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총 설

Synthetic Membranes in Biotechnology: Realities and Possibilities[1]

Georges Belfort

Bioseparation Research Center, Howard P. Isermann Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York 12180-3590, U. S. A.

생물공학에서의 합성막 : 현실과 가능성

Georges Belfort

Bioseparation Research Center, Howard P. Isermann Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, New York 12180-3590, U. S. A.

Abstract: Synthetic membrane processes are being increasingly integrated into existing reaction, isolation, and recovery schemes for the production of valuable biological molecules. In many cases they are replacing traditional unit processes. The properties of membrane systems which are most often exploited for both upstream and downstream processing and their permselectivity, high surface area per unit volume, are their potential for controlling the level of contact and/or mixing between two separate phases. Advances in both membrane materials and module design and operation have led to better control of concentration polarization and membrane fouling. After presenting some recent advances in membrane materials and fluid mechanics, we demonstrate how membranes have been integrated into cellular and enzymatic reaction schemes. This is followed by a review of established and emerging synergism between biological processes and synthetic membranes.

1. Introduction

1. 1. Why Integrate Membranes with Bioprocesses?

Concomitant developments in molecular and cell biology and separation technology during the past thirty years have produced exciting new opportunities in the production of complex mammalian proteins that have the potential to fundamentally alter human healthcare in such areas as diagnostics, prevention, and treatment of disease, is beginning to play an increasingly important role in many aspects of bioprocessing. Not only have membrane processes been used in established separation schemes such as for whole broth clarification and concentration and for purification of macromolecular products, but they are also being used in new emerging schemes for the separation and purification of macro and microsolutes. Well known membrane

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processes such as electrodialysis, reverse osmosis and pervaporation are finding niches in downstream processing of fermentation and cell culture broth. Membrane structures are also being integrated into the bioreactor itself in order to increase volumetric productivity and reduce subsequent recovery requirements. In addition, membranes are being utilized not only for their permselective properties but also for their large internal adsoptive surface areas and excellent mass transfer characteristics. Microporous membranes provide excellent matrices for group and bio-specific adsorption processes such as ion exchange and affinity-based separation, respectively, and for entrapping enzymes. Synergistic advantages of coupling membranes with other unit processes such as affinity ligand adsorption, precipitation, and solvent extraction are also becoming apparent.

Several limitations associated with bioprocessing can be overcome or at least minimized with membrane processes. These include the generation of complex mixtures requiring extensive downstream processing, the production of dilute solutions containing low product concentrations when using suspension cultures, low specific rate constants for biological processes, and contamination and infection of unwanted biological species(especially for mammalian and plant cell cultures). Many of the applications of and recent advances in synthetic membrane technology described below deal with mitigating these limitations. Because of their ability to fractionate and concentrate, membranes are able to separate product from reactant, to increase the concentration of a dilute solution, to reduce the possibility of contamination and infection, and, because they are aften closed processes, to reduce the need for stringent contamination requirements. Membrances can also be used to productivity of a reactor.

1. 2. Recent Advances in Membrane Technology

Novel membrane materials and surfaces: Progress in several areas of membrane development has occurred during the past few years. This has included a better understanding of the influence of chemical structure on function, the incorporation of complexing agents and other affinity groups into membranes to increase selectivity, modification of membrane surfaces to reduce negative effects of fouling, the development of new materials with excellent chemical, thermal and mechanical properties and with narrow molecular weight cut-offs (MWCO), and the preparation of highly versatile particle-containing membrane films. Some examples of these development are discussed below with reference to Fig. 1.

Various modifications [addition of poly(dimethyl siloxane)(PDMS); and replacement of hydrogen for fluorine molecules] of the gas permeable glassy polymer poly(trimethyl silyl propyne) (PTMSP) have resulted in significant increases in the separation factor for pervaporation of ethanol-water mixtures and a better understanding of the relation between chemical structure and function(Fig. 1a)[1-3]. With limited success, complexing agents such as crown ethers have been incorporated into membranes and used to transport oxygen in preference to nitrogen(Fig. 1b). Two approaches have been used to reduce membrane fouling due to protein adsorption: surface modification by chemical and irradiation techniques and the use of new materials with low non-specific protein adsorption and narrow MWCOs. Examples in the development stage or commercially available include asymmetric ceramic microfiltration membranes[5], recrystallized S-layers[6], modified porous glass[7], and polymerized Langmuir-Blodgett(LB) multilayers[8](Fig. 1c & d). A relatively new development is the formation of membrane films with one phase suspended in another phase or with interpenetrating contiguous immiscible phases. Hennepe et al [9] inserted silicalite particle into PDMS and increased the separation factor for ethanol from 12 to 38. Way et al [10] have used a hydrophobic perfluorocarbon membrane containing an interpenetrating sulphonated ion exchange network(hydrophilic) to selectively pass carbon dioxide from methane gas. Others have commercialized "particle membranes" with widely dif

