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Charline Bonatto

Seawater and wastewater from shrimp production as alternatives in the fermentation of fruit residue for ethanol production

Florianópolis, SC, Brazil
2020

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Seawater and wastewater from shrimp production as alternatives in the fermentation of fruit residue for ethanol production

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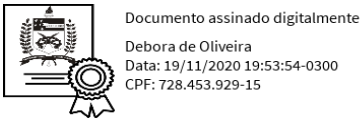

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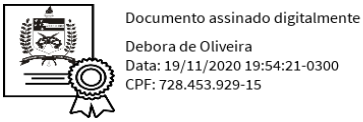

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RESUMO

Na produção de biocombustíveis, a água é consumida principalmente no cultivo das culturas energéticas, extração de carboidratos e fermentação. Estudos recentes mostraram que são necessários aproximadamente 9,8 L de água para produção de 1 L de etanol a partir de biomassa. Com a crescente produção de biocombustíveis espera-se um aumento no consumo da água doce. Relatos da literatura apontam que esse acréscimo pode ser de 5,5% até 2030, o que aplicará uma carga adicional sobre os recursos de água doce. Uma opção frente a essa problemática é o desenvolvimento de sistemas de produção de bioetanol baseado na utilização da água do mar e águas residuárias, o que pode reduzir o estresse sobre os recursos de água doce. Os poucos trabalhos desenvolvidos nessa temática apresentaram resultados promissores ao utilizar a água do mar e águas residuárias em etapas da produção de etanol, portanto outras investigações e contribuições sobre a temática são relevantes. Diante disso, esse trabalho investigou a viabilidade técnica do uso de água do mar (SW) e água da produção de camarão (WSP) para substituir a água doce na fermentação de resíduo industrial de laranja e resíduo de mamão do setor de hortifrúti pelas leveduras *Wickerhamomyces* sp. UFFS-CE-3.1.2 e *S. cerevisiae* CAT-1. O resíduo de mamão apresentou elevadas concentrações de açúcares livres, se mostrando como uma fonte ideal para a produção de etanol. Já o resíduo industrial de laranja apresentou baixas concentrações de açúcares livres, por isso foi submetido a uma etapa de tratamento ácido para solubilização dos açúcares presentes nos polissacarídeos. Os resultados mostraram que a água do mar pode ser uma alternativa promissora à água doce para o setor de biocombustíveis e que os resíduos de frutas são substratos promissores para a produção de etanol.

Palavras-chave: Biocombustível. Resíduo de mamão. Resíduo industrial de laranja. Salinidade.

RESUMO EXPANDIDO

Introdução

Altas quantidades de água são consumidas na produção de etanol, especialmente para o cultivo de culturas energéticas e no processamento de biocombustível, estima-se que são necessários aproximadamente 9,8 L de água para produção de 1 L de etanol a partir de biomassa (ADEN, 2007; INDIRA *et al*., 2018).

Frente à essa questão, a água do mar e água residuária tem sido apontada por alguns estudos como uma alternativa de substituição da água doce na indústria de bioetanol, já que são um recurso abundante se comparado com quantidade de água doce disponível no mundo. Águas residuárias são geradas em grandes volumes diariamente em diferentes setores industriais e agrícolas, necessitando de tratamento específico antes do descarte final. A aplicação dessas águas na cadeia de produção de etanol reduziria a pressão gerada sobre os recursos hidrícos e poderia fornecer água para outros setores mais nobres, como o abastecimento público (GREETHAM *et al*., 2018).

A substituição de culturas energéticas por biomassa celulósica também favorece a redução do consumo de água em biorrefinarias (INDIRA *et al*., 2018), diminuindo o consumo de água utilizada no processo de irrigação das culturas. Dentre essas biomassas, os resíduos de frutas tem se mostrado promissores para a produção de etanol, pois são ricos em açúcares prontamente disponíveis, como glicose, frutose e sacarose, além de polissacarídeos (pectina, celulose e hemicelulose) que podem ser convertidos em açúcares por meio de um pré-tratamento e hidrólise, o que os torna substratos ideais para fermentação (LIAKOU *et al*., 2018; SARKAR *et al*., 2019).

Objetivo

Avaliar a possibilidade de substituição da água doce por água do mar e água residuária da produção de camarão na produção de etanol usando resíduo de laranja industrial e resíduo de mamão vindo do setor de hortifrúti como substrato de fermentação e as leveduras *Wickerhamomyces* sp. UFFS-CE-3.1.2 e *S. cerevisiae* CAT-1 como microrganismos fermentadores.

Metodologia

Inicialmente foi avaliado o potencial da aplicação do resíduo industrial de laranja para produção de etanol pela levedura *Wickerhamomyces* sp. UFFS-CE-3.1.2 usando água do mar e água ultrapura como solvente. Assim, o resíduo industrial de laranja, previamente seco e homogeneizado (mesh 10), foi suspenso em água do mar ou água ultrapura na proporção de 10% (m v^{-1}) (INDIRA *et al*., 2018) para extração e solubilização dos açúcares livres. Por se tratar de um resíduo industrial esgotado, baixas concentrações de açúcares prontamente disponíveis estavam presentes no caldo resultante da extração sólido-líquido. Dessa forma, o resíduo foi submetido a uma etapa de tratamento ácido, usando ácido sulfúrico, para aumentar a concentração de açúcares fermentescíveis e consequentemente o rendimento de etanol. O tratamento ácido foi avaliado por planejamento experimental (DCCR 2^3) e a influência das variáveis concentração de ácido sulfúrico (H_2SO_4) (2, 5, 10, 15 e 18 % (v v^{-1})), temperatura (66, 80, 120 e 134 °C) e relação sólido-líquido (3, 10, 20, 30 e 37 % (m_{seca} v^{-1})) foi estudada. Para fins comparativos e avaliação da influência da salinidade na fermentação, a água ultrapura também foi utilizada nos ensaios. As fermentações foram realizadas utilizando o caldo resultante do processo de extração de açúcares livres e o caldo resultante do processo de tratamento com ácido sulfúrico, considerando a melhor resposta em relação aos açúcares liberados no planejamento experimental. Os experimentos foram conduzidos em Erlenmeyer de 250 mL com 180 mL de meio fermentativo e 20 mL de inóculo da levedura *Wickerhamomyces* sp. UFFS-CE-3.1.2. Amostras foram coletadas em 0, 3, 6, 9 e 12 horas de fermentação para acompanhamento do consumo de açúcares e formação de produtos.

O resíduo de mamão completamente amadurecido, gerado no setor de hortifrúti, também foi aplicado como substrato de fermentação para produção de etanol pelas leveduras *Wickerhamomyces* sp. UFFS-CE-3.1.2 e *Saccharomyces cerevisiae* CAT-1 em um sistema baseado na utilização da água do mar e água residuária da produção de camarão como alternativas à água doce. Para fins comparativos e avaliação do efeito da salinidade a água ultrapura também foi utilizada. Parâmetros fermentativos como agitação (50, 85 e 120 rpm), temperatura (20, 30 e 40 ºC), suplementação com ureia (0, 125 e 250 mM) (LI *et al*., 2017) e relação sólido-líquido (50, 125 e 200 %) foram avaliados em planejamento Plackett-Burmann para avaliar as variáveis significativas para a produção de etanol. Cada ponto do planejamento foi avaliado em cinética, sendo realizada a coleta de amostra em 0, 3, 6, 9, 12, 24 e 48 horas de fermentação. Os

experimentos foram conduzidos em Erlenmeyer de 250 mL com 180 mL de meio fermentativo e 20 mL de inóculo da levedura *Wickerhamomyces* sp. UFFS-CE-3.1.2.

A fim de confirmar a confiabilidade dos resultados obtidos no planejamento a melhor condição foi reproduzida em triplicata utilizando SW, WSP e *Wickerhamomyces* sp. UFFS-CE-3.1.2 como microrganismo fermentador. Para avaliar o efeito da salinidade na fermentação conduzida pela cepa UFFS-CE-3.1.2 também foram realizadas fermentações utilizando água ultrapura (UW). Para fins comparativos, foram realizadas fermentações com *Saccharomyces cerevisiae* CAT-1 como microrganismos fermentador utilizando SW, WSP, UW, como solvente e as mesmas condições experimentais das utilizadas nas fermentações conduzidas com a *Wickerhamomyces* sp. UFFS-CE-3.1.2.

Resultados e discussão

A frutose foi a principal hexose quantificada no caldo do resíduo de laranja, apresentando concentrações de 7,89±0,10 g L^{-1}. Também foram quantificadas concentrações traços de glicose (1,04±0,22 g L^{-1}). Esses açúcares são as principais fontes de carbono para produção de etanol. Uma baixa concentração de açúcares prontamente disponíveis no caldo fermentativo pode afetar negativamente a fermentação alcoólica levando a uma baixa produção de etanol (DAKAL *et al*., 2014; TURHAN *et al*., 2010). Por isso, o resíduo de laranja foi submetido ao tratamento ácido para aumentar a disponibilidade de açúcares.

A liberação de glicose foi favorecida nos ensaios conduzidos com maiores concentrações de ácido, relações sólido-líquido e elevadas temperaturas, resultando em concentrações de até 8,06 g L^{-1} de glicose, 12,47 g L^{-1} de ácido galacturônico, 13,48 g L^{-1} de arabinose, além de 19,45 g L^{-1} de outros açúcares como frutose, xilose e celobiose.

A fermentação conduzida utilizando o caldo resultante do processo de extração de açúcares resultou na produção máxima de 0,61±0,11 g L^{-1} de etanol usando água do mar e 0,57±0,08 g L^{-1} usando água ultrapura. Verificou-se que somente a frutose foi utilizada como fonte de carbono no processo fermentativo e a glicose permaneceu constante ao longo da fermentação. A presença de óleos essenciais na matriz do resíduo de laranja pode ter afetado a fermentação resultando na baixa produção de etanol. Por isso, a remoção desses compostos ou a utilização de microrganismos tolerantes pode ser uma

alternativa para aumentar a produção de etanol a partir de resíduos de laranja (LOHRASBI *et al*. 2010).

Já caldo do tratamento do resíduo de laranja com ácido sulfúrico não foi fermentado pela *Wickerhamomyces* sp. UFFS-CE-3.1.2, apresentando resultado negativo quanto à produção de etanol, mesmo com pH permanecendo em 5,25±0,15 e contendo elevada quantidade de açúcares fermentescíveis (até 51,61±3,67g L^{-1}). O caldo do resíduo de laranja após o tratamento apresentou elevada concentração de ácido galacturônico (13,69±0,91 g L^{-1}) e ácido acético (2,31±0,29 g L^{-1}), além de ácido fórmico (1,22±0,15 g L^{-1}) e ácido cítrico (0,39±0,03 g L^{-1}), que em conjunto podem ter agido como inibidores da fermentação de forma sinérgica.

Os experimentos conduzidos com o resíduo de mamão e diferentes fontes hídricas apresentaram comportamentos fermentativos similares, não apresentando diferença estatística significativa entre os valores máximos alcançados. Concentrações máximas de etanol foram obtidas após 9 horas de fermentação usando a *Wickerhamomyces* sp. (27,31±1,40 g L^{-1}) e 12 horas usando *S. cerevisiae* CAT-1 (24,53±0,68 g L^{-1}). Isso mostra que a *S. cerevisiae* CAT-1 apresentou menor adaptabilidade ao sistema utilizado, resultando na taxa de fermentação mais lenta se comparado com os resultados obtidos na fermentação conduzida pela *Wickerhamomyces* sp. UFFS-CE-3.1.2. Esses resultados também indicam que essas cepas são capazes de tolerar o estresse salino causado pela presença de sais presentes na água do mar e água residuária, sem afetar o comportamento fermentativo.

Com base nos resultados do delineamento experimental, as variáveis relação sólido-líquido e temperatura afetam de forma significativa positiva a produção de etanol e a variável suplementação com ureia ($(NH_2)_2CO$) afetou de maneira significativa negativa ($p<0,05$) tanto ao fermentar usando água do mar quando água da produção de camarão. Já a variável agitação foi significativa positiva ao realizar a fermentação com água do mar ($p<0,05$) e não significativa ao utilizar a água da produção de camarão ($p>0,05$), no entanto a máxima concentração de etanol foi obtida em ambas as fermentações ao utilizar a máxima agitação proposta no planejamento (120 rpm).

Considerações finais

A partir dos resultados obtidos, verificou-se que SW e WSP podem ser utilizadas na produção de etanol como substitutas da água doce sem interferir no seu rendimento ao

utilizar a *Wickerhamomyces* sp. UFFS-CE-3.1.2 ou a *S. cerevisiae* CAT-1 como microrganismo fermentador e resíduo de mamão como substrato.

O tratamento do resíduo de laranja com ácido sulfúrico resultou em altas quantidades de açúcares, mas também levou a formação de elevada concentração ácidos que atuam como inibidores de fermentação.

O resíduo de mamão é uma fonte de substrato promissora para a produção de biocombustíveis, contendo nutrientes suficientes para o processo fermentativo, não sendo necessário suplementá-lo com fontes nutricionais inorgânicas.

ABSTRACT

In biofuels production, water is consumed mainly in the cultivation of energy crops, extraction of carbohydrates, and fermentation. Recent studies have shown that approximately 9.8 L of water are needed to produce 1 L of ethanol from biomass. With the increasing production of biofuels, an increase in freshwater consumption is expected. Literature reports indicate that this increase could be up to 5.5% by 2030, which will apply an additional burden on freshwater resources. One option is developing bioethanol production systems based on seawater and wastewater, which may reduce stress on freshwater resources. The few works set on this topic have presented promising results when using seawater and wastewater in ethanol production stages, so other investigations and contributions on this topic are relevant. Therefore, this work investigated the technical feasibility of using seawater (SW) and shrimp production water (WSP) to replace freshwater in the fermentation of industrial orange and papaya residues from the fruit and vegetable sector with the yeasts *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *S. cerevisiae* CAT-1. The papaya residue presented high concentrations of free sugars, showing itself as an ideal source for ethanol production. The industrial orange residue introduced low concentrations of free sugars, so it was submitted to an acid treatment stage to solubilization of the sugars present in polysaccharides. The results showed that seawater could be a promising alternative to freshwater for the biofuels sector and those fruit residues are promising substrates for ethanol production.

Keywords: Biofuel. Papaya residue. Industrial orange residue. Salinity.

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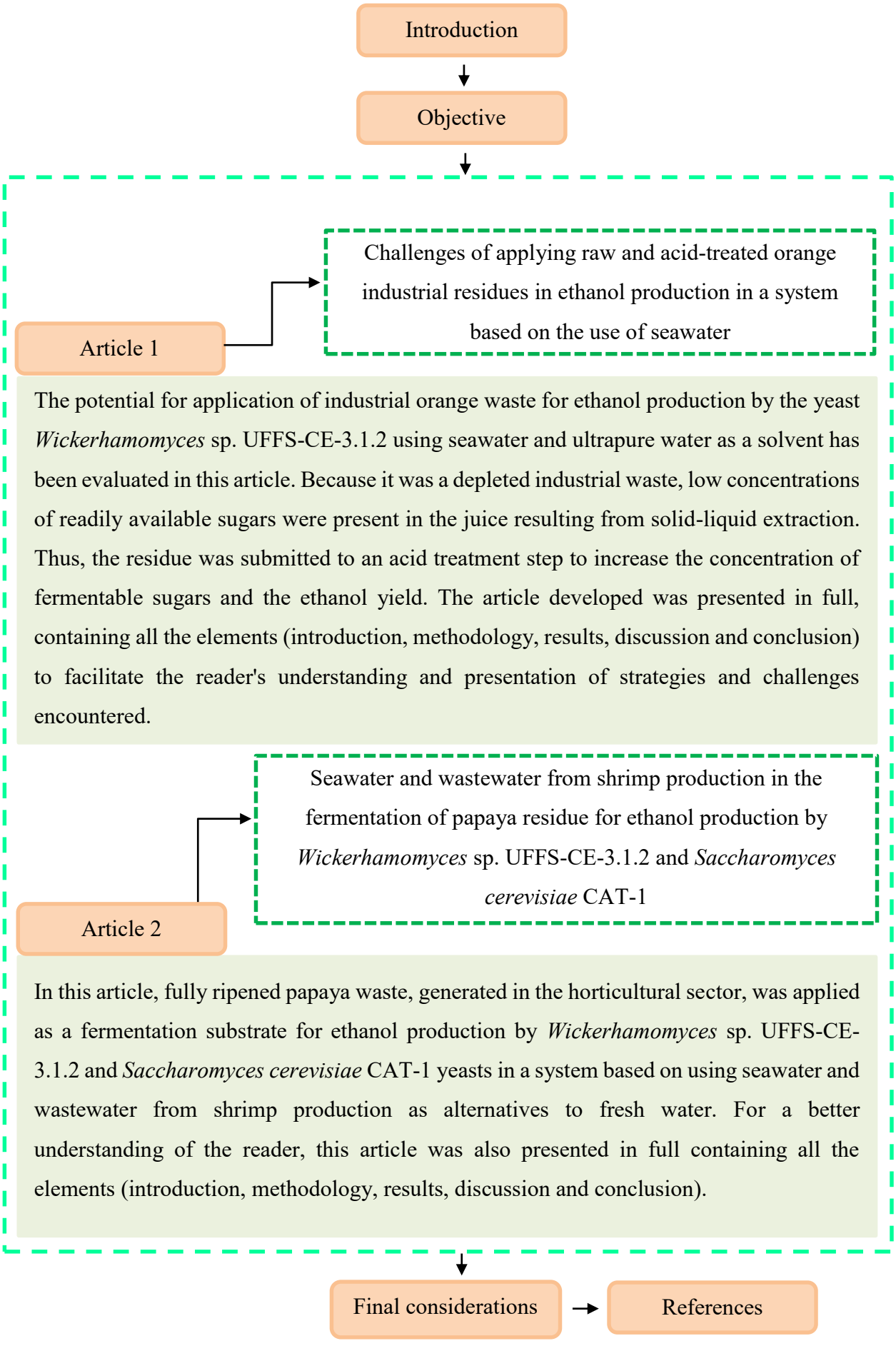
ORGANIZATION OF WORK



CONCEPTUAL DIAGRAM

Evaluate the possibility of replacing freshwater with seawater and wastewater from shrimp production in the ethanol industry using fruit waste as a fermentation substrate

Justification

- A lot of freshwater is used for ethanol production, causing deplete on water resources.
- Use of non-drinking water such as seawater and wastewater in the biofuel production chain can be an alternative to this issue to reduce the water footprint in ethanol production.
- Fruit waste contains high amounts of readily available sugars as well as polysaccharides that can be hydrolyzed, and it is, therefore, a promising substrate for ethanol production.

