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MONITORING AND CONTROL OF A FED-BATCH FERMENTATION

JOSÉ A. TEIXEIRA, MARIA L. SOUSA,
SEBASTIÃO FEYO DE AZEVEDO, MANUEL MOTA
University of Porto
Rua dos Bragas, 4099 Porto Codex Portugal

Fed-batch operation is growing in importance in the fermentation industry. Major biotechnological products such as penicillin and baker's yeast are obtained in units operating under such a regime. Fed-batch culture is an effective means of overcoming inhibition from high initial substrate concentrations. Many authors have reported the use of programmed nutrient feeding to increase the yield and productivity of cells and metabolites.[1-4]

The introduction of equipment for the on-line monitoring and computer control of batch fermentors allows for a several-fold increase in productivity.[5] Fed-batch operation is more complex than the classical batch operation. Exploiting for the former all the flexibility and power of computer control strategies together with innovative fermentation technologies is becoming a necessary feature of operation for competitive production/cost ratios.

As it stands, elucidative (yet simple) experiments dealing with fed-batch operation should be included in the traditional chemical engineering curriculum. The experiments should be designed to help the student develop an understanding of how computers can be used to improve the operation of fermentation processes.

The experiment described below consists of a very simple laboratory-scale fed-batch operation of an alcoholic fermentation. Baker's yeast is the micro-organism and glucose is the carbon source. It enables the students to become familiar with fed-batch operation, on-line monitoring and computer control (*i.e.*, sensing, serial and parallel communications), and model-based control decisions, all at the same time. The experiment is inexpensive and can probably be carried out in chemical engineering departments around the world.

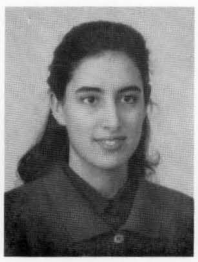
BACKGROUND

In alcoholic fermentation, using *Saccharomyces cerevisiae,* the stoichiometry of glucose conversion to ethanol and CO_2 is given by[1]

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} \text{ -------} \underset{\text{(ethanol)}}{2C_2H_5OH} + \underset{\text{(carbon dioxide)}}{2CO_2} \qquad (1)$$

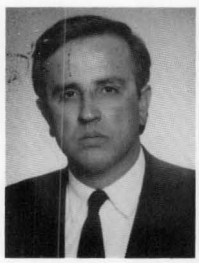
From this equation it may be seen that 0.511 g of ethanol and 0.489 g of CO_2 are produced from each gram of consumed glucose. As some of the glucose is used for the production and synthesis of secondary products and cell components, the real stoichiom-

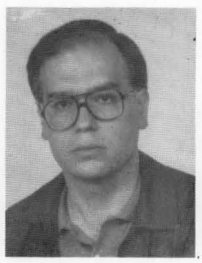
José A. Teixeira *is a Lecturer in the chemical engineering department, University of Oporto where he earned his licenciate in chemical engineering in 1980. He earned his PhD from the University of Oporto in 1988. His main interests are in fermentation and enzymatic technology.*



Maria Luísa Sousa *is a graduate of the chemical engineering department, Oporto University (1990). She is currently a research assistant working for her PhD in flocculation bioreactors.*

Sebastião Feyo de Azevedo *is an associate professor of chemical engineering at the University of Oporto. He is a licenciate in chemical engineering from the University of Oporto (1973) and earned his PhD in chemical engineering from the University of Wales (1982). His interests are in the areas of modeling, optimization, and process control.*





Manuel Mota *is an associate professor in the department of chemical engineering, University of Oporto, where he earned his licenciate degree in 1972. He received his PhD in biochemical engineering from INSA (Toulouse) in 1985. His main interests are industrial microbiology and fermenta-*

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etry yield is known to be 90-95% of those values. Accepting these approximate glucose conversion yields, it is possible to follow the kinetics of a fermentation by measuring the mass of CO_2 released.

