PEROXONE PROCESS APPLIED TO THE DETOXIFICATION OF RICE STRAW HEMICELLULOSIC HYDROLYSATE AS A STRATEGY FOR IMPROVING THE BIOCONVERSION OF PENTOSES

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ABSTRACT

The detoxification of lignocellulosic hydrolysates is an important strategy to improve the bioconversion processes of their sugars. This study investigates the effectiveness of the peroxone process to detoxify rice straw hemicellulosic hydrolysate, comparing it to widely studied methods, such as the use of activated carbon adsorption and overliming, while exploring innovative strategies for their combined application (overliming-peroxone). The processes of detoxification by peroxone, adsorption on activated carbon, overliming and combined provided significant reductions in the concentrations of low molecular weight phenolic compounds (94%, 84%, 44% and 97%, respectively), total phenolic compounds (40%, 31% , 24%, and 58%, respectively) and furans (71%, 63%, 90% and 70%, respectively). Additionally, the effects of these treatments on increasing the bioconversion of sugar present in the hydrolysate by *Scheffersomyces stipitis* NRRL -Y-7124 were evaluated. In general, the treatments by peroxone, overliming and a combination of these, provided notable improvements in cell growth, sugar uptake and ethanol production. Compared to the accumulation of ethanol in the untreated hydrolysate culture (3.8 g/L in 120 h), four times higher concentrations were achieved in hydrolysate treated with peroxone (21.6 g/L) and overliming (18.7 g/L) and six times higher for hydrolysate treated with the overliming-peroxone combination (33.2 g/L). Among the evaluated processes, the overliming-peroxone combination stood out in improving the bioprocess. However, in terms of cost per unit of ethanol produced, the peroxone treatment proved to be the most economically advantageous.

Keywords: Detoxification 1. Peroxone Process 2. Lignocellulosic Biomass 3. Biorefinery 4.

INTRODUCTION

Lignocellulosic materials are an important source of raw material for biorefineries. This biomass can be used by these industrial facilities to process on a large scale, converting it into economically significant products such as biofuels, industrial chemicals, and biomaterials like biopolymers and energy.[1] In Brazil, a wide variety of agricultural waste, including rice straw, could serve as raw material for biorefineries. According to the National Supply Company (CONAB), the Brazilian grain harvest in 2021/2022 was estimated at 270.9 million tons, with rice accounting for 4% of the total at 10.8 million tons, generating a substantial amount of

Lignocellulosic materials are composed of complex heterogeneous polymers of carbohydrates, densely wrapped in lignin, so their use in biotechnological applications requires the separation of carbohydrates from cellulose and hemicellulose through acid or enzymatic hydrolysis processes. However, the separation of these carbohydrates generates a variety of by-products capable of interfering with microbial metabolism, impairing fermentation processes, reducing sugar consumption and, consequently, limiting the productivity of bioconversion processes.[3]

In this way, the detoxification stage is an important aspect to be considered in order to improve fermentation processes using hydrolysates from lignocellulosic materials, and it is important to search for new alternatives that contribute to this stage. There are a variety of methods used to detoxify hydrolysates derived from lignocellulosic materials described in the literature, each of which has different capacities for removing inhibitory compounds.[4] An alternative for detoxification would be the application of Advanced Oxidative Processes (AOPs). In the work by Silva et al. (2013)[5], the procedure was carried out in an alkaline medium (pH 8), in the presence of H_2O_2 as well as ultraviolet radiation, achieving a removal of over 40% of total phenolics, over 95% of low molecular weight phenolic compounds (vanillic acid, vanillin, p-coumaric acid and ferulic acid) and over 52% of furans.

The aim of this study was to evaluate the detoxification treatment by peroxone, a Advanced Oxidative Processes (AOPs), of rice straw hemicellulose hydrolysate, and comparing it with other treatments frequently used in the literature (overliming and activated carbon adsorption) and a combined process (overliming with peroxone), with the aim of improving the biotechnological process of ethanol production by the yeast *Scheffersomyces stipitis* NRRL-Y-7124.

MATERIAL & METHODS

The hydrolysis of rice straw hemicellulose was carried out according to the optimized conditions described by Roberto et al. (2003)[3]. The activated carbon process was carried out according to the methodology of Mussatto and Roberto (2001)[6]. For the Overliming process, the methodology described by Carvalheiro et al. (2005)[7] was used. The hydrolysate had its pH adjusted to 10 with the addition of $Ca(OH)_2$ and then adjusted to pH 5.5 with the addition of H_2SO_4 98% (w/w), so that the precipitate obtained was separated by centrifugation.

The detoxification treatment of the hydrolysate by peroxone was carried out in a bubble column reactor, made of glass and with a useful volume of 150 mL. The reactions were conducted at a temperature of 30 ºC, with an initial concentration of 100 mg/L of H_2O_2 and continuous addition of ozone (supply rate of 2.50 mg of O_3 per minute). An ozonation time of 15 minutes was used in order to achieve ozone dosages of up to 250 mg/L.[5]

To follow up the experiments, the effects of the treatments were evaluated in two respects: (1) the effects of the treatment on the characteristics of the treated hydrolysate; (2) the effects of the treatment on the bioconversion process with the yeast *Scheffersomyces stipitis* NRRL-Y-7124 using the treated hydrolysate as a substrate. The sugar, ethanol, low molecular weight phenolic compounds and furans were quantified using High Performance Liquid Chromatography.

