Reprinted from Biochemical Engineering VI
Volume 589 of the *Annals of the New York Academy of Sciences*
May 20, 1990

An Expert System for Cultivating Operations

H. ASAMA,[a] T. NAGAMUNE,[a] M. HIRATA,[b]
A. HIRATA,[b] AND I. ENDO[a]

[a]*Chemical Engineering Laboratory*
RIKEN (The Institute of Physical and Chemical Research)
Wako-shi, Saitama 351-01, Japan

[b]*Department of Chemical Engineering*
School of Science and Engineering
Waseda University
Ohkubo 3-4-1, Shinjuku-ku, Tokyo 160, Japan

INTRODUCTION

Bioprocess automation has been required in order to shorten the development period of bioproducts, to improve reliability and flexibility of production processes, to improve a product's quality, to save manpower, and to enhance total productivity. Automation technologies in bioprocesses, though, are far behind those in other fields such as the machine industry, the electronic industry, etc. In other words, bioprocess is still controlled by operators who are experts of separating biocatalysts or of cultivation. This means that complicated and diverse bioprocesses are demanded in their operations. Thus, they are based on the operator's empirical and/or heuristic decisions utilizing knowledge stored through past experiments and experiences. Therefore, it is essential to implement the operator's knowledge on experiences and know-how in order to realize a fully automated production system in bioindustries.

On the other hand, in accordance with the progress in the computer science and information processing technologies, knowledge-based systems have been developed in various fields. Even in biotechnology, although being abstract in concept, research works of AI applications for bioprocess design, fermentation control, etc., have been reported.[1,2]

Our objective is to develop new technologies to automate cultivating operations carried out in the stage of research and development of bioproducts. We developed the BIOACS (BIO Advanced Control System[3]), which enables on-line monitoring of various state variables and optimal control based on physiological data of a microorganism, which have been stored in a bio-data base. Moreover, in order to enhance the function of the BIOACS, we are developing expert systems[4] as a supervisory system of the BIOACS by implementing knowledge necessary for bioprocesses. In this report, we discuss a concept of an autonomous and fully automated cultivation system, as well as functions of the expert system (ESCO: Expert System for Cultivating Operations), which support decision making in cultivating operations totally. Then, methods of knowledge representation and the inferring process are proposed, and an implemented prototype system is presented.

AN ADVANCED CULTIVATION SYSTEM

The BIOACS

The ESCO is situated as a supervisor of the BIOACS, which we have developed already. Characteristic features of the BIOACS are summarized as follows:

(1) on-line measurement of cell mass concentration, substrate concentration, and product concentration;
(2) on-line monitoring of physiological activities of microorganisms, which are represented by various specific rates;
(3) optimal control of bioprocesses, which is executed on the basis of the characteristic properties of the bioprocess parameters saved in a data base.

These functions are achieved by the development of an on-line turbidisensor,[5] an on-line sampling unit of cell-free culture medium,[6] accurate estimation of the specific rates via a Kalman filter,[7] and dynamic responses of the specific rates to fed-batch operations.[8]

However, from the supervisory viewpoint, an autonomous and fully automated cultivation system has to guarantee not only advanced control of the cultivation process, but also on-line and real-time diagnosis and dynamic process planning in order to cope with unexpected disturbances to the process, unknown changes of cultivating conditions, unestimable performances of the microorganisms, or any other troubles such as mechanical defects or misoperations. In this sense, the BIOACS is the best system to be upgraded to a more advanced system because it provides an on-line turbidisensor and an on-line sampling unit and it enables us to recognize the dynamic state of the process in real time.

The cultivation process is composed of various operations such as experimental design, installation of sensors, culture medium preparation, sterilization, inoculation of cells, cleaning of the fermentor, and maintenance operations. These operations are, in nature, not always procedural, but require empirical knowledge with heuristic inference. In addition, we first have to determine the cultivating conditions or parameters on process control before the experiment run because these are associated with lots of unknown or ambiguous factors. Therefore, decisions in cultivating operations made by operators are rather trial-and-error than decisive. Moreover, it is difficult to construct a definite model of cultivation with respect to the biochemical reaction. Taking account of these discussions, we present an advanced cultivation system.

