Abstract:
The main aim of this work is the development of mathematical models of aerobic batch fermentations for its use in estimation and control algorithms. Most batch fermentation models are empirical and simple, and do not provide interrelationships between state variables and measurements. In this work such interrelationships are obtained from mass and energy balances of the fermentation components. Since aerobic fermentations with formation of a single metabolite exhibit three degrees of freedom, three independent kinetic equations are necessary to build the state space model. Test results on the batch fermentation of xanthan gum are presented.

1. Introduction

Many publications have applied advanced control to fermentative processes carried out in continuous or fed-batch bioreactors [Wu et al. (1985); Takamatsu et al. (1985); Lim et al. (1986); San and Stephanopoulos (1986); Williams et al. (1986); Modak and Lim (1987); Agrawal et al. (1989); San and Stephanopoulos (1989); Shi et al. (1989); Harmon et al. (1989); Diener and Goldschmidt (1994); reviews by Shioya (1992) and by Shimizu (1993)]. The main obstacle for applying advanced process control to batch fermentations is the poor quality of the processes models, the relative low number of measurements, and the scarcely-known interrelationships between states and measured variables.

In batch fermentations, the main system variables can vary widely along the process. Thus, there is an opportunity for driving the manipulated variables in optimal fashion. Some publications examined the problem of determining optimal control trajectories in fermentors, considering different objective functions and control variables. Constantinides et al. (1970) presented probably the first paper that proposed the use of optimal control in batch bioreactors. Reuss (1986) presented a review on the use of optimal control in fermentative process, and only few of the reviewed works considered batch operations. In recent years, Aseno et al. (1995) and Lee et al. (1999) presented articles on the optimal control of batch reactors, including experimental validations. In spite of the fact that trajectory optimization is a well-known technique, it has not been widely applied to fermentative processes.

The reason is that optimal control results are highly dependent on the process model, and many fermentation models do not accurately represent the dynamic behavior. Erikson et al. (1978) is one of the first publications where mass and energy balances have been used for modeling fermentations. The balances were used to derive optimal operating conditions in continuous single-cell production reactors. Roels (1980) generalized the concepts presented by Erikson et al. (1978) to other fermentations (aerobic with or without product formation and anaerobic fermentations); and outlined a scheme for building models on the basis of the available kinetic information. The relevance of macroscopic principles for modeling biochemical systems was also discussed. Heijnen and Roels (1981) developed a slightly more complex scheme, specific to aerobic fermentations. Simple models were proposed for estimating yield coefficients on substrates with different levels of complexity but assuming that the intracellular reaction rates and the mass transfer mechanisms between the cells and its environment are in the steady state. Batch fermentations are time-varying processes, and we did not find any publication on the use of balances onto batch processes.

In this work, we derive a time-varying state-space model that is applicable to aerobic batch fermentations. This work is organized as follows. In section 2, state-space equations for batch fermentations are derived from a model that involves five partial metabolisms. Microscopic balances are used to calculate the relations between partial metabolisms and the net consumption of the main components. Also, macroscopic balances are used to calculate the relations between the partial metabolism rates, the variation of main component
2. A state-space model for an aerobic batch fermentation

An aerobic fermentation can be seen as a set of parallel "reactions" denoted partial metabolisms. These reactions are not simple chemical reactions. The partial metabolisms either produce or consume carbon dioxide, water, oxygen, main substrate, and nitrogen source. The hydrogen involved in oxidation/reduction reactions (H+ + e-) is bound to electron carriers like NADH2 (nicotinamide adenine dinucleotides). Also, energy carriers like ATP (adenosine 5-triphosphate) transport the produced or consumed free energy.

Since fermentation models should be accurate and of the least possible complexity, the aim is to find the minimum set of differential and algebraic equations that adequately describes the process dynamics. In state-space formulation, a nonlinear model is represented by:

\[
\begin{align*}
\dot{x} &= f(x,u,p) \\
y &= g(x)
\end{align*}
\]

where \(x\) is the state-vector, \(y\) is the vector of measurements, \(u\) is the vector of manipulated variables and \(p\) is a vector of model parameters.

In a homogeneous and constant-volume stirred-tank reactor, the balance equations are expressed as follows [Roels (1980)]:

\[
\begin{align*}
\dot{C} &= r_c + \Phi
\end{align*}
\]

where \(C\) is a vector that represents the concentration of the main components, \(r_c\) is the net conversion rate of these components, and \(\Phi\) is a vector that represents the net transport rate of the system components. To be used as a state-space model, the Eq. (2) must be expressed in the form of Eq. (1). When applied to a specific fermentor, Eq. (2) provides the interrelationships between reaction rates, concentration variations, and net inlet flows. Eq. (2) represents a macroscopic balance where the microbial metabolism determines the conversion rates of the different components \((r_c)\) together with the relations between these rates. Since the microbial metabolism must obey the conservation principles, microscopic balances can be used to derive a minimum number of dynamic equations that describe the process.

