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Lactose continuous fermentation with cells recycled by ultrafiltration and lactate separation by electrodialysis: modelling and simulation

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Summary. Dynamic modelling of a continuous lactose fermentation system for control and optimisation of operation has been carried out. The bioreactor used was coupled with an ultrafiltration module and an electrodialysis unit. A unstructured model taking into account cell growth, substrate consumption, and metabolite (lactic acid) production, has been analysed. The metabolite production model was represented by the Luedeking-Piret equation, modified in order to improve the description of lactic acid production at low dilution rate.

Introduction

This work is part of a more general study on lactose transformation into higher value organic chemicals and deals in great detail with lactose fermentation to lactic acid. In present technology the volumetric productivity of lactic fermentors is known to be rather small due to inhibition of the biomass production rate by accumulated lactic acid (Coulman et al. 1977). Unfortunately, owing to its physico-chemical properties, lactic acid cannot be continuously extracted from the fermentation medium by simple operations, such as distillation, precipitation, or extraction, so a large proportion of the cost of lactic acid production from milk or whey ultrafiltrates is associated with recovery and purification operations (Smith et al. 1977). Membrane processes may lead to both continuous lactic acid extraction and biomass confinement (Coulman et al. 1977) or recycling (Vick-Roy et al. 1983). A membrane bioreactor seems to be the best way to improve the volumetric productivity of lactic fermentation.

Reverse osmosis leads to good lactose and biomass rejection, but lactic acid permeation is rather low and membrane plugging very high (Smith et al. 1977). Electrodialysis (ED) allows good lactic acid separation, but the confinement of biomass and biopolymers yields to fast anionic membrane fouling (Krumphanzl and Dyr 1962). Ultrafiltration (UF) ensures high cell density by recycling (Vick-Roy et al. 1983; Mehaia and Cheryan 1986), but this separation method, besides membrane fouling, suffers from a major drawback: the ultrafiltrate contains both lactic acid and untransformed nutrients. In order to obtain pure lactic acid from the ultrafiltrate, another separation method is needed. Preliminary experiments (Prigent 1983; Prigent and Franco 1984; Boyaval et al. 1987, 1988) have shown that ED is very convenient for this purpose.

Figure 1 displays the flow diagram of a system involving a continuous fermentor with cell recycling by UF, and lactic acid extraction and concentration by ED. The main purpose of this paper is to develop a mathematical model possibly resulting in a better knowledge of this complex system. On the other hand, this model should be a robust analysis tool for process optimization and its automatic control.

Mathematical model

The lactic membrane fermentor illustrated in Fig. 1 involves two feeback loops: the first one deals with cells (UF retentate), and the second one with untransformed nutrient recycling (ED diluate, i.e. ultrafiltrate depleted of lactic acid).

A general model of a cell recycling fermentor is available (Pirt and Kurowski 1970) and this model is independent of the cell separation method involved. This has been shown to agree

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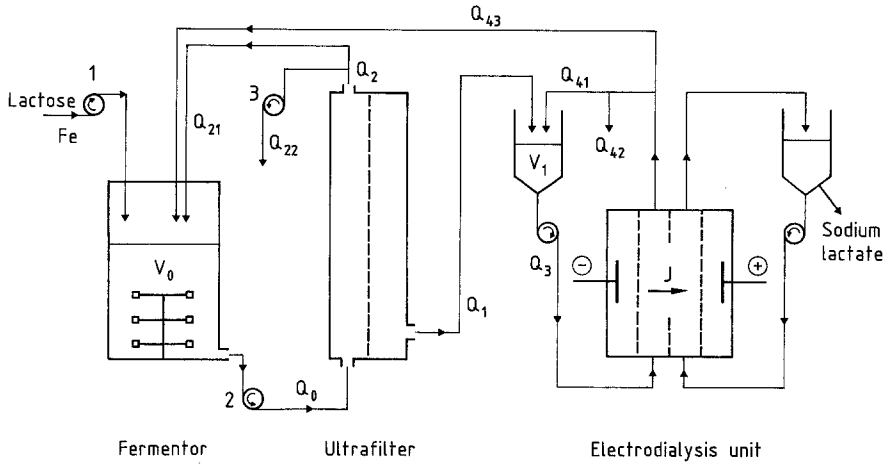

Fig. 1. Schematic flow sheet diagram of the bioreactor process: *1* Feed peristaltic pump; *2* circulation positive-pump; *3* bleed pump (output of concentrated cell suspension)

fairly well with filtration experiments on yeast cultures. Sodium lactate extraction from an ultrafiltred fermentation broth by mean of continuous ED with recycling has also been modelled (Prigent and Franco 1984). The model to be described has to take into account both processes acting simultaneously.

