Growth of Mammalian and Lepidopteran Cells on BioNOC® II Disks, a Novel Macroporous Microcarrier

Jean-Christophe Drugmand[1], Jean-François Michiels J.-F.[2],
Spiros Agathos[1], Yves-Jaques Schneider[2]
Université Catholique de Louvain, Institut des Sciences de la Vie[1] Unit of Bioengineering, Place Croix du Sud, B-1348 Belgium[2], Unit of Cellular Biochemistry, Place Louis Pasteur[1], B-1348 Belgium

Abstract: The use of BioNOC II microcarriers in a fixed bed bioreactor setup allows to produce high protein levels in a CHO-320 cell line expressing constitutively interferon-γ and also with insect cells used with a baculovirus transient expression vector.

Key words: Fixed bed, Microcarriers, BioNOC, CHO cells, Insect cells

1. INTRODUCTION

Microcarrier technology has the advantages of easy sampling, relatively large surface area and is useful in perfusion culture. Insect cells are well-established for the production of recombinant proteins by the baculovirus expression vector system (BEVS). CHO cells are widely used for the production of therapeutic proteins. Plant peptones are the safest supplement for protein-free media. They exert a nutritional effect, and they contain bioactive peptides, responsible for increases in growth and production.

BioNOC II™ (Cesco Bioengineering Co, Taichung, Taiwan) cell culture disks are new 100% pure PET nonwoven macroporous carriers for the growth of animal, mammalian and insect cells. BelloCell-500 is a disposable bioreactor bottle. It consists of a chamber holding 6.5 g of BioNOC II

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carriers and a lower compressible chamber for mixing and surface aeration by an up/down reciprocal motion.

CHO-320 cells producing recombinant interferon-γ were cultivated in BDM serum-free media (Burteau *et al*., 2003) supplemented with 0.5-4 g/l cotton peptone from Quest (Naarden, The Netherlands). High Five™ insect cells were cultivated in YPR serum-free media and infected with a AcMNPV baculovirus r-βgal (MOI 2) (Ikonomou *et al*., 2001). Celligen-Plus 2 L and BelloCell-500 bioreactors were used. The cell biomass was estimated by a protein assay.

2. CHO CELLCULTIVATION IN BelloCell-500

Two BelloCell-500 batch cultivations were conducted simultaneously with and without peptone (1 g/L). Although the growth was slower (Figure 1), interferon-γ production was slightly higher when peptone was added.

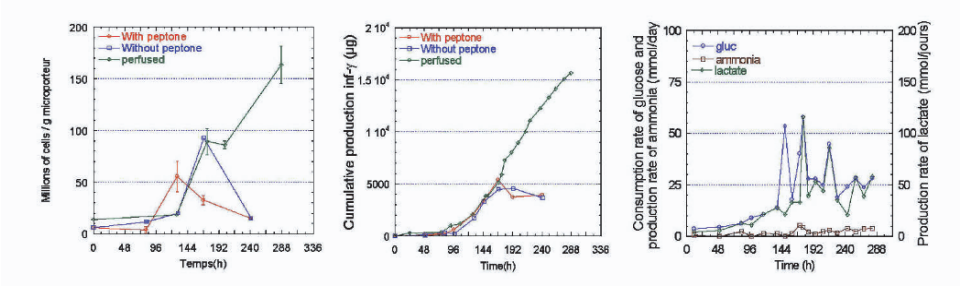

Figure 1. CHO cell cultivation in BelloCell-500. The bioreactor parameters were culture up/down speed = 1/1 mm/s and up/down hold time = 0/3 min.

3.

In a BelloCell-500 perfusion cultivation medium was supplemented with 1 g/L cotton peptone. The medium was changed for the first time after 72 hours of culture, when the glucose concentration was low. After 108 hours, medium was changed twice a day. The time course of glucose consumption and of the lactate and ammonia and ammonia production shows that most of the glucose was converted to lactate which decreased the pH, while ammonia production was low. Interferon-γ production was 3-fold higher than in batch.INSECT CELL cultivation in fixed bed.

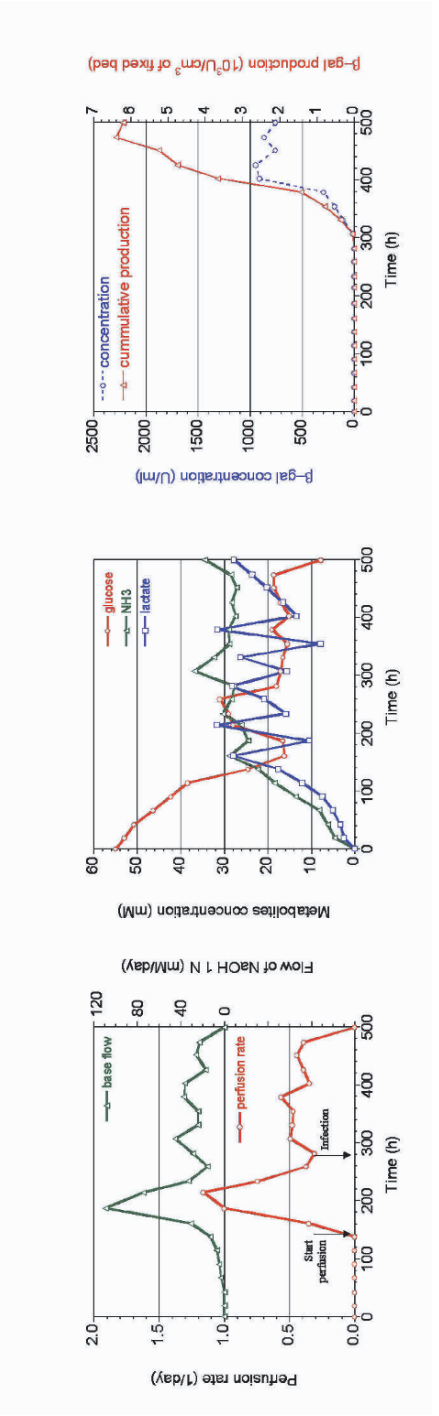

Figure 2. Insect cell perfusion cultivation in Celligen-Plus 2 L bioreactor in fixed bed basket configuration (1.35 L working volume) with 20 g of BioNOC II (bed volume 500 cm3, 80 rpm, 28°C, 60% DO). The pH was controlled with the addition of 1 N NaOH.

A fixed bed reactor in perfusion mode was used with BioNOC II. The cells were cultivated for 6 days in batch before starting the perfusion, and the cells were infected after 11 days (Figure 2). The perfusion rate was adapted to keep not only the glucose concentration below 20 mM (a third of the initial concentration) but also to prevent the accumulation of ammonia and lactate above 25 mM. During infection, this perfusion rate was decreased to prevent the dilution of r-protein and keep by-product concentrations in an acceptable range. Lactate production was high (about 1 mol lactate / 1 mol glucose) probably due to the lack of oxygen and accumulation of CO_2 inside the fixed bed. The ratio oxygen / glucose of 3 supports this observation. The pH was controlled to prevent its dropdue to lactate production.

Cell density (based on metabolite consumption) was estimated at 400.10^6 cells/g of BionocII before infection.

We obtained after 200 hours post infection (hpi) a high production ($3x10^6$ U β-gal /run) i.e. 2.5 fold higher than in batch. In experiments using the same setup but with Fibra-Cell (New Brunswick Scientific, Edison, NJ), we obtained the same level of production.

REFERENCES

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