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Kinetic analysis of hybridoma cell culture in a protein-free medium: Substrate and agitation effects

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Abstract

A kinetic study of a hybridoma cell line that produces monoclonal antibodies against lactoferrin was carried out. A well defined protein-free culture medium was employed to facilitate the subsequent purification of the monoclonal antibodies. It should be highlighted that most of the existing work has been carried out employing culture media enriched with fetal bovine serum (FBS). Cell growth and monoclonal antibody production were monitored and kinetic parameters were determined. Besides, fundamental nutrients such as glucose and glutamine, inhibitory products such as ammonium and lactate, and several amino acids were followed throughout the culture. Additional experiments were carried out supplementing the medium with glutamine and ammonium, none of them resulting the key compound that halted the cell growth under the tested conditions and an unstructured model can be used to describe the system. Finally, agitation of the culture by a rocker set-up has shown high values of the specific death rate.

Keywords: Hybridoma; Kinetics; Monoclonal antibodies; Protein-free; Agitation

1. Introduction

Monoclonal antibodies produced by hybridomas are currently used in many applications, such as the diagnosis and treatment of certain diseases or the purification of substances [1,2]. The growing demand for monoclonal antibodies (MAb) implies that it is necessary to carry out an optimisation of their production processes. Hence, kinetic equations must be developed to describe cell growth, nutrient consumption and product generation. These equations will be very useful in the design and evaluation of large-scale processes and reactors.

To date, many models [3–9] have been proposed to describe in vitro hybridoma growth, but most of them are based on experiments carried out employing culture media enriched with fetal bovine serum (FBS). Fetal bovine serum has been a widely used additive for cultivating hybridoma cells that provides stimulating hormonal factors and proteins to transport hormones, minerals, lipids, etc. However, certain problems arise when FBS is used: its high cost, the lack of homogeneity between different batches and its high protein content, which makes the downstream purification processes more difficult. For all these reasons, the serum in the culture media tends to be substituted by additives such as inorganic ferric salts or transferring [10].

In this paper, a kinetic study has been carried out for hybridoma growth and antibody production in a protein-free culture medium [9,11,12]. The cell line used was HB-8852, which produces monoclonal antibodies against bovine lactoferrin. These antibodies were used in an immunoaffinity chromatography column, showing the ability to separate the protein from whey at a high level of purity.

The concentration and evolution of substrates and products is a key factor in the knowledge of the kinetics of hybridoma culture. Glucose is one of the main carbon and energy sources for hybridoma cells. It is mainly transformed into pyruvate, but part of this glucose is used for the synthesis of biomass through the pentose pathway. Pyruvate is partially converted to CO_2 and H_2O by the TCA cycle, partially converted to lactic acid and partially to fatty acids. Glutamine is another important substrate

Abbreviations: Am, ammonium; Asp, aspartic acid; Glc, glucose; Gln, glutamine; Ile, isoleucine; Lac, lactate; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenilalanine; Pro, proline; S.D., standard deviation; Ser, serine; Tyr, tyrosine; Val, valine

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Nomenclature

C_i	substrate concentration
C_j	product concentration
k_d	specific death rate (h^{-1})
m_i	maintenance energy (mmol $cell^{-1}$ h^{-1})
p_j	specific production rates (μmol $cell^{-1}$ h^{-1})
q_i	specific consumption rates (μmol $cell^{-1}$ h^{-1})
t	time (h)
X_d	dead cell concentration (cell ml^{-1})
X_T	total cell concentration (cell ml^{-1})
X_V	viable cell concentration (cell ml^{-1})
Y_i	yield of cells from substrate (cell $mmol^{-1}$)
Y_j	yield of cells from product (cell $mmol^{-1}$)
Y'_i	apparent yield of cells from substrate (cell $mmol^{-1}$)
$Y_{Am/Gln}$	yield coefficient ammonium/glutamine
$Y_{Lac/Gluc}$	yield coefficient lactate/glucose

Greek letters

μ	specific growth rate (h^{-1})
μ_{app}	apparent specific growth rate (h^{-1})
α, β	Ricatti equation constants

for hybridoma cells. Part of the glutamine is deaminated, yielding ammonium and glutamate, and is later transformed into other amino acids for biosynthesis purposes. Glutamine also enters into the TCA cycle, yielding ATP, CO_2 and H_2O (see Fig. 1). Whereas glucose and glutamine are fundamental nutrients for hybridoma growth, ammonium and lactate are products of cell metabolism that can act as inhibitors when their concentration is high enough [13–15].

