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Plant Cell Tissue Culture— A Potential Source of Chemicals

C. D. Scott
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Chemical Technology Division

PLANT CELL TISSUE CULTURE - A POTENTIAL SOURCE OF CHEMICALS

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ABSTRACT

Higher plants produce many industrially important products. Among these are drugs and medicinal chemicals, essential oils and flavors, vegetable oils and fats, fine and specialty chemicals, and even some commodity chemicals. Although, currently, whole-plant extraction is the primary means of harvesting these materials, the advent of plant cell tissue culture could be a much more effective method of producing many types of phytochemicals. The use of immobilized plant cells in an advanced bioreactor configuration with excretion of the product into the reactor medium may represent the most straightforward way of commercializing such techniques for lower-value chemicals.

Important research and development opportunities in this area include: screening for plant cultures for nonmedical, lower-value chemicals, understanding and controlling plant cell physiology and biochemistry, optimizing effective immobilization methods, developing more efficient bioreactor concepts, and perfecting product extraction and purification techniques.

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PLANT CELL TISSUE CULTURE — A POTENTIAL SOURCE OF CHEMICALS

Charles D. Scott and Donald K. Dougall

1. INTRODUCTION

Higher plants are used extensively for food, fodder, and structural materials. However, many chemical products can also be obtained from plants. Various plant-derived pharmaceuticals have been used for hundreds of years, and other products such as flavors, fragrances, and oils have been and continue to be important plant products.¹ In the past, these materials have been extracted from mature plants. But, more recently, interest has been increasing in obtaining products by culturing isolated plant cells that can then be grown either in suspension or immobilized into or onto solid particulates. The culture of plant cells is under intense study by applied biological scientists, and technologists are beginning to consider these systems as well. The current emphasis seems to be on the use of immobilized plant cells with advanced bioreactor concepts. As a result, such techniques are also being seriously considered for large-scale processing. Several useful reviews of this field have been published in the recent past indicating that this research area is becoming more mature.²⁻⁹

It is now apparent that a wide variety of chemical products can be produced by cultured plant cells; however, most research and development has been conducted with systems that produce high-value, low-volume substances. Fuels and high-energy chemicals can be obtained from some plant cells, but many scientific and technological problems will have to

be overcome before the production of lower-value chemicals by plant cells will have economic feasibility.

This report is not intended as an exhaustive review of plant cell tissue culture. Typical but important publication citations and results are listed, but the several excellent review papers on this subject that are included can be the basis for a detailed understanding of the subject and a more complete bibliography.

2. CONCEPTS FOR PLANT CELL TISSUE CULTURE

The advent of plant cell tissue culture is relatively recent. It has been suggested that the first true plant tissue culture was obtained in 1934.¹⁰ The production of chemicals from plant tissue culture was first detailed by Routien and Nickell in 1956.¹¹ Their concept was based on the well-developed use of microorganisms for fermentation. The art of plant tissue culture has been further developed by many other investigators, and, as of 1979, there were over 22 reports of cultures producing concentrations of chemical products that were equal to or greater than those found in the whole plant.^{12,13}

Recent research has shown that there are five systems where product yields in culture were in excess of 20% of the dry weight of the biomass, at least seven more resulted in yields of 10-20% of biomass dry, and another 12 systems resulted in product yields of 1-10% of the biomass dry weight.¹⁴ Even higher yields of product may be essential for the ultimate production of commodity chemicals.

Organized groups of cells that carry out a multitude of specialized tasks are present in plants. However, plant cell tissue culture is

carried out with unorganized cells that are present as suspensions of individual cells, loose aggregates, or sometimes as an immobilized mass of cells.

2.1 SUSPENSION CULTURES

Typically, the tissue culture is initiated by the isolation of small pieces of plant tissue that upon culturing develop a callus of unorganized cells. The callus is then suspended in agitated nutrient solutions that are contained in enclosed vessels. As the cells grow and subdivide, they result in a suspension of unorganized and viable plant cells that can carry out various chemical transformations to produce primary or secondary metabolites or to produce more biomass.

The tissue culture process can be carried out in a conventional stirred-tank system operating as a batch reactor or chemostat,¹⁵ but, in some cases, more novel bioreactor systems have been used. For example, columnar or tank systems in which air is introduced into a central open tube to induce agitation and mixing have been studied.¹⁶ The bioreactor system must accommodate four characteristics of plant cells: (1) they are more sensitive to damage by turbulence than are microorganisms, (2) they grow more slowly than microorganisms (doubling times 20-30 h), (3) they have lower optimum temperatures (25 to 30°C) and lower thermal death points than do microorganisms, and (4) they have a lower oxygen demand than do microorganisms.

