

SRC-TR-87-98

Advanced Metabolic Control of  
Biochemical Processes

by

Nam Sun Wang

ADVANCED METABOLIC CONTROL OF BIOCHEMICAL REACTORS

Proposal to the SRC for Support  
for the 1987-1988 Academic Year  
from Core NSF Funds

Submitted By:

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(a) Progress Achieved during 1986-1987:

This project is aimed at regulating and controlling the product formation in a bioreactor by manipulating the environment. The first phase of the project deals mainly with the construction of a fully instrumented and controlled fermenter. Various A/D and D/A programs needed for the automatic data acquisition and analysis have been developed and tested on microcomputers (IBM PC/ATs). An integrated graphical software package to control the automatic injection of the filtered fermentation broth into a GC/HPLC and to analyze the resulting chromatogram is near completion. Concurrently, we have constructed a continuous tangential filtration system to generate a stream of cell-free sample for injection into various detectors. The construction and testing of a variety of on-line autoanalyzers have also been completed for the measurement of alpha-amylase, acetate, glucose, and biomass concentrations.

A fluorescence probe has been fully characterized for the first time with a mathematical model so that its signal can be correlated to the level of NADH inside microbial cells even in the presence of other background fluorescent sources and interfering absorbing particulates. As one of the few techniques currently available for providing information at the intracellular level, the culture fluorescence signal, which is indicative of the intracellular oxido-reductive conditions, should prove to be an important tool in the study of the metabolic mechanisms. This model has been experimentally verified to be a superior one through the use of known fluorophore standards. Concurrently, work on the more advanced laser induced fluorescence sensor, which is capable of monitoring a multitude of chemicals, has been started at the National Bureau of Standards.

Finally, a pH probe/controller system and an array of computer controlled pumps to regulate nutrient addition have also been successfully interfaced to microcomputers monitoring our fermentor. Our initial investigation of bacterial growth indicates a strong dependence of the intracellular NADH level on the nutrient composition. Work is currently underway to model this phenomenon.

SRC Related Publications (1986-1987):

"Prediction of Transient Bioreactor Behavior from Steady-State Data," 192nd National ACS Meeting, Anaheim, CA, Sep 7-12, 1986, (with Stephanopoulos, G. N.)

"Bioreactor Stability and Control in the Presence of Time-Lag Effects," 192nd National ACS Meeting, Anaheim, CA, Sep 7-12, 1986, (with Stephanopoulos, G. N.)

"Estimation of the Growth Associated Time-Lag Parameters in a Fermentor," 79th Annual AIChE Meeting, Miami Beach, FL, Nov 2-7, 1986, (with Stephanopoulos, G. N.)

"Characterization of Signals from an On-Line Fluorescence Probe

for the the In Situ Measurement of NADH Concentration," M. B. Simmons and N. S. Wang, (to be submitted to Biotech. Bioeng.)

"The Use of Fluorescence Probe in Modeling Enzyme Production" 1st Annual Mid-Atlantic Region Biochemical Engineering Symposium, Feb 27, 1987, (with Simmons, M. B.)

"A Graphics-Based Program for Chromatographic Analysis," paper accepted for presentation at 194th National ACS Meeting, New Orleans, LA, Aug 24 - Sep 1, 1987, (with Yin, K.)

"Dependence of the Measured Fluorescence Signal on the Fluorophore Concentration," paper accepted for presentation at 194th National ACS Meeting, New Orleans, LA, Aug 24 - Sep 1, 1987, (with Simmons, M. B.)

"Shear Effects on Globular Proteins," paper submitted for presentation at 194th National ACS Meeting, New Orleans, LA, Aug 24 - Sep 1, 1987, (with Chang, K. C. and Calabrese, R. V.)

"Development of an Introductory Biochemical Engineering Laboratory Course," paper accepted for presentation at 80th Annual AIChE Meeting, New York, NY, Nov 15-20, 1987.

"Modeling of a Commercial Fluorescence Probe," paper submitted for 80th Annual AIChE Meeting, New York, NY, Nov 15-20, 1987, (with Simmons, M. B.)

