A model for micro/ultrafiltration cell deactivation in cell-recycle reactors

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Abstract: Mechanical stress due to micro/ultrafiltration has been suggested by a number of authors as one of the sources for cell deactivation in continuous cell-recycle reactors. This work introduces cell deactivation in the modelling of cell-recycle fermenters. A two-population based model is used. It is shown that the kinetic parameters obtained from chemostat fermentations describe accurately the experimental results and the apparent deviations to the Luedeking–Piret relationship at low growth rates are explained. The model is applied to the data of two authors with dilution rates (*D*) ranging 0.2 and 1.05 h^{-1} and using either *Lactobacillus rhamnosus* or *Lactococcus cremoris cells*. Although only data for lactic fermentation were used, the proposed model is of general applicability and not restricted to a type of fermentation, a given apparatus configuration or Newtonian fluids. The development of improved procedures for biological reactor modelling, such as presented in this work, wall allow more adequate and efficient design and operation of these reactors.

Keywords: cell-recycle fermenter; two population model; high cell concentration culture; cell deactivation

NOTATION

D	Dilution rate (h^{-1})
K	Proportionality parameter for cell deactivation (eqn (2))
K_d	Bibal's deactivation constant
K_p	Product inhibition parameter (eqn (6a)) ($m^3 kg^{-1}$)
N	Frequency of passages through the filtration unit (h^{-1})
P	Product concentration ($kg m^{-3}$)
P_c	Critical product concentration (eqn (6b)) ($kg m^{-3}$)
Q_{rec}	Recycling flow rate ($m^3 h^{-1}$)
S	Substrate concentration ($kg m^{-3}$)
S_i	Feed substrate concentration ($kg m^{-3}$)
V_r	Reactor volume (m^3)
t	Time (h)
X_a	Concentration of active cells ($kg m^{-3}$)
X_i	Concentration of inactive cells ($kg m^{-3}$)
X_{tot}	Total cell concentration ($kg m^{-3}$)
$Y_{P/S}$	Yield
α	Luedeking–Piret relationship parameter
β	Luedeking–Piret relationship parameter
γ	Parameter for dependence on viscosity
η	Viscosity ($N s m^{-2}$)
η_w	Water viscosity ($N s m^{-2}$)
μ	Specific growth rate (h^{-1})
μ_{ap}	Apparent specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
v	Specific production ($kg_{product} kg_{biomass}^{-1} h^{-1}$)
v_{ap}	Apparent specific production ($kg_{product} kg_{biomass}^{-1} h^{-1}$)

1 INTRODUCTION

In the last 20 years, there has been considerable effort to develop fermentation processes with high cell density cultures. The increase in the overall process performance, in particular regarding productivity, energy and resources conservation, and the possibility of changing the process from batch to continuous has fuelled the research in this area.[1] The process that generates the higher cell concentration in fermenters involves cell recycling with micro/ultrafiltration membranes.[2] This technology can be used with most sorts of fermentation such as ethanolic, lactic or propionic, SCPs or waste treatment.[1,2] This work will focus on the description of cell deactivation produced by the micro/ultrafiltration recycle.

The loss of cell activity at high biomass concentrations is well known in cell-recycle fermenters.[2–4] Part of the loss may be due to nutrient limitation or toxin accumulation in the broth, but mechanical stress also contributes to cell deactivation. Measurements on cell deactivation due to recycling through a filtration unit

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are reported by Bibal *et al.*[5] Nevertheless, cell deactivation by mechanical stress is not yet widely accepted and more sound experimental evidence seems to be missing. Since no successful model incorporating cell deactivation has been previously described in the literature, a model for total cell-recycle fermenters taking into account cell deactivation by mechanical stress is proposed here.

2 EXPERIMENTAL

This work does not present new experimental data. The data used to test the proposed model have been presented by the authors elsewhere.[5,6] This experimental section aims to give the reader a summary of the organisms and equipment involved in the production of the data used, to test the model.

2.1 Strains and culture media

The lactic acid bacteria used were *Lactobacillus rhamnosus* NRRL B445 by Xavier[6] and *Lactococcus cremoris* by Bibal *et al.*[5]

The fermentation media were $15\,g\,dm^{-3}$ of yeast extract, $0.2\,g\,dm^{-3}$ K_2HPO_4, $0.2\,g\,dm^{-3}$ KH_2PO_4, $0.1\,g\,dm^{-3}$ $MgSO_4\cdot 7H_2O$, $0.03\,g\,dm^{-3}$ $MnSO_4\cdot 7H_2O$, $150\,g\,dm^{-3}$ glucose and 0.1%(v/v) Tween 80 by Xavier[6] and $5\,g\,dm^{-3}$ yeast extract, $7\,g\,dm^{-3}$ bactotryptone $0.2\,g\,dm^{-3}$ $MgSO_4\cdot 7H_2O$ and $50\,g\,dm^{-3}$ lactose by Bibal *et al.*[5] Medium pH was 6.3 and Fermentation temperatures were respectively 42 °C and 27 °C.

