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Mass Cultivation of *Catharanthus roseus* Cells Using a Nonmechanically Agitated Bioreactor

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ABSTRACT

Batch suspension cultures of *Catharanthus roseus G. Don* were grown in a 5 L LKB Ultraferm fermenter, converted to operate as an airlift bioreactor, to test the suitability of such a system for the mass culture of plant cells. Results show that the airlift system has considerable merits as a culture vessel for such a purpose, including: conversion rates of carbohydrate substrate to cell mass equivalent to >50% under optimum conditions. (Operating under these conditions, growth rates of approximately 0.4 d^{-1} are typical). In the absence of the mechanical shear normally associated with mechanically driven bioreactors, the gently agitated environment of the airlift vessel proves to be an ideal system for the growth of fragile plant cells. Use of a nozzle sparger reduces the possibility of a high mass transfer coefficient, except at very high gassing rates, thereby eliminating any interference with the growth rate caused by high rates of gaseous exchange.

Index Entries: Mass culture, of plant cell suspensions; *Catharanthus roseus*, mass culture of; airlift, in plant cell mass culture; bioreactors, for plant cell mass cultures; growth rate, of plant cell mass cultures; biomass yield, of plant cell sugar conversion, in plant cell mass cultures; mixing rate, in plant cell mass cultures; oxygen transfer, in plant cell mass cultures; ventilation, of plant cell mass cultures; oxygen toxicity, in plant cell mass cultures; aeration rate, in plant cell mass cultures; yield, in plant cell mass cultures; plant cell mass cultures, of *Catharanthus roseus*.

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INTRODUCTION

Cultivation of plant cells on a large scale presents problems different from those normally associated with the cultivation of microorganisms. In particular there is a need to achieve a well-mixed culture in the absence of high levels of shear.

Plant cells are surrounded by a cellulose-based wall that has a high tensile strength but low shear resistance. Because of this, difficulties have been experienced with plant cell cultures grown in classical microbial turbine mixed vessels. In these systems, the high shear levels developed in the immediate vicinity of the agitator blades cause rapid cell lysis and culture death.

An alternative approach is through the use of gas-driven vessels (*1*). In such systems the incoming gas stream agitates and mixes the culture broth, and also provides respiratory oxygen, but without development of high levels of mechanical shear. Furthermore, the power demand in such a system is greatly reduced and arises almost entirely from the air compressor.

Circulation and mixing in gas-driven vessels is dependent upon the bulk flow regime, which is itself related to the difference in specific weight between the air-rich volume and air-poor volume. As the culture is aerated, the lower density of the air/liquid mixture creates an upthrust that results in circulation of medium throughout the vessel in a manner dependent upon the vessel configuration. In the present investigation, a simple air-driven vessel incorporating a draught tube was used to study the relationship between the aeration rate and mixing at low shear values. The data presented illustrates the efficiency of air-lift bioreactors for the mass culture of plant cells and the manner in which it is possible to alter the rate of growth and biomass yield by varying the degree of mixing, as effected through alteration in the level of gassing. In addition, the data also highlights some of the problems experienced when plant cell cultures are overventilated.

MATERIALS AND METHODS

Cell cultures (line CllC) of *Catharanthus roseus* were established from leaf explants on Gamborg's B5 medium (*2*) containing sucrose (20 g L^{-1}), 2,4-dichlorophenoxyacetic acid (1.0 mg L^{-1}), and kinetin (0.1 mg L^{-1}). Medium pH prior to sterilization (by autoclaving at 121°C for 15 min) was 5.8. Experiments were carried out with a 5-L LKB Ultraferm fermenter, incorporating a 94-mm diameter draft tube. The dimensions of the vessel are presented in Fig. 1. The working volume of the bioreactor was 4.5 L and all losses in culture volume caused by sampling and evaporation were compensated for by addition of sterile water.

The culture was aerated using a ring sparger (17 holes, 0.533 mm diameter), at rates of 1.5, 2.0, 3.0, and 6.0 L min^{-1}.

