A Novel Membrane Reactor Design for Controlled Studies of Interacting Populations (Simulation of the Interaction Between Microorganism and Plant Suspension Cultures)

L. J. Pestchanker, [1] E. C. Ercoli[2]

Received 9 February 1996: accepted 12 December 1996

ABSTRACT:

The design of a reactor in which two interacting cell populations (microorganisms and plants) could grow under controlled conditions was considered. In this reactor, the cell populations are separated by a membrane which permits semi-in vivo study of induced interaction specific changes in metabolism. In this paper, the interaction of suspension culture of *Nicotiana tabacum* (tobacco) and the Omycete, *Phytophthora nicotiana* was simulated. The results of the computer simulation show the induced metabolic changes as a consequence of the biological interaction. The paper introduces a novel approach in the strategy for the study of interacting population in suspension cultures. This type of system has potential applications in studies of the regulation of secondary metabolism and for the production of high values pharmaceuticals. © 1997 John Wiley & Sons, Inc. *Biotechnol Bioeng* 55: 609-615, 1997.

Keywords: interacting populations; membrane reactor; induced metabolic changes; elicitation.

INTRODUCTION

The in vitro cultivation of plant cells has led to major advances in understanding plant cell biochemistry and in the development of improved plants. Individual strategies to maximize volumetric productivity of plants cells are: strain selection, production medium development,cell immobilization. It is critical to recognize that these strategies are interactive and their integration into a coherent process strategy is problematic (Shuler, 1994). Furthermore, it is difficult to understand the complex processes of infectious plant diseases caused by some pathogenic microorganisms.

Several biochemical responses between microorganisms and plants have been postulated. In many cases, small organic molecules are known to play an important role in the interaction process. In some

---

N. de R. Es ésta la reproducción del paper publicado por Jhon Wiley & Sons, Inc. en el Vol. 55, Nº 4, de agosto de 1997 de la revista Biotechnology and Bioengineering.

1. Centro Regional de Estudios Avanzados, Avenida del Fundador, Puente Blanco 5700, San Luis, Argentina;

2. Facultad de Ingeniería Centro Universitario, C.C. 405, Universidad Nacional de Cuyo, Mendoza 5500, Argentina; Telephone: 061234489; fax 061380120; e-mail: eercoli@raíz.uncu.edu.ar

cases, toxic substances are used in an offensive action by the microorganism, while in other cases, inhibitory substances are released by the plant as defensive substances (Ueno, 1990). Elicitation is the response of many plants to an invasion by a pathogenic or non pathogenic microorganism (fungus, bacterium, or virus). Upon elicitation, the plant cells accumulate phytoalexins, which are low molecular weight compounds that inhibit the growth of microorganisms (Albersheim, 1978; Apostol, 1989; Ayers, 1976; Knogge, 1987). Most of the work done on these interacting popuations has been focused on the interaction itself by exposing plant cell suspension to microbes cell wall extracts of fungi or bacteria (Apostol 1989; Bolwel, 1987; Croteau, 1987; Dudley, 1986; Ebel, 1976; Eilert, 1984; Rokem,1984; Wijns na, 1985). Elucidation of the molecular interaction of phytopathogenic or nonphytopathogenic microorganisms and plants would be greatly facilitated if plant cell suspension and living microorganisms could be co-cultured in a model experimental system.

The need for a controlled experimental system is specially important in biochemical and biological studies aimed at investigating how plant-microbe interaction can modify various metabolic processes. However, to develop a bioreactor system capable of culturing both plant cells and microbes, it is necessary to consider the differences between these cell types. For instance, microbial growth and oxygen consumption rates can be orders of magnitude greater than those for plant cells, while plant cells are considerably more sensitive to shear effects than microbes (Pestchanker et al., 1996). The common observation that plant cell cultures can be contaminated by microbes illustrates the difficulty of controllably co-culturing plant cells with microorganisms.

The purpose of this paper is to consider a membrane bioreactor for the controllable co-cultivation of two interacting cell populations (microorganisms and plants). Such membrane reactors have been used to study interacting populations of microorganisms and to study elicitation processes (Drioli, 1985; Finn, 1986). This type of bioreactor system has potential applications in studies of the regulation of secondary metabolism and for the production of high values pharmaceuticals (Hirasuna et al., 1996 Pestchanker,1996, Srinivasan, 1995). Specifically, a mathematical model was developed to describe metabolic changes induced by the interaction between plant cells and microorganisms.