Another important aspect of alcoholic fermentation, employing glucose as substrate and yeast as microorganism, is the inhibition of glucose consumption at high glucose concentrations.[6] To avoid this inhibition phenomena, the fed-batch fermentation is preferred. In this process, fermentation is started batchwise with a small glucose concentration. When all the initial substrate is consumed, a new addition of fermentation medium is made in an amount such that the glucose concentration level remains just below the point of where it produces inhibitory effects. It may be said that, by operating in this way, the fed-batch fermentation is a sequence of batch fermentations of increasing volumes.

The introduction of equipment for the on-line monitoring and computer control of batch fermentors allows for a several-fold increase in productivity. Fed-batch operation is more complex than the classical batch operation.

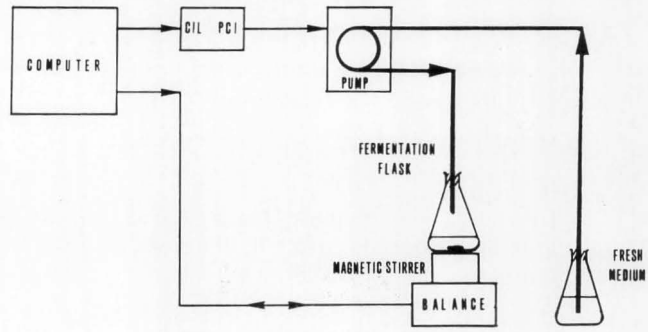

Figure 1. *Experimental set-up.*

EXPERIMENTAL SET-UP AND PROCEDURE

The proposed experimental set-up is shown in Figure 1. Fermentations are carried out in magnetically stirred 1-liter Erlenmeyer flasks. The balance, a Mettler PM4600 device (accuracy of ±0.005 g), is prepared for remote control with its internal commands for bidirectional communication with a computer via serial RS-232 protocol. An IBM-PC compatible microcomputer is employed.

The fed-batch fermentation medium is pumped by a Braun FE411 peristaltic pump. On-off control of the pump is implemented through one of the heavy-duty relay channels of a CIL PCI6380 interface from Microsystems LT. (United Kingdom) connected to the computer via a parallel IEEE port. A Brain Boxes Professional 488 is the internal IEEE interface card inside the computer.

The microorganism employed is baker's yeast. A typical composition of the fermentation medium prepared is presented in Table 1, together with other conditions for the experiment.

The medium is initially autoclaved at 121 °C for twenty minutes, and pH is adjusted to four with H_3PO_4. An initial amount of 50 ml of medium is put into the Erlenmeyer flask, and 5 g of pressed baker's yeast are then aseptically inoculated (for details of aseptical inoculation see reference 7). A good suspension of yeast cells in the medium is obtained by providing some agitation. The flask is then placed on the analytical balance and after a short period for stabilization (approximately two minutes), data acquisition is started. The loss of overall mass observed is due to the CO_2 released. At the end of operation (batch or fed-batch), residual glucose concentrations are determined by the DNS method.[8]

Fed-batch operation can be carried out under different strategies.[9] The initial experiments given to the students correspond to 'constant rate of increase of nutrient feed rate' under the condition of total consumption of glucose in each batch. With the experimental set-up as described, alternative feeding patterns (namely constant flow rate of nutrient feeding and constant stepwise nutrient feed rate) can be readily implemented. The students are encouraged to implement and compare different forms of operation.

The algorithm for the control of the whole operation is straightforward. By continually monitoring the total mass, *i.e.,* the amount of CO_2 released, it is possible to detect the instant corresponding to a residual glucose concentration G_r in the medium. The amount of fresh medium M_f to be pumped inorder to raise the glucose concentration up to a limit G_l is

TABLE 1
Conditions for Fed-Batch Fermentation Experiment

Medium composition (per liter of medium):

KH_2PO_4	5	g
$(NH_4)_2SO_4$	2	g
Carbon source (glucose)	50	g
$MgSO_4.7H_2O$	0.4	g
Yeast extract	1	g

Initial Volume ---------- $V_0 = 0.05$ l
Total volume of added medium ---------- $V_T = 0.5$ l

Glucose concentration limit to stop addition of fresh medium ---------- $G_l = 5\ g.l^{-1}$

calculated and the task is automatically implemented by simple on-off action on the pump. The procedure is stopped optionally where a time limit is observed or when the total volume V_T set for "added fresh medium" is reached.