2.2 IMMOBILIZED PLANT CELLS

Just as with bioreactors utilizing microorganisms, in some cases, there are certain advantages in immobilizing the plant cells onto or

into a suitable particulate.^{4,6-9} This will retard loss of the cells by washout in the effluent during continuous operation, and it will reduce the deleterious effect of the shear forces that are imposed by fluid motion that is required for good biocatalyst contact and mixing. Thus, immobilization can result in much higher concentrations of the plant cells within the bioreactor but in a protected microenvironment. Further, provided the cells are maintained in an adequate physiological state by supplying suitable nutrients, they should continue producing the chemicals far longer than do cells in batch culture. Most research in this area has utilized hydrocolloidal gels such as alginate and carrageenan that were used to entrap the plant cells into a gel matrix while allowing easy access of substrates.

2.3 DISPOSITION OF PRODUCTS

Perhaps the most efficient bioprocessing concepts for the microbial production of commodity chemicals result in the products being excreted into the bioreactor medium where they can be more easily recovered. But, plant cells in culture have some features that are similar to microbiological processes. For example, the product may be a secondary metabolite that is excreted into the reaction medium or the product may normally be accumulated within the intracellular vacuole of the cells. In the former, the plant cell mass operates primarily as a biocatalyst to convert a substrate to a final product, while in the latter case, it will be important to have a significant rate of biomass propagation in order to have high productivity.

In order to optimize the economics of the plant cell tissue culture process, it will be desirable to utilize a system where the product is excreted into the bioreactor bulk medium. One of the most fruitful areas of research for the production of lower-value products must be the study of methods to induce product leakage from cells that normally accumulate the product. Immobilization techniques coupled with nutrient stress, solvent exposure, pH variation, and phytohormone treatment in conjunction with appropriate illumination have been shown to have this desired effect for some applications.^{7,11-20} Also, adsorption on resins to "pull" metabolites from the cells may promote excretion.

2.4 ENERGY AND GROWTH SOURCES

A number of plant cell cultures appear green and contain chlorophyll, but very few have been shown to utilize photosynthetic energy sources. Even when this has been achieved, the rates of photosynthesis have been low.³ This might be an important area for future research, since use of CO₂ would represent the least expensive carbon source and light would be an inexpensive energy source.

Although small quantities of micronutrients and hormones are required, a usable carbon source is the necessary major feed material. This will be required for cell growth and maintenance, but, also, such a material is usually required for the substrate in bioconversion processes. Just as in most microbial fermentation processes, simple sugars are the substrates of choice.³

2.5 BIOPROCESSING SYSTEMS

The basic processing steps required for plant cell tissue culture systems, with an emphasis on bioconversion of a substrate, are shown in Fig. 1. These are quite similar to comparable microbial fermentation systems and will include a means of producing cellular mass either as the crude product or as the necessary biocatalyst for a conversion process. For lower-value, high-volume products, it will undoubtedly be desirable to use a system where secondary metabolites are excreted into the succeeding bioconversion reactor. In this case, the tissue cells will act as the conversion catalyst either in free suspension but more likely in an immobilized form within the bioconversion reactor. Product recovery and purification steps must also be included.

3. PRODUCTS FROM PLANT CELL TISSUE CULTURE

Although productivity from most plant tissue culture processes with today's technology is too low to be economic, except for a few high-valued materials, significant recent advances have been made and additional research will undoubtedly allow even more highly productive systems. Various researchers have demonstrated that a wide variety of chemicals can be produced from plant tissue cultures, at least as many different types as are found in the numerous species of plants.

3.1 HIGH-VALUE PRODUCTS

The entries in Table 1 illustrate the diversity of uses and types of high-value materials that have been detected in plant cell cultures, regardless of their yield. In many of these cases, the yields are

