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Special Edition

Extraction of furfural inhibitor from biomass hydrolysate of rice husk

Extração do inibidor furfural a partir do hidrolisado de biomassa da casca do arroz

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ABSTRACT

The production of second generation ethanol (E2G) has proven to be an alternative to non-renewable fuels, through transforming lignocellulosic waste into renewable fuel. In turn, rice husk has great potential due to its availability and composition. The conversion of lignocellulosic biomass to biofuel comprises a fundamental pretreatment step, however, at this stage, the formation of degradation products (inhibitory compounds) occurs, among them, furfural, which cause negative effects on the viability of fermentative cells, making the production of E2G unfeasible. Given the above, the objective of this work was to remove the furfural inhibitor present in the lignocellulosic broth after the pretreatment process, using oleic acid, through liquid-liquid extraction. The quantification of total reducing sugars in the hydrolysate did not show significant variation between the pre and post extraction stages. Regarding the furfural inhibitor, in tests performed with a solution made in the laboratory, removal of up to 62.30% was obtained when the initial concentration was 5.00 $g.L^{-1}$. With respect to the tests with the hydrolysate from the rice husk pretreatment, the maximum removal observed was 10.40%, but the initial concentration of furfural was 1.64 $g.L^{-1}$. The results obtained indicate the possibility of using oleic acid as an extracting agent of the furfural inhibitor from lignocellulosic hydrolysates.

Keywords: Furfural; Residual biomass; Liquid-liquid extraction

RESUMO

A produção de etanol de segunda geração (E2G), vem mostrando ser uma alternativa aos combustíveis não renováveis, ao realizar a transformação de resíduos lignocelulósicos em combustível renovável. Por sua vez, a casca de arroz possui grande potencial devido a sua disponibilidade e composição. A conversão da biomassa lignocelulósica a biocombustível é composta por uma etapa fundamental de pré-tratamento. Nessa etapa, ocorre a formação de produtos de degradação (compostos inibitórios), os quais causam efeitos negativos na viabilidade das células fermentativas, o que torna inviável a produção

de E2G. Dentre eles, o furfural é um inibidor formado. Diante do exposto, o objetivo do trabalho foi retirar o inibidor furfural presente no caldo lignocelulósico após o processo de pré-tratamento através da utilização do ácido oleico por meio de extração líquido-líquido. A quantificação dos açúcares redutores totais no hidrolisado não apresentou variação significativa entre a etapa pré e pós extração. Com relação ao inibidor furfural, nos testes realizados com solução feita em laboratório, obteve-se uma remoção de até 62,30% quando a concentração inicial dele foi de 5,00 $g.L^{-1}$. Já para os testes com o hidrolisado proveniente do pré-tratamento da casca do arroz, a máxima remoção obtida foi de 10,40%, porém a concentração inicial do furfural era de 1,64 $g.L^{-1}$. Os resultados obtidos indicam a possibilidade do uso do ácido oleico como um agente extrator do inibidor furfural de hidrolisados lignocelulósicos.

Palavras-chave: Furfural; Biomassa residual; Extração líquido-líquido

1 INTRODUCTION

Brazil stands out worldwide in the use of renewable energy, with values equivalent to 48.4% of the country's entire energy supply in 2020 (BALANÇO ENERGÉTICO NACIONAL, 2021). However, the imminent shortage of non-renewable energy and the search for good environmental practices have led the government and society to seek an increase in the percentage of renewable energy used.

A biofuel is a renewable fuel that can be generated from different sources, mainly vegetable sources (MOTA; MONTEIRO, 2013). Among these, the production of cellulosic ethanol or second-generation ethanol (E2G) is an important sustainable alternative (VARGAS BETANCUR; PEREIRA JR, 2013), since it uses energy from biomass from agro-industrial residues (PERES; JUNIOR; GAZZONI, 2005).