"Shear Effects on Enzyme Deactivation," paper accepted for presentation at 80th Annual AIChE Meeting, New York, NY, Nov 15-20, 1987, (with Chang, K. C. and Calabrese, R. V.)

(b) Interdisciplinary Character of the Work:

A collaboration with Dr. T. J. McAvoy on the analysis of fluorescence signals and the development of a prototype probe has been consolidated within the past year. Further collaboration on chemical sensors is being formulated.

Dr. K. Halamane has been consulted in the modeling of a biological cell as a complex chemical reaction network whose natural behavior is explained as the outcome of an optimization strategy adapted by the cell for its maximal growth and survival. Thus, as the environment changes, each individual cell allocates the available energy/chemical resources in such a way that represents the solution to a specific optimization problem.

(c) Experimental Aspects of the Work:

Efforts have been initiated to equip a 14-liter New Brunswick Magnaferm fermentor with the state-of-the-art instrumentation capable of on-line monitoring (and, in certain cases, controlling) of various important biological parameters, such as the pH, temperature, pressure, carbon dioxide evolution rate, oxygen uptake rate, optical density, and concentrations of dissolved

oxygen, dissolved carbon dioxide, carbon and nitrogen sources, inhibitor, inducer, metabolic products, and enzymes in the fermentation broth. The various probes are to be interfaced to an IBM PC/AT. Concurrently, work has been started to develop an integrated software package to control the automatic injection of the filtered fermentation broth into a GC and to analyze the resulting chromatograph.

(d) Work of Students under Supervision:

Michael B. Simmons, Ph.D. (3rd year)

Thesis: "Laser Induced Fluorescence in Advanced Pattern Recognition Process Control of Microbial Fermentations"

Kewen Yin, Ph.D. (2nd year)

Thesis: "Modeling and Control of Intracellular Metabolic Activities through the On-Line Monitoring of the Extents of Reactions through Multiple Pathways"

Kyungmoon Park, M.S. (2nd year)

Thesis: "Modeling and Control of an Aqueous Two-Phase Extractive Fermentation Process Coupled with Cell/Enzyme Immobilization"

Jen-Dar Yang, Ph.D. (1st year)

Thesis: "Separation of Bioactive Materials with Supercritical Fluid"

Philippe Renaudo, M.S. (1st year)

Thesis: "Laser Fluorescence Bio-Sensing"

(e) Work with Visitors and Postdoctoral Associates:

None during 1986-1987.

(f) Proposed Work for 1987-1988:

Dr. Pramod Z. Rao of I.I.T. Madras, India, and Dr. Haiqing Li of Zhejiang University, China, will join our research group this fall as Visiting Associates. The first one will concentrate on the modeling and control of an immobilized enzyme reactor and the latter person on the fermentation instrumentation.

During the second year, our efforts will continue to equip a 14-liter New Brunswick Magnaferm fermentor with the state-of-the-art instrumentation capable of on-line monitoring (and, in certain cases, controlling) of various important biological parameters. The current capabilities will be further expanded to include pressure, carbon dioxide evolution rate, oxygen uptake rate, optical density, and concentrations of dissolved oxygen, dissolved carbon dioxide, carbon and nitrogen sources, inhibitor, inducer, metabolic products, and enzymes in the fermentation broth. These instruments are essential for the accurate on-line tracking of the constantly drifting bioreactor model parameters under adaptation, which are in turn indispensable in calculating

the conditions needed to improve substantially the yield and quality of the desired bioproducts. Our next plan is to incorporate these newly constructed on-line sensors in the monitoring of growth and enzyme formation kinetics of *Bacillus amyloliquefaciens* so that a comprehensive model suitable for the optimal control of the fermentation process can be achieved. It is expected that the control strategy will attempt to minimize the production of the protease enzyme and simultaneously maximize the excretion of alpha-amylase. Furthermore, the allocation of the available nutrient toward biomass production and enzyme formation must be well balanced. Other industrially important microbes such as *Saccharomyces* and *Streptomyces* will also be used as the model biochemical systems.

