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Experimental and Modeling Studies of Diffusion in Immobilized Cell Systems

A Review of Recent Literature and Patents

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Introduction

Many natural and man-made biological materials contain cells that are restricted within a limited region of space owing to cell aggregation; attachment mediated by adhesion molecules; or encapsulation within networks of polymers, foams, or fibers. Examples include biofilms; dental plaque; animal and plant tissues; cell-based artificial organs; and polymer-supported microbial, plant, or animal cells. The use of immobilized cell systems offers a number of advantages over typical suspension culture, including isolation of cells from their surroundings, attainment of higher cell densities, protection from shear forces, and simplification of downstream separations.

This review summarizes the recent research that has been conducted to characterize the processes that control the behavior of immobilized cells. The discussion and analysis focuses primarily on the experimental and modeling approaches which have been used to quantify nutrient and product diffusion, cellular metabolism, and cell proliferation and death. An effort is made to highlight recent findings from similar fields that could be applied to the characterization of immobilized cell systems. Polymer-encapsulated mammalian cell biocatalysts are considered as a basis for discussion. The description of other systems branches out from this one. A number of U.S. patents filed in recent years provide substantial insight into practical applications of immobilized cells and directions for future research. These are summarized at the end of the review.

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From an engineering perspective, immobilized cells may be considered as multiphase materials having a distributed, or cellular, phase surrounded by a continuous condensed phase. The latter phase may consist of a support material, extracellular matrix, or a semisolid surface. Many applications of immobilized cells are affected by insufficient transport of nutrients to the cells. These transport limitations are not restricted to only man-made materials; they are also apparent in many natural biological systems such as tumors and biofilms, for which restrictions in the amount of metabolites available to cells that are positioned far from the external source have often been observed. Most of the literature in this area is concerned with characterizations of a single type of cellular system, and, hence, there has been little cross-communication among researchers working in disparate fields. For example, it has been reported that oxygen can penetrate to a maximum depth of about 50–200 μm in highly packed cellular materials *(1,2)*. These penetration depths are consistent with those encountered in encapsulated microbial cells *(3–7)* and hybridoma cells *(8,9)*. Thus, an attempt is made herein to bridge the gap between similar research areas focusing on the characterization of diffusion, reaction, and cell proliferation in immobilized cell materials. This review by no means provides a complete collection of all research relevant to the characterization of immobilized cells; however, it provides pointers to some of the pertinent research in several areas.

Several excellent reviews covering specific aspects of immobilized cell behavior are available *(10–16)*. The reader is encouraged to consult these reviews to gain a better historical perspective on the use of immobilized cells. The present review focuses primarily on intraparticle transport. An insightful discussion on the effects of external mass transfer limitations may be found in the review by Kasche *(10)*, and Kawase et al. *(17)* provide models for liquid-phase mass transfer coefficients in bioreactors.

Encapsulation of cells for cultivation in bioreactors has several advantages over conventional suspension culture methods. A chief advantage of immobilization is the attainment of higher cell densities. Productivity increases of as much as eightfold have been reported *(18–20)*. Immobilization also can protect fragile mammalian cells from shear forces, serve as a surface on which anchorage-dependent cells may grow, simplify product purification, and permit the introduction of air by bubbling. For continuous operation, higher medium flow rates may be used without risk of reaching washout conditions *(8)*. The cell encapsulates may be easily recovered and used repeatedly. Immobilization has also been shown to increase the specific cellular production rate of desired materials including antibodies *(21)*. These advantages have contributed to the development of several engineering applications in which viable cells encapsulated in polymeric materials are cultivated in bioreactors for the manufacture of valuable biological products.

Despite the numerous advantages of immobilized cell systems, distinct disadvantages are also apparent. The limited space available for cell

growth and proliferation severely restricts their utility for growth-associated products *(14)*. From a characterization standpoint, the cell density cannot be easily quantified by direct and nondestructive methods. By far, the most serious disadvantage is the potential limitation in the supply of nutrients and oxygen to the cells. Owing to the presence of the rigid support material, cells receive nutrients by diffusive mechanisms alone; convective flow within the support is negligible. As the cells proliferate, the total nutrient consumption rate increases, leading sometimes to a demand that cannot be met by the prevailing, and decaying, diffusion rates. These diffusional limitations often lead to uneven distributions of viable cells within the support material *(11)*. When these limitations become extreme, the amount of nutrients available for cells that are far from the nutrient source is so reduced that the cellular metabolic activity is confined to the vicinity of the interface between the growth media and the cell-containing support *(14)*. For example, Karel and Robertson *(4)* observed that active cell growth of *Pseudomonas putida* in microporous hollow fibers occurred only within a distance of <25 μm from the oxygen supply. A thorough characterization of these transport limitations is necessary for the proper design of efficient immobilized cell systems.

Multiple interacting processes control the overall behavior of immobilized cell systems. Metabolites such as carbohydrates, amino acids, oxygen, and cofactors must diffuse through several cell layers that act as diffusional barriers. Cells consume metabolites and generate wastes and desirable products. The cells proliferate at a rate that depends on the supply of metabolites, the accumulation of wastes, and the available space into which progeny may be placed. These processes are intricately linked through the cellular response to its surroundings and hence can be difficult to measure independently.

It is common practice, therefore, to apply methods that seek to decouple these processes to investigate, molecular diffusion in the absence of reaction or cell proliferation. However, the effect of such decoupling approaches on molecular diffusion is not well understood. A more thorough understanding can be gained by evaluating these processes both with and without the confounding effects of interactions. This is possible, in principle, through the use of mathematical models that can describe the overall cellular behavior. These models can become powerful and flexible tools for investigating the system response to a wide range of process variables such as molecular diffusivities, cell densities, cell proliferation rates, and cellular metabolism. Clearly, the usefulness of such models hinges heavily on their validation against experiments. Only after proper validation should these models be used to interpret behavior, support experimental programs, and suggest new research directions.

Suitable models of immobilized cell behavior should include the following basic processes: diffusion of nutrients and products through the support material and the cells, cellular metabolism, and cell proliferation and death. The discussion that follows addresses how these processes are

measured experimentally, how they are physically modeled, and how they are ultimately incorporated into comprehensive multivariate descriptions of the overall cell behavior.

Processes that Regulate Immobilized Cell Behavior

Molecular Diffusion

Experimental Measurements of Diffusion

This review is primarily concerned with immobilized cell systems consisting of cells (microbial, mammalian, or plant) encased in a polymer matrix. For notation purposes, the physical properties characteristic of the cell population and of the polymer matrix are identified with subscripts c and 0, respectively.

Macroscopic diffusion in immobilized cell systems can be reasonably well described as a Fickian process characterized by an effective diffusivity D_{eff} *(12,15)*. This effective diffusivity contains contributions from the polymer and the cellular phases. It therefore depends on the molecule's diffusivity within the cells (D_c) as well as the support phase (D_0). D_c is, in itself, an *effective* diffusivity of a molecule inside the heterogeneous milieu of a cell that takes into account physical constraints, viscous effects that impede molecular movement, and membrane restrictions *(22)*. D_c, which is typically less than D_0, generally depends on the nature of the diffusing species and the type of cell. Measurements of D_c, D_0, and D_{eff} are discussed next.

Measurement Methods

Several techniques are available for measuring molecular diffusivities in immobilized cell systems. They include bead methods *(13)*, diffusion chambers *(23)*, and holographic laser interferometry *(24)*. de Beer et al. *(25)* recently developed a confocal laser microscopic technique, based on the method of fluorescence recovery after photobleaching, to measure diffusion coefficients of fluorescent and fluorescently tagged molecules in biofilms. They found that the diffusivity of phycoerythrin (mol wt = 240,000) was 41% lower in the cell clusters than in the interstitial voids.

The most frequently used approach to quantifying effective diffusivities in immobilized cell systems is based on a diaphragm diffusion cell consisting of two well-mixed compartments separated by a thin sample material through which diffusion occurs *(15)*. The mathematical relations used to calculate the effective diffusivity from experimental data, however, are sensitive to small errors in the evaluation of the species concentration *(26,27)*. Westrin and Zacchi *(28)* evaluated the effect of random and systematic errors on diffusivity measurement techniques and reported that the method of solute uptake by a spherical bead is significantly more sensitive to errors than that of solute dispersal from a spherical bead.

Infrared (IR) microspectroscopy is a relatively new technique that has also been applied to characterize the rates of molecular diffusion in poly-

meric materials. Cameron et al. *(29)* applied this technique to characterize diffusion of bovine serum albumin in amylopectin gels by monitoring the height of the amide I peak at 1650 cm^{-1} as a characteristic of the protein. Challa et al. *(30,31)* applied spatially resolved IR microspectroscopy to characterize the diffusion of liquid crystals into polymers by contacting the pure components of a binary mixture. They collected spectra at varying positions across the polymer surface using microscope stage controls and monitored the 2226 cm^{-1} nitrile peak as a characteristic of the diffusing liquid crystal. IR microspectroscopy has the advantage of providing a noninvasive measurement of the concentration of multiple chemical species simultaneously; however, difficulties in instrument calibration limit its application at this time to more heterogeneous biological systems.

The molecules of greatest interest in immobilized cell systems are cellular nutrients or cellular products that are consumed or produced by the cells. The cell metabolism hence influences measurement of the diffusing species concentration. Three approaches are commonly used to resolve the problem of species reactivity. In the first method, both diffusion and reaction are taken into account. This method is difficult to analyze because reaction kinetics must be explicitly known *(32)* and unaltered by diffusional constraints. However, immobilized cell kinetics in some systems has been reported to differ from free cell kinetics *(9)*, thus invalidating such an approach to characterize the diffusivity. In the second method, the diffusing solute is prevented from reacting owing to some step taken by the experimenter. This can be accomplished by deactivating the cells through heating *(33)* or by treating them with a toxic substance such as glutaraldehyde *(34)* or ethanol *(35)*. In these techniques, the physical characteristics of the cell must be maintained with minimal damage to the membrane, cytoskeleton, and organelles so that a meaningful diffusive behavior is measured. A third method applied to deconvolute reactive from diffusive effects is to evaluate a nonreactive species whose diffusivity can be correlated to that of the solute of interest. Nitrous oxide *(36)* and galactose *(37)* have been employed as diffusive analogs of oxygen and glucose, respectively. The limitations of this technique derive from difficulties in selecting suitable analogs to the species of interest.

