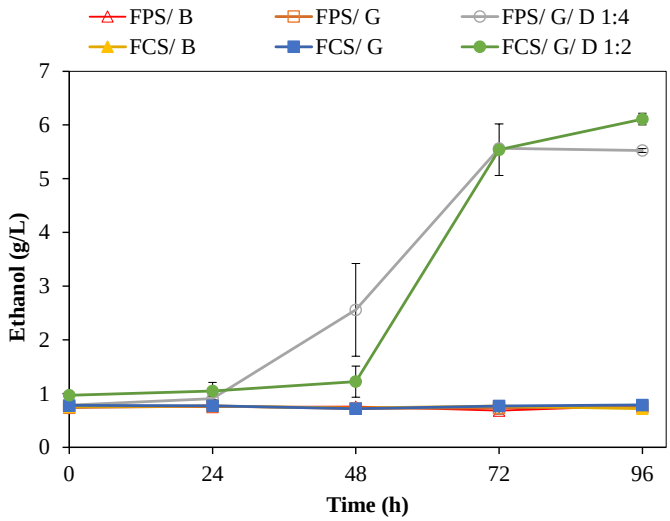
FCS/ B: Fermentation of hull hydrolysate.

FCS/ G: Fermentation of hull hydrolysate supplemented with glucose (10 g/L).

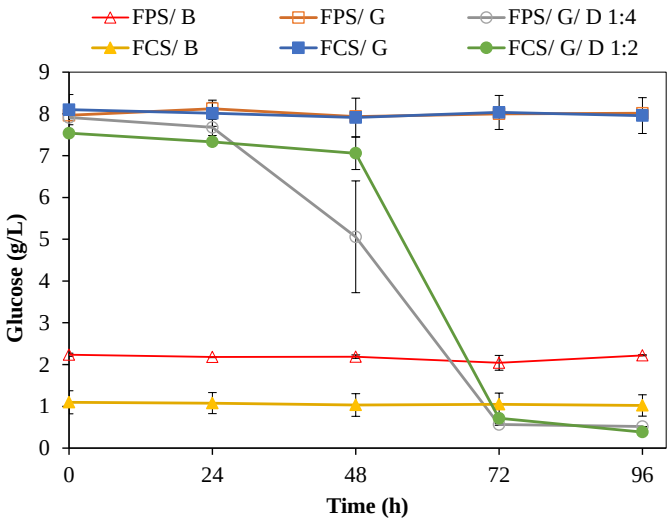
FCS/ G/ D 1:2: Fermentation of hull hydrolysate diluted by 1:2 and supplemented with glucose (10 g/L).

In the kinetic profiles of fermentation (Fig. 1), the total consumption of glucose (Fig. 1B) occurred 72 h and 96 h after starting the fermentation. Consequently, higher ethanol production occurred at 72 h for soybean straw and 96 h for hull hydrolysates (Fig. 1A). The other sugars (xylose and arabinose) were not consumed in fermentation, which remained constant until the end of fermentation (Table 2), corroborating with the study of Bazoti et al. (2017). The authors observed that xylose, arabinose, and cellobiose were not consumed, indicating the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2. preference for glucose.

Figure 1. Kinetic profile of ethanol production and glucose consumption in fermentation by Erlenmeyer flask (30 °C and 50 rpm) for soybean straw and hull hydrolysates obtained by SWH (220 °C, 25 MPa and reaction time of 4 and 3 min, respectively). FPS/ B: Fermentation of straw hydrolysate. FPS/ G: Fermentation of straw hydrolysate supplemented with glucose (10 g/L). FPS/ G/ D 1:4: Fermentation of straw hydrolysate diluted by 1:4 and supplemented with glucose (10 g/L). FCS/ B: Fermentation of hull hydrolysate. FCS/ G: Fermentation of hull hydrolysate supplemented with glucose (10 g/L). FCS/ G/ D 1:2: Fermentation of hull hydrolysate diluted by 1:2 and supplemented with glucose (10 g/L).



(A)



(B)

Another essential factor observed in this work is the ethanol production with organic acid and inhibitors in hydrolysates, indicating the yeast tolerated a moderate presence of inhibitors, mainly the acetic acid. The acetic acid concentrations were 1.74

± 0.01 g/L for straw hydrolysate and 1.48 ± 0.02 g/L for hull hydrolysate (Table 2). These results corroborated with Bazoti et al. (2017) that obtained ethanol production even with the presence of inhibitors in sugarcane bagasse hydrolysate, with approximately 2.57 g/L of acetic acid using the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2.

The assays for straw and hull hydrolysates and the straw and hull hydrolysates supplemented with glucose did not present ethanol production. This is justified by the high presence of inhibitors in the hydrolysates. The inhibitor agent of fermentation was acetic acid (Table 2), which presented concentrations of 7.35 ± 0.65 g/L and 7.83 ± 0.14 g/L in straw hydrolysate and straw hydrolysate supplemented with glucose, respectively. The hull hydrolysate and the hull hydrolysate supplemented with glucose resulted in 2.85 ± 0.05 g/L and 2.83 ± 0.02 g/L of acetic acid. Consequently, the acetic acid inhibited yeast activity during the fermentative process, as seen elsewhere (Olsson and Hahn-Hägerdal, 1996).

The inhibitory action of acetic acid was also observed in other studies. The increase of acetic acid concentration (0.5, 1.5, and 2.5 g/L) increased the inhibition of fermentation, thus reducing ethanol production. 3.5 g/L of acetic acid inhibited completely the fermentation of wheat straw by *Pichia stipitis* (Bellido et al., 2011). Likewise, the mixture of both oat and soybean hull hydrolysates obtained by acid hydrolysis, with a concentration of 2.4 ± 0.5 g/L of acetic acid, 0.19 ± 0.01 g/L of furfural, and 0.47 ± 0.02 g/L of HMF, which were concentrated in sugars (5.4 ± 0.8 g/L of glucose and 57.1 ± 2.1 g/L of xylose), did not present cellular metabolism and any consumption of sugars in fermentations using both recombinant strains of *Saccharomyces cerevisiae* YRH 396 and YRH 400 (Cortivo et al., 2018).

3.3 Ethanol production by bioreactor

The experimental conditions of higher ethanol yield in the fermentation in orbital shaker were selected for the fermentation in the bioreactor. The experimental conditions were: straw hydrolysate diluted by 1:4 and supplemented with glucose (10 g/L) and hull hydrolysate diluted by 1:2 and supplemented with glucose (10 g/L). The dilution and supplementation were based on Bazoti et al. (2017).

The fermentation of straw and hull hydrolysates without changing the pH yielded ethanol productions of 4.66 g/L and 4.77 g/L, respectively (Table 3). In the

kinetic profiles of fermentation of straw (Fig. 2A) and hull (Fig. 2C) hydrolysates without changing the pH, higher ethanol production is seen at 48 h with a pH of 4.2 for both hydrolysates. The total consumption of glucose occurred after 48 h of fermentation (Fig. 2A e 2C) and the yeast did not consume xylose and arabinose in the fermentative process. These results were lower than those presented in the ethanol production with sugarcane bagasse hydrolysate (without changing the pH with a variation of 4.84 to 4.98) by yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2. Ethanol production of 8.35 g/L was obtained. The yeast did not consume xylose, arabinose, and cellobiose, and all glucose was consumed at 72 h (Bonatto et al., 2020). Therefore, the yeast used glucose as the main source of carbon during of fermentation process.

