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Object-oriented fuzzy expert system for on-line diagnosing and control of bioprocesses

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Summary. An object-oriented fuzzy expert system to support on-line control of an automated fermentation plant is described. The major elements of the system consist of a fuzzy inference engine, a database, a knowledge base, and an expression evaluater. The expression evaluater calculates specific rates for growth, and substrate and product formation at different physiological states during the cultivation from the measured data. The specific rates are then compared with the standard target rates stored in the database. If differences outside the set tolerances were observed, the inference engine analyses the reasons for the faults on the basis of the knowledge represented in the form of a knowledge network and fuzzy membership functions of the process variables. The fuzzy expert system was developed on the basis of a shell constructed by using the object oriented Smalltalk/V Mac programming environment, with *Lactobacillus casei* lactic acid fermentation as the example of process application.

Introduction

Automatic on-line diagnosing and control of bioprocesses is frequently difficult owing to the uncertainties involved, to the lack of suitable models and real-time algorithms, and to little available knowledge on the effects of changes in microbial physiology. The lack of appropriate on-line sensors for the measuring of key process variables further limits the real-time information available. In current practice manual bioprocess control is often based on experts' decisions utilizing empirical and/or heuristic knowledge based on past experiments and experiences. However, such methods are not without problems. Process knowledge is difficult to standardize because every expert may have a different basis for process control. Problems associated with uncertainties may be solved by implementing experts' knowledge into intelligent process control systems (Linko 1988), by fuzzy state estimation (Rauman-Aalto 1988; Postlethwaite 1989), by realizing automated cultivation (Endo et al. 1989), and by applying the qualitative physics theory in model construction (Travé-Massuyè et al. 1990). Fuzzy control system principles have been described in detail by Lee (1990) and Abel (1991), and quite recently rule-based fuzzy control has been applied to extrusion cooking (Eerikäinen et al. 1988), glutamic acid fermentation (Kishimoto et al. 1991), and sake brewing (Oishi et al. 1991). Object-oriented programming has been employed in constructing fuzzy expert control systems for extrusion cooking (Aarts et al. 1989), and for enzyme production (Aarts et al. 1990).

In the present work, fuzzy reasoning has been applied for on-line, real-time handling of uncertainties in the measurements, process knowledge and diagnosing as suggested by Aarts et al. (1990). Consequently, an on-line fuzzy expert system was constructed to aid the operator in the diagnosing and control of fermentation processes. Lactic acid fermentation was selected as an example case study because of the available data and expert knowledge at both participating laboratories. The fuzzy LAexpert diagnosing system was based on the physiological activities of the microorganism, characterized by the appropriate specific rates for cell growth, substrate consumption and product formation as determined by an automated on-line HPLC system and a turbidity sensor integrated to the concept of an automated fermentation plant (Endo and Nagamune 1983; Endo et al. 1989; Pokkinen et al. 1992). Although recently both FIA (flow injection analysis) (Nielsen et al. 1989), and FTIR (Fourier transform infrared) spectroscopy (Fairbrother et al. 1991) have been suggested for on-line monitoring both of substrate and product concentrations in lactic acid fermentation, the techniques were not implemented in fully automated intelligent control systems. The paper describes the fuzzy LAexpert as a useful tool for on-line fermentation diagnosis and control.

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Materials and methods

Microorganism. Batch cultivations were carried out with the strain *Lactobacillus casei* (ATCC 27092). The culture was stored at -40°C.

Culture conditions. Seed cultures were grown on Rogosa medium (Constantine and Hansen 1962) at 35°C, pH 6.5. The lactic acid production medium contained per litre: 25 g glucose, 25 g clarified corn-steep liquor, 1 g KH_2PO_4, 1 g K_2HPO_4, and 0.08 g $MnSO_4 \cdot 2H_2O$. The components were sterilized separately and added to the fermentor aseptically using a clean bench. All fermentations were run at 35°C either with 2.5 l (2 l working volume) (Iwashiya, Japan) or 30 l (Komatsugawa Chemical Engineering, Japan) fermentors. The pH was adjusted to 6.5, and the agitation rate to 150 or 200 rpm depending on the volume. On-line data was stored in a specially constructed MS Excel spreadsheet at a sampling-time interval of 30 min. The 30-l fermentor was monitored and controlled by the Bio Advanced Control System (BIOACS) as described by Asama et al. (1990a) and Endo et al. (1989).