Has the subject been studied yet?

- Some studies evaluated the use of seawater for ethanol production and obtained promising results using different microorganisms, especially wild microorganisms.
- No work was found to evaluate the use of wastewater from shrimp production to produce ethanol.
- *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *S. cerevisiae* CAT-1, used as fermenting microorganisms in this study, have never been evaluated in saline system.
- Many works used different fruit residues as fermentation substrate for ethanol production and obtained promising results.

Hypotheses

- The composition of seawater and wastewater from shrimp production does not significantly affect the fermentation process.
- Yeasts produce ethanol even in high relative concentrations of salinity.
- Fruit waste is a good substrate for ethanol production.

1 INTRODUCTION

Freshwater is consumed in ethanol production, especially for energy crops and biofuel processing (INDIRA *et al.*, 2018). A significant increase in ethanol production is expected in the coming years, as through the Energy Independence and Security Act (EISA), the United States Congress, the world's largest ethanol producer, has demanded that by the year 2022 the production of renewable fuels increase to 36 billion gallons per year, limiting the volume of conventional biofuels to 15 billion gallons per year (HOEKMAN; BROCH, 2018), which will increase water consumption in ethanol biorefineries. According to Gerbens-Leenes *et al*. (2012), freshwater consumption for fuel production could increase by 5.5% by 2030, which will apply an additional burden on water resources (INDIRA *et al.*, 2018).

Global water demand increases by 1% per year due to population growth, economic development, and changes in consumption patterns. It is estimated that 3.6 billion people live in areas where water is scarce for at least one month per year (WWAP, 2018). Water scarcity also compromises the ethanol industry's future development since interruptions in the water supply in the process put operations at risk (MOTA-LÓPEZ *et al.*, 2018). Also, it affects the demand-offer balance, causing social discontent and competition for the resource (GAIDAJIS; ANGELAKOGLOU, 2016). The growing concern with the quantity and quality of water has generated questions about ethanol production's feasibility due to the impact the activity generates on water resources (CHEROENNET; SUWANMANEE, 2017).

Some studies have pointed out seawater and wastewater to replace freshwater in the bioethanol industry, as they are abundant compared to the amount of freshwater available globally. Wastewater is generated in large volumes daily in different industrial and agricultural sectors, requiring specific treatment before final disposal. Some of these waters may contain essential components for cell nutrition and growth (NIKOLAOU; KOURKOUTAS, 2018). The application of these waters in the ethanol production chain would reduce the pressure generated on water resources and supply water to other more noble sectors, such as public supply (GREETHAM *et al.*, 2018).

In this sense, some works described the replacement of freshwater by seawater and wastewater in ethanol production stages, using different microorganisms. The few papers presented in the literature that sought to produce ethanol in a system based on seawater and wastewater obtained promising results, some of which are shown in Table 1.1.

Table 1.1 – Successful ethanol production processes from systems based on the use of seawater and wastewater.

Strain used	Water source	Ethanol ($g\ L^{-1}$)	Reference
Zygosaccharomyces bailii MTCC 8177 and *Brettanomyces claussenii* MTCC 7801	Seawater	11.5	Indira *et al.* (2018)
Saccharomyces cerevisiae PE2	Seawater	9.68	Gonçalves *et al*. (2015)
Pichia stipitis Y7124	Seawater	7.34	Gonçalves *et al*. (2015)
Zymomonas mobilis B14023	Seawater	9.44	Gonçalves *et al*. (2015)
Citeromyces matritensis M37	Seawater	6.55	Okai *et al*. (2016)
Candida sp. marine	Seawater	12.3	Khambhaty *et al*. (2013)
S. cerevisiae AZ65	Seawater	52.3	Zaky *et al*. (2016)
Saccharomyces cerevisiae marine	Seawater	97.1	Zaky *et al*. (2016)
S. cerevisiae	Wastewater from olive oil and molasses mill of the raw sugar industry	4.0	Nikolaou and Kourkoutas (2018)
S. cerevisiae	Wastewater from olive oil	52.0	Sarris *et al*. (2013)
Proteus sp. S53Rpdcadh	Digested sludge from urban wastewater	0.34	Godoy *et al*. (2018)
S. cerevisiae	Straw digest	4.9	Stoumpou *et al*. (2020)

The substitution of energy crops by cellulosic biomass also favors the reduction of water consumption in biorefineries (INDIRA *et al.*, 2018), reducing the consumption of water used in crop irrigation. Among these biomasses, fruit residues have proven promising for ethanol production, as they are rich in readily available sugars, such as glucose, fructose, and sucrose. Besides, their polysaccharides (pectin, cellulose, and hemicellulose) can be converted into sugars through pretreatment and hydrolysis, making them ideal substrates for fermentation (LIAKOU *et al.*, 2018; SARKAR *et al.*, 2019).

In general, fruit residue from the processing sector contains lower amounts of free sugars than fruit residue generated in food services (TSOUKO *et al.*, 2020), requiring a pretreatment step to provide higher fermentable sugars concentrations. In this sense, in this

study, two fruit residues with different origins were evaluated as a substrate for ethanol production using seawater and wastewater from shrimp production as a solvent. First, orange residue's potential obtained from the juice industry in fermentation to obtain ethanol using seawater was investigated. The orange residue presented low concentrations of free sugars; for this reason, it was submitted to a treatment step to release higher concentrations of sugars to increase the ethanol yield. However, the treatment step released high amounts of galacturonic acid, due to the pectin, besides other acids that interfered with the fermentation process and affected ethanol production. Based on the results obtained in this phase of the study, it was possible to prepare a manuscript, presented in full on section 2 of this document.

Given the challenges encountered in using orange residue to produce ethanol, papaya residue generated in the fruit and vegetable sector was also investigated as a fermentation substrate for this biofuel production. Papaya, different from the orange residue, had high concentrations of free sugars that favored ethanol production. Based on the results obtained, a manuscript was elaborated and presented in full on section 3 of this document.

1.1 OBJECTIVES

1.1.1 General objective

Evaluate the possibility of replacing freshwater with seawater and wastewater from shrimp production in ethanol industry using fruit waste as a fermentation substrate.

1.1.2 Specific objectives

- Evaluate the fermentation behavior of the yeasts *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *S. cerevisiae* CAT-1 in saline medium (seawater and wastewater from shrimp production).
- Investigate the potential of orange industrial and papaya residue from the fruit and vegetable sector for ethanol production.
- Analyze readily available sugar extraction strategies through statistical planning.
- Evaluate residue treatment techniques to increase the amount of sugars available in the fermentation broth, if necessary.

2 ARTICLE: CHALLENGES OF APPLYING RAW AND ACID-TREATED ORANGE INDUSTRIAL RESIDUE IN ETHANOL PRODUCTION IN A SYSTEM BASED ON THE USE OF SEAWATER

Abstract

Citric hydrolysates contain high concentrations of galacturonic acid, resulting from the solubilization of pectin and also compounds such as essential oils, which can act as fermentation inhibitors. This study evaluated the potential of applying orange residue directly in ethanol production by *Wickerhamomyces* sp. UFFS-CE-3.1.2, using seawater as an alternative to freshwater. The orange residue was suspended in seawater and ultrapure water for solubilization of free sugars and treated with sulfuric acid diluted in seawater to release available sugars in the lignocellulosic and pectin fraction. The acid treatment was evaluated by experimental planning (DCCR 2^3), and the influence of the variables acid concentration, solid-liquid ratio, and temperature on sugar release was studied. 8.35±0.10 g L^{-1} of free sugars were extracted from orange residues, resulting in ethanol production of 0.61±0.11 g L^{-1} and 0.57±0.08 g L^{-1} using seawater and ultrapure water, respectively. The broth resulting from the acid treatment showed high amounts of sugars (51.61±3.67g L^{-1}), including 13.02±1.04 g L^{-1} of galacturonic acid, but they were not fermented. The presence of essential oils and galacturonic acid in the fermentation broths present in citrus residues hydrolysates is a challenge to be overcome in order to enable the direct application of these residues in ethanol production, since these compounds can inhibit cellular functions and consequently negatively affect the fermentation process.

Keywords: fruit waste; galacturonic acid; alcoholic fermentation; *Wickerhamomyces* sp. UFFS-CE-3.1.2; salinity.

2.1 INTRODUCTION

Citrus fruits, predominantly represented by oranges, are among the most produced and consumed crops globally (CYPRIANO; LOPES; TASIC, 2018), constituting a vital processing sector (TSOUKO *et al.*, 2020). World orange production was estimated at 75 million tons in

2018 (FAOSTAT, 2020b). As the world's largest producer, Brazil was responsible for producing 15 million tons in the 2019/2020 harvest, with approximately 10 million tons of oranges destined for processing (USDA, 2020).

After processing and extraction of the fruit juice, the residues generated (peel, internal tissues or bagasse, and seeds) are rich in soluble, fermentable sugars, lignin, proteins, essential oils, and polysaccharides such as pectin, cellulose, and hemicellulose (CHOI *et al.*, 2013; CYPRIANO; LOPES; TASIC, 2018; TSOUKO *et al.*, 2020).

These characteristics aparently make orange residue an ideal source for the production and recovery of different individual compounds, such as ethanol, which can be produced from soluble sugars or by subjecting the residue to a treatment step for the disintegration of polysaccharides into simple sugars for further fermentation (LOHRASBI *et al.*, 2010; POURBAFRANI *et al.*, 2010; TSOUKO *et al.*, 2020). However, some challenges for direct application of orange residue in ethanol production must be overcome. This is because the essential oils present in the orange inhibit microbial growth, affecting ethanol production. Still, pectin, when solubilized, releases high concentrations of galacturonic acid, a sugar that is not assimilated by most yeasts and acts as a fermentation inhibitor (BIZ *et al.*, 2016; CHOI *et al.*, 2013).

Among the types of treatment already studied and applied for sugar solubilization, acid treatment is commonly used for chemical treatment of lignocellulosic biomass. Both organic and inorganic acids are used in this process; sulfuric acid is the most used. Although acid treatment is advantageous because it is a faster process to produce fermentable sugar compared to biological processes and has a high rate of solubilization of cellulose monomers, hemicellulose, and pectin, there are negative impacts related to the use of acid for the treatment of lignocellulosic biomass such as the formation of toxic compounds due to the degradation of sugars released and the need for neutralization of the resulting juice before fermentation that leads to the formation of salts in the fermentation medium (KUMARI; SINGH, 2018; MUSSATTO, 2016; THANGAVELU *et al.*, 2019).

Stages of biomass treatment for ethanol production increase the water demand required for ethanol production. According to Aden (2007), ethanol produced from lignocellulosic biomass has a general water demand twice as high as the process using crops such as corn and sugarcane. The use of non-potable water resources such as seawater in stages of biofuel production can be an alternative to reduce the water footprint in ethanol production, precisely

because it is an abundant resource and used for less noble purposes when compared to the use of freshwater, which allows reducing the impact on water resources (FANG *et al.*, 2015; GREETHAM *et al.*, 2018).

Therefore, this study aims to critically evaluate the challenges of applying industrial orange residue directly, i.e. without removing interfering compounds such as essential oils and galacturonic acid, resulting from the solubilization of pectin, for ethanol production by the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 using seawater and ultrapure as a solvent.

2.2 MATERIAL AND METHODS

2.2.1 Obtaining the industrial orange residue and seawater

The industrial orange residue, basically composed of peel, bagasse (rich in pectin), and seeds were collected in a juice industry located in the South Region of Brazil and kept in a freezer (-80 ºC) until use. The seawater, presenting 35 ppm of salinity, was collected at the Marine Shrimp Laboratory of the Federal University of Santa Catarina, Florianópolis, Brazil.

2.2.2 Microorganism

The yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2, previously isolated from lignocellulosic materials (BAZOTI *et al.*, 2017), was used as a fermenter microorganism. The strain was kept in YPD solid medium (1% yeast extract, 2% peptone, 2% glucose, and 2% agar) for 48 hours in BOD at 30 °C and then transferred to 20 mL liquid YPD medium, where it remained for 12 hours in an orbital agitator at 30 °C and 80 rpm. Finally, it was inoculated in the orange fermentation broth to start the fermentation process.

2.2.3 Preparation and extraction of sugars free of orange residue

The orange residue was dried in a circulating oven for 24 hours at 70 °C and then ground in a knife mill (mesh 10). The residue was suspended in seawater or ultrapure water at the proportion of 10% (m v^{-1}) (INDIRA *et al.*, 2018). Afterwards, it was submitted for 5 minutes to different conditions to evaluate the influence on the release of the sugars: 1) mechanical agitation and temperature of 25 °C; 2) boiling in a thermostatic bath at 100 °C and 3) wet steam

(autoclave) at 120 °C. The best extraction condition was used to conduct the fermentation with *Wickerhamomyces* sp. UFFS-CE-3.1.2. The resulting broth was filtered using a nylon filter and sterilized in an autoclave at 121°C for 15 min before fermentation.

2.2.4 Treatment of orange residue with sulfuric acid

The treatment of orange residue using sulfuric acid was evaluated by statistical planning using a 2^3 central rotational composite design (DCCR). The variables studied were sulfuric acid concentration (H_2SO_4) (2, 5, 10, 15 and 18 % (v v^{-1})), temperature (66, 80, 120 e 134 °C) and solid-liquid ratio (3, 10, 20, 30 e 37 % (m $_{dry}$ v^{-1})). The orange residue was suspended in a sulfuric acid solution, made using seawater, and later submitted to different temperatures for 15 minutes, using a thermostatic bath and autoclave. Afterwards, the content was filtered, neutralized to pH 5 using sodium hydroxide (NaOH) PA and analyzed by High-Performance Liquid Chromatography (HPLC) to quantify the compounds released during the treatment. The broth was then inoculated with the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 to conduct the fermentation.

2.2.5 Alcoholic fermentation

Fermentations were performed using the juice resulting from the extraction process of free sugars and the liquid resulting from the treatment process with sulfuric acid, considering the best response concerning the sugars released in the experimental planning. The experiments were conducted in Erlenmeyer of 250 mL with 180 mL of fermentation medium and 20 mL of inoculum of the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2. performed The fermentations were performed in an orbital agitator at 30 °C and 120 rpm, with 10^6 viable cells per mL of fermentation medium (cell mL^{-1}), counted by optical microscopy in a Neubauer chamber using methylene blue dye. Samples were collected in 0, 3, 6, 9, and 12 hours of fermentation to monitor sugar consumption and product formation.