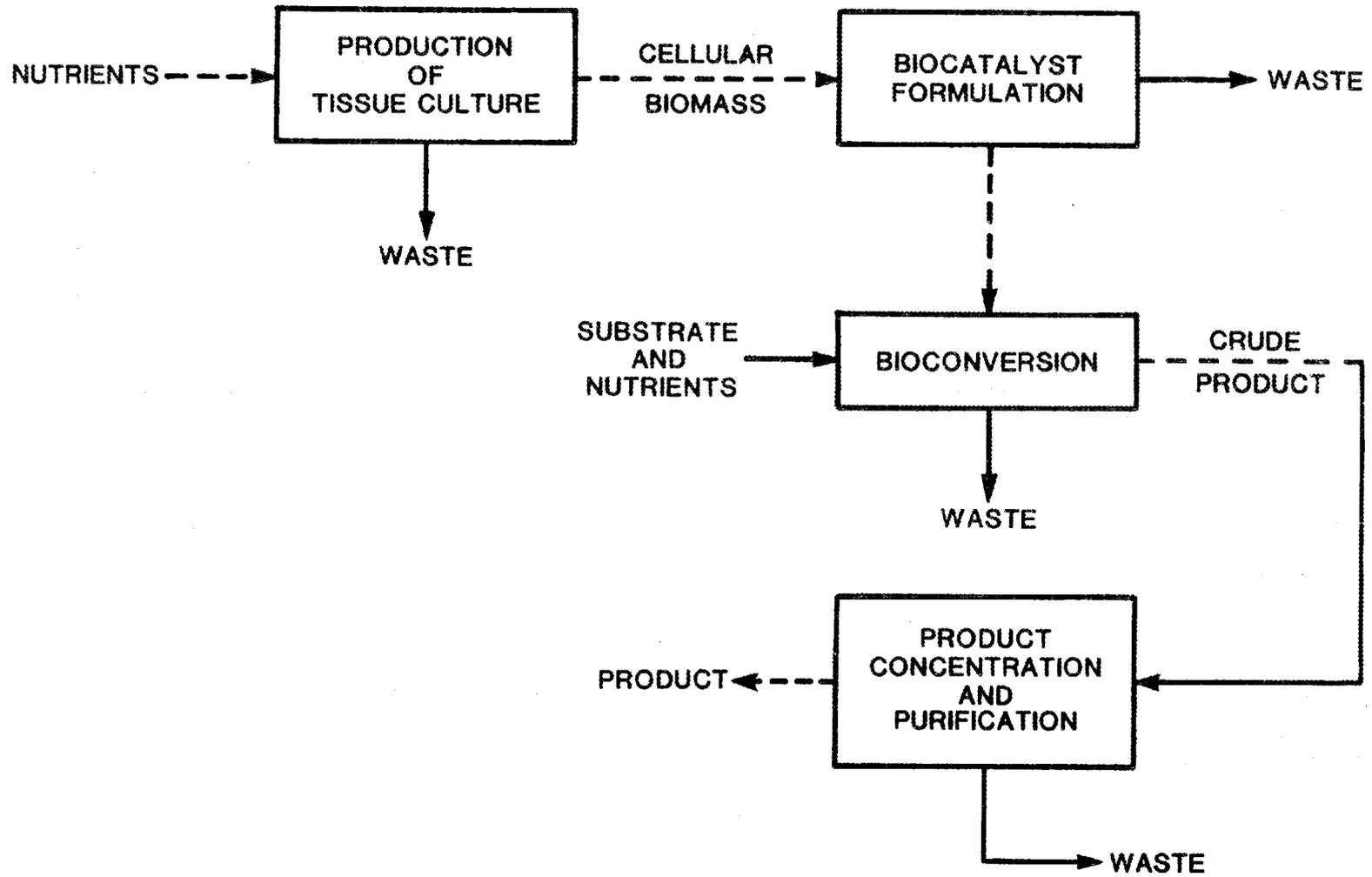


Fig. 1. Typical processing steps in a plant cell tissue culture bioconversion system.

Table 1. Typical high-value substances that have been found in plant cell tissue culture^{1-6,12-14}

Types of substances	Products
Agricultural and chemicals	Growth regulators
	Hormones
	Insecticides
	Plant virus inhibitors
Biochemicals	Amino acids
	Enzymes
	Enzyme inhibitors
	Lipids
	Nucleic acids and derivatives
	Peptides
	Proteins
Vitamins	
Food additives and fragrances	Condiments
	Emulsifiers
	Flavanoids
	Flavors
	Fragrances
	Perfumes
	Spices
Sweeteners	
Medicinals and drugs	Alkaloids
	Allergens
	Anticancer agents
	Antimicrobial agents
	Cardioactive substances
	Opiates
	Immunochemicals
Steroids	

quite low, but where yield improvement has been attempted by optimizing medium and/or strain selection, remarkable increases have been achieved. Now there are reports of ginsenosides at 27% dry wt of Panax ginseng cultures;¹² rosmarinic acid at 23% of dry wt of Coleus blumei;¹⁴ shikonin (naphthoquinones) at 12% dry wt in Lithospermum erythrorhizon;¹² berberine at 10% dry wt in Berberis stolonifera, 8% dry wt in Coptis japonica, and 12% dry wt in Thalictrum minus;¹⁴ and anthraquinones at 27% dry wt in Morinda citrifolia and 22% in Galium glaucum and Rubia fructosa.¹⁴ These yields are, in all cases, increases over those found in the plant by a factor of 5 or more. Thus, some tissue cultures may significantly enhance the yield of desirable plant phytochemicals.