In principle, microscopic balances should be applied to every fermentation component and to every element. However, considering that hundreds of components could participate in the microbial metabolism, it is necessary to limit the analysis to the so-called main components and main elements. The four main elements are \(C, H, O,\) and \(N\); since these elements comprise about 95% of the biological mass [Roels, 1980]. The main components depend on the fermentation type, and they will be identified later for an aerobic batch fermentation with production of a single metabolite.

2.1 Microscopic balances

Figure 1 presents a model for the aerobic growth of biomass (\(X\)) using a single component as carbon and energy source \((S)\), and an independent nitrogen source that can also contain carbon \((S_N)\). The generated metabolic component is denoted \(P\); and \(CO_2, H_2O\) and \(O_2\) are components exchanged between the cells and their media. The growth is assumed to be approximately balanced, in the sense that microorganisms are able to produce exact replicas of themselves. The fermentation is modeled by five partial-metabolisms or pseudo-reactions. Each pseudo-reaction is described by a stoichiometry \(E_r\) and a kinetic rate \(r_c\). This simplified description can be only explained from a stoichiometric point of view, since many biochemical reactions simultaneously participate in different partial metabolisms. The flow of main components can be subdivided according to such pseudo-reactions; and each reaction can be kinetically modeled like an integral unit [Minkevich (1983)]. This description of an aerobic fermentation, although simple, is more complete than most of the empirical models normally found in control and estimation algorithms [Constantinides et al. (1970); Wu et al. (1985); Shimizu et al. (1989)].

The concentration of intracellular components, ATP and NADH2, are assumed to be in the steady state [Roels (1983)]. Therefore, there is no accumulation term in their balances.

Since the element balances must be always satisfied, they represent the constraints to be met by each stoichiometry \(E_r\). The vector of main components is defined as follows:

\[
C^d = [X, S, P, S_N, O_2, CO_2, H_2O]
\]

The composition of components \(X, S, P,\) and \(S_N\) is expressed by their atomic formulae \(CH_bO_cN_d\), \(CaHb2OcNd2\), \(CaHb3Oc3Nd1\) and \(CaHb4Oc4Nd0\) respectively (the metabolite is assumed to be a nitrogen-free component). The coefficients of the stoichiometric equations on Table 1 are expressed as the inverse of the yields \((Y_{ij})\) of product \(I\) on each of the \(J\) components. The coefficients \(Y_{SATP}\) and \(Y_{ATP}\) indicate the moles of energy carriers (ATP) consumed in the anabolism and in the product metabolism respectively; and \(Y_{SARI}\) indicates the moles of ATP generated in the main substrate catabolism.

The production of biomass is a process with a quantified net consumption of ATP \((Y_{SATP} = 10.5 \text{ g X/mol ATP}) [Andrews (1993)]\). The formation of products can either generate energy (e.g. by partial oxidation of substrates in the alcoholic fermentations) or consume
energy (e.g. by generating macromolecules). The net energy demand of each reaction can be known by studying the metabolic pathways that describe the product generation [Andrews (1993)]. Apart from these processes, other numerous degradation and dissipation reactions that imply ATP consumption are also present [Minkevich (1983)]. These so-called “cellular maintenance reactions” imply a non measurable demand of ATP.

![Diagram of metabolic pathways](image)

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**Fig. 1:** The aerobic fermentation model as a network of partial metabolisms. (The grey boxes denote partial metabolisms that consume ATP while the white boxes denote partial metabolisms that produce ATP. The dashed arrows indicate the intracellular NADH2 flow. The continuous arrows indicate the intracellular ATP flow. The bold arrows denote exchange of main components between the biomass and its environment).

