SIMULTANEOUS GENETIC ENGINEERING AND CULTURE MEDIA MANIPULATION IMPROVE RH-EPO PRODUCTIVITY THROUGH LACTATE RE-METABOLIZATION ON CHO CELL CULTURE

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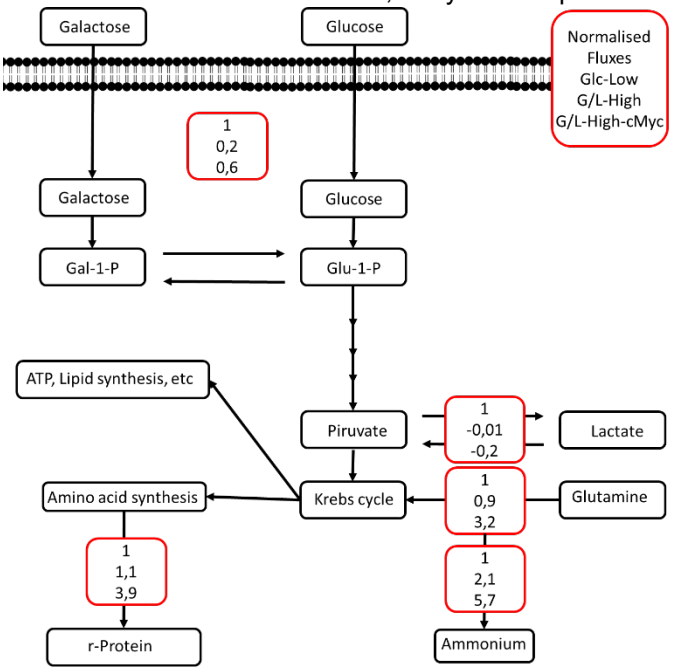
CHO cells are the gold standard for biopharmaceutical production. Different approaches are used to improve the productive and metabolic performance of cells, including genetic engineering and manipulation of operational conditions. The overexpression of c-Myc, as a regulator of cellular metabolism, acting on targets such as hexokinase, lactate dehydrogenase (LDH), and transporters such as GLUT-1, could induce an increase in the flux of carbon towards the production of r-proteins. The replacement of glucose (Glc) by a mixture of galactose and lactate (G/L) as a carbon source in the culture medium has been shown to trigger the lactate metabolic switch, nevertheless the cellular response to these simultaneous manipulations remains unknown.

This work was carried out in batch culture of CHO cells, where an erythropoietin-producing clone was compared with one that also overexpresses the global regulator c-Myc. Glucose was used at a low and a high concentration as a control, which was compared with a galactose-based diet, also at two concentrations. Their kinetic parameters, specific productivity of erythropoietin and expression of LDH-A will be evaluated.
In most of the conditions studied, c-Myc overexpression caused a decrease in the specific rate of cell growth. This overexpression in conjunction with a diet based on a mixture of galactose and lactate at a high concentration generated a 3,9-fold increase in the specific productivity of erythropoietin. Furthermore, in this condition, a decrease in the expression of the enzyme LDH-A was observed, which triggered a metabolic shift towards lactate consumption, being -0,2 times that observed in the control. In the case of the hexose consumption, it decreased to 0,6 times the control, in addition an increase in the consumption of glutamine was seen, which translated into an increase in the production of ammonium.



Finally, it is concluded that the use of galactose and the overexpression of c-Myc in CHO cells improves the specific productivity of r-proteins, decreases the production of toxic metabolites, promotes lactate consumption and improves culture development.

Figure 1 – Metabolic fluxes of extracellular metabolites, normalised by control conditions (CHO-EPO Glc-Low).