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CHAPTER 3

Computer-Assisted Fermentation Developments

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Computer applications in fermentation have been slow in coming. Reasons for this include a reluctance to change, inadequate sensor developments, and the difficulties in analytically modeling fermentation processes. Some companies have utilized computers in a direct digital control scheme as a more economical substitute for analog controls. Others have used computers primarily in data acquisition and analysis schemes. The real power of computers in application to fermentations is yet to be realized. Most probably it will be achieved utilizing computers for on-line analysis of cell biomass concn, growth rate, and substrate uptake through component material balancing. Fermentation companies, in the near future, will begin to use a system of computers in which microprocessers will control fermentation functions and/or individual fermenters. These microprocessers will interface with a minicomputer for management control. In turn, the minicomputer will interface for data transfer and storage with a large frame computer handling many company tasks.

INTRODUCTION

History may never record all those individuals or companies who have contributed to the evolution of computer-assisted fermentation systems. Numerous companies and individuals have been involved. Some of these have been doers and have been limited by company secrecy from describing their systems. Others have been philosophers who have talked about computer-aided fermentations but with little or no operating experience. Each group has made contributions to the evolution of computer-aided fermentation systems. The approaches have been varied, primarily because the various companies and inviduals saw different reasons and evolved different justifications for utilizing computers in fermentation systems. Some saw direct digital control as a more economic alternative to analog control, particularly in new plants. Others felt that computers would be most useful in the task of data acquisition and reduction. Some viewed computers as a means to on-line process optimization. A few saw the computer as a means to on-line monitoring of biomass concn, growth rate, and substrate utilization by indirect measurement through component balancing. A number of papers have reviewed these various approaches to utilizing computers in fermentation systems (Aiba et al. 1973; Armiger and Humphrey 1974; Humphrey 1973, 1974, 1975; Jefferis 1975; Nyiri 1971, 1973, 1974, 1975; Nyiri et al. 1974).

It is my feeling that this latter view in the end will be the most significant role for computers in fermentation systems. The reasons for this belief stem from rapid emergence of high capacity (32K byte chips), cheap (<$300) microprocessers, which will be capable of data acquisition, analysis, and control. Microprocessers will substitute for, and provide greater sophistication than, existing analog controllers. They will do this for the same or cheaper costs.

Future computer-aided fermentations will involve a system of computers. Microprocessers will be used to analyze and control individual functions and/or fermentors. They will interface with minicomputers that will manage a series of microprocesser-controlled fermenters, e.g., the pilot plant, the penicillin plant, etc. In turn, these minicomputers will communicate with a large frame computer, handling many company tasks, which will store and process large files of data and regurgitate these data on demand for analysis and graphical output at minicomputer terminals (Fig. 1). The technical feasibility of such a system exists now. Indeed, the