<table>
<thead>
<tr>
<th>Table 1: Stoichiometric relations of the partial metabolisms (Ei)</th>
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<tbody>
<tr>
<td><strong>Biomass production</strong> (Ex):</td>
</tr>
<tr>
<td>[ \frac{1}{Y_{X/S}} C_{a1}H_{a2}O_{a3}N_{a4} + \frac{1}{Y_{X/S}} C_{a4}H_{a4}O_{a4}N_{a4} + \frac{\text{ATP}}{Y_{X/ATP}} \rightarrow CH_{a1}O_{a1}N_{a1} + \frac{H_2O}{Y_{X/H_2O}} + \frac{CO_2}{Y_{X/CO_2}} ]</td>
</tr>
<tr>
<td><strong>Metabolite production</strong> (Ep):</td>
</tr>
<tr>
<td>[ \frac{1}{Y_{P/S}} C_{p1}H_{p1}O_{p1}N_{p1} + \frac{\text{ATP}}{Y_{P/ATP}} \rightarrow C_{p3}H_{p3}O_{p3} + \frac{\text{ATP}}{Y_{P/ATP}} \rightarrow \frac{H_2O}{Y_{P/H_2O}} + \frac{CO_2}{Y_{P/CO_2}} ]</td>
</tr>
<tr>
<td><strong>Main substrate catabolism</strong> (Ep):</td>
</tr>
<tr>
<td>[ \frac{1}{Y_{S/ATP}} C_{s1}H_{s1}O_{s1}N_{s1} + \frac{H_2O}{Y_{S/H_2O}} \rightarrow \frac{CO_2}{Y_{S/CO_2}} + \frac{\text{ATP}}{Y_{S/ATP}} + \frac{NADH}{Y_{S/NADH}} ]</td>
</tr>
<tr>
<td><strong>Oxidative phosphorylation</strong> (Ep):</td>
</tr>
<tr>
<td>[ \frac{NADH}{2} + \frac{1}{2} O_2 \rightarrow H_2O + \frac{\text{ATP}}{NADH} \cdot \frac{ATP}{2} ]</td>
</tr>
</tbody>
</table>

To satisfy the ATP demand for the biomass production, the metabolite production, and the cellular maintenance, a certain amount of main substrate must be oxidized. But the generated ATP (Y_{ATP/NADH}) is a function of the level of oxidative phosphorylation (P/O). This function depends on the specific metabolic oxidation pathway. Thus, the amount of oxidized main substrate must be calculated from the specific pathway function \( f(P/O) \) and from the ATP balance. The use of stoichiometric relations and balances of intracellular components (ATP and NADH2) allows to write the following relationships.

**ATP balance:**

\[ \frac{r_S}{Y_{S/ATP}} + \frac{r_P}{Y_{P/ATP}} + r_{ATP}^H = \left[ \frac{r_S^Y}{Y_{S/NADH}} + \frac{r_P^Y}{Y_{P/NADH}} \right] Y_{ATP/NADH} \]

\[ Y_{ATP/NADH} = f(P/O) \]
appropriate stoichiometric yields, the knowledge of three kinetic equations with the freedom, and the unknown rates may be obtained from the description of an aerobic fermentation with formation of a single metabolic product has three degrees of processes. Thus, an aerobic fermentation with formation of two interrelationships among the five intracellular components, a stoichiometry for the main substrate oxidation is obtained by reordering the ATP balance (Eq. 3), as follows:

\[
\frac{r_S}{Y_{S/NADH2}} + \frac{r_P}{Y_{P/NADH2}} = \frac{r_{KO}}{Y_{ATP/NADH2}}
\]

(4)

The consumption (or production) of main components exchanged between the cells and their media is calculated writing a mass balance for each component, as follows:

Nitrogen source consumption:

\[
r_N = -\frac{r_Y}{Y_{X/N}}
\]

(5)

Main substrate consumption:

\[
r_S = \frac{r_Y}{Y_{X/S}} + \frac{r_P}{Y_{P/S}} + r_E^S
\]

(6)

Oxygen consumption:

\[
r_O2 = \frac{1}{2} \left( \frac{r_S^E}{Y_{S/NADH2}} + \frac{r_P}{Y_{P/O2}} + \frac{r_E^P}{Y_{P/O2}} \right) \frac{r_S^E}{Y_{S/O2}} + \frac{r_P}{Y_{P/O2}}
\]

with \(Y_{S/O2} = 2Y_{S/NADH2}\) and \(Y_{P/O2} = 2Y_{P/NADH2}\).

Carbon dioxide production:

\[
r_CO2 = \frac{r_Y}{Y_{X/CO2}} + \frac{r_P}{Y_{P/CO2}} + \frac{r_E^E}{Y_{S/CO2}}
\]

(8)

The intracellular balances of ATP and NADH\(_2\) provide two interrelationships among the five intracellular processes. Thus, an aerobic fermentation with formation of a single metabolic product has three degrees of freedom, and the unknown rates may be obtained from the knowledge of three kinetic equations with the appropriate stoichiometric yields \(Y_{E2}\). The presented model provides a fairly accurate description of an aerobic fermentation with formation of a single metabolite, and is applicable to any reactor type. However, the intracellular model parameters (\(P/O, r_{ATP}^M\)) are generally unknown and their values could change due to manipulated or non-manipulated changes in the environment.