Our long-term objective is to model intracellular metabolic activities on-line and to formulate optimal adaptive control strategies based on the estimated model parameters/structures to guide the various metabolic reactions inside a microbial cell along the desired path. The achievement of this goal requires the automation and interfacing of both existing off-line and on-line sensors to computers and the formulation of a unified algorithm for multi-sensor integration, including an algorithmic sensing methodology based on material balance principles, stochastic state and parameter estimation, and gross measurement error detection/identification/rectification. The extents of reactions for various internal cellular metabolic pathways will be continuously monitored to pinpoint the transition between the different phases of cell growth and product formation. The cause-effect relationship between environmental conditions and internal cellular mechanisms will be identified, and the best pairing of the manipulated variables with the controlled variables will be experimentally established.

(g) Industrial Funding & Collaboration through SRC:

As a member of the Chemical Systems Laboratory, the P.I. has actively shared responsibilities in the industrial board meetings and poster presentations aimed at recruiting more participating industrial partners. Two poster papers entitled "A Graphics Based Program for Chromatographic Analysis" and "Modeling of a Fluorescence Probe" were presented. The P.I.'s salary for the summer of 1986 was provided by the industrial fund toward studies in the advanced process control of biochemical reactors.

A mutually beneficial collaboration has been successfully negotiated with the Chemical Process Metrology Division of the National Bureau of Standards (Dr. J. Ulbrecht) to conduct research in chemical/biological sensor development, including the use of semiconductor-based gas/ion detectors and a state-of-the-art tunable nitrogen dye laser for the continuous in situ measurement of various intracellular biological macromolecules. Industrially important Kraft pulping process will be studied

(h) Other Funding through SRC:

None during 1986-1987.

(i) Other Current Funding (1986-1987):

The Chemical Systems Laboratory, \$15,500, 1986.  
DuPont Young Faculty Award, \$25,000, 1986.  
Allied-Signal Corporation Foundation Faculty Support Grant,  
\$10,000, 1986.  
Equipment Grant from the Minta Martin Fund, \$10,000, 1986.  
Designated Research Initiation Fund, \$39,000, 1986.  
Systems Research Center, \$15,000, 1986.

Pending Support for the Current Work

Minta Martin Aeronautical Research Fund, \$10,000, 1987.  
Glen L. Martin Fellowship for C. G. Gingrich, \$10,000, 1987.  
Biomedical Research Support Award, \$11,000, 1987.  
Biochemical Engineering Program Enhancement Grant  
(Dean of Undergraduate Studies), \$2,000, 1987.  
Biochemical Engineering Program Enhancement Grant  
(Dean of College of Engineering), \$11,395, 1987.  
Systems Research Center, Core NSF Fund, \$72,750, 1987.

(j) Plans for Industrial and Other Funding (1987-1988)

A full day industrial seminar on "Fluorescence Measurement in Fermentation Instrumentation and Control" is being organized in collaboration with the Engineering Research Center's Biotechnology Program on June 10, 1987, to address the applications of fluorescence probes in the biochemical and pharmaceutical industries. A short course aimed at the participation of local biotechnology industries is being planned jointly with the American Type Culture Collection of Rockville, MD, on the on-line computer data acquisition and analysis. The short course has the unstated objective of attracting biotechnology companies to our Chemical Systems Laboratory to support our biosensor work. The participation of biotechnology industries in the Chemical Process Systems Laboratory will be sought to support partially projects in sensors and chemical process systems.

Other financial support to supplement the current research projects will subsequently be solicited from the following governmental agencies and industries:

I. Work on the fluorescence biosensor development, in collaboration with Dr. T. J. McAvoy and the National Bureau of Standards (NBS) is well underway. A proposal to the National Science Foundation (NSF) on the deconvolution of fluorescence signals in black liquor is due in one month. Another process of proposal preparation has been initiated on the application of pattern recognition techniques to extract information from array sensors, e.g. thin film chemical sensors; both the Navy and the Army will be approached.