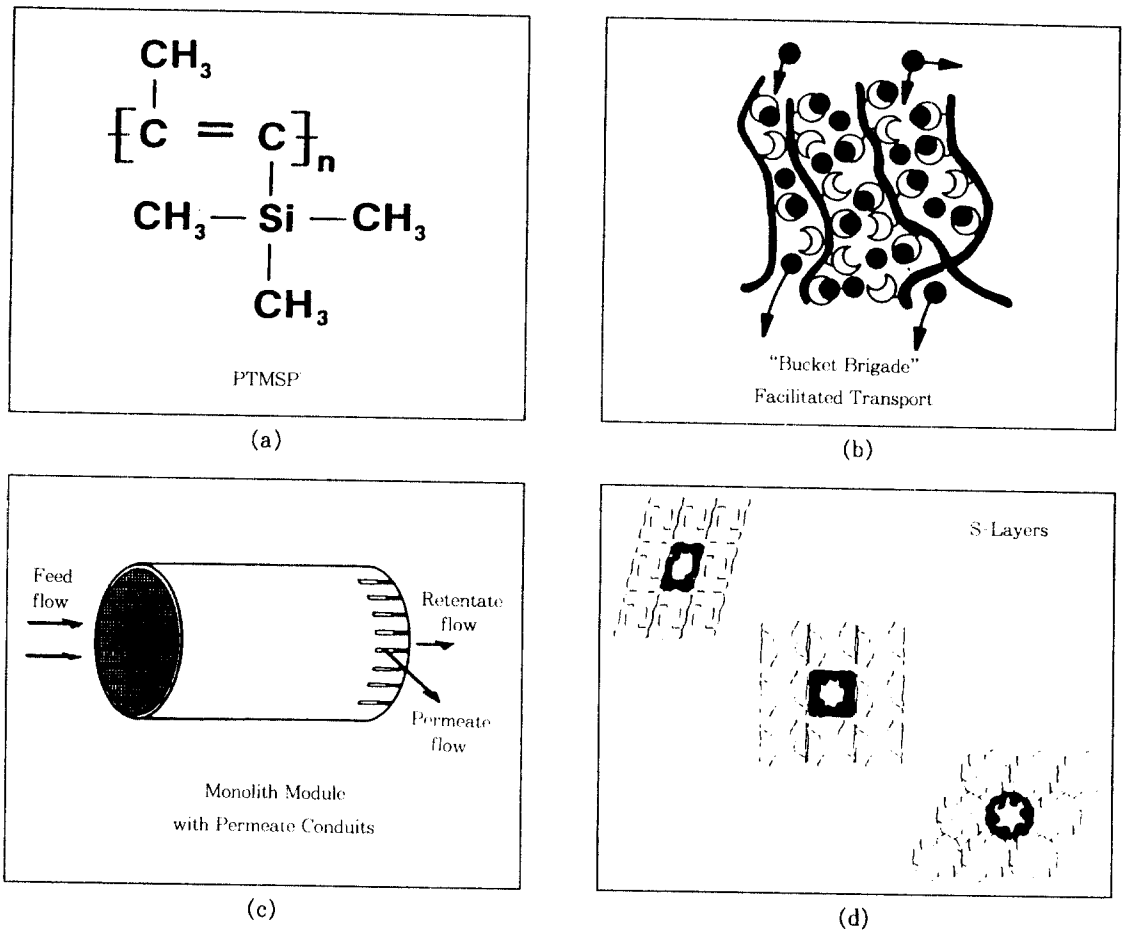

Fig. 1. Novel membrane materials and structures; (a) chemical structure of poly(trimethyl silyl 1-propyne), (b) By covalently attaching affinity or complexing agents to the membrane pore surfaces, the selectivity of membrane pore processes can be increased as molecules are adsorbed onto the surface, (c) Ceramic mainfold and (d) S-layers(after Sara and Sleytr [6]).

fering properties[11].

Fluid management in membrane module develop-ment : Typical commercial membrane modules were originally developed to treat aqueous solutions essentially free of macromolecules(proteins) and suspended particulates. Most manufacturers have, however, adapted these early hollow fiber and spiral wound module designs for treating biological suspensions containing proteins, cells and cell debris. Unexpected and severe fouling problems have occurred as a result of protein adsorption and build-up of particulate matter at the membrane-solution interface. Methods to alleviate these limitations include the development of low non-specific protein adsorbing materials and the design of new modules that induce flow instabilities that could reentrain the so-called "cake" on the membrane surface back into the flowing solution. Modules with furrowed membrane surfaces and others with membranes on the faces of an annulus between a rotating inner cylinder and a stationary outer cylinder have appeared on the market[12-19]. All of these designs use flow instabilities such as vortices to sweep the surfaces of the membranes(Fig. 2). Pulsating axial

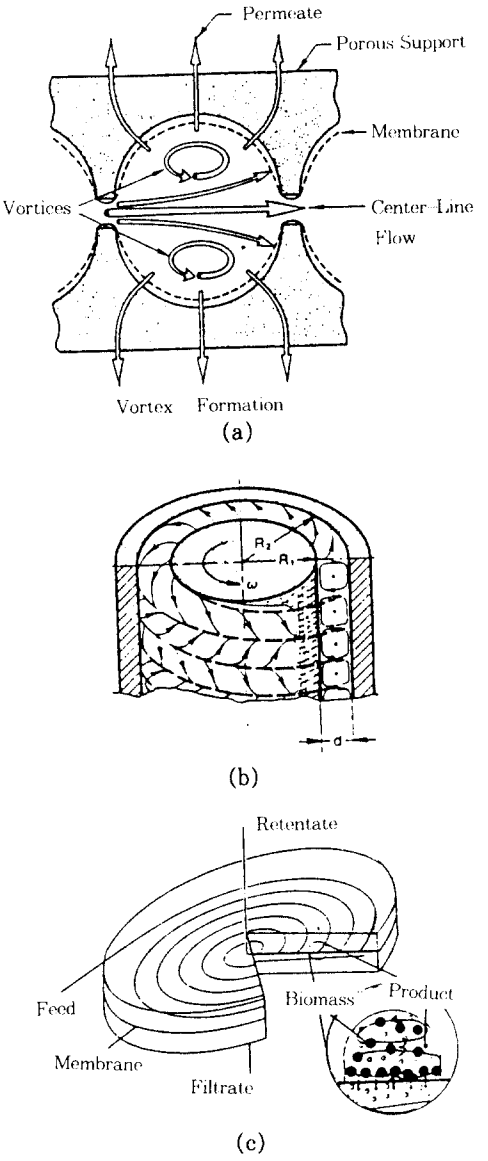

Fig. 2. Membrane module designs that use flow instabilities(vortices) to sweep the surface of the membranes; (a) corrugated surface showing vortex formation(after Stairmand and Bellhouse[12]), (b) rotating inner surface and stationary outer surface resulting in the formation of Taylor vortices. The membrane can be placed onto both or either surface and (c) spiral half cylinder channel showing secondary centrifugal flow with formation of Dean vortices(after Winzeler[18]).