Analyses. Biomass was measured on-line with a sterilizable turbidity sensor at 632.8 nm (Nagamune et al. 1985). Substrate and product concentrations were measured on-line according to Endo et al. (1989) by an HPLC unit (Shimazu, Japan) equipped with a Shodex Sugar SH-1011 column (Showa Denko, Japan). The respective dimensionless specific rates employed in the diagnosis were calculated and scaled within the range of [0.1] in real time according to Endo and Nagamune (1983) as the function of time. The Kalman filter was not employed because it was found to be ineffective when studying culture conditions that differed considerably from the standard conditions. When the specific rates were calculated on the basis of off-line analyses, the results were smoothed by using a first order delay filter with a time constant of about 6 h.

On-line diagnosing and control system. The on-line diagnosis of lactic acid fermentation was physically realized by connecting the process computer Fujitsu A50 through a RS232C communication line to the Macintosh IIci computer running the fuzzy expert system LAexpert (Fig. 1). The process computer received measurement data from the fermentor as the input for the expression evaluater to calculate the specific rates after filtering. The results and the rest of the data were sent to the Macintosh. The inference engine implemented in the Macintosh then applied all available experimental results and knowledge for the on-line diagnosis.

In the on-line diagnosis the process computer sent filtered measurement data and results of the on-line analyses of substrate and product concentrations from the HPLC and of cell mass from the turbidity sensor to the LAexpert every 20 min. The on-line diagnosis started when data was received from the process computer and written into the file. The measurements were compared to the standard data file and, when differences outside the set tolerances were observed, the malfunction diagnosis started. The results were automatically stored into a file named by the operator. The root causes for the faults detected were reported to the BIOACS system for process control and countermeasures.

Programming environment. In the present work a BIOACS for automatic monitoring and control, supported by an on-line turbidity sensor, and an on-line sampling unit for cell-free culture medium coupled with an automated HPLC unit, was employed as described by Asama et al. (1990a). An Expert System for Cultivating Operations (ESCO) built using the Eshell AI-tool in UTILISP on the mainframe computer Fujitsu M780 was employed in the experimental design, and aided in the cultivation operations and data analysis.

Fuzzy object-oriented expert system shell. The knowledge-based system for on-line diagnosis, implemented in a Macintosh IIci computer was constructed to operate integrated with the BIOACS system (Asama et al. 1990b). The Macintosh IIci computer was equipped with a 68020 processor, with an 8 MB memory on the main board extended to 16 MB by using Virtual 0'30 software, and 100 MB hard disc. The Smalltalk/V Mac-(Digitalk, USA) - based object - oriented fuzzy expert system shell and the user interface were modified from those described by Aarts et al. (1990). The object-oriented programming environment enabled flexible modifications. The objects were arranged hierarchically, with one superclass and one or more subclasses. Objects can send and receive messages, and they know how to react or answer to the messages. Subclasses can always do what their superclass can, but they may also have additional capabilities. For example, a *variable* is an object class, which may have subclasses such as *on-line variable* and *off-line variable*. Both subclasses could understand the message, 'compare', asking them, for example, to compare their respective values to the standard values at a certain time point. An *off-line variable* could also reply to the message, 'sampletime', by answering sample time defined for it, etc.

Fuzzy variables. The shell was constructed to handle uncertainties both in knowledge and in expert system shell measurements by fuzzy reasoning. For any given application, fuzzy membership functions for each process variable were defined in addition to the set points, tolerances, and possible ideal (standard reference) profiles. Fuzzy reasoning allowed the use of linguistic descriptions for the variables, and greatly facilitated the transfer of human expert knowledge to the system.