MATERIAL AND METHODS

Cell Characterístics and Cell Interaction

The model is based on the interactions of cell suspension cultures of *Nicotiana tabacum* L (X_A) and *Phytophthora nicotianae* Breda de Haan var. *parasitica* (X_B).Phytophthora nicotianae Breda de Haan var. parasitica is a plant pathogen and produces an extracellular elicitor (Albersheim. 1978; Ayers, 1976). This elicitor was demonstrated to be a polysaccharide of molecular weight 10000 with a composition and structure similar to that of the base insoluble glucan of the mycelia walls of the microorganism (Albersheim, 1978: Ayers, 1976).

Cell cultures of *Nicotiana tabacum L*. Produce farnesyl diphosphate (FPP) as an intermediate of the isoprenoid biosynthetic pathway. Vögeli and Chappel (1988) have studied the regulation of isoprenoid metabolism in plants and reported that FPP P_1) is an important and potential regulatory branch point in isoprenoid biosynthetic pathway. Under normal (uninduced) conditions, FPP is consumed mostly for sterol (P_3) biosynthesis, with the remaining portion being partitioned between ubiquinone (and other phenyl lipid moities) and sesquiterpenoids (P_2) biosynthesis (Bolwel, 1987) (fig.1).

A dramatic accumulation of sesquiterpenoids has been observed in plant cells after elicitation by Phytophthora cell wall extract (Chappell, 1987; Gershenzon, 1989M -Vögeli 1988). Chappell and co-workers suggested that the metabolic changes in tobacco cell suspension cultures upon adition of glucan (P_4) (*Phytophthora nicotianae* Breda de Haan var. *parasitica's* elicitors from cell walls), occurred by a coordinated increase in the 3- hydroxy-3- methylglutaryl coenzyme A reductase and sesquiterpene cyclase and a decrease in squalene sinthetase enzyme activities. P_4 increase enzyme activities of metabolic step from S to P_1 and from P_1 to P_2 and decrease from P_1 to P_3 (figs. 2_a-2_d).

Bioreactor Design

A two-chambered membrane reactor design is proposed. If a 0.1 um pore size membrane is used, then only nutrients and metabolites (S, P_1, P_2, P_3 and P_4) can cross freely through the membrane. Thus this membrane bioreactor separates the cell population while permitting the exchange of metabolites across the membrane (Fig. 3). Basically, the control of interaction is reached through a net interchange of media flowing alternatively in both directions across the membrane. The convective flow of media across the membrane can be controlled by changing the pressures in both compartments. Also, membrane plugging can be prevented by using a large cross flow tangential to the surface of the membrane. Since the flow normal to the filter is small relative to the cross flow, the accumulation of cells at the membrane can be minimized.

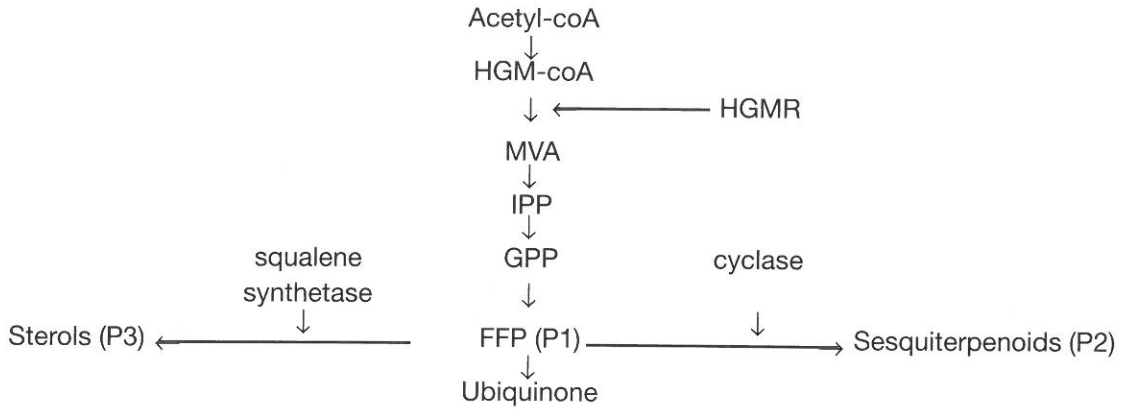

Figure 1. Isoprenoid biosynthetic pathway from N. ταβαχυμ.