II. Proposals in environmental metabolic control of microbial cells will be submitted to biochemical industries, as well as government funding agencies. In particular, a project has been initiated in collaboration with Martek of Columbia, MD, to regulate the environment to achieve a high cell density in the cultivation of an algal culture. Flow Laboratories of Virginia is being contacted to support a project in the dissolved oxygen and pH control studies in mammalian cell cultures. Furthermore, the P.I. has recently visited Eastman Kodak to discuss support for the development of an on-line biomass sensor for single cell microorganisms employed in amino acid production.

(k) Budget Justification for Travel and Equipment

Funds are requested for the support of travel to the upcoming annual national meetings of American Chemical Society (August 87) and American Institute of Chemical Engineers (November 87). Two papers entitled "Dependence of the Measured Fluorescence Signal on the Fluorophore Concentration" and "A Graphics-Based Program for Chromatographic Analysis" have already been accepted for presentation at the ACS meeting, and another two papers entitled "Shear Effects on Enzyme Deactivation" and "Development of an Introductory Biochemical Engineering Laboratory Course" are to be presented at the AIChE meeting. Two additional papers entitled "Shear Effects on Globular Proteins" and "Modeling of a Commercial Fluorescence Probe" are expected to be accepted for these two meetings. Currently manuscripts on each of these topics, all of which are based on the P.I.'s first year work at the University of Maryland, are being prepared for submission to refereed journals for publication. Thus, it is imperative that the P.I. be given the means to present these papers accepted in highly competitive and visible major national meetings. The abstracts of the four already accepted papers are attached.

Partial support for the acquisition of a high performance liquid chromatograph (HPLC), a standard piece of equipment critical to the P.I.'s research in biochemical engineering, is requested. The equipment is central to the current metabolic control work; it is to be used to monitor the concentrations of various chemical species in the growth environment surrounding microbial cells. One third of the fund has been secured from the Designated Research Initiative Grant and another third has been committed by the Department of Chemical and Nuclear Engineering. The remaining third of the required fund is requested from SRC so that the progress on interfacing various instruments to a fermentor need not be overly retarded as a result of the unavailability of the equipment. When approved, the equipment will be made available to all participating SRC members, and the P.I. will assume responsibilities for the subsequent maintenance and servicing.



**SRC-NSF Core Budget Request (Estimate)**  
**for the Period 6/15/87 - 6/14/88**

Name of Individual/group Nam Sun Wang/Chemical Process Systems

Description Amount

Faculty Salary Support, including benefits

	Other Contributing Funding Source		SRC-NSF Core Funds	
	Acct. #	Amount		
Academic Year				
Summer (2 1/2 months)			\$11,842	
<b>Total</b>				<b>\$11,842</b>

Fellowship Support

Name	Other Contributing Funding Source		SRC-NSF Core Funds	
	Acct. #	Amount		
SRC Fellows				
Michael B. Simmons			\$23,418	
<b>Total</b>				<b>\$23,418</b>

GRA

Kewen Yin			\$12,829	
Jeng-Dar Yang			12,829	
<b>Total</b>				<b>\$25,658</b>

Other Student Support (Including Undergraduate Fellowships)


Visitor/Research Associates

<u>Name</u>	<u>Other Contributing Funding Source</u>		<u>SRC-NSF Core Funds</u>	
	<u>Acct. #</u>	<u>Amount</u>		
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				0

Travel

- Domestic		\$2,000		
- Foreign				\$2,000

Equipment/Software

<u>Description</u>	<u>Other Contributing Funding Source</u>		<u>SRC-NSF Core Funds</u>	
	<u>Acct. #</u>	<u>Amount</u>		
	01-1-12080	\$9,832		
Liquid chromatograph	01-3-93207	\$9,832	\$9,832	\$9,832

Other Support

<u>Description</u>	<u>Other Contributing Funding Source</u>		<u>SRC-NSF Core Funds</u>	
	<u>Acct. #</u>	<u>Amount</u>		
				0