2.2 Cell-recycling fermentations

In both sets of experiments[5,6] a $2\,dm^3$ fermenter (SGI, France) was connected with a microfiltration unit. A volumetric pump (Albin Pump AB, SLP 115, Sweden), delivered the fermentation broth from the vessel through one of two modules of ceramic tubular membranes (Tech-Sep, France) where it was tangentially filtered. Fermentations were performed by recycling all the concentrated cell stream and only part of the filtered one. More details can be found elsewhere.[5,7] Before each operation the whole system was cleaned and steam sterilised.

3 THEORY

The model proposed here is based on the consideration that the cell population is composed of two types of cells:

Active cells, either reproducing or fermenting – X_a

Inactive cells, neither reproducing nor fermenting – X_i

The total cell concentration, X_{tot}, is given by:

$$X_{tot} = X_a + X_i \tag{1}$$

The deactivation is postulated to be a consequence of the cell-recycle and should thus be proportional to the frequency of passages through the filtration unit, N ($N = Q_{rec}/V_r$, where V_r is the reactor volume and Q_{rec} the recycling flow rate), the concentration of active cells, X_a, and some function of the viscosity of the fluid $f(\eta)$. This function depends on the design of the filtration unit, the type of cells used and the rheology of the broth, ie it will be different if the fluid is Newtonian or non-Newtonian. For the cases under study the fluid is Newtonian and this dependency is assumed to be represented by $f(\eta) = (\eta/\eta_w)^\gamma$. The variation of the total number of cells is given by:

$$\frac{dX_{tot}}{dt} = \frac{dX_a}{dt} + K\left(\frac{\eta}{\eta_w}\right)^\gamma N X_a \tag{2}$$

where t is the time and K the proportionality parameter describing the cell deactivation.

The Luedeking–Piret model was first proposed for lactic acid formation.[8] This model relates the specific production, ν, and specific growth rate, μ, defined as:

$$\mu = \frac{1}{X_{tot}}\frac{dX_{tot}}{dt} \tag{3}$$

$$\nu = \frac{1}{X_{tot}}\left(\frac{dP}{dt} + PD\right) \tag{4}$$

where P is the product concentration and D the dilution rate. This model will be considered valid for both chemostat and total cell-recycle reactors:

$$\nu = \alpha\mu + \beta \tag{5}$$

The values for the parameters α and β obtained from chemostat fermentations (FQ and FQr[6]) are presented in Table 1.

It is assumed that the only inhibition to growth is caused by the product. Following Xavier[6] and Bibal *et al.*[5] two expressions for the growth rate inhibition due to product formation are used:

$$\mu = \mu_{max} e^{-K_p P} \tag{6a}$$

$$\mu = \mu_{max}\left(1 - \frac{P}{P_c}\right) \tag{6b}$$

Equation (6a) is based on Xavier's data and eqn (6b) on the data of Bibal *et al.* The parameters for these dependencies obtained from chemostat fermentations are also presented in Table 1.

To write the system differential balances based on the total amount of biomass, X_{tot}, it is convenient to

Table 1. Luedeking–Piret and product inhibition parameters

Xavier (1996)[6]	$\alpha = 6.64$	$\beta = 0.79\,h^{-1}$
Bibal *et al* (1991)[5]	$\alpha = 5.15$	$\beta = 0.6\,h^{-1}$
Xavier (1996)[6]	$\mu_{max} = 0.47\,h^{-1}$	$K_p = 0.285\,g^{-1}\,l$
Bibal *et al* (1991)[5]	$\mu_{max} = 0.9\,h^{-1}$	$P_c = 68\,g\,l^{-1}$

define:

$$\mu_{ap} = \mu \frac{X_a}{X_{tot}} \tag{7a}$$

$$\nu_{ap} = \nu \frac{X_a}{X_{tot}} \tag{7b}$$

The advantage of using those apparent specific production and growth rates is that the total biomass concentration is directly accessible, unlike the amount of active, X_a, or inactive cells, X_i.

The Luedeking–Piret relationship expressed as a function of these quantities then becomes:

$$\nu_{ap} = \alpha\mu_{ap} + \beta \frac{X_a}{X_{tot}} \tag{8}$$

where α and β are the parameters obtained for Eq. (5) from chemostat fermentations.