For every process variable fuzzy membership functions were individually defined. All of the variables had two to five fuzzy linguistic values with respective membership functions, such as 'small', 'normal', 'high', 'very low'. The fuzzy membership functions yielded truth values for certain claims.

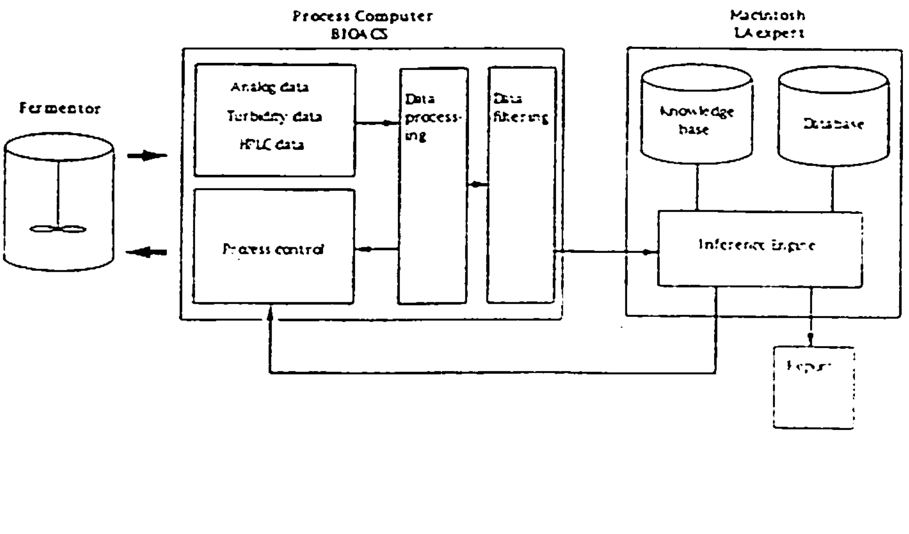

Fig. 1. The configuration of the on-line fuzzy expert system

A fuzzy set A of a universe X is characterised by a membership function $\mu_A(x_i)$, $\forall x_i \in X$ ($\mu_A : X \rightarrow [0,1]$) with a grade of membership of x_i in A, represented by a real number $\mu(x_i)$ within the interval [0,1]. Thus in the case of the claim "*temperature* is 'low'" with the measured value for temperature of (x_i), the respective truth value (grade of membership, $m = \mu_A(x_i)$) could be checked from the fuzzy membership function for the value 'low' for the variable *temperature*. The truth value is 0 [$\mu_A(x_i) = 0$] when the claim is not at all true and 1.0 [$\mu_A(x_i) = 1$] when the claim is absolutely true. The claim is partly true if a truth value is between 0 and 1.0 [$0 < \mu_A(x_i) < 1.0$]. The resulting grade of membership, m, of the 'fuzzy-is' operation can be read directly from the graphical representation of the membership function. For the 'fuzzy-is-not' operation the result is the complement of m, such as $1 - m$.

Given the fuzzy sets A, B on X, the basic operations on A, B employed in the present system were 'not', 'or' and 'and', which can be defined by using the grades of membership (m for A and n for B). The 'fuzzy-and' operation for the truth values gives the minimum as a result. In case of the 'fuzzy-or' operation, the maximum of the truth values participating in the operation is taken as the result. The measured value for a fuzzy variable could also be compared to the membership function with 'fuzzy smaller than' or 'fuzzy greater than' operators. Fuzzy operators used are summarized below.

'fuzzy-is'	$\mu_A(x_i)$	m
'fuzzy-is-not' (complement)	$\mu_{\bar{A}}(x_i) = 1 - \mu_A(x_i)$	$1 - m$
'fuzzy greater than'	$\mu_{\bar{A}}(x_i)$ if and only if $x_i > a_3$	$1 - m$
'fuzzy smaller than'	$\mu_{\bar{A}}(x_i)$ if and only if $x_i < a_2$	$1 - m$
'or' (union)	$\mu_{A \cup B}(x_i) = \max[\mu_A(x_i), \mu_B(x_i)]$	$\max[m, n]$
'and' (intersection)	$\mu_{A \cap B}(x_i) = \min[\mu_A(x_i), \mu_B(x_i)]$	$\min[m, n]$

