**Effect of extracellular pH on lactate metabolism in Chinese Hamster Ovary (CHO) cell cultures**

Victoria Gkoutzioupa1, Edward Close2, Alexandros Kiparissides1,\*

*1 Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, London, UK; 2 Process Systems Enterprise Limited, London, UK*

*\*Corresponding author: alex.kiparissides@ucl.ac.uk*

**Highlights**

* Understanding of the effect of extracellular pH on lactate metabolism, cell viability and product titer
* Improved prediction of lactate concentration including the shift from lactate production to consumption

**1. Introduction**

In spite of the outstanding research developments in biotechnology, there are still several aspects of cellular metabolism whose impact on bioprocess efficiency and product quality we do not fully comprehend. A notable and industrially relevant example is the interplay between glucose, lactate and amino-acid metabolism and in particular the metabolic switch that occurs in high-density cultures of fast growing mammalian cells from lactate production to lactate consumption (Zhou et al., 1997; deZengotita et al., 2000). Recent studies have shown that supplementing CHO cell cultures with lactic acid or artificially altering extracellular pH can promote the effect resulting in the preferential uptake of lactate even in the presence of excessive amounts of glucose (Li et al., 2012). Surprisingly this transition, from lactate production to consumption, results in higher product yields. The aim of the current study is to develop an in depth, quantitative understanding of lactate metabolism in CHO cells and how it responds to variations in extracellular pH. The experimental data will be subsequently used for the development of a predictive unstructured model.

**2. Methods**

GS-CHO cells (kindly provided by Lonza Biologics, Slough, UK) were cultured in 500 mL shake flasks (Presens, Germany) with 110 mL working volume. The flasks had integrated pH and oxygen sensors. The cells were incubated at 5% CO2, 37oC and 180 rpm (ds=19 mm). Viable cell density, total cell density and average cell diameter were measured with the Vi-CELL XR (Beckman Coulter, USA). Metabolites (glucose, glutamate, glutamine, lactate, ammonia) were quantified with the BioProfile FLEX Analyzer (Nova Biomedical, USA). Monoclonal antibody concentration was determined with the use of High Pressure Liquid Chromatography (HPLC) techniques (Agilent Technologies, USA). pH was maintained in predefined levels with the manual addition of 1M HCl and 1M NaHCO3. pH and oxygen were monitored by using a Presens platform (Presens, Germany).

The gPROMS FormulatedProducts© modelling platform was employed for model development and analysis. The parameters of the model were estimated through minimization of the maximum likelihood objective function, where the experimental data were given as inputs.

**3. Results and discussion**

ODEs were constructed for the description of the system’s dynamic response. Monod-type kinetics were mainly used for the development of the model. So far, equations for viable and total cells, glucose, glutamate, lactate and mAbs have been developed and are given by the following expressions:

$\frac{d\left(V∙X\_{V}\right)}{dt}=μ∙X\_{V}∙V-μ\_{d}∙X\_{V}∙V-F\_{out}∙X\_{V}$ (1)

$\frac{d\left(V∙X\_{T}\right)}{dt}=μ∙X\_{V}∙V-F\_{out}∙X\_{T}$ (2)

$μ=μ\_{max}∙\frac{\left[GLC\right]}{\left[GLC\right]+K\_{glc}}∙\frac{\left[GLU\right]}{\left[GLU\right]+K\_{glu}}$ (3)

$μ\_{d}=μ\_{d,max}∙\frac{K\_{d,glc}}{K\_{d,glc}+\left[GLC\right]}∙\frac{K\_{d,glu}}{K\_{d,glu}+\left[GLU\right]}∙\frac{\left[AMM\right]^{3}}{K\_{d,amm}^{3}+\left[AMM\right]^{3}}$ (4)

$\frac{d\left(V∙\left[GLC\right]\right)}{dt}=F\_{in}∙\left[GLC\right]\_{in}-F\_{out}∙\left[GLC\right]-V∙\left(\frac{μ}{Y\_{x,glc}}+m\_{glc}\right)∙X\_{V}$ (Jang & Barford (2000)) (5)

$\frac{d\left(V∙\left[GLU\right]\right)}{dt}=F\_{in}∙\left[GLU\right]\_{in}-F\_{out}∙\left[GLU\right]-V∙q\_{glu,max}∙\frac{\left[GLU\right]}{\left[GLU\right]+K\_{glu,2}}∙\frac{K\_{i,glc}}{K\_{i,glc}+\left[GLC\right]}∙X\_{V}$ (6)

$\frac{d\left(V∙\left[LAC\right]\right)}{dt}=Y\_{lac,glc}∙V∙\left(\frac{μ}{Y\_{x,glc}}+m\_{glc}\right)∙X\_{V}-V∙q\_{lac,max}∙X\_{V}-F\_{out}∙\left[LAC\right]$ (7)

$\frac{d\left(V∙\left[mAbs\right]\right)}{dt}=V∙\left(\frac{μ}{Y\_{x,mab}}+m\_{mab}\right)∙\frac{\left[GLC\right]}{\left[GLC\right]+K}∙X\_{V}-F\_{out}∙\left[mAbs\right]$ (8)

The values of the parameters differ according to the pH of the media. For pH equal to 7, the parameters that were derived from parameter estimation (PE) are μmax = 0.031 h-1, Kglc = 4.098 mM, Kglu = 0.045 mM, μd,max = 0.016 h-1, Kd,amm = 255.711 mM, Kd,glc = 21.8 mM, Kd,glu = 18.284 mM, Yx,glc = 4.34∙108 cells∙mmol-1, mglc = 2.39∙10-11 mmol∙cells-1∙h-1, qglu,max = 3.43∙10-9 mmol∙cells-1∙h-1, Kglu,2 = 19.11 mM, Ki,glc = 0.24 mM, Yx,mab = 3.2∙108 cells∙mg-1, mmab = 2.81∙10-10 mg∙cells-1∙h-1, K = 0.0375 mM, Ylac,glc = 2.0 mmol∙mmol-1, qlac,max = 7.0∙10-11 mmol∙cells-1∙h-1.

The results of the simulation are graphically represented in Figure 1.



 (a) (b) (c)

**Figure 1.** Simulated and experimental profiles of (a) viable cell, (b) mAb and (c) lactate concentrations for different pH levels in batch cultures.

**4. Conclusions**

From Figure 1, it is evident that extracellular pH levels affect cell viability and productivity. Specifically, it is observed that lower pH values, in the culture media, result in higher integral viable cell concentration (IVCC) and higher titer. Additionally, extracellular pH levels can control the lactate switch. In higher pHs, more lactate is accumulated in the system (Figure 1c). Regarding the model, the simulated results are in good agreement with the experimental data. We come to the conclusion that Monod and Hill kinetics can adequately describe the dynamics of the system. However, the equations have to be refined with amino acids data.

**References**

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