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Artificial Intelligence as a Tool for Automatic State Estimation and Control of Bioreactors

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ABSTRACT: Microbial fermentations in real situations are difficult to monitor on-line or describe by mathematical models. This limits the applicability of model-based control under variable conditions. Methods using artificial intelligence (AI) have been very useful in this context. Their strength lies in their ability to learn from process performance, derive inferences akin to human reasoning and anticipate problems. Artificial neural networks, fuzzy logic and expert systems are common forms of AI for bioreactors. Often two or more of them have to be employed together for optimal performance. Sometimes AI is also used in conjunction with mathematical models and/or operations research techniques. With the right combination of AI and modelling, on-line estimation and control of complex fermentation processes can be done more rapidly, accurately and non-invasively than by instrumental or mathematical techniques and conventional control. © 1997 John Wiley & Sons, Inc. Lab Robotics and Automation **9:** 297–304, 1997

INTRODUCTION

Microbial processes are complex in their physiology and performance. For ease of analysis, the complexities may be considered to occur at two levels. One is the microscopic level, which refers to processes inside individual cells; the other level is macroscopic and more easily discernible, and it relates to the interactions between the cells and their environment, i.e., the extra-cellular fluid.

The term "interaction" here is singularly important because the observed behavior of a fermentation process depends strongly on intracellular kinetics, transport across the cell walls, movements of the cells, and spatial heterogeneity in the bioreactor. All of these impose restrictions on the accuracy and detail of measurement and modeling possible. Some of these aspects have been discussed by different contributors to a book edited by Schugerl [1].

In addition to being complex, microbial processes are sensitive to imperfections and disturbances. While this is understandable for living cells from a cybernetic point of view [2], it also exposes the limitations of measurement techniques. Even a basic macroscopic variable such as the biomass concentration cannot yet be measured easily [3] without disturbing the broth. For intracellular variables and even other extracellular variables such as proteins and nutrients, this problem is much more difficult.

Therefore, many important variables of a fermentation are measured by drawing samples and analyzing them off-line. This method, however, has limi-

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tations that increase with the scale of operation and the sensitivity of the process [4], thus making them unsuitable for industrial use. However, large bioreactors are precisely the ones that require good, fast, noninvasive sensors and robust but accurate control because of their susceptibility to disturbances and the large investment for each batch of product. These limitations and the complexity of biological processes, specially in the noisy interactive environment of industrial fermentation, makes it difficult to propose mathematical models that are sufficiently accurate, robust, and simple to permit easy automation and fast responses to the abrupt changes possible due to disturbances and failures [5, 6].

Therefore, the last decade has seen a rapid transition from hardware sensors and algorithmic control to methods based on artificial intelligence built into a "soft" controller. This article provides an overview of the subject, how it has been applied to bioreactors, some important applications and the insight they offer about design, and limitations of artificial intelligence systems.

AI AND EXPERT SYSTEMS

Although artificial intelligence (AI) was developed more than 40 years ago, it has achieved significant application to biotechnological processes only in the past decade. While AI encompasses many other areas such as natural language, robotics, and vision, biotechnology applications have utilized mainly three kinds of AI systems: knowledge-based expert systems (ESs), fuzzy logic (FL), and artificial neural networks (ANN).

Expert systems are essentially computer programs that can use information about a process and make "intelligent" decisions as a human expert would. The advantage of ESs over algorithmic methods is that they can operate even on imprecise data and deduce relationships and consequences for situations not covered by the data. The ability to use inaccurate data, or even incomplete data, is of singular merit because observations of real processes are often corrupted by extraneous factors.

Deduction capability, a key aspect of AI, implies that all ES programs have a formalism of logic and reasoning. Most expert systems are based on Bayesian reasoning and fuzzy logic as these are naturally inclined to accept imprecise data and can also deduce qualitative relationships. It should be clarified that Bayesian theory is not restricted to fuzzy relationships; it represents a form of reasoning that is suitable for the stochastic nature of real-time biological processes. Fuzzy logic forms a part of many ESs because the data of practically useful processes are imprecise or corrupted or qualitative. Zadeh [7] has dealt extensively with the application of fuzzy logic in handling uncertainties in ESs.