The materials produced by these cultures are either high-value compounds with potential commercial value or model compounds chosen for experimental/demonstration purposes. However, there are at least two of these that have been commercialized. One, shikonin, which is traditionally used as a surface antiseptic and a dye, is being produced by tissue culture for use in "biolipstick" in Japan.²¹ The other, ginseng, is being produced for human consumption using tissue cultures in Japan. In the case of ginseng, the processing vessels used are 20 m³ in volume.²²

Most of the past research on plant cell tissue culture has emphasized the production of medicinals and drugs; however, food additives including flavors and essential oils may also become important products, and various other biochemicals and specialty chemicals are likely candidates.

3.2 LOWER-VALUE CHEMICALS

Various oils, carbohydrates, hydrocarbons, and various oxychemicals are also important products of many higher plants. Tissue cultures of such plants may also ultimately produce viable products that could have an important impact on future sources of fuels and energy-intensive chemicals. Some of the types of compounds which fall into this class and which have been detected in plant tissue cultures are listed in Table 2.²³

Table 2. Commodity-type chemicals that have been detected in tissue culture

Commodity chemical	References
Ethylene	24, 25
Latex	26
Oils and lipids	27-31
Organic acids	23, 32, 33
Phenolics	34-41
Sugars and other carbohydrates	32, 42-48
Terpenes	49-55

Ethylene is an important chemical feedstock that is currently produced from fossil materials. However, a whole series of plant tissue cultures have been shown to produce this product. These include mung bean, soybean, rose, flax, wheat, rice, and others.²⁴ Ethylene would be an interesting product since it is a gas and would be relatively easy to recover from the reaction vessel. Yields of this product are quite low

at this stage of development, but some cultures can be stimulated by chemicals such as 2,4-D.²⁵

Various plant latexes have been used for rubber and other important commercial materials. Synthetic latexes are now made primarily in the petrochemical industry. Such material has also been produced as secondary metabolites by plant cultures. There has been a limited amount of work on the activation of such systems by chemical additions.²⁶ Such material would be relatively easy to recover from a bioreactor since it is insoluble in water.

Several different types of lipids and various oils can be produced in plant tissue cultures. These materials will generally have to be recovered from the tissue itself. The lipid content of morning glory cell suspensions and several other species has been studied.²⁷⁻²⁹ Plant oils are produced by several different types of tissues, and there has been a specific interest in palm oil.^{30,31} The consensus as of 1974 was that it would not be possible to economically produce this material on a commercial scale. Certainly, a significant amount of additional research would have to be carried out before technical feasibility is fully established.

Organic acids such as acetic and citric are important products in the conventional fermentation industry, and additional research will make these processes more efficient. At least 18 organic acids can also be produced in plant cell tissue culture,²³ although there has been little recent research in this area. Of those mentioned, citric acid³² and oxalic acid³³ represent industrial chemicals that have large tonnage demand.

Several different reports have indicated that various types of phenolics and polyphenols are constituents of plant cultures.³⁴⁻³⁸ Surprisingly, however, this type of constituent has not been studied extensively. There is evidence that some chemical and environmental factors are critical in enhancing the yield of phenolics.³⁹⁻⁴¹ This can be the basis for an enlightened research effort that might result in viable process concepts.

Although simple sugars are produced in many plant tissues, there are only a few reports on the production of sugars in plant cell tissue culture.^{32, 42, 43} In most cases, these substances are used as the carbon and energy source. Several investigators have reported on the production of extracellular polysaccharides that occur in plant cultures.⁴⁴⁻⁴⁸ Such products could have unique properties with an important industrial market.