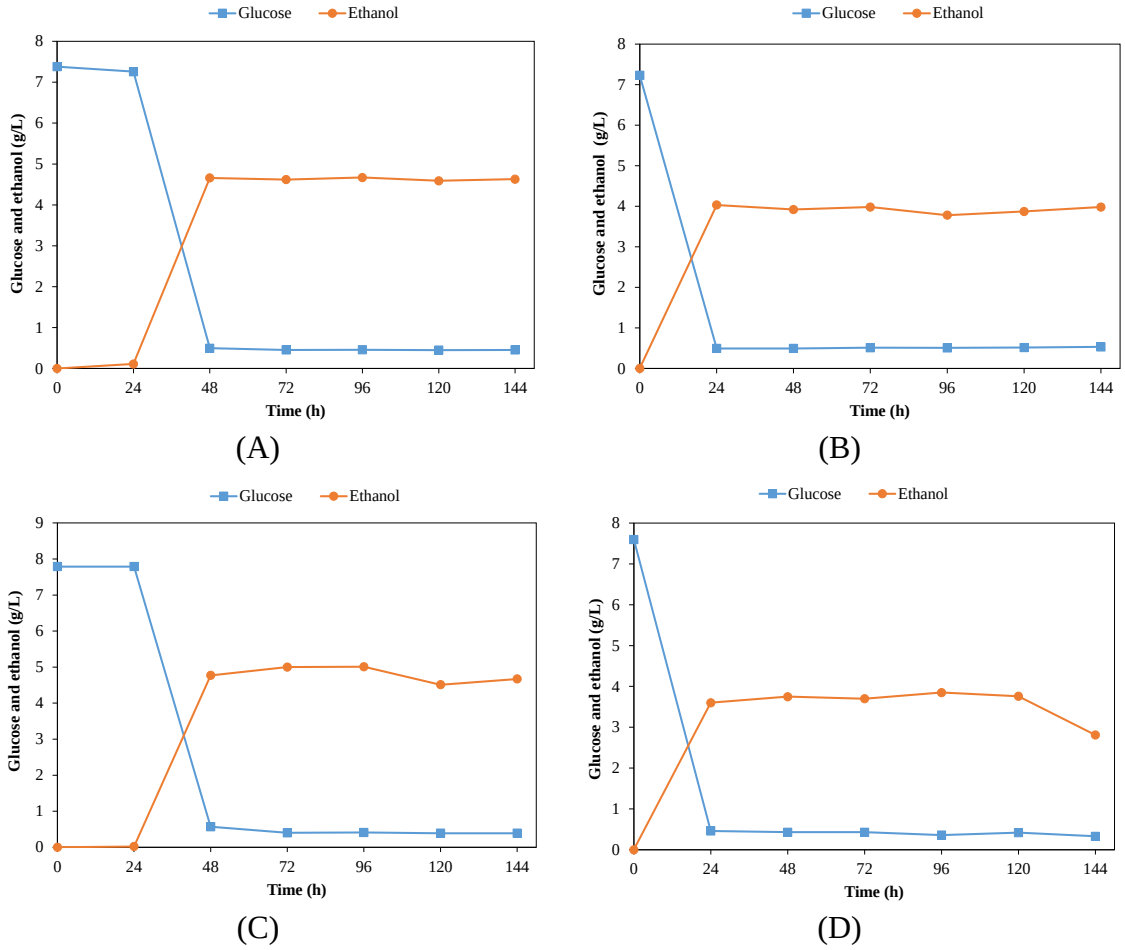
Table 3. Composition of assays of soybean straw and hull hydrolysates using *Wickerhamomyces* sp. UFFS-CE-3.1.2 in bioreactor fermentation.

Assays	Time (h)	Ethanol (g/L)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Acetic acid (g/L)	Formic Acid (g/L)	HMF (g/L)
FPS/without changing the pH	48	4.66	0.50	0.28	-	1.93	1.00	0.03
FCS/without changing the pH	48	4.77	0.57	0.39	0.20	1.39	1.38	0.04
FPS/with changing the pH (7.0)	24	4.03	0.49	0.25	-	2.12	0.86	-
FCS/with changing the pH (7.0)	24	3.60	0.46	0.32	0.16	1.55	1.16	-

FPS: Fermentation of straw hydrolysate.

FCS: Fermentation of hull hydrolysate.

Figure 2. Kinetic profile of ethanol production in fermentation by bioreactor (30 °C and 80 rpm) for soybean straw (A and B) and hull (C and D) hydrolysates without (4.2) and with changing the pH (7.0), respectively.



The concentrations of acetic acid, formic acid, and HMF were approximately the same during the fermentation of both hydrolysates (Table 3). The initial concentration of furfural was 0.18 g/L for straw hydrolysate and 0.10 g/L for hull hydrolysate. The furfural was degraded after 48 h and 24 h for straw hydrolysates and hull hydrolysate without changing the pH, respectively (data not shown). This behavior might be explained by degradation during the fermentative process.

The fermentation of straw and hull hydrolysates with changing the pH to 7.0 (Table 3) resulted in ethanol production of 4.03 and 3.60 g/L, respectively. The main difference in fermentation without and changing the pH of hydrolysates is the faster and, consequently, the maximum ethanol production. The straw and hull hydrolysates with changing the pH (7.0) allowed higher ethanol production at 24 h (Fig. 2B and 2D). Otherwise, the hydrolysates without changing the pH resulted in higher ethanol

production at 48 h of fermentation (Fig. 2A and 2C). This behavior is also observed in ethanol productivity ($P_{ethanol}$: g/L.h^{-1}). The straw and hull hydrolysates without changing the pH presented a $P_{ethanol}$ of 0.10 g/L.h^{-1}. The straw hydrolysate, with changing the pH (7.0), obtained a $P_{ethanol}$ of 0.17 g/L.h^{-1}, which increased 70% in ethanol productivity. For hull hydrolysate with changing the pH, a $P_{ethanol}$ of 0.15 g/L.h^{-1} added 50% in ethanol productivity.

The maximum ethanol production at 24 h for both hydrolysates (straw and hull) with changing the pH (7.0) is directly linked to the rapid glucose consumption by yeast (Fig 2B and 2D). The total glucose consumption occurred at 24 h. The rapid glucose consumption by yeast with changing the pH was also observed in the study reported by Bonatto et al. (2020), where changing the pH of sugarcane bagasse hydrolysate from 4.89 to 7.00 also allowed total glucose consumption at 24 h. Otherwise, the consumption of xylose at pH 7.00 occurred along the fermentative process (120 h) by *Wickerhamomyces* sp. UFFS-CE-3.1.2, in which an ethanol production of 9.25 g/L was achieved. In another work, changing the pH from 5 to 6 increased glucose consumption by the yeast *Saccharomyces cerevisiae* 424A (LNH-ST), in the condition with 7.5 g/L of acetic acid, indicating the increase of pH decreases the inhibitory effect of acetic acid (not dissociated form) (Casey et al., 2010).

The acids (not dissociated) diffuse through of plasmatic membrane of microorganisms. The acid molecules dissociate in cells by a difference of intercellular (neutral) and medium fermentation. Consequently, it decreased the cytosolite pH and inhibited cell activity (Jönsson et al., 2013; Palmqvist and Hahn-Hägerdal, 2000; Taherzadeh and Karimi, 2011). To keep the intercellular pH neutral, the cells pumping protons through ATPase. The use of ATPase to adjustment of intercellular pH can decrease the ethanol production and biomass in solution with a high concentration of acids, but it can benefit the ethanol production with a low concentration of acids (Jönsson et al., 2013; Palmqvist and Hahn-Hägerdal, 2000; Taherzadeh and Karimi, 2011). The inhibitory effect of acids not dissociated is dependent on pH; consequently, the pH is an essential variable in the fermentative process (Palmqvist and Hahn-Hägerdal, 2000).

Table 3 shows the concentration of sugars and organic acids for hydrolysates with changing the pH (7.0) at 24 h. The yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 did not consume other sugars (xylose and arabinose) in both hydrolysates. The concentration of sugars and organic acids were approximately the same over the

fermentative process. In fermentation with changing the pH, HMF and furfural were not detected.

