MODERN BIOCHEMICAL ENGINEERING

Modern Biochemical Engineering

Guest Editor: T. Scheper

Dedicated to Prof. Karl Schügerl on the occasion of his 65th birthday

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With 96 Figures and 27 Tables

Springer-Verlag
Berlin Heidelberg NewYork
London Paris Tokyo
Hong Kong Barcelona Budapest

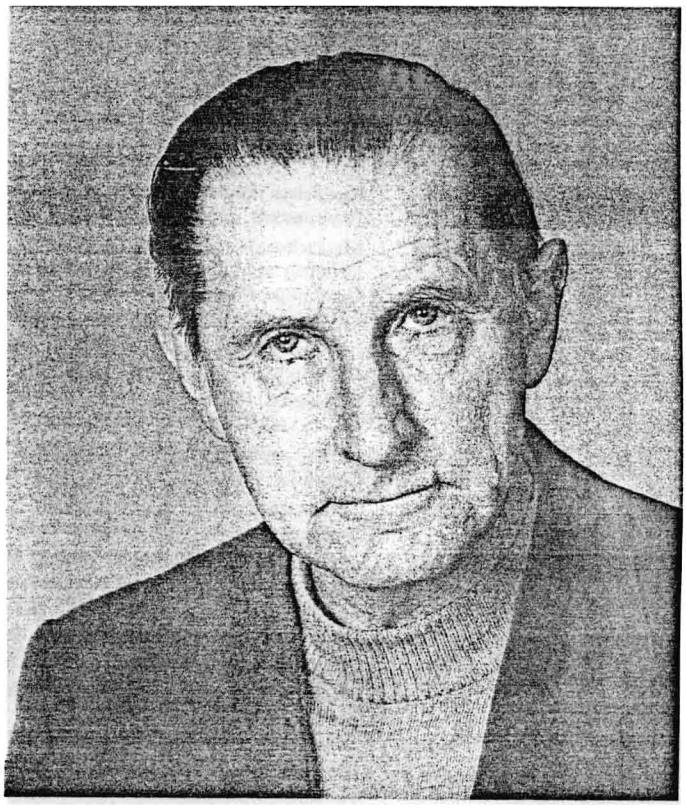
ISBN 3-540-55276-6 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-55276-6 Springer-Verlag New York Berlin Heidelberg

Library of Congress Catalog Coard Number 72-152360
Printed of Germany

Typesetting: Th. Müntzer, Bad Langensalza; Printing: Heenemann, Berlin;
Bookbinding: Lüderitz & Bauer, Berlin
02/3020-5 4 3 2 1 0 – Printed on acid-free paper

46 Advances in Biochemical Engineering/ Biotechnology

Managing Editor: A. Fiechter



Prof. Dr. Karl Schügerl

To Professor Dr. Karl Schügerl on his 65th Birthday

On 22nd June 1992, Karl Schügerl will spend his 65th birthday in the midst of a large assembly of students, PhD students and colleagues in the Faculty of Science. For him, as well as for the Institute of Chemical Engineering of the University of Hanover, it is a day of honour. Those involved in chemical and biological process engineering will join in our congratulations to him on the day, and in our best wishes to him for a happy future.

Karl Schügerl has built up an exemplary career, which began after he completed his chemical engineering studies at Budapest University in 1949. Since that time he has continued to devote himself to research, either in industry or at university. The first steps in his career were in organic chemistry at Budapest University (1949–52); experimental institutions in the organic-chemical industry, Budapest (1952–55); a design studio for bioplant (1955–56). and at Riedel de Haen AG in Seelze, Germany (1956–58). He later completed his PhD degree at Hanover University in the kinetics and rheology of fluid bed systems and spent three years of post-doctorate work at the Universities of New York (high-temperature pyrolysis of hydrocarbons) and Princeton in the USA, where he was closely involved for one and a half years in fundamental investigations of molecular beams for aerospace research. Soon after his return to Germany, he qualified for inauguration as a lecturer in Hanover and shortly afterwards accepted a professorship in process engineering at Braunschweig University. In 1969, he finally returned to Hanover University. In due course, the purposeful nature of his work developed the Institute for Chemical Engineering into a research institute concentrating on biological process engineering. His early work on high-temperature pyrolysis of olefins and on molecular beam ultrasonic applications opened up typical problem areas relating to mass transfer in chemical processes. Hydrodynamics in single drops, three-phase