Rice is part of the diet of most people and Brazil stands out by representing 1.6% of the world production (EMBRAPA, 2017). The rice grain consists of a protective layer, the husk, which accounts for approximately 20% of the grain's weight. The main constituents of the husk are cellulose and lignin, representing 38.4 and 29.4% of its composition, respectively (REYES; PERALTA-ZAMORA; DURÁN, 1998).

Among the steps for converting lignocellulosic biomass to biofuel, the pretreatment step is responsible for cellulose exposure and hemicellulose solubilization (CANILHA *et al*., 2012). At this stage, a liquor, rich in five-carbon sugars (xylose and arabinose), is obtained, and the fermentation of these sugars

must be executed by specific yeasts to obtain ethanol (MARTIN *et al*., 2007; NAKASU *et al*., 2016; NAKANISHI *et al*., 2017; SANTOS *et al*., 2016). Dilute acid pretreatment features high solubilization of hemicellulose, high cellulose digestibility, and an alternative to the hazards presented by concentrated acid pretreatment (CARVALHEIRO; DUARTE; GÍRIO, 2008; GÍRIO *et al*., 2010).

However, the formation of degradation products during the pretreatment stage is one of the biggest problems associated with this process (GÍRIO *et al*., 2010). Inhibitors significantly reduce the cell growth rate, amount of ethanol produced, and/or ethanol production rate in the fermentation stage (PALMQVIST; HAGERDAL, 2000; ALMEIDA; MODIG; PETERSSON, 2007; JAYAKODY; HAYASHI; KITAGAKI, 2011, 2013; LIU, 2011). The main inhibitors generated and which most impact yeast kinetics are: furfural, 5-hydroxymethylfurfural, vanilla, syringaldehyde, and coniferaldehyde (ZAUTSEN, 2011).

When heated in an acidic environment, polysaccharides are hydrolyzed, especially hemicellulose, resulting in free sugars that can be degraded to furfural from pentoses (QIAN *et al*., 2005). An alternative to overcome the problem of inhibitors in the 2G ethanol production process is their removal from the lignocellulosic broth. For this, a liquid-liquid extraction process can be used, in which the organic phase is responsible for extracting part of the inhibitors from the broth.

In general, the organic solvent must be poorly miscible, not extract sugars from the lignocellulosic broth, efficiently extract the inhibitor, and be non-toxic to yeasts (MALINOWSKI, 2001). However, biocompatibility is the most important feature in this type of process (OFFEMAN *et al*., 2010). In this case, as shown in previous studies, oleic acid may be a good option to be tested (BARROS; CABRAL; NOVAIS, 1984; JASSAL; ZHANG; HILL, 1994; LEMOS, 2017; ZAUTSEN, 2011).

Given the above, the search for an effective solvent to remove the furfural inhibitor from lignocellulosic broth is an important step towards improving the 2G ethanol production process. Therefore, the objective of this work is to remove the

furfural inhibitor present in lignocellulosic broth from rice husk biomass after dilute acid pretreatment, through the liquid-liquid extraction process, using oleic acid as an extracting agent.

2 MATERIAL AND METHODS

2.1 Sample preparation

A biomass from a rice processing company in the city of Uberaba/MG was used. The biomass was dried to a humidity below 10% in a forced air circulation oven (Thelga, TE150CD) at 50ºC for 48h (KHAMIS *et al*., 2019; RABELO, 2010). It was later ground in a knife mill (Willye type – Fortinox FT-50). For the sieving of the biomass, a sieve with an opening diameter of 297 µm was used.

2.2 Preparation of furfural solutions

Furfural solutions were prepared at 3 different concentrations, namely 1.00, 3.00, and 5.00 g. L^{-1}. Furfural analytical reagent (purity > 99% - Sigma Aldrich) and distilled water were used.

