Bi-Phasic Culture Strategies Based on Medium Formulation: Substitution of Glucose by Galactose in CHO Culture

C. ALTAMIRANO, J.J. CAIRÓ and F. GÓDIA

1. Introduction

When most animal cell lines are cultured in bioreactors, they have consumption rates for glucose and glutamine very far above the rates strictly required to support the cellular growth, leading frequently to situations of exhaustion of the nutrients, accumulation of toxic compounds or imbalance of the medium, with the consequent initiation of cell death. Substitution of glucose and glutamine in CHO cell cultures has been previously studied (Altamirano et al., 2000). Specifically, it has been observed that the substitution by galactose and glutamate allows to achieve an acceptable cell growth, very low substrate consumption rates with a greater efficiency in their use and lower generation of toxic sub-products.

The observed characteristics of the growth on the two substrates can be exploited to develop new biphasic perfusion strategies based on medium formulation, consisting of a first phase centered in cell growth, which would lead to a high cell concentration, and a second phase in which the cells would be active, with a low (or null) growth, but maintaining their production capacity.

This concept is applied here to a CHO cell line producing recombinant t-PA, and a strategy of perfusion culture in two phases is envisaged, combining the high specific growth rate that cells have when they are cultures in glucose-glutamate with their low specific growth rate when cultured in galactose-glutamate. As a control, a culture using glucose and glutamate during all the experiment is performed.

2. Material and Methods

Cell Culture. Cell line CHO TF 70R (Pharmacia & Upjohn, Stockholm, Sweden) producing t-PA. The basal medium was a proprietary serum-free and low protein medium BIOPRO1 (Bio Whitaker Europe, Verviers, Belgium). BIOPRO1 was supplemented with vitamins (Sigma), lipids and cholesterol (GibcoBRL), proline, serine and aspartic acid (Sigma). This medium was also supplemented with 20 mM of glucose or galactose, and 7 mM of glutamate according to the different experiments. The cell cultures were carried out in spinner flasks (Techne) with a working volume of 250 mL and stirred at 50 rpm in a CO₂ incubator (Forma Scientific CO₂ incubator), at 37.0°C, with 96% relative humidity in an atmosphere of 5% CO₂ in air. The cultures were allowed to grow in batch mode for 3 days before starting the perfusion with a medium change equivalent to a dilution...
rate of $0.5 \text{ d}^{-1}$. Until day 12 both cultures were perfused with a medium which contained glucose and glutamate. Following this, in the bi-phasic perfusion the glucose medium was substituted by a medium that contained galactose and glutamate, while in the control the initial formulation of the perfusion medium was kept.

**Viable and cell concentration.** Cell viability was determined by the trypan blue exclusion method, using a haemacytometer.

**Metabolite determinations.** Glucose and lactate concentrations were determined with a YSI 2700 automated glucose and L-lactate analyser. Ammonium concentration was determined by a flow injection analysis system. Amino acids concentrations were measured by HPLC, and t-PA concentration by ELISA.

3. Results and Discussion

Figure 1a shows the cell growth results obtained, reflecting that, while the same perfusion medium is maintained, cell growth is analogous in both cultures. From this point, when the change of medium is performed for the bi-phasic perfusion, the evolution of each experiment is markedly different. The control culture reached a maximum concentration after 18 days, and then entered in an acute death phase. The abrupt fall in cell growth in the control culture cannot be attributed to the exhaustion of any essential amino acid. Neither can it be attributed to the levels of lactate or ammonium reached (10 mM and 1.4 mM, respectively), notably lower than the values reported as being inhibitory. A possible explanation could be an oxygen limitation, since the aeration capacity of spinner flasks can be too low once the culture reaches a given cell concentration. In contrast to the control experiment, the bi-phasic perfusion culture shows an abrupt arrest of cell growth after the introduction of the carbon source change. The cell level is kept constant for a prolonged period of operation, and it should also be highlighted that the loss of viability is substantially lower that for the control experiment (Figure 1a). Growth arrest in the bi-phasic culture cannot be attributed