fluid beds, cryosorption, residence time distributions, test material mixing, and flash photolysis were typical fields of work in the 1960s and 1970s. Hundreds of experimental publications bear witness to his comprehensive knowledge in these fields. The urge towards constantly new fields of work led Karl Schügerl in 1975 into biotechnology, in which today his institute occupies a dominant position. It has taken a leading role particularly in on-line measurement and control of biological process engineering. Karl Schügerl was one of the first chemical engineers in Germany to conduct extensive analyses of biological aspects. These also include hundreds of papers in the biological field which omit hardly any of the major measuring techniques or process applications. Bacteria, yeasts or fungi are the main agents in this highly developed discipline. New solutions to the problems of future improvements in performance are sought using the most modern methods of analysis and software. His present areas of interest have recently even extended beyond biological process engineering and today cover environmental technology, biosensors, the disposal of highly polluted effluent, soil renewal and hydrometallurgy.

Karl Schügerl has always refused illustrious offers from renowned universities, thereby dedicating himself to developments in Hanover; this was certainly the result of his important recognition that only determined efforts would lead to progress in the rapidly developing field of biotechnology.

It would seem significant in this connection that he willingly accepts many calls on his time to act as consultant and expert. He acts as adviser to countless institutions both in Germany and abroad in the academic and public domain, where his reliable advice and support are highly esteemed. Between 1982 and 1986, he administered the department for biological process engineering at the GBF (Association for Biotechnological Research) in Braunschweig.

Today, 700 publications, including five books, bear witness to the iron discipline by which Karl Schügerl has developed his view of things. Nevertheless he has remained an approachable and cherished colleague, who always finds time for scientific requests. This also holds true for the next generation of scientists for whom he not only proposes attractive subject areas, but also provides support as a helpful adviser in professional and personal problems. There are many of his former PhD students who today are themselves in academic positions,

and who are able to pass on the enthusiasm for science of their former teacher to the coming generations. In industry too, graduates from Hanover have become successful and, with their knowledge and skill, are able to support their companies in these times of stiff economic competition.

Recently, the academic and scientific services of our honoured colleague have been recognized by Budapest University with an honorary doctorate.

This present publication allows the authors to add their appreciation. Their contributions demonstrate the wide interests of Karl Schügerl, which cover many subsidiary fields. We hope in this way to give him great pleasure and thereby to demonstrate the high esteem in which we hold him.

Those who are familiar with the great creative powers of this gifted scientist cannot imagine any slackening of his efforts in the promotion of research and education. We offer our good wishes for his health and the best conditions for the accomplishment of his plans.

Armin Fiechter

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SEND ⟨*filename*⟩	invokes a message with the file "filename",
GET ⟨*filename*⟩	same as "SEND".

Table of Contents

Artificial Neural Networks in Bioprocess State Estimation

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The application of artificial neural networks to the estimation and prediction of bioprocess variables is presented in this paper. A neural network methodology is discussed, which uses environmental and physiological information available from on-line sensors, to estimate concentration of species in the bioreactor. Two case studies are presented, both based on the ethanol production by *Zymomonas mobilis*. An efficient optimization algorithm which reduces the number of iterations required for convergence is proposed. Results are presented for different training sets and different training methodologies. It is shown that the neural network estimator provides good on-line bioprocess state estimations.