Concept of an Advanced Cultivation System

A concept that we propose here is shown in FIGURE 1.[9] The system consists of the following main component systems:

(1) **BIOTRON**, an intelligent fermentation factory, which characterizes microorganisms and generates experimental data. BIOTRON is integrated with robots in order to automate cultivating operations. The BIOACS can be regarded as a primitive system of BIOTRON.

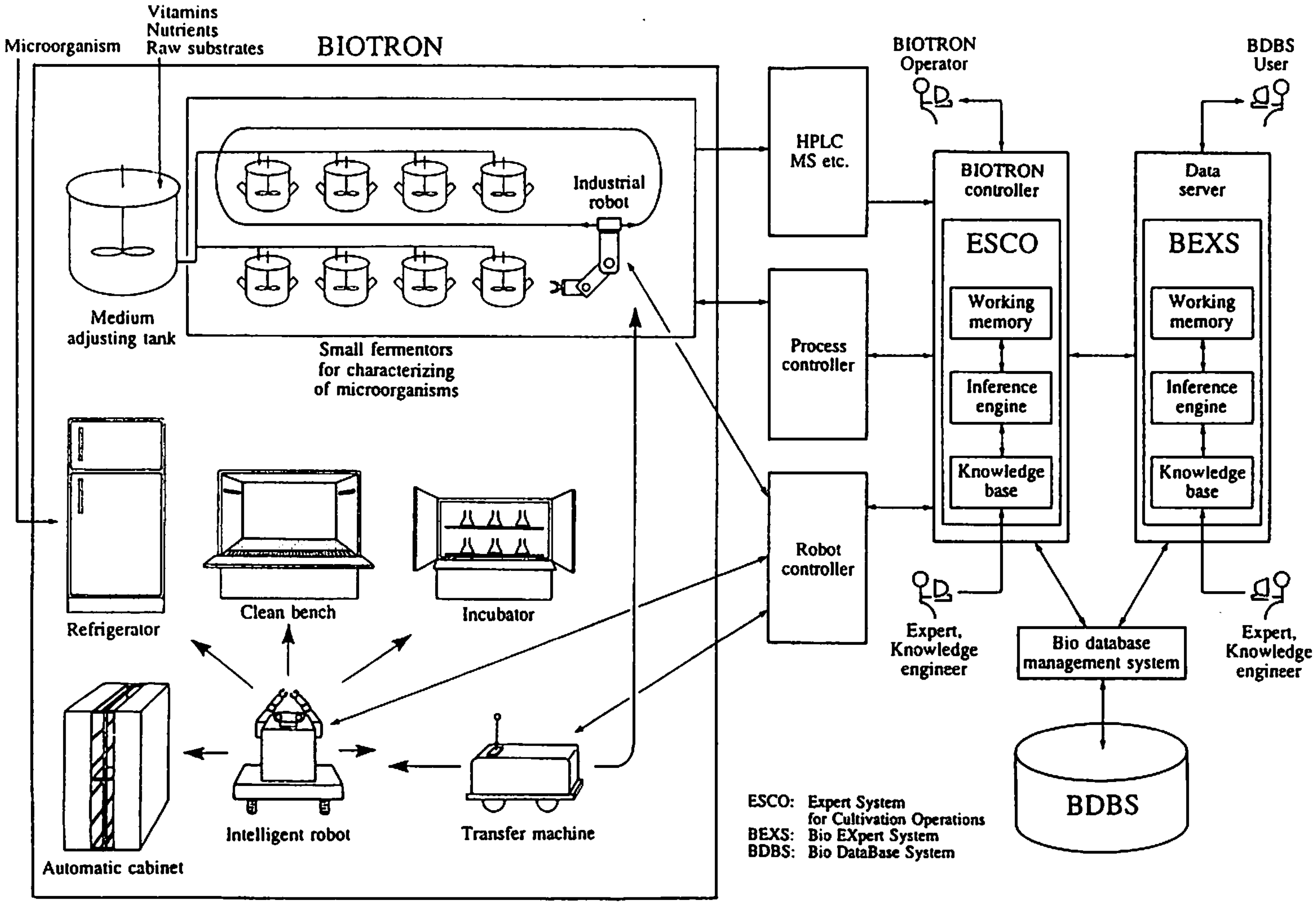

FIGURE 1. Concept of an advanced cultivation system.