Balance Equations (Bailey, 1986; Sinclair, et al. 1987)

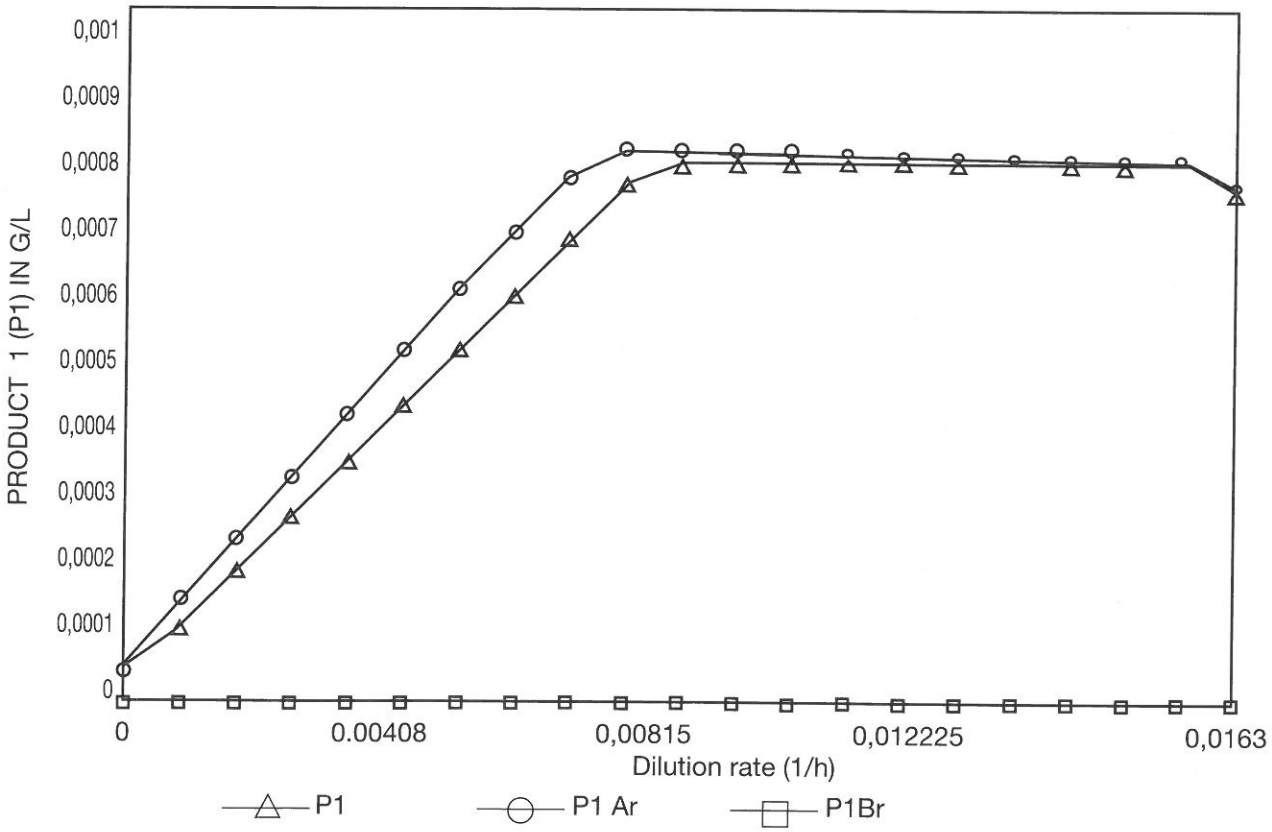

Figure 2a. Product concentration vs. dilution rate, under bath interactive and non-interactive growth conditions.

Balance Equations (Bailey, 1986;Sinclair, et al 1987)

Balance on Cells (1)

Side A

$$D_{Af} \cdot X_A - D_A \cdot X_A + m_A \cdot XA - kdA \cdot XA = 0$$

Side B

$$D_{Bf} \cdot XB - D_B \cdot X_B + m_B \cdot X_B - k_{dB} \cdot X_B = 0$$

Considerations: $X_{Af} = 0$ and $X_{Bf} = 0$

Balance on Death Cells (2)

Side A

$$D_A \cdot X_{dAf} - D_A \cdot X_{dA} + k_{dA} \cdot X_A = 0$$

Side B

$$D_B \cdot X_{dBf} - D_B \cdot E_{dB} + k_{dB} \cdot X_B = 0$$

Considerations: $X_{dAf} = 0$ and $X_{dBf} = 0$

Balance on Substrate (3)

Side A

$$D_A \cdot S_f - D_A \cdot S_A -$$

$$\left[\frac{m_A}{Y_{XA/SA}} + \frac{(a_1 \cdot m_A + b_1)}{Y_{PI/SA}} \cdot \frac{(k_{E1} + P_{4A})}{k_{E1}}\right] \cdot X_A + D_{AB} \cdot (S_B - S_A) = 0$$

Side B

$$D_B \cdot S_f - D_B \cdot S_B -$$

$$\left(\frac{m_B}{Y_{XB/SB}} + \frac{(a_4 \cdot m_B + b_4)}{Y_{P4/SB}}\right) \cdot X_B + D_{AB} \cdot (S_A - S_B) = 0$$