Diffusion in Polymers

A number of polymeric systems have been used to immobilize cells including alginate, agar, agarose, carrageenan, fibrin, and polyacrylamide *(38)*. Alginate polylysine is the most commonly used support material because biocatalysts can be formed through a gentle gelation process that retains high cell viabilities *(39)*. An additional advantage is that polymers formed with calcium as the cation may be selectively dissolved by the addition of calcium chelators such as EDTA, thus providing recovery of the immobilized cells and their products. Temperature-sensitive forms of agarose are also available for rapid gelation *(40)*. Matthew et al., evaluated several polymeric systems for the immobilization of liver endothelial cells

and reported that a combination of carboxymethylcellulose, chondroitin sulfate, chitosan, and polygalacturonate provided superior cell functionality compared with the commonly used alginate-polylysine capsules *(41)*.

The diffusivity of a solute in a gelatinous support or polymer material (D_0) can be measured using cell-free systems. Small molecular weight solutes in dilute (1–4 w/v %, i.e., 10–40 mg/mL) gels of agarose, alginate, or κ-carrageenan diffuse with rates similar to those in water. For example, glucose exhibits diffusivities in the range of $5.0–6.8 \times 10^{-6}$ cm^2/s *(13,42–44)*; corresponding values for oxygen are in the range of $2.0–2.5 \times 10^{-5}$ cm^2/s *(13,33,42,45,46)*. Oyaas et al. *(47)* reported that diffusivities in 2% Ca-alginate beads were only 15% lower than those measured in water. In general, the diffusivity decreases with increasing solute molecular weight and with increasing polymer content. Muhr and Blanshard *(48)* and Tanaka et al. *(42)* provide excellent reviews on the characterization of diffusion of small molecules in polymers.

Diffusion in Cells

Several groups (e.g., *[49,50]*) have reported that the cell membrane represents only a minimal resistance to oxygen transport and that oxygen's diffusivity through the membrane is approximately the same as in the cell interior. Oxygen permeability in the red blood cell membrane is 3.2×10^{-6} mmol/(cm$^2 \cdot$ s$^{-1} \cdot$ mmHg^{-1}), a value that would account for only 5% of the total resistance to oxygen entering the cell *(51)*. However, the rate of oxygen uptake by human erythrocytes is roughly 40 times slower than the corresponding rate of oxygen attachment to free hemoglobin *(52)*. This large decrease in uptake appears to be correlated with the presence of unstirred, oxygen-depleted layers of solvent adjacent to the cell surface whose thickness may be as large as 1.0–5.0 μm *(52)*. Limitations may also be present within the cell cytoplasm. Clark *(53)* has shown that local variations in oxygen concentrations produced by oxygen consumption in isolated mitochondria are small compared with the cellwide variations produced by the collective effect of all the mitochondria consuming oxygen.

Larger molecules encounter a significant transport barrier owing to the cellular membrane. Nonspecific migration of solutes across the membrane is rarely a simple diffusion process. It frequently depends on the presence and activity of specific transporter molecules and the lipophilic nature of the solute. Since the cell membrane is composed of long-chain lipid molecules, highly lipophilic solutes more readily cross the membrane than do lypophobic solutes. The permeability of solutes across plant cells is generally correlated with the solute's oil-water partition coefficient. Other molecules, such as glucose, cross the cell membrane primarily through specific glucose transporters that act in response to a need for an increase in the cellular glucose concentration *(54)*. Ismail-Beigi *(55)* reviewed the metabolic processes that modulate the rate of glucose transport in response to alterations in the cellular metabolism.

Measurements of D_0 have been reported for many molecules in pure polymers *(42,48)*; values of D_c are less well characterized. D_c corresponds to

the *effective* diffusivity of a molecule inside the heterogeneous milieu of a cell, accounting for physical constraints and viscous effects that impede its movement *(22)*. The cell cytoplasm is a complex non-Newtonian fluid consisting of water and electrolytes spread among a convoluted network of proteins and macromolecules. D_c is usually less than D_0 and generally depends on the nature of the diffusing species and the type of cell. For example, the diffusivity of a small fluorescent molecule in the cell cytoplasm relative to that in water was reported as 0.27 *(22)*. D_0 for glucose in gels is about 6.5×10^{-6} cm^2/s *(13,41–43)* whereas D_c for glucose in mammalian tumor cells can be about 2.0×10^{-6} cm^2/s *(55–57)*. This decreased mobility can be attributed to the high viscosity of the cell interior (about 2 to 3 centipoise *[59]*), the transient binding to intracellular components, and collisions with cellular solids *(22,59)*. Clegg *(60)* suggests that the cytoplasm maintains a higher-order structure since cellular water molecules exhibit properties that differ markedly from those of pure water. The water content of a cell may also have a significant effect on diffusion coefficients because a satisfactory correlation may be developed between the diffusivity of various gases and the water content of the medium *(61)*. Models developed to explain experimental observations of diffusion of fluorescent tracers in the cytoplasm of living cells suggest that fluid portions of the cytoplasm may be as crowded as a 13% dextran solution *(62)*. Much of this crowding effect is owing to the large protein content in the cell interior; actively proliferating cells contain between 17 and 26% protein by weight *(63)*, which is only slightly more void than a protein crystal *(63)*.

The cytoarchitecture appears to play a significant role in the reduction of cellular diffusivities of molecules. Diffusivities typically increase on treatment of cells with cytochalasin B, which disrupts actin molecules, suggesting that cellular structural barriers restrict molecular movement *(59)*. Additionally, a decrease in the cell volume owing to hypertonic conditions decreases the translational mobility of molecules and increases the apparent viscosity of the cytoplasm. Most likely, particles larger than 260 Å in radius may not be able to diffuse in the cytoplasm owing to the higher-order intermolecular structure that is not found in simple aqueous solutions *(64)*.

Diffusion in Multicelled Systems

Experimental studies report conflicting trends on the effective diffusivities of molecules in multicellular systems. Kurosawa et al. *(65)* measured oxygen diffusivity in alginate beads entrapping several types of cells. They observed a pronounced nonuniformity in cell proliferation that could be directly related to oxygen transport limitations but reported a lack of influence by cell density, type, or viability on the diffusivity. Hannoun and Stephanopoulos *(35)* reported that diffusivities of glucose and ethanol in calcium alginate membranes were unaffected by nonviable yeast cell concentrations up to 20%. These studies suggest that molecules diffuse at similar rates in the cells as in the polymeric support. However, there are

also numerous reports in the literature indicating that molecules diffuse more slowly within the cells and that the overall effective diffusivity is highly dependent on the cell density in the material *(33,36,37,64,66)*. The effective diffusivity of glucose in tumor masses has been found to be 25–50% lower than the diffusivity of glucose in water *(56)*. Grote *(67)* measured the oxygen diffusion coefficient in tumor tissue at 37°C and determined that $D_{eff} = 1.75 \times 10^{-5}$ cm^2/s. In recent reviews, Karel et al. *(12)* and Westrin and Axelsson *(15)* compared relative diffusivities of several solutes in various cell masses and reported that the effective diffusivity generally decreased with increasing cell volume fraction, regardless of cell type or the nature of the diffusing species. Diffusivity values typically fall within $0.01\ D_0 \leq D_{eff} \leq 1.1 D_0$, in which D_0 is the diffusivity of the solute in water. Siegrist and Gujer *(68)* characterized the mass transfer mechanisms for three molecules in heterotropic biofilms and reported that compared to water, the biofilm structure reduced molecular diffusivities by about 20–50%. Oyaas et al. *(47)* reported that the effective diffusivity of lactose and lactate decreased linearly with increasing immobilized cell concentration.

The diffusivity of molecules in self-aggregated tumors has been characterized. These multicelled tumor spheroids have been used as three-dimensional experimental models of nonvascularized tumors. This lack of vasculature in many fast-growing tumors has several implications. Cells that are distant from blood vessels may be subject to diffusive limitations in the oxygen supply *(1)*, which may reduce the cell growth rate and yield hypoxic cells that are resistant to irradiation *(69)*. The relatively long distances that molecules must traverse to reach cells may also make it difficult to reach cells in poorly vascularized regions with cytotoxic drugs *(69)*. Multicellular tumors and spheroids can develop gradients in oxygen concentration, glucose concentration, and extracellular pH as they grow *(70)*.

Several groups have developed cell culture analogs of the mammalian small intestine by cultivating monolayers of Caco-2 cells on permeable filters. Such model systems may be used to evaluate the uptake of sugars, peptides, and pharmaceuticals. With few exceptions, drugs are transported over the intestinal epithelium by passive diffusion *(71)*. Adson et al. *(72)* systematically evaluated the relative contribution of various molecular routes (transcellular and paracellular) for the transport of weak electrolytes across Caco-2 monolayers. Effective permeability coefficients correlate with the apparent partition coefficient of the diffusant in *n*-octanol/water *(71–73)* used to evaluate the molecular lipophilicity.

Molecular Transport Models

Theoretical Models

Simple correlations have been developed to estimate the effective diffusivity (D_{eff}) of a species in an immobilized cell system as a function of the volume fraction of the material occupied by cells (ϕ), the molecular diffusivity in the cells (D_c), and the molecular diffusivity in the support phase (D_0).

A commonly used theoretical approach for predicting D_{eff} follows from Maxwell's *(74)* equation, which was originally derived to describe electrical conduction in a heterogeneous material. In the context of diffusion, this equation can be interpreted as describing molecules that intermittently diffuse through the polymer support, or continuous phase, and through the cells, or dispersed phase:

$$D_{eff}/D_0 = [2D_0 + D_c - 2\phi(D_0 - D_c)]/[2D_0 + D_c + \phi(D_0 - D_c)] \quad (1)$$

Equation 1 has been frequently used to predict D_{eff} in biological materials *(15)* and serves as a reasonable starting point for estimating effective diffusivities.

In certain situations, molecules diffuse much more slowly in the cell phase than in the support, either owing to large restrictions in the molecule crossing the cell membrane or to large intracellular resistances. In such cases D_c can be assumed to be 0 and Maxwell's model reduces to

$$D_{eff}/D_0 = [(1 - \phi)/(1 + \phi/2)] \quad (2)$$

Equation 2 performs reasonably well in approximating the experimental results of a number of investigators including the studies of lactate diffusion by Chresand et al. *(34)*.

