CULTIVATION OF INSECT CELLS IN AIRLIFT REACTORS: INFLUENCE OF REACTOR CONFIGURATION AND SUPERFICIAL GAS VELOCITY

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***Abstract*- Large-scale cultivation is an essential step towards the feasible production of baculovirus in insect cell cultures. Airlift reactors appear to offer considerable advantages over other insect cell culture systems. In order to evaluate the impact of reactor design on the behavior of insect cell cultures, the IPLB-Sf-21 cell line was cultivated in three different concentric tube airlift reactors that differ in their geometrical parameters. The ratio of downcomer to riser cross sectional areas, the shape of the bottom and the ratio of height to diameter of the reactor proved to be important since them produce significant differences on cell growth behavior. Modifying the reactor design the cellular growth rate could be improved from 0.016/h to 0.031/h, while the maximum viable cell density could be elevated from $9x10^5$ to $2.4x10^6$ cells/ml. Once selected a reactor configuration, the influence of gas flow rate was determined, finding an optimal value of superficial gas velocity that renders sufficient oxygenation without any significant effect on the cellular viability. In addition, the influence of the reactor design on fluid circulation in the reactor was tested.**

***Keywords*: Insect cell culture, Airlift reactor, Geometrical parameters, Gas flow rate, Liquid velocity.**

I. INTRODUCTION

Cultivation of insect cells on a large-scale is an important step towards the in vitro feasible production of entomopathogenic baculovirus used as bioinsecticides. Airlift reactors (ALR) appear to offer considerable advantages over other culture systems for the scale-up of insect cells propagation in suspension cultures (Maiorella et al, 1988; Merchuk, 1990; Shah et al, 1992).

The main aim in the engineering design of a bioreactor for this type of process is to maintain the shear generated in the equipment within the range permitted by the biological process, while reducing at the same time mass transfer limitations. This is possible in small size reactors, but is extremely difficult for large scale cultures. Airlift reactors present an advantage in this aspect. Since the driving force for liquid circulation is not provided by a focal input of energy, but by differences in local fluid density, the shear field is much more homogeneous and easier to set within the range required by the bioprocess.

Concentric tube ALRs can be designed to satisfy simultaneously the oxygen demand and the mildness of flow required for minimal cell damage. Published work on this subject has generally focused on the effect of both the ratio of cross-sectional area of riser to the downcomer (Bello, 1981; Chisti and Moo-Young, 1987), the height of the reactor (Merchuk et al, 1992), the gas-separation section at the top of the reactor (Siegel and Merchuk, 1987), and the design of the bottom section (Blenke, 1979). The evidences stress the importance of geometry in the performance of airlift reactors, since geometrical configuration has a direct effect on the fluid dynamics of the system (Merchuk et al, 1994a). Although the design of an ALR had been pointed out as an important factor to be taken into account (Shuler et al, 1990; Merchuk, 1994a; Merchuk, 1994b; Merchuk and Gluz, 1999), few specific papers are found in the literature that systematically consider the influence of the reactor geometry on the behavior of animal cell cultures in general, and insect cell cultures in particular. A major problem encountered in scaling up insect cell culture systems is the shear sensitivity of these cells (Tramper and Vlak, 1988). Gas flow rate is, in airlift reactors, a parameter closely related to shear, because fluid dynamics depends mainly on the gas flow rate (Merchuk and Berzin, 1995). In addition to the shear in the bulk of the liquid, the bursting of bubbles at the liquid-gas interface at the top of the ALR has been recognized as important cause of cellular death (Murhammer and Goochee, 1988; Tramper and Vlak, 1988; Wu, 1995). On the other hand, if the gas flow rate is too low, cells may fail to be in full suspension. Therefore, the gas flow rate becomes a critical parameter in order to optimize the culture of insect cells in ALRs.