Model assumptions

Continuous fermentation. In the first step, an unstructured model has been retained; in this model the following assumptions have been made:

1. The specific growth rate consists of two terms, a Monod equation (Monod 1942; MacBean et al. 1979) modified for non-competitive inhibition by lactic acid (Tayeb et al. 1984). Such an equation has already been used for the description of a dialysis lactic fermentor (Coulman et al. 1977):

$$\mu = \frac{\dot{X}_0}{X_0} = \mu_{\max} \cdot \frac{S_0}{S_0 + Ks} \cdot \frac{Kp}{P_0 + Kp} \tag{1}$$

2. Lactic acid production is partially associated with growth, so the specific rate of product formation can be represented by the widespread Luedeking and Piret equation (Luedeking and Piret 1959; Coulman et al. 1977; MacBean et al. 1979; Tayeb et al. 1984):

$$q_P = \frac{\dot{P}_0}{X_0} = \alpha \cdot \mu + \beta \tag{2}$$

3. Lactose is converted into both biomass and lactic acid, so there are two contributions to the substrate consumption specific rate:

$$q_S = -\frac{\dot{S}_0}{X_0} = \frac{\mu}{Y_{X/S}} + \frac{(\alpha \cdot \mu + \beta)}{Y_{P/S}} = \mu \cdot \left(\frac{1}{Y_{X/S}} + \frac{\alpha}{Y_{P/S}}\right) + \frac{\beta}{Y_{P/S}} \tag{3}$$

This relationship may be considered as a generalized Pirt equation (Pirt 1975), under conditions of constant substrate to product yield;

4. Supplemented nitrogen is only converted into biomass:

$$q_Z = -\frac{\dot{Z}_0}{X_0} = \frac{\mu}{Y_{X/Z}} \tag{4}$$

Ultrafiltration. The models currently available in the literature do not take into account the reduction of ultrafiltrate flux induced by fouling. Nevertheless, biopolymer absorption on UF membranes may drastically decrease their solvent permeability (Fane et al. 1983; Howell et al. 1981). The application of such models to continuous ultrafiltration of the fermentation broth therefore seems to be hazardous. For these reasons, it was decided to operate at constant pressure and feed rate Q_0. Under these conditions, information about membrane fouling can be obtained form on-line measurement of the permeate flow rate Q_1.

The UF membrane is usually assumed to be completely permeable to low molecular species, such as untransformed nutrients and lactic acid. As the recirculation flow rate Q_2 is usually high (about 800 l/h for our pilot plant) in relation to dead volume (about 2 l), it can be assumed that the ultrafilter always operates at a steady state:

$$Q_0 \cdot X_0 - Q_2 \cdot X_2 = 0 \tag{5}$$

Electrodialysis. Continuous lactate extraction from the ultrafiltered broth has been modelled under the following assumptions (Prigent and Franco 1984): (1) constant volume V_1 in the feed tank:

$$Q_3 = Q_1 + Q_{41} \tag{6}$$

(2) constant recirculation flow rate Q_3; (3) electroosmotic water flux much smaller than Q_1, so it can be neglected:

$$Q_3 = Q_{41} + Q_{42} + Q_{43} \tag{7}$$

(4) negligible lactose transport; (5) under constant current density, constant lactate flux J; (6) negligible mean residence time due to very small dead volume (i.e. steady state conditions hold at all times):

$$Q_3 \cdot P_1 - J - (Q_{41} + Q_{42} + Q_{43}) \cdot P_3 = 0 \tag{8}$$

$$Q_3 \cdot S_1 - (Q_{41} + Q_{42} + Q_{43}) \cdot S_3 = 0 \tag{9}$$

Model development

The mathematical model for a membrane lactic fermentor is based on the following three mass balances.