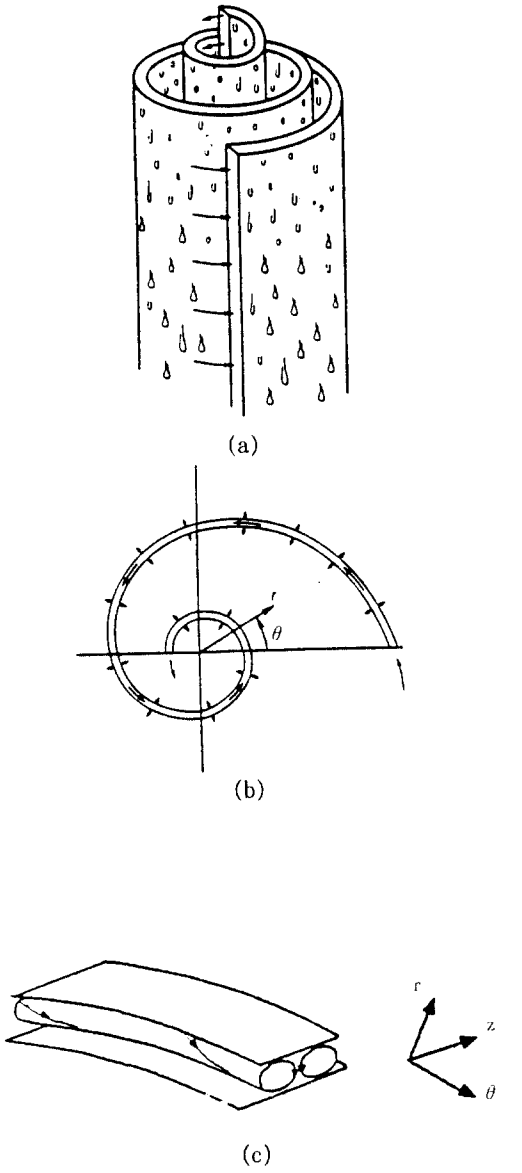

Fig. 3. (a) Schematic drawing of flow through a spiral porous channel, (b) cross-section of spiral channel and (c) schematic drawing of one Dean vortex pair. Brewster et al. [22] have used the neutral stability criterion and a narrow-gap theory to specify the geometric path of these spiral geometries.

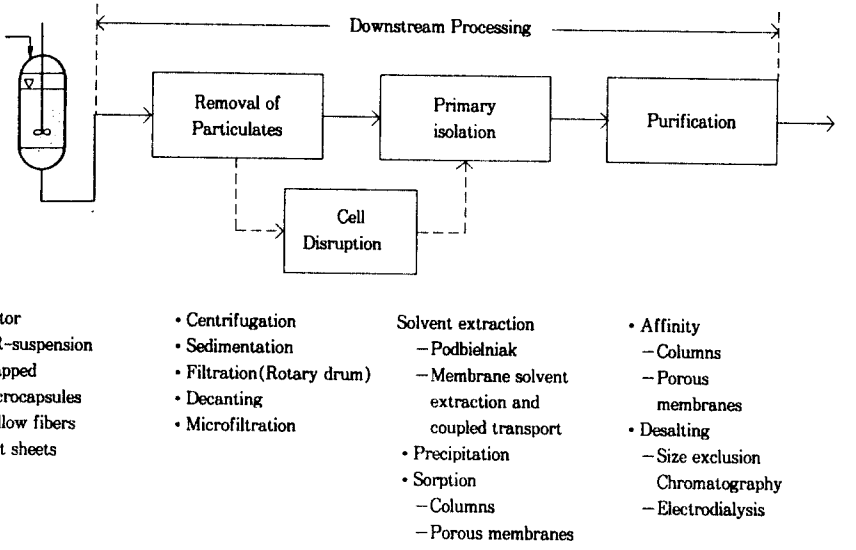

Fig. 4. Classical fermentation/bioconversion and recovery processes showing different unit processes and illustrating the opportunities for synthetic membrane process.

flow and flow backwashing have also been used to clean membrane surfaces and blocked pores[20]. During the past few years, Belfort and coworkers have provided a module design rationale and procedure for capture or non-capture of suspended particles[21]. They have also recently announced the design of a new scalable spiral wound membrane module in which controlled fluid instabilities are used to depolarize and defoul membranes(Fig. 3)[22].

2. MEMBRANE BIOREACTORS

2. 1. Integration of Membrane into Bioprocesses

Biotechnology is based on the use of living organisms and enzymes for the manufacture of commercial products and is exemplified by the recombinant DNA and monoclonal antibody technologies. Besides the bioreactor in which bacterial, yeast, mammalian or other cells are used to convert raw materials and medium to final product, the manufacturing process is similar to those used for the manufacture of small organic drugs and other traditional pharmaceutical products[23]. In classical fementation, bioconversion and recovery processes, there are many opportunities to integrate synthetic membrane(Fig. 4).

2. 2. Immobilized Whole-Cell and Enzyme Reactors

Whole cell reactors: Whole cells have been immobilized in membrane reactors within microcapsules, in the shell space of hollow fibers and behind flat or tubular configurations[24-28]. One difficulty of these systems is the adequate supply of nutrients such as oxygen to all the cells within the dense cultures. Heath and Belfort[29] have shown this to be especially true of large microcapsules(>50㎛ in diameter) and hollow fiber reactors. They emphasize the importance of an even, well-defined spacing between fibers or flat sheets of no more than about 50 ㎛ from the furthest cell. Because of the diffusion limitation, concentric hollow fiber[30], multiple-layer flat sheet reactors[31], and convective-flow membrane reactors[32-35] were designed.

Traditionally, membranes have been inserted into suspension cultures and well mixed reactors[36]. New variants include a porous stainless steel rotating spin filter placed within a draft tube together with an impeller-driven fan[14], and the tubular membrane filter with a strong sterilizable outer jacket into which a tubular membrane is inserted

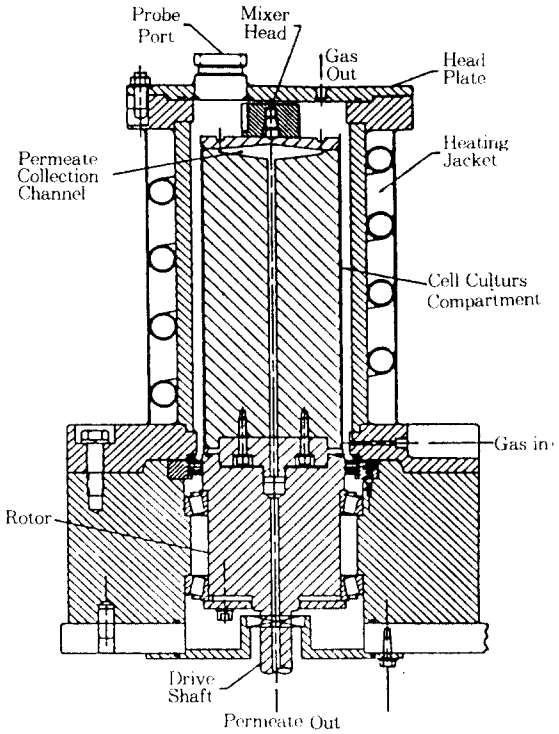

Fig. 5. Perfusion rotating annular bioreactor[38]. Excellent laminar mixing is obtained through the formation of Taylor vortices in the cell culture compartment.

