GENERATING A BUTANOL TOLERANT AND BIOBUTANOL SUPERPRODUCER MUTANT STRAIN OF *Clostridium acetobutylicum*

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Key words: biofuels, ABE fermentation, cheese whey

Introduction. Butanol is an important industrial chemical, considered as a superior liquid fuel with potential to replace gasoline[(1)]. Butanol has many advantages over ethanol: vapor pressure 11 times lower, higher energy content, non-hygroscopic, can be applied pure or blended with gasoline or diesel, can be used in any automobile engine without modifications, and, it´s safer to use.[(2)] Butanol is produced by acetone-butanol-ethanol (ABE) fermentation using various species of solventogenic clostridia. The performance of ABE fermentation process is severely limited by: high cost of substrate, substrate inhibition and low solvent tolerance (max $20g_{solvent}/L$). These limitations result in low butanol concentration productivity and yield, and high downstream processing cost. [(1)]
To reduce the factors that limit the production of biobutanol, this work aims to generate a mutant strain of *Clostridium acetobutylicum* ATCC 824, resistant to the presence of at least $20g_{solvent}/L$.

Methods. Microorganism: *C. acetobutylicum* (CDBB-B-797 National Collection of Microbial and Cell Cultures CINVESTAV-IPN). Culture medium for *C. acetobutylicum* (MPC)[(4)] Fermentation medium: deproteinized whey pH 7.0, added with $FeCl_3$ (10mgFe/L). Undirected mutagenesis: *C. acetobutylicum*, grown in MPC added with butanol 2% v/v (16.20 g/L) was exposed to UV light radiation for periods of 0, 5, 10, 30, 45 and 60 minutes. After each exposure time was reseeded into MPC added with butanol 2.5 % v/v (20.25 g/L) and 3.0% v/v (24.30 g/L) respectively. The plates were incubated in anaerobic conditions at 35-37°C and observed daily for recording the growth of the mutated strains. Batch fermentation: carried out under anaerobic conditions, in volumes of 400 ml, incubated at 35-37°C in orbital shaking at 180 rpm for 28 days. Analytical methods: Solvents were analyzed using a gas chromatograph SRI 310 equipped with a thermal conductivity detector and a Chromosorb® W-AW 80/100 mesh packed with Carbowax®1540 at 10%. Data analyses: STATGRAPHICS centurion®.

Results. Mutants generation: Complete inhibition of growth for *C. acetobutylicum* in butanol concentrations above 16.20g/L. Butanol resistant strains generated after 60 minutes of exposure to UV radiation were selected, for good growth and stability, to evaluate its biobutanol super producer capacity. Mutant strains were designated as M1 and M2, tolerant to 20.25g/L and 24.30g/L of butanol respectively.
Assessment of biobutanol super producer capacity: After 28 days of fermentation production of biobutanol was determined for wild type strain and selected mutants (μ±SD): ATCC824(0.0007±0.0006g/L), M1 (0.9630± 0.2129g/L), M2 (0.6503±0.0438g/L). ANOVA indicates statistically significant difference between the mean biobutanol produced (g/L) from one strain to another at the 95.0% confidence level (p=0.0002). Multiple Range Tests indicate that all strains compared show statistically significant differences at the 95.0% confidence level (Fig.1)

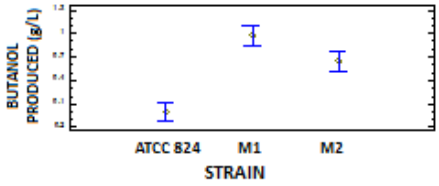

Fig 1. Mean graph. Means and 95% LSD intervals.

Conclusions. Two mutants of *C. acetobutylicum* respectively resistant to concentrations of 20.25 and 24.30 g/L of butanol were generated. Both mutants show a better fermentation performance than the wild strain, butanol concentration achieved is low and fermentation conditions should be improved.

Acknowledgements. To CONACYT for financial support:Scholarship 376812/246886.

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