From a macroscopic point of view and without considering intracellular components, a stoichiometry for the main substrate oxidation is obtained by adding the main substrate catabolism stoichiometry \((E_2^E)\) and the oxidative phosphorylation stoichiometry for the NADH\(_2\) produced by the catabolism of the main substrate \((E_{2P}/Y_{SNADH2})\). This oxidation occurs at a rate \(r_E^S\). Besides, the cellular maintenance ‘reaction’ consumes ATP but not main components. Thus, this reaction can be ignored in the macroscopic mass balances. For both reasons, the three independent processes that are necessary for describing the fermentation are:

- biomass growth \(r_Y\);
- metabolite production \(r_P\); and
- main substrate oxidation \(r_E^S\).

In kinetic models of main substrate oxidation, the unknown parameters \(r_{ATP}^M\) are usually lumped into a ‘maintenance’ coefficient \(K^E\) that models the main substrate oxidation as a first order reaction in the biomass concentration. This assumption is common in empirical models. However, a better model for the main substrate oxidation can be obtained by reordering the ATP balance (Eq. 3), as follows:

\[
r_E^S = \frac{r_X}{Y_{X/ATP}} + \frac{1}{Y_{P/ATP}} \left( \frac{1}{Y_{P/NADH2}} - \frac{r_P}{Y_{P/NADH2}} \right) + \frac{r_{ATP}^M}{Y_{S/ATP}} \]

(9)

Since in batch reactors reaction rates \(r_X\) and \(r_P\) are usually time-varying, Eq. (9) shows that the main substrate oxidation is not strictly first order in the biomass concentration. Therefore, the \(K^E\) parameter from a first order oxidation rate could show strong variations. In effect, when the \(K^E\) coefficients of several experimental fermentations are compared [Heijnen and Roels (1981)], it is observed that their values change for similar fermentation conditions. The assumption of a first order oxidation rate of the main substrate is only valid if the reaction rates and the mass transfer mechanisms between media and cells are in the steady state. In this case, since \(r_X, r_P,\) and \(r_{ATP}^M\) are in the steady state and the yields are constant, then \(r_E^S\) is also in the steady state. However, in batch fermentations, reaction rates and mass transfer processes reach the steady state only when the metabolic activity drops to zero (i.e., at the end of the fermentation).

### 2.2 Macroscopic balances

The previous balance equations are independent of the kinetics and provide relationships between the rates of the different partial metabolisms and between these rates and the net consumption (or production) of main components. These relationships are also independent of the reactor type. Consider now the derivation of macroscopic relations between reaction rates, concentration of main components, and net component flows to the broth. In aerated batch fermentors, \(X, P, S\) and \(S_m\) are not transferred through the surface boundary of the vessel. Thus, the corresponding elements of the transport rate vector \(\Phi\) in Eq. (2) are all zero.

\[
\begin{bmatrix}
\Phi_x \\
\Phi_P \\
\Phi_S \\
\Phi_{S_m}
\end{bmatrix}
= 0
\]

(10)

The oxygen concentration in the broth is considered to be in a quasi steady state because its variations are much faster than the oxygen flow transferred between the gas
The main substrate oxidation is represented by:

\[
\frac{d}{dt} \left[ \frac{O_2}{CO_2} \right] = 0
\]

Thus, in aerated batch bioreactors, Eq. (2) may be written as follows:

\[
\begin{bmatrix}
X \\
P \\
S \\
S_k \\
H \\
O
\end{bmatrix}
= 
\begin{bmatrix}
r_X & r_P & r_O & r_{P2O} & 0 & 0 \\
0 & 0 & 0 & 0 & r_{CO2} & 0 \\
0 & r_{CO2} & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & r_{CO2} & 0 \\
0 & 0 & 0 & 0 & 0 & r_{CO2} \\
0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}
\begin{bmatrix}_X & r_P & r_O & r_{P2O} & 0 & 0
\end{bmatrix}
\]

\[
\frac{d}{dt} \begin{bmatrix} X \ S \\ P \ S_k \ H \ O \end{bmatrix} = \begin{bmatrix} r_X & r_P & r_O & r_{P2O} & 0 & 0 \\ 0 & 0 & 0 & 0 & r_{CO2} & 0 \\ 0 & r_{CO2} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & r_{CO2} & 0 \\ 0 & 0 & 0 & 0 & 0 & r_{CO2} \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} r_X \ r_P \ r_O \ r_{P2O} \ 0 \ 0 \end{bmatrix}
\] (12)

Equation (12) indicates that the oxygen consumption rate \((-r_{CO2})\) and the carbon dioxide production rate \((r_{CO2})\) can be obtained by measuring the oxygen transferred into the broth \((\Phi_{CO2})\) and the carbon dioxide transfer from the broth \((\Phi_{CO2})\). Except for the water, the concentration variation of the remaining main components are obtained from their formation or consumption rates.