Similar exclusion-type models have been developed assuming that $D_c = 0$ and that the presence of impermeable cells simply reduces the volume available for diffusion. This leads to the following relationship:

$$D_{eff}/D_0 = (1 - \phi) \quad (3)$$

Axelsson and Persson *(23)* and DeBacker *(75)* reported that this simple geometric model adequately described their experimental data.

More elaborate capillary models have also been developed to characterize the relationship between effective diffusivity and porosity in constricted pore spaces. Thus, e.g., Wakao and Smith *(76)* produced a phenomenological "random pore" model, to predict D_{eff} in a bimodal porous catalyst having distinct and interconnected population of small and large pores. Translation of such a model to the case of randomly distributed impermeable cells reduces to

$$D_{eff}/D_0 = (1 - \phi)^2 \quad (4)$$

This expression yields a good fit to several sets of experimental data Furusaki and Seki *(33,37,77)*, and its functional dependence on ϕ is nearly the same as a polynomial fit obtained by Sakaki et al. *(78)*.

Empirical Models

Empirical models of diffusion have been developed by fitting polynomial equations directly to experimental data. Using such an approach, Scott et al. *(13)* obtained the following relation for the effective diffusivity of glucose in gel beads containing microbial cells:

$$D_{eff}/D_0 = 1 - 0.9\phi + 0.27\phi^2 \quad (5)$$

For diffusion of galactose in biocatalysts containing *Z. mobilis* cells, Korgel et al. *(37)* developed a relation based on their experimental results:

$$D_{eff}/D_0 = 1 - 2.23\phi + 1.40\phi^2 \quad (6)$$

Similarly, Sakaki et al. *(71)* produced

$$D_{eff}/D_0 = (1 - 0.98\phi)^2 \quad (7)$$

for glucose diffusion in Ca-alginate beads containing *Z. mobilis* cells, and Sun et al. *(33)* generated

$$D_{eff}/D_0 = (1 - 0.333\phi)^2 \quad (8)$$

to describe the diffusion of oxygen in Ca-alginate beads containing microorganisms.

Although all these models follow similar trends in predicting a decrease in D_{eff} with increasing ϕ, there are also some important differences in the predicted results, and the general applicability of these empirical models is limited. The model of Korgel et al. *(37)*, e.g., reaches a minimum at $\phi = 0.8$. This implies that D_{eff} increases as the cell fraction is increased beyond the critical cell fraction of 0.8, and thereby contradicts the trends observed at lower cell fractions. Such a result is clearly a consequence of extrapolation beyond the range of conditions in which the correlation was derived. Accordingly, the development of an accurate predictor method for a wide range of ϕ values requires many measurements over multiple conditions. Furthermore, each model may apply reliably only to the cell type, gel material, and solute for which it was specifically developed. A comparison of these empirical models reveals that the coefficients present in the diffusivity relationships are sensitive to the type of diffusing species, cell type, and gel support material.

An additional complication that arises is that not all experimental results can readily be described by such empirical models. Rizzi et al. *(79)* analyzed the dynamic response of yeast cells to rapid changes in the extracellular glucose concentration and found poor agreement with conventional transport models. These researchers then proposed a mechanism for glucose transport that includes facilitated glucose diffusion superimposed by an inhibition by glucose-6-phosphate. Pu and Yang *(66)* report measurements of the rates of diffusion of sucrose and yohimbine in Ca-alginate beads with and without immobilized plant cells. Their diffusivities are more sensitive to changes in the cell volume fraction than those predicted by several theoretical models.

Clearly, the prediction of effective diffusivities in immobilized cell systems can be improved through the use of more standardized evaluation procedures. Westrin *(80)* recommends correlating the effect of cell concentration to molecular diffusivities through physically based models to avoid errors in interpreting changes in partitioning and the volume accessible to solutes. To this end, Ochoa et al. *(81)* applied mathematical methods of volume averaging to analyze diffusion in cell ensembles in which the pre-

dominant resistance to mass transfer is due to the cell membrane. In general, accurate prediction of overall effective diffusivities in an immobilized cell system requires the characterization of several structural and physical parameters. The volume fraction occupied by cells (ϕ) can be obtained from measurements of the initial cell loading and growth rate data. Once accurate values for D_0, D_c, and ϕ are obtained, a relation is required to describe how these factors affect the overall effective diffusivity. An additional complicating factor is that over time the cells may proliferate, thus increasing ϕ and decreasing D_{eff}.

Mathematical modeling offers the potential for developing more detailed relationships that capture the time-dependent evolution of these systems. Modeling has been successfully used in many branches of science to study transport in multiphase systems, e.g., heterogeneous catalysts, polymer blends, soil, concrete, paper products, and clothing. Several modeling studies relevant to the present discussion are summarized next.

Simulation Methods

The approaches just mentioned provide a suitable basis for understanding the functional dependence of diffusivities in terms of volume fractions, but lack the detail and specificity to describe the actual morphological characteristics observed in actual cell systems. Typically, the cells are distributed neither in regular arrays nor in completely random positions as typically assumed by theoretical models; an intermediate degree of order is often observed. The versatility of Monte Carlo simulations for handling geometric and topologic disorder provide an attractive approach for studying such systems and, as described subsequently, they have indeed been applied with reasonable success. Since a detailed analysis of such methods is beyond the scope of this review, the reader is encouraged to consult some of the following representative studies: Evans et al. *(82)*; Chiew and Glandt *(83)*; Burganos and Sotirchos *(84)*; Rubinstein and Torquato *(85)*; Reyes and Iglesia *(86)*; Tomadakis and Sotirchos *(87)*; Kim and Torquato *(88)*.

Monte Carlo simulation methods have been applied to characterize the rates at which molecules diffuse in biological materials. Bicout and Field *(89)* applied stochastic dynamics simulations of macromolecular diffusion in the cytoplasm of *Escherichia coli*, by modeling the cytoplasm as a polydisperse mixture of spherical particles, accounting for ribosomes, proteins, and tRNA molecules. Northrup and Erickson *(90)* developed similar Brownian dynamics simulations to evaluate purely diffusive effects on the formation of protein-protein complexes. Saxton *(91–95)* has thoroughly investigated diffusion within two-dimensional cell membranes using simulations of particle diffusion on a lattice containing filled and empty sites representing diffusion obstructed by immobile proteins or domains of gel-phase lipids. For random-point obstacles, the diffusion coefficient depends on the size of the tracer only if the obstacles are nonfractal *(93)*. Cluster-cluster aggregates were found to be more effective barriers to lateral diffu-

sion of tracer particles than random-point obstacles, which were in turn, more effective barriers than compact obstacles *(92)*. Saxton *(94)* has also investigated obstructed diffusion on the cell surface showing that the structure of the impediments may govern whether one observes free diffusion, obstructed diffusion, directed motion, or trapping in finite domains.

El-Kareh and coworkers *(96)* evaluated the effect of cell arrangement and of the interstitial volume fraction on the diffusivity of monoclonal antibodies in tissue assuming that antibodies could not enter the cells. Their calculations suggest that antibody transport in the interstitum is one-tenth to one-twentieth the rate of diffusion in water. Riley and coworkers *(97–99)* developed Monte Carlo diffusion techniques to characterize the transport of nonreacting solutes in immobilized cell systems with varying cell volume fractions and diffusivities in each phase. A correlation to predict the effective diffusivity as a function of the cell volume fraction and the molecular diffusivity in the cell and support phases was developed from the results of these simulations:

$$D_{\text{eff}}/D_0 = 1 - [1 - (D_c/D_0)]\,(1.73\phi - 0.82\phi^2 + 0.091\phi^3) \qquad (9)$$

This empirical relation can be used to predict D_{eff} as a function of D_0, D_c, and ϕ and can be used to extrapolate D_{eff} measurements from one cell fraction to any other cell fraction. This approach combines the functional relationship between the effective diffusivity and the cell density as calculated through simulation with experimental inputs for the rate of parameter alteration with the cell density.

Equation 9 has been found to be in good agreement with data from a number of experimental studies *(99)*. DeBacker et al. *(75)* showed that the diffusivity of glucose in an alginate gel decreased with increasing yeast cell concentration. Their data can be described well by Eq. 9 for a value of $D_0/D_c = 3$. Libicki et al. *(36)* reported diffusivities of nitrous oxide, a nonreactive tracer, in aggregates of *E. coli* confined within hollow fiber reactors. Their data lie between predictions for $D_0/D_c = 4$ and 5. Scott et al. *(13)* reported diffusivities of glucose in 4% κ-carrageenan gel beads containing varying microbial cell loadings that represent a value of $D_0/D_c = 2.5$. Axelsson and Persson *(23)* reported diffusivities for glucose in Ca-alginate plates containing varying yeast cell amounts. Their data at low cell fractions approach the theoretical curve for $D_0/D_c = 4$, but at higher cell fractions appear to approach that of $D_0/D_c = \infty$, although the difference is well within experimental error. The case of $D_0/D_c = \infty$ is applicable to situations in which the diffusing molecule is not taken up by the cells. This situation was investigated by Korgel et al. *(37)*, who measured the diffusivity of galactose in gel membranes containing immobilized *Z. mobilis* cells, which do not consume galactose. The data actually fall below predictions for $D_0/D_c = \infty$, but this small deviation could be owing to experimental error. Sun et al. *(33)* reported that the diffusivity of oxygen decreased with cell density in Ca-alginate gels, and their data is closely approximated by a curve corresponding to $D_0/D_c = 2$. Kurosawa et al. *(65)* reported that the diffusivity of

oxygen in cell-containing gel beads was independent of the cell density, type, and viability, and their data would indeed correspond to a horizontal line with $D_0/D_c = 1$.

Cellular Metabolism

Experimental Measurements of Cellular Metabolism

For the purpose of designing immobilized cell systems, it is essential to have an accurate quantification not only of their diffusive characteristics, but also of reactive properties under a wide variety of operating conditions. The literature contains substantially fewer studies on the rate of metabolite consumption by immobilized cells than for studies of the diffusive properties. This more limited amount of work on reactivity likely reflects the complications of evaluating purely reactive properties without the influence of diffusive restrictions and the difficulties in quantifying metabolite concentrations without introducing artifacts through the measurement process itself.