Many different terpenes, similar compounds, and mixtures of terpenes have been important products of plants. These hydrocarbons have properties that could allow them to be considered for chemical feedstocks or fuels if they could be produced economically. Monoterpenes have been reported in some tissue cultures,⁴⁹ but several different di- and triterpenes and terpenoids have also been studied in tissue culture.⁵⁰⁻⁵³ Some sesquiterpenes have also been reported in cultures of Andrographis paniculata.^{54, 55} There has been little research in enhancing yields or studying chemical activators for these products.

There has been no systematic examination of the effects of operating conditions including medium composition on the yield of lower-value chemicals. Despite the absence of such data, it is reasonable to assume

that significant improvements can be made on the production of these industrially important chemicals by plant tissue cultures, especially since intense research on the production of high-value chemicals has resulted in literally order-of-magnitude increases in yield and production rate.

4. CHOICE AND OPTIMIZATION OF PLANT CELL PROCESSES

Although there has not yet been a serious effort to develop plant cell processes for the production of lower-value chemicals, the sequence of development would be similar to that for high-value products (see Fig. 2). This will start with the choice of the product and progress through the actual development of a process and ultimate verification of the integrated system. As these steps are considered for commodity chemicals, past experience with other systems should be very relevant.

4.1 CHOICE OF PRODUCTS AND FEED MATERIAL

Since an economic process will probably demand an excreted product that can be produced in a continuous bioconversion system, our choices should probably be restricted to those types of systems. The choice will also be affected by several market considerations. Although Table 2 does not represent an exhaustive list of such chemicals, it could be a reasonable initial slate of products.

Another way in which candidate chemicals could be identified is to find types of chemicals which are produced widely in whole plants. Their production by whole plants implies the presence of the genetics and of adequate enzymatic machine for synthesis in these plants. These

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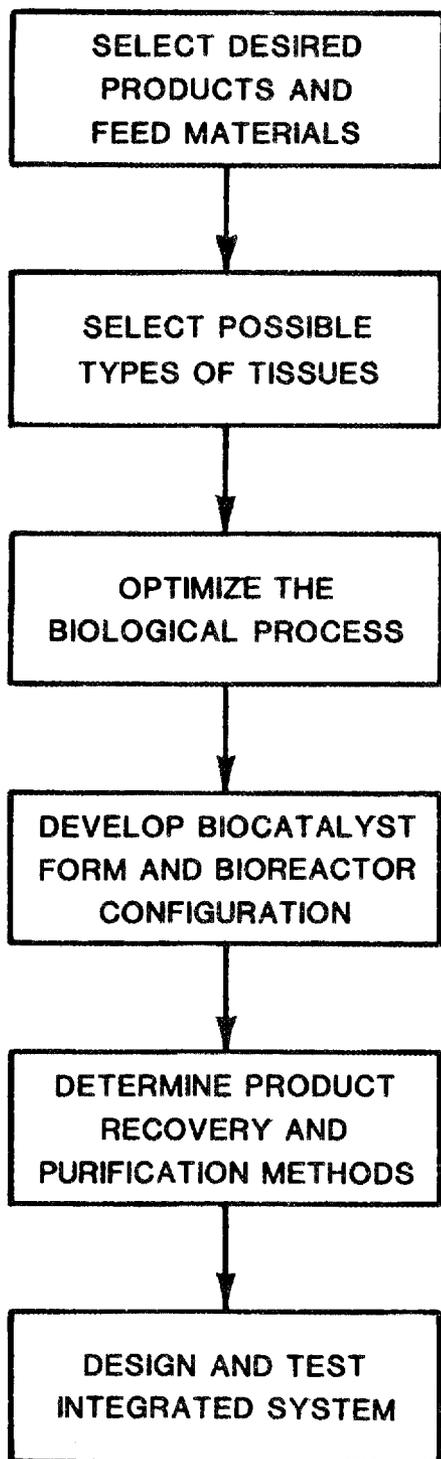


Fig. 2. Steps required in the development of a viable plant cell tissue culture processing step.

capacities should also be present in tissue cultures from the same species. Carbohydrates are extensively present in the cell walls and, thus, could be legitimate target products, but the simple sugars are of very low value. Perhaps unique polymeric carbohydrates would be a better choice.

Plants are also characterized by extensive production of phenolic compounds including not only monomers but also high cross-linked substances such as lignin. These and various chemicals that are produced as intermediates in the biochemical pathway may be useful candidates. The other types of materials listed in Table 2 should be considered, especially if they can be produced continuously as excreted products.

The carbohydrate feed material or substrate chosen for each step should be as inexpensive as possible, probably one of the simple sugars that is commercially available from one of several biomass sources. Photosynthetic systems using CO₂ as the carbon source also represent intriguing potential processing approaches.