Using fuzzy sets $A \subset X$ and $B \subset Y$, a fuzzy relationship, R from X to Y can be defined. The fuzzy relationship can be expressed as a Cartesian product, $R \subset X \times Y$. The corresponding membership function is $\mu_R(x,y) = \min[\mu_A(x), \mu_B(y)]$, where $x \in X$ *and* $y \in Y$ (Linko 1988). This represented the basic rule used in LAexpert, R = *if* A, *then* B, for example "*If* cellmass is 'fuzzy greater than' 'high' *then* measured product formation rate is 'low'." Or in the case of two rules connected to a chain, for example, "*If* the inoculum size is 'small' *then* cell mass is 'low' and *then* measured μ is 'low'." This can be represented as a fuzzy relationship (Dubois et al. 1980):

$$\begin{aligned}
&\mu_{R_1}(\text{inocSize, cellMass, μMeas}) \\
&= \min\{\mu_{SMALL}(\text{inocSize}), \min[\mu_{LOW}(\text{cellMass}), \mu_{LOW}(\text{μMeas})]\} \\
&= \min[\mu_{SMALL}(\text{inocSize}), \mu_{LOW}(\text{cellMass}), \mu_{LOW}(\text{μMeas})]
\end{aligned}$$

Trapezoidal fuzzy membership functions were employed because of their simplicity in design and use, defined with four values (a_{1-4}). Thus a fuzzy set A (*temperature* is 'low') can be defined for example as A = [10°C, 30°C, 33.5°C, 35°C]. The points a_1 and a_4 denote the truth value of 0 and the points between a_2 and a_3 the truth value of unity. The areas a_1-a_2 and a_3-a_4 represent fuzzy regions. Sometimes these regions are not fuzzy but the truth value of unity continues to infinity. Then the corresponding a_i values are denoted by 'nil'. When observing fuzzy variables, the 'fuzzy-is' operator denoted the grade of membership, 'fuzzy-is-not' represented the complement of the grade of membership, 'fuzzy greater than' denoted the complement of the grade of membership when x_i belonged to [a_3, a_4] and 'fuzzy smaller than' stood respectively for the complement of the grade of membership when x_i belonged to [a_1, a_2]. The grade of memberships, $\mu_A(x)$ and $\mu_{\bar{A}}(x)$ for different values of x_i are shown below.

$$\mu_A(x) = \begin{cases} 0 & (x_i < a_1) \\ (x_i - a_1)/(a_2 - a_1) & (a_1 \le x_i < a_2) \\ 1 & (a_2 \le x_i < a_3) \\ (a_4 - x_i)/(a_4 - a_3) & (a_3 \le x_i < a_4) \\ 0 & (a_4 \le x_i) \end{cases}$$

$$\mu_{\bar{A}}(x) = \begin{cases} 1 & (x_i < a_1) \\ (a_2 - x_i)/(a_2 - a_1) & (a_1 \le x_i < a_2) \\ 0 & (a_2 \le x_i \le a_3) \\ (x_i - a_3)/(a_4 - a_3) & (a_3 \le x_i < a_4) \\ 1 & (a_4 < x_i) \end{cases}$$

Process knowledge for fault diagnosis and control. Collecting and organizing process knowledge is one of the most important tasks when developing an expert system. In the present work process knowledge about lactic acid fermentation was collected through several *L. casei* cultivations on different scales. In addition, data available in the literature, and other expert knowledge about bioprocesses was used.

In a typical fuzzy expert system process knowledge is divided into the knowledge base, and the database. The main part of the knowledge in LAexpert was represented as the knowledge network and the fuzzy membership functions of the variables, stored in the knowledge base. Table 1 lists example fuzzy membership functions used in the present knowledge base for the *L. casei* lactic acid fermentation.