The metabolism of cells confined within a polymer layer may be, at times, difficult to predict. Uludag and Sefton *(100)* report that on encapsulating Chinese hamster ovary (CHO) cells, only 5% of the biocatalysts retained a high metabolic activity and up to 40% exhibited undetectable activity. In a tumor cell line that aggregates into spheroids, the glucose consumption rate increased 40% on a reduction in oxygen concentration and decreased when the extracellular pH was decreased *(70)*. pH and oxygen concentrations at which quiescent cells were observed were not low enough to account for the cessation of growth, indicating that the observed quiescence must have been owing to factors other than acidic pH, oxygen depletion, or glucose depletion *(70)*.

Quite often oxygen is the primary limiting species owing to its low solubility in water (6.6 μg/mL at 37°C *[101]*) and its high rate of consumption by the cells. Portner and coworkers *(102)* report that immobilized hybridoma cells are limited predominantly by the supply of oxygen; the supply of glucose or glutamine had a substantially smaller effect on cellular productivity. Several researchers *(1,2,9,19,103,104)* have similarly demonstrated that oxygen is the primary limiting substrate for mammalian cell growth and maintenance in varying types of immobilization materials. Frame and Hu *(105)* report that the oxygen consumption rate by swine testicular cells grown on microcarriers increases as the glucose concentration in the medium is depleted. Oxygen also plays a major role in defining the limitations of cell-based artificial organs. A reduction in oxygen delivery can reduce the attachment and spreading of hepatocytes on microcarriers and collagen layers *(106,107)*.

Several innovative techniques have been applied to characterize oxygen transport and consumption in immobilized cells. Slininger et al. *(108)* developed a colorimetric method based on the consumption of oxygen by glucose oxidase and the production of color formation at a wavelength of

526 nm. Robiolio *(109)* employed an optical method to measure the oxygen concentration based on the ability of oxygen to quench the phosphorescence of selected phosphors in the cell. This method revealed that human neuroblastoma cells consume oxygen at a constant rate when the oxygen pressure is greater than 11 torr. The conversion of glycerol to dihydroxyacetone (1,3-dihydroxy-2-propanone) catalyzed by bacteria has also been employed to indicate the level of oxygen supply to immobilized algae *(110)*. Muller et al. *(111)* developed polarographic microcoaxial needle electrodes to quantify the penetration of oxygen into Ca-alginate beads containing *Saccharomyces cerevisiae* cells. They report that, at steady state, oxygen penetrates only 50–100 μm away from the oxygen supply. These researchers also caution against spurious results that may be obtained with such needle electrodes owing to the formation of pseudo-oxygen gradients created by diffusion barriers at the electrode tip. Several groups report somewhat greater penetration depths of about 200 μm for oxygen into highly packed tumor cell aggregates *(1,2)*.

The cellular consumption of carbohydrates and amino acids can be monitored noninvasively through the use of nuclear magnetic resonance (NMR); however, extremely high cell densities (on the order of 10^8 cells/mL) are required to attain a sizeable NMR signal *(112)*. Cells immobilized within hollow fibers can easily reach such cell densities, thus permitting evaluation of intracellular metabolism under well-defined conditions *(113)*. Recently, NMR has been applied to evaluate the effect of glutamine concentration levels on hybridoma cell metabolism *(114)*. A brief reduction in glutamine could stimulate synthesis of antibodies and alter the consumption of energy-related metabolites *(114)*.

Models of Cellular Metabolism

Owing to the inherent difficulties in experimentally decoupling diffusive effects from reactive effects, mathematical models have been developed to describe the simultaneous diffusion and reaction behavior in immobilized cell systems. Biological models of immobilized cell metabolism can be categorized as unstructured (empirically based on experimental observations), or structured (theoretically based on knowledge of the individual cellular processes). Whereas the former are phenomenological models based on experimental observations, the latter are formulated from a more detailed knowledge of the individual cellular processes. This section summarizes some of the structured and unstructured models that have been proposed to describe metabolism in mammalian cells.

Oxygen is the principal chemical species limiting the activity of most types of immobilized cells. Accordingly, most theoretical studies have focused on predicting the oxygen penetration depth in tissue or other highly packed cellular material. One of the earliest theoretical studies of diffusion and reaction in a heterogeneous biological system is that of Stroeve *(115)*, who evaluated the transport of oxygen into tissue assuming an irreversible reaction on contact with a cell. His predictions of the onset of oxygen partial

pressure leading to anoxic cellular conditions (2 mmHg) were later found to be in excellent agreement with experimental studies conducted by several groups. Chang and Moo-Young *(116)* proposed a method to estimate the oxygen penetration depth by assuming 0th-order kinetics in the presence of several external oxygen transport resistances. Their calculations indicate that penetration depths for immobilized microbial cells (50–200 μm) are significantly less than those for immobilized animal or plant cells (500–1000 μm). Groebe *(117)* proposed an "easy-to-use" model to calculate oxygen penetration depths. This model suggests that for a number of muscle systems, the following effects may be neglected without much loss of accuracy: complexity of oxygen diffusion field near capillaries, deviations of capillary cross sections, oxygen diffusion parallel to the capillary, and oxygen consumption kinetics more complex than 0 order. Neglecting this last effect has the intriguing result of reducing the perceived ability of a cell to adapt to its environment.

Most kinetic analyses of mammalian cells have been carried out for suspension cultures, and few studies have evaluated the interplay of both nutrients and metabolic by-products on cell behavior. Miller et al. *(118)* developed a model for hybridoma growth, death, and monoclonal antibody (MAb) production based on measurements of the viable and dead cell density, metabolite concentrations, and dilution rate. DiMasi and Swartz *(119)* developed an energetically structured model of hybridoma cell metabolism to evaluate the partial substitutability of glucose and glutamine for provision of energy in the cell. Bonarius and coworkers *(120)* applied a metabolic flux analysis to generate a structured model to evaluate the uptake and production rates of glucose, lactate, ammonia, oxygen, carbon dioxide, amino acids, and antibodies. Zeng *(121)* developed a structured kinetic model to describe the effects of multiple factors on MAb production by long-time cultivations. Bree and coworkers *(122)* developed a model for hybridoma cell growth affected by the concentrations of glutamine, lactate, and ammonia. Bibila and Flickinger *(123)* developed a three-compartment model consisting of the endoplasmic reticulum, Golgi, and extracellular medium to describe the transport and secretion of MAbs.

In many hybridoma cell lines, it has been reported that the MAb production rate, q_{MAb}, increases with a decrease in the growth rate *(118)*, Suzuki and Ollis *(124)*; Linardos et al. *(125)*]. Through the use of a structured model for MAb production by hybridoma cells, Suzuki and Ollis *(124)* predicted and experimentally confirmed an enhancement in the MAb production rate with decreasing cell growth rate. Linardos et al. *(125)* developed a model for MAb production based on the cell death rate, $q_{MAb} = \alpha + \beta k_d$, in which α and β are constants for a given set of culture conditions and k_d is the specific cell death rate. Dalili and coworkers *(126)* developed a model to quantify the influence of glutamine on hybridoma growth and MAb production. In agreement with the experimental results of Vriezen and coworkers *(127)*, Dalili's model predicts that the maximum viable cell concentration is obtained when the glutamine levels are in the range of

0.5–2.0 m*M*. Zeng *(121)* provides a more detailed description of the effect of the cell death rate by using the relation $q_{MAb} = \alpha + \beta k_d(B + e^{-A\Delta t})$, in which *A* and *B* are constants that describe the diminishing MAb production with culture time. The effects of stimulating or inhibitory components may be incorporated into the model through the following formulation

$$q_{MAb} = (\alpha + \beta k_d)\,(B + e^{-A\Delta t}) \prod_i \frac{C_i}{C_i + K_i} \prod_j \frac{C_j}{C_j - C_j^* + K_j} \tag{10}$$

in which C_i and C_j are the concentrations of stimulatory and inhibitory components, respectively; K_i and K_j are the corresponding Michaelis constants; and Cj^* accounts for a saturation level that must be reached prior to the presence of inhibitory effects. Zeng *(121)* applied such a model to several hybridoma cell lines and reported that high MAb productivity can be achieved by maintaining low glucose, lactate, and ammonia concentrations.

Several other interesting studies concerning immobilized bacterial cells have been recently published. Adlercreutz *(128)* evaluated the delivery of oxygen to immobilized *Gluconobacter oxydans* cells with the addition of *p*-benzoquinone as an electron acceptor. Both external and internal mass transfer resistances were incorporated into the model, however, most conditions were found to be limited by internal mass transfer restrictions. *p*-Benzoquinone yielded a higher maximal reaction rate than oxygen because of its greater solubility and affinity as an electron acceptor. Cachon et al. *(129)* complemented *in situ* pH measurements with a mathematical model to characterize the microenvironment of *Lactococcus lactis* cells immobilized in alginate gel beads. They found that mass transfer limitations led to a progressive pH acidification within gel beads, which ultimately determined both the cell distribution and the cellular activity of entrapped cells.

Simulation Methods

Several groups of researchers have developed simulation methods to evaluate molecular diffusion and reaction in heterogeneous systems similar to immobilized cells. Much of this work focuses on the calculation of reaction rate constants for molecules diffusing in materials of variable structure. When the rate of molecular diffusion is much slower than molecular reaction, the overall rate constant for reaction follows from the Smoluchowski limit:

$$k_s = 4\pi(D_p + D_t)\,(r_p + r_t) \tag{11}$$

in which D_p is the diffusivity of a molecule with radius of r_p that travels toward a target of radius, r_t, which has a diffusivity of D_t. In this model, it is assumed that the molecular reaction is instantaneous on contact between the target and the traveling molecule. When the target is much larger than the diffusing molecule and has a smaller diffusivity, this relation reduces to

$$k_s = 4\pi D_p r_t \tag{12}$$

The derivation of this simple model requires that targets, or cells, are sparsely distributed and that molecules may react only with one cell. For most immobilized cell systems, these are not valid assumptions because cells are closely spaced and molecules may encounter any number of cells before being consumed.

To evaluate the effects of interactions between neighboring targets, simulation methods have been developed to investigate target placement, molecular diffusivity, and molecular reactivity on the overall rate of reaction *(130–134)*. These studies focused on the extreme scenario of instantaneous reactions. In general, reaction rates increase with increasing structural heterogeneity, or broader distributions in target size and shape. Conditions of near-instantaneous reactions apply reasonably well to the quenching of molecular fluorescence, but in most biological systems, reactions occur much more slowly. Northrup and Erickson *(90)* reported that antibody-antigen interactions occur at a rate that is three orders of magnitude lower than the Smoluchowski limit.

