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Plant Cell Cultures: Some Biotechnological Applications and a Proposed New Bioreactor System

by

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Introduction

The plant kingdom, aside from being an important source of food, produces natural products that are beneficial to man. Most of these products, such as pharmacologically active compounds, essential oils and flavors, and fine and specialty chemicals are impractical or impossible to synthesize. They are part of a large industry. It is difficult to estimate the market value of all these products, although a study in the pharmaceutical industry can give us some idea. The 1973 data for new and refilled prescriptions revealed that about 25 percent of all prescriptions contain active ingredients derived from plants (Fansworth and Morris, 1976). This represents $5-10 billion total sales worldwide. Essential oils and fragrances had a total market of around $200 million in 1979 (Bruns, 1980).

At present, natural products from plants are obtained by extraction from the intact plant, usually cultivated. Aside from being labor intensive, the method is susceptible to political manipulations, and geographical or climatological restrictions. With plant cell and tissue culture, it is now possible to grow plant cells similar to other microorganisms. An attractive alternative, therefore, is to grow plant cells in aseptic fermentors or other bioreactors and recover the products from the medium or the biomass. In the same manner as other microorganisms, plant cells have been immobilized in a number of supports.

Plant Cell and Tissue Culture

Plant cell and tissue culture involve the isolation of a part of a plant and aseptically transferring the isolated segment into a nutrient medium to obtain rapid asexual multiplication of cells.

The simplest method in starting a culture is by forming callus, a mass of undifferentiated cells from any suitable explant such as roots, seeds, embryos, or stems. An excised tissue sterilized of microorganisms is placed on agar medium containing the necessary nutrients and plant growth regulators. Callus develops after four to six weeks. To initiate suspension cultures in liquid media, a piece of callus tissue is transferred to a shake flask agitated on a rotary shaker. Single cells or small clumps of cells eventually dislodge from the friable tissue and propagate in suspension.

Plant cells in culture have some properties similar to microorganisms but there are some differences to be considered when applying them, especially to conventional fermentation processes. They are larger than other microorganisms such as bacteria or yeasts, with sizes ranging from 20 to 150 microns in diameter. With a variable morphology, they are often in

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aggregates of 4 to 200 cells. The aggregation, a function of the type of cells or other factors such as the medium used or culture age, is more often due to the failure to separate after cell division. The clumps often reach sizes up to 2 mm in diameter. Fine suspension could be obtained by physical means such as filtration or using cell wall degrading enzyme (Morris and Fowler, 1981). In *Daucus carota* entrapped in alginate beads, Hamilton (1983) observed fine cells being released into the medium and propagating as free cells. Fine cell suspensions have been produced when cells at the surface of alginate beads are released into the medium (Morris and Fowler, 1981; Morris et al., 1983). This method has a possible use in producing fine cell suspension on a continuous basis. Cells, however, revert to aggregation after some time. Each cell is limited by a cellulose cell wall with a high tensile strength but low shear resistance which poses problems in large-scale culture.

Biotechnological Applications of Plant Cell Cultures

Plant cell cultures have several applications especially in agriculture (Sharp et al., 1977; Sala et al., 1980). Because of the totipotent nature of plant cells, cell cultures are used to propagate rapidly and uniformly food plants, horticultural crops and trees. With protoplast fusion techniques it is possible to produce hybrid crops, improve yields, or develop disease resistant plants. The application of plant genetic engineering is better realized in cell cultures for introducing favorable nuclear and epigenetic factors into plants. With tissue culture it is possible to eradicate some plant diseases and cultivate pathogen-free cell lines. Parasite-host-plant relationships are easily studied using cell cultures mixed with the parasite. Plant cell cultures can be used to screen herbicides or plant growth regulators to some extent.

Much information about the biochemical and physiological properties of plants have been obtained from tissue cultures. They have been used for studies in the biosynthetic pathways, identification of intermediates, and studies in the regulation of synthesis of secondary metabolites (Dougall, 1981). Many studies since the mid-1950s have led to the detection and isolation of secondary metabolites which may or may not be found in the intact plant. Some plant cells can also transform chemicals like other microorganisms. The production of secondary metabolites and biotransformation in large-scale culture are emerging important industrial applications of plant cell cultures.

Synthesized Products

Plants are the sources of many natural products and specialty chemicals. The developments in tissue culture show the possibility of using plant cell culture for the production of metabolites by modified microbiological methods.