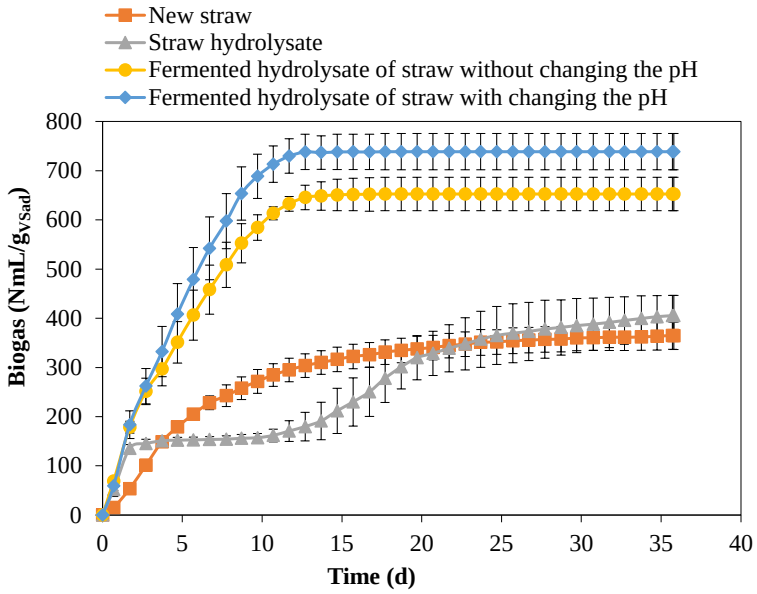
3.4 Biochemical biogas and methane potential

The biogas production of straw and hull hydrolysates are presented in Table 4 and Figure 3. The positive control consisted of microcrystalline cellulose. The accumulated gas production of positive control was 634 ± 32 NmL/g_{VSad} (358 ± 18 NmL_{CH4}/g_{VSad}) at 36 days, corresponding to 85% yield of the reference value of 750 NmL/g_{VSad} (340 and 395 NmL_{CH4}/g_{VSad}). The RSD < 6% was reached, indicating homogeneity and effectiveness of inoculum activity according to Holliger et al. (2016) and Hafner et al. (2020) recommendations. The inoculum production (endogenous respiration) was de 28.4 ± 3 NmL/g_{VSad}.

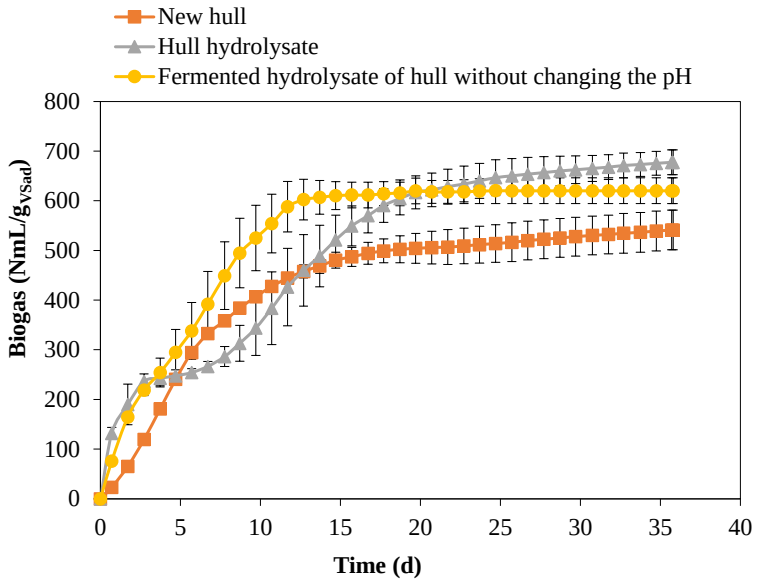
Table 4. Biogas potential (BBP), methane potential (BMP), CH_4 concentration, pH, and characterization of residues and hydrolysates before and after anaerobic digestion.

Samples	Total solids (% m/m)	Volatile solids (% m/m)	pH initial	pH final	BBP (NmL/g_{VSad})	CH_4 (%)	BMP (NmL_{CH4}/g_{VSad})
Cellulose standard	94.62 ± 0.3	94.51 ± 0.9	7.92 ± 0.05	8.06 ± 0.06	634 ± 32	56.4	358 ± 18
New straw	92.51 ± <0.1	86.85 ± 5.5	7.94 ± 0.12	8.23 ± 0.10	365 ± 25	58.5	214 ± 15
Straw hydrolysate	8.15 ± 5.6	7.00 ± 36.5	7.05 ± 0.17	8.16 ± 0.03	406 ± 8	48.4	197 ± 4
Fermented hydrolysate of straw without changing the pH	1.87 ± 1.5	1.62 ± 1.0	7.55 ± 0.15	8.20 ± 0.04	652 ± 34	65.2	425 ± 22
Fermented hydrolysate of straw with changing the pH (7.0)	2.58 ± 2.1	1.28 ± 0.4	7.95 ± 0.01	8.27 ± 0.06	739 ± 37	69.8	516 ± 26
New hull	92.80 ± 0.2	87.20 ± 0.5	7.91 ± 0.03	8.25 ± 0.09	542 ± 39	56.7	307 ± 22
Hull hydrolysate	8.18 ± 0.3	6.46 ± 15.9	7.21 ± 0.20	8.21 ± 0.01	677 ± 35	49.2	333 ± 17
Fermented hydrolysate of hull without changing the pH	2.70 ± 2.4	2.31 ± 22.2	7.49 ± 0.13	8.21 ± 0.03	620 ± 26	61.1	379 ± 16

Figure 3. Biogas production by anaerobic co-digestion of new residues, hydrolysates, and fermented hydrolysates without and with changing the pH (7.0) for soybean straw and hull.



(A)



(B)

After concluding the fermentation process, the fermented hydrolysates were used for the BBP assays. All the evaluated fermented hydrolysates produced biogas. The fermented hydrolysate of straw without changing the pH obtained a biogas production of 652 ± 34 NmL/g_{VSad}, with a gain of 60.59% when compared with straw

hydrolysate. The fermented hydrolysate of straw with changing the pH obtained a biogas production of 739 ± 37 NmL/g_{VSad}, with 82.02% compared with straw hydrolysate. The decrease in the concentration of acetic acid can explain these gains in the biogas production of fermented hydrolysates. This decrease was obtained from the dilution process of hydrolysates before the fermentative process. The biogas production from the fermented hydrolysate of the hull without changing the pH was 620 ± 26 NmL/g_{VSad}, which demonstrated an 8.42% reduction compared with hull hydrolysate.

The production of biogas by the fermentation of residues obtained in this work corroborated with the results reported by Rempel et al. (2019). The fermentation of residues provided higher biomethane production (422 ± 15 NL_{CH4}/kg_{VSad}). The fermentation residues contained high levels of proteins, resulting in the yeast cells obtained in the fermentative process for ethanol production. Also, in another study, using corn stover with different pretreatments (steam explosion and organosolv), the fermentation residue of pretreatment with a steam explosion (0.2% sulfuric acid) obtained higher methane production than fermentation residues of organosolv pretreatment (Katsimpouras et al., 2017).

The biogas production for new straw and hull yielded 365 ± 25 NmL/g_{VSad} and 541 ± 39 NmL/g_{VSad}, respectively (Fig. 3A and 3B). The preparation of new straw and hull for BBP assays only consisted of drying until reaching a constant weight and grinding in a Willey Knife Mills equipment (Solab, model SL 30, Brazil) with a 20 mesh separation grid. The biogas production of new straw and hull was the smallest result for biogas production. However, Venturin et al. (2018) observed that the particle size did not affect the biogas production of corn stalk. Comparing samples sifted and not sifted, production of biogas of 523.5 ± 4.2 NL/kg_{VSad} and 532.4 ± 2.2 NL/kg_{VSad} were obtained, respectively.

After the SWH, the biogas production of straw hydrolysate was 406 ± 8 NmL/g_{VSad}, obtaining 11.23% if compared with the biogas production of new straw. The hull hydrolysate yielded 677 ± 35 NmL/g_{VSad} with a gain of 24.91% if compared with the biogas production of the new hull. These increases in biogas production are related to hydrolysates' carbohydrates after SWH for both residues (Table 1). These results corroborated with other studies. Maciel-Silva et al. (2019) reported that the use of SWH in industries residues of açai processing also provided biogas production by anaerobic digestion with 100 times higher production concerning the assays without the SWH application. Xiong et al. (2020) observed that different thermochemical

pretreatments of soybean straw increased the methane production from 28% to 62% when compared with untreated straw. Methane production of untreated straw was 127 ± 2 NmL/gVS and the highest methane production was 206 ± 2 NmL/gVS for pretreatment with (2% NaOH-4.5%H_2O_2).