The time-course of a fermentation was described with calculated standard specific rates for microbial growth, substrate consumption and product formation, based on typical target curves (Pokkinen et al. 1992). The standard specific rates, dimensionless concentrations, and the respective time-course patterns were included in the database of the system after filtering. The course of the fermentation was divided into three different phases (Endo and Nagamune 1983) on the basis of the different physiological states of the cultivation observed from the standard specific rate patterns.

Results and discussion

Knowledge network

Much of the knowledge in the fuzzy expert system LAexpert was expressed in the form of a knowledge network, a part of which is shown in Fig. 2. The knowledge network is a user-friendly graphical way to represent *if-then* rules typical of expert systems. The Smalltalk pro-

Table 1. Fuzzy membership functions for example process variables. The trapezoidal functions are described by (a_1, a_2, a_3, a_4)

Fuzzy variable	a_1	a_2	a_3	a_4
pH				
Very Low	Nil	Nil	4.0	6.0
Low	4.0	6.0	6.4	6.5
Normal	6.4	6.5	6.5	6.6
High	6.5	6.6	6.8	12
Very high	6.8	12	Nil	Nil
Lactic acid (%)				
Very Low	Nil	Nil	−10	−3
Low	−10	−3	−3	0
Normal	−3	0	0	3
High	0	3	3	10
Very high	3	10	Nil	Nil
ν and π (%)				
Very Low	Nil	Nil	−1.2	−0.95
Low	−1.2	−0.95	−0.75	0
Normal	−0.75	0	0	0.75
High	0	0.75	0.95	1.2
Very high	0.95	1.2	Nil	Nil

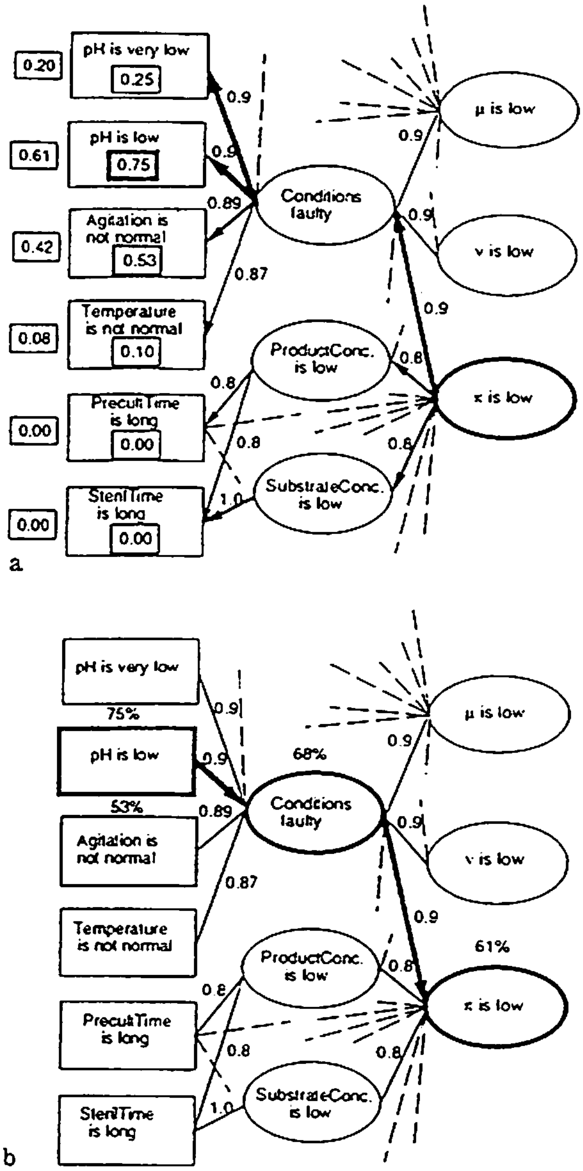

Fig. 2a, b. A simplified example of inferencing is illustrated by a part of the knowledge network for LAexpert. a The inference engine has detected a fault "Specific product formation rate (π) is 'low'." The *heavy lines* illustrate the search for the root cause through backward chaining. b The degree of reliability of the inferencing is reported to the operator according to the certainty factors between the nodes of the whole rule chain, shown in *heavy lines*

