

An auto-regulating expert system strategy for dynamic intelligent system selection for on-line optimization of a bioprocess

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Mathematical models derived through mechanistic information and validated under laboratory conditions do not always portray satisfactorily the behavior of complex microbial processes under realistic conditions. Models based on artificial intelligence (AI) then offer a viable alternative. However, AI models also have limitations, and sometimes different AI methods may be the most effective at different points in time or in different regions of the operating space. The present communication, therefore, presents a supervisory expert system that receives performance data continually and selects that AI module from an on-line library, which maximizes a specified performance index. This ensures that the most efficient AI system is functional at all times. The concept is presently being applied to glucoamylase production in continuous cultivation of a recombinant strain of *Saccharomyces cerevisiae*, and initial results support its feasibility and effectiveness.

Keywords: Artificial intelligence, *Saccharomyces cerevisiae* cultivation, microbial processes, supervisory expert system

Introduction

Living cells often respond to environmental conditions that are often difficult to characterize and describe quantitatively. Nevertheless, since bioprocesses functioning under realistic conditions have to be quantitatively monitored, optimized and controlled, a common approach is to redjust models based on laboratory-scale data to make them (approximately) suitable for real conditions.

Classical mathematical models developed and up-scaled in this manner are not always acceptable, especially when the cells are subject to incomplete fluid mixing, noise from within the cells and from outside, and the presence of complex substrates. The limitations of such attempts have been exposed in a number of studies. Even for a simple cell growth process with one type of cells and one substrate, Ramkrishna¹ showed that the standard Monod equation “fails to describe the drop in biomass concentration as the dilution rate is lowered”. Similar weaknesses when an organism has a choice of two or more substrates have been discussed by Gadkar *et al*².

The presence of noise in a cellular process renders equation-oriented models even more inadequate. Even if the extra-cellular environment is noise-free, the metabolic processes inside living cells are themselves

stochastic in nature^{3,4}. This stochasticity, coupled with the effects of environmental noise, limits the suitability of laboratory-tested mathematical models for complex cellular processes^{5,6}.

Under these circumstances, a common (and reasonable) approach is to describe, optimize and control microbial processes through methods that are independent of mathematical approximations. Methods utilizing artificial intelligence (AI) offer this possibility. They do not depend on phenomenological modeling, they are designed on actual performance data, and they can evolve and improve with usage. Some AI methods commonly applied to cellular processes, especially bioreactors, are overviewed in the next section, followed by a brief discussion of their weaknesses, possible remedies and the conceptual AI framework proposed here.

AI for Cellular Processes

Different sources have defined AI in different ways. One comprehensive definition⁷ states that AI is an area of “computer science that is concerned with the development of computers (and software) able to engage in human-like thought processes, such as, learning, reasoning and self-correction”. The salient feature that differentiates AI from mechanistic models is thus the ability to mimic human thinking. This makes AI suitable for cellular processes because living cells are known to be capable of independent decisions⁸.

Fuzzy Logic

Fuzzy logic employs descriptors and rules to characterize processes that have imprecise or uncertain (“fuzzy”) features. The fuzziness implies that the set of data describing these features does not have a crisp, clearly defined boundary. However, since crisp numbers are required in order to act on the data, fuzzy logic employs “membership functions” and Boolean logic to translate fuzzy inferences into equivalent precise outputs for practical decisions. Applications to sake brewing⁹, alkaline protease synthesis by *Bacillus subtilis*¹⁰ and penicillin G productions by *B. megaterium*¹¹ illustrate the utility of fuzzy logic.

Neural Networks

The structure of neural networks is directly inspired by arrays of neurons in the brain. A neural network comprises an array of information processing elements called neurons. Different designs differ in the number of neurons, their arrangement and how the neurons process incoming information. All neural networks contain a set of input neurons, a set of output neurons, and one or more hidden neurons sandwiched between these two layers. In some cases the outgoing signals from one layer of neurons may be fed back to a proceeding layer; the recycled signals may either be transmitted as such or processed again by so-called recurrent neurons.

The theory and chemical engineering applications of neural networks have been adequately covered in other publications^{12,13}. Biotechnology applications include *Kluyveromyces fragilis* fermentation in fed batch culture¹⁴, on-line monitoring of a noise-affected fermentation by *B. megaterium*¹⁵ and optimization of PCB production by a mixed culture of *Ralstonia entrophia* and *Lactobacillus delbrueckii*¹⁶.