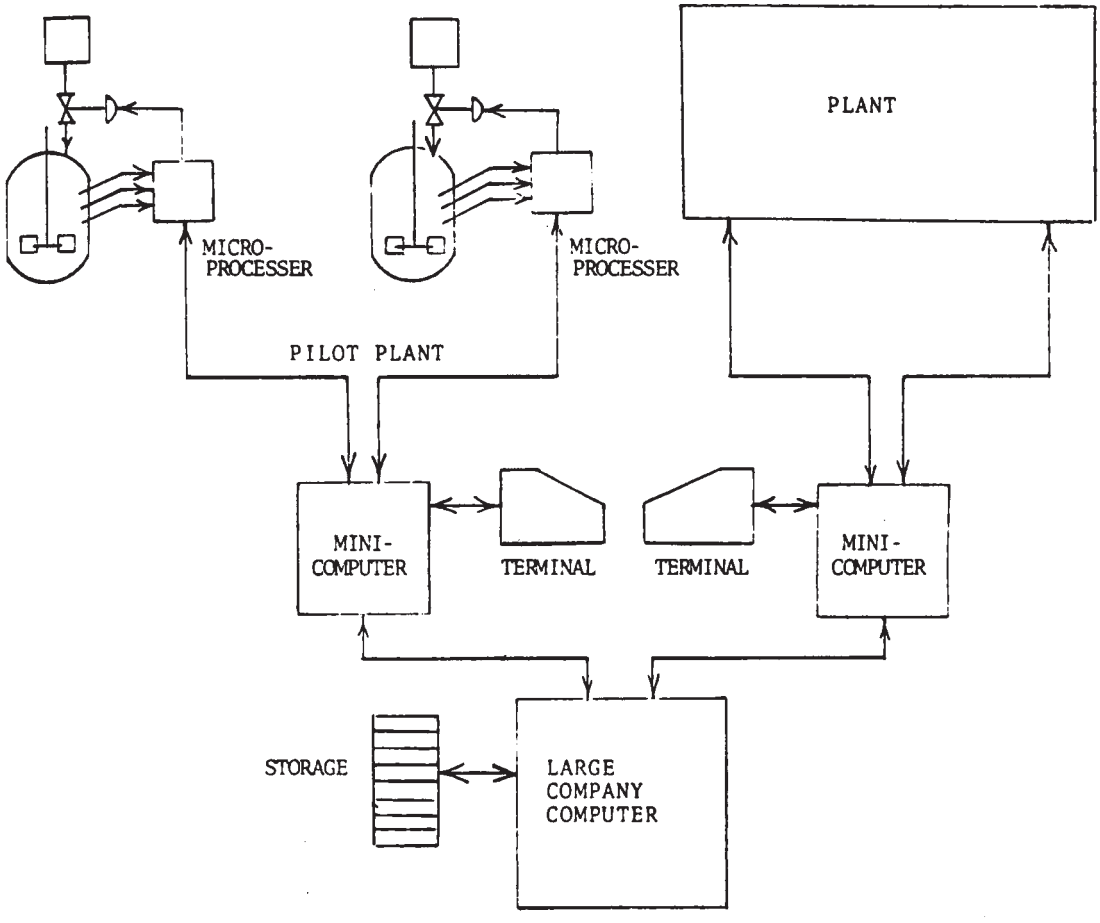

FIG. 1. Future computer-aided fermentations systems.

University of Pennsylvania has a MOS Technology Inc. KIM-1 microprocesser, calculating and transmitting analyzed data to a Digital Equipment Corp. PDP11E10 minicomputer which communicates with a UNIVAC 70/46 computer for purposes of data storage, subroutine transfer, and reprograming of the two 1024 byte ROM in the microprocesser. Within 1 to 3 years microprocessers will begin to impact on the fermentation industry. The bottlenecks to their rapid use will be: (1) hesitancy on the part of older fermentation technologists to accept change; (2) a lack of sensor development to enable full use of the computer analysis power; and (3) limited understanding of fermentative pathways and metabolic control mechanisms so that process modeling is only approximate at best. When the economic pressures become sufficiently great and the need to stay competitive demands it, then computer applications will become more widespread.

Discussion

Historical Application of Computers

The original developments in computer applications to the process industry began in the early 1960s (Trambouze and Mueller 1973). By 1968 publications on computer applications in

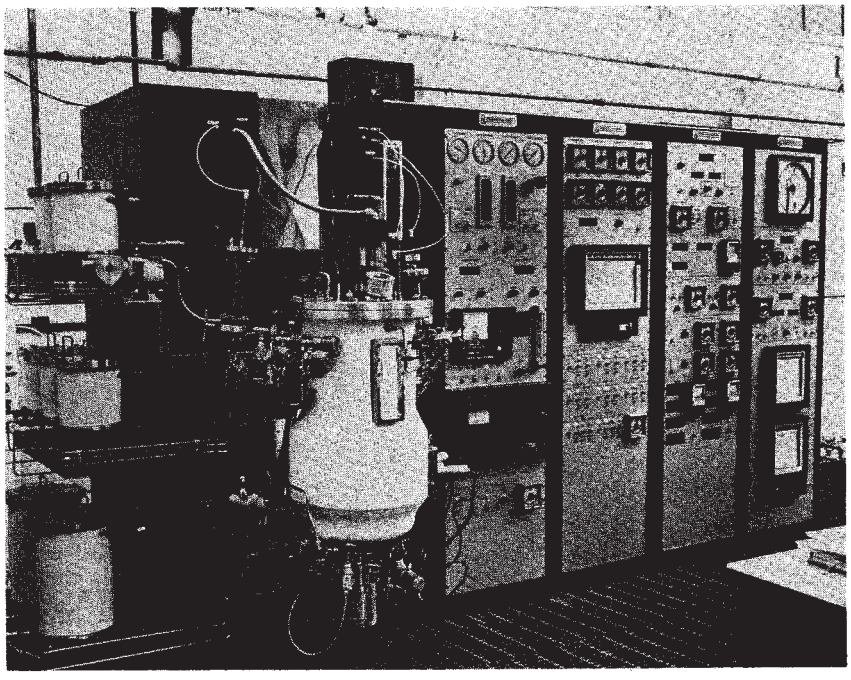

FIG. 2. Highly instrumented fermentation system.

batch process control were fairly common (Huang and Sonn 1972; Itahara 1968; Yamashita et al. 1967, 1968, 1969). Indeed, a tongue-in-cheek article appeared in 1969 entitled, "The computer myth – a way out of the maze," attempting to analyze the reasons for the numerous computer articles and the lack of their application to a large number of processes (Kovac 1969). What was true in 1969 is no longer true today. Computer applications are indeed widespread (Humphrey 1973; Jefferis 1975; Nyiri 1973, 1975; Trambouze and Mueller 1973; Wilson 1975).

The first papers on the use of digital computers specifically relating to fermentation processes appeared in articles by Yamoshita and Murao (1967) and Koga et al. (1967). However, the first real descriptions of computer-coupled fermentation systems were those of Yamoshita et al. (1969), Grayson (1969), and Harrison et al. (1970). From 1971 on numerous publications appeared on computer-aided fermentation system design and philosophy, and in 1973 the first conference on computer process control in fermentation was held in Dijon.