**Grand Total Budget Request****\$ 72,750**

DEPENDENCE OF THE MEASURED FLUORESCENCE SIGNAL  
ON THE FLUOROPHORE CONCENTRATION

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Numerous studies have concentrated on the use of fluorescence measurements to monitor the intracellular NADH level. A rigorous biosensor model was developed for a commercial NADH fluorescence probe to describe the single frequency excitation and emission fluorescence behavior of an aqueous mixture of fluorophores. This model is needed to correlate the measured signals to the concentration of fluorescent compounds in a fermentation broth. The relevant parameters of the model are the absorbance of the medium at both the excitation and the emission frequencies by the solvent and other absorbing species, the background signals, the light path length of the fermentor vessel, the fluorescence yield, the lamp-detector configuration, and a light-scattering coefficient. The effects of temperature, pH, and spatial inhomogeneity (bubbles and insoluble solids) are implicitly included in the model. The model shows that the relationship between the level of fluorescence signal and the concentration of the fluorophore of interest in the presence of other interferences is intrinsically nonlinear. The signal level is independent of the fluorophore concentration for the ideal case of a single fluorophore and an infinitely long light path. The validity of the model was verified experimentally with a system of known fluorophores and microbial cells.

## A GRAPHICS-BASED PROGRAM FOR CHROMATOGRAPHIC ANALYSIS

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An interactive, stand-alone program has been developed to analyze chromatographic data from a laboratory gas chromatograph (GC) and a high performance liquid chromatograph (HPLC). The program, running on the popular IBM-PC or compatibles under DOS, is menu-driven and graphically based. At present, its analytical capabilities are comparable to, and in many aspects surpass, those commercially available products. A set of subroutines callable from FORTRAN have been compiled to handle a wide range of low level I/O functions required by the implementation of A/D-D/A, graphics, and menu-driven interface. Because of the use of device independent techniques, this program can support practically all graphics boards and terminals in high quality modes, as well as provide hardcopy output to printers. The development of this program is an immediate precursor to the objective of demonstrating in situ an advanced scheme for the on-line analysis of chromatographs for the detection of contaminants and abnormal fermentation; work on the scheme is currently underway. The use of semi-continuous chromatographic data should prove indispensable in modeling and applying environmental control to regulate metabolic activities of microorganisms so that the production of the desired fermentation products can be optimized on-line.

## SHEAR EFFECTS ON GLOBULAR PROTEINS

Kuo-Ching Chang, Nam Sun Wang\*, and Richard V. Calabrese  
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Mixing, centrifugation, ultrafiltration, pumping, and passage through pipes are some processing stages in laboratories where globular proteins are subjected to shear. A range of conclusions have been drawn by various investigators on the shear effects on globular proteins, and high shear has often been pointed out as a potential cause of enzyme inactivation. Through rigorous order-of-magnitude analysis based on hydrodynamic, mechanical, energetic, and microscopic considerations, it is concluded, contrary to common beliefs, that shear and/or shear stress alone are not effective in causing protein denaturation or enzyme inactivation. Rather, other secondary effects associated with shear are now suspected as the main causes of enzyme inactivation in a shear flow field.

DEVELOPMENT OF AN INTRODUCTORY  
BIOCHEMICAL ENGINEERING LABORATORY COURSE

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A one-semester undergraduate course in biochemical engineering laboratory is developed at the University of Maryland. The course is designed to introduce chemical engineering seniors to a wide spectrum of biochemical engineering concepts, with an emphasis on quantitative engineering analysis. The experiments covering most of the commonly used laboratory techniques in enzyme and microbial technology are introduced at the rate of one per week. The first half of the semester is centered around enzymatic processes; the topics include enzyme kinetics, production, purification, and immobilization. The second half is focused on microbial processes; the objective is to expose students to aseptic techniques, cell fractionation, fermentation kinetics, and compute data acquisition and control. Various practical applications are interjected to stimulate the students' interest. The criteria used for the selection and design of the experiments will be discussed.