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Immobilization of cultured *Catharanthus roseus* cells using a fibreglass substratum

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Summary. Cultured *Catharanthus roseus* cells were immobilized using geometrically identical needled fibreglass mats prepared with a range of surface coatings. The phenyl (PS), polyglycol (PG), aldehyde (CHO), alkyl (CTMS), and silanol (AW) coatings, along with the untreated glass (HC) surface, produced surfaces with a range of surface tensions. The immobilization efficiency of the substratum, measured as the percentage of cells immobilized, increased with increasing substratum surface tension in the order PS < PG < CHO < CTMS < AW < HC. The dependence of immobilization efficiency on substratum surface tension can be described using a thermodynamic model of adhesion that considers the extent of plant cell adhesion to be a function of the surface tensions of the substratum, the suspending liquid, and the plant cells. In addition, this dependence also demonstrates the fundamental role of adhesion in the immobilization process involving a glass fibre matrix. However, cell entrapment processes are also implicated. The untreated glass fibre substratum (HC), which demonstrated the greatest immobilization efficiency, was used for further characterization of the immobilization strategy. Maximum inoculum biomass was determined to be approximately 1.9 g cells (fresh weight)/g substratum (dry weight) to achieve greater than 90% immobilization efficiency. The growth rate of immobilized cultures was slower than suspension cultures, probably due to mass transfer limitations. Production of the indole alkaloids, tryptamine, catharanthine, and ajmalicine, was also suppressed relative to suspension-cultured cells. These results are considered in relation to other immobilization strategies and their apparent effects on cellular processes.

Introduction

The effectiveness and large-scale applicability of immobilizing cultured plant cells have been issues of considerable debate. In generic terms, immobilization describes the restriction of free movement of any independent biocatalyst such as bacteria, yeast, enzymes, and plant cells. The benefits of immobilizing cultured plant cells, in terms of the development of a commercial-scale bioreactor, can be classified as those resulting from (a) retention of the catalyst within the reactor, (b) high concentration of catalytic activity, (c) microenvironmental control, (d) quantitative and rapid removal of biocatalyst from the product, and (e) uncoupling of growth and production phases. These advantages have been examined in detail in numerous reviews (Brodelius and Mosbach 1982; Rhodes 1985; Rosevear and Lambe 1985).

Despite the advantages of immobilization, the scientific and practical value of the strategy remains controversial (Dainty et al. 1985). From a practical point of view, the present methods of plant cell immobilization are limited in their scale-up capabilities. Two plant cell immobilization techniques which continue to draw attention as well as criticism are entrapment of the cells in a calcium-alginate gel (Brodelius et al. 1979), and retention of the cells within the fibres of a polyurethane foam matrix (Lindsey et al. 1983). Gel methods have been the preferred approach for the immobilization of many types of viable cells. The gel is formed around the cell which remains, in principle, suspended in a normal fluid environment bounded by a hydrogel structure believed by some to be similar to that found in the intact plant. The technique has been used to immobilize a wide variety of cell types which typically show slower growth rates and greater product accumulation than cells cultured in suspension (Brodelius and Mosbach 1982). Besides the limited scale-up capability of a gel system, other concerns include (a) the uncertain metabolic effects caused by the alginate itself, (b) the high calcium content, (c) the required low phosphate levels to reduce chelation of calcium ions and degradation of the gel, and (d) the limited mass transfer capacity of the gel matrix.

Polyurethane foam immobilization overcomes

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many of the limitations of alginate entrapment. This technique exploits the natural tendency of suspension-cultured cells to preferentially aggregate in the pores of a reticulate polyurethane foam matrix (Lindsey et al. 1983). The resulting stable aggregates have been used in small-scale reactor systems demonstrating impressive increases in product synthesis compared to freely suspended cells. The scale-up potential of this system is greater because the substratum is relatively inexpensive (compared with calcium alginate), potentially reusable, and the immobilization method, although slower than gel entrapment, occurs spontaneously. Thus, labour costs will be substantially lower. The most serious limitations of this technique are (a) the possible toxicity of compounds such as polymerizing agents that might be released by the polyurethane polymer, (b) the ill-defined density, distribution, and deposition characteristics of the cells within the polymer matrix, and (c) the unclarified mass transfer efficiency within the cell aggregates.