The straw and hull hydrolysates presented a lag in biogas production (Fig. 3A and 3B). The kinetic behavior of biogas production of straw and hull hydrolysates indicated a production in the beginning. After that, a stabilization occurred until approximately the tenth day. Thereafter, the biogas production returned until the end production at 36 days. The inhibitory substrate effect can justify this lag due to the concentration of organic acids in straw and hull hydrolysates (Table 1). Kothari et al. (2014) reported that the high concentration of organic acids can inhibit or eliminate bacterial activities.

The concentration of acetic acid (Table 1) can be contributed to the lag of biogas production with the inhibition of pathways acetoclastic methanogenesis. Biogas production restarted on the tenth day for both hydrolysates and it can be occurred by hydrogenotrophic methanogenesis pathways. The acetoclastic methanogenesis uses acetate, and hydrogenotrophic methanogenesis usesCO_2 and hydrogen for methane production (Kothari et al., 2014). The inhibitory effect of acetic acid observed in this work was also observed by Xu et al. (2014) in a study of biogas production using kitchen wastes. Acetic acid was the primary inhibitor of methanogenesis. The acetoclastic methanogenesis was more sensible to the acetic acid than the hydrogenotrophic methanogenesis, but the acetoclastic affected the methane production rate, and the hydrogenotrophic could stabilize the anaerobic process until a fixed period by consuming H^+.

The pH of samples in reactors of anaerobic co-digestion was approximate 8.00 (Table 4). The pH values were crucial for microorganisms development and biogas production. The pH is a factor that influences the biogas production because the methanogenic bacteria are sensitive to the acid concentration in the digester; consequently, they preferred a pH neutral (Kothari et al., 2014; Kwietniewska and Tys, 2014). The pH of samples in this work was similar to other studies. Xiong et al. (2020) observed, in anaerobic digestion of soybean straw, that the pH varied from 7.00 to approximately 8.00 for the thermochemical pretreatments, indicating that it was essential for methane production. Also, Zhu et al. (2014) observed the ratio (soybean

processing waste/hay 75:25) of higher methane yield presented a pH of approximately 8.00.

The methane composition of samples is shown in Table 4. They consisted of microcrystalline cellulose obtained a methane concentration was 56.39%. The new straw and hull have a concentration of 58.49% and 56.67% of methane, respectively. The straw and hull hydrolysates provided a methane concentration lower than 50%. The straw and hull hydrolysates obtained 48.36% and 49.21% of methane, respectively. The lower methane concentration for both hydrolysates can be justified by the concentration of organic acids in hydrolysates, as explained previously in biogas production kinetic behavior for hydrolysates. These results of methane concentration for new straw and hull were similar to the study of the co-digestion anaerobic of corn stalk, which provided satisfactory methane levels. Methane concentrations of 57.7% and 57.2% for sifted and not sifted biomass, respectively, were reached. 55.6% and 54.4% of methane after biomass pretreated with H_2SO_4 and H_2O_2, respectively, were also reached (Venturin et al., 2018).

The fermented hydrolysates of straw and hull provided methane concentration higher than 60% and higher methane concentration of positive control with microcrystalline cellulose (Table 4). The fermented hydrolysates of straw without and with changing the pH obtained a concentration of 65.23% and 69.79% of methane, respectively. The fermented hydrolysate of the hull without changing pH obtained 61.10%. Elsayed et al. (2018) reported residual ethanol and free xylose were responsible for the higher methane concentration in fermentation broth without distillation, with a methane concentration for the ethanol stillage and fermentation broth without distillation of 73.4 ± 8.2% and 79.3 ± 9.0%, respectively. Also, they obtained for the alkaline pretreatment 68.9 ± 2.6% using as biomass the rice straw.

4. Conclusion

SWH using soybean straw and hull produced sugars. The hydrolysates diluted and supplemented with glucose produced ethanol with 5.57 ± 0.01 g/L and 6.11 ± 0.11 g/L for hydrolysates of soybean straw and hull, respectively. The fermentation in a bioreactor with pH changing from 4.2 to 7.0 provided the highest ethanol production at 24 h. The fermented hydrolysates of straw increased biogas production by more than 60%, and the fermented hydrolysate of hull obtained a reduction of 8.42% in the biogas

production compared with hydrolysates. Therefore, the use of soybean residues presented the potential for biofuel production.

CRediT authorship contribution statement

Felipe Vedovatto: Conceptualization, Investigation, Writing – original draft. **Charline Bonatto**: Investigation, Formal analysis. **Suzana F. Bazoti**: Formal analysis. **Bruno Venturin**: Investigation. **Sérgio L. Alves Jr**.: Resources. **Airton Kunz**: Resources, Supervision. **Ricardo L. R. Steinmetz**: Writing - review & editing, Investigation, Formal analysis, Resources, Supervision. **Helen Treichel**: Writing - review & editing, Supervision. **Marcio A. Mazutti**: Funding acquisition, Supervision. **Giovani L. Zabot**: Writing - review & editing, Funding acquisition, Supervision. **Marcus V. Tres**: Writing - review & editing, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the National Council of Technological and Scientific Development (CNPq) and Support Foundation of the State of Rio Grande do Sul (FAPERGS) for the support and financial support. They thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for scholarships. The authors also thank the Federal University of Santa Maria, Federal University of Fronteira Sul, and Embrapa Suínos e Aves. M. A. Mazutti (303482/2015-0), M. V. Tres (308936/2017-5), G. L. Zabot (304882/2018-6), and H. Treichel (305258/2018-4) thank CNPq for the productivity grants.

REFERENCES

1. Abaide, E.R., Mortari, S.R., Ugalde, G., Valério, A., Amorim, S.M., Di Luccio, M., Moreira, R. de F.P.M., Kuhn, R.C., Priamo, W.L., Tres, M. V., Zabot, G.L., Mazutti, M.A., 2019a. Subcritical water hydrolysis of rice straw in a semi-continuous mode. J. Clean. Prod. 209, 386–397.

2. Abaide, E.R., Ugalde, G., Di Luccio, M., Moreira, R. de F.P.M., Tres, M. V., Zabot, G.L., Mazutti, M.A., 2019b. Obtaining fermentable sugars and bioproducts from rice husks by subcritical water hydrolysis in a semi-continuous mode. Bioresour. Technol. 272, 510–520.

3. Abdulkhani, A., Alizadeh, P., Hedjazi, S., Hamzeh, Y., 2017. Potential of Soya as a raw material for a whole crop biorefinery. Renew. Sustain. Energy Rev. 75, 1269–1280.

4. Aditiya, H.B., Mahlia, T.M.I., Chong, W.T., Nur, H., Sebayang, A.H., 2016. Second generation bioethanol production: A critical review. Renew. Sustain. Energy Rev. 66, 631–653.

5. APHA, 2012. Starndard Methods for the Examination of Water and Wastewater, American Public Health Association. Washington, DC, USA.

6. Artifon, W., Bonatto, C., Bordin, E.R., Bazoti, S.F., 2018. Bioethanol Production From Hydrolyzed Lignocellulosic After Detoxification Via Adsorption With Activated Carbon and Dried Air Stripping 6, 1–6.

7. Ayodele, B.V., Alsaffar, M.A., Mustapa, S.I., 2020. An overview of integration opportunities for sustainable bioethanol production from first- and second-generation sugar-based feedstocks. J. Clean. Prod. 245, 118857.

8. Bazoti, S.F., Golunski, S., Pereira Siqueira, D., Scapini, T., Barrilli, É.T., Alex Mayer, D., Barros, K.O., Rosa, C.A., Stambuk, B.U., Alves, S.L., Valério, A., de Oliveira, D., Treichel, H., 2017. Second-generation ethanol from non-detoxified sugarcane hydrolysate by a rotting wood isolated yeast strain. Bioresour. Technol. 244, 582–587.