Balance on Product (P_1) (4)

Side A

$$D_A \cdot P_{1Af} - D_A \cdot P_{1A} +$$

$$\left[a_1 m_A + b_1 \cdot \frac{(K_{E1} + P_{4A})}{K_{E1}} - \frac{(a_2 \cdot m_{max2} \cdot P_{1A} + b_2)}{(k_{P1/P2} + P_{1A})} \cdot \frac{(k_{E2} + P_{4A})}{k_{E2}} - \frac{(a_3 \cdot m_{max} \cdot P_{1A} + b_3)}{(k_{P1/P3} + P_{1A})} \cdot \frac{(k_{13})}{(k_{13} + P_{4A})}\right] \cdot X_A + D_{AB} \cdot (P_{1B} - P_{1A}) = 0$$

Side B

$$D_B \cdot P_{1Bf} - D_B \cdot P_{1B} + D_{AB} \cdot (P_{1A} - P_{1B}) = 0$$

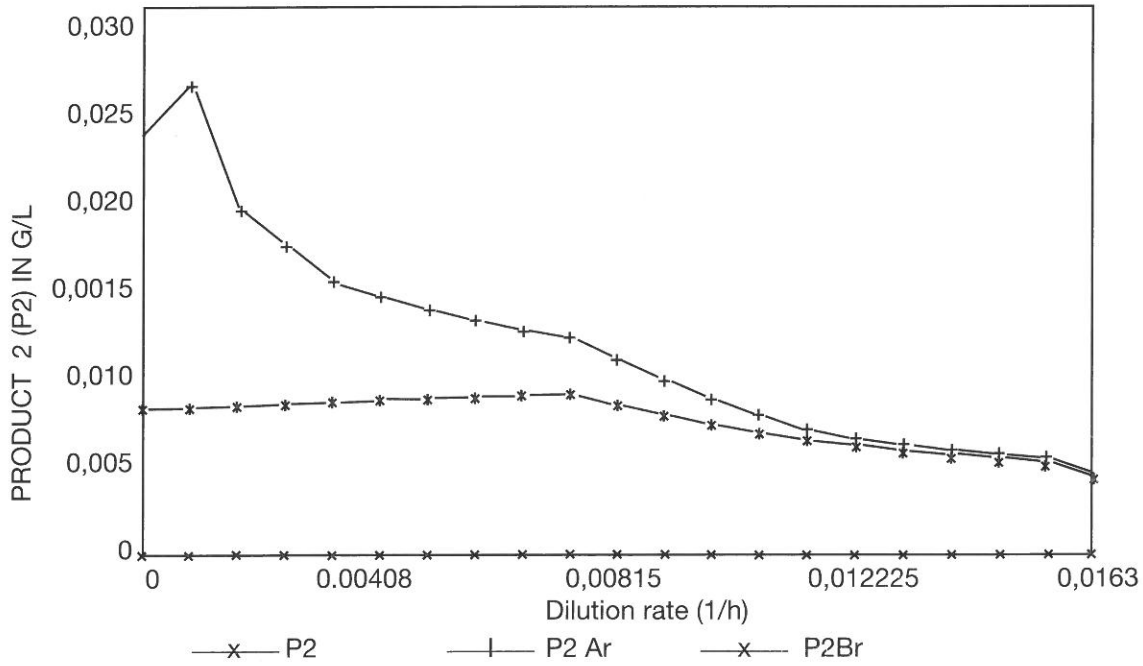

Figure 2b. Product concentration vs. dilution rate, under both, interactive and non-interactive growth conditions.

Considerations: $P_{1Af} = 0$, $P_{1Bf} = 0$

Balance on Product (P_2) (5)

Side A

$$D_A \cdot P_{2Af} - D_A \cdot P_{2A} + \frac{[(\alpha_2 \cdot \mu_{max.2} \cdot P_{1A} + \beta_2) / (k_{P1/P2} + P_{1A})]}{Y_{P1/P2}} \cdot \frac{(k_{E2} + P_{4A})}{k_{E2}} \cdot X_A + D_{AB} \cdot (P_{2B} - P_{2A}) = 0$$

Side B

$$D_B P_{2Bf} - D_B \cdot P_{2B} + D_{AB} \cdot (P_{2A} - P_{2B}) = 0$$

Considerations: $P_{2Af} = 0$, $P_{2Bf} = 0$

Balance on Product (P_3) (6)

Side A

$$D_A \cdot P_{3Af} - D_A \cdot P_{3A} + \frac{[(\alpha_3 \cdot \mu_{max3} \cdot P_{1A} + \beta_3) / (K_{P1/P3} + P_{1A})]}{Y_{P1/P3}} \cdot \frac{(k_{13})}{(k_{13} + P_{4A})} \cdot X_A + D_{AB} \cdot (P_{3B} - P_{3A}) = 0$$

