Multi-element hollow fiber membrane bioreactors for cultivation of Pseudomona putida
by James V Odasso

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University

Abstract:
The goal of this research was to examine growth patterns of Pseudomonas putida immobilized within multi-element hollow fiber membrane (HFM) bioreactofs. Understanding bacterial growth patterns provides important information for modeling and scale up of a bioreactor system and will lead to an improved understanding of cell physiology under possible mass transport limitations. Such information will allow one to also set reasonable performance expectations. Three objectives existed for this project: (1) construction of a multi-element HFM bioreactor system and development of methods for cell inoculation, cultivation, and harvesting, (2) development of methods for obtaining glucose, oxygen, DMA, RNA, and protein profiles within the HFM bioreactor, (3) map the accumulation of biomass within the bioreactor as an indication of reactor performance.

Evaluation of reactor performance through examination of glucose removal rate, RNA/Protein ratio of entrapped cells, and total cell protein within the fibers indicated very poor bioreactor performance. Additionally, cell breakout could not be prevented and the physical dynamics of the HFM bioreactor used for this project were unpredictable. The combined evidence of low biomass retention, limited glucose removal, and small RNA to Protein ratio led to the conclusion that very limited to no growth was taking place in 3 of 4 HFM reactor systems examined for this project. The disadvantages of the hollow fiber reactors used in the project far out number the advantages of such a system at this time.

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APPROVAL

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James V. Odasso

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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The following statement has a special meaning to me because it summarizes the frustrations that a person with Dyslexia faces every day. The author and I share this problem so I understand that to get anywhere in life you must never give up because with enough work things will come out right.

"I think for months and years

ninety nine times the conclusion is false

The hundredth time, I am right."

Albert Einstein

A very special thanks goes out to Mrs. Mary Huenergardt, my life would be very different if not for the kindest and most caring woman I have ever met other than my mom.

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ABSTRACT

The goal of this research was to examine growth patterns of *Pseudomonas putida* immobilized within multi-element hollow fiber membrane (HFM) bioreactors. Understanding bacterial growth patterns provides important information for modeling and scale up of a bioreactor system and will lead to an improved understanding of cell physiology under possible mass transport limitations. Such information will allow one to also set reasonable performance expectations. Three objectives existed for this project: (1) construction of a multi-element HFM bioreactor system and development of methods for cell inoculation, cultivation, and harvesting, (2) development of methods for obtaining glucose, oxygen, DNA, RNA, and protein profiles within the HFM bioreactor, (3) map the accumulation of biomass within the bioreactor as an indication of reactor performance.

Evaluation of reactor performance through examination of glucose removal rate, RNA/Protein ratio of entrapped cells, and total cell protein within the fibers indicated very poor bioreactor performance. Additionally, cell breakout could not be prevented and the physical dynamics of the HFM bioreactor used for this project were unpredictable. The combined evidence of low biomass retention, limited glucose removal, and small RNA to Protein ratio led to the conclusion that very limited to no growth was taking place in 3 of 4 HFM reactor systems examined for this project. The disadvantages of the hollow fiber reactors used in the project far out number the advantages of such a system at this time.

GOALS AND OBJECTIVES

The goal of this research is to examine growth patterns of *Pseudomonas putida* immobilized within a multi element hollow fiber membrane (HFM) bioreactor. Understanding bacterial growth patterns provides important information for modeling and scale up of a bioreactor system. Information on development of the immobilized cells in HFM bioreactors will lead to an improved understanding of cell physiology under possible mass transport limitations. Such information will allow one to also set reasonable performance expectations. Three objectives exist for this project. First, construct a multi element HFM bioreactor system and develop methods for cell inoculation, cultivation and harvesting. Second, develop methods for obtaining glucose, oxygen, DNA, RNA and protein profiles within the HFM bioreactor. Third, map the accumulation of biomass within the bioreactor as an indication of reactor performance.

CHAPTER 1

Introduction

Interest in whole cell biocatalysts has steadily increased over the past two decades and, more recently, due to advances in recombinant DNA and cell fusion technologies (Belford, 1988). Man has used biocatalyst capabilities in nature to improve his well-being throughout history, by collecting, screening, selecting and domesticating living organisms (Akin, 1987). Molecular biology is rapidly creating new tools to design, engineer and modify biocatalytic activities. Whole cell biocatalysis are capable of complex multiple species chemical reactions which no single catalysis, biological or other, could accomplish individually. Application of this technology to any system requires a mode of cell cultivation. Evolution of cell cultivation systems has involved suspended cell bioreactors (i.e., chemostats), biofilm reactors (i.e., Annular reactors), and porous matrix biofilm reactors (i.e., HFM reactors). Each bioreactor type has distinct and inherent advantages and disadvantages when applied to whole cell biocatalyst systems. Cultivation of a biocatalyst is a specialized application where large quantities of biomass accumulates in the smallest reactor volume possible. Immobilization of the whole cells upon a support matrix provides an avenue through which to minimize the bioreactor volume required for a specific reaction. In some phase of their life cycles, most cells tend to locate or attach themselves to a solid surface (Messing, 1985). This attachment and growth of cells is referred to as biofilm development. Biofilms will form on any surface

