Some aspects of hybridoma cell cultivation

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Summary. Two hybridoma cell lines were cultivated in an indirectly aerated 10-l reactor in batch, fed-batch and continuous (perfusion) operations and in spinner flasks. The medium in the reactor was sampled either by an aseptic cross-flow filtration module integrated into a loop or by an in-situ tubular filter. The glucose concentration was monitored by an on-line flow injection analyser and the ammonia concentration by an ion-selective electrode. Since the membrane transmission of the high-molecular components decreased during cultivation, the product, a monoclonal antibody, was enriched in the reactor. During cultivation, the concentrations of cells, viable cells, glucose, lactase, acetate, citrate, ammonia, urea, amino acids, proteins, and monoclonal antibodies were determined off-line. The specific growth rate, specific production, and consumption rates of the medium components were influenced considerably by the medium composition, especially by the type and amount of serum used.

Materials and methods

Cell lines. Two mouse-mouse cell lines (F34 and 3C2) were prepared by P. Nabet (personal communication) by the fusion of myeloma cells of cell line P3X63-Ag8 with activated B-lymphocytes of a mouse. The mouse-mouse 3C2 cells produce monoclonal antibody (MAB) immunoglobulin G-1 (IgG-1) against the hormone Human-Choriongonadotropin. The cell line F34 does not produce antibodies.

The hybridoma cell stock cultures were stored at 37°C and 5% CO2 in air in Roux flasks (Falcon, Cockeyville, USA) in incubators (Heraeus Type B 5060 EK CO2; Hanau) and were diluted twice a week with fresh medium to 10^5 cells ml^-1. These cells were used as seeds for the precultures in Bellco (Vineland, NJ, USA) spinner flasks.

Culture media. The basic medium for F34 and 3C2 was RPMI 1640 powder (Gibco, Grand Island, USA), supplemented with 0.328 g l^-1 glutamine (Serva, Heidelberg, FRG), 0.132 g l^-1 sodium pyruvate (Riedel de Haen, Seelze), 10 μg l^-1 2-mercaptoethanol (Serva), and 2.0 g l^-1 NaHCO3 (Riedel de Haen). For cultivation in the 10-l reactor 0.060 g l^-1 gentamicin (Serva) in distilled water (ASTM-Type I, R= 18 mΩ cm^-1) was also added.

Before inoculating the medium, it was sterile-filtered, and various amounts of either foetal calf serum (FCS; Gibco) or horse serum (HS; Gibco) was added to the medium. The pH value was controlled by addition of gaseous CO2 or 0.1 N NaOH.

Bioreactor. A 10-l bioreactor (Biostat E, Braun, Melsungen, FRG) with a low stirrer speed (<200 rpm), 15.2 m silicone tubing (3 mm diameter and 0.4 mm wall thickness) wound around a cylindrical basket of 16 cm diameter for indirect aeration and 10 m hydrophilized microporous polytetrafluorethylene (PTFE) tubing (Gore, Putzbrunn, FRG) (2 mm diameter 0.4 mm wall thickness) for medium exchange with perfusion, was used for cultivation (Fig. 1). The oxygen transfer rate and pH were controlled by the gas composition (N2:O2:CO2) in the silicone tubing.

On-line analysis. Two aseptic sampling systems were used: (a) a cross-flow flat filtration module (Millipore, Freehold, NJ, USA) (Fig. 2) with a pump (Watson-Marlow 101 UR, Essex, FRG) in the outer loop, and (b) a tubular filter developed in the Technical Chemistry Institute (TCI) at the University of Hannover and sold by ABC (Puchheim, FRG) (Fig. 3). In the Millipore module, a hydrophilic flat membrane (GVWP 04700, Millipore) was used. In the tubular filter, polypropylene microflltration tubing (Enka, Wuppertal, FRG) was used. The sampling modules are characterized in Table 1.
Fig. 1. Experimental set-up: P, pump; V, valve; F, membrane filter; PTFE, polytetrafluoroethylene

Fig. 2. Cross-flow flat membrane filtration module for on-line aseptic sampling

Fig. 3. Tubular filtration module for in-situ sampling

The on-line flow injection analyser (FIA) system for glucose analysis consisted of a 16-channel peristaltic pump (Skalar, Erkelenz, FRG) tygon-tubing, motor valve (Latek-TMV, Eppenheim, FRG), injector valve (Lee Hydraulic Miniature Components, Frankfurt, FRG) and a YSI-glucose analyser (Model 23A, Yellow Spring Instruments, Ohio, USA) modified for on-line operation (TCI, Hannover). The operation of the FIA system was controlled by a microprocessor (Motorola, Type 68000) and a suitable software package (FERAS; Wieneke 1989).