Advances in Biochemical Engineering
Biotechnology, Vol. 46
Managing Editor: A. Fiechter

List of Symbols

In Z. mobilis Kinetic Model:

a		Power of the ethanol inhibition term in μ
b		Power of the ethanol inhibition term in q_p
D	h^{-1}	Dilution rate
F	$l\,h^{-1}$	Permeate flow
F_0	$l\,h^{-1}$	Total feed flow to the system
K_i	$g\,l^{-1}$	Substrate inhibition constant for growth
K_i'	$g\,l^{-1}$	Substrate inhibition constant for ethanol production
K_s	$g\,l^{-1}$	Monod kinetic constant
K_s'	$g\,l^{-1}$	Saturation constant for q_p
p	$g\,l^{-1}$	Ethanol concentration
P_i'	$g\,l^{-1}$	Ethanol threshold concentration for ethanol production
P_m	$g\,l^{-1}$	Maximum ethanol concentration for cell growth
P_m'	$g\,l^{-1}$	Maximum ethanol concentration for ethanol production
q_p	$g\,g^{-1}\,h^{-1}$	Specific ethanol production rate
q_{pm}	$g\,g^{-1}\,h^{-1}$	Maximum specific ethanol production rate
R		Recycle ratio
s	$g\,l^{-1}$	Glucose concentration
S_i	$g\,l^{-1}$	Threshold substrate concentration for cell growth
s_o	$g\,l^{-1}$	Glucose concentration in the feed stream
x	$g\,l^{-1}$	Biomass concentration
x_{max}	$g\,l^{-1}$	Maximum cell concentration
$Y_{p/s}$	$g\,g^{-1}$	Ethanol yield, g ethanol per g substrate consumed
μ	h^{-1}	Specific growth rate
μ_{mo}	h^{-1}	Maximum specific growth rate at zero ethanol concentrations

In Neural Network Theory:

$\mathbf{d}_p$	L-dimensional target vector
E_p	Sum-of-squares error for training example p
$E(\mathbf{w})$	Total sum-of-squares error for all input patterns, function of weight vector $\mathbf{w}$
$\mathbf{G}^{(q)}$	Vector of steepest descent directions in iteration (q)
$\mathbf{g}_p^{(q)}$	Gradient vector of one input-output pattern p
$g_{uv}^{(q)}$	Element of the gradient vector $\mathbf{g}_p^{(q)}$
n	Number of interconnection weights in the network
O_{pj}	Output of neuron j from the training set p
p	number of training pattern
q	number of iteration
$\mathbf{r}^{(q)}$	Vector of conjugate gradient directions in Eq. (17)
S_{pj}	Activation state of neuron j from the training set p
$\mathbf{S}^{(q)}$	Vector of search directions for conjugate gradient algorithm
$\mathbf{w}^{(q)}$	Vector of neural network weights in iteration q

w_{ij} Interconnection weight from node i to node j
$\mathbf{x}_p$ N-dimensional network input vector
$\mathbf{y}_p$ L-dimensional network output vector
$\mathbf{z}^{(q)}$ Vector of conjugate gradient directions in Eq. (16)
$\alpha^{(q)}$ Step size in iteration q used in Eq. (8)
$\beta^{(q)}$ Step size in iteration q used in Eq. (17)
$\gamma^{(q)}$ Step size in iteration q used in Eq. (16)
δ_{pv} Change in E_p due to changes in neural activation state S_{pu}.
σ Argument of the sigmoid function.

1 Introduction

The successful operation, control and optimization of bioprocesses rely heavily on the availability of a fast and accurate evaluation of the system performance. This in turn requires reliable real-time process variable information. Direct on-line measurements of primary process variables, such as biomass, substrate and product concentrations, usually are unavailable. Most industrial bioprocess control policies are based upon the use of infrequent off-line sample analysis, leading to poor process operability and regulation. The state of the cultivation, therefore, has to be inferred from measurements of secondary variables and any previous knowledge of the process dynamics.