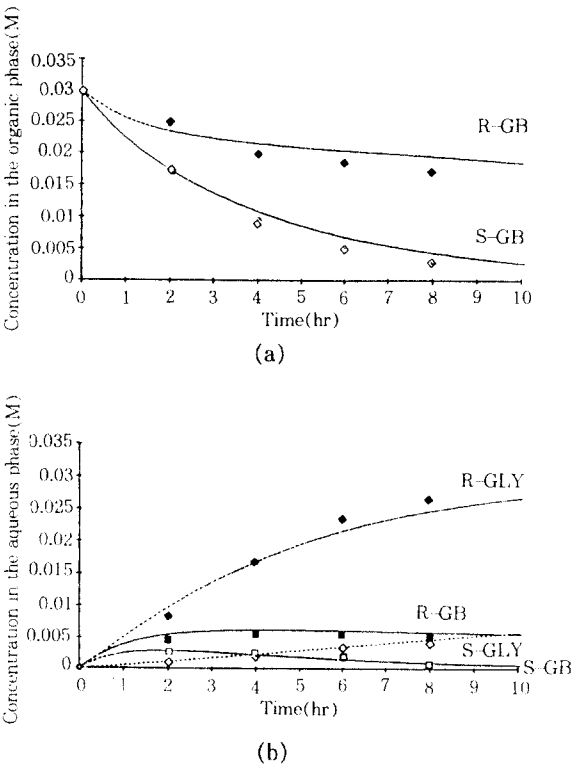

Fig. 6. Comparison of theoretical(lines) and experiment(data points) concentration profiles in (a) organic and (b) aqueous phases. Enzyme loading 0.004㎎/㎖. One fitting parameter was used: k_S=0.025 s^{-1}[58]. R-GB, S-GB are the two substrate glycidyl butyrate enantiomers. R-GYL and S-GLY are the two product glycidol enantiomers. The entrapped enzyme was porcine pancreatic lipase.

[37]. McKinney et al.[38] have developed a new type of Taylor vortex perfusion rotating annular bioreactor for the production of valuable biological molecules. Gasses are supplied though the stationary outer wall and medium is effused through the stainless steel membrane(5㎛ pores) on the rotating inner wall(Fig. 5). Excellent mass transfer of nutrients, gases and products is obtained.

Membrane-immobilized enzyme reactors: Because of their high packing density(large surface area per unit volume of reactor space), hollow fiber membrane systems are frequently used to retain enzymes for bioconversions[39-50]. Membranes have been used as a barrier to retain soluble enzyme catalyst or as a high area per unit volume matrix on which the enzyme is immobilized and called a "reactive membrane"[51]. Microcapsules have been used for retaining enzymes and their cofactors[52-56].

Most enzyme-entrapped membrane bioreactors rely on diffusion of the substrate and/or product over relatively long distances(10-100㎛). Wu et al. [57-58] have used such a system to resolve enantiomers of glycidyl butyrate from an organic feed. The porcine pig lipase was entrapped within the porous part a poly(acrylonitrile) membrane and the organic and aqueous phases were passed across each side of the hollow fiber membrane. With one

fitting parameter, their model was able to predict the temporal concentration changes for the four species(two chiral substrates and two chiral products) in both streams(Fig. 6). van't Riet et al.[59-60] have studied lipase-catalyzed esterification and hydroly-sis reactions in two-phase membrane systems. Taking advantage of the membrane selectivity is an important advantage of these systems. It can increase reaction rate and final enantiomeric excess or product purity.

3. MEMBRANE BIOSEPARATIONS ESTABLISHED PROCESSES

3. 1. Removal of Suspended Matter

In downstream processing it is often necessary to separate the cells from the culture medium either for recovery of the broth(secreted molecules) or for recovery of the cells(cell mass or intracellular heterologous proteins). Microfiltration is a competitive process with sedimentation, rotary drum filtration and decanting(Fig. 4). Usually the membrane process is external to the reactor and the culture broth is fed to the membrane module and recycled back to the reactor after concentration of the cells. Cell recovery can be accomplished in hollow fiber[61-62], plate and frame[61-64] and rotating devices[19, 65]. Cross-flow filtration is widely used for cell harvesting of bacterial cells[64], and animal cells[62-66]. Shear stress due to the pumps and confined laminar flow within the modules are thought to damage the mamallian cells[62]. Nagata et al.[67] studied microfiltration of a bacterial culture and discovered that a precipitate of $MgNH_4PO_4$, formed during sterilization of the medium and was the prime cause of flux decline. They also presented a new simple phenomenological model, the "solids flux" model, to account for the reduction in flux.

3. 2. Removal of Dissolved Components

The recovery of dissolved macromolecules such as heterologous proteins from complex biological fluids, such as cell culture media is a difficult problem. Flaschel et al.[68] have written a review of biocatalyst separation by ultrafiltration. With secreted proteins(i. e. antibodies), separation of the product-containing medium from the undesired cells is usually the first step. This is then followed by concentration of the dilute broth and product isolation. Membranes have been used successfully in all of these steps. With non-secreted products(i. e. for E. coli), it is necessary to disrupt the cells and recover the intra-cellular product from the cell debris. Viscosity and osmotic pressure increase near the membrane surface during microfiltration and can cause problems(i. e. concentration polarization(CP) with variable physical properties). Diafiltration has been used to reduce the unwanted osmotic effects by removing the low molecular weight contaminant[69-71].

Membrane fouling and plugging can be an even more serious problem than CP. Typical polymers used for ultrafiltration and microfiltration include hydrophobic materials such as polysulfone, polypropylene and polyethylene. These materials can foul extensively and can adsorb proteins from solution [72]. Chan et al.[73] used a stainless steel microfiltration membrane to shear recombinant E. coli cells in the presence of EDTA and simultaneously clarify the intracellular heterogolous protein(in the permeate) from the cell debris in one step. Belfort et al. [74-75] have solved the Navier-Stokes equations for Couette flow in a annulus with a porous rotating inner cylinder and used this solution to plot flux -transmembrane pressure data on a universal curve. They then used the analysis with a log-normal pore size distribution to obtain the mean number of adsorbed protein layers in the pores. An important area of research is the quest for membrane materials that exhibit low non-specific protein adsorption.