gramming environment was especially suited to graphical representation. This style of knowledge representation is both illustrative and easy to edit. The expert system shell was equipped with an editor for building such knowledge networks. The network consisted of several nodes of various type, interconnected with arcs. The nodes represented facts about the process, and the arcs connecting the nodes showed the relationship between two facts. The total amount of the nodes in this network is about 100. The complexity of the network correlates with the number of arcs. The weight of the connection was expressed by a factor, a real number, within a range of [0.1]. The type of node illustrated the type of the fuzzy operator in question. The rectangular start-nodes were given a short code written in Smalltalk syntax, which would be evaluated when the node was considered in the diagnosis. The pointed nodes stood for the operator 'and', and the oval nodes for the operator 'or'. The rectangular action-nodes were used to give information for the operator, and were differentiated from other nodes by thinner arcs.

On the left side of the knowledge network the original root causes to the faults and malfunctions were illustrated as start-nodes. All start-nodes were given a truth value for the appropriate root cause in the form of a fuzzy membership function. The oval-type end-nodes with the operator 'or' on right-hand side of the network included in natural language all of the possible faults and malfunctions the system was able to detect. Thus, for example the expression 'vstd is faulty' tells that the standard (std) for the substrate consumption rate (v) is faulty. This might be the case for example if the operator had accidentally loaded standard curves for different cultivations. All of the original causes were given as fuzzy expressions for the appropriate variables such as "pH = 'high'" (measured value for pH is above the tolerance in the range of fuzzy 'high' for pH) or "glucose fuzzySmaller than: 'low'" (measured value for glucose concentration is below the alarm limit in the range of fuzzy 'low' for glucose) or "(self agitation = 'normal') not" (measured agitation rate has changed form the setpoint and does not belong to the membership function 'normal' for the agitation rate any more). Inasmuch as the on-line knowledge-based system for example knows the current value of the pH in real-time, the truth value of the corresponding start-node fact "pH = 'low'" is continuously coupled to the chain from the end-node in the context of the system.

Example rule chains from the LAexpert knowledge network for lactic acid fermentation are expressed below. The start- and end-nodes were connected with arcs to form a detection chain of *if-then* rules through one or more other nodes, such as:

1. "*If* lag phase is longer as compared to standard case *and* conditions have made it longer
then physiological phases are delayed from the standard ones."
2. "*If* pH and/or temperature is not normal
then cultivation conditions are faulty and specific rates for growth, substrate consumption and product formation are low."
3. "*If* lactic acid concentration *and* glucose concentration, both initial *and* current, are higher than standard values
then HPLC calibration is off and *then* the measured specific substrate consumption rate is high."

In some cases technical nodes without any new information are needed in the presenting of the rule and completing the chain.

Inferencing in the knowledge network

An example of inferencing is given in Fig. 2. When the system detects a fault, the inference engine begins backward chaining through the network in order to find the reasons for the fault. In a typical case there are several possible root causes to a certain fault or malfunction. To find the most probable one the inference engine calculates the certainties for each possible root cause by multiplying the certainty factors defined for each arc in the network (Fig. 2a). A node A with a truth value of a, linked with a certainty factor of p to node B represents a rule "If A is true with a value a, then B is true with a value $a \cdot p = b$." In the example case the inference engine has detected a fault 'π is low'. Six possible causes for the fault are found. The inference engine then checks the truth values for all of the potential faults by evaluating the appropriate fuzzy expressions in the order of importance as indicated by the respective weights of the arcs. The grades of membership for the fuzzy sets representing the root causes in question is automatically confirmed on the basis of the current measured value for the variable. The cause with the highest truth value was the most likely root cause for the fault detected. In the example case, when the measured pH, 5.5, was fuzzy 'low', the truth value was found to be $m = 0.75$ [pH 'low' = (4.0, 6.0, 6.4, 6.5), $m = (5.5-4.0)/(6.0-4.0)$] and the overall truth value for the fault observed was 0.61 ($0.9 \cdot 0.9 \cdot 0.75$). If the value obtained was below the given step value (set here to 0.5), the cause was neglected. In this example the causes 'temperature is not normal', 'agitation is not normal', and 'pH is very low' were thus neglected, because their respective overall truth values were less than the set limit value of 0.5 (0.08, 0.42, and 0.20 respectively). Thus, in this case only the 'agitation rate not normal' with the measured value of 142 rpm and the respective truth value $m = 0.53$ from the membership function, was reported to the operator as another possible root cause. If there are several causes with the value higher than 0.5, they all are reported to the operator in the order of decreasing certainty.