Halme and Karim [8] define two ways of reasoning employed by an ES—backward or forward changing of logical connections. In backward chaining, the system tries to answer whether a logical statement concerning the behavior of a process is true or false and why it is so. In forward chaining, the objective is to determine all logical consequences of a particular assertion about a process. Because biological processes are complex, and in a bioreactor they interact with nonbiological features of the flow and mixing [9], in most applications both kinds of reasoning are required.

Similarly, while ES are a major component of AI applications to bioreactors, the other two kinds of AI are also significant, and often a combination of them provides the best solution [10]. ANNs and their applications in biotechnology have been reviewed by Montague and Morris [11]. In an ANN, a number of processing elements (neurons) are connected so as to mimic the flow of signals through the neural cells of the brain. Fuzzy logic aims to process the fuzzy formulations and relationships that are normally used by numans. Rather than precise Yes/No statements about the truth of an assertion, as in deterministic logic, fuzzy logic allows partial degrees of truth through memberships of fuzzy sets. This accommodates subjective reasoning and uncertainties in data. Just as both forward and backward chaining occur in most ESs, so also ESs, FL, and ANN are often combined to portray the performance of a biological system. These connections were discussed by Linko [12] during the early phase of their applications. More recently, Stephanopoulos and Han [13] have placed these three categories of AI in the general framework of intelligent systems for process engineering.

AI FOR BIOREACTORS

The complexity of metabolic processes and their associations with the hydrodynamics of the broth in bioreactors makes it difficult to formulate suitable mathematical models for a realistic situation. Conventional control is thus usually based on corrections to idealized models. AI circumvents this limitation by relying minimally on mathematical models for some data and drawing inferences not through a model but directly from bioreactor performance.

Stephanopoulos and Stephanopoulos [14] have succintly described five key features of biochemical processes that should be considered in the design of AI-based software.

1. The Multiobjective Character of the Problems
Optimal operation of fermentation process often requires a compromise between a number of conflicting objectives. For instance, the large-scale production of therapeutic or diagnostic proteins by recombinant microorganisms requires high cell density, high yield per unit mass of cells, low sensitivity, good stability, and low production cost.

2. The Need to Use Knowledge from Different Domains
Mammalian cell culture is a good example of this. It requires knowledge of molecular biology, reaction engineering, fermentation, and process control. A model that includes all these aspects will be extremely cumbersome.

3. The Need to Articulate Required Expertise
Since real situations are difficult to define exactly and change with time, the formulation and solution of a bioreactor problem often cannot be precise and have to adapt to changing situations. Unlike mathematical models, intelligent systems possess this ability.

4. The Need for Disciplined Use of Assumptions
Any AI method must function on the basis of certain assumptions about the process. However, the assumptions may have to be modified during the course of a process, and this is where AI capability becomes important. For example, the entry of a contaminant or the tripping of a pump will affect the kinetics or the hydrodynamics.

5. The Need to Employ Models and Quantitative Information
Sometimes an AI system may have to be combined with analytical models of some aspects of a process. It may, for instance, be easier to provide equations for cell growth rates and physico-chemical properties rather than have them estimated by an ESs. How much of analytical information is to be combined with an ESs is an important consideration.

With these features included, AI software for bioreactors may perform one or more of the following tasks [8, 15]:

- checking overall process conditions,
- identifying and localizing faults in instruments and equipment,
- clearing alarm blocks in start-up/shut-down operations,
- determining the optimum operating conditions under different situations,
- detecting abnormalities in a fermentation,
- predicting the most suitable harvest time,
- on-line estimation of important properties that are difficult or hazardous to measure,
- supervision of conventional control, and
- optimal scheduling of plant operations.

Although no single AI application will perform all these functions, it is instructive to view how they are interconnected in bioreactor operation. Figure 1 displays a typical multifunctional ES, that was applied to batch fermentations with genetically engineered *Bacillus subtilis* strains producing α-amylase [8]. Note that the box labeled “Expert System” is the core of the software package but not the only AI component. Although Halme and Karim [8] have not provided details, it is conceivable that fault detection and analysis are done by neural networks, and system identification, optimization, and simulation, through fuzzy logic. The bottom row of boxes contain functions performed by conventional sensors and controllers, thus illustrating the remark made earlier that the best configuration may well be a combination of different kinds of AI software with classical estimations and control. Such hybrid systems are becoming increasingly popular, and some applications are discussed later.