2.2.6 Kinetic parameters

The ethanol yield was calculated by the quotient between the amount of ethanol produced and the amount of sugar consumed for this purpose (Equation 2.1). The theoretical maximum yield was estimated by Equation 2.2.

$$Y_{\frac{P}{S}}(gg^{-1}) = \frac{P_f - P_i}{S_i - S_f} \qquad \text{Equation 2.1}$$

$$Y_{máx}(gL^{-1}) = 0.511 \times S_i \qquad \text{Equation 2.2}$$

Where P_f ethanol concentration at the end of the process, P_i ethanol concentration at the beginning of the process, S_i substrate concentration (sugars) at the beginning of the process and S_f substrate concentration (sugars) at the end of the process.

2.2.7 Analytical methods

The levels of glucose, fructose, xylose, arabinose, cellobiose, galacturonic acid, acetic acid, formic acid, citric acid, and ethanol were determined in a High-Performance Liquid Chromatography (HPLC) system equipped with a RID-10A refractive index detector. An AMINEX® BIORAD HPX87H column was used for analysis using 0.005 M sulfuric acid (H_2SO_4) as eluent, 0.6 mL min^{-1} flow rate, and 45 °C temperature. The eluent was vacuum filtered in a 0.45 μm membrane and degassed in an ultrasonic bath (UNIQUE USC-1800A) for 15 minutes before use. The quantified compound were detected based on the specific HPLC standards (Sigma-Aldrich), and the compound concentration was determined by calibration curves built using these HPLC standards. All chromatographic samples were previously diluted (1:5) in 0.005 M sulfuric acid (H_2SO_4) and filtered on a 0.45 μm membrane of 25 mm cellulose acetate (Millipore®) (BAZOTI *et al.*, 2017).

The concentrations of free sugars were also determined by the 3.5-dinitrosalicylic acid (DNS) method (MILLER, 1959) for comparative purposes.

2.2.8 Statistical analysis

The experimental design and statistical analysis of the different responses obtained were performed and interpreted in the Protimiza software. The confidence level used was 90% (p

<0.1). For comparison of the means, the analysis of variance (ANOVA) followed by Tukey's test was applied.

2.3 RESULTS AND DISCUSSION

2.3.1 Extraction of sugars free of orange residue

The concentration of free sugars released in the different extraction processes did not present a significant difference ($p>0.05$), indicating that the temperature does not affect the sugar release process (Table 2.1). Based on this result, the juice orange residue for the fermentation process was obtained by suspending the dry mass of the orange residue in seawater and ultrapure water, using mechanical agitation and a temperature of 25 °C, since it involves lower energy costs if compared with the other processes tested.

Fructose was the main hexose quantified in the orange residue juice, presenting concentrations of 7.89±0.10 g L^{-1}. Trace concentrations of glucose (1.04±0.22 g L^{-1}) were also quantified.

Table 2.1 – Amount of free sugars resulting from the liquid extraction process of orange residue in the proportion of 10 % (m v^{-1}).

Condition	Sum of sugars quantified in HPLC (g L^{-1})	Sugars quantified by DNS (g L^{-1})
Mechanical stirring at 25 °C	8.35±0.10	9.37±0.48
Thermostatic bath at 100 °C	8.84±0.29	9.92±0.36
Autoclave at 120 °C	8.94±0.70	9.61±0.11

Residues produced in the orange processing industry, such as residues from the juice industry, contain smaller amounts of free sugars than orange peel residues from food services. Tsouko *et al*. (2020) separated the free sugars available in the recently collected orange residue by aqueous extraction and obtained mainly glucose (40.6%), fructose (37.3%), and sucrose (20.7%).

These sugars are the primary sources of carbon for ethanol production. A low concentration of readily available sugars in the fermentation broth can negatively affect alcoholic fermentation leading to low ethanol production (DAKAL; SOLIERI; GIUDICI, 2014;

TURHAN *et al.*, 2010). Therefore, the orange residue was subjected to acid treatment to increase the availability of sugars.

2.3.2 Treatment of orange residue with sulfuric acid

In order to ensure a higher sugar concentration in the fermentation broth, since the total amount of free sugars present in the industrial orange residue was relatively low (8.35±0.10 g L^{-1}), the residue was submitted to chemical treatment using sulfuric acid.

The chemical treatment of the orange residue using diluted sulfuric acid has been studied previously by other works. Tsouko *et al*. (2020) used 0.5% w v^{-1} of H_2SO_4 for 30 minutes to treat orange peel residue and subsequently employed enzymatic hydrolysis. Glucose was the main sugar present in the generated hydrolysate, reaching the highest concentration after 8.5 h of hydrolysis (4.9 g L^{-1}).

In our study, glucose release was favored in tests conducted with higher acid concentrations, solid-liquid ratios, and high temperatures, resulting in concentrations of 8.06 and 6.62 g L^{-1} in trials 8 and 14, respectively (Table 2.2).

Hydrolysates of orange residues usually present high levels of arabinose and galacturonic acid (GROHMANN *et al.*, 1994), derived mainly from hemicellulose and pectin (GROHMANN; CAMERON; BUSLIG, 1995). Assay 7, although resulting in a low glucose content (2.87 g L^{-1}) compared to assay 8, showed the highest arabinose release (20.62 g L^{-1}).

However, arabinose is a monosaccharide composed of five carbons (pentose). Most yeasts cannot assimilate arabinose and convert it to ethanol, as is the case of *S. cerevisiae*, the most used yeast in alcoholic fermentation (PATEL; CHAPLA; SHAH, 2017). Other yeasts were investigated to evaluate the production of ethanol from pentoses. For example, *Pichia stipitis* (ATC.58376) was able to convert 1 g arabinose L^{-1} into ethanol, producing ~0.5 g L^{-1} of ethanol (PHAIBOONSILPA *et al.*, 2020). Besides, an up to 50% arabinose assimilation by the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 was also recently reported by adjusting to the fermentation medium to pH 7 (BONATTO *et al.*, 2020).

The sulfuric acid treatment hydrolyzed the pectin structure present in the orange residue since galacturonic acid, the main constituent of pectin, was released at concentrations of 12.47 g L^{-1} in assay 8 (WIDMER; ZHOU; GROHMANN, 2010). In addition to these structures, the acid treatment can reduce cellulose in some conditions (LI *et al.*, 2010), solubilizing mainly

glucose. The conditions used in assays 14 and 8 led to cellulose rupture since they released higher glucose concentrations.

Through the analysis of the effects, with 90% confidence, it was verified that all variables studied (sulfuric acid concentration, solid-liquid ratio, and temperature) and their interactions were significant ($p<0.1$) for the release of glucose. The release of galacturonic acid was affected only by sulfuric acid concentration, temperature, and interactions ($p<0.1$).

From the results obtained from the experimental design, it was possible to propose empirical mathematical models that allow the estimation of glucose concentrations (Equation 2.3) and galacturonic acid (Equation 2.4) released in the system. The mathematical models were validated at a 90% confidence (F_{cal} (13.4) > F_{tab} (2.51) for glucose; F_{cal} (15.5) > F_{tab} (2.48) for galacturonic acid) and can explain 91% (R^2) and 83% (R^2) of the values tested for the release of glucose and galacturonic acid, respectively presenting adequate predictive capacity.

$$G\left(g\ L^{-1}\right) = 0.74 + 0.59\times A + 0.54\times S{:}L + 1.83\times T + 1.08\times T^2 + 0.58\times A\times S{:}L + 0.69\times A\times T + 0.67\times S{:}L\times T \quad \text{Equation 2.3}$$

$$AG\left(g\ L^{-1}\right) = 0.20 + 1.41\times A + 3.85\times T + 3.20\times T^2 + 2.18\times A\times T \quad \text{Equation 2.4}$$

Where: G is the glucose concentration (g L^{-1}), AG the galacturonic acid concentration (g L^{-1}), The acid concentration % (v^{-1}), S:L solid-liquid ratio % (m v^{-1}) and T temperature (°C).

Based on the quadratic model, it is evident that the temperature increase beyond the studied range could return higher concentrations of glucose and galacturonic acid. The literature previously reported that higher glucose yields could be obtained when applying a high temperature and shorter reaction time. Still, the process efficiency becomes low because glucose is degraded at high temperatures (YU; LOU; WU, 2008).

As assay 8 returned a higher sugar concentration (53.96 g L^{-1}), especially concerning glucose, this condition was repeated several times until obtaining the necessary volume for the fermentation.

Table 2.2 – DCCR 2^3 planning matrix with coded and real values used in experiments and experimental responses regarding the release of sugars.

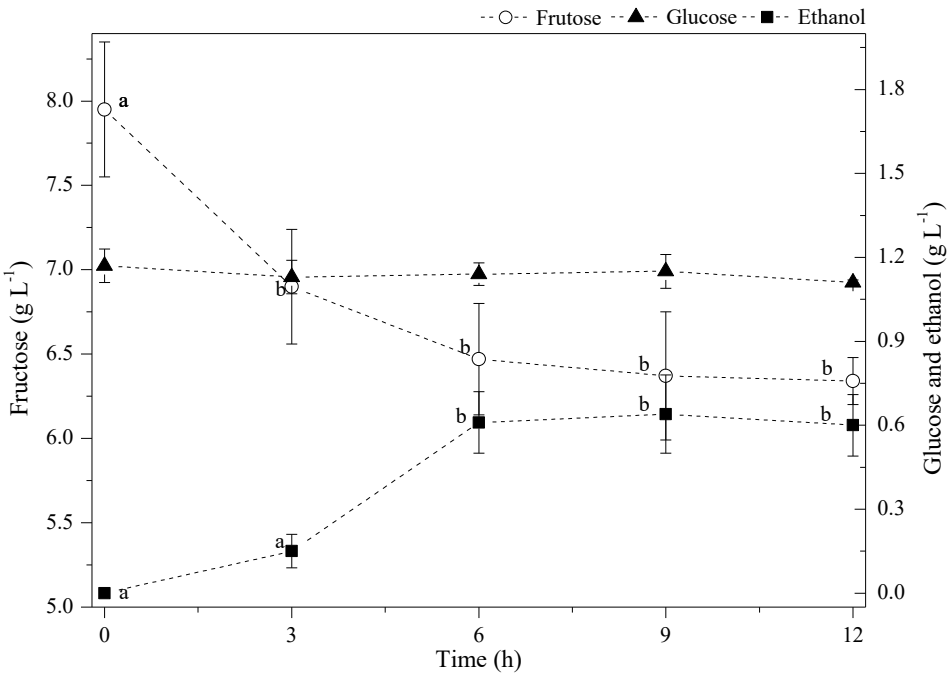
Assay	Acid % (v v^{-1})	Solid-liquid ratio % (m v^{-1})	Temperature (ºC)	Glucose (g L^{-1})	Galacturonic acid (g L^{-1})	Arabinose (g L^{-1})	Other sugars (g L^{-1})*
1	5 (-1)	10 (-1)	80 (-1)	0.09	0.19	1.85	2.00
2	15 (1)	10 (-1)	80 (-1)	0.20	0.13	5.80	1.56
3	5 (-1)	30 (1)	80 (-1)	0.51	0.30	2.39	5.24
4	15 (1)	30 (1)	80 (-1)	0.75	0.88	13.76	13.08
5	5 (-1)	10 (-1)	120 (1)	1.97	0.95	6.54	5.95
6	15 (1)	10 (-1)	120 (1)	2.66	6.67	6.00	4.43
7	5 (-1)	30 (1)	120 (1)	2.87	0.70	20.62	11.34
8	15 (1)	30 (1)	120 (1)	8.06	12.97	13.48	19.45
9	2 (-1.68)	20 (0)	100 (0)	0.24	0.38	0.58	3.05
10	18 (1.68)	20 (0)	100 (0)	1.36	0.82	13.97	13.77
11	10 (0)	3 (-1.68)	100 (0)	0.19	0.11	3.22	1.53
12	10 (0)	37 (1.68)	100 (0)	0.28	0.42	14.79	4.69
13	10 (0)	20 (0)	66 (-1.68)	0.09	0.29	1.59	2.99
14	10 (0)	20 (0)	134 (1.68)	6.62	19.77	9.08	6.73
15	10 (0)	20 (0)	100 (0)	0.53	1.12	10.12	11.32
16	10 (0)	20 (0)	100 (0)	0.42	0.73	9.78	8.57
17	10 (0)	20 (0)	100 (0)	0.49	0.69	10.21	9.50

* Sum of the fractions of fructose, xylose and cellobiose.

2.3.3 Fermentation of free sugar extraction broth

The fermentation conducted by *Wickerhamomyces* sp. UFFS-CE-3.1.2 using the resulting juice from the sugar extraction process using seawater resulted in maximum production of 0.61±0.11 g L^{-1} of ethanol after 6 hours of fermentation and remained constant until the end of the fermentation process. It was verified that only fructose was used as a carbon source in the fermentation process, and glucose remained constant throughout the fermentation, showing no statistical difference between the values quantified during the fermentation ($p>0.05$). The highest fructose consumption rate occurred in the first 3 hours of fermentation, remaining constant until the end of fermentation ($p>0.05$). Even so, only 20% of fructose was consumed, leaving a residual of 6.34±0.14 g L^{-1} fructose at the end of the fermentation process (Figure 2.1).

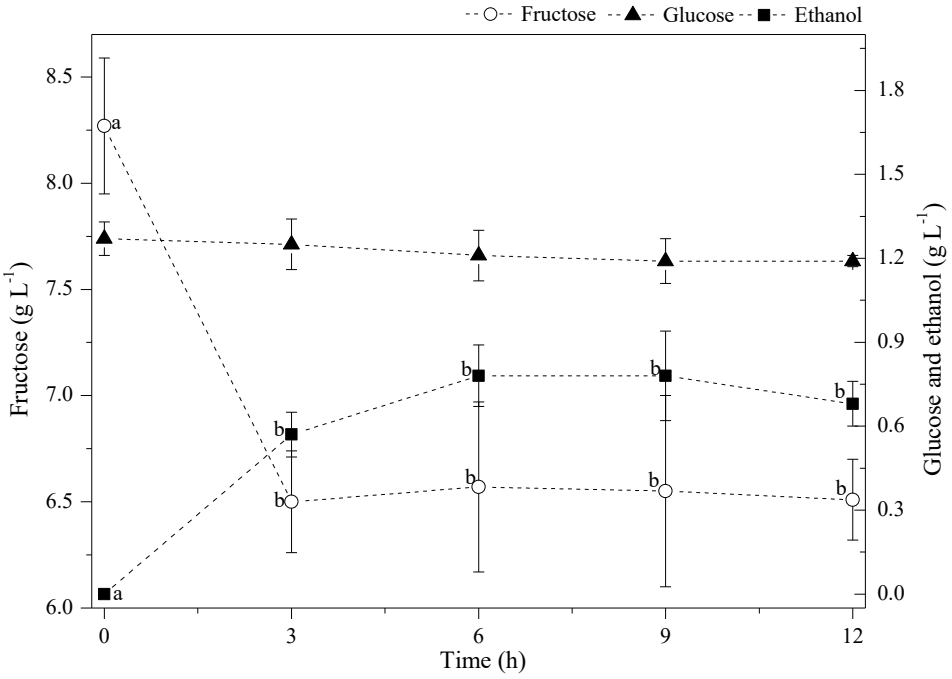
Figure 2.1 – Fermentation using the orange residue broth suspended in seawater. The dashed lines connecting the experimental points are only for a better view of the reader.



The fermentation conducted with the fermentation broth suspended in ultrapure water resulted in similar behavior. The maximum ethanol production occurred in 3 hours of

fermentation, reaching 0.57±0.08 g L^{-1}. Fructose was also the only source of carbon consumed during fermentation (21%), presenting a residual of 6.51±0.19 g L^{-1} at the end of fermentation, while glucose remained constant throughout fermentation ($p>0.05$) (Figure 2.2).

Figure 2.2 – Fermentation using the orange residue broth suspended in ultrapure water. The dashed lines connecting the experimental points are only for a better view of the reader.



Theoretically, the maximum yield of ethanol that could be obtained in fermentation is 0.511 $g_{ethanol}$ g^{-1}_{hexose}. Experimentally, the maximum ethanol yield obtained in the fermentation conducted using seawater was 0.41±0.10 $g_{ethanol}$ g^{-1}_{hexose} and 0.32±0.03 $g_{ethanol}$ g^{-1}_{hexose} using ultrapure water. The amount of fermentable sugars available in the orange residue fermentation broth would be possible to achieve a theoretical ethanol production of 4.66±0.22 and 4.88±0.19 g L^{-1} using seawater and ultrapure water, respectively.

The pH and conductivity of the fermentation remained around 3.54±0.03 and 2.87±0.02 mS cm^{-1} using ultrapure water and 3.17±0.02 and 27.80±1.22 mS cm^{-1} using seawater, respectively. Although the conductivity of the fermentation using seawater is approximately ten times higher than the conductivity of the fermentation using ultrapure water, the fermentation

was not affected by the salinity of the seawater because the same behavior was obtained in the fermentation using ultrapure water.