For diffusion and reaction of oxygen and nutrients in immobilized cells, the rate of reaction on contact with the cell is much slower than instantaneous, resulting in a probability that an individual molecular collision leads to reaction much less than 1. Situations with reaction probabilities less than 1 have been investigated by Fixman *(135)*, Torquato and Avellaneda *(136)*, and Riley et al. *(137,138)*. Employing similar procedures, Axelrod and Wang *(139)* studied the binding of cell surface receptors to ligands with a small success probability of 0.001, thus assuming that only a small fraction of ligand-receptor collisions lead to bond formation. Selection of appropriate reaction probabilities for a complex series of reactions such as cellular metabolism as required for such simulations can be difficult to determine accurately. Therefore, much of the simulation work in this area provides only qualitative information on effects on molecular diffusion and reaction. These studies do reveal that the development of cell clusters or cell aggregates can substantially reduce the overall rate of reaction compared with sparsely distributed cells, and as the reaction probability decreases, the effect of cell clustering is reduced *(137)*.

Cell Proliferation and Death

Experimental Measurements of Immobilized Cell Growth

The rate at which immobilized cells proliferate and die can depend on environmental conditions such as the concentration of available nutrients and the accumulation of wastes. Typically, cell growth occurs within a limited depth from the surface of the support material. This depth clearly depends on the cell type, supply of critical metabolites, accumulation of wastes, rate of surface renewal, and other factors. Franko and Sutherland *(1)* investigated the effects of diffusional limitations of metabolites in tumor microspheres and reported that the depth to which oxygen diffuses controls the development of necrosis whereas low glucose concentrations had

a limited effect on cell viability. Quantifying the spatial variation in the cell density in an immobilized cell system can be particularly difficult because the opacity of immobilization matrices often precludes the use of standard optical techniques.

In some situations, reasonably transparent immobilization supports have been employed so that the cell density could be determined by physically sectioning the biocatalyst, staining the cells, and quantifying the cell density through video microscopy and image analysis. Typically, such studies have revealed that the growth of cells is restricted to short depths away from the oxygen supply, suggesting that at high cell densities only cells at the outer surface received an adequate supply of oxygen. The growth of alginate-entrapped *S. cerevisiae* can be limited to a dense layer near the surface of the support *(3)*. Carlsson and Brunk *(140)* cultivated glioma cells in agarose gels and observed a gradient in cell proliferation that decayed almost exponentially with the distance from the surface. Lim et al. *(141)* cultivated vero and HepG2 cells on macroporous carriers and found that 87% of cells were contained in the outer half of spherical biocatalysts.

The specific cell growth rate in immobilized systems can, in some circumstances, differ significantly from that of suspension cell growth rates. Lefebvre and Vincent *(142)* report that the maximum specific growth rate of immobilized *E. coli* cells is reduced by 33% from that observed with freely suspended cells. Wohlpart et al. *(9)* evaluated the proliferation of hybridoma cells in spherical biocatalyst beads and reported viable cell numbers as a function of incubation time for two bead sizes. Regardless of size, they found that a time lag of about 150 h was required before the cells could begin to reproduce rapidly. They also reported variable cell doubling times of approx 25 and 50 h for 0.5- and 1.95-mm radius biocatalysts, respectively. Both values were significantly longer than those encountered for cells in suspension *(9)*. An increase in the bead radius by a factor of 4 increased the overall cell doubling time by a factor of roughly 2. Similarly, Wolffberg and Sheintuch *(143)* evaluated the distribution of cells in hollow-fiber bioreactors and reported that nutrient diffusion limitation could reduce the cell proliferation rate to half that of free cells. In some cases, it has been found that minimization of cell growth can improve the production of desirable products. Forberg et al. *(144)* applied a minimal supply of glucose to maintain immobilized *Clostridium acetobutylicum* in an active and nongrowing state, which improved production of butanol.

The initial cell seeding or distribution within a material has been shown to significantly impact cell growth. The growth of recombinant *E. coli* in alginate biocatalysts attained higher final cell densities when initially seeded with low cell densities *(145)*. Hollow-fiber bioreactors have been employed to produce large quantities of specific proteins owing to the attainment of high cell densities (up to 10^8 cells/mL) and concomitant high-product concentrations. Sardonini and DiBasio *(146)* evaluated the growth profile of hybridoma cells in the extracapillary space of such bioreactors. Initial seed cells were found to act as nucleation sites for the growth of

individual colonies as a thick cell mass formed near the fiber with cell colonies of diminishing size apparent with increasing distance from the nutrient supply. This same group cultivated SPt20 cells in multifiber bioreactors and showed that the increase in the number of fibers did not correlate with a linear increase in cell density owing to nonuniform distributions of oxygen delivery from the fibers *(147)*. It has been suggested that gravitational forces and convective flow may induce heterogeneous cell distributions that reduce productivities of such hollow-fiber bioreactors *(148)*. Altshuler et al. *(149)* grew hybridoma cells in polysulfone membranes to produce high concentrations of MAbs (740 μg/mL). Belfort *(150)* has thoroughly reviewed the use of membranes and fibers for the cultivation of cells and immobilization of enzymes.

On-line monitoring techniques have been employed to quantify immobilized cell growth. Ruaan and coworkers *(151)* characterized the density-dependent growth of CHO cells through on-line monitoring. Pons et al. *(152)* applied quantitative image analysis to monitor cell growth and cell cluster formation on the surface of microcarriers. Human kidney cells were found to decrease in size during the development of the initial cell monolayer. Cell size remained constant during the stationary phase. NMR techniques have been applied to determine the hybridoma cell density in hollow-fiber bioreactors *(153)* along with simultaneous monitoring of the cell metabolism.

Modeling Immobilized Cell Growth

For most of the applications described so far, the immobilized cells are retained in a viable state and proliferate, provided that an adequate supply of metabolites is available. However, immobilized cells frequently receive a nonuniform supply of nutrients owing to limited penetration of oxygen and cellular nutrients, and the cell growth rate can be affected by the supply of these metabolites. Cells toward the outer regions of the material proliferate rapidly whereas cells toward the inner regions proliferate slowly or not at all. The uneven distribution of cells in the support material results in an uneven distribution of growth rates. Since the cells are stationary, progeny remain in close proximity to the initial seed cells, leading to the formation of compact cell clusters. Numerous models have been developed to describe immobilized cell proliferation under varying environmental conditions to estimate the intrinsic cell proliferation rate while removing the effect of processes such as contact inhibition and to predict the proliferation rate for varying seeding densities and geometries. Such modeling approaches are believed to yield reasonably realistic descriptions of cell growth and death rates, particularly for situations in which cell-cell aggregation is substantial *(154)*.

The presence of cells inside a biocatalyst can be described by either morphologically structured or morphologically unstructured models. Morphologically unstructured models typically represent the cellular material as a uniform distribution of the cell density over a given unit

volume. Structured models account for the individual placement and architecture of single cells or microcolonies of cells. Although both approaches can account for uneven distributions of cells across the biocatalyst dimension, structured models have the ability to characterize nonuniformities at shorter-length scales and to yield more detailed information. Several researchers have ascribed such growth nonuniformities to the formation of microcolonies that germinate from single "seed" cells *(16,155)*.

To evaluate the proliferation of immobilized cells with a morphologically structured model, a set of simple rules must be generated to mimic the physical laws that control cell proliferation. Such rules can be implemented in computational algorithms that discretize time and space to produce a cellular automaton *(156)*. Cellular automata simulations are well suited to characterize contact-inhibited growth of cells immobilized on two-dimensional surfaces *(157–160)*, and in three-dimensional matrices *(97,145,157)*. Such methods have been reviewed by Ermentrout and Edelstein-Keshet *(156)* for application to biological systems.

Cellular automata are a class of computer-simulation techniques in which biological cells (or other individual entities) are represented on a computer as digitized nodes or computational "cells" of a matrix. One node may represent a single biological cell, a small collection of cells, or a fraction of a single cell. When nodes represent a single cell, they are either occupied by a cell or are empty and available for cell growth. An initial seeding of cells is distributed throughout the material in digitized fashion. During a discrete time-step increment, cells can proliferate, die, or remain unchanged. If a cell has a vacant neighboring node, the cell may proliferate with one daughter cell moving into that free node. The decision to proliferate or not can be based on several factors such as cell state, available supply of nutrients, and the presence of inhibiting products. These models often produce an emergent behavior; i.e., a complex form of behavior becomes apparent beyond which was incorporated into the model. Cellular automa simulations have been shown to qualitatively replicate the growth patterns and rates observed for proliferation of anchorage-dependent cells *(158–161)*. Greenberg et al. *(145)* developed a cellular automaton model to characterize the formation of *E. coli* microcolonies in an alginate matrix. The maximum cell density obtained was found to vary with the initial cell density to the –1/6th power, in good agreement with their experimental results.

An important feature of structured morphological models is that they provide a high spatial resolution and thereby have the ability to evaluate processes on the cellular scale. However, the development and implementation of such models can be more difficult and time consuming than simple unstructured models. Using more traditional approaches, models have also been developed to characterize the maximum cell loading in a biocatalyst for different pore sizes *(162)*; the optimum pore size to cell size ratio was reported as approximately 2.5. Qi and coworkers developed a cellular automata model describing the interplay between cancer cells and the immune system following a Gompertz-type relationship.

A commonly used approach for unstructured morphological models is to represent the cell density as a constant value within a given volume element spanning multiple cell layers. The average cell density within a volume element increases or decreases based on the supply of metabolites, the availability of free space, and other factors. In many situations, the cell proliferation rate (μ) is assumed to follow the Monod kinetic form:

$$\mu = \mu_{max} [C/(C + K_m)] \tag{13}$$

in which μ and μ_{max} are the actual and maximum cell growth rates, respectively; C is the concentration of the predominant limiting species; and K_m is the Monod constant.

The cellular proliferation may be limited by multiple species for which one may apply a serial Monod approach:

$$\mu = \mu_{max} [C_a/(C_a + K_{m_a})] [C_b/(C_b + K_{m_b})] [C_c/(C_c + K_{m_c})] \tag{14}$$

in which the indexes a, b, and c identify three independent chemical species, such as glucose, glutamine, and oxygen *(121)*.