Some important secondary metabolites derived from plants include alkaloids, glycosides, and essential oils. The alkaloids are a large group of compounds from plants with over 4,000 structures identified, many with proven medicinal value. Some examples are atropine, strychnine, morphine, and cocaine. The glycosides are used for medicinals, dyes, and food colors. They include phenolics, tannins, saponins, flavonoids, mustard oils, and others. Consisting mostly of terpenoids, the essential oils are used as solvents, perfumes, and flavors. A list of products that have been produced in static tissue cultures or cell suspension systems is shown in Table 1. The potential seems tremendous but not all of them would be practical to exploit. To be economical, the yield of the suspension culture must approach or exceed that of the intact plant (Dougall, 1979). Table 2 shows some examples of plant suspension cultures that yield more natural products than the intact plant. Oftentimes, the compounds of interest are not expressed by the cell culture or the products are found in low yields. In some cases the yield of the product declines after the multiplication of cells and tissue.

There are, however, several methods available to improve production. By starting cultures from intact plants with known high yields, the yield of suspension cultures may increase. It is also necessary to choose high yielding strains from established cultures by analytical or biochemical means to assure that cell suspension cultures will maintain high yields. By manipulating the cultural conditions in terms of the type or concentration of

growth substances, carbon sources, nitrogen source, pH, and illumination among others, yields can be optimized.

At present, the commercial utilization of plant cell cultures can only be applied to high-priced, low-volume products (Fowler, 1981). Thus the production of medicinal substances on an industrial scale is promising. Some of these medicinals will be discussed as examples.

Shikonin, a chemical with anti-bacterial and anti-inflammatory effects derived from *Lithospermum erythrorhizon*, is the first product to be commercially produced from plant tissue culture (Curtin, 1983).

Table 1. Examples of subtances produced by plant cell and tissue cultures

Alkaloids	Immunochemicals
Allergens	Insecticides
Amino acids	Insulin-like compounds
Anthraquinones	Latex
Antileukemic agents	Lipids
Antimicrobial agents	Naphthaquinones
Antitumor agents	Oils, commercial
Benzoic acid derivatives	Oils, volatile
Benzopyrones	Opiates
Benzoquinones	Organic acids
Cardiac glycosides	Peptides
Chalcones	Perfumes
Condiments	Phenolics
Dianthrones	Pigments
Emulsifiers, food	Polysaccharides
Enzyme inhibitors	Spices
Ethylene	Steroids, Sterols
Flavanoids	Saponins, Saponigens
Flavors	Sweeteners
Fragrances	Tannins
Furanochromones	Terpenes, terpenoids
Furanocoumarins	Vitamins

Source: Nickell (1980)

By selecting high-yielding strains and optimizing and medium composition, Mitsui Petrochemical Industry of Japan was able to increase the yield from 1.5 percent in the intact plant to 12 percent in the cell suspension. The present annual demand of the product is about 150 kg at a selling price of $4000 per kg (Curtin, 1983). Part of the demand comes from its use as a dye.

Diosgenin, an important raw material for steroid hormone preparation is obtained from the rhizome of the tropical plant *Dioscorea deltoidea*. Kaul et al. (1969), reported suspension culture producing 1.5 percent dry weight of cell mass. The rhizome takes five years to mature for extraction, making commercial production by suspension culture an attractive alternative. Similarly, *Panax ginseng* plant takes several years of difficult cultivation. Furuya and Ishii (1973) have cultivated *Panax ginseng* cultures yielding as much as 2.1 percent crude saponins. Cultures of *Glycyrrhiza glabra* produced three to four percent dry weight of glycyrrhizin (Tamaki et al., 1973). The extract of the cell culture produced better flavor when mixed with tobacco than the extract from the intact licorice plant. Ubiquinone-10, a cure for congestive heart failure, is produced by cultured tobacco cells in yields higher than microorganisms

Table 2. Examples of products obtained from plant cell cultures in higher yield than the intact plant

Biochemicals	plant species	specific yield of culture / specific yield of intact plant
biscoclaurine alkaloids	*Stephania cepharantha.*	1.22 - 2.79
nicotine	*Nicotiana rustica*	.831-1.16
Anthraquinones	*Morinda citrifolia*	8.18
anthraquinoes	*Cassia tora*	1.60
glutamine	*Symphytum officinale*	264
diosgenin	*Trigonella occulta*	1.16
gitogenin	*Trigonella occulta*	3.50
trigogenin	*Trigonella occulta*	5.00
ubiquinone	*Nicotiana tabacum*	31.3
shikonin derivatives	*Lithospermum erythrorhizon*	12 12
proteinase inhibitors	*Scopolia japonica*	1.11 - 3.28
ginseng saponins	*Panax ginseng*	.115 - 1.27
ginseng saponins	*Panax quinquefolium*	.124 - 1.37
phenolics	*Acer pseudoplatanus*	1.83 - 9.43
flavanols	*Acer pseudoplatanus*	1.79 - 9.71
leucoanthocyanin	*Acer pseudoplatanus*	6.78 - 38.1
serpentine and ajmalicine	*Catharanthus roseus*	5.00

