Unstructured models for batch cultures of *lactobacillus helveticus*

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In the recent year's one can observe a growing interest in lactic acid production, which plays an important role in various applications in food, pharmaceutical and textiles industries More recently, lactic acid fermentation has received much attention because of an increasing demand for new bioengineering materials such as biodegradable polymers and the recent rise in the cost of petroleum, which is usually used as feed stock for production of lactic acid in conventional chemical processes. For a better understanding of the fermentation process and its optimization, a model is of a great help. Owing to the complexity of structured models, unstructured models can be preferred and have proven to accurately describe lactic acid fermentation in a wide range of experimental conditions and media. The aim of this work was to develop an unstructured model based on the experimental results of lactic acid fermentation using *Lactobacillus helveticus* growing on whey permeate supplementation. The first model was developed for cultures without pH control; an additional term to account for the undissociated lactic acid (and pH) inhibition was introduced in the Luedeking-Piret model. The model was found to match both experimental growth and production data. During cultures at pH controlled at 5.9, a corrective term was introduced in the Luedeking-Piret model to account for cessation of production due to carbon substrate limitation. This model matched experimental data accurately. To avoid the use of two expressions for production rate depending on the culture conditions, the above expressions were merged, leading to a unique expression taking into account both effects, a nutritional limitation effect and an inhibitory effect. Results obtained show that the generalized model gave a satisfactory description of experimental data in various culture conditions, since it was validated during cultures at pH control and in absence of pH control, as well as for different nitrogen supplementation of culture media.

1. Introduction

A great interest was reserved for lactic acid production in these last years by several authors. This product plays an important role in various applications mainly in the food industry, but also in the production of pharmaceuticals, cosmetics and textiles industries (Rojan and al., 2007). The continuous increase in its demand has received recently

much attention due to its increasing applications in the preparation of new bioengineering materials such as biodegradable polymers like polylactic acid (PLA), and the more recent rise in the cost of petroleum, which is usually used as feed stock for production of lactic acid in the conventional chemical processes. Lactic acid found also many other applications, such as medical sutures, green solvents (Dutta and Henry, 2006; Wee and al.2006). The industrial production of lactic acid can be carried out by two alternative technologies: chemical synthesis from fossil fuels and biotechnological processes. Nowadays, the fermentative production of lactic acid is the world's leading technology (about 90% of world production).

To increase the efficiency of the lactic acid fermentation processes, various cell culture methods have been investigated (Nandasana and Kumar 2007, Lin and Wang 2007). However, batch fermentation remains the most commonly used approach in industrial lactic acid production. Mathematical models may be useful for understanding the fermentation process and its optimization (modelling experimental data and studying the effects of experimental conditions on cultures kinetics) (Gadgil and Venkatesh, 1997; Amrane and Prigent, 1994a and 1999a). Lactic acid kinetics can be modelled par both structured or unstructured models; structured models have been reported to accurately describe lactic acid fermentation, but are complicated for many normal use (J. Nielsen 1991; Gadgil and Venkatesh, 1997). Unstructured models can be therefore preferred and have proven to accurately describe lactic acid fermentation in a wide range of experimental conditions and media. Some unstructured models were developed in the laboratory based on the partial linking between growth and lactic acid production (Luedeking and Piret, 1959). Additional terms were introduced in the Luedeking and Piret expression, to account for cessation of lactic acid production when carbon became limiting, namely to describe experiments carried out at a constant (and optimal) pH, 5.9 (Amrane and Prigent, 1994a and 1994b; Amrane 2001), or to account for the inhibitory effect of the undissociated lactic acid (and pH), occurring during cultures without pH control, which is the case during seed cultures (Amrane and Couriol 2002). The aim of this work is to develop new models, based on theses previously proposed models.

2. Materials and Methods

2.1. Microorganism

Lactobacillus helveticus strain *milano* used throughout this work was kindly supplied by Dr A. Fur (Even Ltd, Ploudaniel, France). Stock cultures were maintained on 10 % ($w.v^{-1}$) skim milk and deep-frozen at -16°C. As required, these cultures were thawed and reactivated by two transfers in 10 % ($w\ v^{-1}$) skim milk (42°C, 24h).