9. Bellido, C., Bolado, S., Coca, M., Lucas, S., González-Benito, G., García-Cubero, M.T., 2011. Effect of inhibitors formed during wheat straw pretreatment on ethanol fermentation by Pichia stipitis. Bioresour. Technol. 102, 10868–10874.

10. Bonatto, C., Venturin, B., Mayer, D.A., Bazoti, S.F., de Oliveira, D., Alves, S.L., Treichel, H., 2020. Experimental data and modelling of 2G ethanol production by *Wickerhamomyces* sp. UFFS-CE-3.1.2. Renew. Energy 145, 2445–2450.

11. Canabarro, N.I., Alessio, C., Foletto, E.L., Kuhn, R.C., Priamo, W.L., Mazutti, M.A., 2017. Ethanol production by solid-state saccharification and fermentation in a packed-bed bioreactor. Renew. Energy 102, 9–14.

12. Cardenas-Toro, F.P., Forster-Carneiro, T., Rostagno, M.A., Petenate, A.J., Maugeri Filho, F., Meireles, M.A.A., 2014. Integrated supercritical fluid extraction and subcritical water hydrolysis for the recovery of bioactive compounds from pressed palm fiber. J. Supercrit. Fluids 93, 42–48.

13. Casey, E., Sedlak, M., Ho, N.W.Y., Mosier, N.S., 2010. Effect of acetic acid and pH on the cofermentation of glucose and xylose to ethanol by a genetically engineered strain of Saccharomyces cerevisiae. FEMS Yeast Res. 10, 385–393.

14. CONAB, 2020. Follow-up of Brazilian grain crop 2019/2020 - Twelfth survey. National Supply Company 7, 1–68.

15. Elsayed, M., Abomohra, A.E.F., Ai, P., Wang, D., El-Mashad, H.M., Zhang, Y., 2018. Biorefining of rice straw by sequential fermentation and anaerobic digestion for bioethanol and/or biomethane production: Comparison of structural properties and energy output. Bioresour. Technol. 268, 183–189.

16. FAO, 2018. Production of Crops. FAO-Food Agric. Organ. United Nations. URL http://www.fao.org/faostat/en/#data/QC (accessed 10.13.20).

17. Hafner, S., Fruteau, H., Koch, K., Holliger, C., 2020. Improving Inter-Laboratory Reproducibility. Water 12, 1752.

18. Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., Bougrier, C., Buffière, P., Carballa, M., De Wilde, V., Ebertseder, F., Fernández, B., Ficara, E., Fotidis, I., Frigon, J.C., De Laclos, H.F., Ghasimi, D.S.M., Hack, G., Hartel, M., Heerenklage, J., Horvath, I.S., Jenicek, P., Koch, K., Krautwald, J., Lizasoain, J., Liu, J., Mosberger, L., Nistor, M., Oechsner, H., Oliveira, J.V., Paterson, M., Pauss, A., Pommier, S., Porqueddu, I., Raposo, F., Ribeiro, T., Pfund, F.R., Strömberg, S., Torrijos, M., Van Eekert, M., Van Lier, J., Wedwitschka, H., Wierinck, I., 2016. Towards a standardization of biomethane potential tests. Water Sci. Technol. 74, 2515–2522.

19. Jönsson, L.J., Alriksson, B., Nilvebrant, N.O., 2013. Bioconversion of lignocellulose: Inhibitors and detoxification. Biotechnol. Biofuels 6, 1–10.

20. Katsimpouras, C., Zacharopoulou, M., Matsakas, L., Rova, U., Christakopoulos, P., Topakas, E., 2017. Sequential high gravity ethanol fermentation and anaerobic digestion of steam explosion and organosolv pretreated corn stover. Bioresour. Technol. 244, 1129–1136.

21. Kothari, R., Pandey, A.K., Kumar, S., Tyagi, V. V., Tyagi, S.K., 2014. Different aspects of dry anaerobic digestion for bio-energy: An overview. Renew. Sustain. Energy Rev. 39, 174–195.

22. Kucharska, K., Hołowacz, I., Konopacka-Łyskawa, D., Rybarczyk, P., Kamiński, M., 2018. Key issues in modeling and optimization of lignocellulosic biomass fermentative conversion to gaseous biofuels. Renew. Energy 129, 384–408.

23. Kwietniewska, E., Tys, J., 2014. Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation. Renew. Sustain. Energy Rev. 34, 491–500.

24. Lachos-Perez, D., Martinez-Jimenez, F., Rezende, C.A., Tompsett, G., Timko, M., Forster-Carneiro, T., 2016. Subcritical water hydrolysis of sugarcane bagasse: An approach on solid residues characterization, J. Supercrit. Fluids 108, 69–78.

25. Lachos-Perez, D., Tompsett, G.A., Guerra, P., Timko, M.T., Rostagno, M.A., Martínez, J., Forster-Carneiro, T., 2017. Sugars and char formation on subcritical water hydrolysis of sugarcane straw. Bioresour. Technol. 243, 1069–1077.

26. Maciel-Silva, F.W., Mussatto, S.I., Forster-Carneiro, T., 2019. Integration of subcritical water pretreatment and anaerobic digestion technologies for

valorization of açai processing industries residues. J. Clean. Prod. 228, 1131–1142.

27. Olsson, L., Hahn-Hägerdal, B., 1996. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme Microb. Technol. 18, 312–331.

28. Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresour. Technol. 74, 25–33.

29. Paul, S., Dutta, A., 2018. Challenges and opportunities of lignocellulosic biomass for anaerobic digestion. Resour. Conserv. Recycl. 130, 164–174.

30. Qing, Q., Guo, Q., Zhou, L., Gao, X., Lu, X., Zhang, Y., 2017. Comparison of alkaline and acid pretreatments for enzymatic hydrolysis of soybean hull and soybean straw to produce fermentable sugars. Ind. Crops Prod. 109, 391–397.

31. Rempel, A., Sossella, F. de S., Margarites, A.C., Astolfi, A.L., Steinmetz, R.L.R., Kunz, A., Treichel, H., Colla, L.M., 2019. Bioethanol from Spirulina platensis biomass and the use of residuals to produce biomethane : An energy efficient approach. Bioresour. Technol. 288.

32. Cortivo, P.R.D., Hickert, L.R., Hector, R., Ayub, M.A.Z., 2018. Fermentation of oat and soybean hull hydrolysates into ethanol and xylitol by recombinant industrial strains of Saccharomyces cerevisiae under diverse oxygen environments. Ind. Crops Prod. 113, 10–18.

33. Saha, B.C., Nichols, N.N., Qureshi, N., Kennedy, G.J., Iten, L.B., Cotta, M.A., 2015. Pilot scale conversion of wheat straw to ethanol via simultaneous saccharification and fermentation. Bioresour. Technol. 175, 17–22.

34. Salakkam, A., Kingpho, Y., Najunhom, S., Aiamsonthi, K., Kaewlao, S., Reungsang, A., 2017. Bioconversion of soybean residue for use as alternative nutrient source for ethanol fermentation. Biochem. Eng. J. 125, 65–72.

35. Sarkar, N., Ghosh, S.K., Bannerjee, S., Aikat, K., 2012. Bioethanol production from agricultural wastes : An overview. Renew. Energy 37, 19–27.

36. Shrestha, S., Fonoll, X., Khanal, S.K., Raskin, L., 2017. Biological strategies for enhanced hydrolysis of lignocellulosic biomass during anaerobic digestion: Current status and future perspectives. Bioresour. Technol. 245, 1245–1257.

37. Steinmetz, R.L.R., Mezzari, M.P., da Silva, M.L.B., Kunz, A., do Amaral, A.C., Tápparo, D.C., Soares, H.M., 2016. Enrichment and acclimation of an anaerobic mesophilic microorganism's inoculum for standardization of BMP assays. Bioresour. Technol. 219, 21–28.

38. Taherzadeh, M.J., Karimi, K., 2011. Fermentation inhibitors in ethanol processes and different strategies to reduce their effects, in: Biofuels: Alternative Feedstocks and Conversion Processes. Elsevier Inc., pp. 287–311.