Adapted from Dougall (1979)

(Ikeda, 1976). Since tobacco cell suspension has been grown in a 20,000-liter fermentor, prospects for industrial production are bright (Noguchi et al., 1977). Suspension cultures of *Mucuna pruriens* produce L-dopa, released into the medium at a high rate of one percent w/v of medium (Brain,1974). Anthraquinones have been produced by *Morinda citrifolia* suspension cultures at 10 percent dry weight of the cells (Zenk, 1975).

The undifferentiated state of plant cell culture may place the cells in a state of genetic regulation that expresses some metabolic intermediates not normally found in the intact plant. Thus, several novel biochemicals or compounds not found in the intact plant have been detected in cell suspensions. Arens et al., (1982) found a new active indole alkaloid they named pericine from suspension cultures of *Picralima nitida.* Three new sesquiterpine lactones were found in tissue culture of *Andrographis paniculata* (Allison et al., 1968; Butcher and Connoly, 1971). Leistner (1975) found lucidin in suspension cultures of *Morinda citrifolia* not reported to be found in the intact plant.

Biotransformation

An equally important biotechnological application of plant cell cultures is the biotransformation of chemicals to higher costing products. By using synthetic substrate analogues not normally available in whole plants, biotransformation can produce compounds with new chemical and biological properties (Alfermann and Reinhard, 1980). Some specific structural modifications in certain compounds that are difficult to attain by chemical synthesis or microorganisms can easily be carried out by plant cell cultures (Tabata, 1977). For instance, the plant cell reaction for the specific glycosylation of substrates with multifunctional groups has potential commercial application since the glycosides cannot be synthesized chemically or sometimes only by applying protective groups (Barz, et al., 1981).

Some substances that could be biotransformed by plant cell cultures include cardenolides, steroids, terpenoids, alkaloids, and glycosides (Alfermann and Reinhard, 1980). Plant cell cultures have been used to find new cardiac glycosides since some cardenolides often have toxic side-effects in the treatment of heart disorders. *Daucus carota* cell suspension was

able to hydroxylate gitoxigenin and oleandrigenin to new compounds 5-β-hydroxygitoxigenin and 5-β-hydroxyoleandrigenin, respectively (Jones and Velicky, 1981). The same cell line, not known to contain cardiac glycosides in the intact plant, also converted digitoxigenin to periplogenin (Jones, et al., 1978). Some plants that produce cardenolides in nature give rise to cell cultures that transform these compounds. *Digitalis lanata* transforms digitoxin, which is toxic, to digoxin. The transformation, however, is not very efficient. A derivative, methyldigitoxin, is transformed more efficiently to methyldigoxin, a compound as effective as digoxin. A systematic screening program of different cell strains and a suitable bioreactor design has brought the process close to commercialization (Alfermann, 1977; Heins, 1978). Other steroids such as progesterone and pregnenolone could be transformed by several cell cultures such as *Digitalis purpurea, Digitalis lutea, Nicotiana tabacum, Dioscorea deltoidea,* and many others (Reinhard, 1974).

Very few cell lines can metabolize terpenoids which make such cultures interesting. Mentha cell lines biotransformed (-) menthone to (+) neomenthol (Aviv et al., 1983) and (+) pulegone to (+) isomenthone (Aviv and Galun, 1978). Geranial and nerol were biotransformed by *Cannabis sativa* cultures (Itokawa et al., 1976).

Some plant cell cultures increased alkaloid production when some precursors were added to the culture. For instance, *Phaseolus vulgaris* cell cultures produced harmane and norharmane with tryptophane as a precursor (Velicky, 1972).

Large-Scale Mass Culture; Bioreactors

For the industrial exploitation of products from plant cell and tissue culture, large-scale production of cells is necessary. While culturing plant cells on a small-scale does not pose many problems, large-scale culture has constraints on process and equipment design. Based on conventional fermentors, the equipment has to be modified since plant cells have a different "nature".

Plant cells have been grown in batch, semicontinuous, and continuous modes of operation. The choice of mode of operation depends on the nature of culture and capacity of the reactor.