4. MEMBRANE BIOSEPARATIONS EMERGING PROCESSES

Synthetic membranes have traditionally been used as a sieving matrix, however, newer mem-

brane separation processes have emerged that use the membrane's microstructure and surface chemistry. Excellent selectivity and flux have been obtained with immobilized ligand-adsorptive(affinity) membranes or by membranes using an extractant with coupled active transport. Several new approaches are presented below.

Membrane chromatography: Developers of chromatographic packing have realized that diffusion chronically limits the performance and speed of packed bead columns. Attempts to alleviate this and increase the convective flow in such columns has resulted in the development of highly porous beads with perfusion[76]. Porous synthetic membranes have been used as chromatographic supports because of their large surface area density, large range of convective flow rates(i. e. short cycle times), and controllable and predictable pressure drops[77]. Milby et al.[78] have developed hollow fiber ion exchange membranes for the separation of proteins such as immunoglobulins(IgG). Changes in ionic strength or pH enabled elution and recovery of the desired proteins. Ding et al.[79-80], using the inner surface and proteins(Fig. 7a). Low pressure drops characterize such systems. Other have commercialized silica based porous membranes as chromatographic supports[81]. Briefs and Kula[82] have demonstrated fast recycle times and good protein separation with a multiple flat sheet membrane system.

Affinity membranes: Membranes offer a very attractive alternative to conventional packed bead or particle technology for attachment of ligands for affinity separations[83]. The intrinsic compressibility of agarose beads and the need for small particles of silica in order to obtain reasonable surface areas and throughputs can limit scale-up of such systems due to severe pressure-drops. Since most of the ligand is located within the beads or particles, diffusion can seriously limit the speed of the process(and hence the amount of affinity matrix needed). With membrane matrices, convective flow through the pores significant reduce the diffusional path(to about 0.25 of the pore diameter) and hence the process is significantly faster(with less matrix needed) than for packed bed systems[77]. Affinity membranes for fractionation of proteins have been prepared from group-specific ligands such as electrostatic or ion exchange molecules[77, 78, 84]: from pseudo-biospecific ligands such as metal chelates[85-86], dyes such as Cibacron blue[87] and lectins such as concanavalin A for glycoproteins; and from molecular recognition molecules such as protein A for capturing immunoglobulins[88] and receptors for capturing its associated ligands[89-90]. Membranes have also been used to retain dissolved bound ligand complex(also called "affinity escourt") allowing the nonbound solutes to be washed away in the permeate. Then the desired biomolecule is eluted from the complex and collected in the permeate of the second membrane filter. The ligand is retained and recycled for reuse(Fig. 7b)[91]. Concerns that need to be addressed include regulatory approval, minimizing ligand leakage, and development of new and less costly ligands.

Membrane-assisted extraction: With the development of solvent-stable polymer membranes, membrane-mediated extraction has been used to overcome separation difficulties(i. e. phase entrainment, small density and interfacial tension differences) often encountered in conventional solvent extraction[92]. For differential protein extraction, the membrane process is reported to be substantially faster than that conducted in conventional equipment[93]. Applications include: dispersion-free solvent extraction of a pharmaceutical product using pH swing procedures[94], citric acid extraction by reversible chemical complexation in a dual hollow fiber system[95] (Fig. 7c), enzyme facilitated transport through a liquid membrane to separate and purify organic acids[96], isolation of butanol from fed-batch fermentation of Clostridium acetobutylicum[97], and ethanol production from yeast fermentation[98]. In many cases, removal of the product resulted in improved production through