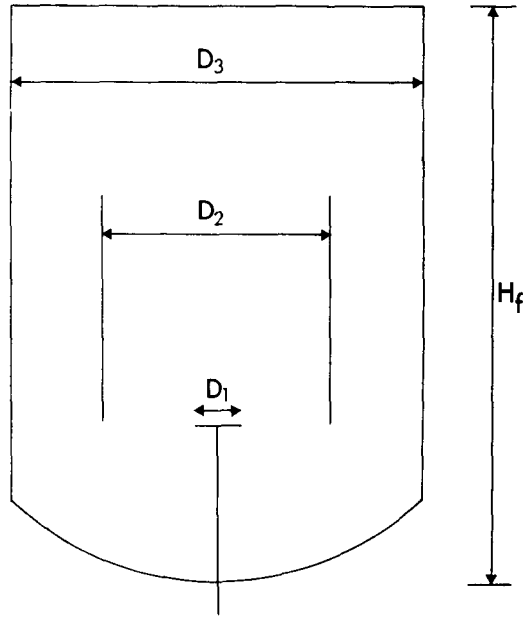

Fig. 1. Schematic representation of the airlift fermenter used in the current studies: D_1, aerator size; D_2, draft tube diameter; D_3, vessel diameter; H_f, fermenter height.

Cultures were initiated with a 10% cell inoculum from a 2 L flask containing 1.2 L of culture. The latter had been grown for 7 d on an orbital shaker (150 rpm) at 25°C. The nutrient broth was B5 (2) with supplements as above. Biomass was measured as both wet and dry weights.

Residual sucrose and fructose in the spent medium were estimated using the anthrone (total carbohydrate) method (3). Residual broth glucose was estimated using Boehringer glucose oxidase/peroxidase test kits. K_La values were measured using the sulfite oxidation method (4).

RESULTS AND DISCUSSION

Experiments were designed to test the effects of the rate of ventilation on the growth profiles of plant cell suspensions. Using airlift configurations, the aeration and agitation are closely interlinked, since they are dependent upon the throughput of gas forced into the system. In such systems, the degree of mixing (as a measure of the agitation), is related to the gas power input, as defined by Eq. (1).

$$\theta = (0.032\ H_f/\sqrt{\mu_{gas}})\ (D_3/D_2)^2 \quad (1)$$

where θ = mixing time, H_f = ferementer height, D_3 = vessel diameter (total), D_2 = draft tube diameter, and μ_{gas} = superficial gas velocity (5).

The major problem with airlift fermentation systems arises from their poor energy utilization. This can cause serious operational prob-

lems connected with fluid movement, particularly during operation at high cell densities.

The optimal gassing rate is dependent upon the total gassed area and is of the order of 0.3–1.0 cm/s. This is optimum in the sense that further increase gives relatively little mixing benefit.

The vessel geometry also plays an important role in the overall mixing process. Two ratios are of particular importance:

First, that of H_F to D_3, where H_F = height of vessel, and D_3 = vessel diameter.

Second, that of $D_2:D_3$, where D_2 = draft tube diameter.

In this case, it has been shown that mixing is expressed by the following relationship (*6*):

$$\theta \alpha H^{1.7} \quad (2)$$

This is obtained by reduction of Eq. (1). Empirically, this confers a height penalty on mixing for this type of system; although conversely, the power-to-gassed-input ratio does increase with increasing height.

With respect to the second point above, the optimal ratio of $D_2:D_3$ is 0.5. In the case of the bioreactor used in the reported work, $D_3:D_2$ has been calculated at 0.58.

The experimental data illustrates the need for a well-mixed environment for optimum productivity. With a progressive increase in the rate of ventilation, there is a corresponding increase in the rate of growth and biomass yield. However, it also illustrates a specific disadvantage of gas-powered bioreactors for the cultivation of plant cell suspensions; that being the ease with which it is possible to overaerate the system. Plant cells have a much lower growth rate and oxygen demand than microorganisms and overventilation of a culture may occur even at relatively low rates of aeration. This is well-illustrated with reference to Experiment 4, Fig. 2, using a gassing rate equivalent to 1.33 vvm. In this example, the biomass yield is severely depressed and is similar to that observed when the aeration rate was 0.44 vvm. The reasons for this depression in biomass yield are not clearly understood; however, two possibilities exist:

1. There is some limitation effect owing to the stripping-off of a key component at high ventilation rates, e.g. carbon dioxide.
2. There is some toxic effect caused by a high concentration of molecular oxygen, probably connected with the formation of peroxide compounds or free radicals. Some preliminary data relating to the problem has already been discussed in an earlier paper (*7*). It should be noted that these effects are not uncommon in microbial and animal fermentation cultures (*8–10*).