Various sizes and shape of bioreactors have been designed to grow plant cells. They include carboys, roller bottles, inverted Erlenmeyer flasks, and Florence flasks, among others.

Several investigators reported successful use of the New Brunswick and LKB fermentors for growing plant cells (Byrne and Kock, 1972; Martin and Rose, 1976; Martin et al., 1977; Rose and Martin, 1974; Fowler, 1976).

Vessels smaller than 15 l were made of glass while the larger ones, of stainless steel. Many investigators have scaled up the batch reactors. Recent designs of bioreactors with capacity of 10 to 100 l are the "air lift" or bubble reactors that involved both the "loop tower vessels" and draft tube fermentors (Fowler, 1981; Wagner and Vogelmann, 1977; Kato, et al., 1975; Smart and Fowler, 1981). Wagner and Vogelmann used reactors with agitators effected by flat-blade turbines, draft tube with turbine, and draft tube with air lift (Wagner and Vogelmann, 1977).

The first successful commercial application of plant tissue culture, the production of shikonin from *Lithospermum erythrorhizon,* uses two batch fermentors in series equipped with agitators. The first vessel (250 l) promotes growth while the second vessel (750 l) uses a medium that encourages shikonin production (Curtin, 1983).

In a semi-continuous operation, Kato et al. (1972) cultured tobacco cells in a 20-l vessel where half of the culture was replaced every day. The cells grew exponentially with a dry cell yield of 120 to 130 g/day.

The fermentors for continuous mode of operation are basically the same vessels used in the batch system with provisions for continuous feed and effluent streams (Miller et al., 1968;

Kurz, 1971; Wilson et al., 1971). Noguchi et al., (1977) used a two-stage flat-blade turbine bioreactors while Kato et al., (1976) used a large-scale bubble fermentor.

In natural product synthesis and biotransformation, the semi-continuous and the continuous culture would be the eventual source of biomass for high capacity production.

Immobilized Enzymes and Cells

The immobilization of enzymes and cells, carried out since the 1970s, is usually based on any of the following procedures: (1) aggregation, (2) adsorption, (3) covalent coupling, or (4) entrapment (Brodelius and Mosbach, 1982).

The aggregation of cells can be effected by heating flocculated cell mass or by using a cross-linking agent such as glutaraldehyde. The adsorption of cells to surface materials involves ionic or less powerful bonds. Some examples of these materials are ion exchange resins, polyvinyl chloride (PVC) fibers, polypropylene web, microporous silica in PVC, and agarose beads. Covalent coupling is a harsh technique usually used when retaining cell viability is not essential. The cells or enzymes are coupled to a support using a binding agent. For instance, the support could be glass or silica with glutaraldehyde as the binding agent. Entrapment within a gel such as polyacrylamide or natural polysaccharides such as carrageenan, alginate, or agarose, is a gentle form of immobilization. Cells can also be entrapped in membranes, such as hollow fiber cartridge. In the production of biochemicals, immobilization permits continuous processing by allowing the re-use of the biocatalyst. Used for the production of aspartic acid, an immobilized system offered 40 percent reduction in costs over the batch process using free cells due to lower catalyst and labor expenses (Chibata, 1980). Immobilized cells for glucose isomerase production have found large-scale application because of low cost of cells, ease of use, and non-toxicity (Bucke, 1983). The present immobilization techniques permit the microorganisms to live and grow within the support increasing the capacity of the biocatalyst.

Advantages and Disadvantages of Immobilizing Plant Cells

Parallel to the application of immobilization for microbial cells (Venkatasubramanian et al., 1979), several advantages can be realized with immobilized plant cells. For example, a reactor with immobilized cells is not subject to complete washout in a continuous culture mode of operation. This is important for plant cells since they grow very slowly. The fullest utilization of the biomass can be effected. Moreover, plant cell clumping, which is a problem in suspension cultures, is less important in immobilized cells. With the protection of the carrier matrix, plant cells are subject to minimal shearing effects caused by fluid motion. Cofactors and coenzymes can be recycled easily. It is also possible to continuously remove inhibitors that affect certain metabolic pathways.

A disadvantage, however, is that the products must be secreted by the cells. With immobilized systems, it is necessary that the cells release the products to the medium where they can be recovered. Some interesting compounds such as alkaloids tend to accumulate in the vacuoles. However, some studies show that it is possible to promote product release by controlling the external pH (Renaudin, 1981). Also, exposure to organic solvents such as chloroform and dimethylsulfoxide makes plant cells "leaky" (Felix et al., 1981, 1982). Investigations are underway in different centers.