The problems associated with these methods have resulted in disagreement concerning the effects of immobilization on cultured plant cell secondary metabolism. The typically observed increase in secondary product accumulation by immobilized plant cells has been attributed to increased intercellular contact (Brodelius and Mosbach 1982; Lindsey et al. 1983; Rhodes 1985; Rosevear and Lambe 1985). It is suggested that this increased cellular organization promotes biochemical differentiation of the cultured plant cells resulting in re-establishment of the full biochemical complexity of the intact plant as represented by a more complete phytochemical profile (Lindsey and Yeoman 1983). However, secondary product accumulation by cultured plant cells is affected by many environmental conditions including the depletion of nutrients such as phosphate, a reduction in the dissolved oxygen concentration caused, for example, by mass transfer limitations, and various media adjuvants that can act as elicitors of secondary metabolism. The gel entrapment and polyurethane foam immobilization methods directly influence these environmental parameters. Therefore, the contribution of cell aggregation toward increasing secondary product levels in cultured plant cells remains uncertain.

A cost-effective immobilization strategy will provide a practical tool to develop a commercial-scale process for the fermentation industry (DiCosmo et al. 1988). Exploiting the spontaneous adhesive behaviour of cultured plant cells to a wide range of surfaces offers potential for the development of a simple and effective immobilized plant cell bioreactor. We have previously investigated the fundamental mechanisms that control the extent of plant cell adhesion to polymer surfaces (Facchini et al. 1988a, b, 1989a, b). Using an adhesion model that considers only thermodynamic and electrostatic interactions the adhesion of suspension-cultured *Catharanthus roseus* cells could be predicted and controlled. These studies were performed using polymer substrata and suspending liquids with well-defined physico-chemical properties. In this paper the adhesion model is used to describe the immobilization efficiency of a fibreglass substratum treated with various coatings to produce geometrically identical substrata with a range of surface properties. The substratum configuration selected was a woven fibreglass mat since this provides a large surface area for plant cell adhesion thereby optimizing the effectiveness of a surface-immobilization strategy. In addition, the inert nature of the fibreglass material and surface coatings provide the potential to examine the effect of immobilization on secondary metabolism in cultured plant cells while minimizing the concerns discussed above. An untreated fibreglass substratum was used to immobilize suspension-cultured *C. roseus* cells and examine the effects on growth and indole alkaloid accumulation.

Materials and methods

Plant cell cultures. Cell suspensions of *C. roseus* (cell line LBE-1) have been maintained for three years in MS (Murashige and Skoog 1962) medium containing 0.5 mg/l α-naphthalenacetic acid, 0.1 mg/l kinetin, and 3.0% (w/v) sucrose. The pH was adjusted to 5.5 before sterilization (121° C, 125 psi, 20 min). Cells were propagated as 65-ml cultures in 250-ml erlenmeyer flasks in the dark at 27° C on a gyratory shaker (120 rpm). Stock cultures were subcultured every 14 days using a one to four dilution of cells to fresh medium.

Fibreglass substrata. The fibreglass substrata used for immobilizing suspension-cultured *C. roseus* cells were approximately 10–15 mm thick needled fibreglass mats with a uniform fibre diameter of 10 μm (PPG Industries, Pittsburgh, Pa, USA). The selected surface coatings provided a range of substratum surface tensions (γ_{sv}) which are listed in Table 1. The surface tensions of the various fibreglass substrata were calculated from an equation-of-state approach (Neumann et al. 1974) using the measured contact angle of double-distilled water on an individual fibre. Briefly, the water contact angle on the 10 μm diameter fibre was determined using a modified Wilhelmy balance method (Li et al. 1984). When a solid, which is bounded by vertical surfaces is partially immersed in a liquid, the liquid will either rise or be depressed along the vertical wall due to capillary effects, thus exerting a force on the solid. The magnitude of the force, F, exerted on the fibre by the liquid is

$$F = p\gamma_{lv}\cos\theta - V\rho g \quad (1)$$

where p is the perimeter of the fibre; γ_{lv} is the liquid surface tension; θ is the contact angle; V is the volume of liquid displaced; ρ is the density of water; and g is the acceleration due to gravity.