Side B

$$D_B \cdot P_{3Bf} - D_B \cdot P_{3B} + D_{AB} \cdot (P_{3A} - P_{3B}) = 0$$

Considerations: $P_{3Af} = 0$, $P_{3Bf} = 0$

Balance on Product (P_4)

Side A

$$D_A \cdot P_{4Af} - D_A \cdot P_{4A} + D_{AB} \cdot (P_{4B} - P_{4A}) = 0$$

Side B

$$D_B \cdot P_{4Af} - D_B \cdot P_{4B} + (\alpha_4 \cdot \mu_B + \beta_4) \cdot X_B + D_{AB} \cdot (P_{4A} - P_{4Bf}) = 0$$

Considerations: $P_{4Af} = 0$, $P_{4Bf} = 0$

Operating Variables

Sf = 20 g/l

$D_A = 1.63 \cdot 10^{-4}\ h^{-1}$ to $1.63 \cdot 10^{-2}\ h^{-1}$

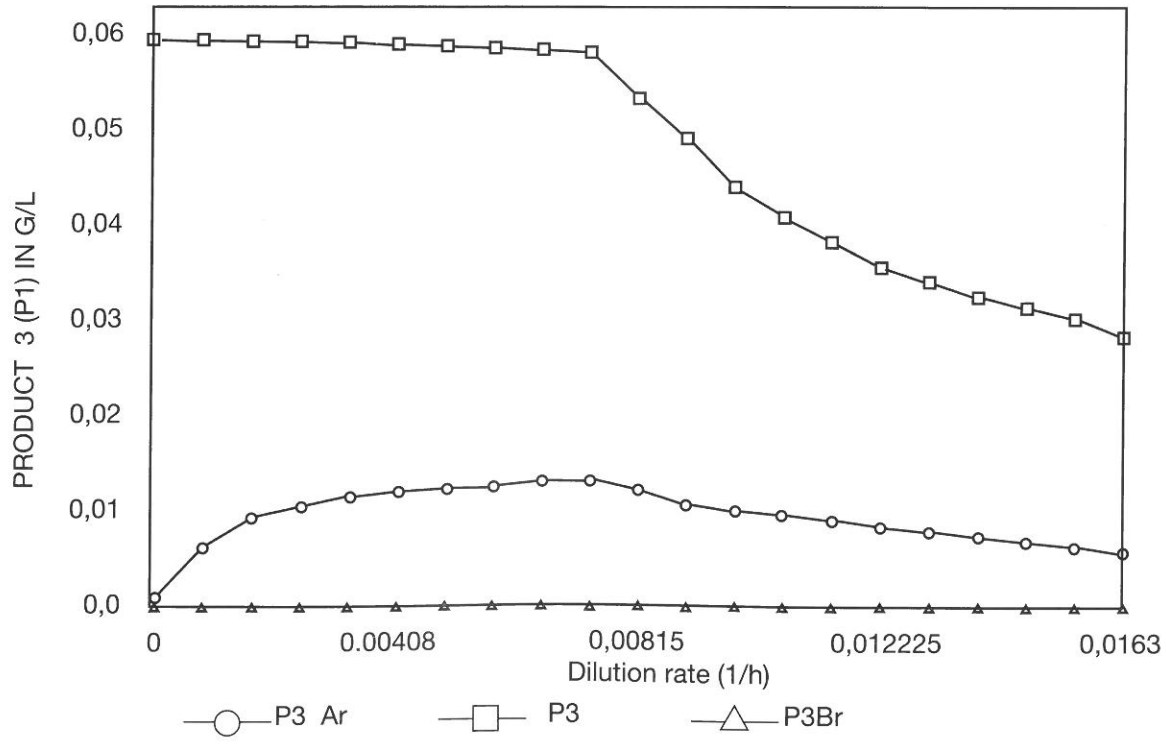

Figure 2c. Product concentration vs. dilution rate, under both, interactive and non-interactive growth conditions.

