XXIII SINAFERM
XIV SHEB
XIV ENZITEC
Búzios, Rio de Janeiro, Brasil
28 a 31 de agosto de 2022
XXIII Simpósio Nacional de Bioprocessos
XIV Simpósio de Hidrólise Enzimática de Biomassas
XIV Seminário Brasileiro de Tecnologia Enzimática

EFFECT OF LACTIC ACID BACTERIA CONTAMINATION ON ETHANOL PRODUCTION USING A MIXED MASH

Mariana Mourani. Buzelli[1], Thamiris Guerra. Giacon [1], Carlos Gonzalo Alvarez. Rosario[1] and Thiago Olitta. Basso [1]

[1] *Department of Chemical Engineering, Escola Politécnica, University of São Paulo*

E-mail: mariana.mourani@usp.br

1. INTRODUCTION

As a result of the large volumes of substrates processed daily in ethanol production plants, non-aseptic conditions are very common. Working conditions such as low pH- and high titers of ethanol-tolerance favor the presence of contaminating bacteria, in particular lactic acid (LAB), belonging to the *Lactobacillus* genus (Basso et al., 2014). Contamination affects directly important fermentation parameters, like reduce yeast viability (Basso et al., 2014), and consequently product yields and productivities (CARVALHO-NETTO et al., 2015). Thus, in this work was studied the influence of lactic acid bacteria on ethanol production.

2. MATERIALS, METHODS AND DISCUSSIONS

The yeast inoculum from the fermentation had its optical density (OD_{600}) measured and the 26mL was centrifuged and the cell biomass was resuspended in the mixed mash composed of corn hydrolyzate and sugarcane syrup. For LAB (*L.plantarum and L.fermentum*), when co-culture was realized, the initial OD_{600} was correlated with the colony forming units (CFU/mL), therefore, for each strain an adequate OD_{600} was used to reach 4.5E+07 CFU/mL and the celular pellet was added to the mash at zero hour together with the yeast cells. Co-cultivation took place for 72 hours at 32°C and 100 rpm in shake-flask. The experiments were performed in triplicate. The collected samples every 24h were subjected to HPLC analysis equipped with a HPX-87H BioRad column (COLA et al., 2020) and HPX-87C BioRad column, to estimate the concentration of metabolities and sugars, respectively throughout the co-cultivation. From the metabolites produced during the fermentations, it was possible to verify a reduction of 3.87% in the ethanol concentration in 24h (maximum concentration obtained) and of 4.68% in 72h between the control and LAB contaminated assays, as shown by the Table and Figure 1.

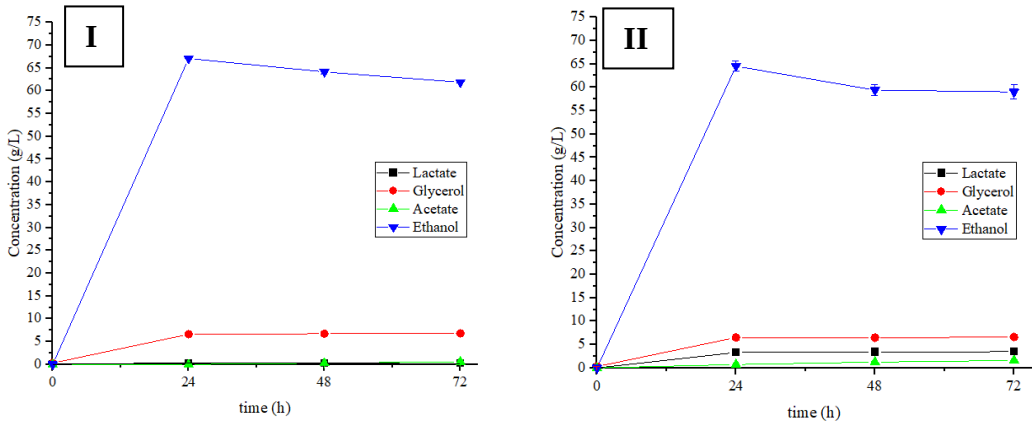

Figure 1. *Production of metabolites in control medium (I) and contaminated medium (II)*