**State dynamics.**

As mentioned before, \(r_X\), \(r_P\), and \(r_{X2}\) are the three independent processes present in an aerobic fermentation with formation of a single metabolite. The dynamics of biomass growth \((r_X)\) and of metabolite production \((r_P)\) are characterized by the evolution of the biomass concentration and the metabolic product concentration. Thus, it is possible to use the following empirical equations:

\[
\frac{d}{dt} \begin{bmatrix} X \\ P \end{bmatrix} = \begin{bmatrix} r_X \\ r_P \end{bmatrix}
\]

(13)

The main substrate oxidation is represented by:

\[
\frac{dS_{X2}}{dt} = -r_{X2} = -r_X - \frac{r_P}{Y_{X/S}} - \frac{r_{P2O}}{Y_{P/O2}}
\]

(14)

Note that the second equality of Eq. (14) is the Eq. (6) reordered. The state associated to this reaction is the amount of oxidized main substrate \(S_{X2}\). Empirical expressions for \(r_X\), \(r_P\), and \(r_{X2}\) with Eq. (14) provides the kinetics of \(r_X\), \(r_P\), and \(r_{X2}\) and therefore complete the fermentation dynamics. Alternatively, other less usual kinetic equations together with the yields \(Y_{ij}\) also can be used to complete the state space model.

**State-measurements relations.**

The theoretical relation between states and measured variables is provided by the stoichiometric coefficients. The microscopic mass balances indicate that the total consumption rate of main substrate and oxygen and the total production rate of carbon dioxide are the sum of their respective evolutions in \(r_X\), \(r_P\), and \(r_{X2}\) (Eqs. 6, 7 and 8). The measurement of \(\Phi_{O2}\) and \(\Phi_{CO2}\) provides more information on the fermentation rates than on the states. Thus, it is preferable to use the cumulative oxygen consumption \((\Delta O_2(t))\) and the cumulative carbon dioxide production \((\Delta CO_2(t))\) at time \(t\) as measurement variables:

\[
\Delta O_2(t) = \int_0^t r_O dt = \int_0^t \frac{r_O dt}{Y_{X/O2}} + \frac{r_O dt}{Y_{P/O2}} + \frac{r_{CO2} dt}{Y_{CO2/O2}}
\]

(15.a)

\[
\Delta CO_2(t) = \int_0^t \Phi_{CO2} dt = \int_0^t \frac{\Phi_{CO2} dt}{Y_{X/O2}} + \frac{\Phi_{CO2} dt}{Y_{P/O2}} + \frac{\Phi_{CO2} dt}{Y_{CO2/O2}}
\]

(15.b)

In these equations, the yield coefficients are extracted from the stoichiometric relations presented in Table 1. The yields of biomass on the other components can be exactly known only in pure cultures of a perfectly identified biomass. Therefore, their values are in general approximate, and can be estimated using some fermentation regularities, element balances and the knowledge of the broth composition [Erikson et al. (1978)].

If the main substrate and the nitrogen source concentrations are measurable, then the following equations provide relationships between the states and the two mentioned main components:

\[
\Delta S(t) = \int_0^t \left[ \frac{r_X dt}{Y_{X/S}} + \frac{r_P dt}{Y_{P/S}} + \frac{r_{X2} dt}{Y_{X2/S}} \right]
\]

(16.a)

\[
\Delta S_{X2}(t) = \int_0^t \left[ \frac{r_X dt}{Y_{X/S}} + \frac{r_P dt}{Y_{P/S}} + \frac{r_{X2} dt}{Y_{X2/S}} \right]
\]

(16.b)

In these expressions, \(X(t)-X_0\), \(P(t)\), and \(S_{X2}(t)\) are respectively the amounts of produced biomass, of produced metabolite, and of oxidized main substrate at time \(t\). Note that when the measured variables belong to the set of main components, then the stoichiometric yields allow to find linear relations between measured variables and state-variables.
2.3 Effect of the control variables and modeling of uncertainties

Batch fermentation models present the following peculiarities:

- The influence of intensive variables (pH, temperature) on the fermentation dynamics is usually expressed as empirical functions $\pi(u)$ that show the dependence of the kinetic parameters $\pi$ on such variables. If the intensive variables can be manipulated, then they can be used as control variables, but in this case important deviations from the empirical functions $\pi(u)$ are to be expected.

- Large changes in the control variables may cause unpredictable changes in the kinetic parameters and even a variation in the structure of the dynamic model. This limits the use of empirical models that cannot quantify the physiological effects.

- Variations in the model parameters from one fermentation to another are to be expected, even when the same control policy is applied.