In the present work we report a set of experiments that were carried out in ALRs of different geometry in order to study the influence of ALR design on the

behavior of an insect cell culture system. The ratio of the cross-sectional area of downcomer to riser, the height of the reactor, and the design of the bottom section of the reactor and its influence on the liquid velocity were examinated. The flow rate into the bioreactor giving the best culture growth was also determined.

II. MATERIALS AND METHODS

A. Airlift Reactors

Cylindrical concentric glass bioreactors of 250 and 500 ml (nominal volume) built at Ben-Gurion University were used in all experiments. The ratio of the cross-sectional area of downcomer/riser ($CSA_{d/r}$) of the ALRs was considered as one of the main important parameters.

All reactors were air-gassed, and oxygen dissolved level was not measured nor controlled. In all experiments, three identical ALRs for each configuration were run simultaneously. The values reported are the mean of samples taken simultaneously from three reactors for each configuration.

B. Liquid Velocity Determination

The three different airlift reactors were filled with culture medium and incubated in a room at 28 °C to simulate the culture conditions. A tracer was introduced in each reactor and air bubbling was started. The tracer was a very small piece of black paper of 1 mm^2 that once wetted was supposed to behave like the liquid. The fall velocity of the tracer in still liquid was very low, and an ideal tracer behavior was supposed. The time for 10 cycles of the tracer were registered, and the average calculated. The same procedure was followed for each superficial gas velocity tested. The mean velocity was calculated from both the circulation time and the distance traveled by the tracer in a complete cycle in the reactor. To minimize errors and to facilitate the calculation of the liquid velocity, it was assumed that, at the bottom, the tracer passed exactly at the middle of the distance from the bottom of the reactor to the draft tube lower rim. The same was assumed at the top.

C. Cells and Culture Medium

The IPLB-Sf-21 insect cell line was used (Vaughn et al, 1977). The cell line was maintained as adherent monolayer cultures in 25 cm^2 T-flasks. Subcultures were made every 4-5 days. The incubation temperature was 28°C. The culture medium used was TC-100 (Gardiner and Stockdale, 1975) (Sigma) supplemented with 10 % fetal bovine serum (Sigma). For cell cultivation in airlift reactors, 150 ppm of silicone antifoam (Antifoam A, Sigma) and 0.20 % w/v of Pluronic F-68 (Sigma) (Murhammer and Goochee, 1988) were added to the culture medium. For all experiments performed, the cells were previously adapted to grow in suspension in 500 ml magnetically agitated spinner-flasks (Techne, U.K.) at 60 rpm. Cell counts were carried out using a Neubauer haemocytometer. Viability was assayed by 0.04 % Trypan Blue dye exclusion. Cells that exclude the colorant were considered viable. Each sample was processed by duplicate counting total and viable cells and taking the mean. Growth rates were evaluated from the linear region of a semilog plot of cell density vs. time.

III. RESULTS AND DISCUSSION

A. Determination of the liquid velocity in airlift reactors

The experimental procedure explained previously was followed to study the behavior of the three different ALRs from the point of view of its circulation velocities. Six different superficial gas velocities ranged from 0.076 to 0.296 cm/min were assayed. The values obtained in the runs are summarized in the Fig. 1. It can be observed that in the range of the superficial gas velocities tested the behaviors of the 3 ALRs were clearly different. For example, at J_G = 0.091 cm/min. the liquid velocity reached in the ALR 2 (4.58 cm/s) and ALR 3 (5.75 cm/s) were 2.57 and 3.22-fold greater, respectively, than liquid velocity in the ALR 1 (1.78 cm/s). This value of J_G is specifically chosen for comparison because being the optimal for growing IPLB-Sf-21 insect cells in our system.