When the accumulation of a metabolic by-product hinders cell growth , the following relation may be used:

$$\mu = \mu_{max} [C_a/(C_a + K_{m_a})] [K_{m_b}/(C_b + K_{m_b})] \tag{15}$$

in which species "a" is a metabolite and species "b" is an inhibitory factor. Such methods have been applied with reasonable success by Zeng *(121)* and by Bibila and Robinson *(163)*.

Biological models have been reported to describe the effects of the cellular environment on proliferation, metabolism, and product generation by hybridoma cells. The cell growth rate and the cell death rate (Ω) can depend on the concentrations of glucose, glutamine, ammonia, and lactate, and this relationship may be described by serial-type equations of the following form:

$$\mu = \mu_{max}\left(\frac{Glu}{K_{Glu} + Glu}\right)\left(\frac{Gln}{K_{Gln} + Gln}\right)\left(1 - \frac{K_{Amm}}{K_{Amm} + Amm^* - Amm}\right)1 - \frac{K_{Lac}}{K_{Lac} + Lac^* - Lac} \tag{16}$$

$$\Omega = k_d\left(\frac{K_{dGln}}{K_{dGln} + Gln}\right)\left(\frac{Amm}{K_{dAmm} + Amm}\right)\left(\frac{Lac}{K_{dLac} + Lac}\right) \tag{17}$$

in which μ_{max} and k_d are the maximal cell growth and death rates, respectively; the K's and K_d's are constants relating the cellular sensitivity to changes in a chemical species concentration; Glu, Gln, Amm, and Lac are the concentrations of glucose, glutamine, ammonia, and lactate, respectively, in solution; and Amm* and Lac* represent saturation concentrations below which the species has no effect on cell growth. The novel forms of the ammonia and lactate terms are required because low levels of these species (below Amm* and Lac*) have no effect on the cell growth rate. Reasonable values of Amm* and Lac* are 4 and 47 mM, respectively, derived from the experimental results of Glacken et al. *(164)* and Ozturk et al. *(165–167)*.

Multivariate Models of Immobilized Cells

The overall behavior of an immobilized cell biocatalyst depends on the interplay of nutrient and product diffusion and reaction, cell proliferation, and cell death. To develop a more thorough understanding of how these processes are related and how the generation of desired products can be maximized, multivariate analyses have been applied. Such multivariate models permit the evaluation of multiple processes and possible interactions. The discussion that follows focuses primarily on processes that occur in the interior of a biocatalyst.

Models of varying degrees of complexity have been developed to simulate the processes of growth, nutrient consumption, and product synthesis by immobilized cells. Such models are used to predict spatial gradients of nutrients and cells and thereby to assess biocatalyst performance.

Multivariate models must incorporate descriptions of nutrient and oxygen diffusion, cell metabolism, cell proliferation, and cell death (where applicable). Such a multivariate description may comprise a mass balance for nutrients, products, and cells. The rates of nutrient and product diffusion may follow from relations such as Eqs. 1–9. The cell metabolism may follow from relations such as Eq. 10. The rate of cell proliferation and death may be approximated by relations such as Eqs. 13–17. The use of multiple dependent variables led to the multivariate terminology. Note that the cell density impacts the diffusivity relation through the cell volume fraction, as well as the overall reactivity through a multiplicative effect. As the cells proliferate, the overall rate of reaction typically increases and the diffusivity decreases. Together these effects reduce the concentration of available nutrients, which often reduces the cell proliferation rate. Therefore, an alteration in one parameter such as the initial cell density can influence multiple processes in a biocatalyst. Numerous examples of such multivariate models have been presented in the literature. Following is a brief summary of some of the more notable works.

Monbouquette et al. *(6)* developed a model of alginate-immobilized *Z. mobilis* cells to predict the pseudo steady-state concentrations of glucose, ethanol, and biomass. Sayles and Ollis *(5)* modeled biocatalyst performance subjected to product inhibition. They investigated the effects of inhibitory product concentrations and cell sensitivities to this product on the cell growth rate, product generation, and thickness of the viable cell layer. Silva and Malcata *(168)* developed a model of aggregated cell growth to determine nutrient feeding schemes to optimize biomass production. Cells at the interior of the aggregate had diminished growth rates and productivities as a result of oxygen and glucose transport limitations into the aggregate.

Sarikaya and Ladisch *(169)* developed an unstructured model for the growth of *Pleurotus ostreatus* in a solid-state fermentation. Their model suggests that substantial competition exists between neighboring cells for available substrate. Lefebvre and Vincent *(142)* modeled diffusion, reaction, and immobilized *E. coli* cell proliferation in agar membranes and

reported that the accumulation of inhibitory products had a larger effect on cell proliferation than did nutrient limitations. Wik and Breitholtz *(170)* developed models for two-species biofilms and determined that thin biofilms yield the most restrictive conditions for steady-state coexistence. Wang and Furusaki *(171)* developed a model consisting of mass balances for glucose, lactate, and ionic species involved in a lactic acid fermentation. A gradient in the cell density became apparent as a function of the accumulation of the inhibitory product and not by substrate limitation. Converti et al. *(172)* evaluated the transient response of immobilized cells during the start-up of a fermentation.

Wijffels et al. *(173)* developed a dynamic model of immobilized *Nitrosomonas europaea* cells accounting for cell growth, substrate diffusion and consumption, and the release of cells from the immobilization support. A comparison of their experimental results and model predictions suggests that cell leakage from the support is owing to the loss of entire colonies and not to single cells. Hunik et al. *(174)* developed a dynamic model for immobilized *N. europaea* and *Nitrobacter agilis* cells accounting for oxygen, ammonia, nitrite, and nitrate diffusion and reaction, along with cell proliferation. Shishido and Toda *(7)* evaluated the effect of oxygen limitations on phenol degradation by entrapped microbes. Their models suggest that the maximum biocatalyst diameter for high cell densities that can be free of oxygen limitations is approx 1 mm. Substrate inhibition sets the depth to which oxygen can penetrate in the biocatalysts.

The aforementioned models permit the evaluation of many properties that can be related to biocatalyst performance. They describe the behavior of immobilized microbial cells, which typically have much higher growth rates than mammalian cells. Much less modeling work has been reported for immobilized mammalian cells. In such systems, the cell density increases slowly and the rate of cell death can become significant during operation. A key design objective is to determine cell loadings, biocatalyst sizes, and feeding schemes for maximal productivity.

Sardonini and DiBasio *(147)* developed a model of immobilized hybridoma cells proliferating around the surface of a hollow fiber that delivers nutrients and removes wastes. This model was used to monitor the penetration depth of oxygen away from the fiber as a function of cell density and oxygen supply. The oxygen penetration depth for a continuous cell mass was predicted to be 110 μm. Experimentally, these researchers reported that for the same conditions, the cell mass thickness was found to be 370 μm; presumably most of these cells were formed before a large cell population developed close to the fiber surface. Riley and coworkers *(99)* developed a model of immobilized hybridoma cell growth to characterize optimal cell loading, biocatalyst diameter, and oxygen supply. Biocatalysts with diameters <1 mm typically were found to be free of significant oxygen transport limitations regardless of the initial cell loading. Tziampazis and Sambanis *(175)* describe a model to characterize nutrient and metabolite concentration profiles in calcium alginate poly(L-lysine) membranes

containing insulin-secreting cells. The model was employed to determine the secretory response for varying biocatalyst sizes and cell loadings. The secretory response of 1 mm or smaller biocatalysts was generally similar to the response of free cells, although with a time delay on the order of several minutes.

While such multivariate models provide valuable understanding of the overall behavior of cell-containing biocatalysts, caution must be exercised when developing such a model. Experimental validation is a critical step that must be carried out to establish the accuracy and suitability of the model. Many of the aforementioned models are empirically based. When this is the case, results must not be extrapolated beyond the valid range of parameters, particularly for the case when relations are developed to correlate the cell density to molecular diffusivities.

Summary

In summary, the overall behavior of immobilized cells is controlled by the interplay of nutrient and product diffusion and reaction, cell metabolism, cell proliferation, and cell death. These process are intrinsically linked, and evaluation of one process independent of other effects can be difficult. Substantial overlap exists between much of the experimental and computational work done in this field over the past 10 yr but with little cross communication. We hope that the areas of research described here will provide a basis for communication between researchers in disparate fields working on similar problems.

The past several years have yielded significant improvements in the development and operation of immobilized cell systems. Possible progress in the near future may develop through the use of new techniques and approaches developed in other research areas. Models and simulations will continue to increase in complexity in proportion to improvements in computer speed, memory, and cost. An increase in simulation sophistication should focus on developing a more thorough understanding of the effect of the accumulation of wastes, deprivation of nutrients, and increased local concentrations of cellular growth factors. Through a combination of experimental and modeling studies, one can thoroughly evaluate the effects of alterations in multiple parameters on the overall productivity of immobilized cell biocatalysts. Possible experimental progress that may impact future use of immobilized cells includes the development of novel polymeric supports that can respond to external stimuli (electrochemical, pH, salt changes); the use of noninvasive monitoring techniques (such as IR microspectroscopy) to directly characterize molecular transport, cellular metabolism, and cell proliferation *in situ*; and the development of improved methods to deliver precise amounts of nutrients and oxygen, possibly through the use of controlled release methods or the use of techniques to increase substantially the oxygen tension in the culture medium since oxygen is predominantly the most frequent limiting factor.

Synopsis of Recent Patents Utilizing Immobilized Cells

The following is a brief synopsis of patents granted from April 4, 1995 to August 12, 1997, for novel uses of immobilized cells.

Bioreactor for Production of Products with Immobilized Biofilm

Inventors: Amihay Freeman
Assignee: Ramot University Authority Ltd., Tel Aviv, Israel
Issued: Apr. 4 1995
Serial Number: 114275

A bioreactor is provided for production of products by biosynthesis or biotransformation using a biofilm of immobilized cells. The bioreactor includes a horizontal cylindrical housing and a central rotatable shaft that extends along the axis of the housing. Connected to the central shaft are one or more screens that are oriented parallel to the shaft, with a small gap between the shaft and the screen. The bioreactor further includes a slidable blade that is mounted on to the shaft, through a set of poles, and located at a fixed distance from the screen so that the blade is made to pass over the screen by the force of gravity as the shaft rotates, as by the action of a rotating external magnet.