Methods for Immobilizing Plant Cells

The first immobilization of plant cells was carried out by entrapping plant cells in alginate beads. It demonstrated the biosynthetic capabilities of immobilized plant cells with the following examples: (1) *Morinda citrifolia* for the *de novo* synthesis of antharaquinones, (2) *Catharanthus roseus* for the formation of indole alkaloids from distant precursors, and (3) *Digitalis lanata* for a specific biotransformation reaction (Brodelius et al., 1979). Filtered cells were added to sterile sodium alginate solution (two to five percent by weight). The suspension was dropped into a medium containing 50 mM calcium salts. The beads formed were left in

solution for 60 minutes and washed. Other investigators also used the alginate method. Alfermann et al., (1980) immobilized *Digitalis lanata* cells for the biotransformation of some cardiac glycosides. They were able to maintain the hydroxylating capability of the immobilized cells for up to 61 days. Veliky and Jones (1981) immobilized *Daucus carota* in alginate beads for the bioconversion of gitoxigenin and digitoxigenin. With the digitoxigenin biotransformation reaction, they examined several parameters such as the effect of temperature, pH, calcium chloride, solvents and detergents, and cell concentration among others (Jones and Veliky, 1981). The alginate method has also been used to entrap protoplasts (Scheurich et al., 1980; Brodelius and Mosbach, 1982).

Other gel-forming polymers such as k-carrageenan, polyacrylamide, gelatin, agar, and agarose as well as combinations of the above materials including alginate have been investigated (Brodelius and Nilson, 1980). Agar, agarose, and k-carrageenan were found to be suitable while polyacrylamide and gelatin failed to maintain viable cells. The cells were found particularly sensitive to glutaraldelhyde used as the crosslinking agent for gelatin.

Jirku et al. (1981) immobilized *Solanum aviculare* by adsorption onto glutaraldehyde activated polyphenylene oxide beads. The cells were able to synthesize steroid glycoalkaloids from solasidine for about 11 days.

Hollow fiber cartridge has also been used to entrap plant cells. Shuler (1981) entrapped *Glycine max* for the production of phenolics. Jose et al. (1983) investigated hollow fiber entrapped *Daucus carota* cells for the hydrolysis of sucrose to glucose and fructose.

A summary of the immobilization techniques is shown in Table 3.

Table 3. Immobilized plant cell systems studied for biochemical production

Species	Compound investigated	Immobilization technique	Reference
Daucus carota	digitoxigenin, gitoxigenin transformed to 5 βhydroxy derivatives	alginate beads	Jones and Veliky, 1981 Veliky and Jones, 1981
	phenolics production, sucrose inversion	hollow fiber system	Jose, et al., 1983
Catharanthus roseus	production of secologanin with tryptamine as precursor	alginate beads	Brodelius, et al., 1979
Digitalis lanata	digitoxin to digoxin, 12 βhydroxylation	alginate beads	Brodelius, et al., 1979
	digitoxin, methyl digitoxin, 12 βhydroxylation	alginate beads	Alfermann, et al., 1980
Morinda citrifolia	*de novo* synthesis of anthraquinone	alginate beads	Brodelius, et al., 1979
Glycine max	production of phenolics	hollow fiber system	Shuler, 1981
Solanum aviculare	production of steroid glycoalkaloids	polyphenylene oxide activated by glutaraldehyde	Jirku, et al., 1981

Bioreactor Systems

Large-scale culture of plant cells has mostly been carried out in fermentor-type suspension systems. Being sensitive to shear stress, plant cells can be protected by carrier matrices. However, the use of immobilized cells in fermentors has not been reported.

Batch reactors have been used for immobilized plant cells (Brodelius et al., 1979; Alfermann et al., 1980) Packed bed reactors have been employed by Veliky and Jones (1981) and Brodelius et al. (1980). In the former case, a column bioreactor was operated in a semi-continuous manner. Straight upward aeration was found to be more efficient than using an airlift pump system. Brodelius et al. (1980) set their system with recirculation that provided an extraction scheme to continuously separate the lipophilic compounds produced by the plant cells. Hamilton (1983) employed fluidized bed by recirculating a large fraction of the liquid stream and sterile air through the bed for a batch recycle system as well as for continuous flow system. The hollow fiber reactors have been employed for batch recycle (Jose et al., 1983) and continuous flow system (Shuler, 1981). The continuous mode of operation will be most suitable for large-scale application of immobilized plant cells.