(2) **BDBS** (Bio-Data Base System), storage of experimental data representing various physiological characteristics and performance of microorganisms in cultivation processes.
(3) **ESCO** (Expert System for Cultivation Operations), an expert system necessary for controlling BIOTRON. ESCO not only controls cultivation processes in BIOTRON optimally, but it also diagnoses the bioreactor system and the cultivation processes and designs the experimental fermentation processes.
(4) **BEXS** (Bio EXpert System), another expert system necessary for intelligent data service to general users. BEXS supports process planning, production design, and scheduling by utilizing characteristic data stored in BDBS.

In this report, we focus on the ESCO.

The ESCO: Expert System for Cultivating Operations

The mechanism of biochemical reaction is so complicated that knowledge and know-how of human operators play a very important part in decision making in cultivating operations. Hence, the ESCO is expected to apply knowledge engineering to various problems from upstream to downstream in the operations. Furthermore, in the stage of research and development of bioproducts, one of the main concerns is to find the optimal fermentation conditions. However, this problem is usually solved by numerous experiments and mathematical modeling. The ESCO should be provided with the following functions:

(1) *Diagnosis of Troubles*: In the case where troubles result from any misoperations or mechanical defects, the system is required to be able to discover them, to specify their root causes, and to instruct countermeasures for repairing or recovering. It is also expected to diagnose contamination by miscellaneous microorganisms.
(2) *Intelligent Process Control*: In the case where unexpected disturbances to the process occur or where variation of cultivating conditions cannot be estimated beforehand, the system is required to be able to modify the controlled process parameters while the process is running and to continue the cultivation process. It is proper that the function for the optimal control of fed-batch operations should also be included.
(3) *Dynamic Experimental Design*: In the case where experimental processes are obliged to be designed based on ambiguous or unreliable information or when data representing the performance of microorganisms are not acquired sufficiently previous to the process run, the system is required to store the observed data and to redesign the process dynamically by taking account of the data acquired in real time.
(4) *Simulation of Operations*: In order to realize these functions, it is indispensable for the system to refer to the data base on past experiments. In addition, when detecting troubles, sensed data in the cultivation process should be monitored by comparison with results from a simulator that estimates the performance of microorganisms referring to the data base.

A DIAGNOSING SYSTEM

Diagnosis of Fermentation System

Here, we discuss the methodology of knowledge representation and the inference process with respect to the function of diagnosing the bioreactor system. As mentioned above, in the case of diagnosis of fermentation processes, we should take account of not only recovery of troubles by detecting any mechanical defects or misoperations, but we also should place emphasis on intelligent control by monitoring unsuitable cultivating conditions and on data base construction by acquiring data of unknown performances of the microorganism. Thus, the system diagnosing the bioreactor system should have the function to recognize the state of the bioreactor system and processes, which is considered to be intelligent control or dynamic experiment planning. In other words, this system has a learning function in the narrow sense that it accumulates all data automatically in the data base so that it can be applicable to other cultivating operations.

In developing a prototype system, the BIOACS is assumed to be a diagnosed object. As the BIOACS has a typical structure as a bioreactor system, this system can be applied to any general bioreactor system. However, we have the necessary conditions that physiological activities can be monitored and that the system is integrated with the data base, which both have already been realized in the BIOACS. FIGURE 2 shows the flowchart of the BIOACS.

The diagnosing system should be provided with the following functions:

(1) efficient reasoning with an expert's heuristics,
(2) deep reasoning on the basis of the structure of the bioreactor system (BIOACS),
(3) evaluation of the effects caused by trouble,
(4) utilization of available characteristic data.

Furthermore, the system requires the following items:

(1) extensibility and maintainability,
(2) generality to be applied to any bioreactor system,
(3) generous framework for redundant knowledge,
(4) reliability of diagnosed results,
(5) combination with sensors and other software resources.

From the above-mentioned requirements, it is concluded that the system is required to be highly flexible by apt modulization of knowledge bases.