4.2 CHOICE OF PLANT CELL CULTURES

In a few cases, there is some very preliminary information on the production of commodity-type products in tissue culture, but the yield in plant tissue cultures is somewhat correlated with the yield in the whole plant, so some of the preliminary screening can be carried out with the whole plant tissue. Thereafter, those that have the highest yields should be further tested in tissue culture.

In such a screening of species and varieties, one must consider that some media (particularly depending on the growth regulators used) may allow no detectable production of the target compound even in cultures from plants known to be genetically competent to produce the target compound. In those cases, the impact of a variety of growth regulators on the growth of this tissue or on target product production rate must be determined.

4.3 OPTIMIZING THE BIOLOGICAL PROCESSES

At least two biological processes must be considered: (1) the production of the tissue cells that will act as the bioconversion catalyst or even the source of the end product, and (2) the bioconversion process itself where the tissue cells carry out the chemical transformation. Optimization of these steps will require extensive study of the substrate composition including carbon sources, nitrogen sources, phosphate concentration, pH, and a detailed study of growth regulators for steps (1) and (2) and metabolic regulators for step (2). Quite likely, the optimum parameters for these two steps will be different.

Cultures consist of a mixture of cells with different levels of accumulation of chemicals or metabolic rates. As a consequence, improvements in productivity can be achieved by selection from within the original culture. Improvement has been achieved by examining subpopulations and by examining single cells for their yields. Improved yields from subpopulations appear to be stable on subsequent passaging of the culture, whereas improved yields from single cells appear to be unstable. However, this correlation is not firmly established nor is its basis understood.

The possibility that some cultures or strains may not be entirely stable over long term is a cause for concern; however, this can be partially accommodated in systems where the bioconversion process is not coupled with growth. There must be attention to detail in the maintenance of cultures to minimize variations. Further, with selected strains, the rate of decline in yield in one case was the result of differences in media composition. It is difficult to determine if changes in the chemical environment result in preferential selection of unstable components of a population or if spontaneous mutations are occurring. One would expect to be able to minimize changes in yield by using some sort of selective agent in the cultures. However, methods have not been designed to link the growth of cells to their ability to produce a target compound and, thus, favor the high-producing cells in the population. It is clear that, while uncertainty concerning yield stability exists and knowledge of its mechanistic basis is nonexistent, a significant problem of long-term stability of the use of plant tissue cultures for any process will continue to exist.

4.4 CHOICE OF BIOCATALYST FORM AND BIOREACTOR CONFIGURATION

For bioconversion processes, the plant cells can be used as suspended cultures or immobilized into a protected environment that remains resident in the bioreactor. Much of the recent research with plant cell systems has been directed toward the use of cells immobilized into gel beads or entrapped on one side of a membrane. Where possible, these immobilization techniques appear to be superior to suspended cultures. Since the cells are more protected, a higher cell concentration can be maintained and higher production rates are anticipated.

This technique would not be useful if the cell mass had to be harvested in order to recover the product.

There is some experience in the use of large, stirred-tank bioreactors to grow tobacco cells. Here a marine impeller was used at very low speed (~30 rpm) with a reactor having a height/diameter ratio of unity.⁵⁶ These conditions reflect characteristics of plant cell cultures, namely large cells > 100 μm which are damaged by high turbulence in the vessel, but for which low oxygen exchange rates are needed because of slow metabolism of slow-growing cells. An additional consequence of the slow growth rates of plant cells is that great attention has to be devoted to the achievement and maintenance of sterility in bioreactors, especially where suspension cultures are used. Immobilized cells may have some degree of microasepsis that would represent another advantage of immobilization.

There has been some concern and examination of problems which occur as the density of the culture increases.⁵⁷ These are associated with the consequent viscosity of the suspension resulting in incompletely reversible effects of shear force on the fluidity of the suspension. Thus, an exploration of cylindrical vessels rotated on their side has been performed.⁵⁸ In addition, tall vessels designed to enhance the stirring action of the air stream and with no mechanical stirring have been used. These, however, appear to be ineffective at higher culture densities and require additional mechanical agitation.⁵⁹

In cases where a two-stage fermentation is used, one for biomass production followed by one for chemical production by cells, the requirements of the bioreactors for each stage will probably be different. For

example, in the second stage a very high loading of biomass with consequent effects on viscosity, stirring characteristics, and gas transfer may be desirable to increase the productivity of the second stage. If immobilized cells are used for production of the chemical, then the effects of factors such as tissue density in beads, bead size, as well as agitation, aeration, etc., in bioreactors would need to be examined to develop optimum conditions for maximum productivity. In that case, the resulting bioreactor configuration might be a fixed bed or fluidized bed.

