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COMPARING PERFORMANCE OF *Saccharomyces cerevisiae* AND *Kluyveromyces marxianus* ON SSF PROCESS USING HYDROTHERMALLY PRETREATED SUGARCANE STRAW

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ABSTRACT

Saccharomyces cerevisiae and Kluyveromyces marxianus were used as fermenting microorganism during the simultaneous saccharification and fermentation (SSF) of hydrothermally pretreated sugarcane straw. A pre-saccharification (PS) step prior to SSF process was carried out for 12h at 50°C and pH 4.8, with solid load of 15.0 % ($m_{solid}/m_{solution}$) and 20 FPU/$g_{cellulose}$. After PS, the temperature was reduced and the yeast was inoculated (5 g/L). Then, the SSF process was performed for 48h. Different temperatures were evaluated during SSF assays: 34 and 40 °C for S. cerevisiae, and 40 and 45 °C for K. marxianus. Four fermentation parameters were assessed: ethanol concentration, volumetric ethanol productivity, relative and overall ethanol yields. The results demonstrated that both microorganisms exhibited their best results at 40 °C. However, S. cerevisiae showed better performance than K. marxianus, indicating that its use is promising for SSF process from lignocellulosic feedstocks, even not being a thermotolerant yeast.

1. INTRODUCTION

Among many possibilities and combinations of hydrolysis and fermentation strategies, the pre-saccharification (PS) followed by simultaneous saccharification and fermentation (SSF) process is a very promising alternative. Using this process configuration, enzyme inhibition by sugar and

equipment investments are greatly reduced. However, the main limitation during SSF process is the temperature. Enzymatic hydrolysis of cellulose is carried out at 50 ºC, while most of yeasts present an optimal fermentation temperature in the range between 30 and 34 ºC. One of the ways to mitigate this limitation is using thermotolerant yeasts (Silva et al., 2015). Thus, the objective of this work is to evaluate, at different temperatures, the performance of two yeasts: *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*.

2. MATERIALS AND METHODS

2.1. Materials

Enzyme and substrate: Commercial complex of cellulases Cellic®CTec2, donated by Novozymes Latin America (Araucária, PR, Brazil), was used in this work.
Hydrothermally pretreated sugarcane straw was employed as substrate for PS and SSF experiments. The biomass was pretreated in a Parr Reactor (1:10 ($m_{dry\ straw}/m_{H2O}$), 195ºC, 10 min and 200 rpm).

Microorganisms and growth conditions: Lyophilized *Saccharomyces cerevisiae* yeast (Y-904, AB Brasil, Pederneiras, SP, Brazil) and *Kluyveromyces marxianus* (a thermotolerant yeast, isolated at an industrial mill located in SP, Brazil) were used in SSF experiments. Cells of *K. marxianus* were cultivated in 500 ml Erlenmeyer flasks containing 50 mL of YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose) in stirred shaker incubator at 40ºC and 200 rpm. After 10h of cultivation, the medium was centrifugated (10,000 rpm, 10 min, 4 ºC) and cells (approx. 5 g/L dry cell weight) were resuspended in the hydrolyzed.

2.2. Methods

SSF process: The PS step was conducted prior to SSF for 12h at 50 ºC, pH 4.8, solid loading of 15.0 % ($m_{solid}/m_{solution}$) and enzyme dosage of 20 FPU/$g_{cellulose}$. After PS step, the hydrolysate was supplemented with (g/L) yeast extract (6.8), urea (5.32), $MgSO_4$ (1.4) and KH_2PO_4 (5.6), and the yeast was inoculated (5 g/L), which turned the process into SSF. The SSF process was performed during 48h. Different temperatures were evaluated during SSF assays: 34 and 40 ºC for *S. cerevisiae*, and 40 and 45 ºC for *K. marxianus*. The experiments were carried out in 200 mL bench scale reactors with 50 mL of total reaction medium at 250 rpm. All assays were performed in duplicate.

Analytical methods and calculations: Samples were withdrawn during SSF process from 0 to 48h and analyzed using HPLC for glucose, ethanol and acetic acid quantification.