Knowledge Representation

In designing the configuration of knowledge bases, information or knowledge as shown below should be provided in order to diagnose a bioreactor system and cultivation processes:

(1) structural model of the bioreactor system,
(2) states or conditions of the bioreactor system and cultivation processes,
(3) heuristic logics extracted from diagnosing experts,

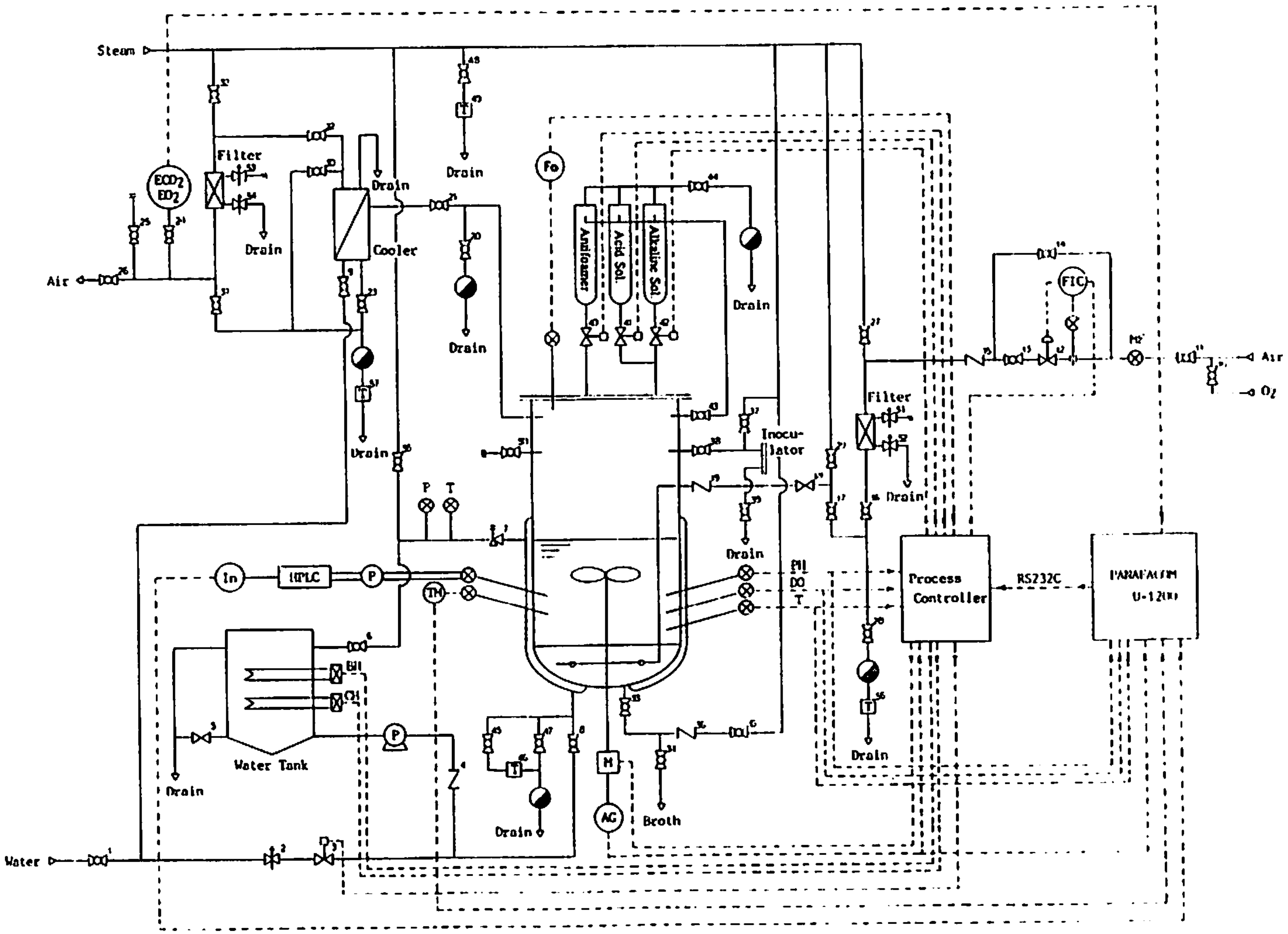

FIGURE 2. Flowchart of BIOACS. Abbreviations: BH = base heater; CH = control heater; AG = agitation speed meter; DO = dissolved oxygen concentration; HPLC = high performance liquid chromatograph; TM = turbidity meter; In = integrator; Fo = foam sensor; MF = mass flow meter.