Table 1. Production of metabolites during the tests in the absence and presence of LAB

	Metabolites (g/L)				
time (h)	Ethanol	Lactate	Acetate	pH	LAB
0	0 ± 0	0 ± 0	0 ± 0	5.21 ± 2E-02	-
	0 ± 0	0 ± 0	0 ± 0	5.19 ± 3E-02	+
24	67.075 ± 7.5E-01	0.300 ± 1.0E-02	0 ± 0	3.270 ± 1.5E-02	-
	64.5 ± 1.1	3.337 ± 9.7E-02	0.740 ± 6.4E-02	3.30 ± 6E-02	+
48	64.165 ± 4.9E-01	0.255 ± 5E-03	0.295 ± 5E-03	3.540 ± 5E-03	-
	59.4 ± 1.2	3.410 ± 2.1E-01	1.257 ± 9.7E-02	3.42 ± 5E-02	+
72	61.85 ± 1.8E-01	0.215 ± 5E-03	0.590 ± 1.0E-02	3.600 ± 2E-02	-
	59.0 ± 1.5	3.503 ± 1.70E-01	1.610 ± 9.2E-02	3.500 ± 6.1E-02	+

$^{-}$- No Lactobacillus contamination. The initial density of S. cerevisiae was approximately 2.4E+07 cel/mL.
$^{+}$-Initial density of Lactobacillus strains in co-culture was approximately 5E+07 CFU/mL, inoculated at t=0h together with the yeasts. Data are presented as the mean ± the standard deviation of experiments performed in triplicate.

Possibly this decrease occurs because, when present in the fermentation medium, lactic acid bacteria compete with the yeast for available nutrients and carbon sources, especially sugars (glucose, maltose, fructose and sucrose). This competition causes the diversion of these substrates for the production of the final LAB metabolites: lactic and acetic organic acids and ethanol, which inhibit the growth and metabolism of the yeast, thus causing a drop in the ethanol yield, once that the acids have the ability to diffuse across the yeast cell membrane, where they will then dissociate and release H^+ ions, acidifying the cell cytoplasm (BECKNER; IVEY; PHISTER, 2011). In fact, it was possible to notice a more pronounced production of these acids throughout the co-culture, different from what occurs in the control culture, in which very low concentrations of these metabolites are reported (Table and Figure 1).

3. REFERENCES

BASSO, T. O.; GOMES, F. S.; LOPES, M. L.; AMORIM, H. V.; EGGLESTON. G.; BASSO, L.C. Homo- and heterofermentative lactobacilli differently affect sugarcane-based fuel ethanol fermentation. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, v. 105, n. 1, p. 169–177, 2014.

BECKNER, M.; IVEY, M. L.; PHISTER, T. G. Microbial contamination of fuel ethanol fermentations. **Letters in Applied Microbiology**, v. 53, n. 4, p. 387–394, 2011.

CARVALHO-NETTO, O.V. et al. Saccharomyces cerevisiae transcriptional reprograming due to bacterial contamination during industrial scale bioethanol production. **Microbial Cell Factories**, v. 14, n. 1, p. 1-13, 2015.

COLA, P.; PROCÓPIO, P.D.; ALVES, A.T.C.; CARNAVALLI, L.R.; SAMPAIO, I.V.; COSTA, B.L.V.; BASSO, T.O. Differential effects of major inhibitory compounds from sugarcane-based lignocellulosic hydrolysates on the physiology of yeast strains and lactic acid bacteria. *Biotechnology Letters*, v. 42, n. 4, p. 571-582, 2020.

4. ACKNOWLEDGMENTS

The authors are grateful to SINOCHEM Petróleo Brasil LTDA and Embrapii for funding.