2.2. Media

Whey permeate powder (SIAB, Chateaubourg, France) was used as a carbon source; the powder was reconstituted at 57 g L^{-1}, corresponding to a lactose concentration of 48 g L^{-1}. Before use, and after clarification, the solution was pumped through two heat exchangers at 80 and 16 °C respectively (mean residence time: 20 seconds). The solution was left to decant overnight at 4 °C, and the supernatant was then supplemented with a 5 g L^{-1} of yeast extract or the following *RM* supplementation (g L^{-1}): yeast extract (*YE*), 20; trypsic and pancreatic casein peptones, 5 each (all from Biokar, Pantin, France).

2.3. Culture conditions

Batch culture was carried out in a 2 L fermentor (SET 2M; SGI, Toulouse, France), magnetically stirred (300 rpm), at 42°C and without pH control. Seed culture was carried out in a 0.25 L laboratory-designed glass fermentor. Both fermentors were equipped with an aseptic recirculation loop (Watson-Marlow 501 U peristaltic pump; Volumax, Montlouis, France) incorporating a laboratory-made turbidimeter. As the turbidity was continuously recorded, the total biomass could be calculated on-line after dry weight calibration; the observed standard deviation was ±0.2 g L^{-1}.
Bacteria were precultivated by inoculating sterile culture medium with 0.8 % (v/v) of the second skim milk transfer. Then, 1.6 L of pasteurised culture medium was inoculated with 0.2 L seed culture (11% v/v), and the reaction proceeded.

At the end of both preculture and culture, the final biomass and lactic acid concentrations were determined as previously described (Amrane and Prigent, 1994a).

3. Results

3.1 Model without pH control (inhibition model)

During lactic acid fermentation, the accumulated lactic acid decreases the pH value. The acidic pH inhibits fermentation (Luedeking and Piret, 1959, Amrane and Prigent, 1994b). To overcome this inhibition, the pH is maintained during culture at its optimal value for lactic acid production (Hanson and Tsao 1972, Venkatesh et al 1993), at which the final free lactic acid concentration (approximately 0.3 g/l) is below the inhibitory threshold (Gätje and Gottschalk 1991). Since the positive effects of precultivating without pH control was shown (Amrane and Prigent 1996, Amrane and Prigent 1998), the above models are not convenient for seed culture. Indeed, they do not take into account the inhibition observed in absence of pH control. Several models involving lactic acid inhibition can be found in the available literature, which consider non-competitive product inhibition (Dutta et al. 1996, Ohara et al. 1992), or other types of inhibition (Pinelli et al. 1997, Biazar et al. 2003) by the total lactic acid produced. It is now recognised that the main inhibitory species is the undissociated form of the lactic acid. From this, the Luedeking-Piret model (1959) was modified by introducing an additional term to account for the undissociated lactic acid inhibition (Balannec et al. 2007):

$$\frac{dp}{dt} = A * \frac{dx}{dt} + B * x * \left(1 - \frac{[HL]}{[HL]_{inh}} \right) \quad (1)$$

Where $[HL]$ and $[HL]_{inh}$ were the undissociated lactic acid concentration and its inhibitory concentration.
The Verlhust model which proved to satisfactory describe growth kinetic (Moraine and Rogovin 1996, Pandey 2000) was used in this work:

$$\frac{dx}{dt} = \mu_{max} * \left(1 - \frac{x}{x_{max}} \right) * x \quad (2)$$

Integration of equation (2) gave:

$$x = x_0 * x_{max} * \frac{e^{\mu_{max} * t}}{x_{max} - x_0 + x_0 * e^{\mu_{max} * t}} \quad (3)$$

Where x_0 and x_{max} were the initial and maximal values of the biomass concentration and μ_{max} was the maximal specific growth rate.