(4) causality of events of troubles,
(5) data on characteristic properties of bioprocess parameters and physiological activities.

Taking into account these discussions and requirements mentioned in the previous section, we adopted the schemes of knowledge representation and the configuration of knowledge bases as shown in FIGURE 3.

Logics to diagnose the bioreactor system and cultivation processes are represented by production rules and they are modulized into several knowledge sources, each corresponding to the monitoring or controlling parameters such as temperature, pH, DO, etc. Knowledge on the structure of the bioreactor system composed of various equipment and knowledge on the states of the processes are represented by a network of frames, which is necessary for the application of deep reasoning. Moreover, knowledge on the causality of events of troubles is also represented by linkages of frames.

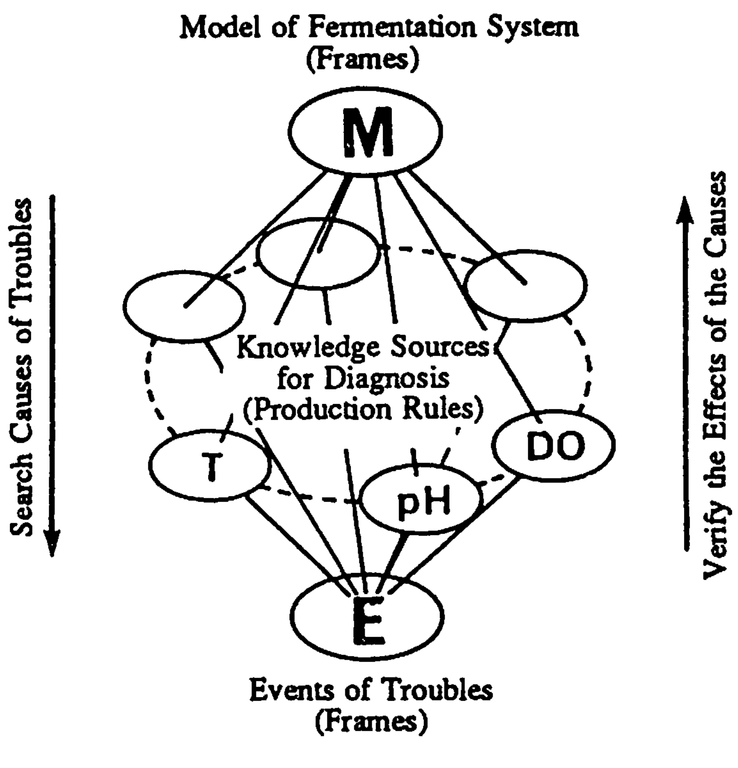

FIGURE 3. Configuration of knowledge bases.

Inferring Process

The inferring process for diagnosis is shown as follows:

(1) specification of a diagnosing item that is one of the monitoring or controlling parameters,
(2) entry of an observed unusual phenomenon, which is a trigger of diagnosis,
(3) activation of knowledge sources for diagnosing possible hypotheses, along with judgement effects with an operator's interaction,

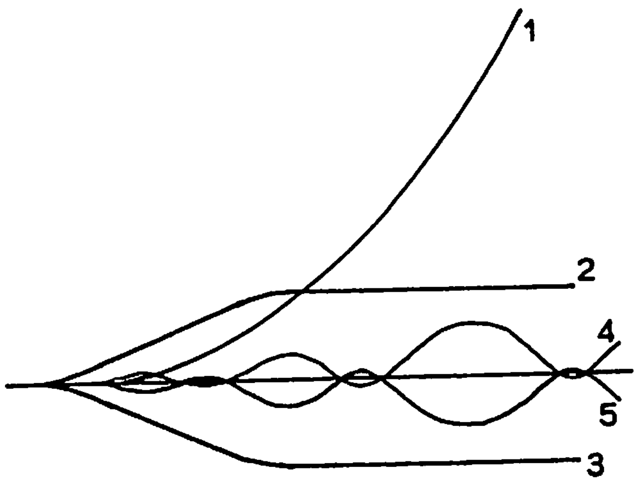

FIGURE 4. Typical troubled patterns of the time course (triggers of diagnosis).