In recent years, several techniques have been developed for indirect estimation of process variables and data analysis of biological systems. Approaches range from statistical methods, including linear and nonlinear regression, to artificial intelligence techniques, for determination of rules for expert systems from on-line data. Estimation techniques generally are used to predict unmeasurable process variables, and/or to identify process model parameters, by establishing the structure of a process model. However, it is well-known that models of real nonlinear systems possess a great amount of uncertainty. This is due in part to imprecise measuring devices, environmentally dependent system parameters, or disturbances inherent in the plant. However, the most important cause of process/model mismatch is perhaps our incomplete knowledge of the system dynamics. The quality of the estimation depends greatly on the depth of understanding of the process. Methods which can provide an adequate estimation of process states and parameters in spite of incomplete process knowledge could be successfully applied in control and optimization of bioprocesses.

The use of artificial neural nets for identification and control of chemical and biochemical plants has recently been the focus of some research groups [1, 2, 3, 4, 5]. Artificial neural nets are highly interconnected networks of non-linear processing units arranged in layers and having adjustable connection strengths (weights) [6]. Neural networks can approximate large classes of nonlinear functions by changing the strengths of the connections on the links, a procedure which is

called *learning*. Neural networks are well-known for their ability to adjust dynamically to environmental changes, to perform useful generalization from specific examples, and to find relevant regularities in the data. Recent applications of neural nets have been mostly in the areas of speech recognition, image processing, optimization problems, robotics, decision making, and identification and control. However, no non-trivial experimental validation of state estimation in biological systems using neural networks has been reported in the literature.

In this paper, we will present some applications of artificial neural networks to the estimation and prediction of bioprocess variables. Biological kinetic models seldom include the influence of environmental factors, like pH and temperature. The development of quantitative models which simulate how cells respond to various environmental changes, will help in better utilizing the chemical synthesis capabilities of the cells. We will discuss a neural network methodology which will use environmental and physiological information available from on-line sensors, to estimate concentrations of species in the bioreactor.

Two case studies will be presented, both based on ethanol production by *Zymomonas mobilis*. Yeasts and bacteria have been successfully used in the past for bulk production of ethanol. Reported data on ethanol productivities for yeasts and *Z. mobilis* have demonstrated that *Z. mobilis* is superior to yeasts as far as productivity is concerned [7]. Both the specific ethanol productivity and specific glucose uptake rate are several times larger in *Z. mobilis* than in yeasts; this is associated with lower levels of biomass formation during the cultivation. Given the obvious advantages of *Z. mobilis* for industrial ethanol production, it is worthwhile to investigate ways of improving the bioprocess evaluation, by means of better process variable predictions. Neural networks applied in this context will demonstrate to be a powerful tool for identification and control in biological systems.

2 Estimation Methods in Bioprocesses

In terms of control and optimization, bioprocesses are not more complex or unique than any other chemical process. However, species are ill-defined and interact, and the kinetics is poorly understood [8]. Both process and disturbance dynamics are uncertain and variable. This lack of knowledge of the underlying principles, together with the scarcity of appropriate biosensors, is what makes the identification procedure so difficult.

Different estimation-filtering techniques have been developed to aid in the prediction of the biological and physicochemical parameters needed for control and regulation. Identification techniques may be divided into *parameter estimation* and *functional estimation* [9]. The parameter estimation approach is simple, if one can safely assume a known system function with only unknown parameters. Functional estimation deals with the estimation of the system function, as well as its parameters.

Model-based estimation methods, are parameter estimation techniques, where usually simplified nonlinear kinetic models and/or material balance equations [10,

11] are defined. This approach is useful in cases when all the species involved are known and their elemental composition is completely defined and time-invariant. Otherwise, changes in growth rates or in the nature and composition of the medium may affect the chemical composition of the cells [12], invalidating the process description.

Other parameter estimation techniques include the least-squares fitting of linear, time series models, valid only in a certain operating range. The success of this approach depends on the match between the actual system dynamics and the linearized approximation. Adaptation can provide some improvement in the estimation, but in general, these models do not represent the dynamic nonlinear behavior of the process accurately. Several examples of this approach exist in the literature [13, 14, 15].