As mentioned earlier, it is not clear just which companies first utilized computers in fermentation processes because of secrecy policies of various companies. It is now known that in 1966 the Ajinomoto Company in Japan utilized a YODIC-500 computer, developed by Yokogauo Electric Works, for direct digital control of their glutamic acid fermentations (Yamashita et al. 1969; Yamashita 1972). Also, in 1966, Dista Products entered into detailed studies with Elliott-Automation which resulted in a decision to put 114 control loops in the new fermentation plant at Speke under the direct digital control of an ARCH 102 computer (Anon. 1967). Glaxo Laboratories, Ltd., in 1969, on the basis of cost considerations, converted a fermentation plant with 200 bits of analog data output to direct digital control. In 1969 the University of Pennsylvania under a NIH contract began the construction of a highly instrumented fermentation system (Fig. 2) which was interfaced in the spring of 1973 with a

PDP 11E10 computer (Armiger and Humphrey 1974; Armiger et al. 1975; Harrison et al. 1970; Harrison and Harmes 1972). This was a research-oriented pilot plant system, focusing on data acquisition, analysis, and digital set point control. Similar research-oriented systems were assembled during this same period at E. R. Squibb (Moes et al. 1971; Young and Koplove 1972), The Lord Rank Research Center (Flynn 1974),.Station de Gene Microbiologique, Dijon (Blachere 1973; Corrieu et al. 1974; Lane 1973), Karolinska Institute (Unden and Heden 1973), Gessellschaft für Biotechnologische Forschung mbH (Jefferis 1975), and many others (Anon. 1973; Cooney et al. 1975; Jefferis 1973, 1975; Jefferis et al. 1973; Nyiri et al. 1975; Ohashi et al. 1976). Figure 3 illustrates a typical pilot plant system.

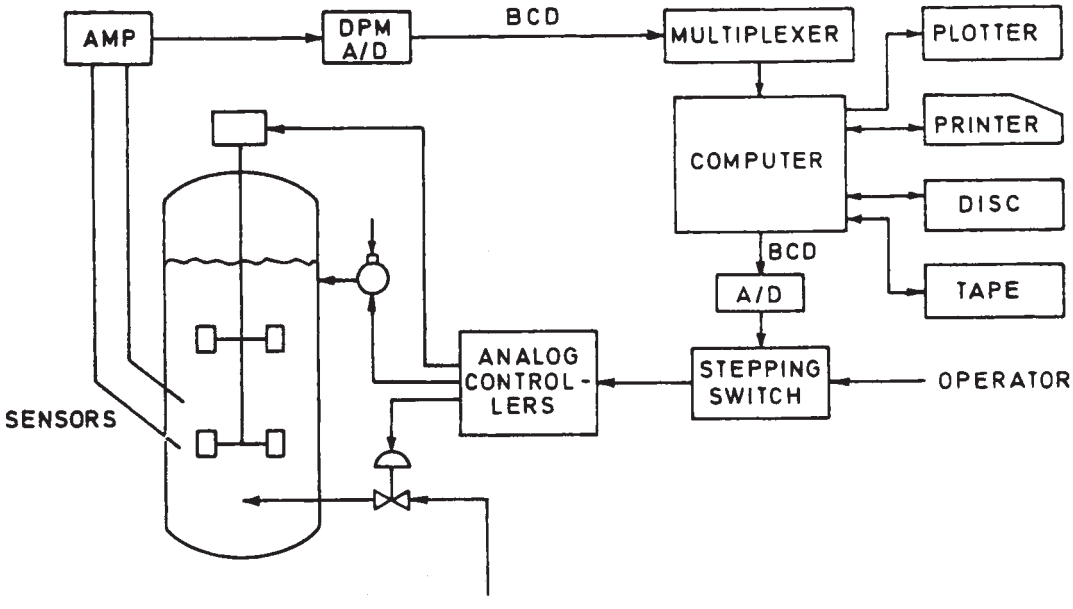

FIG. 3. Typical pilot plant computer-coupled fermentation system.

Indirect Measurement Concept

The possibility of using a computer to estimate, on-line by substrate consumption measurement, the biomass concn and cell growth rate and then, from a simulated model, to predict the product formation rate was first described by Yamashita and Murao (1967). The idea was expanded upon in 1969 (Yamashita et al. 1969). However, it wasn't until 1971 in publications by Humphrey (1971) and Nyiri (1971) that the full possibility of using component balancing around the fermentation to estimate biomass concn and growth rate was outlined. The first on-line application of component balancing utilizing oxygen was described in a paper by Jefferis et al. (1972). The idea is embodied in French and U.S. patents on the technique (Wilson 1975; Wilson et al. 1974, 1975).

The basis of the technique is illustrated in Fig. 4. From the consumption rate, Q_iX, it is possible to describe the biomass concn and growth rate by taking into account that the component is required both for growth and for maintenance of the biomass, i.e.