RESULTS & DISCUSSION

Table 1 presents the characterization results for untreated rice straw hemicellulose hydrolysate, as well as the hydrolysate after undergoing various treatment processes, including the peroxone process, overliming, activated carbon adsorption and combined process (overliming-peroxone). The rice straw hemicellulose hydrolysate concentrated had a total of sugar of approximately 163.8 g/L, distributed in a ratio of 16:3:1 for xylose (124.3 g/L), arabinose (31.6 g/L) and glucose (7.9 g/L). In addition to sugars, the presence of compounds such as aliphatic acids (2.6 g/L), furans (87 mg/L furfural and 27.9 mg/L hydroxymethylfurfural) and low molecular mass phenolic compounds (syringaldehyde 1736, 8 mg/L, ferulic acid 1661.7 mg/L, syringic acid 30.3 mg/L, vanillin 33.8 mg/L, furoic acid 276.2 mg/L, gallic acid 38.6 mg/L, vanillyl alcohol 408.2 mg/L, vanillic acid 37.0 mg/L, p-coumaric acid 146.2 mg/L).

Regarding the xylose concentration, the overliming and combined process (overliming-peroxone) treatments resulted in a reduction of approximately 18%. Conversely, the reductions observed with the activated carbon (3.3%) and peroxone process (1.6%) treatments are considered insignificant due to their proximity to the analytical error associated with the quantification method. About phenolic compounds and furans, the treatment of the hydrolysate by peroxone process provided a reduction of over 95% for furoic acid and ferulic acid. The compounds furfural, vanillin, p-coumaric acid and syringaldehyde were reduced by between 70% and 94%. It is important to note that these reductions in the concentrations of potentially inhibitory compounds were accompanied by the maintenance of sugar concentrations in the hydrolysate, thus demonstrating an important selectivity of the detoxification treatment process, a very desirable characteristic for such purposes.

Treatment by the combined process (overliming and peroxone process) provided practically complete reduction (equal to or greater than 97%) of vanillyl alcohol, furoic acid, syringaldehyde and ferulic acid. For the compound's vanillin, furfural and syringic acid, the treatment reduced 85%, 74% and 90%, respectively. For HMF and gallic acid, the treatments provided reductions of around 57% and 26%, respectively. About furan compounds, the activated carbon treatment showed the greatest reduction, around 90%, followed by the peroxone process with a 71% reduction in furan concentration.

Table 1 Composition of untreated concentrated rice straw hemicellulose hydrolysate (RSHH-NT) and after treatment by peroxone process, overliming, activated carbon and combined process (overliming-peroxone)

Compounds	Composition in terms of concentration				
	RSHH-NT	Peroxone Process	Overliming	Activated carbon	Overliming-Peroxone
Carbohydrates and aliphatic acids (g/L)					
Xylose	124,3	122,0	101,4	120,2	101,3
Glucose	7,9	8,3	9,4	8,0	9,7
Arabinose	31,6	31,0	22,5	30,1	22,5
Acetic Acid	2,6	2,6	2,0	2,4	2,2
Furans (mg/L)					
Furfural	87	26	31	5	23
Hydroxymethylfurfural	28	7	12	6	12
Low molecular mass phenolic compounds (mg/L)					
Syringaldehyde	1737	97	233	1116	51
P-coumaric acid	146	18	49	108	13
Gallic acid	39	44	11	31	29
Vanillyl alcohol	408	24	79	61	0
Vanillic acid	37	4	30	31	4
Furoic acid	1662	48	263	1072	34
Syringic acid	30	3	17	23	3
Vanillin	34	5	19	9	5
Furoic acid	276	14	0	14	0
Total low molecular mass phenolic compounds (mg/L)	4369	257	701	2465	139
Total phenolics (mg/L)	9572	5790	6570	11964	4059
Total inorganics (g/L)	39	140	35	124	98

With regard to the reduction in total low molecular mass phenolic compounds, we can see that the greatest reductions, 94% and 97%, were achieved using the peroxone process and combined overliming-peroxone treatment processes, respectively. The reductions achieved by overliming and activated carbon treatment were 84% and 44%, respectively. Similar reduction results were also observed for total phenolic compounds, with the greatest reduction obtained for the combined process (58%), followed in descending order by the peroxone process (40%), overliming (31%) and activated carbon (25%) treatments. In general, the treatments evaluated also had a very varied influence on the content of total soluble inorganic compounds present in the hydrolysate.

To assess the fermentability of rice straw hydrolysate, 120-hour cultivation trials were carried out with the yeast *Scheffersomyces stipitis* NRRL-Y-7124 at initial xylose concentrations of 80 g/L and yeast extract supplementation of 3 g/L. The results are shown in Figure 1.