It has been previously reported that there may be no difference in ethanol yield when fermenting using saline or freshwater as a solvent. Many yeasts can tolerate saline stress (ANDREISHCHEVA; ZVIAGIL'SKAIA, 1999; INDIRA *et al.*, 2018)

Besides, the citric matrix contains compounds that act as fermentation inhibitors, such as essential oils (CHOI *et al.*, 2013). Citric essential oils are a complex mixture of volatile compounds that present inhibitory activity of microbial growth. This activity may result from a single compound or the synergistic or antagonistic effect of several compounds, such as D-limonene, linalool, and citral (JING *et al.*, 2014; VIUDA-MARTOS *et al.*, 2008).

For this reason, the strategy presented by some works in the literature has been the removal of D-limonene before fermentation. Choi *et al*. (2013) reported that reducing D-limonene concentration in mandarin peel from 0.21% to less than 0.01% allowed an increase in ethanol concentration from 39.8 to 46.2 g L^{-1}. Wilkins *et al*. (2007) also removed 90% of the D-limonene present in citrus fruit residues by a steam explosion process. They found that ethanol concentrations were lower when the D-limonene concentration was greater than or equal to 0.33% (v v^{-1}). However, in this study, D-limonene was not monitored or removed from the orange residue and may have acted as an inhibitor of the fermentation process.

In view of this, both the low concentration of sugars available in the fermentation broth and the presence of compounds that may have acted as interfering agents, such as D-limonene, may have affected the fermentation resulting in low ethanol production.

2.3.4 Fermentation of the juice resulting from the treatment of orange residue with sulfuric acid

From the amount of fermentable sugars available in the juice resulting from the treatment of orange residue, it would be possible to achieve a theoretical ethanol production of up to 19.42±0.30 g L^{-1}, disregarding galacturonic acid and up to 25.54±2.80 g L^{-1} considering the use of galacturonic acid in fermentation (Table 2.3). However, the juice of the orange residue treatment with sulfuric acid was not fermented by *Wickerhamomyces* sp. UFFS-CE-3.1.2, presenting negative results regarding ethanol production, even with pH remaining at

5.25±0.15 and containing a high amount of fermentable sugars (up to 51.61±3.67g L^{-1}, including glucose, fructose, xylose, arabinose, cellobiose, and galacturonic acid).

A factor that may have interfered with the process is the neutralization reaction of the juice resulting from acid treatment (H_2SO_4) with sodium hydroxide (NaOH) that results in the formation of sodium sulfate salt (Na_2SO_4), increasing the salinity of the fermentation medium. The presence of high salt concentrations in hydrolysates restricts the conversion of sugars into ethanol due to the increased intracellular concentration of Na^+ ion (ANDREISHCHEVA; ZVIAGIL'SKAIA, 1999; GREETHAM; ZAKY; DU, 2019). By this statement, the formation of crystals in the juice resulting from the acid treatment process after neutralization was verified and high conductivity was verified both using seawater (42.47±0.83 mS cm^{-1}) and ultrapure water (42.87±0.32 mS cm^{-1}) as a solvent in the treatment and fermentation process, indicating that the system has saturated.

Table 2.3 – Fermentation of the orange residue broth resulting from the sulfuric acid treatment using seawater.

Time (h)	Total sugars * (g L^{-1})	Formic acid (g L^{-1})	Acetic acid (g L^{-1})	Citric acid (g L^{-1})	Ethanol (g L^{-1})
0	47.83±3.97	1.12±0.10	2.19±0.17	0.29±0.02	0.00±0.00
3	51.97±0.76	1.24±0.02	2.39±0.05	0.35±0.03	0.09±0.02
6	51.61±3.67	1.25±0.09	2.38±0.05	0.37±0.02	0.08±0.03
9	51.13±2.19	1.22±0.15	2.31±0.29	0.38±0.04	0.08±0.02
12	47.93±2.71	1.20±0.07	2.36±0.10	0.39±0.03	0.09±0.01

* Sum of glucose, fructose, xylose, arabinose, cellobiose and galacturonic acid fractions.

In addition, the orange residue broth also had a high concentration of galacturonic acid (13.69±0.91 g L^{-1}) and acetic acid (2.31±0.29 g L^{-1}), in addition to formic acid (1.22±0.15 g L^{-1}) and citric acid (0.39±0.03 g L^{-1}). However, concentrations of up to approximately 2.5 g L^{-1} of acetic acid have been reported not to interfere with ethanol production in fermentations conducted by *Wickerhamomyces* sp. UFFS-CE-3.1.2 (BAZOTI *et al.*, 2017; BONATTO *et al.*, 2020), but these acids together may have acted as inhibitors of fermentation synergistically. Because it is a molecule smaller than acetic acid, formic acid diffuses more easily through the

plasma membrane. It has been seen that 3.9 mM of formic acid causes the same inhibition rate as 90.6 mM of acetic acid (LARSSON *et al.*, 1999).

Galacturonic acid, even though it is a sugar acid composed of six carbons as glucose and fructose, is not fermented by most yeasts such as *S. cerevisiae* (BIZ *et al.*, 2016). Moreover, it inhibits other sugars' fermentation, such as galactose, xylose, and arabinose, even at low concentrations (HUISJES *et al.*, 2012). When galacturonic acid enters the cytoplasm of yeast, several inhibition mechanisms are possible. In the cytosol, the galacturonic acid dissociates due to the almost neutral intracellular pH. The yeast does not metabolize it. The anion accumulates, generating high turgor pressure, and the proton can acidify the cytosol, inhibiting metabolic functions. To maintain the pH homeostasis, the anion and proton are excreted and exported through a plasma membrane at ATP's expense. The need for energy expenditure to transport the ions out of the cell, coupled with the low rate of substrate uptake due to competitive inhibition, results in weak cell growth and consequently affects the fermentation process (HUISJES *et al.*, 2012; PIPER *et al.*, 2001).

Therefore, there are challenges to be overcome in the direct use of orange residue as a substrate for ethanol production, since galacturonic acid represents a significant amount of sugars in the hydrolysate from pectinolytic residue (18% (w w^{-1})). However, the inhibition of the fermentation process caused by galacturonic acid limits the use of residues rich in pectin as raw material for biorefinery (BIZ *et al.*, 2016). Some microorganisms such as *Escherichia coli* can metabolize galacturonic acid but do not efficiently convert it into ethanol (GROHMANN *et al.*, 1994); or this reason, genetic engineering has presented itself as an alternative (JEONG *et al.*, 2020).

Another option is to apply the residue to integrated processes, initially removing the pectin fraction present in the residue and later submitting only the cellulose and hemicellulose fractions to the treatment stage for fermentable extraction sugars for ethanol production. Pourbafrani *et al*. (2010) and Lohrasbi *et al*. (2010) developed an integrated process for D-limonene, pectin, methane, and ethanol from citrus peel residues. They reported that a biorefinery using citrus peel residues would be economically feasible by developing an integrated system to produce multiple compounds.

2.4 CONCLUSION

The treatment of orange residue with sulfuric acid resulted in high amounts of sugars and led to the formation of high concentration acids that act as inhibitors of fermentation. Also, the neutralization step increased the salinity in the system affecting the fermentation process. However, it was possible to produce 0.61±0.11 and 0.57±0.08 g L^{-1} of ethanol by *Wickerhamomyces* sp. UFFS-CE-3.1.2 from the free sugars present in the orange residue using seawater and ultrapure water, respectively. The presence of essential oils in the orange residue matrix may have affected the fermentation resulting in low ethanol production. Therefore, the removal of these compounds or the use of tolerant microorganisms may be an alternative to increase ethanol production from orange residue. The fermentation behavior obtained using seawater, and ultrapure water suggests that the salinity did not adversely affect the fermentation process.

3 ARTICLE: SEAWATER AND WASTEWATER FROM SHRIMP PRODUCTION IN THE FERMENTATION OF PAPAYA RESIDUE FOR ETHANOL PRODUCTION BY *Wickerhamomyces* sp. UFFS-CE-3.1.2 AND *Saccharomyces cerevisiae* CAT-1

Abstract

Seawater (SW) and wastewater from shrimp production (WSP) were used as solvent for the fermentation of papaya residue by *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *Saccharomyces cerevisiae* CAT-1. For comparative purposes and evaluation of the effect of salinity ultrapure water was used as control. Fermentative parameters such as agitation, temperature, solid-liquid ratio, and urea supplementation were evaluated in Plackett-Burman planning to assess ethanol production's significant variables. Urea supplementation was the only variable not significant for the proposed process, suggesting that papaya residue contains all the nutrients needed for fermentation. The experiments conducted with the different water sources resulted in similar concentrations of ethanol. Maximum ethanol concentration was obtained after 9 h of fermentation using UFFS-CE-3.1.2 (27.31±1.40 g L^{-1}) and 12 h using CAT-1 (24.53±0.68 g L^{-1}). This study demonstrated that fresh water can be replaced by SW and WSP, without affecting ethanol production. Papaya residue from the fruit and vegetable sectors can be considered a promising substrate source for ethanol production.

Keyword: Yeast. Solid-liquid ratio. Salinity. Fruit waste. Bioprocess.

3.1 INTRODUCTION

Corn and sugarcane are examples of raw materials, rich in starch or sugar, for large-scale ethanol production. However, these raw materials are also food sources, and many researchers have questioned their use in biofuel pathways, because other biomasses are available, such as waste, and are good alternatives for the production of biofuels since they do not compete with food crops (TOMÁS-PEJÓ *et al.*, 2012).

Fruit residue is generated in large quantities worldwide, mainly by the fruit and vegetable sectors and household waste, but available information on discarded amounts is limited (LIAKOU *et al.*, 2018). The most considerable fruit losses occur during harvest and

consumption, due to the quality standards of the demanding foods established in the fruit and vegetable sectors and requested by consumers. Fruits are rich in readily available sugars such as the hexoses glucose and fructose, as well as the disaccharide sucrose (composed of two hexoses aforementioned), which can make them ideal substrates for fermentation (LIAKOU *et al.*, 2018; SARKAR *et al.*, 2019).

Despite its potential for bioproduct production, landfill ends up being the most common form of disposal (ESPARZA *et al.*, 2020). Papaya fruit, grown mainly in tropical and subtropical countries, with world production exceeding 13.3 metric tons in 2018, has a disposal rate of 35-50% of the total harvest (FAOSTAT, 2020a; HELLER *et al.*, 2015). Thus, exploratory studies using this waste are necessary to evaluate its potential for bioproducts production, adding value to it (HAN; PARK; SU, 2018; HELLER *et al.*, 2015).

Ethanol production consumes high amounts of water, especially for the cultivation of energy crops and biofuel processing. Approximately 9.8 L of water is needed to produce 1 L of ethanol from biomass (ADEN, 2007; INDIRA *et al.*, 2018). However, according to Aden (2007), ethanol produced from waste has an overall water demand two times higher than the process using crops such as corn and sugarcane, since additional steps are commonly needed. Therefore, it is necessary to develop strategies to supply the demand for drinking water in the biofuels sector in an environmentally efficient manner. Its application represents a potential damage to global water security (ADEN, 2007; INDIRA *et al.*, 2018).

The evaluation of non-potable water resources as a reaction medium for biofuel production seems to be a promising field of research, which may result in integrative systems (FANG *et al.*, 2015). The use of seawater and wastewater may be an alternative to reduce the water footprint in ethanol production. Unlike freshwater, seawater is an abundant resource, representing 97% of the total water in the world, and is used for less noble purposes (GREETHAM *et al.*, 2018). Wastewater is present in several sectors and is produced in large quantities daily, requiring specific treatment before release to the hydrous bodies. In addition to being a water source, this wastewater may contain essential nutrients and cell growth components (NIKOLAOU; KOURKOUTAS, 2018). Thus, seawater and wastewater in stages of ethanol production are an attractive approach to biofuels obtaining that can improve the economy and reduce the impact on water resources (GREETHAM *et al.*, 2018). The few studies developed on this theme (GREETHAM; ZAKY; DU, 2019; INDIRA *et al.*, 2018; INDIRA; JAYABALAN, 2020; NIKOLAOU; KOURKOUTAS, 2018) showed promising results when

seawater and wastewater were used as solvents in ethanol production stages, so other investigations and contributions on the issue are relevant.

Based on all these aspects, this study evaluated for the first time in the open literature the potential of ethanol production in a system using seawater and wastewater of shrimp production in fermentation conducted by *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *Saccharomyces cerevisiae* CAT-1 using papaya residue generated in the fruit and vegetable sector, fully matured, as substrate.

3.2 MATERIAL AND METHODS

3.2.1 Microorganism and inoculum preparation

Wickerhamomyces sp. UFFS-CE-3.1.2 and *Saccharomyces cerevisiae* CAT-1 were the fermenting microorganisms used in this study. For inoculum preparation, the yeasts were maintained in solid YPD medium and incubated in BOD for growth for 48 hours at 30 °C. Subsequently, the yeasts were transferred to 20 mL of liquid YPD and incubated in an orbital agitator for 12 hours, 30 °C, and 80 rpm. After that, the volume of inoculum was transferred to the fermentative medium. Samples were collected to estimate the viable cells present in fermentation by optical microscopy in the Neubauer chamber using methylene blue dye. All fermentations were conducted with 10^6 cells per mL of fermentative medium (cell mL^{-1}). All the materials and media were previously sterilized at 121 °C, 1 bar for 15 minutes.

3.2.2 Papaya residue

The papaya residue used as substrate is constituted by peel, pulp, and seeds, which is discarded by the fruit and vegetable sectors for several reasons, the most common being the advanced stage of maturation. Papaya residue, completely matured and presenting rot characteristics, was collected in a supermarket in the Southern Region of Brazil, ground in a mixer, homogenized to standardize the aspects, and stored in a freezer (-80 ºC) until use.

3.2.3 Seawater and wastewater of shrimp production

Seawater (SW) and wastewater from shrimp production (WSP) were collected at the Marine Shrimp Laboratory of the Federal University of Santa Catarina, Florianópolis, Brazil. The SW is captured from *Barra da Lagoa* beach, Florianópolis, Brazil, and stored by the laboratory for shrimp, algae, and fish cultivation. The WSP is the result of shrimp cultivation by the bioflocs system (LEGARDA *et al.*, 2019; PURDUE, 2014). At the end of the cultivation process, the shrimp production water is sent to a conventional treatment system and later released to the water body. Seawater and wastewater presented 35 ppm of salinity and 16.63±0.61 mS cm^{-1} of conductivity. These parameters were measured using a manual salinometer and electrical conductivity meter (Gehaka CG 2000), respectively.

3.2.4 Ethanol production from papaya residue

The effect of fermentative parameters on ethanol production was studied by the Plackett-Burman experimental design, resulting in 11 assays (Table 3.1) (RODRIGUES; IEMMA, 2014). The parameters studied were agitation (50, 85 and 120 rpm), temperature (20, 30 and 40 ºC), urea ($(NH_2)_2CO$) supplementation (0, 125 and 250 mM) (LI; WANG; SHI, 2017) and solid-liquid ratio (50, 125 and 200 %). Seawater (SW) and wastewater from shrimp production (WSP) were used as a solvent for the extraction of soluble sugars present in papaya residue. Papaya residue was mixed with SW or WSP at room temperature (20-25 °C), in the proportion established in the experimental design. The mixture was stirred for 5 min, filtered using a nylon filter, sterilized at 121 °C for 15 min and cooled. The experiments were conducted in Erlenmeyer of 250 mL with 180 mL of fermentative medium and 20 mL of inoculum of yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2. Each point of the planning was evaluated in kinetics, and sample collection was performed in 0, 3, 6, 9, 12, 24 and 48 hours of fermentation.

To confirm the reliability of the results obtained in the planning, verification experiments were carried out in the best experimental condition using SW, WSP and the strain UFFS-CE-3.1.2. To evaluate the effect of salinity on ethanol production by UFFS-CE-3.1.2, fermentations were also performed with ultrapure water. For comparative purposes, fermentations were also carried out with *S. cerevisiae* CAT-1 using SW, WSP, and ultrapure water (UW). Fermentations were conducted in triplicate for statistical treatment, and samples were collected in 0, 3, 6, 9, 12, 24 and, 48 hours of fermentation to monitor the fermentation kinetics.