EQUATIONS FOR MONITORING AND CONTROL

All the equations for monitoring and for control decisions are obtained by manipulation of the mass balance equations. In the following,

G_M = concentration of glucose in the fresh medium

ρ_M = density of the medium

Y_{CO2} = theoretical stoichiometry mass yield of glucose conversion to CO_2 (0.489 g of CO_2/g of glucose)

η = conversion yield factor (considered as 0.95) assumed constant throughout the operation

Also, and assuming that the fed-batch is a sequence of batch operations, the following variables are defined:

(i) $G_t^{(i)}$ is the concentration of glucose in batch i, at instant t (referred to the beginning of that batch). In particular, $G_o^{(i)}$ represents the concentration just after fresh medium has been added.

(ii) $M_t^{(i)}$ is the total mass of batch i at instant t (referred to the beginning of the batch). $M_o^{(i)}$ represents the initial mass, after fresh medium has been added. $M_t^{(i)}$ is the variable monitored in the whole process.

(iii) $M_f^{(i)}$ represents the mass of fresh medium added at the end of batch i, *i.e.,* in preparation for batch i+1.

For batch i, employing the yield definition, the amount of CO_2 released is related to the glucose consumption by the mass balance equation

$$M_o^{(i)} - M_t^{(i)} = \left(M_o^{(i)} G_o^{(i)} / \rho_M - M_t^{(i)} G_t^{(i)} / \rho_M\right) \eta\, Y_{CO_2} \quad (2)$$

Rearranging Eq. (2), the concentration of glucose at any instant $G_t^{(i)}$ can be related to the monitored variable $M_t^{(i)}$ by the equation

$$G_t^{(i)} = \frac{K\, M_o^{(i)} G_o^{(i)} - \left(M_o^{(i)} - M_t^{(i)}\right)}{K\, M_t^{(i)}} \quad (3)$$

where

$$K = \eta\, Y_{CO_2} / \rho_M \quad (4)$$

The instant corresponding to total consumption of glucose (*i.e.,* $G_{tr}^{(i)} = G_r = 0$) corresponds to a total amount of CO_2 released $M_{CO_2}^{(i)}$ in batch i, given by

$$M_{CO_2}^{(i)} = \left(M_o^{(i)} - M_{tr}^{(i)}\right) = K\, M_o^{(i)} G_o^{(i)} \quad (5)$$

where the subscript tr means time corresponding to residual G_r.

For the first batch (i = 1)

$$M_o^{(i)} = V_o \rho_M \quad (6a)$$

and

$$G_o^{(i)} = G_M \quad (6b)$$

where V_o is the initial value.

For a fed-batch operation where each batch is to be carried out up to the point of total consumption of glucose, Eq. (5) gives the reference for addition of fresh medium. The total amount $M_f^{(i)}$ to be added at the end of the batch in order to start batch i+1 with a glucose level given by G_l is obtained from a mass balance to glucose

$$M_{tr}^{(i)} G_{tr}^{(i)} + M_f^{(i)} G_M = \left(M_f^{(i)} + M_{tr}^{(i)}\right) G_l \quad (7)$$

which can be appropriately rearranged as

$$M_f^{(i)} = M_{tr}^{(i)} \frac{G_l - G_{tr}^{(i)}}{G_M - G_l} \quad (8)$$

For the particular case of $G_r^{(i)} = 0$, then

$$M_f^{(i)} = M_{tr}^{(i)} \frac{G_l}{G_M - G_l} \quad (9)$$

$M_f^{(i)}$ is the set-point for addition of fresh medium. Due to the natural lag in the pump response time, the mass of fresh medium effectively added tends to be slightly higher than the value set by the computer. This little problem is overcome by programming the computer to use the values effectively added. This means that for batch (i+1), the computer gives a direct reading of $M_o^{(i+1)}$ and the following values should be calculated:

(i) Mass of fresh medium effectively added

$$\left(M_f^{(i)}\right)_e = M_o^{(i+1)} - M_{tr}^{(i)} \quad (10)$$

(ii) Glucose concentration at the beginning of batch i+1

$$G_o^{(i+1)} = \frac{M_{tr}^{(i)} G_{tr}^{(i)} + \left(M_o^{(i+1)} - M_{tr}^{(i)}\right) G_M}{M_o^{(i+1)}} \quad (11)$$

Under this assumption, the reference value for the amount of CO_2 to be released in batch i+1 is given by

$$\left(M_{CO_2}^{(i+1)}\right)_{tr} = K \left(M_f^{(i)}\right)_e G_M \quad (12)$$

Equations (5), (9), (10), and (12) are the ones to be employed in the programming of the algorithm.

ASPECTS OF IMPLEMENTATION AND SAMPLE RESULTS

The experiment described is routinely carried out in the authors' laboratory by students taking the biotechnology option. In order to run the experiment the students are given the main specifications. They become conversant with the problems of data acquisition and write and implement the software. Compiled QUICK BASIC (version 4.5) is currently a good option since it is a structured programming language. The conditions given in Table 1 are only suggestions and obviously can be changed. The software should allow for the required flexibility; examples of parameters to be supplied by the user in each experiment are glucose concentration in the initial medium and in the medium to be added, glucose limits, and total value and/or time for end of operation.

The experiment lasts for about twenty-four hours, but since it is computer controlled the students spend only two hours in the laboratory during the first day (for preparation and start-up) and two hours during the second day (to collect data and conclude the work). This time aspect in itself demonstrates to the students the advantage of computer-controlled operations, especially for processes which are known to take a long time, as is typically the case for fermentation processes.

Figure 2 shows a print screen of the monitor display for a case study conducted with the conditions presented in Table 1. The evolution of CO_2 agrees with that predicted by theoretical considerations; the rate of CO_2 production is nearly constant. The students can also check and find that the mass of added culture medium increases as fermentation proceeds, and that a fed-batch fermentation is a sequence of several "increasing volume" batch fermentations.

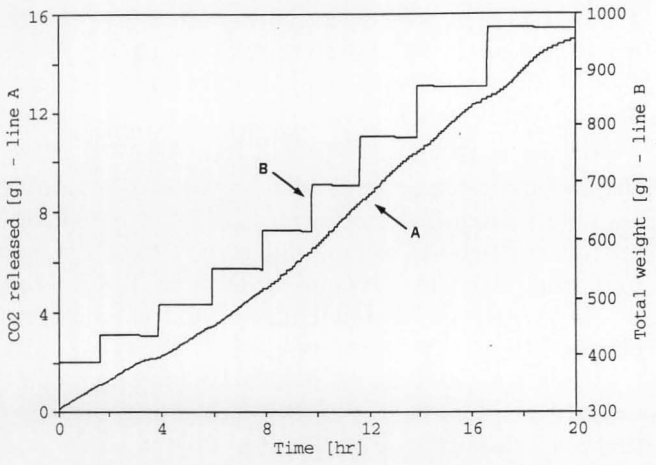

Figure 2. *Print screen of the monitor display*

Besides fitting the theoretical model, validation of these experiments can also be made by confirming that the mass of CO_2 released compares well (within 5%) to the one estimated by assuming the stoichiometric conversion yield of glucose to CO_2.

Inclusion of this experiment in the laboratory practice has undoubtedly helped students to understand a controlled operation of fed-batch processes.

