PIBIC CNPq

Fermentation processes evaluation for the production of acetic and lactic acids using pretreated elephant grass liquor

LEB Laboratório de Enzimas e Biomassas

PRONEM2

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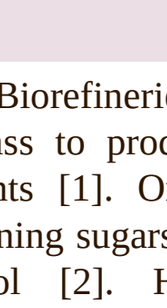
INTRODUCTION / OBJETIVE

Biorefineries correspond to industrial sectors that employ waste biomass to produce bioproducts such as biofuels, plastics, food and solvents [1]. One strategy for biorefineries is to fully convert the remaining sugars from the pretreated lignocellulosic biomass to generate ethanol [2]. However, during second generation (2G) ethanol fermentation, pentoses in the liquor are not metabolized by most microorganisms. In this study, the liquid fraction from the physicochemical pretreatment of elephant grass (EG) was used in the production of acetic acid (AA) and lactic acid (LA) using wild type bacteria and analyzing different concentrations of nutrients. Therefore, the main objective of this work is to evaluate ways to reduce the production cost of these organic acids.

MATERIALS & METHODS



Detoxified and filtered liquor (2.5% *w/v* activated carbon, 50 °C, 200 rpm, 1 h)

Acetic and lactic fermentation through *Acetobacter cerevisiae* AV and *Lactobacillus brevis* OR (10% *v/v*) at 28 °C, 120 rpm, pH 6.0, for 48 h, in 50 mL vials (40 mL reaction volume).

Experimental design for fermentation processes

The factors analyzed were: proteose peptone; yeast extract; ammonium citrate; and dipotassium phosphate.

Central Composite Rotational Design (CCRD):

- 2^4,
- 3 replicates at central point,
- 27 experiments.

Results after 48 h of fermentation were evaluated using Statistica 7.0 software (StatSoft, Tulsa, Oklahoma, USA)

Table 1 Values used in the central composite rotational design to determine the yield of organic acids

Variables	Levels				
	-2	-1	0	+1	+2
Proteose peptone (g L^{-1})	5.0	7.5	10.0	12.5	15.0
Yeast extract (g L^{-1})	2.5	3.75	5.0	6.25	7.5
Ammonium citrate (g L^{-1})	1.0	1.5	2.0	2.5	3.0
Dipotassium phosphate (g L^{-1})	1.0	1.5	2.0	2.5	3.0

Quantification of analytes

High Performance Liquid Chromatography (HPLC) – (Shimadzu Corporation, Japan). Aminex® HPX-87H column; 60 °C; 5.0 mmol L^{-1} H_2SO_4; 0.6 mL min^{-1}.

Glucose | Xylose | AA | LA

RESULTS & DISCUSSION

Fermentations were started with a cell concentration of 2.4×10^{13} CFU mL^{-1} for *A. cerevisiae* AV and 4.9×10^{11} CFU mL^{-1} for *L. brevis* OR. After 24 h, using experiment 14 of *A. cerevisiae* AV, there was a production of up to 8.18 g L^{-1} of LA and 6.15 g L^{-1} of AA from 9.16 g L^{-1} of initial sugars (Fig. 1a). In relation to experiment 22 of *L. brevis* OR, there was a production of up to 6.89 g L^{-1} of LA and 5.39 g L^{-1} of AA in 12 h of fermentation, from 9.23 g L^{-1} of sugars (Fig. 1b).