The operating mode of the bioreactor must also be optimized. Continuous operation is preferred, but where batch reactors are considered, the substrate addition techniques must be established. The optimum process will probably include a two-step configuration with the growth of biomass in a stirred tank followed by a continuous columnar or membrane reactor utilizing high concentrations of immobilized cells for the conversion process.

4.5 PRODUCT RECOVERY AND PURIFICATION

The preferred process will result in the target chemical being excreted into the bioconversion reactor fluid stream, either as a gas or liquid. In the former case, simple phase disengagement followed by adsorption or membrane processes should result in a useful product. Where the product is dissolved in the liquid phase, some of the same processing techniques that are found to be effective in the fermentation industry will be useful here.

Most product concentration and purification techniques will require a post bioreactor stage where preferably low-energy-requirement separa-

tion processes will be used such as adsorption, ion exchange, and solvent extraction. In some cases, these same techniques may be incorporated into the bioconversion reactor itself if the separation reagent does not interfere with the bioconversion kinetics. This complicates the design and operation of the bioreactor usually by adding another phase but it does reduce the number of processing steps. It may also be effective in cases where product inhibition of the bioconversion step is a problem or where the product must be protected from oxidation or destruction.

5. RESEARCH AND DEVELOPMENT OPPORTUNITIES

Since the use of plant tissue cultures is at a very early stage of development and because the production of commodity-type chemicals imposes additional requirements on the system, several important research and development needs can be identified. Some of these can be addressed using model systems before a target compound is identified, others may more properly be addressed in the context of the study of process concepts for the production of specific chemicals.

5.1 SCREENING PLANT CELLS FOR CHEMICAL PRODUCTION

It is important to expand the very limited data base on plant cell tissue cultures that can produce lower-value chemicals (Table 2). At the very least, much additional screening work should be conducted on plant cells from plants that are known to accumulate such chemicals. A serious effort should result in the identification of several more species that can be considered for process applications.

5.2 RELEASE OF CHEMICALS FROM CELLS

Economic plant tissue cultures for the production of lower-value chemicals or fuels will probably require a bioconversion process that results in the release of the product from the plant cell to the bioreactor environment, but many compounds are stored in the unique vacuole internal to plant cell walls. Limited data suggest that the membrane around the vacuole, separating it from the rest of the cell's contents, is different from the membrane surrounding the cell and delineating it from the environment. Some empirical probing of these differences with the objective of enhancing transfer of compounds from the vacuole to the culture medium would be very useful. This could include the development of additional chemical or environmental agents to induce such excretions. A better understanding of elicitors could also result in the control and enhancement of product formation.⁶⁰

A systematic study of the isolation and purification of the two membrane systems together with studies of their composition, function, and structure may be even more productive. A study of the intracellular compartments in which synthesis of chemicals occurs may also be necessary, because if synthesis occurs in one location in the cell and is followed by transport to the vacuole for accumulation, then an alternative to be seriously considered is prevention of vacuolar accumulation and consequent enhancement of release into the medium.

5.3 CONTROL AND ENHANCEMENT OF CELL GROWTH

The growth of plant cells will certainly be an important step in plant-tissue culture processes. Therefore, studies on the control and

enhancement of cell growth are very appropriate. Several types of chemicals are known to affect cell growth. Analogs of compounds with growth regulator activity, such as those that include chlorine or other halogens, may have very large effects on the yields of chemicals with only minor effects on biomass accumulation.⁶¹ Information on the effects of such analogs on phytochemicals production is urgently needed to provide a systematic body of data. How these analogs achieve such remarkable differences in yield is not understood. The mechanisms may be associated with the amounts of essential enzymes in the biosynthetic pathways or it may be associated with the supply of enzyme-synthesized precursors. It is tempting to speculate that these compounds control the activity and expression of the genes for biosynthesis. However, in the case of most phytochemicals, a great deal of knowledge about the biochemistry and regulation of synthesis will be needed before questions of gene responses can be addressed.