2.3 Pretreatment sample

For the pretreatment step, the methodology proposed by Santos (2014) was used with adaptations. Initially, 25 g of crushed and sieved biomass with a diameter smaller than 297 µm were weighed and then 100 mL of 0.1 M sulfuric acid solution were added (ratio of 1:5) in a 500 mL Erlenmeyer flask. With the Erlenmeyer properly closed, it was taken to an autoclave at a pressure of 1 atm and 121°C for 30 minutes. The sample was subsequently removed, filtered on filter paper, and then the hydrolysate was obtained for the extraction step.

2.4 Inhibitor extraction process

The liquid-liquid extraction process was carried out both for the synthetic furfural solution and for the hydrolysate obtained after pretreatment according to the methodology adapted from Lemos *et al*. (2017).

For the extraction process with an oleic acid/sample ratio of 1:1, 5 mL of furfural or hydrolysate solution were pipetted and placed in a 50 mL Falcon-type plastic tube. Next, 5 ml of the oleic acid extracting agent were measured and added to the tube. This mixture was stirred for 60 seconds in a vortex mixer to ensure complete mixing of the two components, before being left to stand at room temperature for 6 hours to separate the two phases. Subsequently, a very thin syringe needle (to avoid disturbing the system) was used to remove an aliquot of the residual aqueous phase, in order to verify the amount of furfural that remained there after extraction.

For the furfural inhibitor removal tests from previously prepared “synthetic” solutions, the extraction process was used in two different proportions of oleic acid/solution, 1:1 and 1:2.

In the process containing the hydrolysate obtained after pretreatment, the quantification of total reducing sugars and furfural inhibitor were performed before and after extraction. However, at this stage, we chose 3 different proportions of oleic acid/hydrolysate, being 1:1, 1:2, and 1:3.

2.5 Analytical methodologies

The analysis of total reducing sugars (TRS) and furfural inhibitor was carried out before and after extraction. All tests were performed in duplicate.

Sugars were measured using the 3,5-dinitrosalicylic acid (DNS) method according to modified Bernfeld (1955). Initially, the lignocellulosic hydrolysate sample was diluted 1:10 in distilled water so that the value was within the previously made calibration curve. Afterwards, the sample and DNS reagent were

added to a test tube that was placed in a bath with boiling water for 5 minutes. To stop the reaction, the test tube was placed in an ice bath until it cooled down and then distilled water was added to the tube. Finally, the sample was placed in a glass cuvette and the reading was taken in a digital spectrophotometer (UV-VIS with automatic scanning, Instrutherm) at 540 nm to quantify the total reducing sugar present.

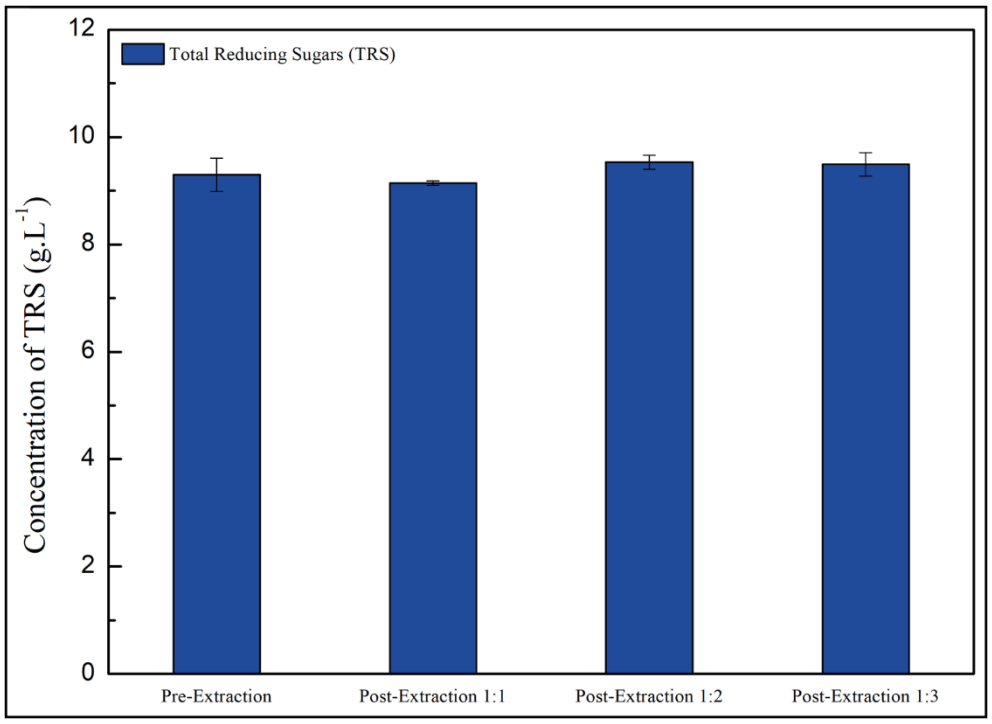
The quantification of furfural inhibitor was adapted from the methodology of Khabarov *et al*. (2006). Initially, 1 mL of the sample and 5 mL of mercury acetate were measured in a 50 mL volumetric flask and the volume was completed with distilled water. From this solution, 1 mL was removed, and 0.3 mL of sulfuric acid P.A. were added in a test tube and placed in a bath with boiling water for 30 minutes. After heating, the solution was transferred to a 100 ml volumetric flask and made up to volume with distilled water. The sample was placed in a quartz cuvette and read in a digital spectrophotometer (UV-VIS with automatic scanning, Instrutherm) at 238 nm to quantify the furfural present.