Thus, if the perimeter of the immersed fibre and the surface tension of the liquid are known, the contact angle between the

Table 1. Surface coatings, water contact angles, and surface tensions of the fibreglass substrata used for immobilization experiments

Coating	Chemical nature	Contact angle θ H_2O (degrees)	Surface tension (mJ m^{-2})
PS	Phenyl	85.7±2.6	31.5±1.7
PG	Polyglycol	73.7±7.9	36.8±7.4
CHO	Aldehyde	62.5±3.8	45.7±2.2
CTMS	Alkyl	52.5±1.6	51.1±1.3
AW	Silanol	48.4±6.1	53.9±3.6
HC	Glass	37.5±4.4	60.1±2.2

fibre and the liquid matrix can be calculated by measuring the pulling force, F. The value of F was determined for four individual fibres of each sample. Each fibre was mounted with adhesive tape to a glass micropipette. The fibre extended approximately 2 cm past the end of the micropipette. This assembly was suspended from a microbalance by bending the end of the pipette into a hook. The fibre was positioned precisely perpendicular to the surface of the liquid prior to immersion. Since

$$F = \Delta m g \quad (2)$$

the change in mass (Δm) of the fibre after immersion in the liquid was used to calculate F. The fibre was slowly immersed through the air-liquid interface with Δm continuously monitored along the length of the fibre as it entered the liquid.

Determination of fibreglass substratum immobilization efficiency. Immobilization efficiency is defined here as the capacity of the substratum to retain a growing biomass in the immobilized state measured as the percentage of immobilized cells versus the total biomass (i.e., immobilized and freely-suspended cells) in the culture system. In order to test the immobilization efficiency of the coated fibreglass samples, each sample mat was cut into a 3 cm × 3 cm square, accurately weighed, rinsed in distilled water and placed into a 250-ml erlenmeyer flask containing 50 ml fresh culture medium. The dry weight of each fibreglass sample was approximately 1.4 g. The medium and fibreglass substratum were autoclaved together as described above.

Each flask was inoculated with 1.2 g (fresh weight) of 14-day-old suspension-cultured *C. roseus* cells. Control flasks did not include a fibreglass sample and were cultivated as suspension-cultures. Culture conditions were as described above (i.e., in the dark at 27° C on a gyratory shaker at 120 rpm) unless otherwise noted. After 7 days some of the cells in each flask were immobilized on the fibreglass substratum while others remained in suspension. At this time the fibreglass substrata, with cells attached, were removed from the flask, washed once with distilled water, and partially dried using a vacuum filtration apparatus. Cells in suspension were collected by vacuum filtration and kept separate. All cells were dried for 48 h at 60° C prior to dry weight determination. The dry weight of the immobilized cells was calculated by subtracting the weight of the fibreglass substratum. All experiments were performed in triplicate.

Immobilization experiments. The loading capacity and time course experiments were all performed using the untreated fibreglass substratum. For all experiments a 3 cm × 3 cm square piece of the fibreglass mat was weighed and sterilized together with the culture medium as described above. Loading capacity experiments were performed by inoculating triplicate flasks with a range of inoculum densities (measured as fresh weight) and collected after 96 h in culture. The percentage of cells immobilized relative to total biomass was determined as described above. Time course experiments were performed using free-cell suspensions and immobilized cultures collected at 2-day intervals during the course of a 16-day growth cycle.

Analytical methods. Cell viability tests were performed by dye exclusion of a 0.5% (w/v) solution of Evan's Blue stain (Taylor and West 1980) and by using the fluorescein diacetate method (Widholm 1972). Fresh weight was determined by collecting cells on Whatman No. 1 filter paper using a vacuum filtration apparatus.

Alkaloids were extracted from samples that had immediately been frozen in liquid nitrogen after fresh weight determination and stored at −20° C for not longer than 14 days. After thawing, the cell sample was extracted in 50 ml methanol in a sonicating bath for 15 min. The methanol extract was reduced to dryness under vacuum, and the residue taken up in 20 ml bicarbonate buffer (sodium carbonate/sodium bicarbonate 6:4 (w/w), pH 10), and partitioned three times into ethyl acetate. The ethyl acetate phase was evaporated under vacuum, and the final extract taken up in 1.0 ml methanol.

Quantitative determinations by the method of external standards were performed using a Hewlett-Packard (Mississauga, Ontario, Canada) 1090 HPLC system, equipped with a UV detector. Separation was accomplished using a Pierce (Rockford, Ill, USA) RP-18 Spheri-5 (220 × 4.6 mm) column. Initial conditions of 60:40 methanol/water (v/v) containing 0.1% triethylamine were maintained for 4 min, followed by a convex solvent gradient to 90:10 methanol/water over 9 min. These final conditions were maintained for a further 2 min. Alkaloid elution was routinely monitored at 230 nm, and alkaloids tentatively identified on the basis of retention times and UV spectra.