The differential balances for total biomass, X_{tot}, product, P, and substrate, S, are thus:

$$\frac{dX_{tot}}{dt} = \mu_{ap} X_{tot} \tag{9}$$

$$\frac{dP}{dt} + PD = \nu_{ap} X_{tot} \tag{10}$$

$$\frac{dS}{dt} + DS = DS_i - \frac{\nu_{ap} X_{tot}}{Y_{P/S}} \tag{11}$$

where S is the substrate concentration in the reactor, S_i the feed substrate concentration and $Y_{P/S}$ the yield. The proposed model is defined by Eqns. (2), (6) and (8)–(11).

Although defined for total cell-recycle fermentations, this model can easily be extended to cell-recycle fermenters with biomass bleed.

4 RESULTS AND DISCUSSION

4.1 Determination of constant values

If there are experimental data for cell deactivation, eqn (2) can be fitted to it, and, in the absence of other inhibitions not common to both the chemostat and cell-recycle fermenter, the cell-recycle model could be predictive. Unfortunately no data are available in the literature with all the necessary information: data by Xavier[6] have no deactivation information; the data by Bibal *et al*[5] have some information on deactivation but not on viscosity or on the recirculation conditions. In the absence of other data the deactivation parameters K and γ were fitted to the available ν_{ap} versus μ_{ap} data:

(i) For Xavier[6] $\gamma = 0.2$ and $K = 10^{-4}$. As expected, since the same experimental apparatus and micro-organism are used, the parameters are valid for all the data sets by this author. The frequency of passage through the filtration unit *(N)* is $371.7\,h^{-1}$.

(ii) For Bibal *et al*,[5] since no information on viscosity was available, and some measurements presented by the authors indicate that their cells were rather viscosity-insensitive, no viscosity dependence was considered $\gamma = 0$, and $K = 7.5 \times 10^{-4}$.

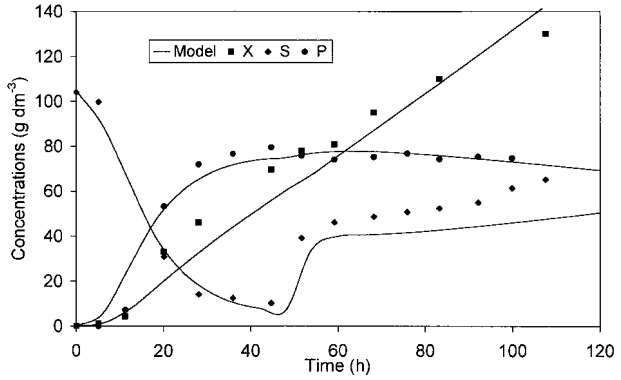

Figure 1. Comparison between experimental data of substrate (*S*), product (*P*) and *Lactobacillus rhamnosus* (*X*) concentrations and the model results for UF18. $D = 0.4\,h^{-1}$.

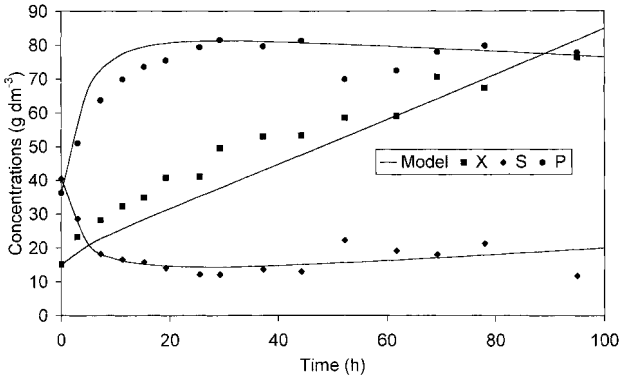

Figure 2. Comparison between experimental data of substrate (*S*), product (*P*) and *Lactobacillus rhamnosus* (*X*) concentrations and the model results for UF7. $D = 0.2\,h^{-1}$.