(4) output of all the causal relations from the inferred cause until the observed event,
(5) output of the countermeasure.

The diagnosing item is specified as a typical troubled pattern against time, which is shown in FIGURE 4. The system infers to search for causes of troubles by activating knowledge sources that orderly refer to the structural model in order to obtain information on the state of the bioreactor system. When the system succeeds in searching a root cause, it estimates the effects of the cause on other parameters by tracing the causality. The system compares the real states with the estimated effects and verifies the diagnosed results. The inferred process and the assumed hypotheses are recorded on the blackboard and a simple explanation function can be achieved by displaying the contents on the blackboard before the system stops.

Prototype System

Based on the above-mentioned discussion, we prototyped an expert system for diagnosing the BIOACS with respect to temperature control, pH control, and DO

```
RULE# <1>
 IF
   (LESSP (@FGET 'PANEL-AG 'SV '(O N)) *AGSVMIN)
 THEN
  1 PROPOSE
    CHANGE TYPE:  ADD
    EVENT NAME :  PRINT-CONCLUSION
    LEVEL      :  DIAGNOSTIC-STATE
    ATTRIBUTES : DIAGNOSTIC-ELEMENTS =  'AG-FAULT
                  <CF>  0.9
                 IDENTIFY            =  'AG-SV-LOW
```

FIGURE 5. An example of production rules for diagnosis.

```
(@DEFRAME
   'FERMENTOR-A
   '(CLASS (¥VALUE (INDIVIDUAL)))
   '(AKO (¥LINK (FERMENTOR)))
   '(BROTH (¥IF-NEEDED
            ((#INPUT-HIGH-LOW
              "Input a level of broth. (HIGH/LOW)"))))
   '(AGITATOR (¥LINK (AGITATOR-A)))
   '(THERMOMETER (¥LINK (THERMOMETER-A))))
```

FIGURE 6. An example of frames representing equipment.

control. We implemented a prototype system with AI tool *Eshell* in UTILISP on a main frame computer, namely, Fujitsu M780.

An example of production rules for diagnosis, an example of frames representing equipment, an example of frames representing events causality, and an example of frames representing root causes are shown in FIGURES 5, 6, 7, and 8, respectively. An executed example of the diagnosing system is shown in FIGURE 9.

The diagnosing system is a stand-alone system in the present stage. Therefore, the reasoning starts with a manual entry of an observed troubled pattern and the human operator should answer interactively to the questions on states of the bioreactor system according to the system prompt. A data base for characteristic data on the fermentation can be prepared, but it has not been implemented yet in the prototype system.

Concerning the specification of the expert system for diagnosing the BIOACS with regard to the control of temperature, pH, DO, and agitation speed, the number of rules for diagnosis is 141 and the number of frames is 70 for a structural model and 140 for relations between events.

CONCLUSIONS

We presented a concept of an advanced cultivation system where robots, expert systems, and a data base system are introduced. We discussed functions for the ESCO, focusing first on the diagnosis of a bioreactor system and cultivation processes. Then, taking account of requirements for the diagnosing system, we discussed the knowledge representation and the inferring process. Finally, based on these discussions, we

```
(@DEFRAME
   'ACID-ADDITION-INSUF
   '(CLASS (¥VALUE (GENERIC)))
   '(SUPERC (¥LINK (ACID-ADDITION-FAULT)))
   '(SUBC (¥LINK (ACID-CONC-INSUF) (ACID-TIMER-UNSUIT-CLOSE)))
   '(MESSAGE
      (¥VALUE ("Therefore, acid cannot be added sufficiently. "))))
```

FIGURE 7. An example of frames representing events causality.

```
(@DEFRAME
   'V42-AO-LONG
   '(CLASS (¥VALUE (GENERIC)))
   '(SUPERC (¥LINK (ALKALINE-TIMER-UNSUIT-OPEN)))
   '(MESSAGE (¥VALUE ("Open-time of VALVE-42 (AO) is set too long. ")))
   '(COUNTERMEASURE (¥VALUE ("Set shorter time to AO. "))))
```