Medium alkaloid levels were determined by adjusting the spent medium to pH 10 with 1.0 *M* NaOH, and partitioning three times into equal volumes of ethyl acetate. The ethyl acetate phase was reduced to dryness under vacuum and the final extract taken up in 1.0 ml methanol. Quantitative determinations were performed as above.

Results and discussion

Adhesion of suspension-cultured *C. roseus* cells to individual fibres of the various substrata plays a fundamental role in the immobilization process. This is demonstrated by the results presented in Fig. 1 which illustrate the relationship between the surface tension of the fibreglass substratum (γ_{sv}) and its inherent immobilization efficiency measured as the percentage of cells immobilized. The immobilization efficiency increased with increasing fibreglass γ_{sv}. These results are consistent with predictions of a thermodynamic model, considering only van der Waals interactions and used previously to describe the extent of plant cell adhesion to polymer surfaces (Facchini et al. 1988a). Our previous work has demonstrated that the extent of adhesion can be predicted using the thermodynamic relationship

$$\Delta F^{adh} = \gamma_{cs} - \gamma_{cl} - \gamma_{sl} \quad (3)$$

where ΔF^{adh} is the change in the free energy of the system due to the process of adhesion, γ_{cs} is the cell-substratum interfacial tension, γ_{cl} is the cell-liquid interfacial tension, and γ_{sl} is the substratum-liquid interfacial tension. The experimentally observed pattern in the extent of plant cell adhesion per unit surface area as a function of the surface tensions of the substratum (γ_{sv}),

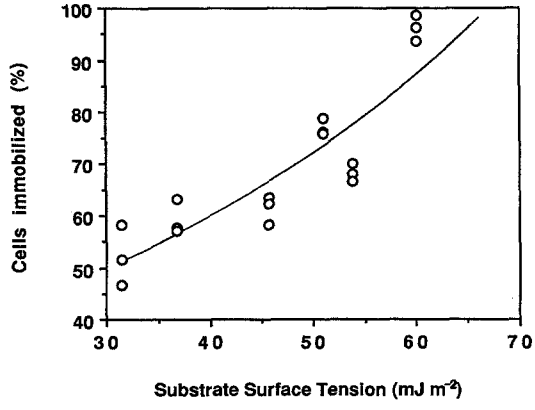

Fig. 1. Percentage of *Catharanthus roseus* cells immobilized as a function of the glass fibre substratum surface tension (γ_{sv}). *Line* was drawn as the best fit curve

the suspending liquid (γ_{lv}), and the plant cells (γ_{cv}) follows the same trend as the theoretical calculation of ΔF^{adh}. Briefly, when $\gamma_{lv} > \gamma_{cv}$ the extent of plant cell adhesion decreases as γ_{sv} is increased. When $\gamma_{lv} < \gamma_{cv}$ the opposite pattern of adhesion is observed, i.e., increased adhesion with increasing γ_{sv}. In the present study the γ_{lv} of the suspending liquid (i.e., Murashige and Skoog nutrient medium) was approximately 47 mJ m^{-2} (data not shown) and the γ_{cv} of the cultured *C. roseus* cells approximately 55 mJ m^{-2} (Facchini et al. 1988a). From thermodynamic model considerations the extent of adhesion should increase with increasing γ_{sv} since $\gamma_{lv} < \gamma_{cv}$ (Facchini et al. 1988a). These predictions have been confirmed experimentally as shown in Fig. 1.

The role of adhesion in immobilizing suspension-cultured *C. roseus* cells using a fibrous substratum is evident from the influence of γ_{sv} on immobilization efficiency. However, the fibrous nature of the substratum suggests that other factors are involved in the immobilization process. Passive entrapment of cells by the fibreglass matrix probably functions in immobilizing a substantial number of cells. In addition, adherent or entrapped cells grow during the course of the culture period and fill the interfibrous spaces (Lindsey et al. 1983). This has two effects on the immobilization process. First, it results in a generally high biomass loading of all fibreglass substrata after approximately 2 weeks in culture. However, the higher surface tension substrata will achieve maximum biomass accumulation more rapidly. Secondly, the increased cell density within the fibreglass matrix prevents any further entrapment of cells remaining in suspension.