Genetic Algorithms

Genetic algorithms (GA) qualify as an AI method since their methodology and terminology are borrowed from the natural selection literature. Like classical optimization methods, GA determine the optimal solutions by search techniques. However, they differ from classical optimization in that the initial solutions are random numbers, the search techniques are stochastic, and the starting solutions undergo probabilistic transformations that lead eventually to the optimal solution. Each solution is encoded as bit strings called *chromosomes*, the position of each bit in a string is called a *gene* and the entire set of strings constitutes a *population*. Three

basic steps are involved in the evolution of each intermediate solution from the proceeding one: reproduction, cross-over and mutation¹⁷.

A good number of applications of GA to microbial systems have established their credibility. Two recent applications are Patnaik's¹⁸ use of GA to optimize the PHB fermentation process studied earlier¹⁶ through neural networks, and the biotransformation of crude polydatin to resveratrol by *Aspergillus niger* AN-2436¹⁹.

Expert Systems

Like fuzzy logic, expert systems also rely on rule-based learning. However, they have a more elaborate topology and wider applications. Typically, an expert system contains: (a) a knowledge base editor, (b) an explanation system and (c) an inference engine. These three interact with the user on one side and with problem-specific data on the other side. The inference engine reasons with the knowledge base and with the data to formulate and apply an appropriate set of rules. Since this reasoning is in coded terminology, the explanation system deciphers the codes and explains them to the user. The knowledge base editor helps the experts to update and check the knowledge base continually.

The complexity of waste-water treatment processes makes them good candidates to apply expert systems, and numerous applications confirm their effectiveness^{20,21}. Expert systems have been effective in other applications too, such as, the cultivation of desulfurizing cells of *Rhodococcus erythropolis* for petroleum refining²². The versatility of expert systems enables them to be used in supervisory functions to control the performance of other AI methods.

Limitations of AI Methods

Even though AI techniques circumvent the rigidity and rigorous requirements of mathematical equations, they are not without weakness and limitations. Fuzzy logic, for example, relies on the ability to formulate “correct” membership functions and rule bases to generate crisp, usable numbers from fuzzy data and inferences. For complex biological processes, neither the appropriate membership functions nor all the relevant rules are obvious, and sometimes two or more functions may perform comparably well within the uncertainties of the data²³.

In spite (or perhaps because) of having been designed by analogy with the neural cells of the brain, neural networks are not fool-proof devices. One major

weakness is the large amounts of data often required to train a network adequately. Another limitation is that as with fuzzy logic, more than one design may represent the data equally well, and the optimum number of neurons is a trade-off between predictive capability and the avoidance of imbibing spurious features of a process in addition to the relevant features. Being “black box” devices, neural networks have poor extrapolation capability. These kinds of weaknesses get exacerbated with data corrupted by noise, as in many real processes^{12,24}.

A major difficulty in a genetic algorithm is the choice and proper representation of the *fitness function*. This function is roughly the equivalent of an objective function in that both determine the suitability of a solution. This apart, the choice of other parameters, such as, the population size, the rates of mutation and cross-over, and the type and strength of selection, are not always straightforward. In particular, one ought to be wary of “deceptive” fitness functions, which provide misleading information about where the optimum solution is likely to be found. Another well-known problem is that of *premature convergence*. This can occur if an early solution is so much more fit than its competitors that it narrows down the diversity of the population too soon, resulting in convergence to a local optimum instead of the global optimum^{17,25}.

Even though an expert system operates on the basis of knowledge provided by human experts, it lacks the decision making capabilities, creative responses and intuitive abilities of a human being. Since the system relies on a knowledge base, it is important that this base be maintained up-to-date. Nevertheless, the advantages of expert systems often outweigh their disadvantages, thus justifying their wide usage for complex (biological) problems^{26,27}.