39. VDI 4630, 2016. Fermentation of organic materials. Characterization of the substrate, sampling, collection of material data, fermentation tests, Verein Deutscher Ingenieure - VDI. Germany.

40. Vedovatto, F., Ugalde, G., Bonatto, C., Bazoti, S.F., Treichel, H., Mazutti, M.A., Zabot, G.L., Tres, M. V., 2021. Subcritical water hydrolysis of soybean residues for obtaining fermentable sugars. J. Supercrit. Fluids 167, 105043.

41. Venturin, B., Frumi Camargo, A., Scapini, T., Mulinari, J., Bonatto, C., Bazoti, S., Pereira Siqueira, D., Maria Colla, L., Alves, S.L., Paulo Bender, J., Luís Radis Steinmetz, R., Kunz, A., Fongaro, G., Treichel, H., 2018. Effect of pretreatments on corn stalk chemical properties for biogas production purposes. Bioresour. Technol. 266, 116–124.

42. Xiong, J., Hassan, M., Wang, W., Ding, W., 2020. Methane enhancement by the co-digestion of soybean straw and farm wastewater under different thermo-chemical pretreatments. Renew. Energy 145, 116–123.

43. Xu, Z., Zhao, M., Miao, H., Huang, Z., Gao, S., Ruan, W., 2014. In situ volatile fatty acids influence biogas generation from kitchen wastes by anaerobic digestion. Bioresour. Technol. 163, 186–192.

44. Yu, Y., Lou, X., Wu, H., 2008. Some Recent Advances in Hydrolysis of Biomass in Hot-Compressed Water and Its Comparisons with Other Hydrolysis Methods. Energy and Fuels 22, 46–60.

45. Zabed, H., Sahu, J.N., Suely, A., Boyce, A.N., Faruq, G., 2017. Bioethanol production from renewable sources : Current perspectives and technological progress. Renew. Sustain. Energy Rev. 71, 475–501.

46. Zheng, Y., Zhao, J., Xu, F., Li, Y., 2014. Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog. Energy Combust. Sci. 42, 35–53.

47. Zhu, J., Zheng, Y., Xu, F., Li, Y., 2014. Solid-state anaerobic co-digestion of hay and soybean processing waste for biogas production. Bioresour. Technol. 154, 240–247.

CAPÍTULO 5 - CONSIDERAÇÕES FINAIS

5.1 DISCUSSÃO

Os resultados obtidos neste trabalho demonstram que a palha e a casca de soja têm potencial como matéria-prima para produção de açúcares fermentescíveis e para produção de biocombustíveis (etanol e biogás).

A hidrólise com água subcrítica em modo semi-contínuo da palha e da casca de soja foram avaliadas para obtenção de açúcares fermentescíveis. As condições de 220 °C/R-18 e 220 °C/R-15 apresentaram o maior rendimento de açúcares redutores (Y_{RS}) e a maior eficiência (E). A palha obteve um Y_{RS} de 9,56 ± 0.53 g/ 100 g em 4 min e a casca obteve um Y_{RS} de 10,15 ± 0.50 g/100 g em 3 min de reação de hidrólise. Já a eficiência foi de 23,65 ± 1,32 g/100g de carboidratos em 4 min para palha e para a casca a eficiência foi de 23,04 ± 1,14 g/100 g de carboidratos em 3 min. O rendimento de açúcares redutores e a eficiência está associado a dissociação dos componentes dos resíduos pelo processo de hidrólise com água subcrítica.

Na quantificação dos componentes dos hidrolisados foram obtidos açúcares (glicose, xilose, arabinose, celobiose), ácidos orgânicos (ácido acético e ácido fórmico) e inibidores (HMF e furfural). As condições que obtiveram o maior rendimento de açúcares (soma de glicose, xilose, arabinose e celobiose) foram a condição de 180 °C/R-9 com uma concentração de 9,67 ± 1,99 g/L a 4 min e a condição de 220 °C/R-7,5 com uma concentração de 9,46 ± 1,87 g/L a 3 min. O aumento de temperatura de 180 °C para 220 °C aumentou a produção de açúcares, ácidos orgânicos e inibidores. Mas, quando aumentado a temperatura de 220 °C para 260 °C, houve uma diminuição da produção de açúcares e um aumento na produção de ácidos orgânicos e inibidores, indicando que temperaturas muito altas favorecem a degradação dos açúcares em outros bioprodutos.

Quando analisados os sólidos através do FT-IR e MEV, após o processo de hidrólise com água subcrítica, foi possível observar a remoção de diferentes componentes e a modificação da estrutura dos resíduos. Na análise de FT-IR, a condição de 260 °C apresentou uma maior intensidade entre os picos de 2900 a 3500 cm^{-1} e de 800 a 1700 cm^{-1}, indicando maior remoção dos componentes. A condição de 180 °C apresentou uma menor intensidade, indicando uma baixa remoção dos componentes e a condição de 220 °C apresentou uma remoção intermediária dos componentes. A intensidade dos picos, consequentemente a

remoção dos componentes é influenciada pela ação da temperatura na dissociação da celulose e hemicelulose. Na análise de MEV as amostras das condições de 220 °C/R-18 e 220 °C/R-18 após o processo de hidrólise apresentou modificação da estrutura morfológica com vasos rompidos e torcidos.

As condições de maior rendimento de açúcares redutores (220 °C/R-18 e 220 °C/R-15) foram selecionadas para avaliação da produção do etanol e do biogás. Na produção do etanol foi selecionada a levedura *Wickerhamomyces* sp. por ser uma levedura recentemente descrita e apresentar possível potencial de produção de etanol na presença do ácido acético.

Os resultados de produção de etanol em frasco de Erlenmeyer foi possível nas condições diluídas e suplementadas com glicose. O hidrolisado da palha diluído 1:4 e suplementado com 10 g/L de glicose obteve uma produção de 5,57 ± 0,01 g/L de etanol em 72 h de fermentação. O hidrolisado da casca diluído 1:2 e suplementado com 10 g/L de glicose obteve uma produção de 6,11 ± 0,11 g/L de etanol em 96 h de fermentação. Os hidrolisados possuíam 1,74 ± 0,01 g/L de ácido acético para o hidrolisado da palha e 1,48 ± 0,02 g/L de ácido acético para o hidrolisado da casca. Estes resultados indicam que a levedura tolerou uma moderada presença do ácido acético. Os autores também observaram que a levedura preferiu consumir a glicose e os outros açúcares não foram consumidos.

Na fermentação em biorreator foram selecionadas as condições de maior rendimento de etanol do ensaio em frasco de Erlenmeyer para avaliação da produção de etanol com e sem ajuste de pH no hidrolisado. Os hidrolisados sem ajuste de pH produziram 4,66 g/L e 4,77 g/L de etanol, para os hidrolisados da palha e da casca em 48 h de fermentação, respectivamente. Os hidrolisados da palha e da casca com ajuste de pH (7,0) apresentaram uma produção de etanol de 4,03 g/L e 3,60 g/L de etanol em 24 h de fermentação, respectivamente. A máxima produção de etanol em 24 h de fermentação está associada ao ajuste de pH dos hidrolisados para 7,0, indicando a diminuição do efeito do ácido acético (forma não dissociado), como visto na literatura.

Na avalição do potencial bioquímico de biogás, os hidrolisados fermentados da palha de soja sem e com ajuste de pH (7,0) apresentaram uma produção de biogás de 652 ± 34 NmL/g_{SVad} e 739 ± 37 NmL/g_{SVad}, e quando comparado com a produção do biogás do hidrolisado da palha representa um ganho de 60,59% e 82,02% na produção de biogás, respectivamente. O hidrolisado fermentado da casca sem ajuste de pH obteve uma produção de 620 ± 26 NmL/g_{SVad} e quando comparado com o hidrolisado da casca isso representa uma redução de 8,42%. Os hidrolisados da palha e da casca proporcionaram uma produção de 406

± 8 NmL/g_{SVad} e 677 ± 35 NmL/g_{SVad} em 36 dias. Na avaliação da produção dos hidrolisados da palha e da casca foi observado um retardamento da produção do biogás até o décimo dia do processo de co-digestão anaeróbica. Isto pode ter ocorrido pelo efeito inibitório do substrato, devido à concentração de ácidos orgânicos no hidrolisado. A concentração de ácido acético nos hidrolisados pode ter inibido a via metagênese acetoclássica contribuindo com retardamento da produção de biogás até o décimo dia. A produção de biogás pode ter retornado via metanogênese hidrogenotrófica após o décimo dia do processo de co-digestão anaeróbica.