The trends shown in Fig. 1 are enhanced by the combination of passive cell adhesion and entrapment processes. The high surface tension substrata allow for greater initial cell loading and adherent-cell density than the low surface tension substrata. The greater density of the adherent cells implies that more cells are immobilized by the adhesion process. Consequently, fewer suspended cells remain in flasks containing the high γ_{sv} substratum because more cells were initially immobilized by adhesion to fibres. Since fewer cells remain in suspension the probability is greater that all of these cells will be immobilized by entrapment. Selection of the high surface tension substratum makes the most efficient use of the available inoculum.

Cell viability tests revealed that cultures immobilized using any of the various glass fibre substrata, and cultures cultivated as suspensions, contained approximately 80%–85% viable cells. Figure 2a and b illustrates the effect of immobilizing *C. roseus* cells using the various glass fibre substrata on total culture growth (i.e., including immobilized and non-immobilized cells), the actual biomass immobilized by each substratum, and the accumulation of three indole alkaloids (tryptamine, catharanthine, and ajmalicine) by cells immobilized using the various substrata after 7 days in culture.

The fibreglass-substratum surface coatings did not affect cell viability or cell growth relative to the untreated fibreglass material demonstrating their inert nature. Generally, however, greater total culture growth

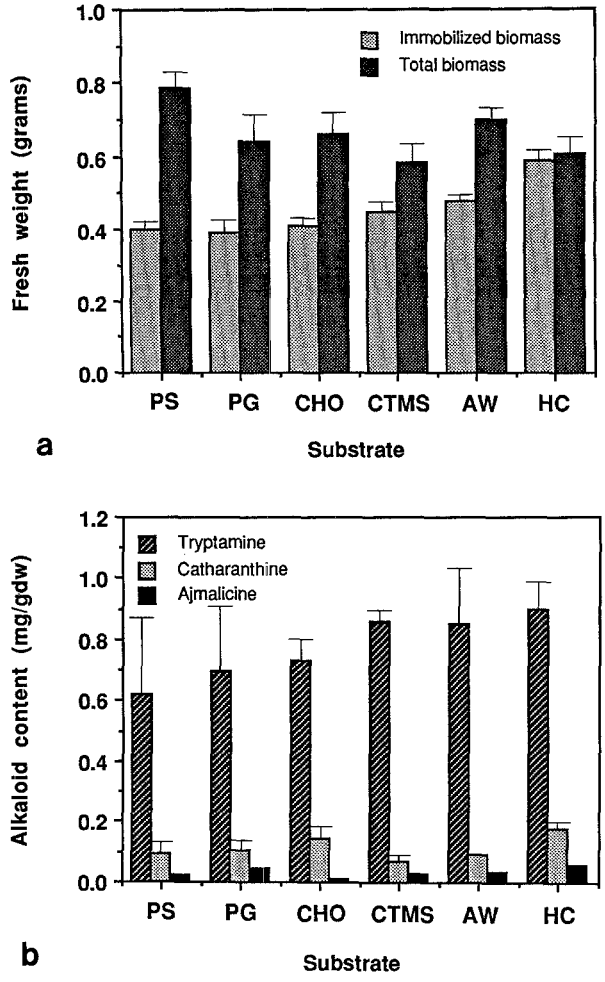

Fig. 2. Effect of the glass fibre surface coating on **(a)** total culture growth (i.e., including immobilized and non-immobilized cells) and the actual immobilized biomass, and **(b)** secondary metabolite production 7 days after inoculation with *C. roseus* cells. Surface coatings were as follows: PS, phenyl; PG, polyglycol; CHO, aldehyde; CTMS, alkyl; AW, silanol; HC, untreated glass. *Error bars* are standard deviations

was observed when the percentage of cells immobilized was lower. This is especially apparent when the culture containing the phenyl-coated substratum (PS) is compared to the culture containing the untreated fibreglass material (HC). These results are explained by the differential growth rates of immobilized and freely-suspended cells. Immobilized cells of *C. roseus* exhibit slower growth than cells in suspension (Brodelius et al. 1979; Majerus and Pareilleux 1986; Payne et al. 1988); this phenomenon will be considered in more detail shortly. The number of non-immobilized cells decreased with increasing immobilization-efficiency. Therefore, the total culture biomass accumulation also decreased with increasing immobilization-efficiency of the substratum.