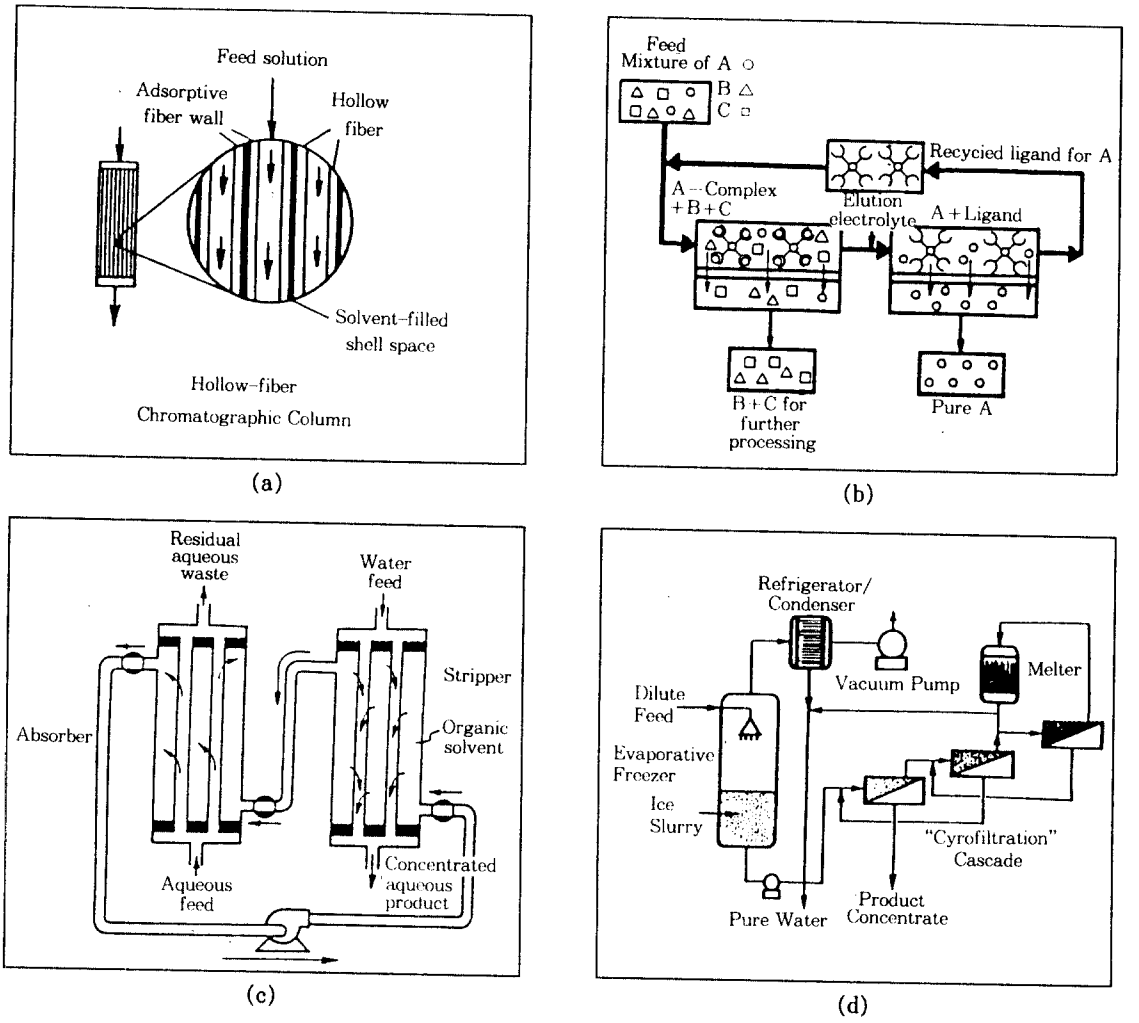

Fig. 7. (a) Hollow fiber chromatographic column[79, 80]. (b) Continuous affinity ligand membrane extraction showing the filtration unit where the non-bound microsolutes are washed out of the feed stream and the stripping unit where the desired biomolecule is released from its ligand. The biomolecule then permeates through the membrane leaving behind the ligand which is recycled for further complexation(after Mattiasson ad Ramstorp[91]). (c) Membrane solvent extraction showing the adsorber in which the desired solute is extracted from the feed stream through the membrane into the organic extracted and the stripper in which the product is re-extracted out of the organic solvent into a concentrated aqueous stream(after Sengupta et al.[95]). (d) Concentration by cryofiltration showing the recovery of ice crystals from the membrane cascade in the retentate and the ion ice-forming product concentrate in the permeate(after Michaels[108]).

a lessening of feed-back inhibition. Liquid membrane emulsions containing aqueous inner cores with enzymes were used for the continuous production of L-isomers of amino acids from a racemic mixture[99].

Electrically-driven membrane processes: Electrodi-

alysis across ion-selective semipermeable membranes has been used to induce isoelectric precipitation of proteins from human plasma[100]. Amino acids such as alanine have been isolated from other amino acids following acid hydrolysis under different pH values using electrodialysis[101]. Superposition of electric forces on a pressure-driven membrane process has been shown to alleviate fouling and enhance cross-flow filtration rates[102]. Grimshaw et al.[103], using electrical forces have deformed a charged polyelectrolyte membrane and purposely effected transport across the membrane. The process has the potential to effect selective dynamic control of solute transport.

Membranes and precipitation: Pretreatment of protein solutions by precipitation prior to membrane filtration have resulted in improved performance as compared to that without such pretreatment[104-105]. Inclusion bodies resulting from overproduction of heterogolous proteins in E. coli have been recovered by crossflow filtration and washed by diafiltration[106]. Membrane-modulated precipitation has been proposed to overcome the difficulties in controlling the concentration of the precipitating agent(electrolyte or solvent) during the formation of precipitates[107]. Controlled transfer of electrolyte or solvent into a protein solution by electrodialysis or dialysis can result in higher efficiency and greater selectivity.

Cryofiltration with membranes: In conjunction with an evaporative freezer, ice particles rejected by a membrane filter can be separated from the permeable non-ice-forming solvent(containing the desired product)[108]. The ice can then be melted for recovery of pure water from the ice-forming crystals(Fig. 7d).

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REFERENCES

1. Y. Nagase, Y. Takamura, and , K. Sugimote, Improved alcohol permselectivity for pervaporation with modified poly(1-trimethylsilyl-1-propyne) membranes, Proceedings from the International Congress on Membranes and Membrane Processes(ICOM), August 1990, Chicago, Vol. 1, p. 331(1990).
2. R.H. Sedath, E.W. Funk, and Li, N.W., Reducation of fouling in ultrafiltration membranes via surface fluorination. Proceedings ICOM, August 1990, Chicago, Vol. 1, p. 106 (1990).
3. Nagase et al., *J. Polym. Sci.*, Part B. Polymer Physics **28**, 377(1990).
4. Le, M.S. and K.L. Gollan, *J. Memb. Sci.* **40**, 231 (1989).
5. W.M. Clark,, A. Bausal, M. Soutakke, and Ma, Y.H., *J. Memb. Sci.* **55**, 21(1991).
6. M. Sara, and U.B. Sleytr, *J. Memb. Sci.* **33**, 27 (1987).
7. R. Schnabel, P. Langer, and E. Bayer, Application oriented treatment of inorganic membranes, 6th International Symposium on Synthetic Membranes in Sciences and Industies, Sept. 4-8, 1989, Tubingen(1989).
8. O. Albrecht, A. Laschewsky, and H. Ringsdorf, H., *J. Memb. Sci.* **22**, 187(1985).
9. H.J.C. Hennepe, D., Bargeman, M.H.V. Mulder, and C.A. Smolders, *J.Memb. Sci.*, **35**, 39(1987).
10. J.D. Way, R.D. Nobel, D.L. Reed, G.M. Ginley, and L.A. Jarr, *AIChE J.* **33**, 480(1987).