Thus, the control variables affect the system via the kinetic parameters. Calling $p(u,t)$ the function of the kinetic parameters with respect to the control variables $u$, then the batch reactor dynamics can be described through:

$$x = f(x, p(u,t))$$

(17)

Usually only a crude empirical function $\pi(u)$ which is an approximation of the real $p(u,t)$ is known. If an accurate knowledge of the parameter values is required, then an estimation algorithm can be used, expressing $p(u,t)$ as follows:

$$p(u,t) = \pi(u) + \Delta \pi(u,t)$$

(18)

where $\Delta \pi(u,t)$ is an unknown deviation between $p(u,t)$ and $\pi(u)$. To identify this deviation, the control variable must vary along the whole range of variation. In contrast, when the control variable is kept in a narrow interval during the whole fermentation time, then it is not possible to identify $\Delta \pi(u,t)$. In this case, $p(u,t)$ can be written as follows:

$$p(u,t) \equiv \pi(u) + \Delta p(t)$$

(19)

The sum of $\pi(u)$ and the estimated value of $\Delta p(t)$ provides a better representation of $p(u,t)$ than $\pi(u)$.

3. A batch kinetic model

A common practice in batch fermentations models is to use the logistic equation for the biomass growth together with the Luedeking-Piret equation for the product and main substrate kinetics [Weiss and Ollis (1980)]:

$$r_p = \mu \left(1 - \frac{X}{X_s}\right) X$$

(20.a)

$$r_s = -\frac{a}{b} \frac{dX}{dt} + bX$$

(20.b)

$$r_s = -\left(\frac{a}{b} \frac{dX}{dt} + bX\right)$$

(20.c)

Consider the development of a state-space model that uses this set of kinetic equations. As explained in section (2.1), the biomass growth, the product formation and the main substrate oxidation describe an aerobic fermentation with production of a single metabolite. For the first two processes, Eqs. (20.a) and (20.b) can be used as dynamic models. Equation (20.c) corresponds to the total main substrate consumption (the sum of the substrate consumption in each of the three independent processes). Thus, a model for the main substrate oxidation may be obtained by subtracting Eqs. (20.a) and (20.b) divided by their respective stoichiometric yields $Y_{XS}$ and $Y_{FS}$ from Eq. (20.c). Alternatively, it can be assumed that this process is first order in the biomass concentration:

$$\frac{dS^k}{dt} = K^k X$$

(21)

When empirical equations that express the kinetic parameters ($\mu, X_p, a, b$ and $K^k$ in this case) as functions of the control variables are available, these equations together with Eqs. (20.a), (20.b) and (21) constitute the dynamic model $f(x, p(u,t))$. But as explained in section 2.3, it is convenient to add a deviation term or ‘disturbance-parameter’ to each parameter. These disturbances include the errors of the functions $\pi(u)$. Thus, the process dynamics can be expressed as:

$$dX^p = \left(\begin{array}{c}
X \\
p \\
p^* \\
x \\
x^* \\
\mu^* \\
\Delta \mu \\
\Delta \pi \\
\Delta \pi^* \\
\Delta K^* \\
\end{array}\right)$$

(22)

The uncertainty in the ‘disturbance-parameters’ dynamics suggests to model them as constants with a real-time state and parameter estimator estimating their values.

4. Application to the batch production of xanthan gum. Results and discussion.

Xanthan gum is an extracellular polysaccharide produced by Xanthomonas campestris. This gum has numerous applications and is produced in large quantities. The kinetic model of Eqs. (20) has been used by Weiss and Ollis (1980), Pinches and Pallent (1986) and Shu and Yang (1991) to describe the production of xanthan gum in batch fermentors. Since these authors do not provide a complete information on the main
component dynamics, the model of section 2 is used to complete the fermentation description. Pons et al. (1989) developed a structured model for this fermentation and estimated the stoichiometric relations for the gum production, the catabolism of the main substrate (glucose), and the oxidative phosphorylation. The chemical formula of a “mol” of xanthan gum proposed by Pons et al. (1989) is $C_{32.34}H_{48.58}O_{27.36}Na_{1.38}$.

The stoichiometry of xanthan gum production provides the values $Y_{P/S} = 0.917$ g P/g S, $Y_{P/CO_2} = 34.326$ g P/g CO$_2$ and $Y_{P/O_2} = 15.820$ g P/g O$_2$. Also, $Y_{S/O_2} = 0.937$ g S/g O$_2$ and $Y_{SCO_2} = 0.682$ g P/g CO$_2$ are derived from the stoichiometry of the glucose oxidation. The stoichiometry of biomass production was not reported but can be estimated from some regularities of aerobic fermentations, element balances and the knowledge of the broth composition [Erikson et al. (1978)]. Then, the yields $Y_{X/S}$, $Y_{X/O_2}$ and $Y_{X/CO_2}$ can be calculated from the experimental value of $Y_{X/N}$.