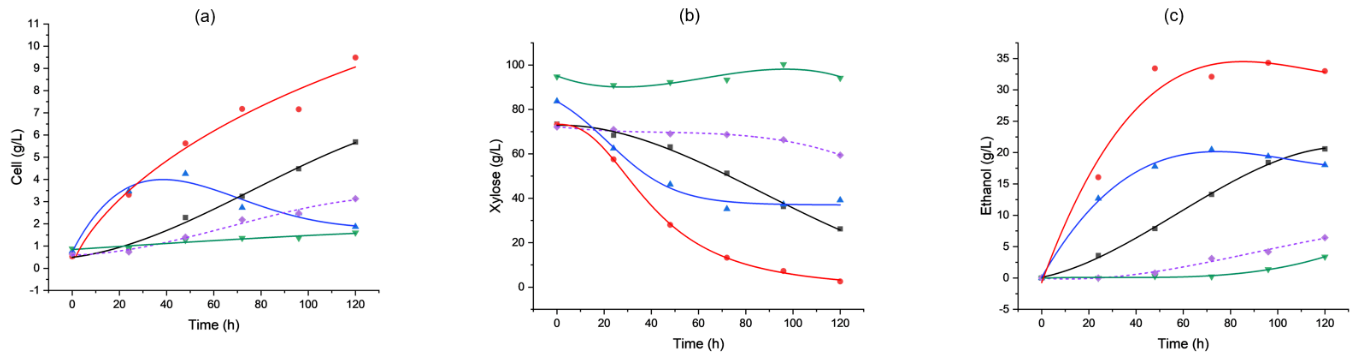

Figure 1- Cell growth (a), xylose consumption (b) and ethanol production (c) during cultivation by *Scheffersomyces stipitis* NRRL-Y-7124 on rice straw hemicellulose hydrolysate untreated with about 80g/L initial xylose (♦) and after treatments: peroxone (■), combined (●), activated carbon (▼), overliming (▲).

In general, during the cultivation time observed, the yeast was able to show cell growth in all the hydrolysates treated. However, the type of treatment used had a major influence on the yeast's cell growth profile. This influence is shown by detoxification capacity varied greatly between the different treatments evaluated. The lowest cell growth being observed in the yeast cultivated in hydrolysate treated with activated charcoal, reaching only 1.6 g/L after 120h of cultivation, as well as the lowest ethanol production. The lower formation of cell biomass may indicate that the removal of inhibitory compounds was not sufficient to reduce inhibition. In general, it was found that substrate consumption was influenced by the type of detoxification treatment evaluated. In yeast cultivations in hydrolyzate treated by overliming, peroxone process and combination of these, the yeast was able to consume practically all the glucose in 24 h of cultivation. In the same conditions of cultivation, in untreated hydrolysate, glucose was only completely consumed by yeast in 72 h (data not shown). During cultivation in hydrolyzate treated by overliming or the peroxone process, the yeast was able to consume approximately 60% of the initial xylose in 120 h. This data shows a significant increase in relation to that observed in the untreated hydrolyzate, which reached 15% in the same period. During cultivation in hydrolyzate treated by overliming or peroxone processes, the yeast reached a maximum accumulation of 18.7 g/L and 21.6 g/L of ethanol, respectively, in periods of 72 hours and 120 hours. Although the ethanol concentrations achieved are close, the overlapping treatment made it possible to obtain a volumetric ethanol productivity (Q_P = 0.26 g/L.h) higher than that obtained with the Peroxone treatment (Q_P = 0.18 g/L.h). It is important to highlight that, despite the higher productivity achieved with the Overcaling treatment, it resulted in a loss of 69% of the hydrolyzate volume, while the hydrolyzate treated with peroxone provided almost complete recovery of the treated volume.

In addition, for a better comparison of the detoxification methods, a simplified economic analysis based on estimates of the operating costs of inputs for a 1000 L scale of ethanol produced was also carried out. The costs of each process in relation to the quantity of product obtained can provide an important point of comparison, especially for the relevance that such costs can have on the applicability and competitiveness of such processes on an industrial scale. Among the treatments studied, the treatment using peroxone process had the lowest cost per volumetric unit of ethanol produced (USD 859.47/m^3) and was the only one with a lower cost than that estimated for the process without detoxification (USD 1,481.23/m^3). Thus, these results show the competitive potential of this process compared to techniques more frequently studied for this purpose, such as the use of activated carbon (USD 17,238.29/m^3) and overliming (USD 4,356.93/m^3).

CONCLUSION

In this study it was possible to verify that all the processes evaluated (peroxone process, overliming, adsorption on activated carbon and overliming-peroxone combined treatment) provided reductions in the concentration of potentially inhibitory compounds. Treatment of the hydrolysate by peroxone process, overliming and the combined process (overliming-peroxone) mainly reduced the concentration of lignin-derived compounds (reductions of over 95% for compounds such as ferulic acid, syringaldehyde, vanillyl alcohol and furoic acid).

Among these detoxification methods, the combined overliming-peroxone process proved to be the most effective in improving the bioconversion stage of sugars into ethanol by yeast. Even so, when comparing the processes in terms of operating costs, it was found that the peroxone process proved to be more economically advantageous than the other scenarios evaluated, with the lowest cost per volumetric unit of ethanol produced.

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