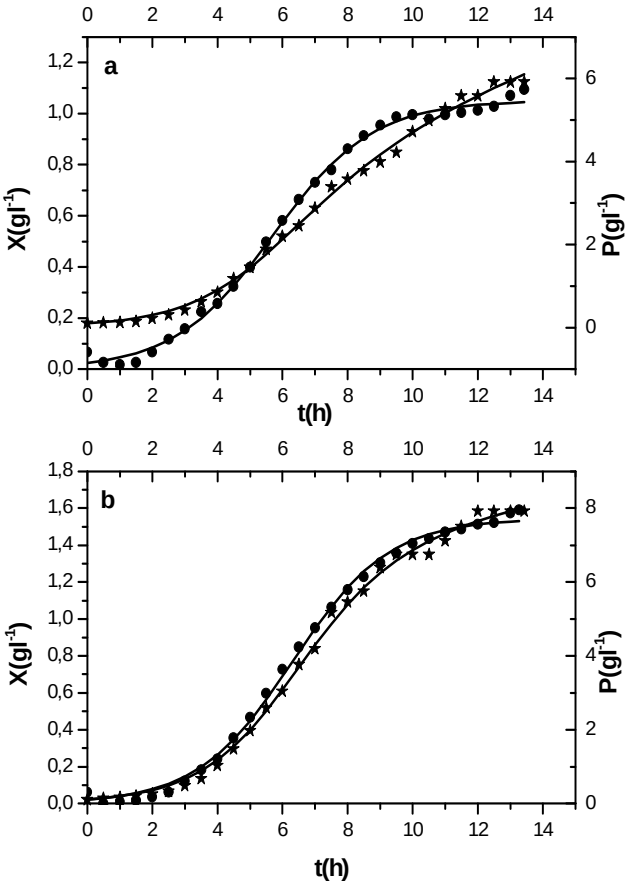

Figure 1. Growth (•) and lactic acid production (*) kinetics during batch cultures of L. helveticus *growing without pH control on whey supplemented with 10 g* L^{-1} *yeast extract (a) and the RM supplementation (b); calculated data (—) by means of the growth model (Eq.3) and the product inhibition model(Eq.1).*

The model was found to match both experimental growth and production data, and was validated in various culture conditions, namely for a large range of nitrogen supplementations of whey permeate (Balannec et al 2007).

3.2 Model at pH controlled at 5.9 (substrate limitation model)

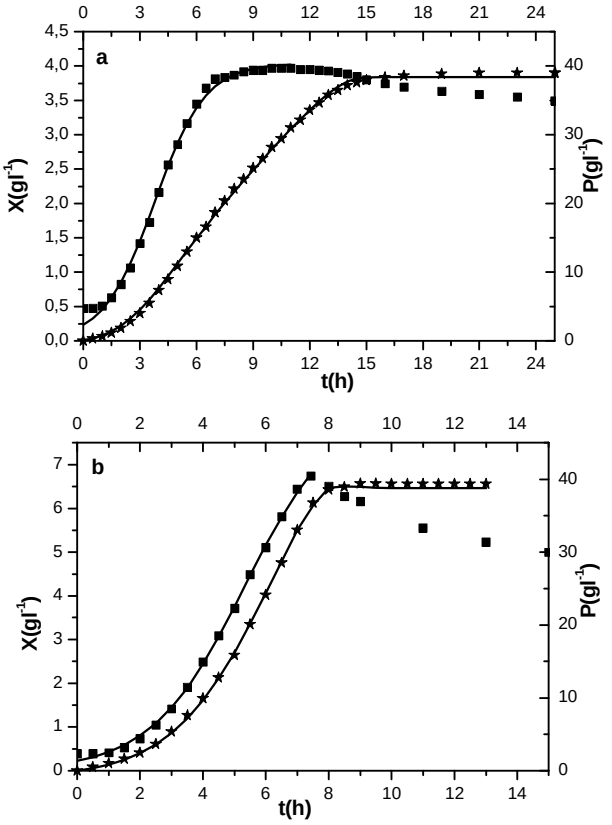

Figure 2. Growth (•) and lactic acid production (*) kinetics during batch cultures of L. helveticus *growing at pH controlled at 5.9 on whey supplemented with 10 g* L^{-1} *yeast extract (a) and the RM supplementation (b); calculated data (—) by mean of the growth model (Eq.1)and the substrate limitation model for production (Eq.4).*

As previously shown lactic acid production ceased at the beginning of cell death irrespective of the considered nitrogen supplementation, namely when carbon became limiting, since bacteria are unable to use the carbon content of autolysed cell (Amrane 2001). Cessation of production was therefore concomitant to cessation of lactose consumption. The corrective term s_{res} was thus replaced by an expression taking into account the carbon substrate limitation.

$$\frac{d\,p}{d\,t} = A^* \frac{d\,x}{d\,t} + B^* x^* \left(1 - \frac{s_{Lim}}{s}\right) \qquad (4)$$

Where s and s_{lim} were the lactose concentration at time t and the end of batch (the limiting carbon concentration), respectively. This model was successfully tested for a large range of nitrogen supplementation (Bouguettoucha et al.2007); the model accounted for the whole production kinetics.