3 RESULTS AND DISCUSSION

The quantification of total reducing sugars using the DNS reagent method showed that the sugar concentration in the lignocellulosic broth was 9.30±0.30 $g.L^{-1}$ before the extraction process. After extraction by oleic acid, the sugar concentration in the lignocellulosic broth was 9.14±0.06 $g.L^{-1}$ at a ratio of 1:1. Subsequently, at a ratio of 1:2, the result was 9.53±0.11 $g.L^{-1}$ of total reducing sugar remaining in the analyzed sample. Finally, at a ratio of 1:3 of broth and extracting agent, the result obtained was 9.49±0.19 $g.L^{-1}$ of sugar. These results are presented in Figure 1, which clearly demonstrates that oleic acid did not remove the reducing sugar during the extraction step, since there were no significant variations in the values of the pre- and post-extraction sugar concentrations. Validation was

performed using the Tukey Test, which showed that there was no variation in a 95% confidence interval.

Figure 1 – Concentrations of total reducing sugars (TRS) before and after liquid-liquid extraction with volume ratios of 1:1, 1:2, and 1:3 aqueous solution/extracting agent



Source: Authors, 2021

Thus, the results obtained demonstrate that the liquid-liquid extractive process using oleic acid as an extracting agent did not remove sugar from the hydrolysate, which is a relevant characteristic for the process according to OFFEMAN *et al*. (2010). This property is important, since by not removing sugars from the hydrolysate, there will be no loss of substrate from future fermentation. Other works that used liquid-liquid extraction in fermentation processes for 1G ethanol also did not show sugar removal by the extracting agent (LEMOS, 2017; LEMOS *et al*., 2018, 2020; ZAUTSEN, 2011).

The quantification of furfural in the water and furfural solution before extraction presented values of 1.26, 3.66, and 5.01 $g.L^{-1}$ (samples 1, 2, and 3, respectively).

The values of furfural concentration after extraction with a 1:1 ratio, for samples 1, 2 and 3, were, respectively; 0.88, 2.17, and 3.31 $g.L^{-1}$. These values represent removal of 30.2% of furfural present in sample 1 compared to the initial solution, 40.7% in sample 2, and 33.9% in sample 3.

Next, the same initial samples of furfural solution were used, but with an oleic acid / solution ratio of 1:2. The results for furfural concentrations after extraction were 0.66 gL^{-1} for sample 1, 1.40 $g.L^{-1}$ for sample 2, and 1.89 $g.L^{-1}$for sample 3. Thus, the removal from each of the samples in relation to the initial solution was 47.6, 61.7, and 62.3%, respectively.