With respect to the cell yield, Table 1 and Fig. 3 show this to be a function of the aeration rate. In the case of the K_La, cell yield correlation, Fig. 3, subsequent experiments carried out at intermediary aeration rates to those quoted confirm the effect giving results broadly contained within

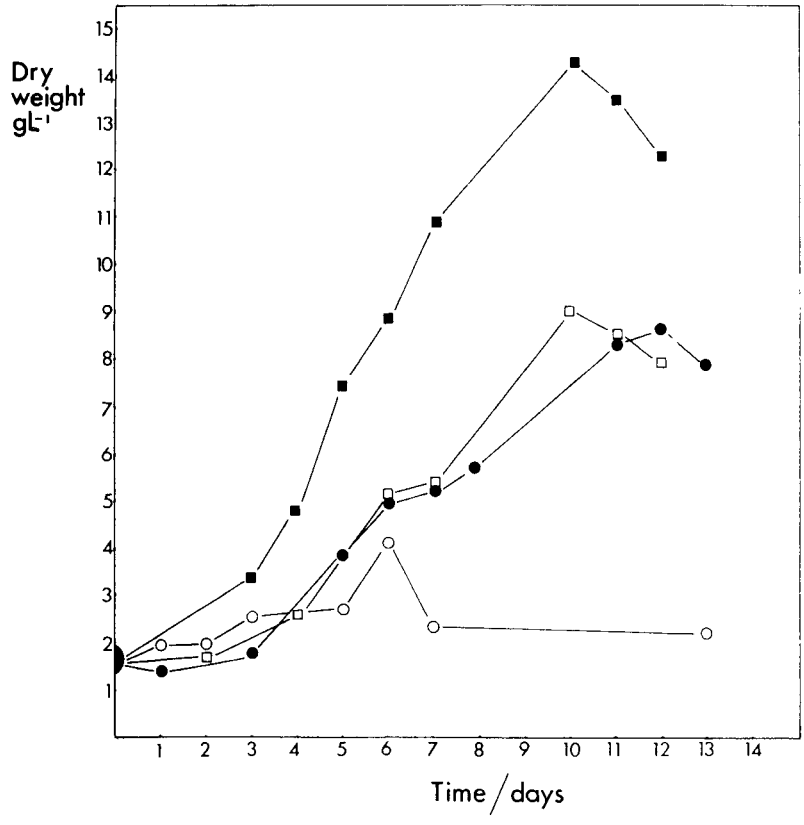

Fig. 2. Comparison of biomass dry weights at different rates of aeration: ○, 1.5 L min^{-1}; ●, 2.0 L min^{-1}; ■, 3.0 L min^{-1}; □, 6.0 L min^{-1}.

the relationship developed for the present data. From Fig. 2, it is apparent that the cultures reach a critical biomass concentration above which the flow is limited. This is particularly marked in the case of Experiment 1, Fig. 2, which shows indications of oxygen limitation.

In the case of Experiment 3, the fluid movement and aeration rate would appear to be optimal, since the conversion of sucrose to cells is at its maximum efficiency. The decline in efficiency in the case of Experiment 4 is probably a result of the effects mentioned earlier.

TABLE 1
Experimental Parameters and Results for *Catharanthus roseus* Growth Experiments

Expt. no.	Aeration rate, L min^{-1}	vvm	K_La, h^{-1}	Growth rate, d^{-1}	Biomass yield (dry wt), g L^{-1}	Time to maximum yield, d	Sucrose conversion, %
1	1.5	0.33	3.5	0.18	4.3	7	24
2	2.0	0.44	6.0	0.28	8.6	12	36
3	3.0	0.66	14.5	0.38	14.3	10	59
4	6.0	1.33	39	0.32	8.9	10	37