although in some fermentors and in waste treatment systems flocculation, pellet formation, can also be viewed as a form of immobilization. For a formal definition, immobilized cell, biofilm, biocatalysis can be designated as, "cells which are physically confined or localized in a defined region or space with retention of their catalytic activities or selected portions thereof, for repeated and continuous use" (compare with Klein and Wager, 1983). The application of a bioreactor for treatment of a contaminated waste stream or production of a specialty chemical involves cultivation of biomass as well as production of effluent devoid of cellular material. HFM bioreactors have the potential to effectively remove the pollutant while effectively retaining the biocatalyst in the reactor.

Suspended vs. Immobilized Cell Bioreactor Systems

Immobilized cell reactor systems have distinct advantages over suspended (or planktonic) cell cultivation systems including improved product yield and process efficiency. The use of cheap, easily obtainable materials for construction of the reactors is another advantage. Support material for cell immobilization can be composed of almost anything. The "quick vinegar" process, invented in 1823 by Scheulzenback (Biofilms, P.736), makes use of wood chips as the biofilm support substance. A list of support materials and the bacteria used for ethanol production is provided in Table 1. Immobilized cells allow the reactor to be operated at residence times well below the generation

time of the microbial species. Suspended continuous flow bacterial cultures,i.e., chemostats, are limited by the growth rate of the cells; when the dilution rate of a

Table 1
Immobilized Cells for Ethanol Production

Immobilization Method	Organism	Reference
Adsorption to wood chips	Saccharomyces cerevisiae	Gencer, 1983
Adsorption to ion exchange resins	S. cerevisiae	Daugulis, 1981
Adsorption to glass fiber filters	Zymomonas mobilis	Arcuri, 1982
Adsorption to gelatin-coated supports	S. cerevisiae	Sitton, 1980
Entrapment in polyurethane foam or stainless steel mesh	S. cerevisiae S. uvarum	Black, 1984
entrapment in κ-carrageenan	S. cerevisiae	Wanda M, 1980
co-entrapment in Ca alginate with β-D-glucosidase	Z. mobilis	Lee, 1983
co-entrapped in Ca alginate with magnetite	S. cerevisiae	Lasson P.O., 1981
entrapped in polyacrylamide	S. formosensis	Furuasaki, 1983
entrapped in Na alginate	Kluveromyces marxianus	Margaritis, 1982
entrapment in polyacrylamide or in κ-carrageenan	Kl.fragi	King, 1983
entrapped in pectin	S. cerevisiae	Navarro, 1983
containment by hollow fiber membranes	S. cerevisiae	Inloes, 1983
containment of cells with cellulase and cellobiose by means of a liquid-liquid phase boundary	S. cerevisiae	Hahn-Hagerdal, 1982
Flocculation of yeast	Schizosaccharomyces pombe	Hsiao, 1983

suspended cell bioreactor exceeds the maximum culture growth rate, the cells

will wash out of the bioreactor. Immobilized cell bioreactors physically retain the cells and thus they can handle higher flow rates than suspended cell reactors. Immobilized cell bioreactor systems also provide an avenue through which multiple bacterial species can be cultured in a synergistic environment. Advantages of cell immobilization also include reduction in cost of bioprocessing because of repeated and continuous use of the biocatalyst, reduction in required separation processes and protection of shear-sensitive cells such as plant and animal. Recent advances in genetic engineering and cell fusion technologies have produced a class of biocatalyst which benefit from immobilization through improved plasmid stability (de Taxis du Poet, Dhulster, Tomas, 1984 and C.T. Huang, 1992). Immobilized cell bioreactors do have serious limitations including: (1) excessive internal mass transfer resistance due to excessive cell densities, (2) destruction of the cell support system by growing cells and, (3) cell sloughing from the cell support. A challenge of immobilized bioreactor systems is to develop a versatile, general cell support capable of retaining a wide variety

Table 2
Categories of Support Systems

1	Solid supports or matrices for adhesion or adsorption of cells.
2	Solid supports or matrices for cross linking with cells.
3	Directly cross linked cells.
4	Gel and other polymers for entrapment or encapsulation of cells.
5	Combinations of gels and other polymers for encapsulation or entrapment of cells.
6	Composite immobilization matrix.
7	Hollow structures, fibers and plates (membrane reactors) for physical retention of cells.