METABOLIC ENGINEERING FOR PRODUCTION OF 2,3-BUTANEDIOL IN *Klebsiella oxytoca* B199 UNDER NITRATE RESPIRATION

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Introduction. 2,3-Butanediol (BDO) is a chemical compound that can be employed in the production of synthetic rubber, plastics and solvents. *Klebsiella oxytoca* is the most studied of the producing microorganisms for BDO production. The balance of coenzymes ($NADH_2$/NAD) produced in glycolysis and utilized in fermentation, is a determinant factor in BDO production, where one of the two produced $NADH_2$ is required for reduction of BDO to acetoin; while the other one is recycled in the respiratory chain. Dissolved oxygen control is difficult to attain, specially in industrial fermenters. One alternative is to use another electron acceptor such as nitrate. The purpose of this work is to explore this possibility.

Methods. *Bacterial strain.* The *Klebsiella oxytoca* B199 strain employed was obtained from the collection of the Department of Agriculture (ARS) USA and used for obtaining the improved strain *K. oxytoca* GR7.

Method of obtaining improved strain. The method for producing improved strains was proton suicide (2) modified, which consists of supplementing the growth medium with NaBr and $NaBrO_3$. The culture medium used was a minimal medium known as PA, which contains glucose (8.64 g/L), K_2HPO_4 (5 g/L), KH_2PO_4 (1 g/L), $(NH_4)_2SO_4$ (0.25 g/L), $MgSO_4$-$7H_2O$ (0.25 g/L), $FeSO_4$ (0.05 g/L), $ZnSO_4$-$7H_2O$ (0.001 g/L), $MnSO_4$-H_2O (0.001 g/L), EDTA (0.05 g/L), K_2MoO_4 (0.005 g/L) and $NaNO_3$ (1.02 g/L) as electron acceptor, bromothymol blue was employed as a pH indicator. In order to obtain an improved non acids production strain, five successive passages where done with a molar concentrations of NaBr and $NaBrO_3$ of 100 mM and 25 mM, respectively, at 37 ° C and 150 RPM for 48 h with a 5% inoculum. After five passes the concentrations were increased to 150 mM and 37.5 mM, respectively and finally to concentrations of 175 mM and 43.75 mM. After every different treatment, determinations of optical density (600 nm), glucose (DNS technique) and pH were done.

Results. Figure 1 shows the kinetics of growth of *K. oxytoca* B199 with a molar ratio of glucose/nitrate of 4, which corresponds to the theoretical requirement of nitrate to maintain redox balance in the production of BDO. The specific growth rate (μ_{max}) was 0.47 h^{-1}. The production of acids is the principal products accumulated, where acetic acid showed the highest accumulation (21.17 mM). BDO production was scare, 0.66 mM, nitrate was reduced without accumulation of nitrite.

To obtain the improved strain of *Klebsiella oxytoca* B199 with low acid production, the molar concentrations 100/25 and150/37.5 of NaBr/NaBrO3 were useful, because the culture was still growing, but slower than the wild type. When the molar concentration 175/43.75 was employed the culture did not show any growth.

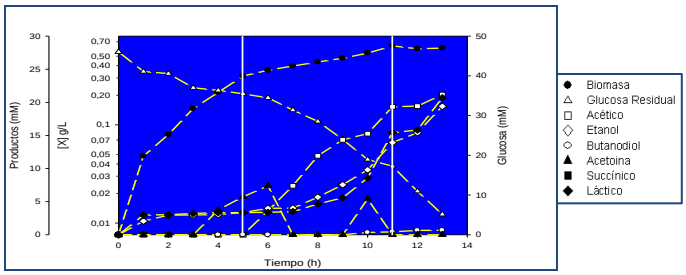

Fig.1 Growth kinetics of *K. oxytoca* B199 in PA medium with molar ratio glucose/nitrate 4.

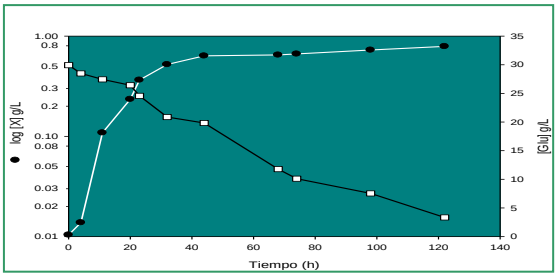

Fig. 2 Growth kinetics of *K. oxytoca* GR07

Conclusions. *Klebsiella oxytoca* B199 presented a preference towards the production of acetic acid because it is an energetically favorable route due to ATP is produced. The improved strain GR07 of *K. oxytoca* obtained from molar concentration 150/37.5 showed a smaller μ_{max} (0.13 h^{-1}) compared to the parental strain, which was due to the decrease production of acetic acid, reducing the energy obtained in this way. The proton suicide method was efficient in obtaining an improved strain by adaptive evolution. Metabolic changes will be discussed in the improved strain.

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