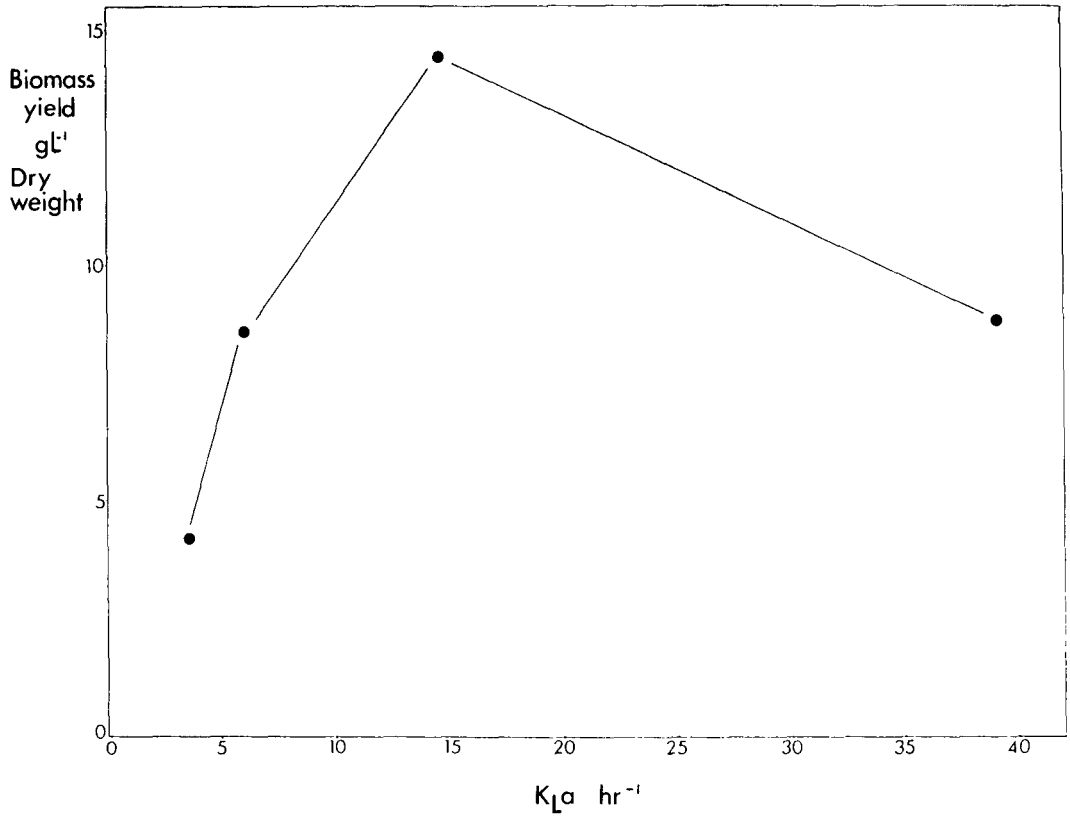

Fig. 3. Correlation of biomass yield with K_La (oxygen transfer coefficient).

Growth rate and sugar conversion follow similar trends to those observed above. In the case of the sugar supply, the conversion into biomass is most efficient in Experiment 3 and least efficient in Experiment 1, Table 1. In terms of the relative uptake of the separate monosaccharide components, there appears to be a decoupled assimilation that varies according to the rate of ventilation (Figs. 4–7). In the case of lower aeration rates (Fig. 7), there is a faster assimilation of fructose from the medium than for glucose. However, in the case of higher aeration rates (Fig. 4), assimilation of both components appears to be similar. This suggests that as the growth rate is altered as a result of the ventilation rate, there is a shift in the cell's carbon metabolism. Although no confirmatory evidence is available, a plausible hypothesis is to suggest that the cells close down all catabolic pathways, other than those involved in the primary fructose pathway, when oxygen is limiting. Currently, this is being investigated in some detail.

As seen in many cell suspension cultures, there is usually a short period of continued growth in terms of the fresh weight as the dry weight starts to decline (Fig. 8). This is probably caused by the assimilation of the starch grains within the cell's vacuoles (which are usually quite large), when the broth sugar supply is depleted. Microscopic exam-