$D_B = 2.4 \cdot 10^{-2}\ h^{-1}$

$D_{AB} = 2.075 \cdot 10^{-4}\ h^{-1}$

Kinetics Parameters

$\mu_{maxA} = 1.66 \cdot 10^{-2}\ h^{-1}$

$\mu_{maxB} = 2.5 \cdot 10^{-1}\ h^{-1}$

$\mu_{max2} = 5 \cdot 10^{-2}\ h^{-1}$

$\mu_{max3} = 8 \cdot 10^{-2}\ 10^{-1}$

$K_{dA} = 3.33 \cdot 10^{-2}\ h^{-1}$

$K_{dB} = 1 \cdot 10^{-2}\ h^{-1}$

$K_{sA} = 8 \cdot 10^{-3}$ g/l

$K_{sB} = 1 \cdot 10^{-2}$ g/l

$K_{P1/P2} = 4\ 10^{-2}$ g product 1/g product 2

$K_{P1/P3} = 1 \cdot 10^{-2}$ g product 1/ product2

$K_{E1} = 3 \cdot 10^{-1}$ g/l

$K_{13} = 1 \cdot 10^{-1}$ g/l

$K_{E2} = 1 \cdot 10^{-1}$ g/l

$\alpha_1 = 1 \cdot 10^{-2}$ g product / g. cell h

$b_1 = 0$ g product/g cell

$\alpha_2 = 1 \cdot 10\text{-}^2$ g product/ g cell h

$b_2 = 0$ g product/g cell

$\alpha_3 = 1 \cdot 10^{-2}$ g product/ g cell h

$b_3 = 0$ g product/g cell

$\alpha_4 = 1 \cdot 10^{-1}$ g product/ g cell h

$b_4 = 0$ g product/g cell

Stoichiometry Parameters

$Y_{XA/SA} = 4 \cdot 10^{-1}$ g cell/g substrate

$Y_{XB/SB} = 4 \cdot 10^{-1}$ g cell/g subtrate

$Y_{P1/SA} = 1 \cdot 10^{-1}$ g product/g substrate

$Y_{p4/SB} = 9.5 \cdot 10^{-1}$ g product/g substrate

$Y_{P1/P2} = 8.5 \cdot 10^{-1}$ g product 1/g product 2

$Y_{P1/P3} = 8.5 \cdot 10^{-1}$ g product /g prod. 3

Nomenclature

D_A and D_B	dilution rate on sides A and B
D_{AB}	dilution rate through membrane
K_{sA} and K_{sB}	Michaelis constant of specie A and B
K_{dA} and K_{sB}	death rate constant of specie A and B
P_{1A} P_{2A} P_{3A} P_{4A}	product concentrations on side A (g/l)
P_{1AF} P_{2AF} P_{3AF} P_{4AF}	inlet product concentrations on side A (g/l)
P_{1B} P_{2B} P_{3B} P_{4B}	Product concentrations on side B (g/l)
P_{1BF} P_{2BF} P_{3BF} P_{4BF}	inlet product concentrations on side B (g/l)
S_A and S_B	limiting substrate concentration on side A and B
S_r	inlet limiting substrate concentration
X_A and X_B	cell concentration of specie A and B (g/l)
X_{AF} and X_B	inlet cell concentration of specie A and B (g/l)