ILLUSTRATIVE APPLICATIONS

Fuzzy Logic

Japanese schools appear to be at the forefront of research in fuzzy estimations and control. As early as 1985, Sugeno [16] introduced the concept of fuzzy control and discussed possible industrial applications. Its usefulness for fermentation control was exemplified by Jin and Shimizu [17].

Their aim was to maximize the production of the enzyme β-galactosidase through cells of *Escherichia coli* JM103 harboring the plasmid pUR2921. The fermentation was carried out in fed-batch mode with glucose being fed continually to a complex fermentation medium, whose composition is described by the authors. The key to maximum productivity is the time-dependent feed rate of glucose so as to minimize acetate formation and maximize the expression of β-galactosidase.

Conventionally, the pH of the broth is used as a measure of glucose concentration. Jin Shimizu’s [17] data, however, showed that at low concentrations of glucose, the CO_2 concentration in the exhaust gas is

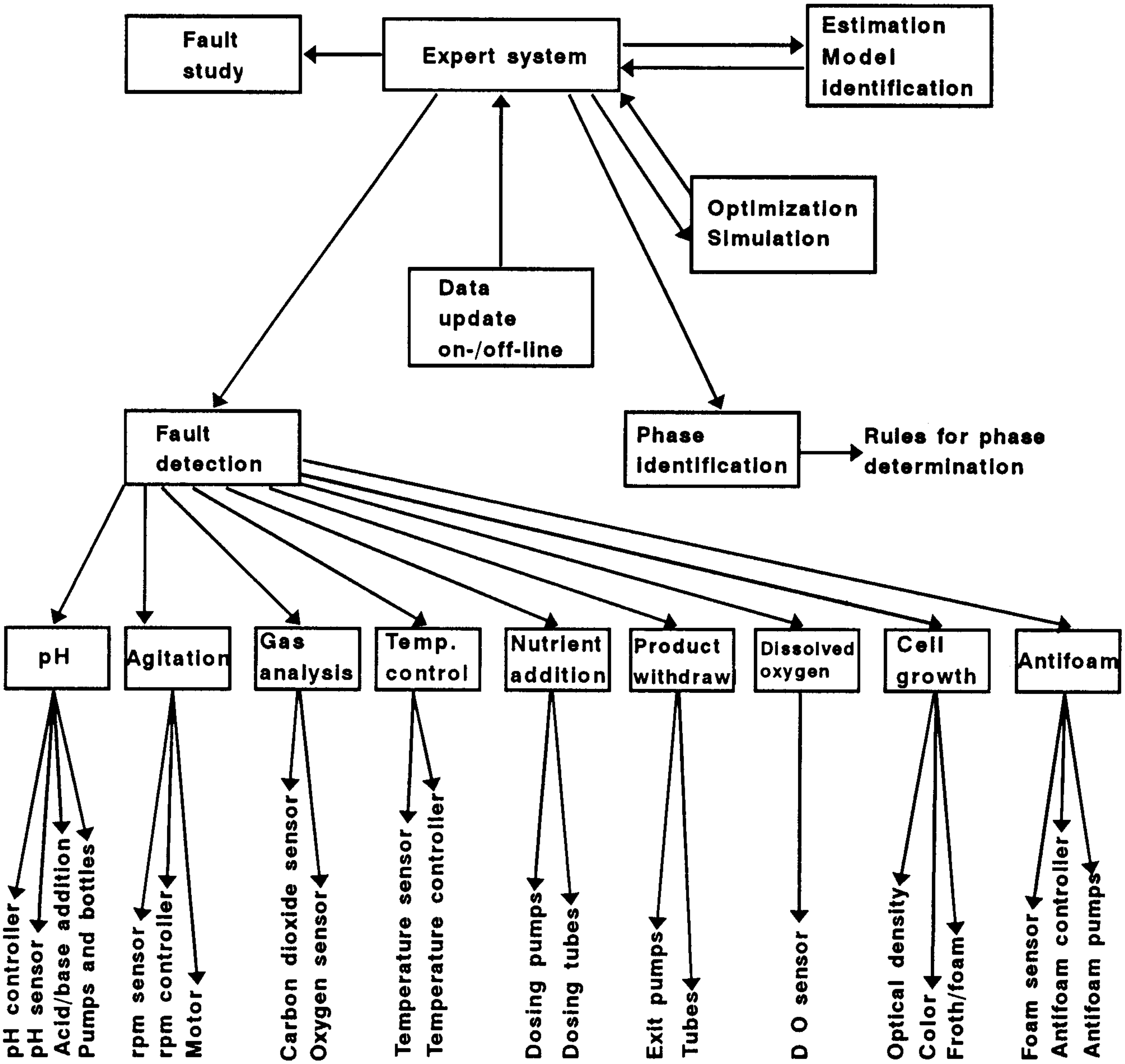

Figure 1. An expert system network showing sensors, interconnections, rules, and inference engine. (Reprinted from Halme and Karim [8] with permission by VCH Publishers, Weinheim, Germany © 1991.)

a more sensitive index than pH. Therefore, both variables were considered in the linguistic rule:

IF $\Delta CO_2(k) < O$ and $\Delta pH(k) > O$ THEN $S(k) = O$

where

$$\Delta CO_2(k) = CO_2(k) - CO_2(k-1)$$
$$\Delta pH(k) = pH(k) - pH(k-1)$$

Here k denotes the kth sampling instant and $S(k)$ is the residual glucose concentration.

The feed rate of glucose was calculated as

$$F(k) = F(k-1) + (1 + \lambda)\, \Delta F(k)$$

The correction factors λ and $\Delta F(k)$ at each instant of time were calculated according to fuzzy rules.

In the first scheme, λ was set to zero for simplicity. The maximum dry cell mass attained was 36 g/dm^3, and that for β-galactosidase, 1380 IU/cm^3, both occurring after about 30 hours. When λ also was adjusted on-line, the cell mass concentration doubled, and the protein activity increased by a factor of 3. Moreover, the improved values were attained between 21 and 22 hours. By contrast, model-based pH control could achieve no more than 600 IU/cm^3 of β-galactosidase.

Neural Networks

Zhang et al.'s [18] study highlights some important features of the development and design of artificial neural networks for bioreactors. *Bacillus thuringiensis* was cultivated in a batch process, and the objective was to maximize its concentration in the shortest possible time.