Relative ethanol yield and overall ethanol yield were calculated by Equations 1 and 2, respectively.

$$Relative\ ethanol\ yield\ (Y_{E/G}) = \frac{[E]}{[G]} \quad (1)$$

where: [E] is the ethanol concentration produced during SSF and [G] is the glucose concentration produced at the same experimental conditions of PS and SSF with no microorganism inoculation (control experiment).

$$Overall\ ethanol\ yield\ (Y_{E/PG}) = \frac{[E]}{91.6} \quad (2)$$

where: 91.6 g/L is referred to the potential glucose (PG) from hydrothermally pretreated sugarcane straw. This value was calculated considering the cellulose content (55%) in the pretreated biomass and a solid loading of 15% ($m_{solid}/m_{solution}$).

Volumetric ethanol productivity (Q_P) was calculated by dividing the final ethanol concentration by 48h.

3. RESULTS AND DISCUSSION

The values obtained for ethanol ([E]) and acetic acid [Ac] concentrations, volumetric ethanol productivity (Q_P) and relative ($Y_{E/G}$) and overall ($Y_{E/PG}$) ethanol yields, are presented in Table 1.

Table 1. Parameters obtained from the SSF process of hydrothermally pretreated sugarcane straw by *S. cerevisiae* and *K. marxianus* at 48h of fermentation.

Yeast	[E]* (g/L)	[Ac]* (g/L)	Q_P (g/L.h)	$Y_{E/G}$ (g/g)	$Y_{E/PG}$ (g/g)
S. cerevisiae - 34 °C	22.02±1.72	4.49±0.04	0.46	0.40	0.24
S. cerevisiae - 40 °C	26.56±0.86	5.33±0.33	0.55	0.43	0.29
K. marxianus - 40 °C	23.86±0.06	3.37±0.03	0.50	0.38	0.26
K. marxianus - 45 °C	22.62±0.07	2.15±0.05	0.47	0.35	0.25

* Values listed above are the average values ± standard deviations (measured in duplicates).

The results showed that for *S. cerevisiae* the temperature increase improves all parameters. On the other hand, *K. marxianus* exhibited contrary behavior. The highest ethanol concentrations were found at 40 °C for both strains.

In all evaluated conditions, overall ethanol yields were low (<57% of theoretical overall ethanol yield). This behavior was attributed to the fact that the enzymatic hydrolysis took place at

temperatures below its optimum temperature (50 ºC), reducing the hydrolysis reaction rate (so, not all potential glucose was actually available to fermentation). But contrary to expectations, SSF process conducted at 40 and 45 ºC with *K. marxianus* had an overall ethanol yield virtually equal to the value obtained at 34 ºC with *S. cerevisiae*. Regarding the relative ethanol yield, it was expected that this parameter would also be higher at 45 ºC. However, the relative ethanol yield obtained with *K. marxianus* at 45 ºC (0.35 g/g) was the lowest value. Probably, these facts are more related to *K. marxianus* metabolism than enzymatic hydrolysis efficiency.

Better results could be achieved if there was not the possibility of ethanol lost by evaporation during sample withdraws at 40 and 45 °C. Besides that, high temperatures are a potential cause of microorganism stress which has great influence on cellular processes such as: inhibition of cell division, imbalance of protein homeostasis and difficulty on coupling of oxidative phosphorylation (Kelbert et al., 2016).

In all experiments, the values of acetic acid concentration were not sufficiently high (<100 mM or 6 g/L) to reach toxic levels to the yeast metabolism (Jönsson et al., 2013). Higher ethanol concentrations were found in higher acetic acid concentrations. Similar behavior was reported by Jönsson et al. (2013) and Silva et al. (2015).

4. CONCLUSION

The results demonstrated that both microorganisms exhibited their best results at 40ºC. However, *S. cerevisiae* showed better performance than *K. marxianus*, indicating that its use is promising for SSF process from lignocellulosic feedstocks, even not being a thermotolerant yeast.

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