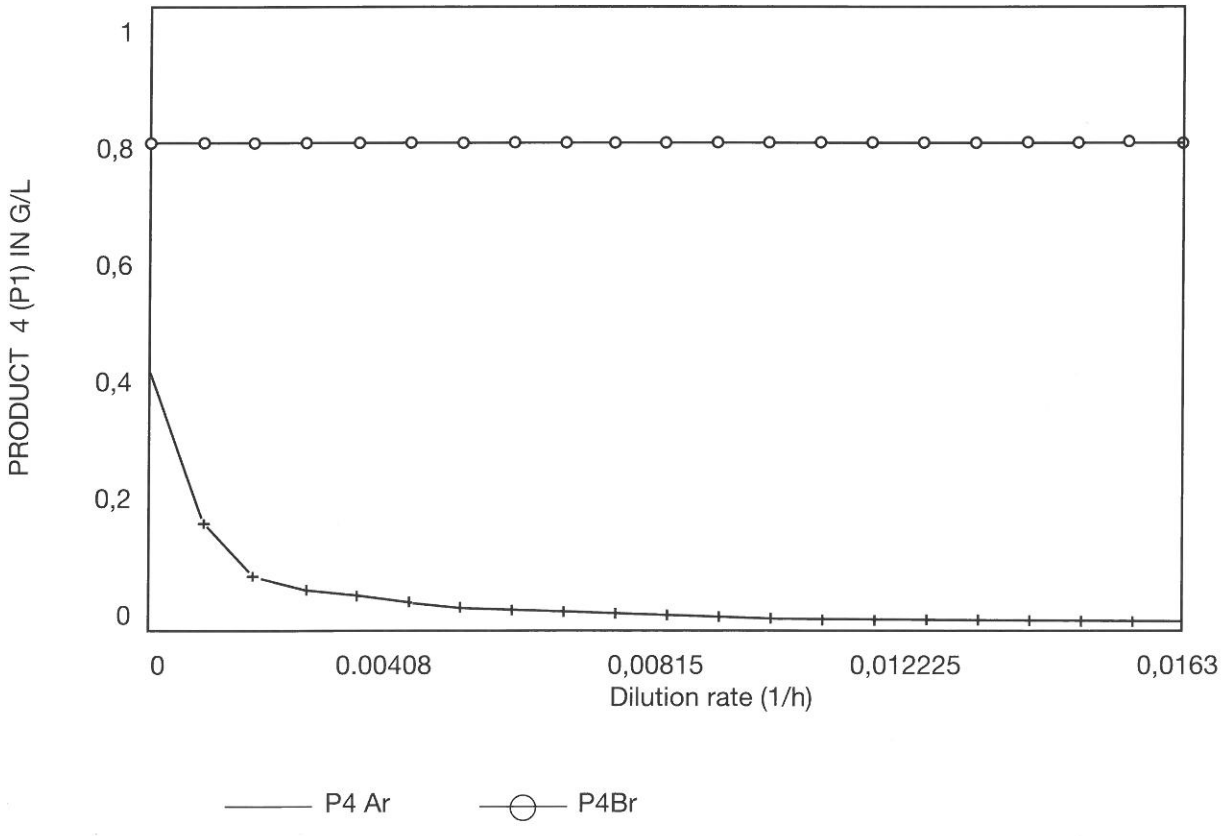

Figure 2d: Product concentration vs. dilution rate, under both, interactive and non-interactive growth conditions.

$Y_{XA/SA}$ $Y_{P1/SA}$ $Y_{P1/P2}$ $Y_{P1/P3}$ Yield factors of specie A (g/g S)

$Y_{XB/SB}$ $Y_{P4/SB}$ Yield factors of specie B (g/g S)

K_{13} Inhibition constant (g P/l)

K_{E1}, K_{E2} exaltation constant (g P/l)

α_1, α_2, α_3, and α_4 growth related product formation coeficient (g P/g X)

β_1, β_2, β_3 and β_4 non-growth related product formation coefficient (g P/g X - h)

μ_{maxA} and μ_{maxB} top specific growth rates of species A and B (h^{-1})

μ_{max2} and μ_{mac3} top specific production rate of product 2 and 3 (h^{-1})

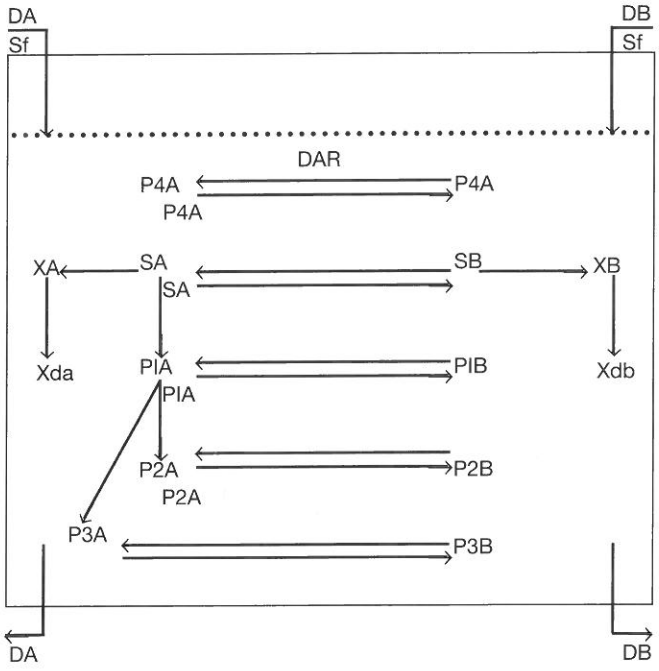

Figure 3. Schematic diagram of the reactor.

Conclusions

Both microorganism and cell plant populations can be individually controlled by the proposed reactor design. Specifically, it es possible to independently control the growth rate of the population by modifying the dilution rate in each compartment. Also, various types of plants cultures (e,g. suspension culture immobilized and organ culture) can be studied in the plant cell compartment.

Furthermore, since it is possible to alter the flow rate through the membrane, it is possible to alter the concentration of metabolites experienced by each population and therefore to alter the interaction between the two populations. Elucidation of the kinetic parameters of the molecular interaction between microorganisms and plants could be obtained by using different dilution rates through membrane and determining metabolite concentrations in both compartments.

As an industrial system for the production of certain metabolites that are induced by pathogens, it might be more efficient just to add the fungal elicitor to the plant cell suspension culture. Productivity could be incrreased if more than one elicitor are added.