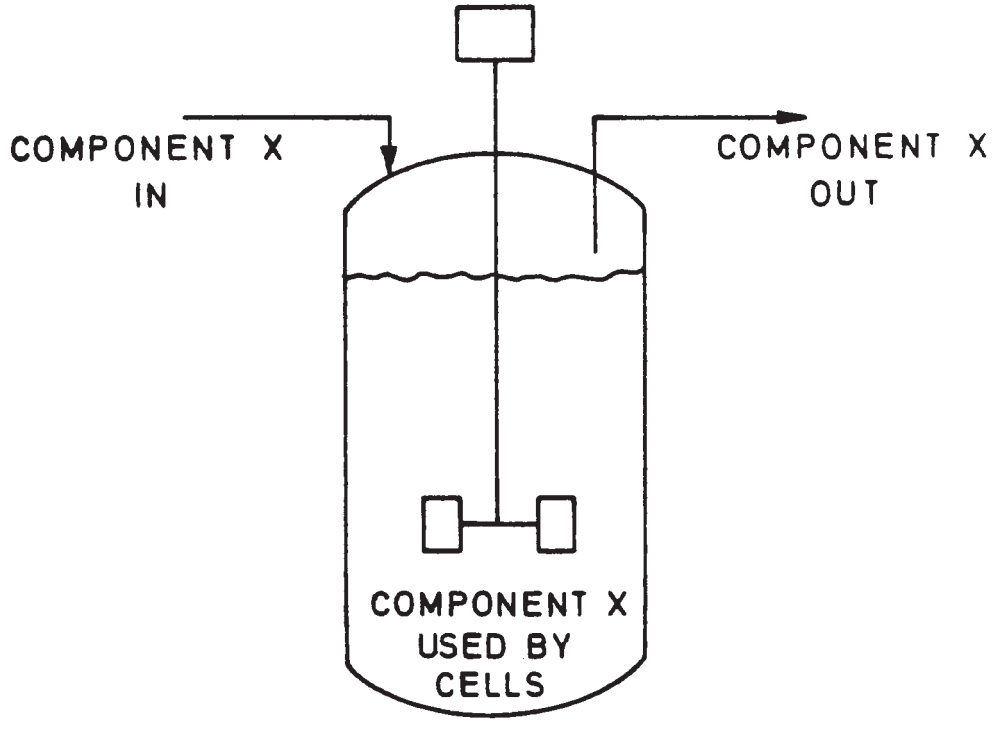

FIG. 4. Component balance around fermenter.

$$Q_i X = \frac{1}{Y_{x/i}} \frac{dX}{dt} + mX \tag{1}$$

where

Q_i = specific consumption rate of component *i*, g *i*/g cell-h,
m = specific maintenance requirement for component *i*, g *i*/g cell-h,
X = biomass concn, g cell/liter,
t = time, h,
$Y_{x/i}$ = maximum growth yield, g cell/g *i* used for growth.

The constants m and $Y_{x/i}$ are obtained from yields vs. growth rate data (Fig. 5). Equation (1) can be rearranged to give the growth rate, i.e.

$$\frac{dX}{dt} = Y_{x/i}(Q_i X - mX) \tag{2}$$

The idea behind this concept is to obtain the component consumption rate by an on-line computer-aided component balance around the fermentor. Then from a point at which the biomass concn has been initialized, a continuous estimation of biomass concn and cell growth rate can be made (Cooney et al. 1976; Humphrey 1975). Either the biomass concn or the

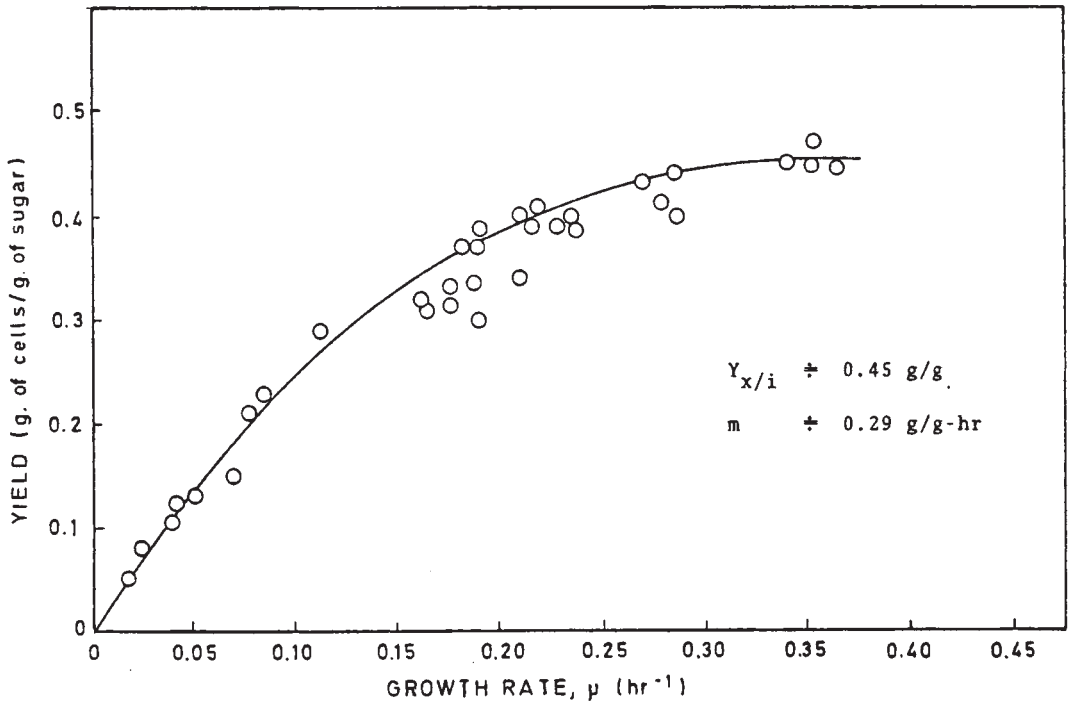

FIG. 5. Typical yield data for baker's yeast grown on sugar-limited medium (data from Wang et al. 1976).

