FERMENTATION OF GLUCOSE/XYLOSE MIXTURE BY *Scheffersomyces stipitis*: EVALUATION OF ETHANOL TOLERANCE

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ABSTRACT

The yeast *Scheffersomyces stipitis* has been identified as a promising microorganism for ethanol production from lignocellulosic biomass due to its ability to ferment glucose and xylose with relatively high yields. However, the ethanol production from this yeast still faces some challenges to become viable, especially regarding ethanol tolerance. The present study aims to evaluate the ethanol tolerance of *S.stipitis* in medium containing glucose:xylose mixture (75:30 g/L) as carbon sources. Fermentations were carried out in 125 mL Erlenmeyer flasks containing 50 mL medium at 30ºC, 150 rpm for 48h. Ethanol concentrations ranging from 10 to 50 g/L were added to the media. In the absence of ethanol, the yeast was able to consume all glucose and 20% xylose after 48h producing about 27 g/L of ethanol (Yp/s= 0,39 g/g Qp = 0,54 g/L/h). However, with increased initial ethanol concentration in the medium, the rate of sugars consumption, growth and ethanol production gradually decreased. According to results, the ability of fermentative yeast was strongly inhibited in the presence of 30 g/L ethanol indicating that *S.stipitis* has low ethanol tolerance as compared to the level tolerated by industrial yeast strain *S.cerevisiae*.

Keywords: *Scheffersomyces stipitis*. Fermentation, Glucose/xylose mixture, Ethanol tolerance.

1 INTRODUCTION

Lignocellulosic biomass, including corn straw, rice straw and sugarcane bagasse, is a sustainable feedstock for fuel ethanol production. Due to its composition rich in polysaccharides (cellulose and hemicellulose) and polyphenolics (lignin), they represent significant sources of fermentable sugars and aromatic compounds[1]. Consequently, the efficient use of these fractions play an essential role to ethanol production as well as attaining value-added chemicals within the context of biorrefinary[2]. Brazil's ethanol industry, primarily based on starchy and sugary feedstocks (first-generation ethanol), faces the challenge of transitioning to green ethanol production using lignocellulosic biomasses (second-generation ethanol). This transition involves development of main steps of biomass processing, including pre-treatment, saccharification, fermentation, and distillation[3].

The advantage of using lignocellulosic biomass for ethanol production at large scales is due the fact of this material to do not compete for food with people or animals, directly or indirectly, using arable land as that might occur in fuel ethanol production from sugar and grains. Thus, lignocellulosic biomass is a sustainable feedstock for producing 2G fuel ethanol[4]. However, one of the determining factors for reducing 2G ethanol costs is the total utilization of sugars available in lignocellulosic biomasses. The two main fermentable sugars present in these biomasses are glucose and xylose, originating from cellulosic and hemicellulosic fractions, respectively. Utilizing both sugar fractions in the fermentation stage is one of the major challenges for making ethanol production from lignocellulosic materials viable.

Glucose fermentation is a well-established process and traditionally conducted with the yeast *Saccharomyces cerevisiae*, due to its high fermentative efficiency, high production rates, and tolerance to stress conditions imposed by the process itself, including ethanol tolerance[6]. However, *S.cerevisiae* is unable to assimilate xylose, which is the main sugar present in the hemicellulose fraction of lignocellulosic biomasses. The yeast *Pichia stipitis*, currently classified as *Sheffersomyces stipitis*, has been identified as a promising microorganism for ethanol production from lignocellulosic biomass due to its ability to ferment xylose and glucose with relatively high yields[7]. However, the ethanol production by *S.stipitis* still faces some challenges to become viable, especially due to ethanol tolerance. Although the fermentative capacity of *S.stipitis* under different environmental/nutritional conditions has been widely studied in recent years, there are few reports in the literature regarding the ethanol tolerance level of this yeast. Therefore, this study aims to obtain relevant data regarding the yeast's tolerance to ethanol. For this study the effects of added initial ethanol on the fermentation of a glucose/xylose mixture by *S.stipitis* Y 7124 was evaluated. The sugar mixture with glucose as the major component was chosen as a model of a cellulosic hydrolysate obtained from rice straw.

2 MATERIAL & METHODS

Sheffersomyces stipitis Y-7124 was the yeast strain used in this study. The cells were cultured in 125 mL Erlenmeyer flasks containing 50 mL of medium with the following composition (g/L): 75 glucose, 30 xylose, 1.0 $(NH_4)_2SO_4$, 1.5; KH_2PO_4, 0.1 $MgSO_4.7H_2O$, and 3 yeast extract. Aiming to evaluate the ethanol tolerance of *S.stipitis* on fermentative process, ethanol concentrations varying from 10 to 50 g/L were added to the medium. The flasks were inoculated with 1 g/L of cells, and incubated on a rotary shaker at 30°C under 150 rpm for 48 hours. Samples were taken periodically to monitor cellular growth, sugars consumption, and ethanol production. Cellular growth was determined by measuring optical density of cells at 600 nm, which was correlated with dry weight in g/L by means of a standard calibration curve previously established. Sugars and ethanol concentrations were determined by high performance liquid chromatography (HPLC).

