GROWTH OF HYBRIDOMA CELLS UNDER DIFFERENT AGITATION CONDITIONS

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SUMMARY

The effect of mechanical agitation on hybridoma cell growth was examined in laboratory scale vessels. At an agitation rate four times that required to keep the cells in suspension, both growth rate and growth extent were reduced. However, using the minimum agitation rate required to suspend cells, no adverse effect on cell growth was observed even with a turbine agitator.

INTRODUCTION

A question often raised in using a stirred tank bioreactor for cell culture is how shear force generated by agitation will affect cells. It has been reported that the growth rate of hybridoma cells is 15% lower in a stirred vessel than in a stationary culture (de St. Groth, 1983). This was observed for ten different cell lines even with the lowest agitation rate required to suspend cells. However, the data presented were insufficient to conclude that shear force caused by agitation affects cell growth. Other possibilities, such as localized concentrations of “conditioning factors” in the stationary culture that might improve cell growth, cannot be excluded. Nevertheless, the effect of shear force on cell growth and antibody production cannot be overlooked when addressing the problems of scale-up. As part of our investigation on the cultivation of hybridoma cells in different reactor types, we examined the growth of hybridoma cell line HB8178 under different agitation conditions in our laboratory scale stirred vessels ranging in size from 250 ml to 2 l. We observed no detrimental effect of agitation at a stirring rate which is required to maintain cells in suspension.

MATERIALS AND METHODS

The hybridoma cell line HB8178 (American Type Culture Collection) which produces a monoclonal antibody against the K99 pilus of Escherichia coli, was chosen due to the availability of antigen for the assay of the antibody. Cells were maintained in one liter roller bottles at 37 °C in a 5% CO2 in air atmosphere using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (by volume) horse serum (Gibco Laboratories, Grand Island, NY). The medium was supplemented with 100 units/ml of penicillin G and 100 µg/ml of streptomycin.

Inocula were obtained from exponentially growing stock suspensions in roller bottles. Cultures with a 100 ml volume were performed in 250 ml spinner vessels (5.5 cm diameter) with a side-arm for culture sampling (Wilbur Scientific, Boston, MA). A suspended magnetic stirring bar (0.9 cm x 4.5 cm) placed about 1 cm from the bottom of the flask was used for agitation. Unless otherwise noted, the agitation speed used was approximately 60 rpm. The flask was placed on a magnetic stirrer in a humidified CO2 incubator at 37 °C.

Cultures with a 500 ml volume were carried out in a one liter round bottom vessel (10 cm diameter). The suspended impeller was modified by the addition of two 45° pitched blades (2.5 cm x 2.5 cm). Stainless steel rods (0.125” diameter) were placed around the periphery of the vessel to support 80 cm of silicone rubber tubing wound around the rods. The tubing is used for both aeration and pH control. If pH falls below the set point, the controller triggers a solenoid valve allowing air to pass through the tubing and vent into the headspace, thereby reducing dissolved CO2 concentration and raising the pH.

Growth was monitored by counting total and viable cells with a hemacytometer. Viability was determined by the dye exclusion method using trypan blue. The culture supernatant was kept frozen until the measurement of glucose, lactate, and antibody. The K99 antibody was quantitated using an
Figure 1. Growth kinetics of Hybindoma HB8178 in spinner and in stationary culture. Open symbols spinner culture; filled symbols, stationary culture; (○, ●): cell concentration; (∆, ▲): antibody concentrations; (□, ■): glucose concentration; (○, ●): lactate concentration.

Figure 2. Growth kinetics of HB8178 under different agitation rate. (○): 60 rpm; (∆): 120 rpm; (□): 240 rpm. The concentration of nonviable cells was negligible before 45 hr. In the cell concentration curves, open symbols are viable counts and filled symbols represent total cell concentration.