1. The rate of biomass production can be obtained from the microorganism balance inside the fermentor and the ultrafilter:

biomass accumulation = growth − decay − output + feedback.

Assuming a first order decay (Chiu et al. 1972), in the infinitely small time interval dt, the balance in the fermentor will be:

$$V_0 \cdot \dot{X}_0 \cdot dt = \mu \cdot X_0 \cdot V_0 \cdot dt - Kd \cdot X_0 \cdot V_0 \cdot dt - Q_0 \cdot X_0 \cdot dt + Q_{21} \cdot X_2 \cdot dt \quad (10)$$

Taking into account Eq. (5) and the ultrafilter volume balance $Q_{21} = Q_0 - Q_1 - Q_{22}$, and dividing by $V_0 dt$, the following expression is obtained:

$$\dot{X}_0 = \left[\mu - Kd - \frac{Q_0 \cdot Q_{22}}{(Q_0 - Q_1) \cdot V_0}\right] \cdot X_0 \quad (11)$$

2. The lactic acid balance may be described as follows:

fermentor and ultrafilter:

accumulation = production − output + feedback

$$V_0 \cdot \dot{P}_0 \cdot dt = q_P \cdot X_0 \cdot V_0 \cdot dt - Q_0 \cdot P_0 \cdot dt + Q_{21} \cdot P_0 \cdot dt + Q_{43} \cdot P_3 \cdot dt \quad (12)$$

electrodialyser feed tank:

accumulation = input − output + feedback

$$V_1 \cdot \dot{P}_1 \cdot dt = Q_1 \cdot P_0 \cdot dt - Q_3 \cdot P_1 \cdot dt + Q_{41} \cdot P_3 \cdot dt \quad (13)$$

The volume balance for the fermentor leads to:

$$Q_{43} = Q_1 + Q_{22} - Fe \quad (14)$$

On substituting Q_{41} and Q_{43} from Eqs. (6) and (14), P_3 from (7) and (8), and q_P from (2), Eqs. (12) and (13) become respectively:

$$\dot{P}_0 = (\alpha \cdot \mu + \beta) \cdot X_0 - \left(\frac{Q_1 + Q_{22}}{V_0}\right) \cdot \left(P_0 - P_1 + \frac{J}{Q_3}\right) - \frac{Fe}{V_0} \cdot \left(P_1 - \frac{J}{Q_3}\right) \quad (15)$$

$$\dot{P}_1 = \frac{Q_1}{V_1} \cdot \left(P_0 - P_1 + \frac{J}{Q_3}\right) - \frac{J}{V_1} \quad (16)$$

3. The balance for the growth-limiting substrate in the fermentor is given by:

fermentor and ultrafilter:

accumulation = input − consumption − output + feedback

$$V_0 \cdot \dot{S}_0 \cdot dt = Fe \cdot Se \cdot dt - q_S \cdot X_0 \cdot dt - Q_0 \cdot S_0 \cdot dt + Q_{21} \cdot S_0 \cdot dt + Q_{43} \cdot S_3 \cdot dt \quad (17)$$

electrodialyser:

accumulation = input − output + feedback

$$V_1 \cdot \dot{S}_1 \cdot dt = Q_1 \cdot S_0 \cdot dt - Q_3 \cdot S_1 \cdot dt + Q_{41} \cdot S_3 \cdot dt \quad (18)$$

From Eqs. (7) and (9) we obtain: $S_3 = S_1$.

By insertion of the relevant expressions into Eqs. (17) and (18), and substituting q_S from Eq. (3), the mass balance model may be represented by:

$$\dot{S}_0 = -\left[\mu \cdot \left(\frac{1}{Y_{X/S}} + \frac{\alpha}{Y_{P/S}}\right) + \frac{\beta}{Y_{P/S}}\right] \cdot X_0 + \frac{Fe}{V_0} \cdot Se - \frac{Q_1 + Q_{22}}{V_0} \cdot S_0 + \frac{Q_1 + Q_{22} - Fe}{V_0} \cdot S_1 \quad (19)$$