4.2 Application of model to experimental data

The proposed model was used to describe a series of experiments with dilution rates (D) ranging between $0.2\,h^{-1}$ for UF7 and UF8[6] to $1.05\,h^{-1}$ for Bibal *et al.* [5] The results for some of the fermentations by Xavier[6] are presented in Figs 1–3. The model results show a

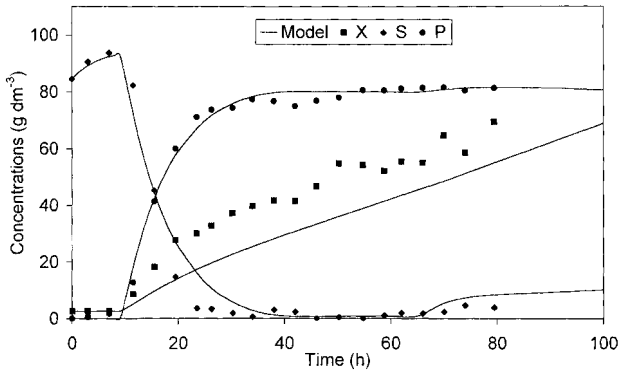

Figure 3. Comparison between experimental data of substrate (*S*), product (*P*) and *Lactobacillus rhamnosus* (*X*) concentrations and the model results for UF8. $D = 0.2\,h^{-1}$.

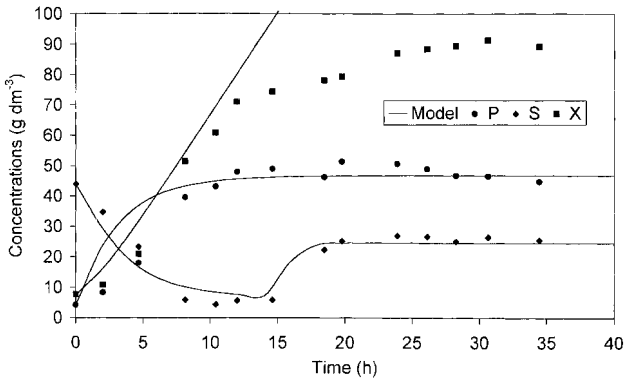

Figure 4. Comparison between experimental data on substrate (*S*), product (*P*) and *Lactococcus cremoris* (*X*) concentrations and the model results for Bibal *et al.* $D = 1.05\,h^{-1}$.

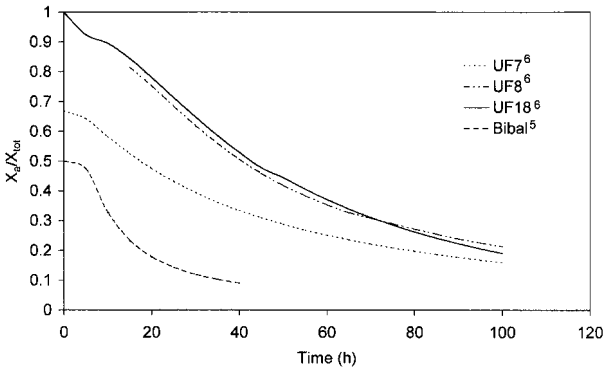

Figure 5. Predictions of the evolution of the fraction of active cells by the proposed model for the fermentations studied.

very good agreement with the experimental data. Results for other fermentations by the same authors are equivalent. The proposed model provides a better description of the data with a smaller number of fitting parameters than the model used by Xavier.[6]

Results for another fermentation by Bibal *et al*[5] are reported in Fig 4. Again an excellent description of the fermentation is achieved with the exception of the total biomass concentration. For this quantity the model is not able to accurately describe the evolution above 12h, indicating that either a biomass-dependent inhibition on the growth rate or some viscosity dependence for the deactivation has to be included in the fermentation modelling. The lack of experimental data does not allow us to go any further in the description of the data without making unsubstantiated assumptions.

The fermentations presented in Figs 1, 3 and 4 have their feed substrate composition changed during the experiment. This feature was incorporated in the model by changing the S_i value and it results in a 'jump' in substrate composition presented by both experimental and model results. It is interesting to remark that, from both an experimental and modelling point of view, this change in composition has no effect on the other variables (product and biomass). It is also important to notice that a lag of about 8h was used in the modelling of UF8 to match an 8h lag in the cells' development shown by the experimental data.

4.3 Prediction of active cell concentration

The variation of the fraction of active cells with time, predicted by the model for the various fermentations under study, is shown in Fig 5. The fraction of active cells quickly falls to levels below 50% and it seems not to be uncommon that values as low as 20% may be attained. This explains the apparent deviations to the