Na avaliação da composição do metano do biogás, os hidrolisados fermentados da palha sem e com ajuste de pH (7,0) obtiveram uma concentração de metano de 65,23% e 69,79%, respectivamente. O hidrolisado fermentado da casca sem ajuste de pH obteve uma concentração de 61,10%. Já os hidrolisados da palha e a casca a concentração de metano foi de 48,36% e 49,21%, respectivamente.

5.2 CONCLUSÕES

A partir dos resultados obtidos nesta Tese de Doutorado sobre a avaliação da hidrólise com água subcrítica da palha e da casca de soja e produção de etanol e biogás, podemos concluir que:

- A hidrólise da palha e da casca com água subcrítica apresentou potencial para a produção de açúcares fermentescíveis.

- Na caracterização dos hidrolisados foram quantificados os açúcares (glicose, xilose, arabinose e celobiose), ácidos orgânicos (ácido acético e ácido fórmico) e inibidores (HMF e furfural).

- Na análise de FT-IR e MEV foi possível observar a modificação da estrutura da palha e casca após o processo de hidrólise com água subcrítica, indicando a ação da temperatura na dissociação dos componentes dos resíduos.

- A produção de etanol foi possível a partir dos hidrolisados diluído e suplementado com glicose da palha e da casca usando a levedura *Wickerhamonyces* sp..

- Os hidrolisados fermentados apresentaram potencial bioquímico para produção de biogás e de metano via co-digestão anaeróbica.

- Após a finalização do trabalho, concluiu-se que os resíduos da cultura da soja (palha e casca) juntamente com a hidrólise com água subcrítica e os processos de produção de biocombustíveis são alternativas promissoras para a produção de energias renováveis.

SUGESTÕES PARA TRABALHOS FUTUROS

- Avaliar o pré-tratamento dos resíduos (palha e casca) antes do processo com hidrólise com água subcrítica.

- Avaliar o aumento de escala (*scale-up*) na produção de açúcares fermentescíveis.

- Realizar uma análise econômica do processo de hidrólise e da produção de biocombustíveis.

REFERÊNCIAS

ABAIDE, Ederson R. et al. Reasons for processing of rice coproducts: Reality and expectations. **Biomass and Bioenergy**, v. 120, p. 240–256, 2019a.

ABAIDE, Ederson R. et al. Subcritical water hydrolysis of rice straw in a semi-continuous mode. **Journal of Cleaner Production**, v. 209, p. 386–397, 2019b.

ABDULKHANI, A. et al. Potential of Soya as a raw material for a whole crop biorefinery. **Renewable and Sustainable Energy Reviews**, v. 75, p. 1269–1280, 2017.

ADITIYA, H. B. et al. Second generation bioethanol production: A critical review. **Renewable and Sustainable Energy Reviews**, v. 66, p. 631–653, 2016.

ALVIRA, P. et al. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. **Bioresource Technology**, v. 101, n. 13, p. 4851–4861, 2010.

ARTIFON, W. et al. Bioethanol Production From Hydrolyzed Lignocellulosic After Detoxification Via Adsorption With Activated Carbon and Dried Air Stripping. **Frontiers in Bioengineering and Biotecnology**, v. 6, p. 1–6, 2018.

CABRERA, E. et al. Comparison of industrially viable pretreatments to enhance soybean straw biodegradability. **Bioresource Technology**, v. 194, p. 1–6, 2015.

CANABARRO, N. I. et al. Ethanol production by solid-state saccharification and fermentation in a packed-bed bioreactor. **Renewable Energy**, v. 102, p. 9–14, 2017.

CAO, G. et al. Bioabatement with hemicellulase supplementation to reduce enzymatic hydrolysis inhibitors. **Bioresource Technology**, v. 190, p. 412–415, 2015.

CARDENAS-TORO, F. P. et al. Integrated supercritical fluid extraction and subcritical water hydrolysis for the recovery of bioactive compounds from pressed palm fiber. **Journal of Supercritical Fluids**, v. 93, p. 42–48, 2014.

CASSALES, A. et al. Optimization of soybean hull acid hydrolysis and its characterization as a potential substrate for bioprocessing. **Biomass and Bioenergy**, v. 35, n. 11, p. 4675–4683, 2011.

COCERO, M. J. et al. Understanding biomass fractionation in subcritical & supercritical water. **Journal of Supercritical Fluids**, v. 133, p. 550–565, 2018.

CONAB. Compania Nacional de Abastescimento. Acompanhamento da safra Brasileira de cana-de-açúcar, v. 7 - Safra 2020/21, n. 3 - Terceiro levantamento, Brasília, p. 1-62, dez. 2020.

CONAB. Companhia Nacional de Abastecimento. Acompanhamento da safra Brasileira de grãos, v.8 - Safra 2020/2021, n. 7 - sétimo levantamento, Brasília, p. 1-116, abr. 2021.

COOPER, J. et al. Treatment and resource recovery options for first and second generation bioethanol spentwash – A review. **Chemosphere**, v. 241, p. 124975, 2020.

DA CUNHA-PEREIRA, F. et al. Liberation of fermentable sugars from soybean hull biomass using ionic liquid 1-butyl-3-methylimidazolium acetate and their bioconversion to ethanol. **Biotechnology Progress**, v. 32, n. 2, p. 312–320, 2016.

DALL CORTIVO, P. R. et al. Conversion of fermentable sugars from hydrolysates of soybean and oat hulls into ethanol and xylitol by *Spathaspora hagerdaliae* UFMG-CM-Y303. **Industrial Crops and Products**, v. 146, p. 112218, 2020.

EPE. Empresa de Pesquisa Energética. Nota Técnica DEA 15/14 - Inventário Energético de Resíduos Rurais, Rio de Janeiro, out. 2014.

FAO. **Production of Crops**, 2018. Disponível em: http://www.fao.org/faostat/en/#data/QC. Acesso em: 13 out. 2020.

FARDIN, J. F.; BARROS Jr, O. de; DIAS, A. P. F. Biomass: Some Basics and Biogas. In: YAHYAOUI, I. **Advances in renewable energies and power technologies:** Biomass, Fuel Cells, Geothermal Energies, and Smart Grids. Elsevier Inc., 2018. v. 2, p. 1–37.

GNANASAMBANDAM, Ravin; PROCTOR, A. Preparation of soy hull pectin. **Food Chemistry**, v. 65, n. 4, p. 461–467, 1999.

KNEZ, Ž. et al. Chemicals and value added compounds from biomass using sub- and supercritical water. **Journal of Supercritical Fluids**, v. 133, p. 591–602, 2018.

KOOPMANS, A.; KOPPEJAN, J. Agricultural and forest residues - generation, utilization and availability. **Regional consultation on modern applications of biomass energy**, v. 10, p. 1–23, 1997.

KOTHARI, R. et al. Different aspects of dry anaerobic digestion for bio-energy: An overview. **Renewable and Sustainable Energy Reviews**, v. 39, p. 174–195, 2014.

KOVAČIĆ, Đ. et al. Electroporation of harvest residues for enhanced biogas production in anaerobic co-digestion with dairy cow manure. **Bioresource Technology**, v. 274, p. 215–224, 2019.

KUNZ, A.; STEINMETZ, R. L. R.; AMARAL, A. C. do. **Fundamentos da digestão anaeróbia, purificação do biogás, uso e tratamento do digestato**. Concórdia: Sbera: Embrapa Suínos e Aves, 2019. 209 p.