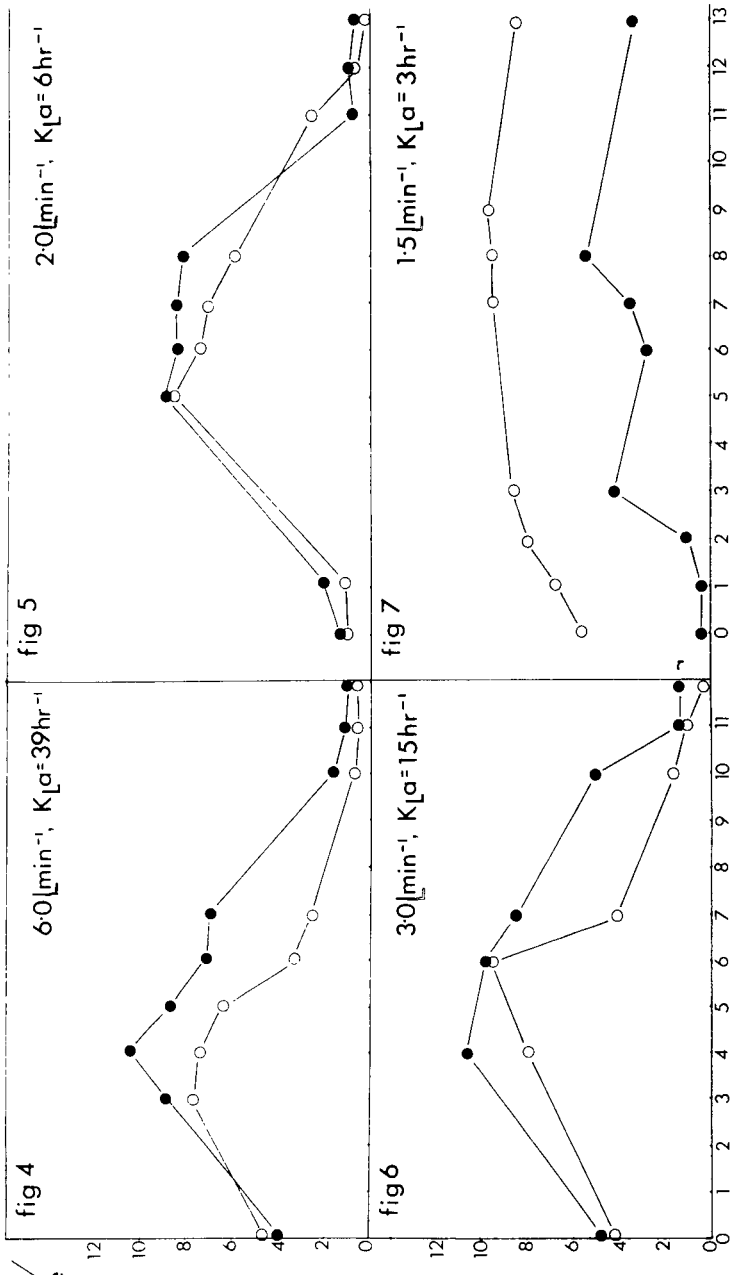

Fig. 4–7. Monosaccharide assimilation at different aeration rates: ●, fructose; ○, glucose.

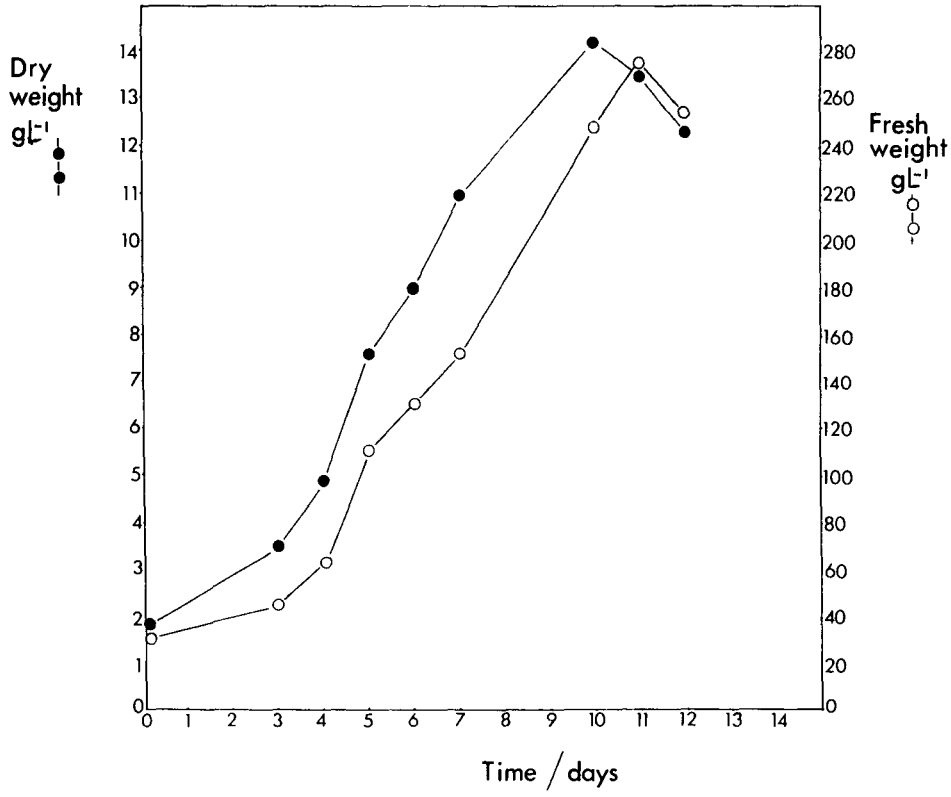

Fig. 8. Relationship between fresh weight and dry weight for a typical batch suspension culture. Aeration rate, 3 L min^{-1}.

ination of the cells during this decoupled period would appear to support this hypothesis.

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