The cellular maintenance coefficient of Eq. (21) is estimated using Eq. (14). By modelling $r_P$ and $r_S$ through Luedeking-Piret type equations, then $K^E$ results:

$$K^E = \beta - \frac{b}{Y_{P/S}}$$

Kinetic expressions for $r_{O_2}$ and $r_{CO_2}$ can be calculated replacing Eqs. (20.a), (20.b), and (21) into Eqs. (7) and (8). Since $r_P$ is modeled by the Luedeking-Piret equation, then the ‘calculated rates’ $r_{O_2}$ and $r_{CO_2}$ will also have the same structure.

In Table 2, the resulting values of some kinetic parameters are compared with experimental values by Pinches and Pallent (1986); Pons et al. (1989); and Peters et al. (1992). As the rates $r_{O_2}$ and $r_{CO_2}$ depend on the biomass concentration, the evolution of this concentration must be simulated for driving these rates. The fermentation conditions of the cited papers and the kinetic equations used for simulating the evolution of the biomass concentration are also summarized in Table 2. Computer simulations are presented in Figs. 2 to 5. In these figures the evolution of the biomass concentration was simulated; the model-predicted evolution of the oxygen consumption (computed as explained in the previous paragraph) is compared with their simulated evolution using the experimental kinetic parameters (Eq. (15) with experimental parameters of Table 2). The predicted carbon dioxide production is also presented. Unfortunately, there is not experimental information on the evolution of this main component.

For the fermentation presented in Pinches and Pallent (1986) with sodium glutamate as nitrogen source, the experimental and theoretical evolution of consumed oxygen are quite similar. Interestingly, the theoretical value of $c$ is smaller than the experimental value, while the opposite occurs with the parameter $d$, indicating a compensation of values. This can be attributed to errors in the graphical method used by Pinches and Pallent (1986) to identify the experimental parameters. For the fermentation of Peters et al. (1992), the agreement between experimental and theoretical parameters is quite good, as it can be seen in the simulation of Fig. 5.
Table 2: Experimental and calculated values of yields and kinetic parameters for four xanthan gum fermentations carried out in batch reactors (The reported experimental values are given in normal font. The calculated values are given in Italics, and below the experimental values.)

<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>N-source</td>
<td>Sodium glutamate</td>
<td>Peptone</td>
<td>Peptone + Yeast</td>
</tr>
<tr>
<td>Temperature</td>
<td>30 °C</td>
<td>30 °C</td>
<td>29 °C</td>
</tr>
<tr>
<td>pH</td>
<td>7</td>
<td>6.9</td>
<td>7</td>
</tr>
<tr>
<td>$r_s$</td>
<td>0.106X $(X \leq 1.77)$</td>
<td>0.283 $\frac{1-X}{2.145}$ $X$</td>
<td>0.084X $(X \leq 4.12)$</td>
</tr>
<tr>
<td>$X(0)$</td>
<td>0.250</td>
<td>0.140</td>
<td>0.063</td>
</tr>
<tr>
<td>Stoichiometric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yields</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Y_{X/N}$</td>
<td>12.02</td>
<td>10.03</td>
<td>6.35</td>
</tr>
<tr>
<td>$Y_{X/S}$</td>
<td>2.69</td>
<td>$\infty$</td>
<td>$\infty$</td>
</tr>
<tr>
<td>$Y_{X/O_2}$</td>
<td></td>
<td>25.45</td>
<td>1.23</td>
</tr>
<tr>
<td>$Y_{X/CO_2}$</td>
<td>9.47</td>
<td>9.66</td>
<td>0.878</td>
</tr>
<tr>
<td>Kinetic Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$a$</td>
<td>1.314</td>
<td>0.474</td>
<td>N/A</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>2.93</td>
<td>1.243</td>
<td>N/A</td>
</tr>
<tr>
<td>$\beta$</td>
<td>2.025</td>
<td>0.517</td>
<td>---</td>
</tr>
<tr>
<td>$c$</td>
<td>0.386</td>
<td>---</td>
<td>N/A</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>N/A</td>
<td>0.144</td>
<td>N/A</td>
</tr>
<tr>
<td>$\delta$</td>
<td>N/A</td>
<td>0.117</td>
<td>---</td>
</tr>
<tr>
<td>$b$</td>
<td>0.107</td>
<td>0.148</td>
<td>0.250</td>
</tr>
<tr>
<td>$K^G$</td>
<td>0.052</td>
<td>0.045</td>
<td>0.158</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.169</td>
<td>0.206</td>
<td>0.431</td>
</tr>
<tr>
<td>$d$</td>
<td>0.0554</td>
<td>0.0719</td>
<td>0.128</td>
</tr>
<tr>
<td>$\delta$</td>
<td>N/A</td>
<td>0.0622</td>
<td>0.0574</td>
</tr>
<tr>
<td>$\delta$</td>
<td>N/A</td>
<td>0.0794</td>
<td>0.0703</td>
</tr>
</tbody>
</table>

N/A: not available.