3.3 Generalized model

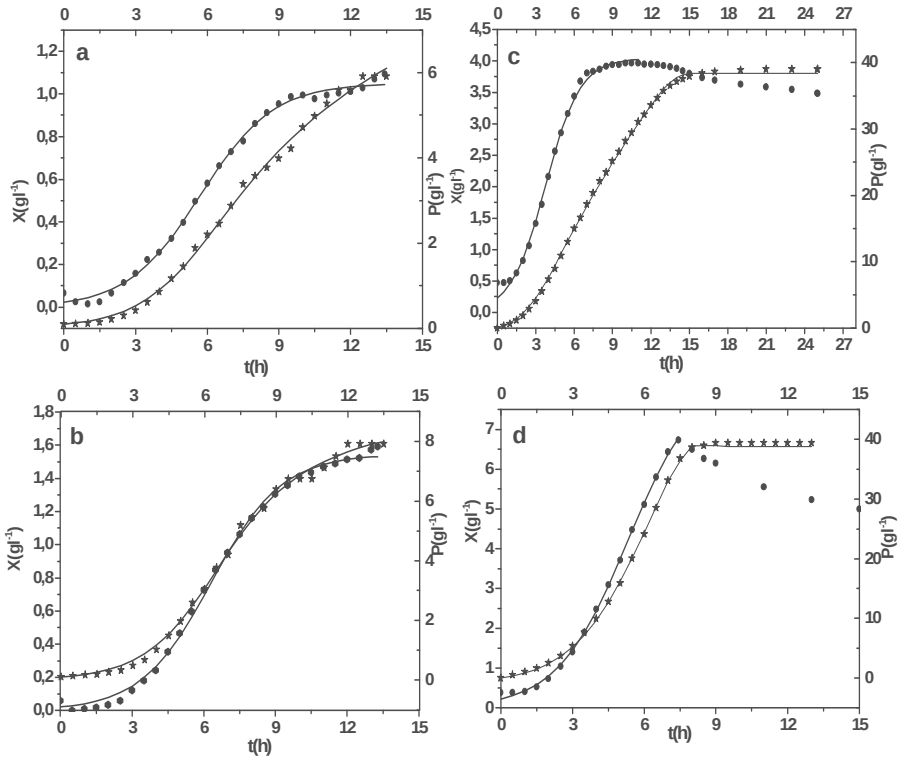

Figure 3. Growth (•) and lactic acid production (*) kinetics during batch cultures of L. helveticus *growing without pH control (a, b) and at pH controlled at 5.9 (c, d) on whey supplemented with 10 g L^{-1} yeast extract (a, c) and the RM supplementation (b, d); calculated data (—) by means of the growth model (Eq.1) and the generalized model for production (Eq.5).*

To avoid the use of two expressions to describe production rate (involving nutritional limitations, Eq.1 or an inhibitory pH effect, Eq.4), depending on culture conditions, the above expressions were merged, leading to an unique expression taking into account both effects, a nutritional limitation effect and an inhibitory effect:

$$\frac{d\,p}{d\,t} = A * \frac{d\,x}{d\,t} + B * x * \left(1 - \frac{s_{lim}}{s}\right) * \left(1 - \frac{[HL]}{[HL]_{inh}}\right) \qquad (5)$$

To describe the growth rate, the Verlhust model (eq.3) was considered. The generalized model gave a satisfactory description of experimental data in various culture conditions, since it was validated during cultures at *pH* controlled (Fig. 3a et b) and in absence of *pH* control (Fig. 3c et d), as well as for different nitrogen supplementation of culture media (Bouguettoucha et al. 2007).

4. Conclusion

The inhibition model was found to match both experimental growth and production data recorded without pH control, namely in the case of an inhibitory effect of the undissociated lactic acid (and the pH); the model was validated in various culture conditions, namely for a large range of nitrogen supplementation of whey permeate. The substrate limitation model was successfully tested for a large range of nitrogen supplementation; the model matched whole production kinetics recorded during cultures at pH controlled, namely in the case of nutritional limitations. Satisfactory results were also obtained by considering the generalized model, which matched experimental data in various culture conditions, since it was validated during cultures at pH controlled and in absence of pH control, as well as for different nitrogen supplementation of culture media.

5. References

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