The various surface coatings were selected to examine the effect of a wide range of γ_{sv} on the immobilization efficiency of the substratum. All of the coatings had the effect of lowering the value of γ_{sv} for the bulk

fibreglass material and reducing the immobilization efficiency of the substratum. However, the presence of free functional groups on the surface of the coated fibres could result in a specific interaction with the cells at the point of contact. This may lead to an altered accumulation of secondary metabolites relative to the untreated glass fibre material. This type of interaction has been observed in surface-immobilized cells of *Solanum aviculare* covalently bound to porous, high-surface-area beads of polyphenylene oxide that had been pre-activated with glutaraldehyde (Jirku et al. 1981). The situation in the present study is similar with respect to the absence of low molecular weight toxins and the mildness of any chemical interaction which prevented the loss in viability that might be expected from direct reaction with cell-wall components. However, as shown in Fig. 2a and b, the various surface coatings had no influence on cell viability, culture growth, or secondary-product accumulation as measured by the analysis of tryptamine, catharanthine, and ajmalicine levels after 7 days, relative to the untreated fibreglass. After 14 days in culture the alkaloid levels remained essentially unchanged.

The untreated fibreglass material with a high γ_{sv} value of approximately 60 mJ m^{-2} demonstrated the greatest potential as an immobilization substratum by making the most efficient use of the available cell inoculum. The various surface coatings offer no practical benefit in terms of improving the immobilization efficiency or secondary product accumulation. Therefore, the untreated material was selected for further characterization. In order to optimize efficient use of the cell inoculum, the loading capacity of the fibreglass material was determined. Figure 3 shows the percentage of cells immobilized as a function of the inoculum biomass (measured as fresh weight) after 4 days incubation with a 1.3-g sample of the fibreglass support in a 250-ml erlenmeyer flask containing 65 ml medium with orbital shaking at 120 rpm. More than 92% of the cells were immobilized when 2.5 g (fresh weight) or less of cells were inoculated into flasks containing the 1.3 g fibreglass sample. When the inoculum contained 3 g (fresh weight) of cells, approximately 55% were immobilized while the percentage immobilized decreased to less than 40% when the inoculum biomass was increased to 5 g (fresh weight). The fibreglass support demonstrates an initial cell loading threshold, to achieve greater than 90% loading efficiency, of approximately 1.9 g cells (fresh weight)/g support (dry weight). All experiments described herein used an inoculum biomass approximately one half of the threshold value. The initial biomass loaded onto the fibreglass material will grow and eventually fill the spaces between the fibres. The amount of time required to achieve maximum biomass accumulation by the substratum will decrease with greater initial inoculum densities.

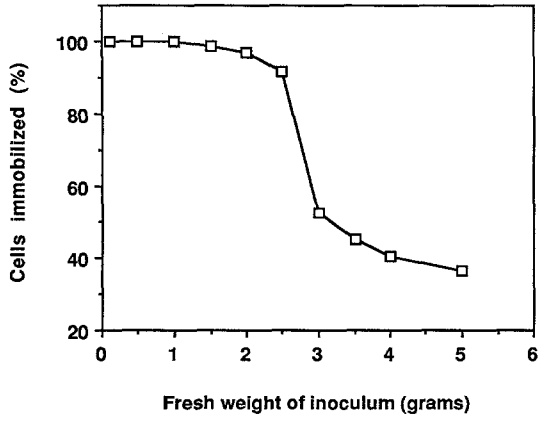

Fig. 3. Percentage of *C. roseus* cells immobilized using the untreated fibreglass substratum (HC) after 4 days in culture as a function of initial inoculum biomass measured as fresh weight. Errors are ±5% of mean value

The immobilization of cultured plant cells on an inert support has been recognized as a means by which the environment of the cells can be easily manipulated (Brodelius and Mosbach 1982; Rosevear and Lambe 1985). Two general responses have been implicated as resulting from the immobilization process: (1) a slower growth rate of the immobilized cells relative to suspended cells under otherwise identical conditions, and (2) increased yields of specific secondary metabolites relative to those in suspension-cultured cells. The proposed reasons for such behaviour have been examined in considerable detail but can be summarized in two points. First, the immobilization techniques usually result in mass transfer limitations of essential nutrients to the cells and waste products from the cells into the bulk medium. Secondly, the increased cell contact and organization of the culture result in the re-establishment of a rudimentary degree of differentiation (Rhodes 1985; Rosevear and Lambe 1985). This results in the expression of necessary secondary metabolic enzymes and, ultimately, in greater yields of specific products (Lindsey and Yeoman 1983). The species-specificity of this phenomenon must be recognized since the response is variable among different species tested (Brodelius et al. 1979; Lindsey et al. 1983; Payne et al. 1988).