Stephanopoulos and San [16] integrated the concept of material balance with estimation using a Kalman filter, for the simultaneous state and parameter estimation of a bioprocess system. Kalman filtering is a technique for estimating the underlying trend in noisy measured data. Due to the iterative nature of the filter, an inaccesible state variable can be estimated via an imperfect process model [8]. An extension to the Kalman filtering technique involves the use of nonlinear models and their subsequent linearization. Several applications of the Extended Kalman Filter (EKF) for state estimation in biotechnological processes have been reported [17, 18, 19]. However, in spite of the apparent success of this widely used estimation technique, care should be exercised due to its inherent limitations. Assumptions of the EKF include the use of a linear process model (or a linearized version of a nonlinear model), previous knowledge of the covariance matrices of the state and output noise, and a good initial value for the filter covariance. Sensitivity problems in biological systems due to deviations from this assumptions may cause the performance of the estimator to deteriorate, resulting in biased estimates of the states.

Since the biochemical process is non-linear, a better estimation is to be expected from exploiting a nonlinear structure for designing the estimator. Bastin and Dochain [20] applied asymptotic observers for the estimation of specific growth rate. These are a class of estimators that can handle nonlinearities in the process model. Improvements to this algorithm have been proposed [21], but two main disadvantages remain: high sensitivity to wrong initial guess of the biomass concentration and the use of off-line measurements to define the state of the process.

Therefore, it appears that a major improvement in bioprocess control and optimization can be made if one has an estimation algorithm which can effectively reduce the off-line analysis frequency, while maintaining the quality of the information available.

3 Overview of Neural Networks

Neural networks offer the opportunity to directly model nonlinear processes, and estimate or predict the values of relevant process variables. Artificial Neural Networks (ANNs) models consist of a large number of simple interconnected

nonlinear computational elements *(neurons)*. The strength of the links between nodes is typically adapted during use to improve performance. Neural networks have received wide interest during the last years due to their abilities of learning, generalization and abstraction.

At present, in chemical and biochemical engineering applications, the most widely used neural net training method is *backpropagation*, a generalization of the Least Mean Square Error algorithm. Backpropagation uses an iterative gradient search technique to minimize a performance function, equal to the mean square difference between the desired and the actual net outputs. Several applications of ANNs trained by backpropagation have been reported in the literature.

ANNs have been applied as a method for identifying regular patterns in data. Qian et al. [22] presented a new method for predicting the secondary structure of globular proteins based on nonlinear neural network models. Network models learned from existing protein structures how to predict the secondary structure of local sequences of amino acids. The use of this technique provided a significant improvement over existing methods for nonhomologous proteins. In another application, McAvoy et al. [23] used neural nets to deconvolute fluorescence spectra obtained from solutions of amino acid fluorophores. They showed that neural nets are superior to standard linear techniques in estimating the solution concentrations, due to the adequate handling of the nonlinearities in the spectral data.

Neural networks are well-suited to learn and retrieve correlations between measurements and faults or responses, and are particularly useful when the measurements are incomplete or inaccurate [2, 24]. A technique for the application of neural nets for diagnosing process failures during process transients has been recently investigated [3]. The symptom patterns were presented to the network in two ways: by using a raw time-series process sensor data and by using a moving average value of the same time-series data. Both methods were successful in detecting simple and interactive faults, even when they were trained only in single fault cases. This demonstrates the neural networks ability for generalization.

Other recent research deals with the feasibility of using neural networks for estimation and control of nonlinear plants. Bhat et al. [5] investigated the use of neural nets trained by backpropagation to estimate and control the variables of a simulated pH CSTR. They extended the backpropagation approach to enable the dynamic modeling of a nonlinear process by including past values of process variables as inputs to the network. Their results showed a better overall estimation compared to traditional linear ARMA (autoregressive moving average) modeling. This approach, using a *temporal* or *sliding window*, has also been investigated by other researchers [25, 26, 27]. Since only simulation analysis were performed, it was assumed that all the required process variables were available on-line, an assumption which is not always valid. One of the disadvantages of using *temporal windows* is that process delays should be known so that an adequate number of past observations can be used, in order to include the influence of all input variations on the prediction of the outputs.

Adaptive learning is one of the most attractive features of neural networks, since they learn how to perform certain tasks by undergoing training with