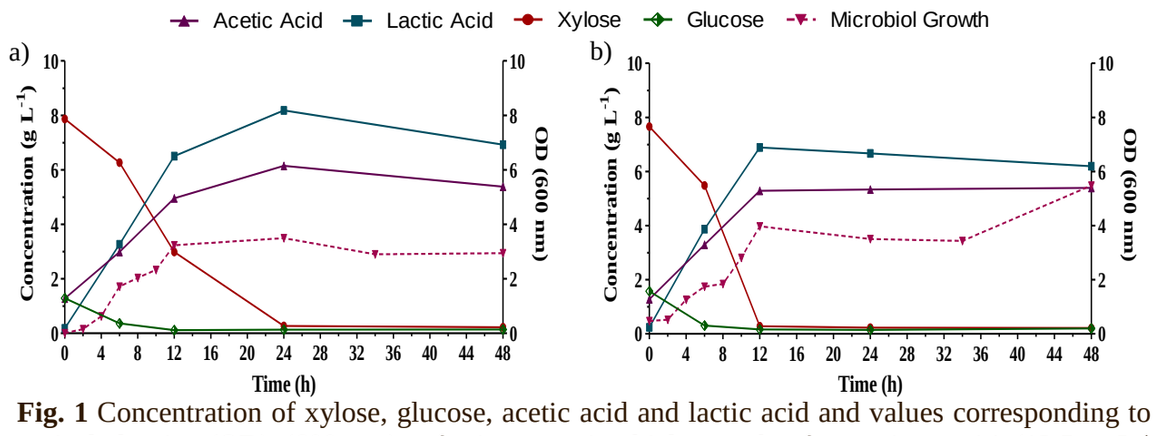


Fig. 1 Concentration of xylose, glucose, acetic acid and lactic acid and values corresponding to optical density (OD) (600 nm) referring to microbial growth of experiment 14 (12.5 g L^{-1} proteose peptone; 3.75 g L^{-1} yeast extract; 2.5 g L^{-1} ammonium citrate; 2.5 g L^{-1} dipotassium phosphate) of *A. cerevisiae* AV (a); and experiment 22 (10 g L^{-1} proteose peptone; 5 g L^{-1} yeast extract; 3 g L^{-1} ammonium citrate; 2 g L^{-1} dipotassium phosphate) of *L. brevis* OR (b) using pretreated elephant grass liquor.

The Pareto diagrams for *A. cerevisiae* AV and *L. brevis* OR assays (Fig. 2) show the *t*-test values for each of the independent variables, as well as their interactions. In all situations, only ammonium citrate (linear) had statistical significance ($p<0.05$) and positive effect on acetic and lactic fermentations. As indicated by Camu *et al*. (2007), this is because some bacteria can metabolize citrate and produce AA and LA [3]. The data indicates that lower contents of the other studied nutrients can be used in the current proposal, reducing costs in the industrial production of these chemical inputs.

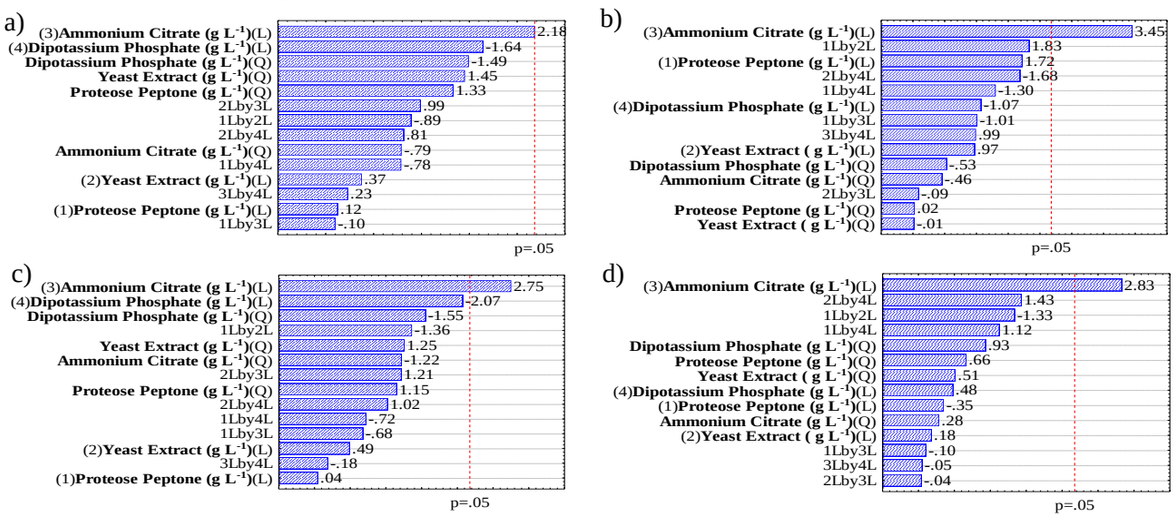


Fig. 2 Pareto diagrams obtained from the central composite rotational design involving fermentation tests with *A. cerevisiae* AV and the production of acetic (a) and lactic (c) acids as response variables; as well as with *L. brevis* OR and the production of acetic (b) and lactic (d) acids.

CONCLUSIONS

Promising results can be obtained relating the production of AA and LA in pretreated elephant grass liquor, even employing wild type bacteria. The production of these organic acids in synthetic medium with ammonium citrate, as the only carbon source, will be further evaluated to better understand how this nutrient is metabolized by the studied microorganisms.

REFERENCES

[1] Ohara, 2003. **Appl. Microbiol. Biotechnol**. 62, 474-477.
[2] Montipó *et al*., 2018. **Cellulose**. 26, 7309-7322.
[3] Camu *et al*., 2007. **Appl. Environ. Microbiol**. 73, 1809-1824.