and

$$\dot{S}_1 = \frac{Q_1}{V_1} \cdot (S_0 - S_1) \quad (20)$$

4. As that of lactose (limiting substrate), nitrogen balance may be drawn in the same way, using Eq. (4) instead of (3) in Eq. (17):

fermentor and ultrafilter:

$$\dot{Z}_0 = -\frac{\mu \cdot X_0}{Y_{X/Z}} + \frac{Fe \cdot Ze}{V_0} - \frac{Q_1 + Q_{22}}{V_0} \cdot Z_0 + \frac{Q_1 + Q_{22} - Fe}{V_0} \cdot Z_1 \quad (21)$$

electrodialyser:

$$\dot{Z}_1 = \frac{Q_1}{V_1} \cdot (Z_0 - Z_1) \quad (22)$$

Results and discussion

The model developed above has been implemented into a computer programme and tested under different fermentation conditions; the Sirena programme allows simulation of complex non-linear systems.

Batch fermentation seems to be the best way to check the self-consistency of the non-linear model. For this purpose, the following set of data has been used:

$$\begin{bmatrix} Ks = 0.35 \text{ g/l}; \; Kp = 20 \text{ g/l}; \; Se = 50 \text{ g/l}; \\ V_0 = 3 \text{ l}; \; Y_{S/X} = 1.32; \; Y_{S/P} = 1; \; Y_{Z/X} = 1; \\ \beta = 2.2 \text{ h}^{-1}; \; \alpha = 0.55; \\ Kd = 0.04 \text{ h}^{-1}; \; \mu_{max} = 1 \text{ h}^{-1}. \end{bmatrix}$$

These values of biological parameters have been estimated from preliminary batch experiments. Figure 2a and b show the kinetics of specific growth rate μ and amino nitrogen concentration Z_0 for batch cultivation, calculated from the model. It can be seen that this model yields kinetics conflicting with the batch assumption (negative values for μ and Z_0; amino nitrogen production for $t > t_1$). This unexpected behaviour leads us to question the validity of the Luedeking and Piret assumption (Eq. 2) for lactic acid production.

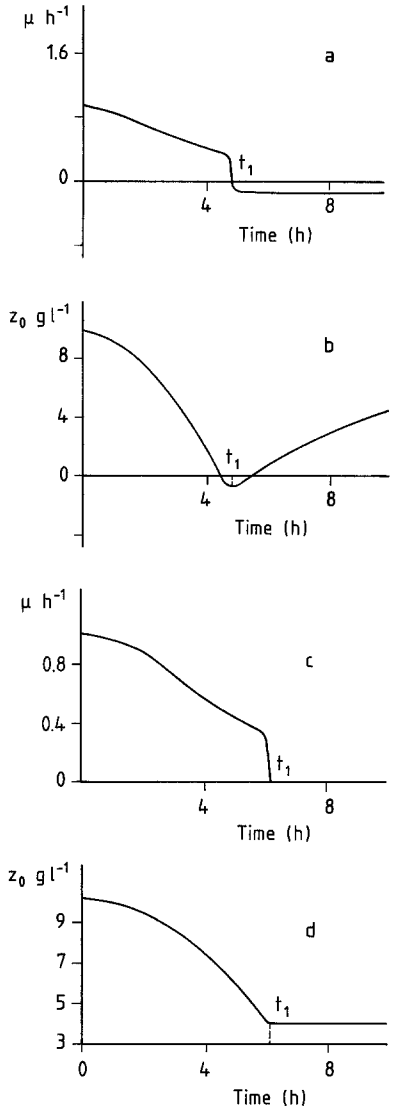

Fig. 2a–d. Kinetics of specific growth rate μ, (**a** and **c**), and nitrogen concentration Z_0, (**b** and **d**) for batch cultivation, using first the Luedeking-Piret production model (**a** and **b**), and secondly the modified model (**c** and **d**)

Figure 3 displays experimental values from batch experiments of specific lactic acid production rate ($q_P = dP_0/X_0 dt$) versus specific growth rate ($\mu = dX_0/X_0 dt$). As seen in Fig. 3, the Luedeking-Piret assumption (Eq. 2) holds only for the higher values of μ, i.e. at the beginning of batch fermentation or at low biomass recycling (high removal flow rate Q_{22}).