11. D.F. Hagen, S. Craig, G. Markell, G. Schmitt, and D. Blevins, *Anal. Chima, Acta*, **236**, 157 (1990).
12. J.W. Stairmand, and B.J. Bellhouse, *Int. J. Heat Mass Transfer*, **27**, 1405(1985).
13. K.H. Kroner, B. Reismeier, V. Nissinen, and M. R. Kula, Recent studies on dynamic filtration in enzyme recovery. Engineering Foundation Conferences on Recovery of Bioproducts, Uppsula, May 1986, Sweden(1986).
14. W. Beyeler, T. Thales, and R. Clement, Proceedings of 2nd Annual Meeting of Japanese Association for Animal Cell Technology, November 20-22, 1989, Tsukuba, Ibaraki, Japan(1989).
15. A.P. Davidson, M.P. Thomas, D.C. Azubike, and P.M. Gallagher, Proceedings of Vth World Filtration Conferences, Nice, France, p. 235 (1990).
16. M.F. Edwards and W.L. Wilkinson, *Trans. Inst. Chem. Engng.a* **49**, 85(1971).
17. I. Sobey, *J. Fluid Mech.* **96**, 1(1980).
18. H. Winzeler, Poster presentation at the 5th European Biotechnology Conference, Copenhagen, Denmark(1990).
19. K.H. Kroner, V. Nissinen, and H. Ziegler, *Bio/Technol.* **5**, 921(1987).
20. S. Ilias, and K. Govind, *Sep. Sci. Tech.* **25**, 1307 (1990).
21. M.E. Brewster, K.Y. Chung, and G. Belfort (1992), Dean vortex flow in a curved membrane channel:1. A new approach to membrane module design, *J. Memb. Sci.*, submitted.
23. R.L. Garnick, N.J. Solli, and P.A. Papa *Anal. Chem.* **60**, 2546(1988).
24. C.A. Heath, and G. Belfort, *Int. J. Biochem.* **22**, 823(1990).
25. S.F. Karel, S.B. Libicki, and C.R. Robertson, *Chem. Engng. Sci.* **40**, 1321(1985).
26. H.H. Chang, *Biotechnol. Adv.* **5**, 129(1987).
27. G. Belfort, *Biotechnol. Bioeng.* **33**, 1047(1989).
28. M. Cheryan, and M.A. Mehaia, Membrane, bioreactors. IN:W.C. McGregor (ed.) "Membrane Sepatations in Biotechnology", Marcel Dekker, NY(1986).
29. C.A. Heath, and G. Belfort, *Adv. Biochem. Enjg./Biotechnol. 34*, 1(1988).
30. L. Curter, Physiological studies of hydridoma cultivation in hollow fiber bioreactors, PhD Thesis, University of California at Berkeley, CA, (1988).
31. L. Rainen, *Am. Biotechnol. Lab.* **6**, 20(1988).
32. J.P. Tharakan, and Chau, P.C., *Biotechnol. Bioeng.*, **28**, 329(1986).
33. J. Feder and W.R. Tolbert, *Scientific Amer.*, **248**, 36(1983).
34. S.L. Gallagher, J.T. Tharakan, and P.C. Chau, *Biotechnol. Tech.*, **1**, 91(1987).
35. S.S. Ozturk, B.O. Palsson, A.R. Midgley and C. R. Halberstadt, *Biotechnol. Tech.*, **3**, 55(1989).
36. A. Margaritisand C.R. Wilke, *Biotechnol. Bioeng.*, **20**, 727(1978).
37. H. Markl, H. Kurosawa, C. Neibuhr-Redder, and Kasche, V., New membrane bioreator for the cultivation of animal cells, Proceedings ICOM, August, 1990, Chicago, Vol. 2, p. 968 (1990).
38. K.L. McKinney, R. Dilwith, and G. Belfort, Methods to improve cellular productivity and improve bioreator performance for hydridoma cell cultures. 9th Int. Biotechn. Symp. and Exposition, Am. Chem. Soc., Crystal City, VA, August 16-21, 1992.
39. T.K. Ghose and J.A. Kostick, *Biotechnol. Bioeng.*, **12**, 921(1970).
40. P.R. Rony, *J. Am. Chem. Soc.*, **94**, 8247(1972).
41. J.C. Davis, *Biotechnol. Bioeng.*, **16**, 1113(1974).
42. W. Lewis, and S. Middleman, *AIChE J.* **20**, 1012 (1974).
43. L.R. Waterland, C.R. Robertson and A.S. Michaels, *Chem. Eng. Commun.*, **2**, 37(1975).
44. R. Korus and A. Olson, *Biotechnol. Bioeng.*, **19**, 1(1977).
45. I. Ohlson, G. Tragardh and B. Hahn-Hagerdal, *Biotechnol. Bioeng.*, **26**, 647(1978).
46. J. K. Kan and M.L. Shuler, *Biotechnol. Bioeng.*, **20**, 217(1978).

47. J.P. Roozen and W. Pilnik, *Enz. Microb. Technol.*, **1**, 22(1979).
48. R.G. Henley, R.Y.K. Yangand P.F. Greenfield, *Enz. Microb. Technol.*, **2**, 206(1980).
49. V.C. Gekas, *Enz. Microb. Technol.*, **8**, 450(1986).
50. M. Pizzichini, C. Fabiani, A. Adami, and V. Cavozzoni, *Biotechnol. Bioeng.*, **33**, 955(1989).
51. S.L. Matson and J.A. Quinn, Membrane reactors in bioprocessing Biochemical Eng. Ⅳ (New York Academy of Sciences, NY), Vol. 49 (1986).
52. J. Cousineau and T.M.S. Chang, *Biochem. Biophys. Res. Commun.*, **79**, 24(1977).
53. T.M.S. Chang and C. Malouf, *Trans. Am. Sco. Artif. Intern. Organs*, **24**, 18(1978).
54. E. Ilan and T.M.S. Chang, *Appl. Biochem. Biotechnol.*, **13**, 221(1986).
55. H.P. Walh, and T.M.S. Chang, *J. Mole. Catal.*, **39**, 147(1987).
56. K.F. Gu and T.M.S Chang, *Appl. Biochem. Biotechnol.*, **13**, 221(1990).
57. Wu, D.-R., G. Belfort and S.C. Cramer, *I & EC Res.*, **29**, 1612(1990).
58. Wu., D.-R., S. Cramer and G. Belfort, *Biotechnol. Bioeng.*, submitted(1992).
59. Pronk, Wo, P.J.A.M. Kerkhof, van Helden, C. and van't Riet, K., *Biotechnol. Bioeng.*, **32**, 512 (1988).
60. van der A. Padt, M.J. Edema, J.J.W. Sewalt and van't K. Riet, *Amer. Oil Chem. Soc.*, **67**, 347 (1990).
61. J. Shiloach,, J.B. Kaufman and R.M. Kelly, *Biotechonol. Prog.* **2**, 230(1986).
62. B. Mairella, G. Dorin, A. Carion and D. Herano , *Biotechnol. Bioeng.*, **37**, 121(1991).
63. J. Sheehan, W. DeVane, J. Shiloach, Ha, Y., E. Surette, M. Weinstein, Bench and pilot scale recovery of animal cells grown in suspension culture using a plate and frame crossflow filter system. Proceedings ICOM, August 1990, Chicage, Vol. 1, p. 610(1990).
64. W. Hanisch, Cell harvesting. In, W.C. McGregor (ed.) "Membrane Separations in Biotechnology", Marcel Dekker, NY(1986).
65. P. Roghigo, private communication(1991).
66. R. van Reis,, L.C. Leonard, Hsu, C. C. and S.E. Builder, *Biotechnol. Bioeng.*, **38**, 413(1991).
67. N. Nagata, K.J. Herouvis, D.M. Dziewulski and G. Belfort, *Biotechnol. Bioeng.*, **34**, 447(1989).
68. E. Flaschel, C. Wandrey and M-R. Kula, *Adv. Biochem. Engng./Biotechnol.*, **26**, 74(1983).
69. W.F. Blatt, A. Dravid, A.S. Michaels, and L. Nelson, Solute/polarization and cake formation in membrane ultrafiltration:causes, consequences and control techniques. Ins J.E. Flinn, (ed.) "Memb. Sci. and Techn"., Plenum, NY, (1970).
70. M.C. Porter, *Ind. Eng. Chem. Prod. Res. Dev.*, **11**, 234(1972).
71. V.L. Vilker, C.K. Colto and K.A. Smith, *AIChE J.*, **27**, 637(1981).
72. O. Velcangil and J.A. Howell, Protein ultrafiltration:theory of membrane fouling and its treatment with immobilized proteases. In: Cooper A. (ed.) "Ultrafiltration Membranes and Applications", Plenum, NY, p. 217(1980).
73. W.K.Y. Chan, M. Belfort and G. Belfort, *J. Biotechnol.*, **18**, 225(1991).
74. G.Belfort, J.M. Pimbley, A. Greiner and K-Y Chung, Diagnosis of membrane fouling using rotating annular filter 1. Cell culture media, J. Membrane Sci., in press, (1992).
75. G. Belfort, P. Mikulasek, J.M. Pilbley and K-Y Chung, Diagnosis of membrane fouling using a rotating annular filter, 2. Dilute particle suspensions of known particle size, J. Membrane Sci., in press(1992).
76. N.B. Afeyan, N.F. Gordon, I. Mazsaroff, L. Varady, S.P. Fulton, Y.B. Yang and F.E. Regnier, *J. Chrom.*, **519**, 1(1990).
77. S. Brandt, R.A. Goffe, S.B. Kessler, J.L. O' Connor and S.E. Zale, *Bio/Technology.*, **6**, 779 (1988).
78. K.H. Milby, D.E. Steinmeyer and M.K. Tripodi, Ion exchange hollow fiber membranes for protein purification. Proceedings ICOM, **1**, 625, Chicago(1990).