Composite AI Designs and Conceptual Generalization

One method to minimize the weaknesses of individual AI techniques and optimize their advantages is to use a combination of two or more AI techniques. The most common combination of AI systems for bioprocesses seems to be fuzzy logic with neural networks or expert systems. In one of the early studies, van Can *et al*²⁸ used a neural network in series with macroscopic balance equations for the biosynthesis of penicillin G from 6-aminopenicillanic acid and phenyl acetic acid. Later work by Galvanauskas and coworkers²⁹ expanded this idea to investigate different combinations of serial and

parallel components of AI modules and mathematical equations. Yet another variation was that of Zuo *et al*³⁰, whose work pertained to an airlift bioreactor with four hydrodynamically different regions to maximize bacterial cellulose production.

These authors did not incorporate fuzzy logic because they did not consider noise and uncertainties in the variables. Hanai *et al*⁹ included these aspects for sake brewing through fuzzy neural networks. So did Punal *et al*²⁰ and Cakmakci³¹ for waste-water treatment; the former combined fuzzy logic with an expert system and the latter with an adaptive neural network. Simple combinations of AI modules in series or in parallel cannot, however, portray all the important features of complex cellular process. This was illustrated by Patnaik³², who had to employ fuzzy logic, two kinds of neural networks and a PID controller in tandem to optimize *Sacharomyces cerevisiae* cultivation with oscillating outputs.

Arrays of multiple AI systems are however not easy to design because little is known on how to evolve the best architecture systematically. Since reasonably simple models are practically desirable for these so-called hybrid systems, it becomes necessary to lump many of the complex details of a multi-cellular processes. As a result, more than one hybrid architecture may represent and regulate a process comparably well and evolving the best design is largely problem-specific and heuristic. The fed-batch production of poly- β -hydroxybutyrate by *R. entrophia*³³ illustrates these limitations.

Since no single AI model or a defined combination of AI systems is always the best for a class of bioprocesses or even for a particular process under different conditions, a conceptual strategy to implement the best AI method at all times is to design AI framework that continually evaluates a library of modules and selects at different points in time the particular module that generates the best performance. This concept is represented schematically in the information flow diagrams of Figs 1 and 2. Fig. 1 is essentially a topological representation of an expert system, whose main components have been explained in section ‘Expert Systems’. The expert system functions here as an evaluation and selection routine that receives performance data during successive intervals of time, distributes them to the other AI modules, evaluates their relative optimized predictions in terms of a specified performance index and selects the best AI strategy during each time interval. By analogy with supervisory control

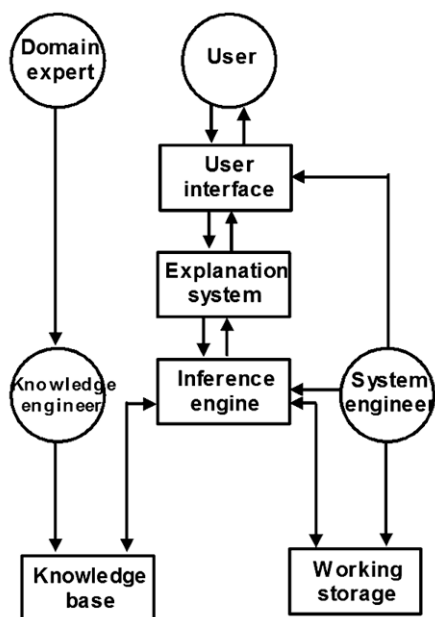


Fig. 1—Schematic representation of an expert system for a bioprocess.

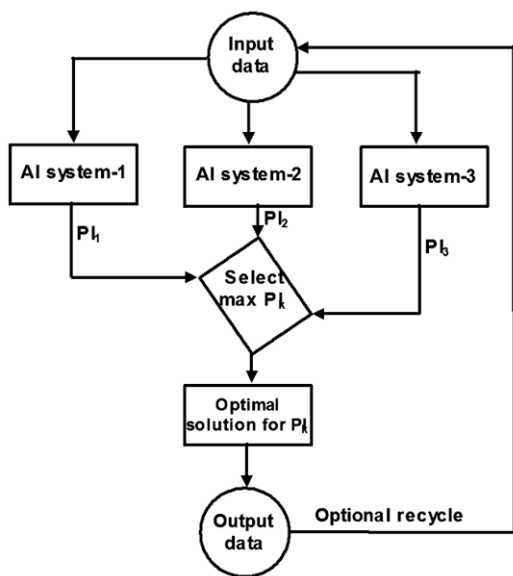


Fig. 2—Detailed architecture of the inference engine.

systems³⁴, the expert system functions as a master that decides how the other slave AI systems will operate from time to time.