The experimental arrangement is shown in Figure 2, and it is conceptually general because the box labeled "Neural Network Supervisory Unit" (NNSU) may be replaced by a fuzzy logic unit or an expert system. The NNSU was used to estimate process conditions and supervise the digital measurement and control unit according to the estimated values of the optical density (OD), which was a measure of cell mass concentration.

The starting ANN was a feed-forward network with six input nodes and one output, the optical density. The inputs were the type of inoculum, time, temperature, pH, the partial pressure of oxygen, and the current OD. It may be noted that the OD is an input as well as an output. The input is the OD at the kth (current) instant, and the output is the neural prediction at the $(k + 1)$th point. This strategy overcomes the inherently static nature of a feed-forward network when applied to dynamic problems [11].

The NNSU in Figure 2 contained on ANN, based on whose predictions of OD the supervisory controller searched for desired set points (temperature and pH) and then tuned the digital control unit (a PID controller). Thus, the supervisory controller does not replace but enhances the performance of the PID controller. While PID control achieved a peak OD of 12 units in 9 hours, the combined system attained 13 units in less than 7 hours. Moreover, in the latter case, the OD remained constant after the peak was reached, whereas it decreased for PID control alone.

Expert Systems

The use (and limitations) of an ES for fermentation control and fault diagnosis is illustrated by the work of Halme and Karim [8]. As mentioned before, α-amylase was synthesized in batch fermentation by *B. subtilis* containing the *B. amyloliquefaciens* gene as a single-copy chromosomal integrate.

pH is a key variable, and it is regulated by dosing either acid or alkali. The ES was designed to detect and report any fault in the pH-control system. It operated at two levels. Checking the overall condition of the measurement and control functions in the bioreactor was done by level-1 rules. The ES continuously checked these rules, and if a fault was detected in any element of the measurement and control loops, then it activated the level-2 rules, which analyzed the problem and made recommendations to the operator. This logic is displayed in Figure 3.