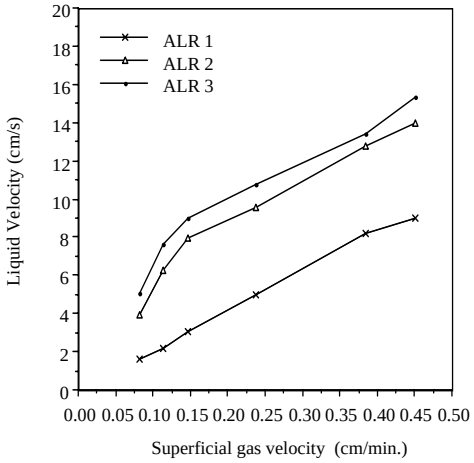

Figure 1. Liquid velocity as a function of the J_G for the three ALRs. Experimental points represent the tracer average time for ten cycles.

The difference in the liquid velocity observed between ALR 1 and ALR 2 was larger than the observed between ALR 2 and ALR 3. The reason of this behavior was a consequence not only of the bottom design (flat in the ALR 1 versus round in ALR 2 and ALR 3) but also bottom clearance, which contributed to produce a better fluidization, avoiding cell accumulation in the bottom of the reactor. On the other hand, the difference in the liquid velocity observed between ALR 2 and ALR 3 was a consequence of the

height difference between them. This height was traduced in the ALR 3 into a larger driving force for circulation between riser and downcomer as consequence of a larger hydrostatic pressure difference, rendering a larger liquid velocity.

Taking into account that ALRs are pneumatic reactors this fact is very important, because at the same gas input per unit area it will be possible to obtain a larger liquid velocity without generating additional bubbles and foam into the reactor, providing better mixing without increasing the damage to the cells. Additionally, it is expected that the better mass transference into the ALR can also improve the transference of nutrients and oxygen from the medium to the cells.

B. Air flow rate influence on insect cell growth in airlift reactors

The influence of gas flow rate on IPLB-Sf-21 culture behavior was studied. Five different superficial gas velocities, J_G, were tested: 0.076, 0.091, 0.115, 0.178 and 0.296 cm/min.

Figure 2 shows the maximum values of viable cells reached in the ALR 3 at different gas flow rates. The minimum gas flow rate chosen for these experiments was close to that required for avoiding the sedimentation of the cells at the bottom of the reactor (determined in preliminary experiments). The cells were seeded simultaneously in three airlift reactors of design ALR 3 at initial cell densities that ranged from 2.4 to 2.6 x 10^5 viable cells/ml. Cultures were followed until the mortality of the cells reached approximately 90%.

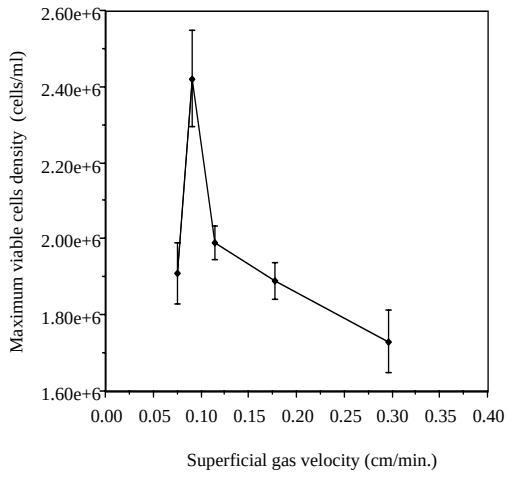

Figure 2. Evolution of the maximum viable cell density as a function of the superficial gas velocity for IPLB-Sf-21 cultures in ALR 3.

The existence of an optimum value of gas flow rate (0.091 cm/min.), corresponding to a larger maximum cell density, was evident. This optimum can be explained as a compromise between the cellular requirement for adequate mixing and oxygenation, on the one hand, and the cellular sensitivity to the shear stress caused by the gas flow, on the other hand. J_G values below the optimum would lead to an insufficient oxygenation and an inadequate mixing, while values above the optimum could produce diminution in the cellular division frequency as a result of shear stress (Tramper and Vlak, 1988; Merchuk, 1991a; Merchuk, 1991b; Murhammer, 1991). In addition, higher values of J_G could produce levels of shear stress that will lead to mechanical damage and increase in mortality (Tramper and Vlak, 1988; Merchuk, 1991a; Merchuk, 1991b).