Hollow Fiber Reactors

The hollow fiber membrane reactor is part of the membrane separation technology that has found wide applications in biotechnology as a means of effecting phase or molecular separations such as: (1) separation of colloidal solids from suspensions, (2) concentration of solutes, (3) macrosolute/microsolute separation, (4) fractionation of macrosolutes, (5) selective confinement of enzymes or cells, and (6) membrane barriers for sensors (Michaels, 1980).

The hollow fibers are made up of asymmetric membrane structures with effective pore diameters ranging from 10 - 200 Angstroms and are designed for ultrafiltration/dialysis processes. The membranes are made by solution spinning in water and then densified to produce a monolithic asymetric membrane (Porter, 1972). The material can be made up of regenerated cellulose or non-cellulosic materials such as polysulfone. The hollow fiber consists of a cylindrical tube divided into three regions: (1) a porous sponge section of fiber wall, (2) a dense ultrathin membrane and (3) the tube lumen. Typical dimensions of the fibers are 350 μ m OD and 250 μ m ID. It consists of 0.5 m thick semipermeable skin covered by 75 μ m thick sponge with pore size 5 - 10 μ m. The sponge, very permeable, is 80 - 90 percent void and serves as a mechanical support for the membrane lining for the fiber lumen. The pores in the membrane are large enough to fully pass substrate and product. A reactor consists of a bundle of fibers assembled within a shell similar in appearance to a shell and tube heat exchanger. (See Figure 1.)

The use of hollow fiber reactors is particularly suited to the fragile plant cells. The cells are simply entrapped outside the fibers and no special carrier matrix is needed. The medium flows inside the fibers and provides the necessary nutrients and subtrates. The reactants and the products, with molecular weights less than a few thousand Daltons are freely exchanged. Growth can be maintained if the essential nutrients are provided or it can be suppressed by altering the composition of the medium. Unlike other entrapment procedures, the diffusional limitation imposed by the carrier is minimal and the pressure drop across the reactor is small.

A Proposed Design for a Large-Scale Plant Cell Bioreactor System

This section presents a new design of a bioreactor based on the hollow fiber reactor experiments carried out by Jose (1983). The hollow fiber reactor is suitable for plant cells as shown by the following advantages:

(1) Compared to other immobilization techniques where a chemical reaction or harsh condition is involved, the entrapment is gentle.

(2) The membrane barrier prevents any cell wash out.

(3) Fresh batches of cells can be economically pumped in and out of the reactor with a properly designed hollow fiber assembly.

(4) Large-scale hollow fiber units for ultrafiltration are readily available or can easily be manufactured.