The effect of immobilizing cultured *C. roseus* cells using a fibreglass support on growth is shown in Fig. 4a and b which illustrates fresh weight and dry weight, respectively. Over the 16 days examined, the immobilized cells grew at a slower rate than the suspension-cultured cells. Suspension cultures reached the stationary growth phase after approximately 8 days. The immobilized cultures appeared to be in a slowly decelerating growth phase for the entire 16 days but continued to increase in mass to the end of the period. Extrapolation of the line suggests that the immobilized cultures would eventually have reached the same biomass as the suspension cultures. These results are consistent with a mass transfer limitation of essential nutrients and dissolved oxygen to the cells (Robins et al. 1986). Although this immobilization technique produces cultures that remain friable, the high cell densities achieved will invariably limit mass transfer capacity to some degree.

The species-specificity of the immobilization response may also be associated with the immobilization

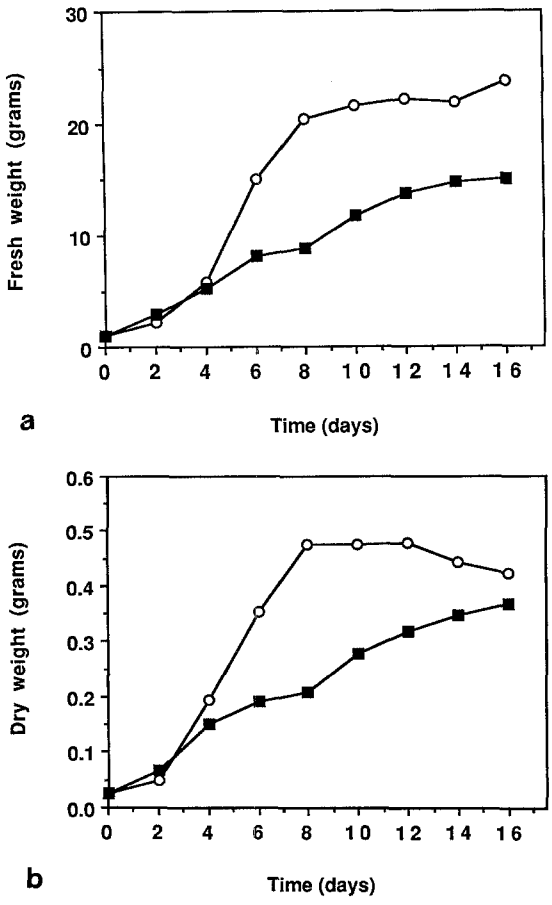

Fig. 4. Growth of suspension (○) and immobilized (■) cultures of *C. roseus* cells determined as **(a)** fresh weight and **(b)** dry weight. For immobilized cultures in Figs. 4 and 5 the untreated fibreglass substratum was used