The heart of this optimization strategy, in the present context, is the inference engine, which generates the optimal decisions of the expert system; its proposed architecture is depicted in Fig. 2. Three AI systems have been shown here as an example. This number may, however, change according to the extent of

search required for a particular application. Each AI module receives the same input data and generates during each succeeding interval of time an optimal performance index PI .

The optimal performance index for each AI module may typically be the concentration of a desired product or the yield of biomass. These indexes, PI_1 , PI_2 and PI_3 in Fig. 2, are compared and the AI system then selects the highest PI_k for a given interval of time; the k -th AI method, corresponding to this PI_k , is then implemented over the next time slot. This process is carried sequentially across successive intervals of time by a forward marching process.

Two aspects of this conceptual design may be highlighted. One is that since different AI methods may be the best during different intervals of time, the overall performance of the bioprocess is likely to be superior to that generated by a fixed AI strategy. Results from Galvanauskas *et al*²⁹ and Patnaik³³ support this inference. The second aspect pertains to the forward marching technique itself, which has been applied successfully in many previous studies^{6,16,35} covering different microbial systems, thus establishing its feasibility and effectiveness.

The conceptual framework embodied in Figs 1 and 2 is presently being applied to a continuous flow bioreactor with immobilized recombinant *S. cerevisiae* synthesizing glucoamylase from glucose as the carbon substrate. This system was studied recently by Schapper *et al*³⁶, who compared a reactor with homogeneously distributed pellets with one that had a structurally optimized nonuniform distribution. Further details are available in their publication. The present application combines fuzzy logic with an array of neural networks, similar to that employed by previous studies^{15,16,33,35}, and a genetic algorithm as the third choice. This design thus extends the format of Fig. 1 by considering one of the AI systems to be not a single neural network but a committee of networks. Initial results for the best two optimization methods are compared in Fig. 3 as time-domain plots of the glucoamylase output rate for: (i) a low concentration of 5 g glucose/L, and (ii) a 20-fold higher concentration in the feed stream. The exact values at the steady state are summarized in Table 1. These results show that while Schapper *et al*³⁶ achieved substantial increases in glucoamylase productivity by a nonuniform distribution itself, further significant improvements are seen to be possible through a judicious application of AI methods. The analysis is

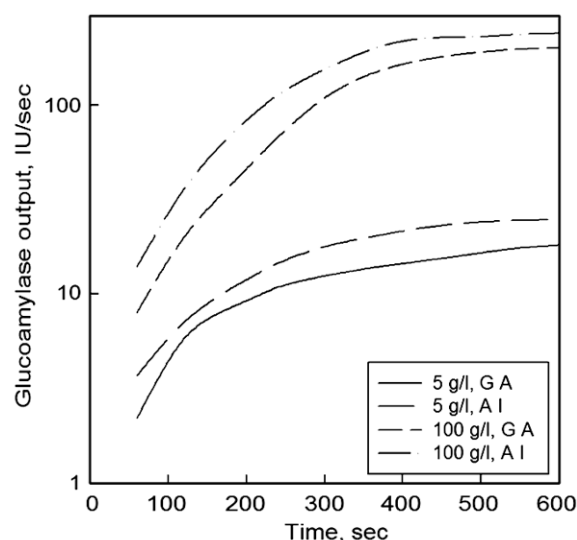


Fig. 3—Comparison of the time-domain plots of the glucoamylase output determined by the best two optimization methods. The numbers in the legends refer to glucose concentration in the feed; GA = genetic algorithm, AI = expert system based on artificial intelligence method.

Table 1—Comparison of the optimized glucoamylase productivities by recombinant *S. cerevisiae* under different conditions.

Glucose feed conc. mg/L	Glucoamylase flow at the reactor outlet, IU/sec				
	Homogeneous reactor	Structurally optimized	Neural networks	Genetic algorithm	Composite AI system
5	4.1	12.9	16.7	18.2	24.8
100	17.6	170.3	195.8	202.4	241.5

continuing and the complete results will be presented in detail in a later article. The aim of the present communication was to establish the concept that an expert system can intelligently select the best AI method, or a combination of methods, to optimize a complex bioprocess better than mathematical optimization or a stand-alone AI method.

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