growth rate can be used as a control variable. Application of this technique has been described in the penicillin fermentation (Ryu and Humphrey 1971, 1973) and in the baker's yeast fermentation (Wang et al. 1976).

Two very excellent theses have recently appeared on the use of component balancing to follow and control various fermentations. One was by Zabriskie (1976) at Pennsylvania, in which applications of oxygen balancing around three different fermentations, one utilizing baker's yeast, another utilizing a thermophilic actinomycetes, and a third utilizing a streptomyces, were described. The advantages to the method were obvious. In one system a CSL medium was employed in which turbidometric methods could not be used to estimate biomass concn. In another a solid cellulose substrate was used. Indirect measurement of biomass by material balancing was the only reliable means of estimating biomass concn. The other thesis by Wang (1976) at M.I.T. was concerned with computer-aided carbon and nitrogen balancing for estimating the yeast concn and growth rate and then using this information in the optimal control of a baker's yeast fermentation. The success of the system was evidenced by the fact that 80-100 g/liter of yeast could be consistently obtained in a batch process. A computer algorithm for such a system evolved by Wang (1976) is shown in Fig. 6.

In the work by Minkevich and Eroshin (1974) and later by Zabriskie (1976) and by Wang (1976) it was shown that since yeasts have several energy yielding metabolic pathways, the consumption both of oxygen and of sugar differs with each metabolic route. Both the yield and maintenance coefficients have to be expressed as functions of the various pathways. Zabriskie suggested that one approach would be to define the yield and maintenance constants as those expected when cellular energy is entirely derived by metabolizing 1 mole of glucose to 6 moles of CO_2 and H_2O and 36 moles of ATP by the glycolytic and tricarboxylic

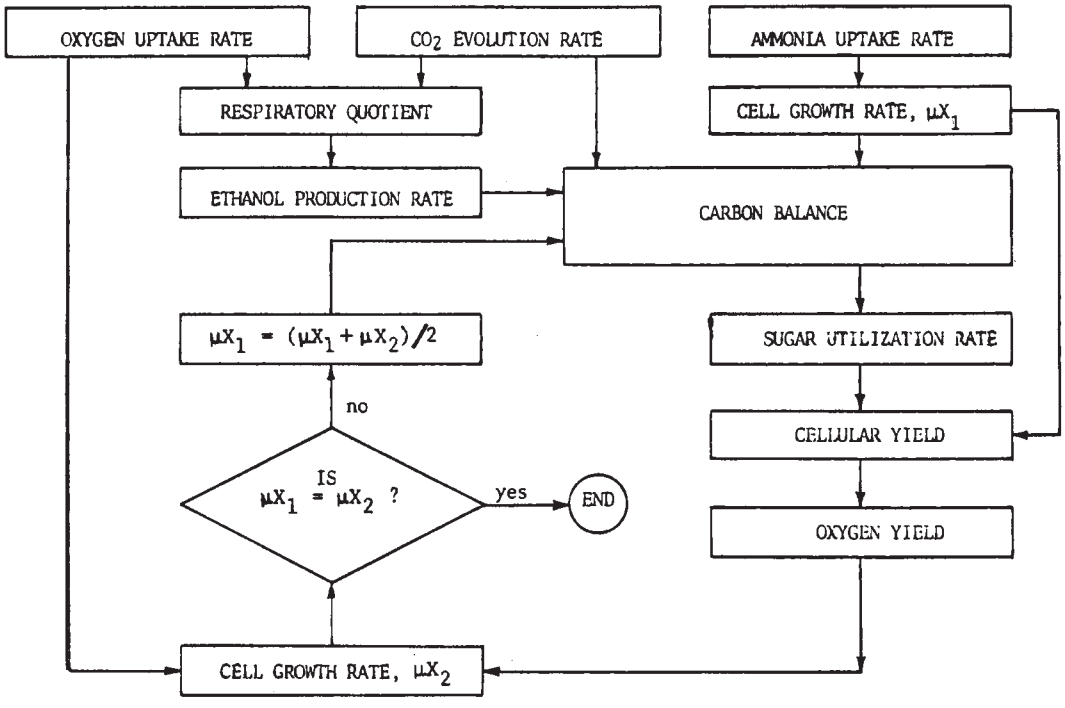

FIG. 6. Computerized indirect measurement scheme by component balancing (Wang et al. 1976).

acid cycle sequences. Then the consumption rate would be corrected by a metabolic correction function, β, whose value depends on the relative rates of other significant energy yield metabolic pathways, i.e.

$$\beta\,(Q_i X) = \frac{1}{Y_{x/i}}\,\frac{dX}{dt} + mX \tag{3}$$

For that period of aerobic baker's yeast growth under high glucose concn and accumulating ethanol as a product, Zabriskie showed by ATP energy balancing that the oxygen consumption correction could be given by:

$$\beta = \frac{\text{R.Q.} + 5}{6} \tag{4}$$

where R.Q. = CO_2 evolution rate/O_2 c onsumption rate. This suggests that material balances must be coupled to measurements of respiratory quotients if meaningful control is to be achieved. For dauxic growth on ethanol and in the absence of glucose, he concluded that the metabolic correction factor was constant and approx. equal to 0.78.