Contrary to a sistem in which isolated elicitors are added to plant cell suspension culture, it would not be possible to prove, using the described system in this paper, which would be the true biological elicitor. However, the power of this novel reactor design may be not only to produce elicitation effect, but also could be helpful for studying nutrient exchange between pathogens and hosts. It would be possible to identify new compounds with effect on the interaction by extracting them from the culture and using them in a single reactor or by changing the flow rate through the membrane.

We thank Dr. Gregory F. Payne for helping us to edit this paper and Mr. F. Pestchanker for his technical assistance in the computer simulation process.

References

Albersheim. P., Valent, S. 1978. Host-phatogen interactions in plants. The

Journal of Cell Biology 78: 627.

Apostol I., Low P. S., Heinstein P. 1988. Effect of age of cell suspension culture on susceptibility to a fungal elicitor. Plant Cell Rep. 692.

Apostol I. Heinstein , P., Low. P. S. 1989. Rapid stimulation of an oxidative burst during elicitation of cultured plant cells. Plant Physiol. 90: 109.

Ayers, A. R. et al. 1976. Host-pathogen interaction. Plant Physiol. 57:751.

Bailey, J. E., Ollis, D. 1986. Biochemical engineering findamentals. 2nd.

edition. McGraw Hill Book Company.

Bolwel, G.P. 1987. Planta. 185

Chappell, J., Nable, R. 1987. Induction of sesquiterpenoid biosynthesis in tobacco cell suspension cultures by fungal elicitor. Plant Physiol. 85: 469

Croteau. R.,Gurkewitz, S., Johnson, M. A., Fisk, H.J. 1987. Biochemistry

of oleoresinosis. Plant Physiol. 1123.

Dudley, M. W., Dueber, M.; West, A. 1986. Biosynthesis of the macrocyclic diterpene casbene in castor bean. Plant Physiol. 81:335.

Drioli, E. 1985. Membrane processes in the separation, purification and concentration of bioactive compounds from fermentation broths. ACS symposium series 314.52.

Ebel. J., Ayers. A., Albersheim, P. 1976. Host-pathogen interaction. Plant Physiol. 57:775.

Eilert, U., Ehmke, A., Wolters, B. 1984. Elicitor-induced accumulation of acridone alkaloid exposides in ruta graveolens suspensión cultures. Planta Medica. 508

Finn, R. K., Ercoli, E. 1986. A membrane reactor for simultaneous production of anaerobic single cell protein and methane. ACS Symposium series 314.43

Gershenzon, J., Maffei, M., Croteau, R. 1989. Biochemical and histochemical localization monoterpene biosynthesis in the glandular trichomes of spearmint (mentha spicata). Plant Physiol. 89: 1351.

Hirasuna, T., Pestchanker, L. J., Srinivasan, V., Shuler, M. L. 1996. Taxol production in suspension cultures of Taxus baccata. Pl. Cell tis. Org. Cult. 44:95.

Knogge, W., Kombrink, E., Schmelzer, E., Halhbrock, K. 1987. Occurence of phitoalexins and other putative defense- related substances in uninfected parsley plants. Planta 171: 279.

Payne, G.T. 1987. Large Scale Cell Culture Technology, 195.

Pestchanker, L. J., Moser, S. C., Shuler, M.L. 1996. Kinetics of taxol production and nutrient use in suspension cultures of Taxus and Wilson-type bioreactor. Enz. Microb. Technol.

Rokem, J. S., Schwarzberg, J., Goldberg, I. 1984. Autoclaved fungal mycelia increase diosgenin production in cell suspension culture of dioscorea deltoidea. Plant Cell Rep. 3: 159.

Shuler, M.L. 1994. Strategies for improving productivity in plant cell, tissue, and organ culture in bioreactors. Personal communication.

Sinclair, C. G., Kristiansen, B. 1987. Fermentation kinetics and modeling. Edited by J. D. Bu Lock, Open University Press, Milton Keynes and Taylord Francis, New York- Philadelphia.

Srinivasan, V., Pestchander. L. J., Moser, S., Hirasuna, T. J., Taticek, R. A., Shuler, M. L. 1995. Biotechnol. Bioeng. 47:666.

Ueno, T. 1990. Secondary metabolites related to host selection by plant pathogenic fungy. Pure and Appl. Chem. 62 (7): 1347.

Vögeli, U., Chappell, J. 1988. induction of sesquiterpene cyclase and suppression of squalene synthetase activities in plant cell cultures treated with fungal elicitor. Plant Physiol. 88: 1291.

Wijnsma, R., Go, J.T.K.A., van Weerden. I.N. Harkes, P.A.A., Verpoorte, R., Svendsen B. 1985. Anthraquinones as phytoalexins in cell and tissue cultures of Cinchona spec. Plant Cell Rep. 4: 241.