C. Influence of ALR design on cell growth

One of the central points in this work was to measure the influence of ALR geometrical design on the growth of IPLB-Sf-21 insect cell cultures. Two 250 ml ALRs (ALR 1 and ALR 2) and one 500 ml ALR (ALR 3) with different geometrical characteristics were run employing the same superficial gas velocity (J_G = 0.090 cm/min.).

Figure 3, where growth curves obtained in each reactor are drawn, shows that Sf-21 growth kinetics was strongly influenced by ALR design.

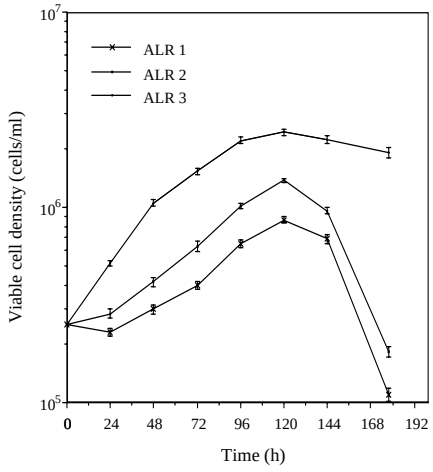

Figure 3. Comparison of the growth curves of IPLB-Sf-21 insect cell cultures in three airlift reactors of different design.

The growth rate and the maximum cell density were consistently influenced by changes in the geometrical configuration of ALRs (Table **1**).

Considering the geometrical characteristics of ALR 1 and ALR 2, it becomes clear that culture performance was enhanced by reactor parameters that improve the liquid circulation: smaller $CSA_{d/r}$, and round bottom. The improvement of liquid circulation is responsible of both better fluidization of the cells and an even distribution of all nutrients, including oxygen. In parallel, oxygen absorption rate is closely related to gas flow rate. The influence of liquid circulation appears to

be confirmed by the results attained in ALR 3, where a higher ratio of height to diameter increased the difference of hydrostatic pressure generated between riser and downcomer, leading ultimately to a higher driving force. In addition, an increase in the height results in a reduction of the time spent by the cells at the top of the reactor, where adverse phenomena can take place as a result of foaming and bubble burst. Foam is capable of traping until 30% of whole cells in suspension culture (Ghildyal et al, 1988; Aunis et al, 1989; Bavarian et al, 1991; Wu, 1995). Bubble bursting has also been pointed out as a cause of death cells in sparged reactors (Handa-Corrigan et al, 1989).

Table 1. Biologicals parameters tested as a function of bioreactor design. Mortality values were registered after 120 hours.

Parameter	ALR 1	ALR 2	ALR 3
Maximum viable cell density (10^5 cells / ml)	8.60 ± 0.35	13.7 ± 0.41	23.6 ± 0.92
Mortality (%)	39.0 ± 3	18.1 ± 0.9	3.2 ± 0.17
μmax. (h^{-1})	0.016	0.020	0.031

Culture viability was also clearly affected by reactor design, as can be observed in Table **1**. The high mortality observed in the ALR 1 could be a consequence of the stress suffered by the cells in the bottom of the reactor as consequence of its flat design (poorer fluidization). This last phenomena had been described previously (Merchuk et al, 1994b).

IV. CONCLUSIONS

In the present work it was shown how through an efficient reactor design the growth rate, the density and the viability of cultures of insect cells in airlift reactors can be improved. As a consequence of better design, the viable cell counts in the ALR almost triplicated at the same fermentation time. In addition, the mortality decreased from 39 to 3 % from the ALR 1 to ALR 3, confirming the improvement of the design from the point of view of cell damage prevention. The enhanced performance of the ALR seems to be closely related to fluid circulation. A superficial gas velocity equal to 0.091 cm/min. was found as the best for our system.

NOTATION

$CSA_{d/r}$	Cross-sectional area of downcomer/riser (-)
J_G	Superficial gas velocity (cm min^{-1})
μmax	Maximum specific growth rate (h^{-1})

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