LACHOS-PEREZ, D. et al. Subcritical water hydrolysis of sugarcane bagasse: An approach on solid residues characterization. **Journal of Supercritical Fluids**, v. 108, p. 69–78, 2016.

LI, D. et al. Effects of feedstock ratio and organic loading rate on the anaerobic mesophilic co-digestion of rice straw and cow manure. **Bioresource Technology**, v. 189, p. 319–326, 2015.

LI, Y. et al. Optimization study on the hydrogen peroxide pretreatment and production of bioethanol from seaweed *Ulva prolifera* biomass. **Bioresource Technology**, v. 214, p. 144–149, 2016.

LIMAYEM, A.; RICKE, S. C. Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects. **Progress in Energy and Combustion**

Science, v. 38, n. 4, p. 449–467, 2012.

MAITAN-ALFENAS, G. P.; VISSER, E. M.; GUIMARÃES, V. M. Enzymatic hydrolysis of lignocellulosic biomass: Converting food waste in valuable products. **Current Opinion in Food Science**, v. 1, n. 1, p. 44–49, 2015.

MATSUMURA, Y. et al. Supercritical water treatment of biomass for energy and material recovery. **Combustion Science and Technology**, v. 178, p. 509–536, 2006.

MERCI, A. et al. Properties of microcrystalline cellulose extracted from soybean hulls by reactive extrusion. **Food Research International**, v. 73, p. 38–43, 2015.

MIELENZ, J. R.; BARDSLEY, J. S.; WYMAN, C. E. Fermentation of soybean hulls to ethanol while preserving protein value. **Bioresource Technology**, v. 100, n. 14, p. 3532–3539, 2009.

MOHAN, D.; PITTMAN, C. U.; STEELE, P. H. Pyrolysis of wood/biomass for bio-oil: A critical review. **Energy and Fuels**, v. 20, n. 3, p. 848–889, 2006.

MUÑOZ, M. J. et al. Comparison of industrially viable pretreatments to enhance soybean straw biodegradability. **Bioresource Technology**, v. 194, p. 1–6, 2015.

MUSSATTO, S. I.; DRAGONE, G. M.. Biomass Pretreatment, Biorefineries, and Potential Products for a Bioeconomy Development. In: MUSSATTO, S. I. **Biomass fractionation Technologies for a lignocellulosic feedstock based biorefinery**. Elsevier Inc., 2016. p. 1-22.

MUSSATTO, S. I. et al. Technological trends, global market, and challenges of bio-ethanol production. **Biotechnology Advances**, v. 28, n. 6, p. 817–830, 2010.

NAIK, S. N. et al. Production of first and second generation biofuels: A comprehensive review. **Renewable and Sustainable Energy Reviews**, v. 14, n. 2, p. 578–597, 2010.

NESHAT, S. A. et al. Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. **Renewable and Sustainable Energy Reviews**, v. 79, p. 308–322, 2017.

OGEDA, T. L.; PETRI, D. F S. Hidrólise enzimática de biomassa. **Quimica Nova**, v. 33, n. 7, p. 1549–1558, 2010.

ONTHONG, U.; JUNTARACHAT, N. Evaluation of Biogas Production Potential from Raw and Processed Agricultural Wastes. **Energy Procedia**, v. 138, p. 205–210, 2017.

PITARELO, A. et al. Effect of moisture content in the steam treatment and enzymatic hydrolysis of sugarcane bagasse. **Química Nova**, v. 35, n. 8, p. 1502–1509, 2012.

PRADO, J. M. et al. Sub- and supercritical water hydrolysis of agricultural and food industry residues for the production of fermentable sugars: A review. **Food and Bioproducts Processing**, v. 98, p. 95–123, 2016.

PRASAD, R. K. et al. Bioethanol production from waste lignocelluloses: A review on microbial degradation potential. **Chemosphere**, v. 231, p. 588–606, 2019.

QING, Q. et al. Comparison of alkaline and acid pretreatments for enzymatic hydrolysis of soybean hull and soybean straw to produce fermentable sugars. **Industrial Crops and Products**, v. 109, p. 391–397, 2017.

RABELO, S. C. et al. Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide. **Biomass and Bioenergy**, v. 35, n. 7, p. 2600–2607, 2011.

REDDY, N.; YANG, Y. Natural cellulose fibers from soybean straw. **Bioresource Technology**, v. 100, n. 14, p. 3593–3598, 2009.

RISBERG, K. et al. Biogas production from wheat straw and manure - Impact of pretreatment and process operating parameters. **Bioresource Technology**, v. 149, p. 232–237, 2013.

ROJAS, M. J. et al. Sequential proteolysis and cellulolytic hydrolysis of soybean hulls for oligopeptides and ethanol production. **Industrial Crops and Products**, v. 61, p. 202–210, 2014.

SAHA, B. C. et al. Pilot scale conversion of wheat straw to ethanol via simultaneous saccharification and fermentation. **Bioresource Technology**, v. 175, p. 17–22, 2015.

SANTOS, M. S. N. dos et al. Optimization of subcritical water hydrolysis of pecan wastes biomasses in a semi-continuous mode. **Bioresource Technology**, v. 306, p. 123129, 2020.

SANTOS, F. A. et al. Otimização do pré-tratamento hidrotérmico da palha de cana-de-açúcar visando à produção de etanol celulósico. **Quimica Nova**, v. 37, n. 1, p. 56–62, 2014.

SARKAR, N. et al. Bioethanol production from agricultural wastes: An overview. **Renewable Energy**, v. 37, n. 1, p. 19–27, 2012.

SHRESTHA, S. et al. Biological strategies for enhanced hydrolysis of lignocellulosic biomass during anaerobic digestion: Current status and future perspectives. **Bioresource Technology**, v. 245, p. 1245–1257, 2017.

SINAG, A.; KRUSE, A.; SCHWARZKOPF, V. Formation and Degradation Pathways of Intermediate Products Formed during the Hydropyrolysis of Glucose as a Model Substance for Wet Biomass in a Tubular Reactor. **Engineering in Life Sciences**, v. 3, n. 12, p. 469–473, 2003.

SINDHU, R. et al. Development of a combined pretreatment and hydrolysis strategy of rice straw for the production of bioethanol and biopolymer. **Bioresource Technology**, v. 215, p. 110–116, 2016.

SU, T. et al. Lignocellulosic biomass for bioethanol: Recent advances, technology trends, and barriers to industrial development. **Current Opinion in Green and Sustainable Chemistry**, v. 24, p. 56–60, 2020.

TAVVA, S. S M. D. et al. Bioethanol production through separate hydrolysis and fermentation of *Parthenium hysterophorus* biomass. **Renewable Energy**, v. 86, p. 1317–1323, 2016.

VAN DYK, J. S.; PLETSCHKE, B. I. A review of lignocellulose bioconversion using

enzymatic hydrolysis and synergistic cooperation between enzymes-Factors affecting enzymes, conversion and synergy. **Biotechnology Advances**, v. 30, n. 6, p. 1458–1480, 2012.

VENTURIN, B. et al. Effect of pretreatments on corn stalk chemical properties for biogas production purposes. **Bioresource Technology**, v. 266, p. 116–124, 2018.

XU, Z. et al. Enzymatic hydrolysis of pretreated soybean straw. **Biomass and Bioenergy**, v. 31, p. 162–167, 2007.

YU, Y.; LOU, X.; WU, H. Some Recent Advances in Hydrolysis of Biomass in Hot-Compressed Water and Its Comparisons with Other Hydrolysis Methods. **Energy and Fuels**, v. 22, p. 46–60, 2008.

ZHENG, Y. et al. Pretreatment of lignocellulosic biomass for enhanced biogas production. **Progress in Energy and Combustion Science**, v. 42, n. 1, p. 35–53, 2014.

ZHU, G. et al. Recovery of biomass wastes by hydrolysis in sub-critical water. **Resources, Conservation and Recycling**, v. 55, n. 4, p. 409–416, 2011.

ZHU, J. et al. Solid-state anaerobic co-digestion of hay and soybean processing waste for biogas production. **Bioresource Technology**, v. 154, p. 240–247, 2014.