3.2.5 Kinetic parameters

The ethanol yield was calculated by the quotient between the amount of ethanol produced and the amount of sugar consumed for this purpose (Equation 3.1).

$$Y_{\frac{P}{S}}\ (gg^{-1}) = \frac{P_f - P_i}{S_i - S_f} \qquad \text{Equation 3.1}$$

The ethanol productivity was calculated by the quotient between the concentration of ethanol produced and the time to reach this concentration (Equation 3.2).

$$P\ (gL^{-1}h^{-1}) = \frac{P_f - P_i}{t_p} \qquad \text{Equation 3.2}$$

Where P_f is the concentration of ethanol at the end of the process, P_i is the concentration of ethanol at the beginning of the process, S_i is the concentration of substrate (sugars) at the beginning of the process, S_f is the concentration of substrate (sugars) at the end of the process e t_p process time to achieve the ethanol concentration P_f.

3.2.6 Analytical methods

Glucose, fructose, sucrose, glycerol, and ethanol quantification was performed by High-Performance Liquid Chromatography (HPLC), using a Shimadzu chromatograph equipped with RID-10A refraction index detector, operated with AMINEX® BIORAD HPX87H.

To quantify fermentable sugars with the AMINEX® BIORAD HPX87H column, samples containing sucrose were pretreated with invertase before being chromatographed. This was necessary because the acid conditions of 5.0 mM sulfuric acid (H_2SO_4), used as an eluent, catalyzed the hydrolysis of α-1,2-β glycosidic bond (between glucose and fructose) of sucrose during the run in the column, so that none of the sugars (glucose, fructose and sucrose) could be quantified accurately. Sucrose hydrolysis was performed by incubating 0.4 mL of sample with 0.4 mL of invertase at ~ 100 U mL^{-1} in 100 mM sodium acetate buffer, pH 4.5, at 50 °C for 30 min. The enzymatic activity of invertase was determined by the standard p-hydroxybenzohydrazide reagent 0.5%, according to the Sigma-Albrich method. At the end of

the incubation, all sucrose was hydrolyzed into glucose and fructose and could thus be separated and quantified by HPLC in the AMINEX column (FISH; BRUTON; RUSSO, 2009).

The hydrolysed samples were previously diluted (1:10) in sulfuric acid (H_2SO_4) 0.005 M and filtered in a membrane of 0.45 μm cellulose acetate 25 mm (Millipore®). Samples of 20 μL were chromatographed using sulfuric acid (H_2SO_4) 5.0 mM as eluent, a flow rate of 0.6 mL min^{-1} and temperature of 45 °C. The eluent was vacuum filtered in a 0.45 μm membrane and degassed in an ultrasonic bath (UNIQUE USC-1800A) for 15 min. The compounds' concentration was determined by calibration curves constructed using specific standards (Sigma-Aldrich) for HPLC (BAZOTI *et al.*, 2017).

3.2.7 Statistical analysis

The experimental design was performed and interpreted by the Protimiza software, and the statistical analysis of the different responses obtained was performed in the Statistica software (Statsoft, Tulsa, USA). The confidence level used was 95% ($p<0.05$). To compare the means, the variance analysis (ANOVA) followed by the Tukey test was applied.

3.3 RESULTS AND DISCUSSION

3.3.1 Effect of process parameters on ethanol production

The kinetic behavior of ethanol production by *Wickerhamomyces* sp. UFFS-CE-3.1.2 at the experimental conditions tested was similar using SW and WSP. It took at least 9 hours of fermentation to obtain the maximum ethanol concentration in the planning of ethanol using SW (20.95 g L^{-1}) and WSP (22.14 g L^{-1}) (Figure 3.1). Thus, the experimental design was evaluated using ethanol production results obtained in 9 hours of fermentation (Table 3.1).

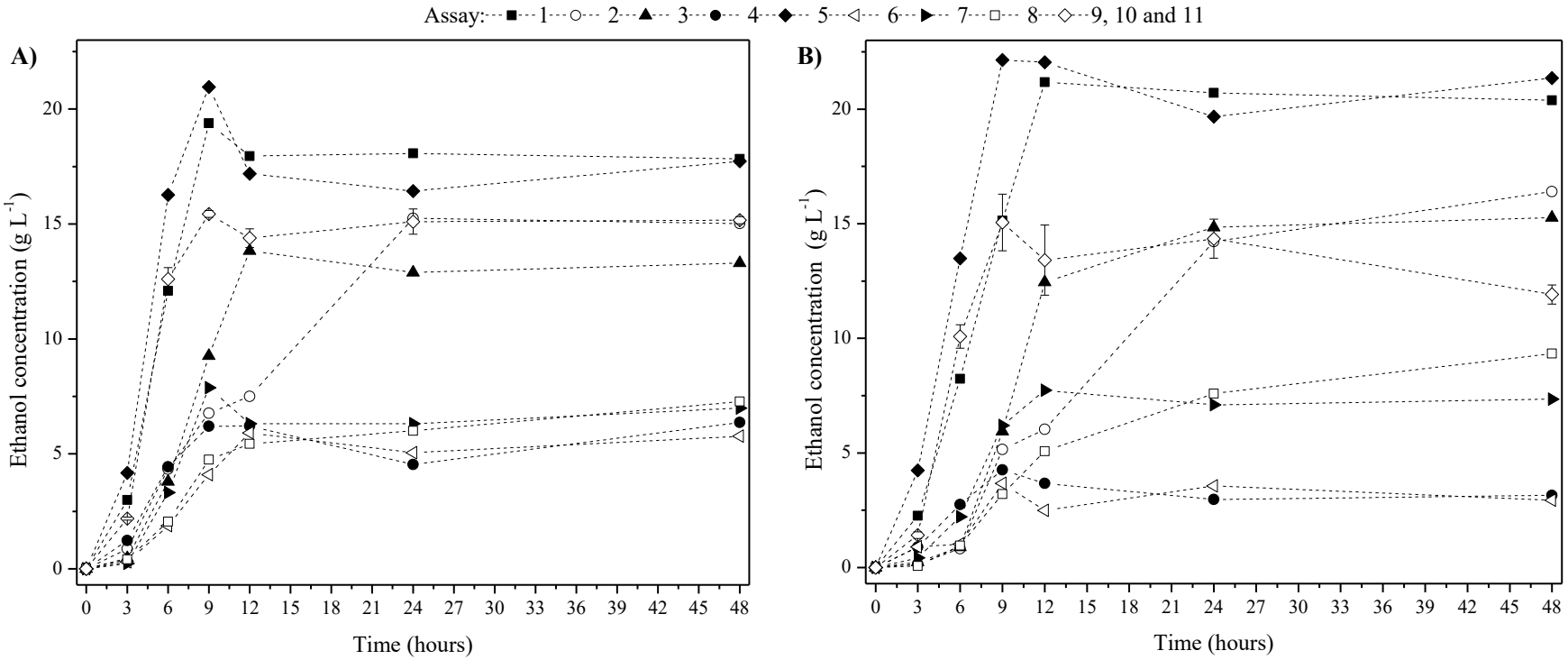
Based on the results of the experimental design, the parameters solid-liquid ratio and temperature significantly positively affect ethanol production, and the variable urea supplementation ($(NH_2)_2CO$) significantly negatively affected ($p<0.05$), both when using SW and WSP. The agitation variable was significantly positive when seawater was used ($p<0.05$) and not significant when using shrimp production water ($p>0.05$).

The solid-liquid ratio was statistically significant for ethanol production because, as expected, the higher the solid-liquid rate, higher the sugar concentration was released to the

fermentative medium. Consequently, higher ethanol production was achieved in the experiments with higher substrate availability (Table 3.1). The lowest sugar concentration was obtained using a solid-liquid ratio of 50% (18.61±1.81 g L^{-1}) and the largest was obtained with solid-liquid ratio of 200% (47.87±5.38 g L^{-1}). Sugars are the main sources of carbon and cellular energy for yeasts. Thus, higher ethanol concentrations were obtained by using a solid-liquid ratio of 200%, while low substrate concentrations led to an inferior product concentration (Figure 3.1), which becomes economically unfeasible (DAKAL; SOLIERI; GIUDICI, 2014). Higher ethanol yields and productivity were also obtained in assay with higher solid-liquid ratios (Table 3.1). These data show that the solid-liquid ratio is an essential parameter for sugar extraction and affects the carbon source concentration and, consequently, the ethanol production, product yield, and productivity (TURHAN *et al.*, 2010).

Urea was not adequate for use as a nitrogen supplement in papaya residue broth for ethanol production by the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2, negatively affecting the fermentation process at both concentrations used in fermentation (125 and 250 mM). The highest ethanol concentration was obtained in the experiment carried out without urea supplementation (assay 5 - see Table 3.1).

Figure 3.1 – Ethanol production kinetics by *Wickerhamomyces* sp. UFFS-CE-3.1.2 in each condition of Plackett-Burman planning (assays 1-11, according to Table 3.1) using SW (A) and WSP (B).



Note: the lines connecting the experimental points are illustrative for a better understanding of the reader. The lower case letters of the experimental points indicate that the samples are different when the letters are different and equal when the letters are equal.

Table 3.1 – Plackett-Burman planning matrix and responses obtained in relation to initial concentration of substrate and products generated after 9 hours of fermentation by *Wickerhamomyces* sp. UFFS-CE-3.1.2.

Assay	Solid-liquid ratio (% m/v)	Urea (mM)	Agitation (rpm)	Temperature (°C)	SW						WSP					
					Sugars ($g\ L^{-1}$)	Ethanol ($g\ L^{-1}$)	Glycerol ($g\ L^{-1}$)	Yield ($g\ g^{-1}$)	Productivity ($g\ L^{-1}\ h^{-1}$)	pH	Sugars ($g\ L^{-1}$)	Ethanol ($g\ L^{-1}$)	Glycerol ($g\ L^{-1}$)	Yield ($g\ g^{-1}$)	Productivity ($g\ L^{-1}\ h^{-1}$)	pH
1	200 (1)	0 (-1)	50 (-1)	40 (1)	45.07	19.39	0.87	0.51	2.15	3.90	53.72	15.14	0.86	0.41	1.68	3.98
2	200 (1)	250 (1)	50 (-1)	20 (-1)	41.96	6.77	0.81	0.33	0.75	6.28	50.16	5.15	1.18	0.21	0.57	6.34
3	200 (1)	250 (1)	120 (1)	20 (-1)	41.89	9.26	1.24	0.37	1.03	7.18	50.61	5.95	1.30	0.23	0.66	6.90
4	50 (-1)	250 (1)	120 (1)	40 (1)	18.59	6.20	0.90	0.33	0.69	6.16	16.31	4.26	1.06	0.32	0.47	6.20
5	200 (1)	0 (-1)	120 (1)	40 (1)	43.86	20.95	0.76	0.48	2.33	3.99	55.70	22.14	1.20	0.40	2.46	4.01
6	50 (-1)	250 (1)	50 (-1)	40 (1)	21.17	4.09	0.78	0.30	0.45	8.31	17.60	3.67	1.08	0.21	0.41	8.37
7	50 (-1)	0 (-1)	120 (1)	20 (-1)	17.38	7.87	0.64	0.45	0.87	5.01	20.41	6.20	0.67	0.35	0.69	5.15
8	50 (-1)	0 (-1)	50 (-1)	20 (-1)	17.07	4.74	0.44	0.46	0.53	3.87	20.34	3.21	0.37	0.28	0.36	4.00
9	125 (0)	125 (0)	85 (0)	30 (0)	37.33	15.48	1.51	0.41	1.72	6.10	43.27	13.63	1.58	0.31	1.51	5.98
10	125 (0)	125 (0)	85 (0)	30 (0)	36.04	15.27	1.48	0.42	1.70	8.56	45.38	15.65	1.92	0.34	1.74	6.06
11	125 (0)	125 (0)	85 (0)	30 (0)	35.74	15.56	1.57	0.44	1.73	6.12	44.91	15.87	1.81	0.35	1.76	6.08

Other studies have also evaluated ethanol production in fermentation with and without urea supplementation and obtained higher ethanol concentrations in fermentation conducted without adding extra nutrients, indicating that the substrate used has nitrogen concentration and other nutritional components in sufficient quantity for yeast growth and alcoholic fermentation (KUNDIYANA *et al.*, 2010; TOMÁS-PEJÓ *et al.*, 2012).

The use of urea caused a change in the medium's pH, making it more basic, which may have interfered with cellular metabolism and caused more significant osmotic stress (LI; WANG; SHI, 2017). Studies indicate that the ideal pH of alcohol fermentation is around 4 (AKIN-OSANAIYE; NZELIBE; AGBAJI, 2008), although recent research has shown that the increase in the pH of the fermentative medium has favored the consumption of sugars such as xylose (BONATTO *et al.*, 2020; CASEY *et al.*, 2010). In order to balance intracellular osmotic pressure caused by the external environment, yeasts synthesize osmolytes or compatible solutes such as glycerol, which was produced in concentrations of up to 1.92 g L^{-1} in fermentations (Table 3.1) (CHEN *et al.*, 2019; INDIRA *et al.*, 2018). Also, urea can react with ethanol resulting in ethyl carbamate (urethane) as a product, consequently, a lower concentration of ethanol is obtained (COULON *et al.*, 2006; LAOPAIBOON *et al.*, 2009). However, the formation of the urethane was not monitored in this experiment.

Temperature is another critical parameter for fermentative processes and can determine the performance of ethanol production (ABDEL-BANAT *et al.*, 2010), directly affecting the fermentation rate and ethanol yield (ABDEL-BANAT *et al.*, 2010; BAI; ANDERSON; MOO-YOUNG, 2008; PATTANAKITTIVORAKUL *et al.*, 2019). In the tests carried out with temperature at 20 °C, a lower ethanol concentration was obtained in 9 hours of fermentation when compared to the experiments carried out with higher temperatures (30 and 40 °C), and the highest levels were obtained in the experiments performed with a temperature of 40 °C and higher solid-liquid ratio. This result indicates that the strain UFFS-CE-3.1.2 is thermotolerant.

Many researchers have explored yeast strains that ferment ethanol at high temperatures from various substrates. The yeast *S. cerevisiae* DMKU3-S087, for example, was selected among 168 other ethanol-producing strains in the study by Pattanakittivorakul et al. (2019) because it produced the highest concentration of ethanol at 40 °C (58±0.24 g L^{-1}). One can see other yeast strains, such as *S. cerevisiae* KKU-VN8, *S. cerevisiae* DBKKU Y-53, and *Kluveromyces marxianus* MTCC 1389 indicated in the literature as thermotolerant for fermenting at 40 °C (NUANPENG *et al.*, 2016; RAJA SATHENDRA *et al.*, 2019;

TECHAPARIN; THANONKEO; KLANRIT, 2017). The growing interest in yeasts with high thermotolerance is due to their potential application in saccharification and simultaneous fermentation (SSF). The saccharification temperature is higher (up to 60 °C) compared to the fermentation temperature (30 °C - 35 °C), inducing that the saccharification and fermentation are performed separately. Thus, yeasts capable of fermentation at high temperatures are advantageous for SSF and contribute to the reduction of costs associated with the use of enzymes and process time (ABDEL-BANAT *et al.*, 2010).

The agitation had a significant effect on ethanol production using SW and although it had a non-significant impact on fermentation using WSP, the maximum ethanol production was obtained in both fermentations under the conditions using agitation of 120 rpm. Agitation induces movement of the fermentative medium, increasing the permeability of nutrients presents uniformly. Thus, the contact of the cells with the substrate is improved and the rate of consumption is increased. Agitation also reduces toxic inhibition of ethanol and other components in cells (RAJA SATHENDRA *et al.*, 2019).

The variable agitation and temperature were not statistically significant in ethanol production using SW and WSP in 48 h of fermentation. As shown in Figure 3.1, assays 2 and 3, using a solid-liquid ratio of 200%, a delay on ethanol production was observed compared to experimental conditions 1 and 5. Assay 2 reached the maximum ethanol production in 24 h using SW (15.24 g L^{-1}) and 48 h using WSP (16.40 g L^{-1}). Assay 3 reached the maximum ethanol production in 12 h using SW (13.84 g L^{-1}) and 48 h using WSP (15.27 g L^{-1}). Agitation and temperature have a mainly influence on the fermentation rate, causing a reduction in the time of maximum ethanol production and affecting ethanol productivity. Higher ethanol yields were obtained by using higher agitation and temperatures (2.33 g L^{-1} h^{-1}, using SW and 2.46 g L^{-1} h-1, using WSP) (Table 3.1).