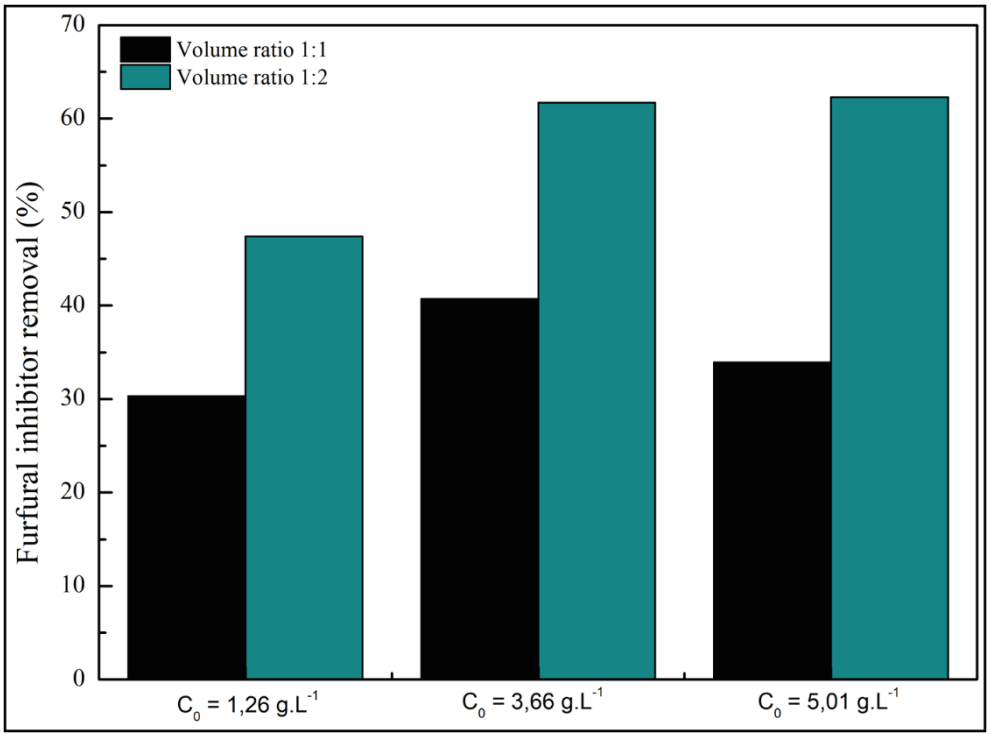
These results show that oleic acid was able to extract furfural from an aqueous solution at concentrations close to those found in real hydrolysates (from residual biomass), reaching values greater than 60% removal. It was also noticed, as can be seen in Figure 2 and Table 1, that the increase in the ratio between the aqueous solution and extracting agent resulted in greater removal in all cases, especially for the initial concentration of 5.01 $g.L^{-1;}$ by doubling the amount of oleic acid used, approximately twice the removal of furfural inhibitor was obtained.

Table 1 – Furfural concentrations before and after liquid-liquid extraction with aqueous solution/solvent volume ratios of 1:1 and 1:2

	Furfural concentration Pre-extraction ($g.L^{-1}$)	**Post-extraction furfural concentration (1:1) ($g.L^{-1}$)**	**Post-extraction furfural concentration (1:2) ($g.L^{-1}$)**
Furfural solution (1 $g.L^{-1}$)	1.26 ± 0.01*	0.88 ± 0.09*	0.66 ± 0.02*
Furfural solution (3 $g.L^{-1}$)	3.66 ± 0.09*	2.17 ± 0.08*	1.40 ± 0.04*
Furfural solution (5 $g.L^{-1}$)	5.01 ± 0.01*	3.31 ± 0.05*	1.89 ± 0.02*

*S.D. - Standard deviation
Source: Authors, 2021

Figure 2 – Percentage of furfural inhibitor removal after liquid-liquid extraction with aqueous solution/solvent volume ratios of 1:1 and 1:2 at different initial solution concentrations



