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Performance of a membrane-dialysis bioreactor with a radial-flow fixed bed for the cultivation of a hybridoma cell line

Abstract A bioreactor system for the continuous cultivation of animal cells with a high potential for scale-up is presented. This reactor system consists of radial-flow fixed-bed units coupled with a dialysis module. The dialysis membrane enables the supply of low-molecular-weight nutrients and removal of toxic metabolites, while high-molecular-weight nutrients and products (e.g. monoclonal antibodies) are retained and accumulated. This concept was investigated on the laboratory scale in a bioreactor with an integrated dialysis membrane. The efficiency of the reactor system and the reproducibility of the cell activity (hybridoma cells) under certain process conditions could be demonstrated in fermentations up to 77 days. Based on model calculations, an optimized fermentation strategy was formulated and experimentally confirmed. Compared to chemostat cultures with suspended cells, a ten-times higher mAb concentration (383 mg 1⁻¹) could be obtained. The highest volumetric specific mAb production rate determined was 6.1 mg mAb (1 fixed bed)⁻¹ h⁻¹.

Introduction

For large-scale cultivation of animal cells special attention has to be paid to the reactor design and the process strategy (Griffiths 1988, 1992; Mizrahi 1986; Tyo and Spier 1987). Animal cells are very sensitive to mechanical forces, require a well-defined medium and are characterized by low specific growth rates and production rates. In particular, fixed bed reactors with immobilized cells enable the cultivation of high-volume specific cell concentrations under low shear conditions (Ong et al. 1994; Racher et al. 1990). If a radial-flow geometry is applied (Fig. 1), oxygen is not limited along the radius and high fixed-bed units can be built (Kurosawa et al. 1991; Matsumura et al. 1993; Yoshida et al. 1993).

High-volume specific concentrations of cells require high perfusion rates to supply nutrients and to remove toxic metabolites. Consequently the product concentration (e.g. monoclonal antibodies) remains low even at high volume-specific production rates. The product concentration in the harvest stream can only be increased if devices for product retention are installed.

Possible devices for product retention are filtration units with a transmembrane hydraulic flux (Altshuler et al. 1986; Belfort 1989; Faust and Kopf 1989). But the hydraulic flux induced by a pressure difference over the membrane leads to fouling and clogging of the membrane, especially if the medium contains serum (McDonogh et al. 1992; Pörtner et al. 1991). Therefore such processes do not provide a stable flow during long-term cultivation.

As an alternative, dialysis membranes without a hydraulic flux over the membrane offer the possibility of supplying low-molecular-weight nutrients and removal of toxic metabolites, while high-molecular-weight nutrients or products are retained (Adamson et al. 1983; Kurosawa et al. 1991; Linardos et al. 1992; Sjögren-Jansson and Leansson 1985). The transmembrane exchange of components is accomplished by diffusion through the membrane, driven by the concentration difference over the membrane. There is no hydraulic transmembrane flux. Therefore dialysis membranes do not change their properties even during long-term cultivation under high protein titers, as will be demonstrated in this paper.

In Fig. 2 a reactor concept for the cultivation of immobilized cells is shown that offers a high potential...
for scale-up. The cultivation unit consists of a radial-flow fixed bed, which is coupled with an aeration unit, a dialyzer module and a medium reservoir. Low-molecular-weight nutrients are supplied, and low-molecular-weight metabolites are removed via the membrane. Because high-molecular-mass components cannot pass through the membrane, serum is added to the cultivation unit directly and products (e.g. monoclonal antibodies) are accumulated.

This concept was investigated on a laboratory scale in a reactor with an integrated dialysis membrane (Fig 3). The reactor consists of two chambers separated by a cylindrical membrane. In the inner chamber a radial-flow fixed bed containing porous carriers for cell immobilization is introduced, which has the same geometry as the fixed-bed element indicated in Fig. 2. Therefore data obtained with this reactor are representative of the large-scale situation. In contrast to the concept shown in Fig. 2, in the laboratory reactor aeration was performed in the outer chamber (medium reservoir) for practical reasons, and oxygen had to permeate through the membrane. It could be confirmed during the experiment that the membrane area was sufficient to supply enough oxygen to the inner chamber.

In earlier publications (Bohmann et al. 1992; Kurosawa et al. 1991) we have reported on successful fermentations with the membrane dialysis reactor using a hybridoma cell line producing monoclonal antibodies against penicillin-G amidase (Niebuhr-Redder and Kasche 1990). Porous Siran carriers had been found to be suitable for cell immobilization (Bohmann et al. 1992). Siran carriers of 3–5 mm diameter showed an advantage compared to those of 1–2 mm diameter. For the 3- to 5-mm carrier the specific glucose uptake rate and the mAb production rate were constant, when the superficial flow velocity of the liquid through the fixed bed was varied between 0.09 mm s$^{-1}$ and 0.75 mm s$^{-1}$. At higher flow velocities cells were washed out of the bed.

The intention of this work was to evaluate the long-term stability of the reactor system during continuous operation and to examine the influence of the concentrations of low- and high-molecular-weight medium components on the volume-specific substrate uptake and metabolite production rates of the immobilized cells, especially the antibody productivity. From these data an optimized fermentation strategy with respect to the dilution rates in the inner and outer chamber was derived and experimentally tested.

**Materials and methods**

**Cell line and culture medium**

A hybridoma cell line producing monoclonal antibodies against penicillin-G amidase (Niebuhr-Redder and Kasche 1990) was cultivated in a 1:1 mixture of Iscove's modified Eagle medium MEM and Ham's F12 medium supplemented with 3% fetal calf serum (all chemicals purchased from ICN Flow, Germany), 2 mmol l$^{-1}$ L-glutamine (Gibco, Germany) and 2.5 g l$^{-1}$ NaHCO$_3$ (Merck, Germany).