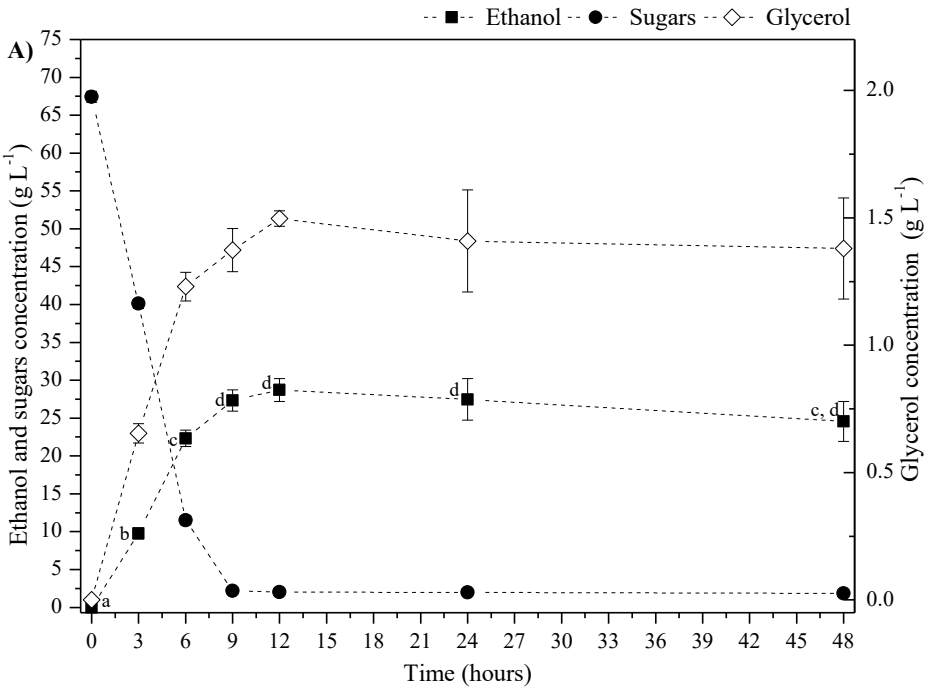
Considering that the best ethanol production in the fermentation conducted with a solid-liquid ratio of 200% at 120 rpm and 40 ºC, without urea, the next stages of the work were carried out using these conditions.

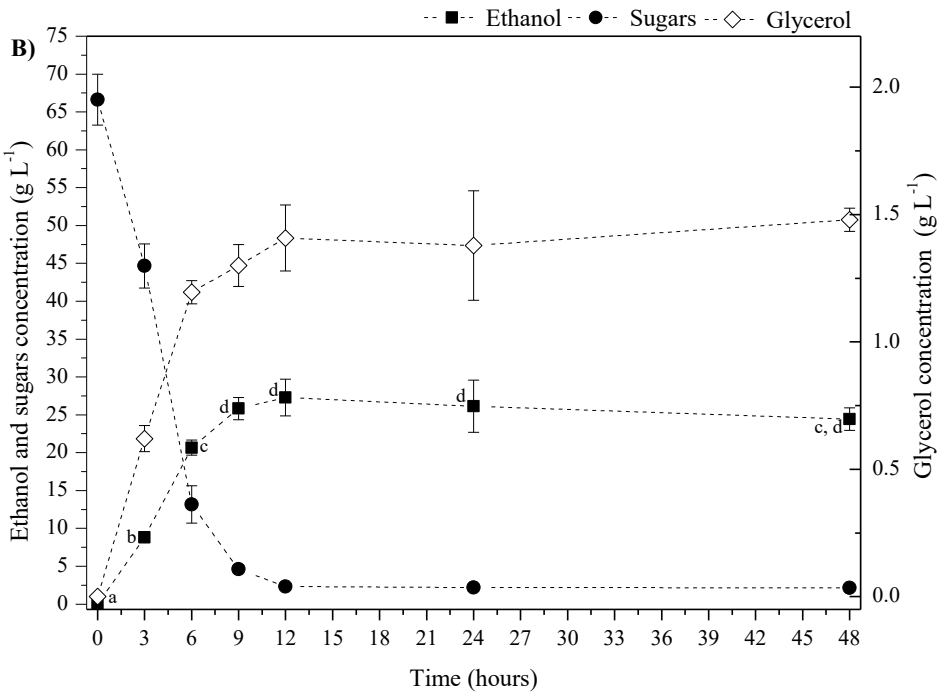
3.3.2 Influence of salinity on fermentation and comparison with *S. cerevisiae* CAT-1

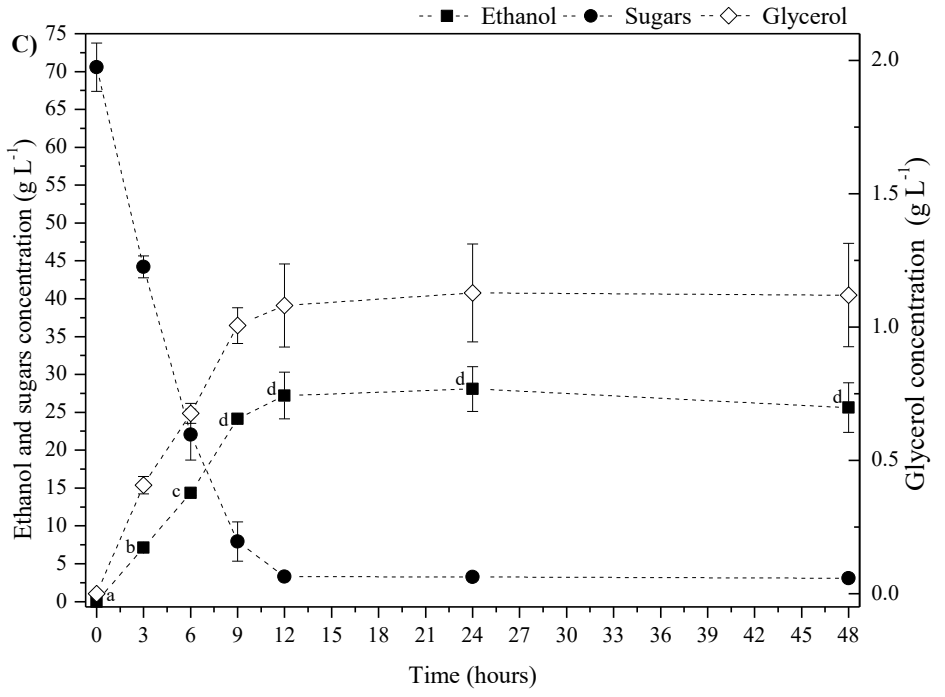
In the fermentation carried out with *Wickerhamomyces* sp. UFFS-CE-3.1.2, ethanol production was started after 3 h of fermentation and reached a maximum production in 9 h,

remaining the same in 12, 24, and 48 h of fermentation ($p>0.05$). The fermentation carried out with SW, WSP, and UW resulted in 27.31±1.40 g L^{-1}, 25.82±1.46 g L^{-1}, and 24.12±0.23 g L^{-1} of ethanol, respectively and are statistically equal ($p>0.05$) (Figure 3.2).

Figure 3.2 – Ethanol and glycerol production and carbohydrate consumption in fermentation conducted by *Wickerhamomyces* sp. UFFS-CE-3.1.2 using SW (A), WSP (B) and UW (C).







Note: the lines connecting the experimental points are illustrative for a better understanding of the reader. The lower case letters of the experimental points indicate that the samples are different when the letters are different and equal when the letters are equal.

The ethanol yield of the fermentations using *Wickerhamomyces* sp. UFFS-CE-3.1.2 showed values close to the theoretical maximum yield (0.511 $g_{ethanol}\ g^{-1}_{hexose}$): around 0.42±0.04 g of ethanol per g of sugar present in the fermentation broth (glucose, fructose and sucrose). Ethanol productivity also showed no significant difference between fermentations, reaching a maximum value of 3.03±0.16 g $L^{-1}h^{-1}$ using seawater (Table 3.2).

Table 3.2 – Yield and productivity of ethanol obtained in the fermentation of papaya residue by *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *S. cerevisae* CAT-1 yeasts using different hydric sources.

Kinetic parameters	***Wickerhamomyces* sp. UFFS-CE-3.1.2**			***S. cerevisiae* CAT-1**		
	SW	**WSP**	**UW**	**SW**	**WSP**	**UW**
Yield ($g\ g^{-1}$)	0.42±0.02	0.42±0.04	0.39±0.02	0.42±0.04	0.39±0.06	0.46±0.04
Productivity ($g\ L^{-1}\ h^{-1}$)	3.03±0.16	2.87±0.16	2.68±0.03	2.04±0.06	1.82±0.22	2.06±0.12

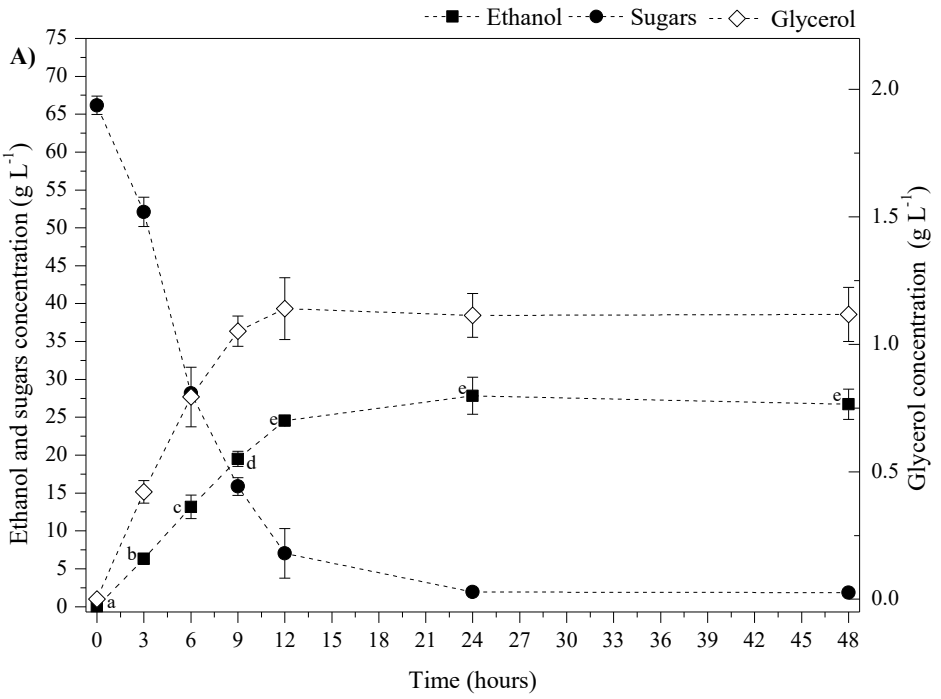
Similar behavior was obtained with yeast *S. cerevisiae* CAT-1. All fermentations conducted with CAT-1 achieved ethanol yield and productivity comparable with fermentations conducted with UFFS-CE-3.1.2 (Table 3.2). However, it took 12 h of fermentation to produce an ethanol concentration similar to that obtained in the fermentation conducted by *Wickerhamomyces* sp. UFFS-CE-3.1.2 ($p>0.05$), resulting in 24.53±0.68 g L^{-1} (using SW), 21.84±2.62 g L^{-1} (using WSP), and 24.73±1.49 g L^{-1} (using UP) (Figure 3.3).

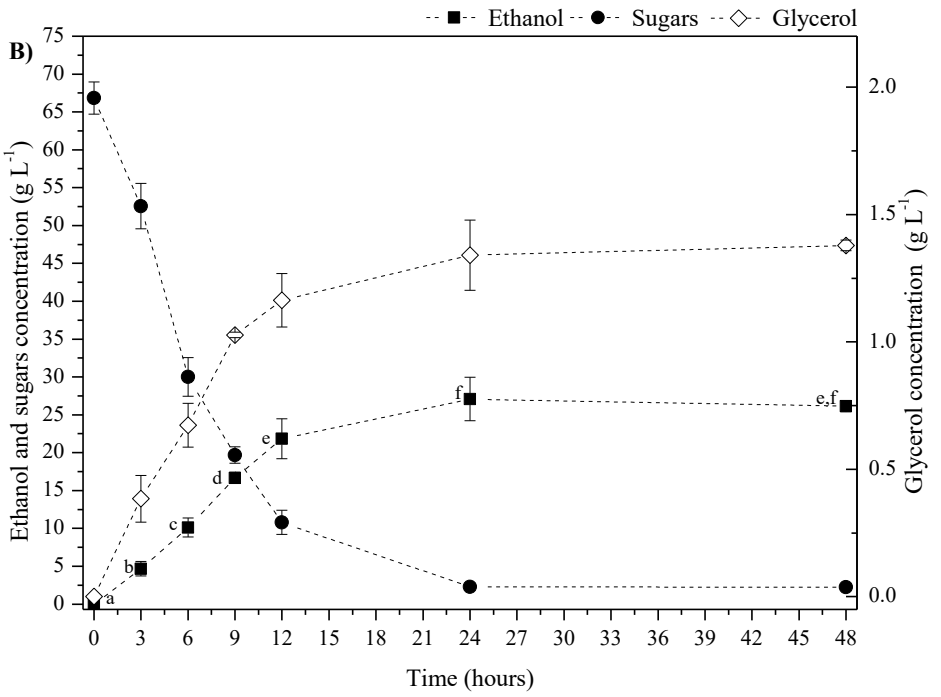
S. cerevisiae CAT-1 showed lower adaptability to the system used, resulting in a slower fermentation rate than the results obtained in fermentation conducted by *Wickerhamomyces* sp. UFFS-CE-3.1.2.

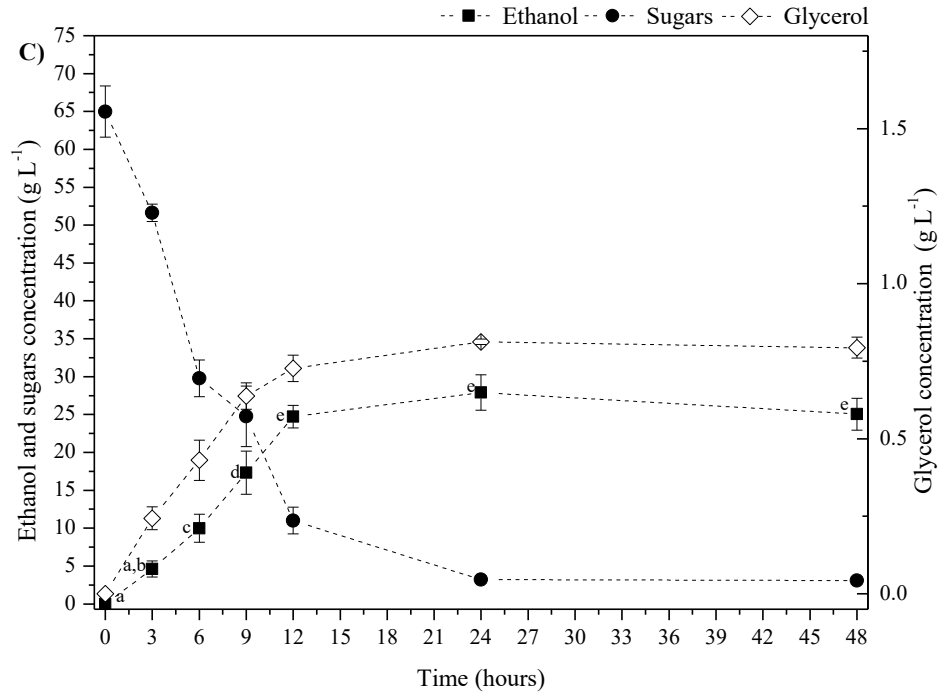
A recent study by Greetham, Zaky and Du (2019) showed that *Wickerhamomyces anomalus* M15 marine yeast is more tolerant to inhibitors and salt than industrial yeast *S. cerevisiae* NCYC2592. However, the fermentative behavior of *S. cerevisiae* CAT-1 is not associated with low tolerance to the saline system, because this fermentative delay was also observed in fermentation conducted with ultrapure water. This suggests that this strain is more sensitive to the components of papaya residue than the yeast *Wickerhamomyces* sp. UFFS-CE-3.1.2 and even to pH, which remained around 3.79±0.08, lower than the

recommended pH range of 4.5 to 5 to ensure optimal ethanol yield by *S. cerevisiae* (AKIN-OSANAIYE; NZELIBE; AGBAJI, 2008; GRAVES *et al.*, 2006).

Figure 3.3 – Ethanol and glycerol production and carbohydrate consumption in fermentation conducted by *S. cerevisiae* CAT-1 yeast using SW (A), WSP (B) and UW (C).







Note: the lines connecting the experimental points are illustrative for a better understanding of the reader. The lower case letters of the experimental points indicate that the samples are different when the letters are different and equal when the letters are equal.