FIGURE 8. An example of frames representing root causes.

```
 INPUT DIAGNOSTIC ITEM  (1. TEMP /2. pH /3. DO) > 2
 INPUT TRIGGER > 1
 Input a state of PANEL-SWITCH NC for pH. (ON/OFF) > OFF
 Is the solution for pH control added to broth? (YES/NO) > NO
 Is there acid solution within the acid pot? (YES/NO) > YES
 Input a state of PANEL-SWITCH BSW. (AUTO/OFF/MANUAL) > AUTO
 Input a state of VALVE-43. (OPEN/CLOSE) > OPEN
 Turn the switches BSW to MANUAL and ASW to OFF. Is acid added to
broth? (After tested, turn both switches to AUTO.)  (YES/NO) > NO


      *** RESULTS OF DIAGNOSIS ***
  There is a trouble in VALVE-41.
  VALVE-41 is closed.
  Therefore, acid can not be added.
  Because acid isn't added to broth functionally,
  pH can not be controlled.

      *** COUNTERMEASURE ***
  Repair VALVE-41.


  ** 異常診断 の CURRENT ノード **
  NODE NAME <診断項目>
    PROTO: 異常診断
  ** DIAGNOSTIC-STATE の CURRENT ノード **
  NODE NAME <DIAGNOSTIC-STATE1>
    PROTO: DIAGNOSTIC-STATE
    DIAGNOSTIC-ELEMENTS = ((PH-CONTROL-MODE-UNSUIT -1.0))
  NODE NAME <DIAGNOSTIC-STATE2>
    PROTO: DIAGNOSTIC-STATE
    DIAGNOSTIC-ELEMENTS = ((ACID-ADDITION-FAULT 1.0))
    IDENTIFY            = (BSW-V41-TROUBLE-CLOSE)
```

FIGURE 9. An executed example of the diagnosing system.

prototyped an expert system for diagnosing cultivation processes and verified its efficiency.

SUMMARY

A new concept of a fully advanced cultivation system aiming at bioprocess automation is presented, which is composed of BIOTRON (a robotized cultivation factory), ESCO (Expert System for Cultivating Operations), BEXS (Bio EXpert System), and BDBS (Bio-Data Base System). Especially functions required of ESCO such as diagnosis of trouble, intelligent process control, and dynamic experimental design are discussed in this report. In designing an expert system for diagnosing a bioreactor system and cultivation processes, information and knowledge necessary for diagnosis are clarified and a new methodology for knowledge representation and a strategy of inferring processes are proposed, based on functional requirements and system requirements. Finally, specification of a developed prototype system is reported and its efficiency is verified.

ACKNOWLEDGMENT

We wish to acknowledge Noriko Koizumi at Fuji Facom Company, Limited, for her kind advice in the implementation of a prototype system using *Eshell.*

REFERENCES

1. STEPHANOPOULOS, G. & G. STEPHANOPOULOS. 1986. Trends Biotechnol. **32:** 241–249.
2. O'CONNOR, G. M. & C. L. COONEY. 1986. ACS Meeting, Anaheim, MBTD paper no. 85.
3. ENDO, I. & T. NAGAMUNE. 1987. Bioprocess Eng. **2:** 111–114.
4. ENDO, I., H. ASAMA & T. NAGAMUNE. 1989. *In* Bioproducts and Bioprocesses. A. Fiechter, H. Okada & R. D. Tanner, Eds.: 337–346. Springer-Verlag. Berlin/New York.
5. NAGAMUNE, T., *et al.* 1985. Japan patent laid-open publication no. 57–201, 954; United States patent no. 4,561,799.
6. ENDO, I., *et al.* 1985. Japan patent laid-open publication no. 57–68, 781; United States patent no. 4,501,161.
7. ENDO, I., T. NAGAMUNE & I. INOUE. 1983. Ann. N.Y. Acad. Sci. **431:** 228–230.
8. NAGAMUNE, T., I. ENDO & I. INOUE. 1984. Operation charts for a successive fed-batch fermentation of alcohol. Kagaku Kogaku Ronbunshu **10:** 506–512.
9. ASAMA, H., *et al.* 1988. Japan-Finland Symposium on Automation Technologies and AI Applications for Bioprocesses, p. 1–3.