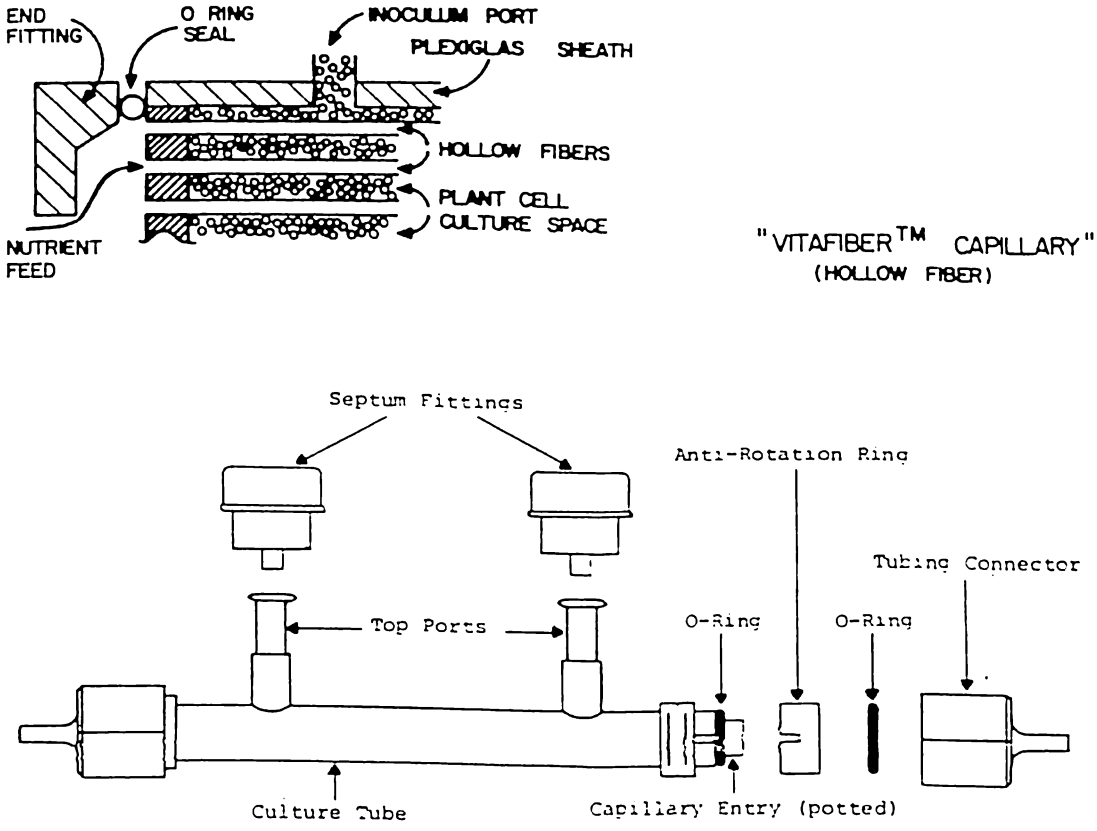

Figure 1. Schematic diagram of the hollow fiber reactor

Some shortcomings however, are:

(1) To take full advantage of the hollow fiber systems, special units have to be designed for plant cells.

(2) The hollow fibers are fragile and the possibility of fiber rupture or snapping is great when cleaning the system or flushing out the cells.

(3) Plant cells have a tendency to settle which limits the use of the large surface area that is available. Changing the cartridge orientation may be a solution but the inefficiency is quite obvious.

(4) The present hollow fibers are intended for ultrafiltration and the pore size could pose diffusional limitation to some substrates and products. Since plant cells are larger than 25 μm, more porous membranes can be used.

(5) Oxygen supply is usually provided by saturating the input medium to the reactor with oxygen. There is no method for direct aeration with the present design.

(6) For short-term usage, the hollow fiber units are costly. Units for hydrogen separation processes are designed to last for five years. Certainly, it would be desirable not to use a unit that is disposed of after one-time use.

With the above advantages and disadvantages in mind, the following design is proposed. The same basic configuration of a bundle of tubes within a shell where the plant cells are confined is adapted. The hollow fibers are replaced by porous tubes with pore size in the

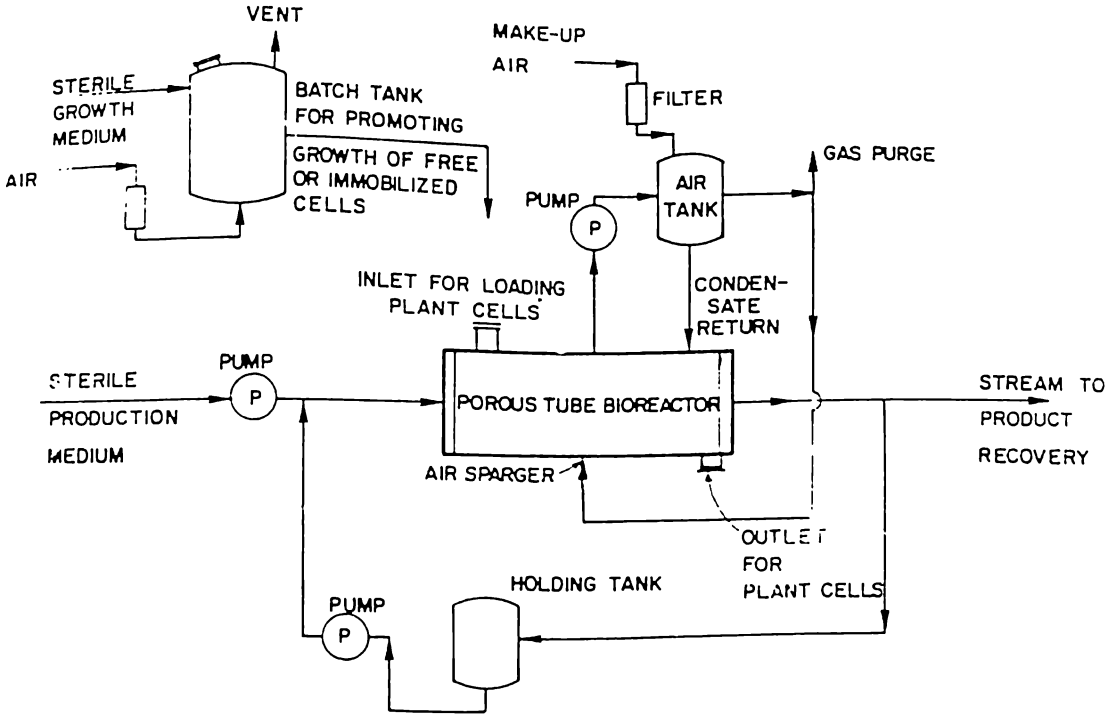

Figure 2. Proposed new bioreactor system. The porous tube bioreactor is the heart of the system. The production medium passes thru the porous tube in the bioreactor. The outlet stream is sent to product recovery with part recycled back. The plant cells are loaded in the shell side, with air bubbled through to provide oxygen and agitation.