Previous studies evaluated papaya residue regarding the potential of ethanol production from *S. cerevisiae*. These studies used papain-free papaya residue (AKIN-OSANAIYE; NZELIBE; AGBAJI, 2008) and subjected it to saccharification (AKIN-OSANAIYE; NZELIBE; AGBAJI, 2005; JAYAPRAKASHVEL *et al.*, 2014; PARAMESWARI *et al.*, 2015) or acid hydrolysis (ABDULLA *et al.*, 2018). The pH of the resulting papaya broth was adjusted in a range of 4.2 to 5, even though low ethanol yields were obtained (0.036 g g^{-1}) (ABDULLA *et al.*, 2018), and the maximum ethanol production occurred in 72 h of fermentation (AKIN-OSANAIYE; NZELIBE; AGBAJI, 2008). Therefore, papaya residue from the fully matured fruit and vegetable sector is a promising substrate source for ethanol production, available locally, inexpensively. With a simple technology, it is possible to extract the available fermentable sugars and produce ethanol efficiently.

The results obtained also permitted us to conclude that SW and WSP can be used in the production of ethanol as substitutes for freshwater without interfering in its yield when using *Wickerhamomyces* sp. UFFS-CE-3.1.2 or *S. cerevisiae* CAT-1 as a fermenting microorganism and papaya residue as substrate. Previous studies have also stated that there is no significant difference between ethanol yields in the freshwater or saline-based system, probably because yeasts have salt tolerance or ability to metabolize it (GONÇALVES; DOS SANTOS; DE MACEDO, 2015; INDIRA *et al.*, 2018; INDIRA; JAYABALAN, 2020). High salinity in the fermentative medium can cause loss of cell turgor and inhibit a set of functions due to increased intracellular concentration of Na^+ (ANDREISHCHEVA; ZVIAGIL'SKAIA, 1999). Nevertheless, many yeasts can tolerate saline stress by different mechanisms (INDIRA *et al.*, 2018). Salt tolerance studies have revealed that yeast cells can tolerate up to 9% NaCl in the fermentative medium, a concentration higher than the concentration of NaCl present in seawater (approximately 2.71% m v^{-1}) (LIN *et al.*, 2011; ZAKY *et al.*, 2018).

Hortaea werneckii black yeast is one of the most salt-tolerant eukaryotic organisms described so far. Hypersaline water is its natural ecological niche, being able to grow in an almost saturated saline solution (30% or 5.1 M) as well as in unsalted media (GUNDE-CIMERMAN *et al.*, 2000; KOGEJ *et al.*, 2007). The stress response involves changes in gene expression, modulation of enzymatic activities, restructuring of lipid composition, modification of plasma membrane transport systems to expel Na^+ ion from the cell and increased production and accumulation of compatible solutes, especially glycerol, to counterbalance the increase in external osmotic pressure (ANDREISHCHEVA; ZVIAGIL'SKAIA, 1999; DAKAL;

SOLIERI; GIUDICI, 2014; GOSTINČAR *et al.*, 2008) and maintain cellular redox balance in anaerobic conditions (PETROVIC˘; GUNDE-CIMERMAN; PLEMENITAS˘, 2002; YANCEY, 2005). Higher concentrations of glycerol were obtained in the fermentations carried out with sea water and shrimp production water if compared to the fermentations conducted with ultrapure water, suggesting that glycerol was produced as a response to salt stress by the yeasts *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *S. cerevisiae* CAT-1.

The use of wastewater for ethanol production is an expanding research area due to the possibility of using the compounds present in water as a source of nutrient or water source. Many toxic compounds and microbes may be present in wastewater, interfering with the fermentative process. Therefore, some wastewater is only used in ethanol production after submission of a sterilization stage, water dilution and/or cellular immobilization to provide protection against environmental factors (IURCIUC (TINCU) *et al.*, 2016; NIKOLAOU; KOURKOUTAS, 2018). Nikolaou and Kourkoutas (2018) found that the dilution of the wastewater of olive oil mill and molasses of the sugar industry in the proportion of 1:1 with tap water increased the production of ethanol from 4 to 49.8 g L^{-1} when compared to the use of raw residual water alone.

The WSP for ethanol production was investigated for the first time when its insertion in ethanol production and the data showed that it could be used crudely without interfering with the fermentation process, resulting in ethanol concentrations close to the one obtained using ultrapure water independent of the yeast employed (Figure 3.2 and 3.3).

3.4 CONCLUSION

The presence of salinity in fermentation can affect cell growth and metabolism. However, fermentation by *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *S. cerevisiae* CAT-1 was successfully implemented using seawater and shrimp production water with 35 ppm salinity, indicating that freshwater can be replaced by seawater and wastewater without affecting ethanol production. Based on the fermentative results obtained, the papaya residue from the fruit and vegetable sector is an effective source of substrate for the production of ethanol, containing sufficient nutrients for the fermentation process, and it is not necessary to supplement it with inorganic nutritional sources. This indicates the possibility of producing ethanol from locally available fruits using simple technology and at a reduced cost, as it is a waste.

4 FINAL CONSIDERATIONS

Seawater and wastewater from shrimp production can be used as alternatives to freshwater in the ethanol production chain without affecting its yield using *Wickerhamomyces* sp. UFFS-CE-3.1.2 and *S. cerevisiae* CAT-1 strains as fermenting microorganisms. The promising results obtained using wastewater in ethanol production indicate the possibility of developing integrated processes, increasing the life cycle of wastewater. Also, reducing the amount of fresh water used in the process reduces the pressure on water resources for nobler purposes such as fresh water, which is of great relevance to the environment and is the primary source of public supply.

The industrial residues of fruits present low concentrations of free sugars since it is a residue in which most of the fruit was used. Therefore, to increase the amount of sugars available for the fermentation process, the residue needs to be submitted to a treatment stage to solubilization of the sugars present in polysaccharides. However, in this treatment step, many unwanted compounds can be formed and act as inhibitors interfering with the fermentation process.

Being specifically treated for residues rich in pectin, such as orange, the treatment can break up the pectin structure and release high amounts of galacturonic acid sugar, which can be fermented or act as a fermentation inhibitor, depending on the yeast strain used. In this work, we used the acid treatment to solubilize the sugars present in the polysaccharides and obtained high concentrations of galacturonic acid, which may have acted as an inhibitor, negatively affecting ethanol production.

The choice of treatment may not have been the most appropriate because the neutralization phase led to the formation of salts, in addition to the formation of galacturonic acid. However, an enzymatic process would also hydrolyze the pectin releasing the galacturonic acid to the fermentation medium through pectinase's action on the pectin.

In this sense, this challenge can be overcome by developing an integrated process, performing the removal of pectin before application for the production of biofuels, or finding an yeast strain capable of fermenting and tolerating the galacturonic acid, which would be more viable to apply the orange residue or any other pectin residue directly in ethanol production.

On the other hand, fruit residues, generated in the horticultural sector, represent an essential source of substrate for the production of biofuels, precisely because they present high concentrations of readily available sugars, and it is not necessary to perform pretreatment or hydrolysis to solubilize sugars present in polysaccharides so that fermentation occurs efficiently.

This indicates the possibility of producing ethanol from locally available fruits using a simple sugar extraction technology and a reduced cost because it is a residue. Besides, fruit residues' application in obtaining bioproducts allows minimizing impacts on the environment and food safety, while waste is avoided.

REFERENCES

ABDEL-BANAT, Babiker M. A. *et al.* High-temperature fermentation: how can processes for ethanol production at high temperatures become superior to the traditional process using mesophilic yeast? **Applied Microbiology and Biotechnology**, [*s. l.*], v. 85, n. 4, p. 861–867, 2010. Available at: https://doi.org/10.1007/s00253-009-2248-5

ABDULLA, Rahmath *et al.* Fuel ethanol production from papaya waste using immobilized Saccharomyces cerevisiae. **ASM Science Journal**, [*s. l.*], v. 11, n. 2, p. 112–123, 2018.

ADEN, Andy. Water usage for current and future ethanol production. **Southwest Hydrology**, [*s. l.*], v. 6, n. 5, p. 22–23, 2007.

AKIN-OSANAIYE, B.C.; NZELIBE, H.C.; AGBAJI, A.S. Ethanol Production from Carica papaya (Pawpaw) Fruit Waste. **Asian Journal of Biochemistry**, [*s. l.*], v. 3, n. 3, p. 188–193, 2008. Available at: https://doi.org/10.3923/ajb.2008.188.193

AKIN-OSANAIYE, B. C.; NZELIBE, H. C.; AGBAJI, A. S. Production of ethanol from Carica papaya (pawpaw) agro waste: Effect of saccharification and different treatments on ethanol yield. **African Journal of Biotechnology**, [*s. l.*], v. 4, n. 7, p. 657–659, 2005. Available at: https://doi.org/10.5897/AJB2005.000-3118

ANDREISHCHEVA, E N; ZVIAGIL'SKAIA, R A. [Adaptation of yeasts to salt stress (review)]. **Prikladnaia biokhimiia i mikrobiologiia**, [*s. l.*], v. 35, n. 3, p. 243–256, 1999. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10496006

BAI, F.W.; ANDERSON, W.A.; MOO-YOUNG, M. Ethanol fermentation technologies from sugar and starch feedstocks. **Biotechnology Advances**, [*s. l.*], v. 26, n. 1, p. 89–105, 2008. Available at: https://doi.org/10.1016/j.biotechadv.2007.09.002

BAZOTI, Suzana F. *et al.* Second-generation ethanol from non-detoxified sugarcane hydrolysate by a rotting wood isolated yeast strain. **Bioresource Technology**, [*s. l.*], v. 244, p. 582–587, 2017. Available at: https://doi.org/10.1016/j.biortech.2017.08.007

BIZ, Alessandra *et al.* The introduction of the fungal d-galacturonate pathway enables the consumption of d-galacturonic acid by Saccharomyces cerevisiae. **Microbial Cell Factories**, [*s. l.*], v. 15, n. 1, p. 144, 2016. Available at: https://doi.org/10.1186/s12934-016-0544-1

BONATTO, Charline *et al.* Experimental data and modelling of 2G ethanol production

by Wickerhamomyces sp. UFFS-CE-3.1.2. **Renewable Energy**, [*s. l.*], v. 145, p. 2445–2450, 2020. Available at: https://doi.org/10.1016/j.renene.2019.08.010

CASEY, Elizabeth *et al.* Effect ofacetic acid and pH on the cofermentation of glucose and xylose to ethanol bya genetically engineered strain of Saccharomyces cerevisiae. **FEMS Yeast Research**, [*s. l.*], v. 10, p. 385–393, 2010. Available at: https://doi.org/10.1111/j.1567-1364.2010.00623.x

CHEN, Dai *et al.* Effects of diammonia phosphate addition on the chemical constituents in lychee wine fermented with Saccharomyces cerevisiae. **LWT**, [*s. l.*], v. 105, p. 224–232, 2019. Available at: https://doi.org/10.1016/j.lwt.2019.02.018

CHEROENNET, Nichanan; SUWANMANEE, Unchalee. Net Energy Gain and Water Footprint of Corn Ethanol Production in Thailand. **Energy Procedia**, [*s. l.*], v. 118, p. 15–20, 2017. Available at: https://doi.org/10.1016/j.egypro.2017.07.003

CHOI, In Seong *et al.* Bioethanol production from mandarin (Citrus unshiu) peel waste using popping pretreatment. **Applied Energy**, [*s. l.*], v. 102, p. 204–210, 2013. Available at: https://doi.org/10.1016/j.apenergy.2012.03.066

COULON, Joana *et al.* Metabolic Engineering of Saccharomyces cerevisiae to Minimize the Production of Ethyl Carbamate in Wine. **American Journal of Enology and viticulture**, [*s. l.*], v. 57, p. 113–124, 2006.

CYPRIANO, Daniela Z; LOPES, Lucimara; TASIC, Ljubica. High value-added products from the orange juice industry waste. **Waste Management**, [*s. l.*], v. 79, p. 71–78, 2018. Available at: https://doi.org/10.1016/j.wasman.2018.07.028

DAKAL, Tikam Chand; SOLIERI, Lisa; GIUDICI, Paolo. Adaptive response and tolerance to sugar and salt stress in the food yeast Zygosaccharomyces rouxii. **International Journal of Food Microbiology**, [*s. l.*], v. 185, p. 140–157, 2014. Available at: https://doi.org/10.1016/j.ijfoodmicro.2014.05.015

ESPARZA, Irene *et al.* Fruit and vegetable waste management: Conventional and emerging approaches. **Journal of Environmental Management**, [*s. l.*], v. 265, p. 110510, 2020. Available at: https://doi.org/10.1016/j.jenvman.2020.110510

FANG, Chuanji *et al.* Seawater as Alternative to Freshwater in Pretreatment of Date Palm Residues for Bioethanol Production in Coastal and/or Arid Areas. **ChemSusChem**, [*s. l.*], v. 8, n. 22, p. 3823–3831, 2015. Available at: https://doi.org/10.1002/cssc.201501116

FAOSTAT. **Crops: papayas**. [*S. l.*], 2020a. Available at:

http://www.fao.org/faostat/en/?#data/QC.

FAOSTAT. **Data production: orange**. [*S. l.*], 2020b. Available at: http://www.fao.org/faostat/en/#data/QC. Acesso em: 12 set. 2020.

FISH, Wayne W; BRUTON, Benny D; RUSSO, Vincent M. Watermelon juice: a promising feedstock supplement, diluent, and nitrogen supplement for ethanol biofuel production. **Biotechnology for Biofuels**, [*s. l.*], v. 2, n. 18, p. 1–9, 2009. Available at: https://doi.org/10.1186/1754-6834-2-18

GAIDAJIS, Georgios; ANGELAKOGLOU, Komninos. Sustainability of Industrial Facilities through Water Indicators. **Environmental Processes**, [*s. l.*], v. 3, p. 91–103, 2016. Available at: https://doi.org/10.1007/s40710-016-0158-y

GERBENS-LEENES, P W *et al.* Biofuel scenarios in a water perspective : The global blue and green water footprint of road transport in 2030. **Global Environmental Change**, [*s. l.*], v. 22, p. 764–775, 2012. Available at: https://doi.org/10.1016/j.gloenvcha.2012.04.001

GODOY, Patricia *et al.* Microbial Production of Ethanol From Sludge Derived From an Urban Wastewater Treatment Plant. **Frontiers in Microbiology**, [*s. l.*], v. 9, n. 2634, p. 1–6, 2018. Available at: https://doi.org/10.3389/fmicb.2018.02634

GONÇALVES, Fabiano Avelino; DOS SANTOS, Everaldo Silvino; DE MACEDO, Gorete Ribeiro. Alcoholic fermentation of Saccharomyces cerevisiae, Pichia stipitis and Zymomonas mobilis in the presence of inhibitory compounds and seawater. **Journal of Basic Microbiology**, [*s. l.*], v. 55, n. 6, p. 695–708, 2015. Available at: https://doi.org/10.1002/jobm.201400589

GOSTINČAR, C. *et al.* Expression of fatty-acid-modifying enzymes in the halotolerant black yeast Aureobasidium pullulans (de Bary) G. Arnaud under salt stress. **Studies in Mycology**, [*s. l.*], v. 61, p. 51–59, 2008. Available at: https://doi.org/10.3114/sim.2008.61.04

GRAVES, Tara *et al.* Effect of pH and lactic or acetic acid on ethanol productivity by Saccharomyces cerevisiae in corn mash. **Journal of Industrial Microbiology & Biotechnology**, [*s. l.*], v. 33, n. 6, p. 469–474, 2006. Available at: https://doi.org/10.1007/s10295-006-0091-6

GREETHAM, Darren *et al.* A brief review on bioethanol production using marine biomass, marine microorganism and seawater. **Current Opinion in Green and Sustainable Chemistry**, [*s. l.*], v. 14, p. 53–59, 2018. Available at:

https://doi.org/10.1016/j.cogsc.2018.06.008

GREETHAM, Darren; ZAKY, Abdelrahman Saleh; DU, Chenyu. Exploring the tolerance of marine yeast to inhibitory compounds for improving bioethanol production. **Sustainable Energy & Fuels**, [*s. l.*], v. 3, n. 6, p. 1545–1553, 2019. Available at: https://doi.org/10.1039/C9SE00029A

GROHMANN, K. *et al.* Fermentation of galacturonic acid and other sugars in orange peel hydrolysates by the ethanologenic strain of Escherichia coli. **Biotechnology Letters**, [*s. l.*], v. 16, n. 3, p. 281–286, 1994. Available at: https://doi.org/10.1007/BF00134626

GROHMANN, K.; CAMERON, R.G.; BUSLIG, B.S. Fractionation and pretreatment of orange peel by dilute acid hydrolysis. **Bioresource Technology**, [*s. l.*], v. 54, n. 2, p. 129–141, 1995. Available at: https://doi.org/10.1016/0960-8524(95)00121-2