The shape of q_P vs μ suggested that we should modify the Luedeking-Piret assumption (Eq. 2) in the following manner:

$$q_P = \frac{dP_0}{X_0 \cdot dt} = \alpha \cdot \mu(t) + \beta_0 \cdot [1 - e^{-\mu(t)/\mu_0}] \qquad (23)$$

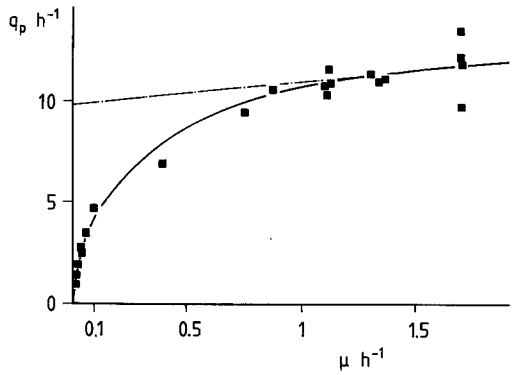

Fig. 3. Specific lactic acid production rate, q_P, as a function of the specific growth rate μ, drawn from a batch culture experiment — · — Luedeking and Piret approximation (Eq. 2)

Such a model, in which β_0 and μ_0 are constant, should lead to a better description of lactic acid production at low specific growth rate (i.e. end of batch fermentation, or continuous fermentation at high cell recycling).

Figure 2c and d show the kinetics of μ and Z_0 calculated from the modified model (Eq. (23) instead of Eq. (2)). The new model seems to be self-consistent, as the amino nitrogen is no longer consumed after growth stops ($t > t_1$). This new model would appear attractive for simulation of the complex system (continuous fermentor + UF + ED, with cell concentrate and ED diluate recycling) outlined in Fig. 1. The main objective is to simulate the dynamic response of the system, initially working at a steady state, to any perturbation.

Hereafter are discussed, as especially striking examples, the simulated responses to the following perturbations: (i) a step-shaped increase of the biomass removal rate Q_{22}, the most important control variable for the system, (ii) a ramp shaped decrease in the permeate efflux rate Q_1 out of the ultrafilter. Such a parameter perturbation simulates fairly well the observed UF membrane fouling.

Figure 4 has been drawn with the following data:

$$\begin{bmatrix} V_1 = 1\ \text{l};\ Q_0 = 800.75\ \text{l/h};\ Q_1 = 0.75\ \text{l/h}; \\ Q_2 = 800\ \text{l/h};\ Q_3 = 500\ \text{l/h};\ \mu_0 = 0.1\ \text{h}^{-1}; \\ J = 20\ \text{g/h};\ Fe = 1\ \text{l/h};\ Q_{22} = 0.1\ \text{l/h}. \end{bmatrix}$$

At zero time, Q_{22} is settled at 0.4 l/h, and the other parameters are kept constant throughout the simulation. As Q_0 is very much higher than Q_1, Eq. (11) predicts that the specific growth rate μ,