Labels: black 1:1 ratio, blue 1:2 ratio.
Source: Authors, 2021

Finally, after analyzing the extraction with solutions prepared in the laboratory, the tests were carried out using the hydrolysate coming from the rice husk biomass after pretreatment with dilute acid. The value found for the furfural inhibitor concentration before extraction was 1.64 $g.L^{-1}$. This value is close to that found in the literature, since Khamis *et al*. (2019) studied the concentrations of furfural present in rice husks and straw using different types of pretreatments and found values of furfural concentrations from these biomasses ranging from 0.3 to 3.5 $g.L^{-1}$. For other biomass, such as the sugarcane industry, values in the range of 0.1 to 0.6 $g.L^{-1}$ are found (ROQUE *et al*., 2019; DA SILVA MARTINS *et al*., 2015).

The furfural value obtained after extraction in the 1:1 ratio was 1.62 $g.L^{-1}$, showing a removal of approximately 1.2%. For the ratio of 1:2, the value of 1.54 $g.L^{-1}$ of residual furfural in the hydrolysate was found, with removal of 6.1%.

In the ratio of 1:3, the value of 1.47 $g.L^{-1}$ was found for the residual furfural, accounting for removal of 10.4%.

The final ratio, 1:3, was only tested with the hydrolysate to verify if the tendency to increase extraction with an increase in the oleic acid/hydrolysate ratio would be maintained, since the values were much lower with the hydrolysate.
The values of residual furfural concentration and percentage of furfural removal from the hydrolysate can be seen in detail in Table 2.

Table 2 – Furfural concentrations before and after liquid-liquid extraction with aqueous solution: solvent volume ratios of 1:1, 1:2, and 1:3

Furfural Concentration Pre-extraction ($g.L^{-1}$)	**Post-extraction furfural Concentration (1:1) ($g.L^{-1}$)**	**Post-extraction furfural Concentration (1:2) ($g.L^{-1}$)**	**Post-extraction furfural Concentration (1:3) ($g.L^{-1}$)**
1.64 ± 0.03*	1.62 ± 0.13*	1.54 ± 0.08*	1.47 ± 0.04*

*S.D. - Standard deviation
Source: Authors (2021)

Finally, furfural removal maintained the same trend as the results obtained in solutions prepared in the laboratory, but did not present a significant difference when evaluated by the Tukey test with a 95% confidence interval. This fact may be related to the fact that the removal efficiency in lignocellulosic hydrolysate was lower than those previously found in solutions prepared with analytical reagent, in which when there was a higher proportion of extracting agent, there was a more significant removal of the inhibitor. One possible explanation is that the furfural in the hydrolysate is mixed with other substances, and this could compromise its affinity with oleic acid and end up reducing its removal (Lemos *et al*., 2017).

However, even at a low concentration in the hydrolysate, oleic acid proved to be an option for removing furfural, and furthermore, it did not remove the sugar in the solution. Roque *et al*. (2019) also studied the removal of the furfural inhibitor, among others, by means of liquid-liquid extraction with three different extracting

agents. In their case, they used different process conditions, such as hydrolysate concentration, pH change, and series process (evaporation and extraction), obtaining removals of up to 99%. However, the presence of one of the extracting agents interfered with the fermentation process. Therefore, new process alternatives and new extracting agents could still be tested to improve the process.

4 FINAL CONSIDERATIONS

The results obtained were satisfactory and indicated the possibility of using oleic acid as an extracting agent for the furfural inhibitor of lignocellulosic hydrolysates. It was possible to extract the inhibitor with no removal of the substrate (reducing sugar) from the medium, which favors the subsequent fermentation to produce second-generation ethanol. Despite the low values, this study represents a starting point to obtain better conditions, such as by concentrating the hydrolysate before the extraction process or even looking for another solvent that could better meet the extraction demand.

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