Both Wang and Zabriskie noted that the yield and maintenance coefficients were strong functions of temperature and pH and hence had to be controlled. These observations suggest that future computer-controlled fermentation systems will have to have the means for measuring and/or controlling at least pH, temperature, air flow rate, and O_2 and CO_2 concn in the entering and exit gasses. Further, it will be useful if the carbon and nitrogen substrate additions can be monitored, as well as the dissolved oxygen. Figure 7 is an illustration of this ideal computer-aided fermentation system.

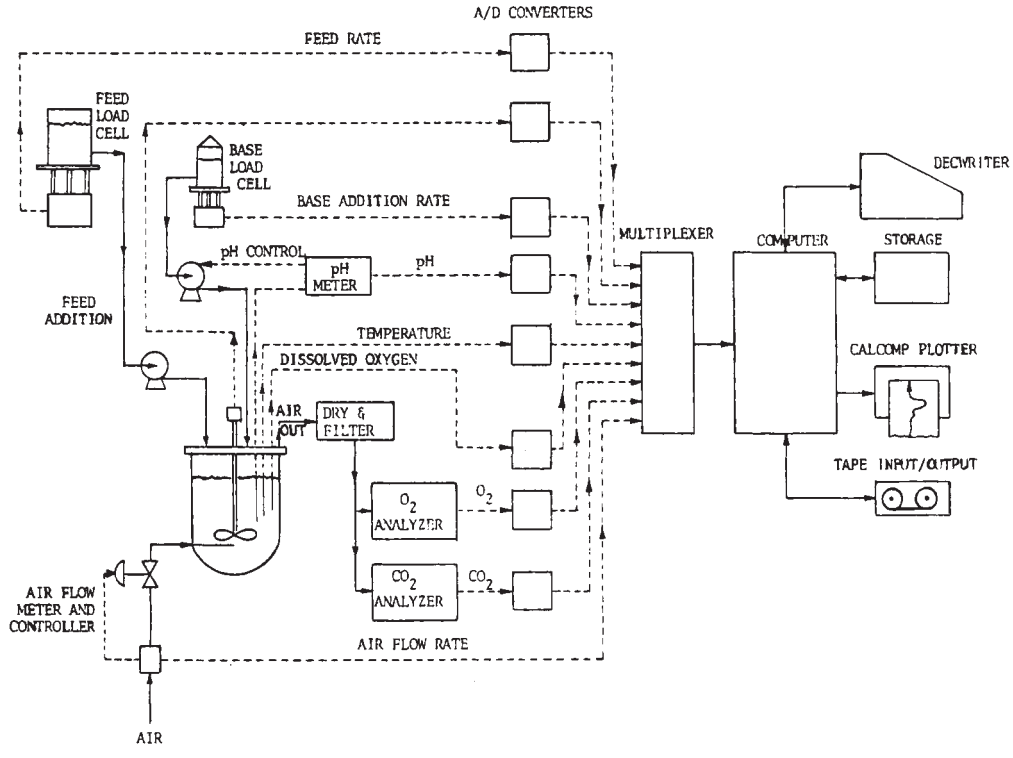

FIG. 7. Ideal computer-coupled, highly instrumented fermenter.

Recently, Harima (1976) expanded on the idea that since consumption rate is related to production rate, one ought to be able to measure the CO_2 evolution rate and relate it to biomass concn and growth rate. For the growth of an organism such as *Tricoderma viride* on glucose or cellulose, which has a relatively simple metabolic pathway and in which the R.Q. is nearly 1, i.e. $Q_{CO_2} = Q_{O_2}$, Harima obtained the following relationships between CO_2 evolution, glucose consumption, and growth rate:

$$Q_{CO_2}X = Q_{O_2}X = 0.0245X + 0.423\frac{dX}{dt} \tag{5}$$

and

$$Q_{\text{glucose}}X = 0.0428X + 1.68\frac{dX}{dt} \tag{6}$$

These relationships allow biomass concn, growth rate, and glucose consumption to be estimated only from the monitoring of CO_2 evolution. Figure 8 illustrates the kind of on-line computer fit equations (5) and (6) will give. In the case of a more metabolically complex organism such as baker's yeast, Harima concluded that independent measures of CO_2 evolution and O_2 uptake were needed to estimate cell growth rate and glucose consumption. He obtained correlations of the following form:

$$\frac{dX}{dt} = 0.484\,X\,Q_{CO_2} + 0.886\,X\,Q_{O_2} - 0.0191X \tag{7}$$

and

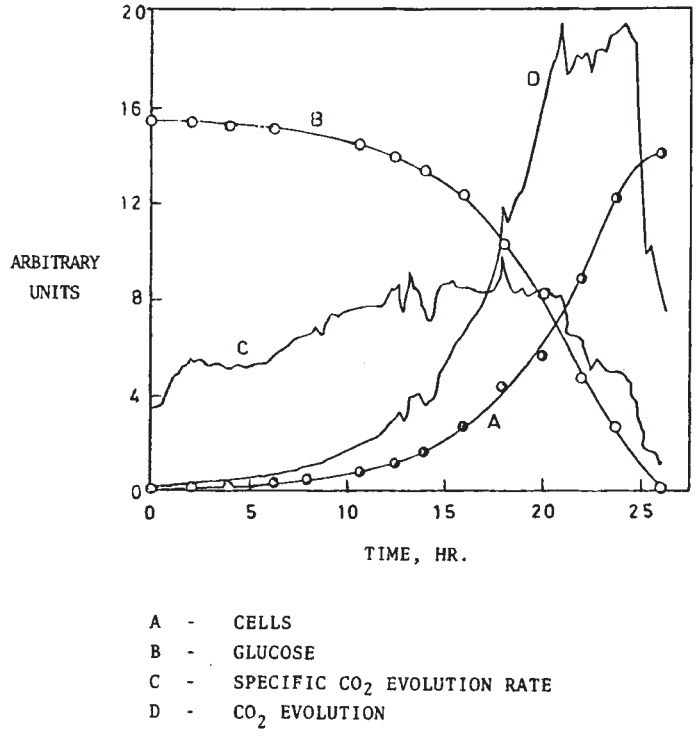

FIG. 8. On-line computer estimate of cell and glucose concn for growth of *T. viride* on glucose (after Harima 1976).

$$Q_{\text{glucose}}X = 4.02\,X\,Q_{CO_2} - 2.68\,X\,Q_{O_2} + 1.36\frac{dX}{dt} + 0.746X \qquad (8)$$

for the portion of growth in which appreciable concn of glucose occur in the fermentation medium.

Process Optimization Using Computers

A number of researchers has viewed the ultimate utility of the computer in fermentation processes for on-line optimization of the process (Aiba and Okabe 1974; Anon. 1973; Bourdaud and Foulard 1973; Jefferis 1973; Nyiri 1974, 1975; Shu 1972; Unden and Heden 1973). The objective has been to optimize product concn (Nyiri 1974; Ohasha et al. 1976; Wang et al. 1976) or product productivities (Calam and Russell 1973; Ryu and Humphrey 1971, 1973). The difficulty has been that, while models have been evolved to simulate the various fermentation processes, a real understanding of these fermentation processes has been lacking, hence, making it difficult to evolve a sound optimal strategy. Successful attempts at optimization can be found in the works of Ohashi et al. (1976) and Wang (1976). These researchers noted that a relationship exists between R.Q., oxygen yield, and cellular yield. By monitoring R.Q. via a computer-aided system, they were able to evolve an optimal strategy for control of a batch fed baker's yeast fermentation in order to maximize biomass yield.

In some fermentations, particularly antibiotic fermentations, the objective function to be optimized is not simply product concn nor product productivity; it is usually profit. Some day the computer will take over plant scheduling function as part of its dynamic optimization

function. Before that occurs, an almost order-of-magnitude improvement in process reliability will have to be made to make such a venture worthwhile. The bottlenecks to this are limited understanding of the process kinetics, sensor accuracy, and reliability. Greater effort is needed here.

As mentioned earlier the practical utilization of computers in plant production units has been in substitution of direct digital control for analog control. The motivation for the substitution has been largely a cost factor (Blachare 1973; Calam and Russel 1973; Flynn 1974; Grayson 1969; Yamashita et al. 1969; Yamashita 1972). Computer systems have been utilized in glutamic acid fermentations (Yamashita et al. 1969; Yamashita 1972), in baker's yeast fermentations (Nyiri 1974; Ohashi et al. 1976; Wang 1976), in single cell protein fermentations (Armiger et al. 1975; Flynn 1974; Nyiri and Jefferis 1973), in penicillin fermentations (Calam and Russell 1973; Ryu and Humphrey 1971, 1973), and in other antibiotic fermentations (Anon. 1967, 1973; Grayson 1969; Lane 1973).