A salient feature of the ES was its use of a sliding window concept. The time span of observation and inference is moved from one phase of fermentation

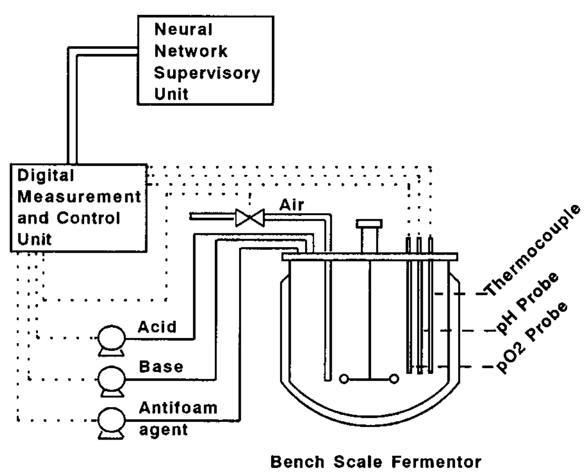

Figure 2. Neural network supervised fermentation control system. (Reprinted from Zhang et al. [18] by permission of John Wiley & Sons, Inc., New York, NY 10158, U.S.A. © 1994.)

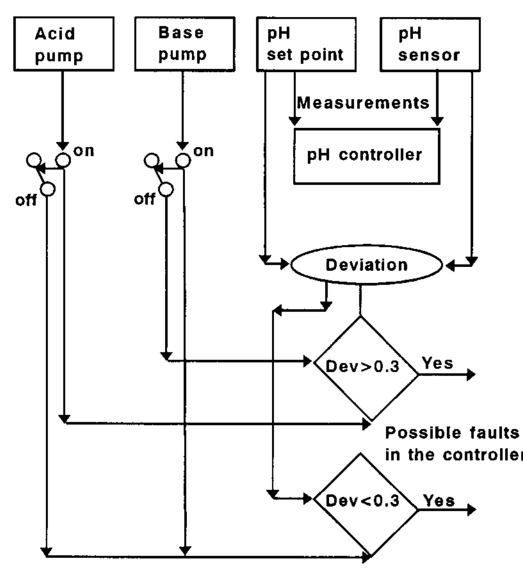

Figure 3. Logic for finding faults in the pH controller. (Reprinted from Halme and Karim [8] with permission by VCH Publishers, Weinheim, Germany © 1991.)

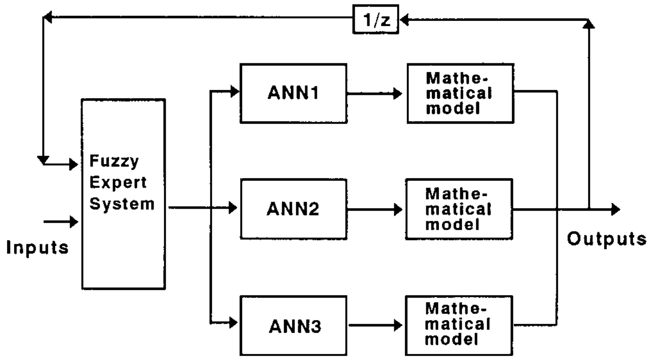

Figure 4. Schematics of a combination of a fuzzy expert system, several neural networks and mathematical models. (Reprinted from Schubert et al. [21] with kind permission from Elsevier Science-NL, 1055 KV Amsterdam, The Netherlands © 1994.)

to another. This avoids storage of huge amounts of data, enables accurate interpolation and smoothening, and allows the use of modest PC-based software. The usefulness of a moving window has also been demonstrated for neural networks [19].

Since the measured signals may be noisy, a moving average filter was employed to screen out the noise before the data are processed. This too was done by the ES, which decided which variables were to be filtered. Since this was done in real time, the choice of variables was time dependent according to the disturbances.

In a test run, faults were introduced deliberately in the pH controller by adding a bias in the acid pump at 3.5 hours into the fermentation, and in the CO_2 sensor, after 6.35 hours by injecting a pure CO_2 pulse. The ES predicted correctly the faults in the pH-control system but was misled by the consequences of the CO_2 perturbation. Introduction of CO_2 disturbed the measurement of the oxygen uptake rate (OUR). The ES's heuristic rules led it to believe that both OUR and CO_2 sensors had behaved identically, their sharp increases indicating rapid cell growth. Since the output from the dissolved oxygen probe did not (rightly) conform to this pattern, the ES reasoned (incorrectly) that the gas sensors were functioning properly and that the fault lay in the controller for the speed of the stirrer.