3 RESULTS & DISCUSSION

The fermentative profile of *S.stipitis* in medium containing a mixture of glucose and xylose supplemented with increasing ethanol concentrations is shown in Figure 1a-c. As can be seen, ethanol concentration in the medium affected sugars consumption, as well as growth and ethanol production by *S.stipitis*. In fermentation without ethanol addition (control), glucose was fully consumed after 48h and only 20% xylose was used. In these assays, ethanol production achieved about 27 g/L, but decreased to 22 g/L in the presence of 10 g/L ethanol. At initial ethanol concentration of 30 g/L, the fermentative process was strongly inhibited, and above this ethanol level no cellular activity was observed. These results are accordingly with the observation reported by others work that at higher glucose concentrations (>50 g/L), *S.stipitis* NRRL Y-7124 is unable to consume xylose efficiently. These found could be explained by an ethanol concentration inhibition effect which attained a maximum value of 27g/L when the medium containing glucose/xylose mixture ratio was 70/30.

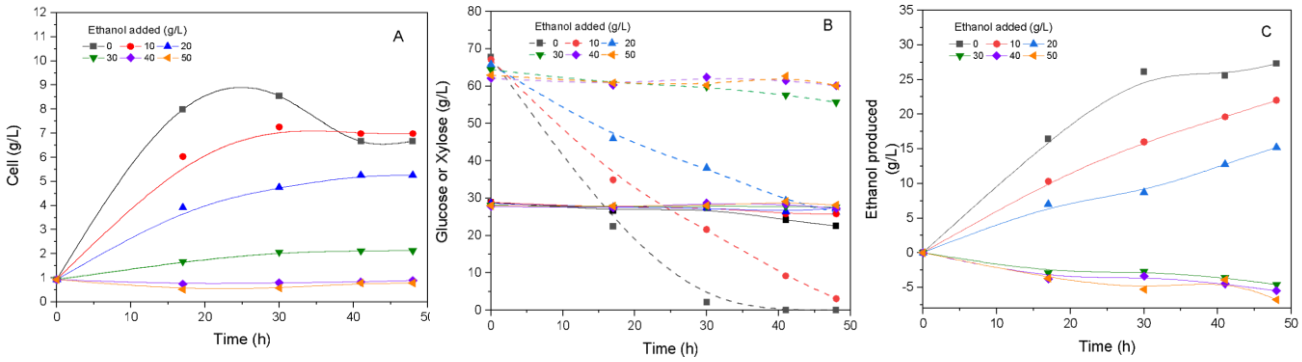

Figure 1. Effect of different initial ethanol concentrations on fermentative profile of *S.stipitis* in medium containing glucose/xylose mixture.

The effect of initial ethanol concentration on the fermentation parameters of *S.stipitis* is reported in Table 1. As can be seen, up to 30 g/L initial ethanol level, cell yield factor was similar to the control, whereas the ethanol yield factor, and volumetric productivity were reduced, indicating that fermentative metabolism was more sensitive to ethanol toxicity than the cell growth process.

Table 1. Fermentation parameters of *S.stipitis* from glucose/xylose mixture in the presence of different initial ethanol concentration (E_0).

E_0 (g/L)	Fermentation parameters*					
	Consumed Sugars (%)		Produced ethanol (g/L)	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	Q_P (g/L/h)
	Glucose	Xylose				
0 (control)	100	20.7	27.3	0.12	0.39	0.54
10	95.4	10.3	22.0	0.11	0.33	0.45
20	60.0	4.2	15.2	0.11	0.35	0.31
30	13.5	1.8	0	0.13	0	0
40	3.2	1.8	0	0	0	0
50	4.4	0	0	0	0	0

*Parameters calculated in 48h.

Aiming to establish the concentration of ethanol required to inhibit the cell's metabolic activity (growth and ethanol production) by 50% (IC50), the added ethanol in the medium was correlated with cell and ethanol produced as shown in Figure 2. The IC50 values for growth and ethanol production were of 22 and 25 g/L ethanol, respectively.

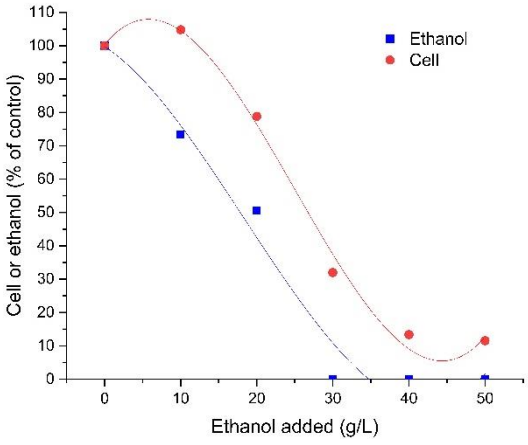

Figure 2. Effect of initial ethanol concentration on metabolic activity of *S.stipitis* in glucose/xylose mixture fermentation.

4 CONCLUSION

Based on the results obtained, it can be concluded that the metabolism of the yeast *S.stipitis* is affected by the presence of ethanol in the culture medium, and that ethanol tolerance limit of the yeast is relatively low (~30 g/L) as compared to the ethanol level tolerated by *S.cerevisiae* (~100 g/L) used in the alcoholic fermentation industry. However, to overcome this limitation of *S.stipitis*, the literature has highlighted alternatives such as the use of more ethanol-resistant strains, selected by adaptation to increasingly higher concentrations of ethanol.

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