Immobilized Biocatalysts and Their Preparation and Use

Inventors: Abraham Harder, Ben R. DeHaan, Johannes B. Van der Plaat, Marsha Cummings
Assignees: Gist-Brocades N.V., Netherlands
Issued : Apr. 11, 1995
Serial Number: 225392

Immobilized water-insoluble biocatalysts in particulate form comprise living cells, particularly yeast, dispersed in a crosslinked gelling agent. An enzyme, particularly amyloglucosidase, may be coimmobilized in the particles. These particles are prepared by suspending the living cells in an aqueous solution of a gelling agent, dispersing this suspension in a water-immiscible organic liquid to form a suspension in the liquid of aqueous particles comprising the living cells and gelling agent, gelling the gel, and crosslinking the gelling agent. It is found that when living cells such as microbial cells and especially yeast are immobilized in this way, surprisingly, not only is their viability retained but the ability of yeast cells to produce ethanol under continuous fermentation conditions is significantly improved.

Apparatus and Process for Continuous In Vitro Synthesis of Proteins

Inventors: Bobak R. Mozayeni
Assignees: The United States of America as represented by the Department of Health and Human Services, Washington, DC
Issued: July 18, 1995
Serial Number: 017062

An apparatus and process for continuous, cell-free, in vitro synthesis of peptides, particularly peptide-major histocompatibility complexes make use

of a novel bioreactor flow cell that allows for the reproducible, systematic variation of single parameters in order to optimize translation processes. The bioreactor flow cell includes a pair of substantially parallel membranes positioned within a chamber, which permits high perfusion rates through the system with lower flux rate per membrane area.

Method and Compositions for the Degradation of Tributyl Phosphate in Chemical Waste Mixtures

Inventors: Daphne L. Stoner, Albert J. Tien
Assignees: Lockheed Idaho Technologies Company, Idaho Falls, ID
Issued: Sept. 26, 1995
Serial Number: 108345

This is a method and process for the degradation of tributyl phosphate in an organic waste mixture and a biologically pure, novel bacteria culture for accomplishing the same. A newly discovered bacteria is provided that is combined in a reactor vessel with a liquid waste mixture containing tributyl phosphate and one or more organic waste compounds capable of functioning as growth substrates for the bacteria. The bacteria is thereafter allowed to incubate within the waste mixture. As a result, the tributyl phosphate and organic compounds within the waste mixture are metabolized by the bacteria, thereby eliminating materials that are environmentally hazardous. In addition, the bacteria is capable of degrading waste mixtures containing high quantities of tributyl phosphate.

Process for the Biotechnological Preparation of L-Thienylalanines in Enantiomerically Pure Form from 2-Hydroxy-3-Thienylacrylic Acids, and Their Use

Inventors: Gerhard Kretzschmar, Johannes Meiwes, Manfred Schudok, Peter Hammann, Ulrich Lerch, Susanne Grabley
Assignees: Hoechst Aktiengesellschaft, Frankfurt am Main, Federal Republic of Germany
Issued: Jan. 2, 1996
Serial Number: 099352

L-Thienylalanines are prepared via the hydantoin or the azlactone route. The starting substances used for the biotransformation are 2-hydroxy-3-thienylacrylic acids. The innovative step consists in the transamination of the enol form of the 2-hydroxy-3-thienylacrylic acids to give L-thienylalanines with the aid of biotransformation.

Adsorbent Biocatalyst Porous Beads

Inventors: Thomas I. Bair, Carl E. Camp
Assignees: E. I. Du Pont de Nemours and Company, Wilmington, DE
Issued: Jan. 23, 1996
Serial Number: 205689

Highly porous, adsorbent biocatalyst beads of synthetic organic polymer have powdered activated carbon dispersed throughout the polymer and a biocatalyst located within macropores of the beads. The beads are used for remediation of contaminated aqueous streams. The biocatalyst can consume

adsorbed organic contaminants, while continuously renewing the adsorptive capacity of the activated carbon.

Microcapsule-Generating System Containing an Air Knife and Method of Encapsulating

Inventors: Randel E. Dorian, Kent C. Cochrum
Assignees: The Regents of the University of California, Oakland, CA
Issued: May 28, 1996
Serial Number: 185709

Spherical microcapsules containing biological material such as tissue or living cells are formed with a diameter of <300 μm using a microcapsule-generating system containing an air knife. The air knife is formed by an air sleeve positioned eccentrically around a needle. An encapsulating material such as an alginate solution containing the biological material to be encapsulated is forced through the needle, while pressurized air is introduced into the air sleeve and flows out an end opening of the sleeve in which the needle is positioned. The pressurized air breaks up the alginate being discharged from the needle. The resultant alginate droplets fall into a collecting tank, where they contact a gelling medium, such as $CaCl_2$, so that the outer surface of these droplets hardens and microcapsules are formed.

Metal Accumulation

Inventors: Rosemary E. Dick, Lynne E. Macaskie
Assignees: British Nuclear Fuels PLC, Warrington, England
Issued: May 28, 1996
Serial Number: 436205

Metals having phosphates of low water solubility are removed from water by reaction with phosphate produced by enzymatically cleaved polyphosphate that has been accumulated by one or more polyphosphate-accumulating microorganisms.

Method of Removing Protein from a Water-Soluble Gum and Encapsulating Cells with the Gum

Inventors: Heather A. Clayton, Roger F. L. James, Nicholas J. M. London
Assignees: University of Leicester, Leicester, England
Issued: June 25, 1996
Serial Number: 196072

Contaminating protein is removed from a water-soluble gum such as alginate by dialyzing a solution of the gum against a solution of a disulfide bond reducing agent. Purifying the gum by removing antigenic protein improves biocompatibility of the gum making biocompatible capsules containing cells such as mammalian cells. Dialyzing is preferably carried out for more than 1 h and preferably twice, each time for 2 h. Cells are encapsulated by forming a suspension of cells in an aqueous solution of the gum; forming droplets from the suspension; gelling the droplets with a multivalent cation; contacting the gelled droplets with a polymer containing cationic groups, such as poly-L-lysine chloride, that crosslink with anionic groups of the gum to form a semipermeable membrane around the droplets; and coating the

membrane with a layer of the dialyzed gum. Islets of Langerhans cells can be encapsulated for implanting to produce insulin.

Microbubble Generator for the Transfer of Oxygen to Microbial Inocula, and Microbubble Generator Immobilized Cell Reactor

Inventors: Ralph J. Portier, Huazhong Mao
Assignee: Louisiana State University Board of Supervisors, a governing body of Louisiana State University, Agricultural and Mechanical College, Baton Rouge, LA.
Issued: Jul. 9, 1996
Serial Number: 378072

A microbubble generator is disclosed for optimizing the rate and amount of oxygen transfer to microbial inocula or biocatalysts. The microbubble generator, and an associated immobilized cell reactor, are useful in the detoxification and cleanup of nonvolatile polymeric– and volatile organic–contaminated aqueous streams. In particular, they are useful in the continuous mineralization and biodegradation of toxic organic compounds, including volatile organic compounds, associated with industrial and municipal effluents, emissions, and groundwater and other aqueous discharges.

Glucose-Responsive Insulin-Secreting β-Cell Lines and Method for Producing Same

Inventors: Megan E. Laurance, David Knaack, Deborah M. Fiore, Orion D. Hegre
Assignees: CytoTherapeutics, Inc., Providence, RI
Issued: July 9, 1996
Serial Number: 208873

A method of selecting cells with enhanced secretion of a product is disclosed. The method comprises exposing a population of cells to a secretagogue to result in the secretion of a product from the cells and selecting from the population cells that exhibit increased amounts of intracellular free calcium when exposed to the secretagogue. The method enables the selection of correctly regulated β-cells that secrete appropriate amounts of insulin in response to varying glucose levels.

Method for Implanting Encapsulated Cells in a Host

Inventors: Laura M. Holland, Joseph P. Hammang, Seth A. Rudnick, Michael J. Lysaght, Keith E. Dionne
Assignees: CytoTherapeutics, Inc., Providence, RI
Issued: Aug. 27, 1996
Serial Number: 228403

This invention provides methods for implanting encapsulated cells in a host comprising exposing cells to restrictive conditions for a period of time to establish a desired cell property in response to the restrictive conditions and implanting the encapsulated cells in a host.

Processes for Production of Optically Active 4-Halo-3-Hydroxybutyric Acid Esters

Inventors: Akinobu Matsuyama, Akira Tomita, Yoshinori Kobayashi
Assignees: Daicel Chemical Industries, Ltd., Osaka, Japan
Issued: Sept. 24, 1996
Serial Number: 180420

A microorganism capable of acting on a 4-halo-acetoacetic acid ester to produce an optically active 4-halo-3-hydroxybutyric acid ester or a preparation thereof is permitted to act on said 4-halo-acetoacetic acid ester, and the product optically active 4-halo-3-hydroxybutyric acid ester is harvested. Thus, the desired optically active 4-halo-3-hydroxybutyric acid ester of high optical purity can be produced with commercial efficiency.

Extractive Fermentation Using Convoluted Fibrous Bed Bioreactor

Inventors: Shang-Tian Yang
Assignees: The Ohio State University Research Foundation, Columbus, OH
Issued: Oct. 8, 1996
Serial Number: 101926

Apparatus and method for converting organic materials such as sugars and acids into other organic materials such as organic acids and salts other than the starting materials with immobilized cells. The invention is applicable to the conversion of the lactose content of whey, whey permeate, or other lactose-containing solutions and wastes into lactic acid, propionic acid, acetic acid, and their salts. The cells are immobilized onto the surface of and within convoluted sheets of a fibrous support material, and reactant-bearing fluids are caused to flow between the opposing surfaces of such convoluted sheets. Lactose-containing solutions such as whey and whey permeate may be cofermented with homolactic and homoacetic bacteria to acetic acid or acetate. The product may be extracted from its aqueous media by high-distribution coefficient solvents, particularly trioctylphosphine oxide and long-chain aliphatic secondary, tertiary, and quaternary amines.