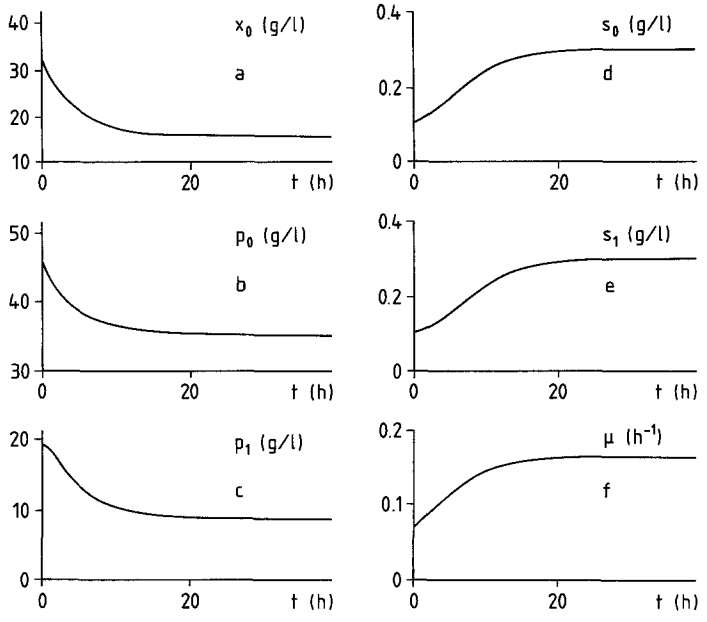

Fig. 4a-f. Q_{22} step-input simulated responses of the bioreactor process. **a** Biomass concentration X_0 ($g \cdot l^{-1}$). **b, c** Lactic acid concentration in the bioreactor, P_0 ($g \cdot l^{-1}$), and in tank D of the electrodialysis (ED) unit, P_1 ($g \cdot l^{-1}$). **d, e** Lactose concentration in the bioreactor, S_0 ($g \cdot l^{-1}$), and in tank D of the ED unit, S_1 ($g \cdot l^{-1}$). **f** Specific growth rate, μ (h^{-1})

proportional to Q_{22}, must reach a new stationary value higher than the previous one. Such behaviour is indeed observed in Fig. 4f.

As expected (Fig. 4a), a rise in the biomass removal rate Q_{22} at constant feed rate Fe yields a drop in the biomass concentration. Consequently, at constant substrate input rate, the lactic acid concentrations in the fermentor and in the electrodialyser decrease (Fig. 4b, c) while the substrate concentrations increase (Fig. 4d, e). Such behaviour is due to the constant residence time in the electrodialyser (constant Q_1).

Figure 5 has been drawn with the following data:

$$\begin{bmatrix} V_1 = 1\ \text{l};\ Q_0 = 801\ \text{l/h};\ Q_2 = 800\ \text{l/h}; \\ Q_3 = 500\ \text{l/h};\ \mu_0 = 0.1\ \text{h}^{-1};\ J = 20\ \text{g/h}; \\ Fe = 1\ \text{l/h};\ Q_{22} = 0.1\ \text{l/h};\ Q_1 = 1\ \text{l/h}. \end{bmatrix}$$

At zero time, Q_1 decreases linearly at the rate $-0.025\ \text{l/h}^2$, keeping the other parameters constant. Figure 5a and f show that the biomass concentration X_0 and the specific growth rate μ do

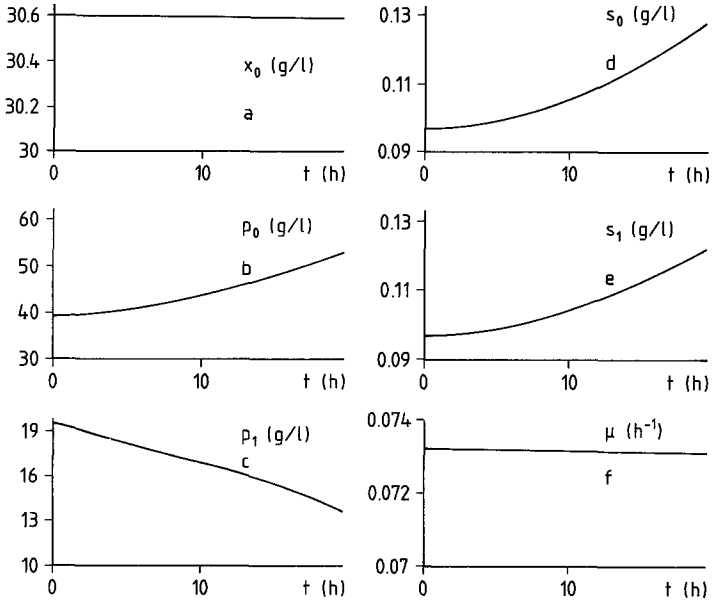

Fig. 5a-f. Q_1 ramp-perturbation simulated responses of the bioreactor process. **a** Biomass concentration X_0 ($g \cdot l^{-1}$). **b** and **c** Lactic acid concentration in the bioreactor, P_0 ($g \cdot l^{-1}$), and in tank D of the ED unit, P_1 ($g \cdot l^{-1}$). **d, e** Lactose concentration in the bioreactor, S_0 ($g \cdot l^{-1}$), and in tank D of the ED unit, S_1 ($g \cdot l^{-1}$). **f** Specific growth rate, μ (h^{-1})

not depend on this parameter perturbation. This can be explained by the negligible rôle of Q_1 in the biomass balance (Eq. (11)).