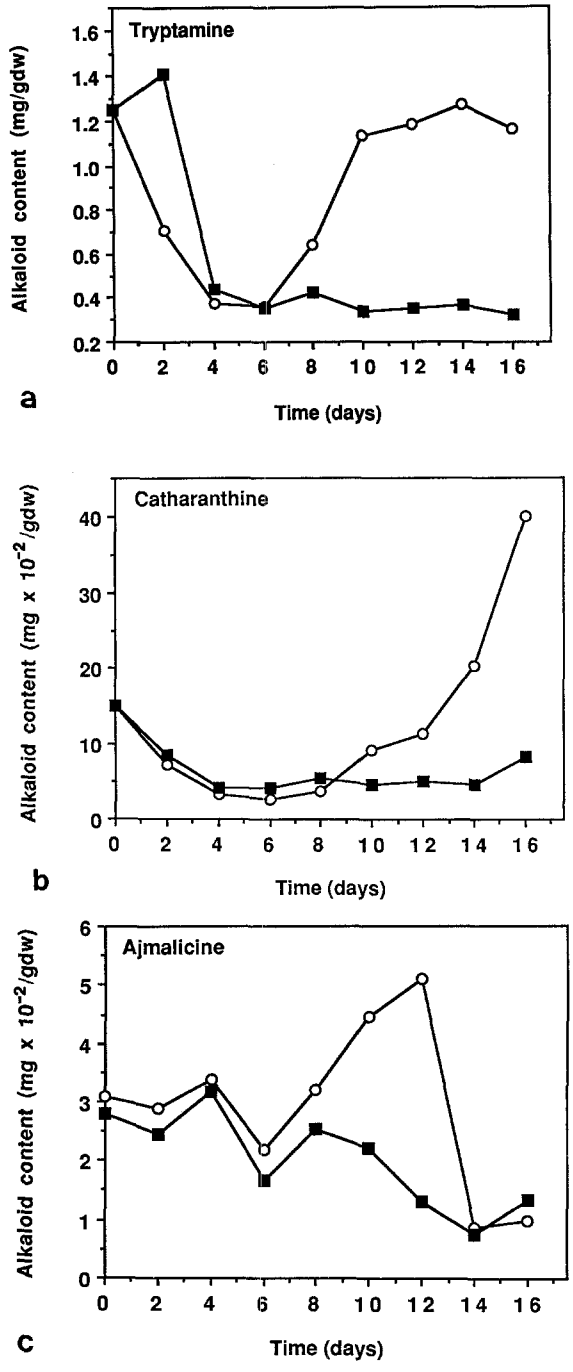

Fig. 5a–c. Comparison of alkaloid accumulation in *C. roseus* cells grown in suspension (○) and as immobilized cultures (■). **a** Tryptamine. **b** Catharanthine. **c** Ajmalicine

technique employed. For example, *C. roseus* cells have now been immobilized using the techniques of gel-entrapment (Brodelius et al. 1979; Majerus and Pareilleux 1986), membrane-entrapment (Payne et al. 1988), passive immobilization on cotton fibres (Payne et al. 1988), and immobilization on glass fibres as reported in this study. Only alginate entrapment has been reported to increase levels of indole alkaloids in immobilized cultures relative to suspension cultures (Brodelius et al. 1979; Majerus and Pareilleux 1986). A complete inhibition of secondary metabolic activity was observed for membrane-entrapped cells and cotton-fibre-immobilized cultures (Payne et al. 1988). However, when these same immobilized *C. roseus* cells were transferred to suspension culture the typical product yields of cells in suspension returned. In the present study, immobilizing *C. roseus* cells using glass fibres resulted in a decreased specific accumulation of tryptamine, catharanthine, and ajmalicine, relative to suspension cultured cells (Fig. 5a, b, and c). No alkaloids were detected in the culture media indicating that cell leakage did not occur.

Despite the difficulties in comparing data, there are similarities in the membrane entrapment and cotton fibre immobilization techniques, and the fibreglass immobilization method reported here, especially in terms of the inert nature of the substratum materials used. Moreover, the response of the cultures to immobilization by these methods, i.e., suppression of secondary metabolic activity relative to suspension cultures, suggests that the direct cell-to-cell contact achieved by these methods inhibits secondary metabolic processes (Payne et al. 1988). The immobilization methods used in this study and by Payne et al. (1988) ensure a direct cell-to-cell contact which is considerably different from calcium alginate immobilization procedures (Brodelius

and Mosbach 1982). These results also support the suggestion that the observed increases in secondary metabolite accumulation by calcium-alginate-immobilized cells are caused by properties of the polysaccharide matrix or ionic environment of the alginate gel. Apparently, these conditions are not reproduced by immobilization techniques employing inert substrata such as fibreglass or cotton fibres. We also suggest that the variable response of cultured plant cells to widely different immobilization techniques in terms of secondary metabolite accumulation implicates a species-specific phenomenon. This may be most important when considering the role of direct cell-to-cell contact in immobilized plant cell cultures. A better understanding of this phenomenon may elucidate some of the regulatory control mechanisms of cultured plant cell secondary metabolism. This knowledge is essential if the biosynthetic potential of cultured plant cells is to be commercially exploited.

Although the surface-immobilization of cultured *C. roseus* cells using a fibreglass support resulted in decreased secondary metabolic activity relative to suspension cultures, we believe that the strategy deserves further consideration. The material possesses excellent scale-up potential because it is inexpensive, inert, reusable, and can be produced in any geometric configuration desired. The immobilization technique relies on the passive processes of cell adhesion and entrapment, thus labour costs are reduced. The inert nature of the substratum eliminates the concern of the indeterminate variables associated with other immobilization techniques. This will provide the opportunity to investigate the true nature of cell-to-cell contact phenomena. Additional benefits of, and aspects concerning, the surface immobilization of plant cell cultures are given by Archambault et al. (1989). Further research must be directed toward evaluating the effects of fibreglass immobilization on other commercially interesting cultured plant cell species.

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