ACKNOWLEDGEMENT

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NOMENCLATURE

G_l = Limit for glucose concentration in the fermentation medium to stop addition of fresh medium ($g.l^{-1}$)

G_M = Concentration of glucose in the fresh medium ($g.l^{-1}$)

G_r = Residual glucose concentration ($g.l^{-1}$)

$G_t^{(i)}$ = Concentration of glucose in batch i, at instant t (referred to the beginning of that batch)($g.l^{-1}$)

K = Constant (Eq. 2)

$M_f^{(i)}$ = Mass of fresh medium added at the end of batch i (g)

$M_t^{(i)}$ = Total mass of batch i at instant t (referred to the beginning of that batch)(g)

$M_{tr}^{(i)}$ = Mass of fermentation medium in batch i, corresponding to glucose concentration G_r (g)

$\left(M_{CO_2}^{(i)}\right)_{tr}$ = Mass of CO_2 released in batch i set-point to start addition of fresh medium (g)

V_o = Initial volume (l)

Y_{CO2} = Stoichiometric yield of glucose conversion to CO_2 ($g\ g^{-1}$)

ρ_M = Density of fresh medium ($g.l^{-1}$)

η = Conversion yield factor

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references he used to prepare the figures in this chapter nor in subsequent chapters; however, he does provide a bibliography at the end of each chapter.

The third chapter is on the thermodynamics of electrochemical cells and includes a section on Pourbaix diagrams which is very useful for understanding phase equilibria and cathodic protection. This chapter should be studied by all chemical engineering students.

Chapter Four presents discussions of phase equilibria and the concepts of electrochemical potential and mean activity coefficients solutions containing ionic species. The author also includes in this chapter a detailed discussion on the Debye-Huckel theory for electrolytic solutions. The author finishes this chapter with discussions on the two concepts of a potential in an electrolytic solution and liquid junction potentials.

The fifth chapter is on electrode kinetics. The author begins the chapter by presenting a useful description of the electric double layer on an electrode. The author continues this chapter by presenting a derivation of the Butler-Volmer equation, which is the commonly used reaction rate expression for electrochemical reactions. He then presents and discusses simplified forms of the Butler-Volmer equation: the so-called linear and Tafel forms of the Butler-Volmer equation. He continues by presenting a practical description of reference electrodes and their use in measuring potential distributions in electrolytic cells. He also presents in this chapter a description of a study of the reaction mechanism for the anodic reaction of zinc in an alkaline electrolyte. He presents a reaction rate expression for this reaction which is similar to the Butler-Volmer equation but includes a potential-dependent pre-exponential term. Finally, the author presents a very useful discussion of the kinetics of corrosion processes and Evans' diagrams. Finally, he provides a lucid description of simplified forms of the reaction rate expressions for corrosion reactions and associated expressions for the corrosion potential.

Chapter Six contains a very useful presentation of the fundamental equations used to describe mass transfer in electrolytic solutions. This chapter should be required reading for all chemical engineers. The author uses the rotating disk electrode to demonstrate how electrochemical reactions can be used to develop mass transfer correlations in the form of the Sherwood number as a function of the Reynolds and the Schmidt number, for example. The final section in Chapter Six is a brief discussion of how to treat the time dependence of a simple electrochemical reaction.

In Chapter Seven, the author presents a classification scheme for the types of current distribution problems that have been modeled in the past. He also presents a discussion of the Wagner number which can be used as a characterizing parameter for current distributions in electrochemical cells. Next, the author presents a summary of analytical and numerical methods that can be used to predict current distributions. The next topic in this chapter is on gas-evolving electrodes, which are found in many electrochemical cells used in industry (*e.g.,* chlor-alkali cells), and the author presents a mass transfer correlation for vertical, gas-evolving electrodes for such cells. The final section in this chapter contains a presentation of the equations that are used for mass and charge transfer in porous electrodes, which are important in such areas as batteries and fuel cells.

Chapter Eight is entitled "Experimental Methods" and presents material on several popular experimental systems used in electrochemical engineering. These are the rotating disk electrode, the rotating ring-disk electrode, rotating cylinder electrode, and parallel plate electrode systems.

The last chapter in the book contains descriptions of several applications of electrochemical engineering principles. These include energy storage and conversion, electric vehicles, thermally regenerative electrochemical systems, and the electrochemical production of adiponitrile. The author also includes descriptions of monopolar and bipolar electrochemical cells, the chlor-alkali process, and thermal management of electrochemical cells. The final section of this last chapter is on future developments in which the author speculates that "the premium on efficiency will stimulate additional research on electrochemical energy conversion and storage." I hope he is right. ❐

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