INFLUENCE OF OXYGEN SUPPLY ON THE METABOLIC FLUXES OF *Azotobacter vinelandii* ATCC9046: STUDIES IN SHAKEN FLASK CULTIVATIONS

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Introduction. *Azotobacter vinelandii* is a fixing-N_2 bacterium which produces two polymers of industrial interest: alginate (extracellular polysaccharide) and poly-3(hydroxybutyrate) (PHB), which is an intracellular reserve material [1]. Metabolic flux analysis (MFA) allows understanding the intracellular carbon fluxes and its regulation [2]. The aim of this study was to evaluate the carbon flux in *A .vinelandii* under two aeration conditions.

Methods. Two aeration conditions were evaluated in shaken flasks cultivations, by changing the gas-liquid mass transfer area and the type flask. *A. vinelandii* ATCC9046 was grown on Burk's medium without N_2 source. Growth, glucose consumption, alginate and PHB production were evaluated. The MFA was done based on the mathematical simulation model described by Yang et al [3]. In addition, enzymatic activities of pyruvate dehydrogenase (PDH), isocitrate dehydrogenase (ICDH) and isocitrate lyase (ICL) were evaluated.

Results. Changes in aeration conditions mainly affected the carbon fluxes through the tricarboxylic acids (TCA) cycle, glyoxylate shunt, the pentoses phosphate pathway (PPP), as well as those fluxes involved in acetyl CoA metabolism. Under the high aeration condition the carbon flux toward TCA cycle was up to 2.6 fold times higher in nodes such as ICDH. On the other hand, under low aeration condition, there was an increase on the fluxes through glyoxylate shunt, the pathways involved in acetyl CoA metabolism and PPP (Table 1).

Table 1. Relative carbon fluxes (mole-%) related to glucose uptake rates (100%) in parallel cultivations of *A. vinelandii* ATCC 9046 grown on [1-^{13}C] - glucose under low and high aeration conditions.

Pathway	Node	Low aeration	High aeration
TCA cycle	ICDH	61.3	157.2
Glyoxylate shunt	ICL	41.9	15.0
Acetyl-CoA metabolism	PDH	213.1	193.1

These changes in metabolic fluxes had an important impact on growth, glucose consumption as well as, in biosynthesis of alginate and PHB. However, the specific activities of the PDH, ICDH and ICL did not show significant differences between the aeration conditions evaluated (Fig. 1). This suggests that fluxes through those enzymes could be modulated by cofactors such as NADPH and NADP.

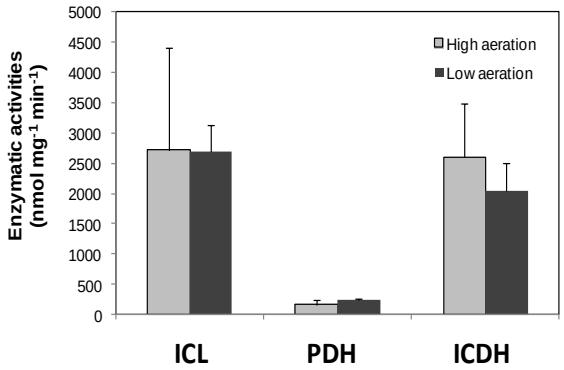

Fig.1 Specific enzymatic activities of the enzymes ICL, PDH and ICDH from cultivations of *A. vinelandii* grown under high and low aeration conditions.

Conclusions. Changes in aeration conditions affected metabolic fluxes in *A. vinelandii.* Differences in metabolic fluxes had an important effect on growth, and production of PHB and alginate. However, the specific enzymatic activity at nodes such ICL, PDH and ICDH was not affected.

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