of diploid[†]). Cryopreservation of cell lines is not possible in all cases, and many cell lines must be maintained through routine subculture.

This loss of activity can be counteracted in many cases by the active selection of productive subclones. Selection is facilitated when the product of interest is readily visible. In cases where extensive screening and selection have been done, stable, high-producing clones have been reported. A stable, high-producing cell line is essential to any commercial process.

One strategy that has proved to be useful in increasing productivity has been the use of a two-phase culture. The first phase uses a medium optimized for growth, while a second phase uses a different medium optimized for product formation. The first commercial process with plant cell culture has been shikonin production, which utilizes two batch reactors in series.

13.3.2. Reactors Using Cell Immobilization

Further improvements in productivity may be possible due to cell immobilization. Immobilized-cell reactors inherently follow the two-phase approach. Cells are grown and then immobilized; once immobilized, conditions for product formation are optimized. Immobilized-cell systems are advantageous when continuous operation is possible and if the product is, or can be made, extracellular. Some advantages and disadvantages of immobilization are given in Table 13.3. Probably the most important advantage is that the degree of cell-to-cell interaction can be manipulated.

Plant cells will often self-immobilize by preferentially attaching to or within a porous matrix. The resulting biofilm (if on a surface) has been shown to be very effective in a number of cases. Plant cells have also been entrapped in gels or between membranes. Immobilization generates concentration gradients that alter the biosynthetic capacity of the culture (sometimes negatively and sometimes positively). The cell-to-cell contact due to immobilization or the contact of the cell surface with the surrounding gel phase may also alter cell physiology. In some cases, immobilization has improved intrinsic production rates by more than an order of magnitude.

Plant cell cultures can be cultivated at three levels of cell-to-cell communication. In a fine suspension, aggregates are small and mass transfer gradients are unimportant. Any diffusable species that might act as chemical messengers are diluted to a low concentration. Plasmodesmata interconnect only a small fraction of the cells. If cells are concentrated and entrapped, they form a pseudotissue; since the entrapping matrix is typically on the order of 1 to 10 mm, mass transfer gradients become important. Diffusable chemical species can build to high local concentrations because of mass transfer resistances. However, plasmodesmata are relatively unimportant in pseudotissues. If a few cells are immobilized (say, between two membranes or in the pockets of a foam matrix) and allowed to grow in place, they will develop a tissuelike structure. Not only can diffusable species accumulate, but cells will be interconnected through the plasmodesmata. Then immobilization (and the method used for immobilization) can be used as an engineering design parameter to alter the degree and type of cell-to-cell communication. Immobilization can be coupled with other strategies to enhance product formation.

[†]Recall that a diploid cell has two copies of each chromosome; a tetraploid cell has four copies.