Because the faults were artificially created, we know that the ES's inference was wrong. However, if the faults were unknown (as in a real situation), then the ES's inference would be logical and hard to identify as being implausible. This underlines the importance of data analysis, design, logic, and training of an ES. Indeed, this is true of other forms of artificial intelligence also [10, 13], and lack of care and sufficient understanding of the process can result in an AI system that is seemingly reasonable but malfunctions in a crucial situation.

Hybrid Systems

Sometimes it is useful to combine two AI systems, or an AI system with a mathematical model, so that the weaknesses of one may be compensated by the advantages of the other. Among the more popular of these are fuzzy neural nets, i.e., neural networks that operate through fuzzy logic.

Shi and Shimizu [20] applied this idea to the fermentative production of ethanol by the yeast *Saccharomyces cerevisiase.* In a fed-batch operation, the inflow rate of substrate was regulated by a fuzzy controller whose membership functions were adjusted on-line by two ANNs, one for dissolved oxygen concentration and the other for ethanol. The work of Zhang et al. [18] cited earlier combined FL and ANN in a different way. It was observed that the ANNs could not portray the decline in optical density in the stationary phase of growth. Normalization of the input variables did not help. So the output variable, the OD predicted one time step ahead of the current state, was graded into nine qualitative classes, each represented by a triangular membership function. The estimated values of the actual ODs were then the weighted sums of the median values of each class. This method achieved a faithful representation of the full range of OD variation.

There are also other ways to combine ANNs and fuzzy-rule systems: fuzzy decisions as inputs to neural networks, scheduling of different ANNs by fuzzy decisions, or compensating for the difficulties with the networks when they have to function in a domain outside the state space where they were trained [10].

Halme and Karim's [8] representation of an ES as part of an overall optimization-cum-control configuration (Figure 1) shows how mathematical models and hardware sensors can provide basal data for an expert system's decisions. Schubert et al. [21] have gone further and integrated a set of dynamic differential equations, an ANN and a fuzzy ES. Like Shi and Shimizu [20], they chose the production of ethanol by *S. cerevisiae.* The hybrid system performed better in state estimations than either a differential equation model or an ANN separately; it also required fewer data and shorter training times. This estimator was then used as a reference model of the fermentation system, and a neural network controller was employed to regulate the substrate feed rate. It is interesting to compare this with the approach of Shi and Shimizu [20], who employed ANNs for state estimation and FL for control.

CONCLUDING REMARKS

Artificial neural networks, fuzzy logic, and expert systems are only some forms of artificial intelligence, even though they are the most commonly used for bioreactors. While each of them offers many choices of design and deduction, and combinations among them are possible, lately there have been forays into other areas in conjunction with AI. The use of mathematical models with AI systems has been cited. For the scheduling of a set of operations, either with a single reactor and in a process plant, AI has been integrated with operations research techniques [22].

Indeed, the many combinations of AI techniques now being employed has given birth to the generic terms "intelligent systems" and "intelligent controllers." Broadly, an intelligent controller has the following features [13]:

1. it uses logic, sequencing, reasoning, or/and heuristics in addition to numerical algorithms;
2. it is essentially a nonlinear controller with wider autonomy than a conventional controller; and
3. it relies on representational forms and decision-making procedures designed to mimic human/biological systems.

With the tremendous computation power now available even in PCs and workstations, it has become possible to study complex biological processes in depth through AI without having to formulate detailed mathematical models. This is useful from an application point of view because it enables the rapid development of commercial scale technologies. Mammalian cell culture [14] and recombinant protein synthesis [10] have reached the industry even while scientific insights are still being probed. However, as recent studies [18, 20, 21] have shown, no particular form of AI is sufficient by itself and neither can mathematical models be dispensed with. The best solution to a bioprocess estimation and/or control problem is often a combination of the two approaches; this has been depicted lucidly by Schubert and coworkers [21] in Figure 4.

The impact of artificial intelligence on research and industry is evident from the prolific growth of publications (15 books, 65 reviews, and more than 700 papers in 10 years), and the projected value of the sale of intelligent systems for process applications ($2.0 billion in 1997) [13].

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