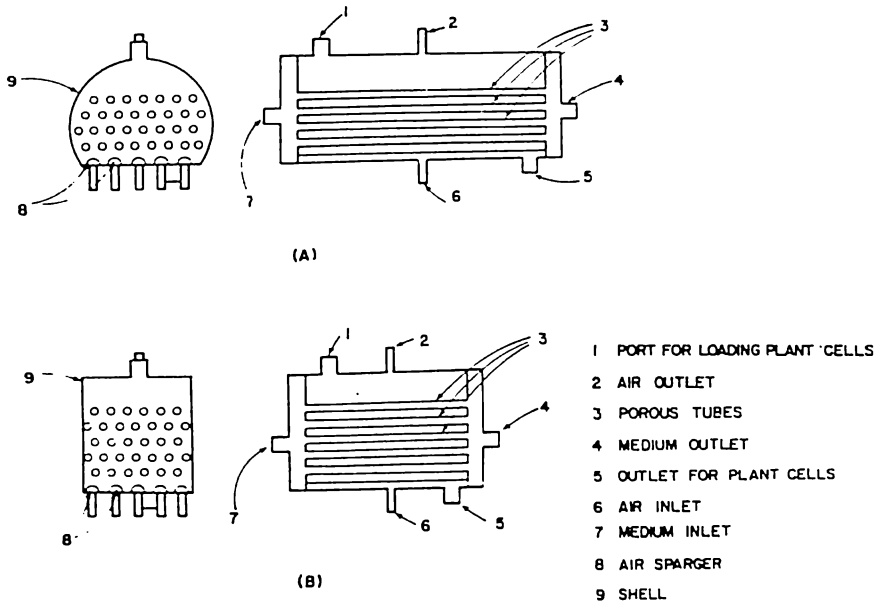

Figure 3. Porous tube bioreactor. (a) Unit for free cells. (b) Unit for alginate-immobilized cells. The diagrams on the right represent the front view cross-section along the porous tube length. The diagrams on the left represent the cross-section across the tubes and shell. High density free cell suspension is loaded at the top port and fills the shell to the level of the top row of tubes. The unit for immobilized cells is actually a packed bed with the bank of tubes providing the nutrient medium and the air agitating the particles. Inlet and outlet ports are provided to withdraw and replenish the plant cells periodically. Large cell aggregates that settle can be withdrawn at the outlet port.

range of 15 to 50μm. The material of construction is porous ceramic, stainless steel or plastic materials such as polypropylene. Commercially available materials are preferable. The bioreactor system is shown in Figure 2. Two types of bioreactors are proposed: one to handle free cells (Figure 3a), and the other, immobilized cells (Figure 3b). Production medium is passed through the tubes with partial recycle. The cell suspension or the entrapped cells are loaded in the shell side. An outlet is provided to be able to remove part of the plant cells, and an inlet port to replenish fresh cells. Air is bubbled across the shell from the bottom of the horizontally-oriented unit to provide agitation and necessary oxygen. To avoid loss of key volatiles such as CO_2 and perhaps, essential oils, and control the oxygen supply, the air is recirculated with fresh feed make-up, a condensate trap returns evaporated liquid to the reactor, and a purge stream is provided (See Figure 2). The unit for free cells should be able to handle high cell density loading as long as uniform air input for agitation is provided. The large cell aggregates that settle at the bottom should be discarded and replaced by fresh cells. On the other hand, the unit for the immobilized cells is essentially a packed-bed (rectangular) reactor with the medium perfusing through the tubes and air bubbled upwards the bed. Problems in non-homogeneity in the reactor, diffusional limitations, possible formation of dead zones, inefficient dispersion of oxygen, and maintaining asepsis may be encountered. However, construction of a prototype in the near future will help us realize whether this proposed design is suitable or not.

Summary

The potential applications of plant cell and tissue culture are tremendous. The synthesis of natural products and biotransformation are the emerging important industrial applications of plant cells. However, further developments in bioreactors are still needed for enhanced productivity to make the process as economical as other microbial fermentations.

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