PRELIMINARY STUDY ON IMMOBILIZATION OF *P. stipitis* ACL 2-1RD ON UNTREATED AND ON CHEMICALLY PREHYDROLYZED SUGARCANE BAGASSE FOR BIOETHANOL PRODUCTION

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Introduction. Bioethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by worldwide utilization of fossil fuels[1]. Therefore, bioethanol production from energy renewable sources such as sugarcane bagasse has taken relevance in recent years because of its lignocellulosic nature and availability as sugar mill residue from sugar production. These features make it feasible for its use as support for yeast immobilization which presents several advantages over free cells conventional systems, for example: cellular function stability, higher cell concentration in reduced volumes leading to an increase in productivity and decrease in time and fermentation costs.[2]

The aim of this work is to establish *P. stipitis* ACL 2-1 RD immobilization conditions on sugarcane bagasse for ethanol production.

Methods. *P. stipitis* ACL 2-1 RD, provided by UNIDA-ITV, was kept and activated in synthetic medium with 70 gL^{-1} glucose[3]. Immobilization conditions were established evaluating the supports: untreated sugarcane bagasse (USB) and prehydrolyzed sugarcane bagasse (PSB) (2 % w/v H_2SO_4, 121°C, 40 min). Yeast growth was determined by dry weight correlated to optical density (620nm), viability and cellular count on Thoma camera by methylene blue method. Substrates and products were determined by HLPC. Box Benkhen experimental design with 3 factors: bagasse:culture medium ratio (1:16, 1:20 and 1:24); contact time (16, 20 and 24 h) and inoculum size ($3x10^5$, $3x10^7$, $3x10^9$ $celmL^{-1}$) were performed using Minitab software. Response variables were viability, efficacy and cell yeast retention.

Results. Individual effects and interactions of conditions on the immobilization process were evaluated for each type of lignocellulosic support: USB and PSB. USB (1:23.35 solid:liquid ratio; 24 h contact time and $1.22x10^7$ cel mL^{-1} inoculum size) exhibited 89% efficacy, 95% viability and 6 mg cell/g of support retention. In the case of PSB, (1:20.68 solid:liquid ratio; 16 h contact time and $3x10^6$ cel mL^{-1} inoculum size), 95% efficacy, 92% viability and 20 mg cell/g of support were obtained.

The stability of *P. stipitis* ACL 2-1 RD immobilized in PSB was evaluated at the best immobilization conditions. Figure 1 shows the results obtained during 32 fermentation cycles. Ethanol concentration was maintained at 4.4gL^{-1} with 10.3 gL^{-1} residual glucose; consequently, the concentration of free cells was maintained with the stable process.

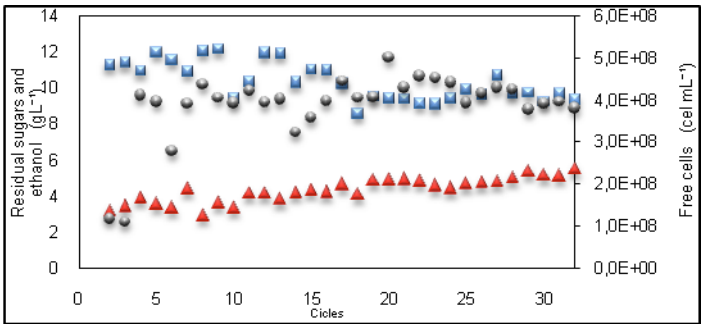

Fig.1 Stability of *P. stipitis 2-1 ACL RD* immobilized on sugarcane bagasse prehydrolysate during 32 cycles. (▲) ethanol concentration (■) residual sugar concentration (●) free cell concentration.

Conclusions. This study demonstrated that use of prehydrolyzed sugarcane bagasse is a good support for *P. stipitis* ACL 2-1 RD immobilization (95% efficacy and 92% viability). PSB showed more cellular adsorption due to acid pretreatment which modified fiber structure resulting in the formation of a porous surface.

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