The only expected changes concern the concentrations of the product and substrate able to go through the UF membrane. During Q_1 lowering, with *Fe* and Q_{22} constant, the ED diluate recycling rate Q_{43} has to decrease to ensure a constant level in the fermentor (Eq. (14)). So the mean residence time both in the reactor and electrodialyser becomes longer. Thus at constant biomass concentration X_0, and specific growth rate μ, the product concentration P_0 in the reactor increase (Fig. 5b), because the biomass stays in contact longer with the substrate. Under constant current conditions in the electrodialyser, a longer residence time leads to a better exhaustion of the product, so P_1 decreases (Fig. 5c).

The shape of Fig. 5d can be explained by Eq. (1) which forces the substrate concentration S_0 to rise in order to keep μ constant when P_0 decreases. As the substrate does not go through the ion exchange membranes, its concentration S_1 in the electrodialyser (Fig. 5e) varies like S_0.

In conclusion, the present paper is mainly concerned with development and testing a mathematical model for a continuous lactic fermentor with cell recycling by UF, and product extraction by ED. A first version of the developed model for the production rate involves the widespread Luedeking and Piret equation (production partially associated with growth). By the way of dynamic simulation, the first version has been improved. The novel production model has also been checked by simulation, and leads to more sensible results than the first version.

The importance of permeate flow rate Q_1 (membrane fouling correlation), and its noticeable effect upon the knowledge model have been emphasised. This parameter has therefore to be taken into account in the elaboration of the process optimization and its automatic control.

The next step will consist of identifying the continuous lactic bioreactor in order to estimate the biological parameters of the unstructured model. This step will so allow a better understanding of the different phenomena involved in lactose fermentation.

Nomenclature

Fe: bioreactor influent flow rate (l/h); *J:* lactate flux in the ED unit (g/h); *Kd*: mortality constant (h^{-1}); *Kp*: product inhibition constant (g/l); *Ks*: substrate saturation constant (g/l); P_0: product concentration in the bioreactor (g/l); P_1: product concentration in tank *D* (g/l); P_3: product concentration in the effluent of the ED unit (g/l); Q_0: retentate flow rate (UF influent) (l/h); Q_1: permeate flow rate (l/h); Q_2: retentate flow rate (UF effluent) (l/h); Q_{21}: cell recycling flow rate (l/h); Q_{22}: cell bleed flow rate (l/h); Q_3: recycling flow rate in the ED unit (influent) (l/h); Q_{41}: recycling flow rate in the ED unit (effluent) (l/h); Q_{42}: tank *D* overflow (l/h); Q_{43}: recycling flow rate to the ED unit to the bioreactor (l/h); q_P: specific rate of product formation (h^{-1}); q_S: substrate consumption specific rate (h^{-1}); q_Z: nitrogen consumption specific rate (h^{-1}); *Se*: substrate concentration in the influent (g/l); S_0: substrate concentration in the bioreactor (g/l); S_1: substrate concentration in tank *D* (g/l); S_3: substrate concentration in the effluent of the unit (g/l); *t*: time (h); t_1: time when $\mu = Kd$ (h); V_0: fermentation broth volume (l); V_1: tank *D* volume (l); X_0: biomass concentration in the bioreactor (g/l); X_2: biomass concentration in the retentate UF flow rate (g/l); $Y_{P/S}$: ($=1/Y_{S/P}$) lactic acid yield coefficient (g lactic acid/g substrate consumed); $Y_{X/S}$: ($=1/Y_{S/X}$) cell yield coefficient (g cells produced/g lactose consumed); $Y_{X/Z}$: ($=1/Y_{Z/X}$) second cell yield coefficient (g cells produced/g nitrogen consumed); Z_e: nitrogen concentration in the influent (g/l); Z_0: nitrogen concentration in the bioreactor (g/l); Z_1: nitrogen concentration in tank *D* (g/l); α, β: constants of the Luedeking and Piret model; β_0, μ_0: coefficients in Eq. (23); μ: specific growth rate (h^{-1}); μ_{max}: maximum specific growth rate (h^{-1}).

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