For the fermentations of Pinches and Pallent (1986) with peptone as nitrogen source and Pons et al. (1989), the results are not too good. Nevertheless, the differences can be explained by the possible generation of by-products from a complex nitrogen source. Some experimental information by Flores Candia (1994) supports this statement.

In summary, the agreement between the calculated and experimental parameters is acceptable, considering the large uncertainties in this process and the poor measurements of some of the key variables. The use of ‘disturbance-parameters’ seems appropriate in this case.

5. Conclusions

A procedure was outlined for building dynamical models by combining macro and microscopic balances with available kinetic information. A model of the microbial metabolism that is based on elemental and intracellular component balances was presented. The model divides the metabolism into five partial metabolisms or pseudo-reactions. The balances allow to
calculate how errors in the kinetics parameters or in the stoichiometry of a partial metabolism influence the remaining process, and also allow to calculate some of the unknown rates. This is useful in a batch process where the biomass grows in a changing environment that can generate strong kinetic disturbances. Kinetic information on fermentations is generally scarce, particularly in the case of the gaseous components interchanged between the broth and the gas phase (O₂ and CO₂). A procedure was proposed to derive interrelationships between these measured variables and the state variables.

As it was seen in section 2.1, in batch fermentations the main substrate oxidation rate can show considerable variations. Contrary to what is usually assumed, it was shown here that this rate is not first order in the biomass concentration.

Some modern control strategies require of dynamical models. In batch fermentations the kinetic rates are generally unreliable, and for this reason the concept of disturbance-parameters estimated via an estimation algorithm were introduced to improve the quality of the kinetic information.

The proposed procedure was applied to xanthan gum batch fermentations, and the gum production was modeled through the Luedeking-Piret equation. The equation parameters and some stoichiometric coefficients were used to predict the oxygen consumption and the carbon dioxide production rates. A fairly good agreement with experimental data was observed.

Acknowledgments

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Nomenclature

- μ: specific biomass growth on the logistic equation (h⁻¹)
- α: growth associated to the specific main substrate consumption (g substrate/g biomass h)
- β: steady specific main substrate consumption (g substrate/g biomass)
- γ: growth associated to the specific carbon dioxide production (g CO₂/g biomass h)
- δ: steady specific carbon dioxide production (g CO₂/g biomass h)
- Δp: vector of disturbance-parameters
- ΔCO₂: cumulative main substrate consumption
- ΔS₅: cumulative nitrogen source consumption
- ΔO₂: cumulative oxygen consumption
- φᵢ: net flow rate of component I into the reactor (g I/L h)
- α: growth associated specific metabolite production (g metabolite/g biomass)
- ATP: adenosine 5-triphosphate (energy carriers)
- h: steady specific metabolite production (g metabolite/g biomass h)
- c: growth associated specific oxygen consumption (g O₂/g biomass)
- C: vector of main components concentration
- d: steady specific oxygen consumption (g O₂/g biomass h)
- Eₚ: stoichiometry of the metabolite production
- Eₚ𝙤: stoichiometry of the oxidative phosphorylation
- Eₛ: stoichiometry of the main substrate catabolism
- Eₓ: vector of dynamic functions for the states
- f(●): vector of state -measurements relations
- g(●): macroscopic specific coefficient of maintenance (g main substrate/g biomass h)
- Kₑ: reduced equivalents (electron carriers)
- NADH₂: concentration of metabolic product (g metabolite/L)
- p: vector of model parameters
- p/P/O: level of oxidative phosphorylation (ratio between ATP formed and oxygen consumed on Eₒₚ)
- rₐₜₚ: ATP consumption for maintenance (moles ATP/g biomass h)
- r₉: vector of conversion rates for the main components
- r₀₂: production rate of carbon dioxide (g CO₂/L h)
- r₀ₙ: consumption rate of the nitrogen source (g nitrogen source/L h)
- r₀₂: consumption rate of oxygen (g O₂/L h)
- rₚ: production rate of metabolite (g Metabolite/L h)
- rₒₔ: oxidative phosphorylation rate (moles ATP/L h)
- rₛ: consumption rate of main substrate (g main substrate/L h)
- rₚₛ: main substrate concentration (g main substrate/L)
- Sₙ: concentration of nitrogen source (g Nitrogen source/L)
- u: vector of control variables
- X: biomass concentration (g biomass/L)
- x: vector of state variables
- Xₛ: stationary biomass concentration (g biomass/L)
- Y: vector of measurements
- Yᵢ: stoichiometric yield of component I on component J (g I/g J)

References:


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