The doubling time of plant cell cultures is generally 24–36 h when they have been well adapted. With improvements in medium, 18 h has been reported for tobacco.⁶² Since the cells in the root tips of most whole plants have cell division times of 10 to 14 h, this may also be possible in tissue culture. Decreasing the doubling time of plant cell cultures to 12 h would have an important impact on the rate at which biomass could be generated. As a first step, medium optimization should be performed followed by determination of whether oxygen availability further limits growth rate. There is sporadic information in the literature to suggest that environmental limitations are occurring. An

additional area of investigation could include application of a selection pressure to cultures for increased growth rate.

5.4 MECHANISM BY WHICH YIELD CHANGES OCCUR IN CELLS IN CULTURE

Wide variations in phytochemical yield have been seen when cells are taken from a culture and grown separately. The range of yields does not significantly decrease even if the high-yielding cultures are passed through the same cycle. Thus, there appears to always be a heterogeneous population. Clearly, populations in which all cells are not producing the maximum amount of chemical are less productive than those which are.

The nature of the differences between high- and low-producing cells, the mechanism or mechanisms by which changes in cellular yield occur, and methods of preventing these changes need to be identified. Such an effort would call for quantitative investigations of individual cells and their behaviors, for biochemical analysis of the differences between high- and low-yielding cells, for studies of the molecular mechanisms by which the changes occur, and for the design and testing of methods to prevent high-yielding cells changing to low-yielding cells.

5.5 INCREASING THE RATE OF PRODUCTION OF PHYTOCHEMICALS

The rate or the amount of a chemical produced by a culture may be limited by the availability of enzymes or precursors required. Research is needed to develop methods for activating or maintaining the synthesis of required enzymes. In some cases, this will require development of the necessary biochemical knowledge of the pathways. If precursors are limiting, the problem is similar in that their supply

must be enhanced. The rate of accumulation or excretion of a chemical may be limited by its rate of destruction in the cell. Rates of synthesis and destruction of chemicals in cells can be measured. If destruction is significant, then removal of the enzyme catalyzing the first degradative reaction would be needed. Knowledge of the biochemistry of this reaction would be required for progress in this direction whether through design of inhibitors of the enzyme or via removal of the gene for its synthesis.

5.6 CELL IMMOBILIZATION CONCEPTS

Preliminary results indicate that for many applications, the immobilization of the plant cell biocatalyst could result in better operation with better productivity. This is especially true for advanced bioreactor concepts such as continuous fixed beds and fluidized beds. However, the immobilization techniques currently used have not been optimized for plant cell use, but rather they have been adapted from similar research with microbial systems. The effect of cell-to-cell contact could be very important, and immobilized cells may behave much differently from suspended cells. Thus, a more systematic investigation of immobilization would be very useful. Encapsulation of the cells into gel matrices, entrapment into meshes and porous materials, and the isolation by membranes should all be more thoroughly studied.

5.7 ADVANCED BIOREACTOR CONCEPTS

Much work has already been carried out on advanced bioreactor concepts for other bioprocessing applications. Many of these results can be directly adapted for plant tissue use. Bioreactors for cellular

growth will likely be relatively conventional stirred tanks, hopefully operating continuously, or more efficient configurations such as air-lift systems. Optimization of the operation of this type of reactor for specific application will be required.

On the other hand, the bioconversion step for production of lower-value chemicals or fuels will probably be most efficiently done with continuous bioreactors that allow high biomass loading and that can accommodate the fragile plant cells. Fixed-bed, fluidized-bed, or membrane bioreactors with immobilized plant cell biocatalyst would probably be the most appropriate for this application. A thorough study of this class of bioreactors for plant cell applications should be carried out. Important factors to consider should include:

(1) minimization of the effects of shear force, (2) optimization of the immobilization technique, (3) enhancement of necessary transport phenomena, and (4) the potential for the use of a combined bioconversion and product recovery system.

6. CONCLUSIONS

Plant cell tissue culture is progressing from an art to an understandable science and technology. At this stage of development, commercialization of two plant tissue culture processes for high-value products has occurred. Information on plant tissue systems for the production of lower-value, commodity-type chemicals or fuels is very sketchy, but there is an indication that a variety of such chemicals can be produced by plant tissues. These processes are not well understood, and yields and production rates are far less than required for economic

consideration. However, some of the same research approaches used for high-value products coupled with advanced bioprocessing concepts, such as cell immobilization and continuous bioreactor systems, should materially enhance the productivity.

This is certainly not a short-term research task, and ultimate economic success cannot be completely ensured for any particular product, but a concerted research effort in this area should make valuable additions to our scientific and technical data base and provide future options for chemical and fuel production that are not now available.

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