Design of Computer-Coupled Fermentation Systems

A number of papers have been devoted primarily to the design of computer-coupled systems (Cooney et al. 1975; Corrieu et al. 1974; Flynn 1974; Harmes 1972; Harrison et al. 1970; Jefferis 1973; Lane 1973; Moes et al. 1971; Nyiri 1971, 1972; Nyiri et al. 1975; Trambouze and Mueller 1973; Wilson 1975a,b; Wilson et al. 1974, 1975; Yamashita 1972; Young and Koplove 1972). Some have focused on the instrumentation and equipment (Cooney et al. 1976; Harmes 1972; Harrison et al. 1970; Lane 1973; Moes et al. 1971; Trambouze and Mueller 1973; Wilson 1975a,b; Yamashita 1972; Young and Koplove 1972); others have focused on the control strategies and software (Corrieu et al. 1974; Flynn 1974; Jefferis 1973;

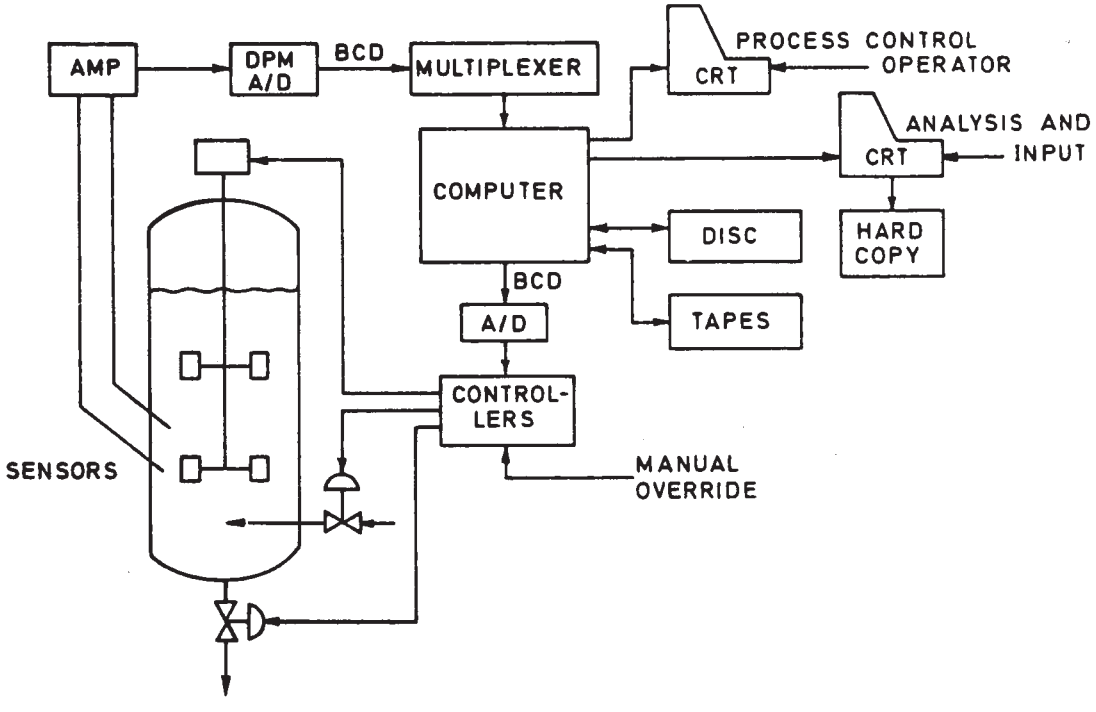

FIG. 9. Future computer-coupled fermentation system interface.

Jefferis et al. 1973; Nyiri 1971, 1972, 1974; Wilson et al. 1974, 1975; Yamashita et al. 1969). It is now obvious from the success of the indirect measurement techniques that key instrumentation on a computer-coupled system includes means for monitoring: pH, temperature, vessel pressure, gas flow rate, gaseous O_2 concn, gaseous CO_2 concn, dissolved oxygen, sugar addition rate, nitrogen substrate addition rate. Control systems needed include means for controlling: pH, temperature, vessel pressure, gas flow rate, agitation speed, dissolved oxygen, sugar addition rate, nitrogen substrate feed rate, foam. These systems will be interfaced to give the means for continuously monitoring: cell biomass concn, cell growth rate, O_2 uptake rate, CO_2 evolution rate, respiratory quotient, sugar consumption rate, and combining them in some kind of an algorithm for the control and optimization of the fermentation process. Both the direct digital control, primarily for plant scale equipment, and digital set point control, primarily for research and pilot plant scale equipment, will be used. The latter is preferred on the research scale because it can be made modular, expanded at will, and instruments readily substituted as improvements are made.

The one design topic about which little has been written is the man-computer interface. My own feeling is that the visual, cathode ray tube (CRT) terminals are much better than printers. Not only are they faster but one has the possibility of combining alpha numerics with graphical displays. For example, the process flow diagram can be displayed and the settings of each valve or instrument shown. This allows for faster operator-system interaction plus faster system diagnostics when there are process problems.

In Fig. 9 a future or ideal system interface is indicated. It uses one CRT for the process control operator. A second CRT is utilized for process diagnostics and control modification input. The latter CRT need not be located in the control room. Indeed, for management reasons it may well be located in a remote office so as not to disturb the process operator functioning.

Conclusions

Computer-assisted fermentation technology has developed to the point where it is now ready for general application to the fermentation industry. The key development has been the indirect measurement by component balancing of such process variables as cell biomass, cell growth rate, substrate consumption rate, and respiratory quotient. Future fermentation processes will have a system of computers that consist of microprocessers managed by minicomputers connected to a large frame computer for data storage and processing.

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