Plant Germinants Produced from Analogs of Botanic Seed

Inventors: William C. Carlson, Jeffrey E. Hartle, Barbara K. Bower
Assignees: Weyerhaeuser Company, Tacoma, WA
Issued: Oct. 15, 1996
Serial Number: 423965

An analog of botanic seed is disclosed that comprises a plant embryo preferably encapsulated in a hydrated oxygenated gel. The gel can be oxygenated by passing oxygen through a gel solution before curing the gel or by exposing the gel to oxygen after curing. The gel is preferably oxygenated by adding to an uncured gel solution a suitably stabilized emulsion of a perfluorocarbon compound or a silicone oil, whose compounds are capable of absorbing large amounts of oxygen and are nontoxic and inert. The seed analog can further comprise an outer shell at least partially surrounding the gel and embryo, thereby forming a capsule. Other shell materials are selected

to provide requisite rigidity to the capsule while imparting minimal restriction to successful germination.

Biocatalysts Immobilized in a Storage-Stable Copolymer Gel

Inventors: Toshiaki Doi, Hiroyasu Bamba, Kouzou Murao
Assignees: Nitto Chemical Industry Co., Ltd., Tokyo, Japan
Issued: Oct. 22, 1996
Serial Number: 370254

Biocatalysts such as cells and enzymes are immobilized in a polymer gel by forming a mixture containing a biocatalyst, an *N*,*N*-dialkylacrylamide monomer, a cationic acrylamide monomer, and a water-soluble crosslinking monomer, and copolymerizing the monomers to produce a polymer gel entrapping the biocatalyst. The polymer gel containing a biocatalyst has excellent storage stability and does not putrefy even after 1 mo of storage at ordinary temperature.

Photopolymerizable Biodegradable Hydrogels as Tissue-Contacting Materials and Controlled-Release Carriers

Inventors: Jeffrey A. Hubbell, Chandrashekhar P. Pathak, Amarpreet S. Sawhney, Neil P. Desai, Jennifer L. Hill-West
Assignees: Board of Regents, The University of Texas System, Austin, TX
Issued: Oct. 22, 1996
Serial Number: 468364

Hydrogels of polymerized and crosslinked macromers comprising hydrophilic oligomers having biodegradable monomeric or oligomeric extensions whose biodegradable extensions are terminated on free ends with end cap monomers or oligomers capable of polymerization and crosslinking are described. Macromers are polymerized using free-radical initiators under the influence of long wavelength ultraviolet light, visible light excitation, or thermal energy. Biodegradation occurs at the linkages within the extension oligomers and results in fragments that are nontoxic and easily removed from the body. Preferred applications for the hydrogels include prevention of adhesion formation after surgical procedures, controlled release of drugs and other bioactive species, temporary protection or separation of tissue surfaces, adherence of sealing tissues together, and prevention of the attachment of cells to tissue surfaces.

Gels for Encapsulation of Biological Materials

Inventors: Jeffrey A. Hubbell, Chandrashekhar P. Pathak, Amarpreet S. Sawhney, Neil P. Desai, Jennifer L. Hill-West, Syed F. A. Hossainy
Assignees: Board of Regents, The University of Texas System, Austin, TX
Issued: Nov. 12, 1996
Serial Number: 024657

Water-soluble macromers are modified by the addition of free-radical polymerizable groups, such as those containing a carbon-carbon double or triple bond, which can be polymerized under mild conditions to encapsulate tissues, cells, or biologically active materials. The polymeric materials are

particularly useful as tissue adhesives, coatings for tissue lumens, coatings for cells such as islets of Langerhans, supports or substrates for contact with biological materials such as the body, and as drug delivery devices for biologically active molecules.

Support Containing Particulate Adsorbent and Microorganisms for Removal of Pollutants

Inventors: Louis J. DeFilippi
Assignees: AlliedSignal Inc.
Issued: Dec. 3, 1996
Serial Number: 174587

A biologically active support for removing pollutants from a fluid stream such as wastewater is prepared. The support is formed of a polymeric foam substrate coated with a composition containing a particulate adsorbent that adsorbs and then releases pollutants, and a polymeric binder that binds the adsorbent to the surface of the substrate. The binder contains a suspension aid, and one or more pollutant-degrading microorganisms are adhered to the surface of the coated support. To remove pollutants, the biologically active support is placed in a reactor and a fluid stream containing a pollutant such as phenol is passed through the reactor, where the pollutant is degraded by the microorganism and adsorbed to the adsorbent. The adsorbent acts as a buffer by adsorbing excess pollutant from solution when the pollutant concentration increases, and when the pollutant concentration decreases, it releases pollutant into solution, where the microorganism degrades the pollutant.

Immobilized Cell Bioreactor

Inventors: Cheryl A. Plitt, W. J. Harris
Assignees: none
Issued Dec. 17, 1996
Serial Number: 538976

An immobilized cell bioreactor is disclosed wherein the cells are harbored within or on an immobilization matrix including cell support sheets comprising common textile fabric. The cell support sheets are oriented in a vertical parallel layered array with a gas phase substantially surrounding each sheet. The vertical orientation allows nutrient culture supply and product recovery to be assisted by gravity. The vertical orientation also allows the sheets to extend into unused vertical space, producing a space-efficient bioreactor.

Biodesulfurization of Bitumen Fuels

Inventors: James M. Valentine
Assignees: none
Issued: Jan. 14, 1997
Serial Number: 538254

A simple and effective biochemical process solves the problems associated with sulfur in bitumen by removing sulfur from active participation in SOx-producing combustion reactions. In one aspect, an emulsion of bitumen

and water is contacted with a microbiological desulfurization agent for a time and under conditions effective to reduce the oxidizable sulfur content of the bitumen. The preferred agents do not affect the heating value of the fuel, but selectively oxidize organic sulfur to water-soluble sulfates.

Immobilization of Microorganisms on a Support Made of Synthetic Polymer and Plant Material

Inventors: Anthony L. Pometto III, Ali Demirci, Kenneth E. Johnson
Assignees: Iowa State University Research Foundation, Inc., Ames, IA
Issued: Jan. 21, 1997
Serial Number: 254476

A solid support for immobilization of microorganisms cells is made of a synthetic polymer such as a polyolefin, in a mixture with an organic polymeric plant material such as corn fibers, oat hulls, starch, and cellulose. The plant material may be a mixture including a plant material that functions as a nutrient to enhance growth of the microorganism on the support. The supports are useful for immobilizing living cells of a microorganism to form a biofilm reactor for use in continuous fermentations, in streams for bioremediation of contaminants, and in waste treatment systems to remove contaminants and reduce biochemical oxygen demand levels.

Metal Removal from Aqueous Solution

Inventors: Mark R. Tolley, Lynne E. Macaskie
Assignees: British Nuclear Fuels plc, Cheshire, England
Issued: Jan. 28, 1997
Serial Number: 525633

Removal of a target metal having an insoluble phosphate is effected by passing the solution through a bioreactor containing an immobilized phosphatase-producing microorganism that has been cultivated using a culture medium containing an assimilable organic source of phosphorus and that has been primed with an element having an insoluble phosphate so as to deposit the phosphate of the priming element on cell surfaces of the microorganism.

Bioreactor Device with Application as a Bioartificial Liver

Inventors: Wei-Shou Hu, Frank B. Cerra, Scott L. Nyberg, Matthew T. Scholz, Russell A. Shatford
Assignees: Regents of the University of Minnesota, Minneapolis, MN
Issued: Feb. 25, 1997
Serial Number: 376095

This is a bioreactor apparatus comprising two chambers: a feed and waste chamber and a cell chamber separated by a selectively permeable membrane. Within the cell chamber, a biocompatible three-dimensional matrix entraps animal cells or genetic modifications thereof. Owing to the presence of this biocompatible matrix, the cell chamber generally has a gel phase, i.e., the biocompatible matrix and cells, and a liquid phase containing a concentrated solution of the cell product to be harvested. A bioartificial liver is based on a bioreactor of the type having two fluid paths separated by a permeable

medium. The bioreactor can be of either hollow-fiber or flat-bed configuration. In the configuration using hollow fibers, the two fluid paths correspond to the cavity surrounding the hollow fibers (the extracapillary space) and to the lumens of the hollow fibers themselves. The gel subsequently contracts, leaving an open channel within the hollow fiber adjacent to the gel core–entrapped hepatocytes.

Centrifugal Fermentation Process

Inventors: Heath H. Herman
Assignees: Kinetic Biosystems, Inc., Tucker, GA
Issued: Apr. 22, 1997
Serial Number: 412289

This invention comprises a novel culture method and device in which living cells or subcellular biocatalysts are immobilized by the opposition of a centrifugal force field and a liquid flow field. The immobilized cells or biocatalysts are ordered into a three-dimensional array of particles, the density of which is determined by the particle size, shape, and intrinsic density as well as by the selection of combinations of parameters such as liquid flow rate and angular velocity of rotation.

Synthesis of Catechol from Biomass-Derived Carbon Sources

Inventors: John W. Frost, Karen M. Draths
Assignees: Purdue Research Foundation, West Lafayette, IN
Issued: May 13, 1997
Serial Number: 122919

A method is provided for synthesizing catechol from a biomass-derived carbon source capable of being used as a host cell having a common pathway of aromatic amino acid biosynthesis. The method comprises the steps of biocatalytically converting the carbon source to 3-dehydroshikimate (DHS), biocatalytically converting the DHS to protocatechuate, and decarboxylating the protocatechuate to form catechol. Also provided is a heterologous *E. coli* transformant.

Compositions and Methods for the Delivery of Biologically Active Molecules Using Cells Contained in Biocompatible Capsules

Inventors: Edward E. Baetge, Joseph P. Hammang, Frank T. Gentile, Mark D. Lindner, Shelley R. Winn, Dwaine F. Emerich
Assignees: CytoTherapeutics, Inc., Providence, RI
Issued: Aug. 12, 1997
Serial Number: 449946

This invention provides improved devices and methods for long-term, stable expression of a biologically active molecule using a biocompatible capsule containing genetically engineered cells for the effective delivery of biologically active molecules to effect or enhance a biological function within a mammalian host. The novel capsules of this invention are biocompatible and are easily retrievable. This invention specifically provides improved methods and compositions that utilize cells transfected with recombinant

DNA molecules comprising sequences coding for biologically active molecules operatively linked to promoters that are not subject to downregulation in vivo on implantation into a mammalian host. Furthermore, the methods of this invention allow for the long-term, stable, and efficacious delivery of biologically active molecules from living cells to specific sites within a given mammal.

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