After this, the inference engine follows the chain back to the fault and reports the reliability for the fault reasoning by informing the operator about certainty factors in the chain and the truth value of the original cause/causes (Fig. 2b). Furthermore, it is possible to check the truth value for the fault detected, to see how severe the problem is. The whole chain, and the reliability of the inferencing is reported to the operator, in the example case as: "pH is low 75%, conditions are faulty 68%, specific growth rate is low 61%. Another possible cause: agitation is not normal 53%."

Fault diagnosis

In the fault diagnosis, the inference engine used both the knowledge base and the database. Timing was controlled by the process computer. The diagnosis was performed using on-line filtering for data sets because the diagnosing method was very sensitive to any noise in the measurements. At every sample time the system compared the incoming data set to the corresponding standard (target) data, which should be the same within a certain given average tolerance, usually in this work ±15%. The tolerance could be adjusted by the operator at will. Furthermore, the phase of the cultivation defined on the basis of the measured data should be same as the one defined for the respective time by the standard database. The phases were defined on the basis of the first derivative calculated from the moving average of four subsequent values of specific rates. If the derivative was positive, the phase in question could be for example exponential; if the derivative was zero, the report to the operator indicated that the phase is changing, and so on. If any differences beyond the allowed limits were observed, the inference engine started to search for the reasons for the deviations found. Typical example causes for faults are given in the following: (a) μ and/or π is 'low' when (1) pH is 'low' or 'high'; (2) inoculum size is 'small'; (3) precultivation time is 'long'; (4) cell mass is 'high'; (5) HPLC calibration is off; or (b) phases are delayed when (1) inoculum size is 'small'; (2) precultivation time is 'long'; (3) HPLC calibration is high, substrate and product concentrations are high.

Because the automatic fault diagnosis was based on the use of on-line HPLC and a turbidity sensor, their correct calibration and stability were essential for the proper functioning of the system. This was taken into account in the knowledge network, which was also capable of detecting calibration-related faults.

It can be concluded that the on-line diagnosing system was successfully tested by running experiments with the 30 l fermentor controlled by BIOACS. The communication interface constructed by using Smalltalk to control the external data exchange of the Macintosh operating system worked well. The fuzzy expert system developed proved to be able to communicate with the process control computer, and to perform on-line diagnosis. We are currently further expanding the system to provide real-time control, by adding direct control functions to the knowledge network.

Nomenclature: A, B fuzzy sets [$A = (a_1, a_2, a_3, a_4)$]; $\overline{A}$, complement of A; $A \times B$, cartesian product of two sets A and B; AI, artificial intelligence; a_1-a_2, cornerpoints for trapezoidal fuzzy membership function; m, grade of membership in fuzzy set A; n, grade of membership in fuzzy set B; R, a fuzzy relation; std, standard; X, Y, classical sets of objects (universe of discourse); x, y measurable variables; x_i, measured value for variable x; μ, specific growth rate (s^{-1}); $\mu_A(x_i)$, $\mu_A(x)$ fuzzy membership functions for fuzzy set A; $\mu_B(x_i)$, $\mu_B(y)$ fuzzy membership functions or fuzzy set B; μMeas, μ calculated from measurements; $\mu_R(X, Y, Z)$, fuzzy membership function for the fuzzy relation R; ν, specific substrate consumption rate ($g\,s^{-1}$); π, specific product formation rate ($g\,s^{-1}$); $\in$, belongs to; $\subset$, non-strict inclusion; $\cup$, union; $\cap$, intersection; $\forall x$, universal quantifier (for all x).

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