With regards to culture conditions, hybridoma culture has been traditionally carried out in static flasks. However, several authors have described the influence of agitation on hybridoma growth [16,17]. Agitation ought to be positive for cell growth since it improves nutrient and oxygen transport towards the cells, although the lack of a protective cell wall in mammalian cells makes them more sensitive to shear stress and bursting bubbles. Some authors have studied the addition of protective agents such as fetal bovine serum or surfactants [18]. There is no previous data regarding agitation for the specific cell line studied in the present work. Therefore, the possibility of improving cell growth employing gentle agitation has also been considered.

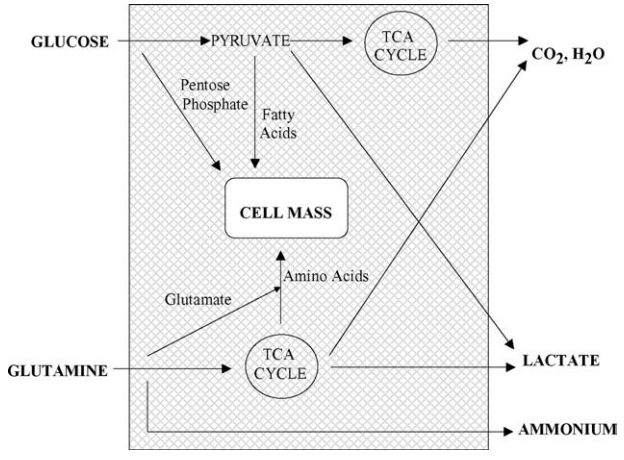

Fig. 1. Main metabolic routes for hybridoma cells.

2. Materials and methods

The hybridoma cell line studied, HB-8852, was supplied by the American Type Culture Collection (ATCC). It produces IgG_1 monoclonal antibodies against bovine lactoferrin. Experiments were carried out employing a protein-free culture medium supplied by SIGMA (Hybrimax Serum-Free and Protein-Free Hybridoma Medium, Ref.: S2897).

Initially, cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 20% (v/v) of FBS. Later, a gradual adaptation to DMEM with 10% (v/v) FBS was carried out. Once in this medium, the cells were passed to Hybrimax with 10% (v/v) FBS and later adapted so as to grow in Hybrimax with no serum. The pH of the culture medium was controlled by a buffer of MOPS and bicarbonate so as to maintain the pH near to 7.2. Cells were cultured in a CO_2 incubator.

Media supplemented with glutamine and ammonium were prepared as follows: in the experiments with initial addition of ammonium, 10 ml of a concentrated solution of NH_4Cl (18 mM) were added to 100 ml of Hybrimax, obtaining a final excess concentration of 1.6 mM in the medium. The enriched glutamine medium was prepared in a similar manner, but the final excess concentration was 1.4 mM. To dismiss any dilution effect, an experiment was carried out in parallel adding to 100 ml of Hybrimax 10 ml of distilled water (control).

Cell adaptation to a protein-free culture medium was carried out in T-25 flasks containing 10 ml of medium and without agitation. Once adapted to grow in a protein-free medium, the cells were expanded to a T-75 flask containing 50 ml of medium and afterwards to a T-150 flask containing 100 ml. Once in the T-150 flask, the cells were centrifuged at 1200 rpm for 10 min and used to inoculate the medium, the initial cell concentration being approximately 10^5 cell ml^{-1}. Regarding agitated experiments, a rocker was used as a simple equipment to promote mixing in the system (10 oscillations per minute and a tilt angle of 7°).

Samples of 5 ml were taken daily and centrifuged at 3000 rpm for 5 min. This sampling frequency and size was chosen so that the change in the relation air/culture medium did not affect the kinetic results. The supernatants were filtered through 0.45 μm pore membranes, divided into 1 ml aliquots and frozen at −20 °C for later analysis. Simultaneously, total and viable cells were counted in a hemocytometer by the trypan blue exclusion method (standard deviation $<10^5$ cell/ml). Glucose concentration