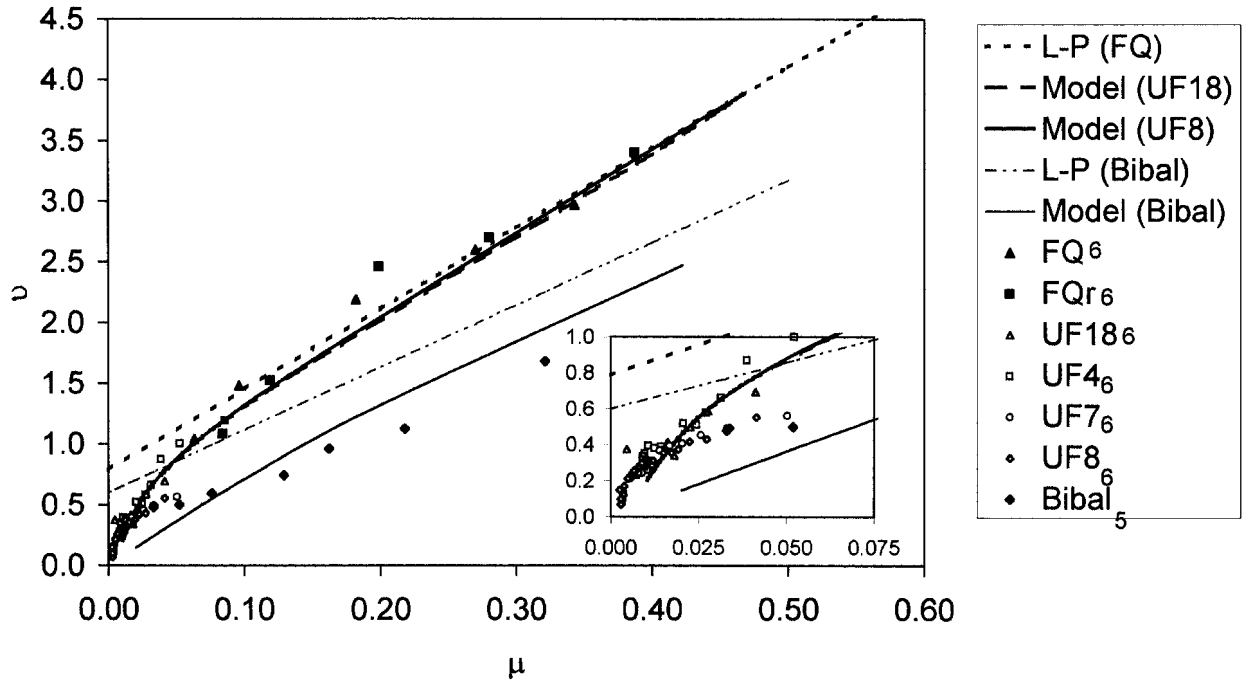

Figure 6. Comparison of v_{ap} and μ_{ap} with model results and the Luedeking–Piret relationship (v vs μ) for the studied fermentations. FQ and FQr are the chemostat fermentations used by Xavier,[6] to obtain the Luedeking–Piret parameters.

linear Luedeking–Piret relationship at low growth rates. For a continuous total cell-recycle fermenter, only the values of liter are reported v_{ap} and μ_{ap} in the literature, since only the total biomass is measured. Unlike eqn (5), eqn (8) is not linear due to the evolution of the fraction of active cells. As shown in Fig 6, the behaviour of the apparent production rate, v_{ap}, as a function of the apparent growth rate, μ_{ap}, is, as expected, well described by eqn (8). It must be noted that eqn (8) is based on the Luedeking–Piret parameters obtained for chemostat fermentation and assumes that the linear relationship between the real v and μ, based on the concentration of active cells, is valid.

Bibal *et al*[5] report some data on the influence of recycling on cell activity. The cells' activities were measured during cell-recycle cultures functioning in a chemostat mode at low cell concentrations and quantified using a parameter defined by the authors, the deactivation constant, K_d (K_p in the original paper). Although the data used in this work present biomass concentrations much higher than those used by Bibal *et al* to assess K_d, the deactivation constant obtained from the active cell concentrations predictions reported on Fig 5, $K_d = 1.3 \times 10^{-4}$, compares well with the value of K_d presented by Bibal *et al*,[5] 1.78×10^{-4}. The deactivation constant for the fermentations by Xavier[6] is not much different. It has a value of 4×10^{-5} and the difference can be justified by the utilisation of a different organism and the introduction of a viscosity-dependence which was not used in Bibal's data.

5 CONCLUSIONS

It is shown that a two-population model accounting for cell deactivation due to mechanical stress can well describe continuous total cell-recycle fermenters using parameters obtained from chemostat fermentations without the introduction of any inhibitions other than product inhibition. The apparent deviations to the linear Luedeking–Piret relationship at low growth rates can also be explained.

It must be emphasised that these results do not prove that there is cell deactivation by mechanical stress in the studied fermentations. Neither do they prove that a cell concentration dependent inhibition, or other, does not exist sometimes. Still they show that a model based on these assumptions provides a very good description of the experimental data available. Further work on the deactivation of cells by mechanical stress in cell-recycle fermenters leading to a definite conclusion is required.

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