79. H. Ding, M.C. Yang, D. Schisla and E.L. Cussler, *AIChE J.*, **35**, 814(1989).
80. H. Ding and E.L. Cussler, *Biotechnology Prog.*, **6**, 472(1990).
81. S. Nochumson, *Biotechniques*, **12**(3), 436(1992).
82. K.G. Briefs and M.R. Kula, *Chem. Engr. Sci.*, **47** (1), 141(1992).
83. E. Klein, "Affinity Membranes", John Wiley & Sons, NY, (1991).
84. B. Goldberg, FMC Corp., private communication.
85. H. Iwata, K. Saito, S. Furasaki, T. Sugo, J. Okamoto, Development of immobilized metal affinity hollow fiber membrane to adsorb proteins, Proceedings ICOM, **1**, 163, Chicago(1990).
86. A. Serafica and G. Belfort, Manuscript in preparation, (1992).
87. S. Krause, K.H. Kroner and W.D. Deckwer, **5**, 199(1991).
88. S.E. Zale, J.L. O'Connor and S.L. Matson, Hollow fiber affinity membrane separations in downstream processing, Proceedings ICOM, **1**, 60, Chicago(1990).
89. M. Nachman, A.R.M. Azad and P. Bailon, *J. Chrom.*, **597**, 155(1992).
90. M. Nachman, *J. Chrom.*, **597**, 167(1992).
91. B. Mattisson and M. Ramstorp, *J. Chrom.*, **283**, 322(1984).
92. R. Prasad and K.K. Sirkar, *J. Memb. Sci.*, **50**, 153(1990).
93. L. Dahuron and E.L. Cussler, *AIChE J.*, **34**, 130 (1988).
94. R. Prasad and K.K. Sirkar, *J. Memb. Sci.*, **47**, 235(1989).
95. A. Sengupta, R. Basu, and K.K. Sirkar, *AIChE J.*, **34**, 1698(1988).
96. J.S. Dordick, D.G. Rethwisch and T. Gao, Enzyme-facilitate transport of organic acids through a liquid membrane, Proceedings ICOM, **1**, 673, Chicago(1990).
97. Y.J. Jeon and Y.Y. Lee, *Enz. Microb. Technol.*, **11**, 575(1989).
98. G.S. Efthymiou and M.L. Shuler, *Biotechnol. Prog.*, **3**, 259(1987).
99. T. Scheper, W. Hawachs and K. Schugerl, *Chem. Eng. J.*, **29**, B31(1984).
100. D.H. Bing, A.C. DiDonno, M. Regan, and C.J. Strang, Blood Plasma Processing by electodiaylsis, W.C. McGregor (ed.), "Membrane Separations in Bioechnology", Marcel Dekker, NY(1986).
101. C. Gavach, R. Sandeaux and J. Sandeux, Electrotransport of amino acids through ion exchange membranes, Separation of amino acids by electrodiaysis, Proceedings ICOM, **2**, 870., Chicago(1990).
102. A. Brors and K.H. Kroner, Electro-crossflow filtration of microbial suspensions, Proceedings ICOM, **1**, 616, Chicago(1990).
103. P.E. Grimshaw, A.J. Grodzinsky, M.L. Yarmush and D.M. Yarmush, *Chem. Engng. Sci.*, **44**, 827(1989).
104. N. Devereux, M. Hoare and P. Dunnill, *Chem. Eng. Commun.*, **45**, 255(1986).
105. A.C. Benthan, M.J. Treton, M. Hoare. and P. Dunnill, *Biotechnol. Bioeng.*, **31**, 984(1988).
106. S.M. Forman E.R. DeBrnardez, R.S. Feldberg and R.W. Swartz, *J. Memb. Sci.*, **48**, 263(1990).
107. A.S. Michaels and S.L. Matson, *Desal.*, **53**, 231 (1985).
108. A.S. Michaels, *Desalination*, **77**, 5(1990).