

TABLE 13.3 Potential Advantages and Problems of Large-scale Immobilized Plant Cell Cultures

Potential advantages

- Continuous operation facilitated
- High cell concentrations
- Cell reuse may lead to increased efficiency
- Cells can be protected from shear
- Once immobilized, the slow growth and strain instability of plant cells are no longer problems
- Media can be easily changed, which would be important for processes that require a series of media for optimal production
- Continuous removal of inhibitory metabolites may enhance the overall cellular metabolism or unmask biochemical pathways
- May be able to better exploit the biological relationships between aggregation, morphological differentiation, and secondary metabolite production

Potential problems

- Large-scale aseptic immobilization procedures must be developed
 - Mass transfer limitations may significantly affect cell metabolism (positively and adversely)
 - Products must be produced by nongrowing cells
 - Products must be released from the cell into the medium
 - Experience in the scale-up of immobilized-cell systems is limited
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With permission, from G. F. Payne and others, in B. K. Lydersen, ed., *Large Scale Cell Culture Technology*, John Wiley & Sons, Inc., New York, 1987.

Consider the production of ajmalicine from periwinkle (*Catharanthus roseus*). Ajmalicine is stored primarily in the vacuole in cells in suspension culture, although a small amount (10%) is excreted. Ajmalicine has a pKa of 6.3, so that at the growth pH (5.6) much of the alkaloid is in the neutral form. A neutral resin can be used to remove ajmalicine *in situ*. The *in situ* removal of the product can enhance formation of a product by relief of feedback inhibition or protection from degradation or further conversion.

Figure 13.6 shows the results of experiments involving combinations of *in situ* adsorption, use of a fungal elicitor, immobilization in calcium alginate gels, and the use of a production medium. Using all four approaches increases extracellular concentrations almost 100-fold. The purity is high. The closely related alkaloid, serpentine, has a much higher pKa (10.8) and will not adsorb into the resin. The adsorption of ajmalicine increases its synthesis preferentially. Under some circumstances, the intracellular levels of ajmalicine and serpentine are nearly equal. In this system, the ratio of extracellular ajmalicine to serpentine was 60-fold, with serpentine accounting for a vanishingly small amount of the adsorbed alkaloid. Perhaps the most important result of such an experiment is that at least some normally intracellular compounds can be excreted from viable cells when environmental conditions are correctly manipulated.

13.3.3. Bioreactors for Organized Tissues

In many cases, neither suspension nor immobilized-cell cultures will produce satisfactory amounts of a desired metabolite. However, organ cultures from the same plant may give good yields (Table 13.4). In addition to high yields, organ cultures have a number of distinct advantages (Table 13.5).