GUNDE-CIMERMAN, Nina *et al.* Hypersaline waters in salterns - natural ecological niches for halophilic black yeasts. **FEMS Microbiology Ecology**, [*s. l.*], v. 32, n. 3, p. 235–240, 2000. Available at: https://doi.org/10.1111/j.1574-6941.2000.tb00716.x

HAN, Zhenlin; PARK, Alex; SU, Wei Wen. Valorization of papaya fruit waste through low-cost fractionation and microbial conversion of both juice and seed lipids. **RSC Advances**, [*s. l.*], v. 8, n. 49, p. 27963–27972, 2018. Available at: https://doi.org/10.1039/C8RA05539D

HELLER, Wade P. *et al.* Utilization of papaya waste and oil production by Chlorella protothecoides. **Algal Research**, [*s. l.*], v. 12, p. 156–160, 2015. Available at: https://doi.org/10.1016/j.algal.2015.08.013

HOEKMAN, S. Kent; BROCH, Amber. Environmental implications of higher ethanol production and use in the U.S.: A literature review. Part II – Biodiversity, land use change, GHG emissions, and sustainability. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 81, p. 3159–3177, 2018. Available at: https://doi.org/10.1016/j.rser.2017.05.052

HUISJES, Eline H. *et al.* Galacturonic Acid Inhibits the Growth of Saccharomyces cerevisiae on Galactose, Xylose, and Arabinose. **Applied and Environmental Microbiology**, [*s. l.*], v. 78, n. 15, p. 5052–5059, 2012. Available at: https://doi.org/10.1128/AEM.07617-11

INDIRA, Dash *et al.* Investigation on the production of bioethanol from black tea waste biomass in the seawater-based system. **Bioresource Technology Reports**, [*s. l.*], v. 4, p. 209–213, 2018. Available at: https://doi.org/10.1016/j.biteb.2018.11.003

INDIRA, Dash; JAYABALAN, R. Saccharification of lignocellulosic biomass using

seawater and halotolerant cellulase with potential application in second-generation bioethanol production. **Biomass Conversion and Biorefinery**, [*s. l.*], v. 10, n. 3, p. 639–650, 2020. Available at: https://doi.org/10.1007/s13399-019-00468-4

IURCIUC (TINCU), Camelia Elena *et al.* Yeast cells immobilized in spherical gellan particles cross-linked with magnesium acetate. **Journal of Biotechnology**, [*s. l.*], v. 236, p. 45–56, 2016. Available at: https://doi.org/10.1016/j.jbiotec.2016.08.002

JAYAPRAKASHVEL, M. *et al.* Production of bioethanol from papaya and pineapple wastes using marine associated microorganisms. **Biosciences Biotechnology Research Asia**, [*s. l.*], v. 11, p. 193–199, 2014. Available at: https://doi.org/10.13005/bbra/1410

JEONG, Deokyeol *et al.* Simultaneous fermentation of galacturonic acid and five-carbon sugars by engineered Saccharomyces cerevisiae. **Bioresource Technology**, [*s. l.*], v. 295, p. 122259, 2020. Available at: https://doi.org/10.1016/j.biortech.2019.122259

JING, Li *et al.* Antifungal Activity of Citrus Essential Oils. **Journal of Agricultural and Food Chemistry**, [*s. l.*], v. 62, n. 14, p. 3011–3033, 2014. Available at: https://doi.org/10.1021/jf5006148

KHAMBHATY, Yasmin *et al.* Bioethanol from Macroalgal Biomass: Utilization of Marine Yeast for Production of the Same. **Bioenergy Research**, [*s. l.*], v. 6, n. 1, p. 188–195, 2013. Available at: https://doi.org/10.1007/s12155-012-9249-4

KOGEJ, Tina *et al.* Osmotic adaptation of the halophilic fungus Hortaea werneckii: role of osmolytes and melanization. **Microbiology**, [*s. l.*], v. 153, n. 12, p. 4261–4273, 2007. Available at: https://doi.org/10.1099/mic.0.2007/010751-0

KUMARI, Dolly; SINGH, Radhika. Pretreatment of lignocellulosic wastes for biofuel production: A critical review. **Renewable and Sustainable Energy Reviews**, [*s. l.*], v. 90, p. 877–891, 2018. Available at: https://doi.org/10.1016/j.rser.2018.03.111

KUNDIYANA, Dimple K. *et al.* Influence of temperature, pH and yeast on in-field production of ethanol from unsterilized sweet sorghum juice. **Biomass and Bioenergy**, [*s. l.*], v. 34, n. 10, p. 1481–1486, 2010. Available at: https://doi.org/10.1016/j.biombioe.2010.04.022

LAOPAIBOON, Lakkana *et al.* Ethanol production from sweet sorghum juice using very high gravity technology: Effects of carbon and nitrogen supplementations. **Bioresource Technology**, [*s. l.*], v. 100, n. 18, p. 4176–4182, 2009. Available at: https://doi.org/10.1016/j.biortech.2009.03.046

LARSSON, Simona *et al.* The generation of fermentation inhibitors during dilute acid

hydrolysis of softwood. **Enzyme and Microbial Technology**, [*s. l.*], v. 24, n. 3–4, p. 151–159, 1999. Available at: https://doi.org/10.1016/S0141-0229(98)00101-X

LEGARDA, Esmeralda Chamorro *et al.* Integrated recirculating aquaculture system for mullet and shrimp using biofloc technology. **Aquaculture**, [*s. l.*], v. 512, p. 734308, 2019. Available at: https://doi.org/10.1016/j.aquaculture.2019.734308

LI, Chenlin *et al.* Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification. **Bioresource Technology**, [*s. l.*], v. 101, n. 13, p. 4900–4906, 2010. Available at: https://doi.org/10.1016/j.biortech.2009.10.066

LI, Zhaofeng; WANG, Donghai; SHI, Yong-Cheng. Effects of nitrogen source on ethanol production in very high gravity fermentation of corn starch. **Journal of the Taiwan Institute of Chemical Engineers**, [*s. l.*], v. 70, p. 229–235, 2017. Available at: https://doi.org/10.1016/j.jtice.2016.10.055

LIAKOU, Vasiliki *et al.* Valorisation of fruit and vegetable waste from open markets for the production of 2,3-butanediol. **Food and Bioproducts Processing**, [*s. l.*], v. 108, p. 27–36, 2018. Available at: https://doi.org/10.1016/j.fbp.2017.10.004

LIN, Carol S.K. *et al.* A seawater-based biorefining strategy for fermentative production and chemical transformations of succinic acid. **Biofuels, Bioproducts and Biorefining**, [*s. l.*], v. 4, p. 1471–1479, 2011. Available at: https://doi.org/10.1002/bbb.328

LOHRASBI, Mehdi *et al.* Process design and economic analysis of a citrus waste biorefinery with biofuels and limonene as products. **Bioresource Technology**, [*s. l.*], v. 101, n. 19, p. 7382–7388, 2010. Available at: https://doi.org/10.1016/j.biortech.2010.04.078

MILLER, G. L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. **Analytical Chemistry**, [*s. l.*], v. 31, n. 3, p. 426–428, 1959. Available at: https://doi.org/10.1021/ac60147a030

MOTA-LÓPEZ, Dulce Rocío *et al.* Evaluation the impact of water supply disruptions in bioethanol production. **Computers & Industrial Engineering**, [*s. l.*], v. 127, p. 1068–1088, 2018. Available at: https://doi.org/10.1016/j.cie.2018.11.041

MUSSATTO, S.I. Biomass Pretreatment With Acids. *In*: BIOMASS FRACTIONATION TECHNOLOGIES FOR A LIGNOCELLULOSIC FEEDSTOCK BASED BIOREFINERY. [*S. l.*]: Elsevier, 2016. p. 169–185. Available at: https://doi.org/10.1016/B978-0-12-802323-5.00008-6

NIKOLAOU, Anastasios; KOURKOUTAS, Yiannis. Exploitation of olive oil mill wastewaters and molasses for ethanol production using immobilized cells of Saccharomyces cerevisiae. **Environmental Science and Pollution Research**, [*s. l.*], v. 25, n. 8, p. 7401–7408, 2018. Available at: https://doi.org/10.1007/s11356-017-1051-6

NUANPENG, Sunan *et al.* Ethanol Production from Sweet Sorghum Juice at High Temperatures Using a Newly Isolated Thermotolerant Yeast Saccharomyces cerevisiae DBKKU Y-53. **Energies**, [*s. l.*], v. 9, n. 253, p. 1–20, 2016. Available at: https://doi.org/10.3390/en9040253

OKAI, Masahiko *et al.* Citeromyces matritensis M37 is a salt-tolerant yeast that produces ethanol from salted algae . **Canadian Journal of Microbiology**, [*s. l.*], v. 63, n. 1, p. 20–26, 2016. Available at: https://doi.org/10.1139/cjm-2016-0259

PARAMESWARI, K *et al.* Isolation of yeast and ethanol production from papaya (Carica papaya) and grape (Vitis vinifera) fruits. **International Journal of Scientific and Engineering Research**, [*s. l.*], v. 6, n. 2, p. 100–104, 2015. Available at: http://www.ijser.org/researchpaper%5CIsolation-of-yeast-and-ethanol-production-from-papaya.pdf

PATEL, Harshvadan; CHAPLA, Digantkumar; SHAH, Amita. Bioconversion of pretreated sugarcane bagasse using enzymatic and acid followed by enzymatic hydrolysis approaches for bioethanol production. **Renewable Energy**, [*s. l.*], v. 109, p. 323–331, 2017. Available at: https://doi.org/10.1016/j.renene.2017.03.057

PATTANAKITTIVORAKUL, Sornsiri *et al.* Selection of thermotolerant Saccharomyces cerevisiae for high temperature ethanol production from molasses and increasing ethanol production by strain improvement. **Antonie van Leeuwenhoek**, [*s. l.*], v. 112, n. 7, p. 975–990, 2019. Available at: https://doi.org/10.1007/s10482-019-01230-6

PETROVIC˘, Uros˘; GUNDE-CIMERMAN, Nina; PLEMENITAS˘, Ana. Cellular responses to environmental salinity in the halophilic black yeast Hortaea werneckii. **Molecular Microbiology**, [*s. l.*], v. 45, n. 3, p. 665–672, 2002. Available at: https://doi.org/10.1046/j.1365-2958.2002.03021.x

PHAIBOONSILPA, Natthanon *et al.* Fermentation of xylose, arabinose, glucose, their mixtures and sugarcane bagasse hydrolyzate by yeast Pichia stipitis for ethanol production. **Energy Reports**, [*s. l.*], v. 6, p. 710–713, 2020. Available at: https://doi.org/10.1016/j.egyr.2019.11.142

PIPER, Peter *et al.* Weak acid adaptation: the stress response that confers yeasts with resistance to organic acid food preservatives. **Microbiology**, [*s. l.*], v. 147, n. 10, p. 2635–2642, 2001. Available at: https://doi.org/10.1099/00221287-147-10-2635

POURBAFRANI, Mohammad *et al.* Production of biofuels, limonene and pectin from citrus wastes. **Bioresource Technology**, [*s. l.*], v. 101, n. 11, p. 4246–4250, 2010. Available at: https://doi.org/10.1016/j.biortech.2010.01.077

PURDUE, Robert Rode. **Marine Shrimp Biofloc Systems: Basic Management Practices**. [*S. l.: s. n.*], 2014.

RAJA SATHENDRA, E. *et al.* Bioethanol production from palm wood using Trichoderma reesei and Kluveromyces marxianus. **Bioresource Technology**, [*s. l.*], v. 271, p. 345–352, 2019. Available at: https://doi.org/10.1016/j.biortech.2018.09.134

RODRIGUES, M.I.; IEMMA, A.F. **Experimental Design and Process Optimization**. first ed.ed. [*S. l.*]: CRC Press, 2014.

SARKAR, Debapriya *et al.* Production of ethanol by Enterobacter sp. EtK3 during fruit waste biotransformation. **International Biodeterioration & Biodegradation**, [*s. l.*], v. 145, n. 104795, p. 1–7, 2019. Available at: https://doi.org/10.1016/j.ibiod.2019.104795

SARRIS, Dimitris *et al.* Conversions of olive mill wastewater-based media by Saccharomyces cerevisiae through sterile and non-sterile bioprocesses. **Journal of Chemical Technology & Biotechnology**, [*s. l.*], v. 88, n. 5, p. 958–969, 2013. Available at: https://doi.org/10.1002/jctb.3931

STOUMPOU, Vasileia *et al.* Assessing straw digestate as feedstock for bioethanol production. **Renewable Energy**, [*s. l.*], v. 153, p. 261–269, 2020. Available at: https://doi.org/10.1016/j.renene.2020.02.021

TECHAPARIN, Atiya; THANONKEO, Pornthap; KLANRIT, Preekamol. High-temperature ethanol production using thermotolerant yeast newly isolated from Greater Mekong Subregion. **Brazilian Journal of Microbiology**, [*s. l.*], v. 48, n. 3, p. 461–475, 2017. Available at: https://doi.org/10.1016/j.bjm.2017.01.006

THANGAVELU, Saravana Kannan *et al.* Microwave assisted acid hydrolysis for bioethanol fuel production from sago pith waste. **Waste Management**, [*s. l.*], v. 86, p. 80–86, 2019. Available at: https://doi.org/10.1016/j.wasman.2019.01.035

TOMÁS-PEJÓ, E. *et al.* Effect of nutrient addition on preinoculum growth of S. cerevisiae for application in SSF processes. **Biomass and Bioenergy**, [*s. l.*], v. 45, p. 168–174,

2012. Available at: https://doi.org/10.1016/j.biombioe.2012.06.002

TSOUKO, Erminta *et al.* Integrated biorefinery development for the extraction of value-added components and bacterial cellulose production from orange peel waste streams. **Renewable Energy**, [*s. l.*], v. 160, p. 944–954, 2020. Available at: https://doi.org/10.1016/j.renene.2020.05.108

TURHAN, Irfan *et al.* Ethanol production from carob extract by using Saccharomyces cerevisiae. **Bioresource Technology**, [*s. l.*], v. 101, n. 14, p. 5290–5296, 2010. Available at: https://doi.org/10.1016/j.biortech.2010.01.146

USDA. **Citrus: World Markets and Trade**. [*S. l.*], 2020. Available at: https://www.fas.usda.gov/data/citrus-world-markets-and-trade. Acesso em: 12 set. 2020.

VIUDA-MARTOS, M. *et al.* Antifungal activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils. **Food Control**, [*s. l.*], v. 19, n. 12, p. 1130–1138, 2008. Available at: https://doi.org/10.1016/j.foodcont.2007.12.003

WIDMER, Wilbur; ZHOU, Weiyang; GROHMANN, Karel. Pretreatment effects on orange processing waste for making ethanol by simultaneous saccharification and fermentation. **Bioresource Technology**, [*s. l.*], v. 101, n. 14, p. 5242–5249, 2010. Available at: https://doi.org/10.1016/j.biortech.2009.12.038

WILKINS, Mark R.; WIDMER, Wilbur W.; GROHMANN, Karel. Simultaneous saccharification and fermentation of citrus peel waste by Saccharomyces cerevisiae to produce ethanol. **Process Biochemistry**, [*s. l.*], v. 42, n. 12, p. 1614–1619, 2007. Available at: https://doi.org/10.1016/j.procbio.2007.09.006

WWAP. **The United Nations World Water Development Report 2018: Nature-based solutions for water**. [*S. l.*], 2018.

YANCEY, P. H. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. **Journal of Experimental Biology**, [*s. l.*], v. 208, p. 2819–2830, 2005. Available at: https://doi.org/10.1242/jeb.01730

YU, Yun; LOU, Xia; WU, Hongwei. Some Recent Advances in Hydrolysis of Biomass in Hot-Compressed Water and Its Comparisons with Other Hydrolysis Methods †. **Energy & Fuels**, [*s. l.*], v. 22, n. 1, p. 46–60, 2008. Available at: https://doi.org/10.1021/ef700292p

ZAKY, Abdelrahman Saleh *et al.* A new isolation and evaluation method for marine-derived yeast spp. with potential applications in industrial biotechnology. **Journal of**

Microbiology and Biotechnology, [*s. l.*], v. 26, n. 11, p. 1891–1907, 2016. Available at: https://doi.org/10.4014/jmb.1605.05074

ZAKY, Abdelrahman Saleh *et al.* The establishment of a marine focused biorefinery for bioethanol production using seawater and a novel marine yeast strain. **Scientific Reports**, [*s. l.*], v. 8, n. 1, p. 1–14, 2018. Available at: https://doi.org/10.1038/s41598-018-30660-x