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## Fed-batch Fermentation of Lactic Acid Bacteria to Improve Biomass Production: A Theoretical Approach

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**Abstract:** Recently, fed-batch fermentation has been introduced in an increasing number of fermentation processes. Previous researches showed that fed-batch fermentation can increase the biomass yield of many strains. Improvement of the biomass yield is interested because biomass from lactic acid bacteria (LAB) fermentation is widely used in food and pharmaceutical industry. The aim of this research is to study the ability and feasibility of fed-batch fermentation to improve biomass production of LAB. Appropriate model has been selected from literature. Monod equation described the substrate limitation of LAB and the product inhibition of LAB follows a non-competitive model. Furthermore, the lactic acid production follows Luedeking and Piret model. Then the models are applied to simulate the fermentation of batch and fed-batch cultures by using MATLAB. From the results of simulation, fed-batch fermentation showed that substrate limitation and substrate inhibition can be avoided. Besides that, the variable volume fed-batch fermentation also showed that product inhibition can be eliminated by diluting the product concentration with added fresh feed. However, it was found that fed-batch fermentation is not economically feasible because large amount of substrate is required to reduce the product inhibition effect. Therefore, fed-batch fermentation plays more importance role if the fermentation strain has high  $K_s$  value or low  $K_p$  value.

**Key words:** Lactic acid production, Improvement of biomass yield, Growth kinetics, Kinetic parameters, Model development and simulation

### INTRODUCTION

Lactic acid bacteria (LAB) have been used for the fermentation of food and feed products since ancient days and today their major applications are still in the food and feed industry as starter cultures (Desmons *et al.*, 1998; Mallika *et al.*, 2002; Somkuti, 2000). Microbial biomass of LAB also has attractive attributes as sources of protein for human and animal consumption (Blanch and Clark, 1996). Lactic acid has wide application and increasing demands in food, cosmetics and biochemical industry, so the microbial lactic acid production is one of the most commonly studied processes (Ana *et al.*, 2001; Appadurai *et al.*, 1999; Kaiming, Jin and Shimizu., 1996). Besides, the other metabolites from LAB also has been used in pharmaceutical and food industry in recent years. For instance, bacteriocins were used to control the growth of spoilage and pathogenic microorganisms in food (Desmazeaud, 1996).

In order to improve productivity of biomass, lactic acid and other metabolites, high cell density in a fermentor is required. Besides, microbial growth and product

formation are influenced by fermentation medium conditions such as pH, temperature, substrate concentration and product (Blanch and Clark, 1996).

Batch fermentation to produce lactic acid gives low productivity due to end-product inhibition (Hujanen *et al.*, 2001). Numerous studies on various fermentation approaches have successfully obtained higher lactic acid yield than batch mode (Karin and Barbel, 2000). Besides that, fermentor with cross-flow filtration has been used to improve the productivity of cell-mass by separation of inhibitory metabolites (Masayuki *et al.*, 1987). On the other hand, it was also found that higher biomass yield can be obtained when the fermentation was pH controlled and aerated (Ha *et al.*, 2003; Wenge and Mathews, 1999).

Besides, fed-batch fermentation successful showed improvement of productivities and final product yield in shorter fermentation time in various fermentation broths in past researches. More than 100% improvement in cell growth rate and approximately 2 times higher final cell concentration can be achieved by fed-batch mode compare to batch mode (Desmazeaud, 1996; Dong *et al.*, 2003; Korz *et al.*, 1995).

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In a nutshell, the concern of this research was to understand the ability of fed-batch fermentation to overcome substrate limitation, initial substrate inhibition and product inhibition by using computer simulation.

**MODELLING**

**Model strain:** Lactobacillus casei has been used as model strain for the model development and simulation. *L. casei* is a homofermentative Lactobacillus strain which produces mainly L(+) lactic acid from glucose.

**Batch fermentation model:** Since the *L. casei* was simulated at low initial concentration of glucose, so the substrate inhibition term is not included in the growth kinetics models. *L. casei* is homofermentative and L(+) lactic acid producer and follow semi-empirical equation in Davide *et al.* (1997). Terms for all these developed models are fully defined in the Nomenclature.

The rate of biomass production:

$$\frac{dX}{dt} = \frac{\mu_{max} \cdot S}{(K_{sx} + S)} \cdot \frac{K_{px}}{(K_{px} + p)} \cdot X \tag{1}$$

Lactic acid production rate of *L. casei* can be described by the Luedeking and Piret model. Since *L. casei* produces more primary metabolites than secondary metabolites, so  $\alpha$  is greater than  $\beta$ .

The rate of lactic acid formation:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \frac{q_{p,max} \cdot S}{(K_{sp} + S)} \cdot \frac{K_{pp}}{(K_{pp} + P)} \cdot X \tag{2}$$

The rate of glucose utilization:

$$\frac{dS}{dt} = \frac{1}{Y_{xs}} \frac{dX}{dt} + \frac{1}{Y_{ps}} \frac{dP}{dt} \tag{3}$$

**Fed-batch fermentation model:** The fed-batch fermentation profiles can be simulated based on the kinetic model developed for the batch fermentation. Considering that no fermentation broth was withdrawn, the reactor volume continuously increased and the feed flow rate is derived based on the rate of substrate utilization. The model equations are derived from the batch model equations with addition of dilution rate term.

The feed flow rate:

$$F = \frac{\frac{V}{Y_{xs}} r_x + \frac{V}{Y_{ps}} r_p}{(LS - S)} \tag{4}$$

The rate of biomass production:

$$\frac{dX}{dt} = \left( \frac{\mu_{max} \cdot S}{(K_{sx} + S)} \cdot \frac{K_{px}}{(K_{px} + S)} \cdot X \right) - D \cdot X \tag{5}$$

The rate of lactic acid formation:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \frac{q_{max} \cdot S}{(K_{sp} + S)} \cdot \frac{K_{pp}}{(K_{pp} + P)} \cdot X - D \cdot P \tag{6}$$

The rate of substrate utilization:

$$\frac{dS}{dt} = D(LS - S) - \frac{1}{Y_{xs}} \left( \frac{dX}{dt} \right) - \frac{1}{Y_{ps}} \left( \frac{dP}{dt} \right) \tag{7}$$

**SIMULATION**

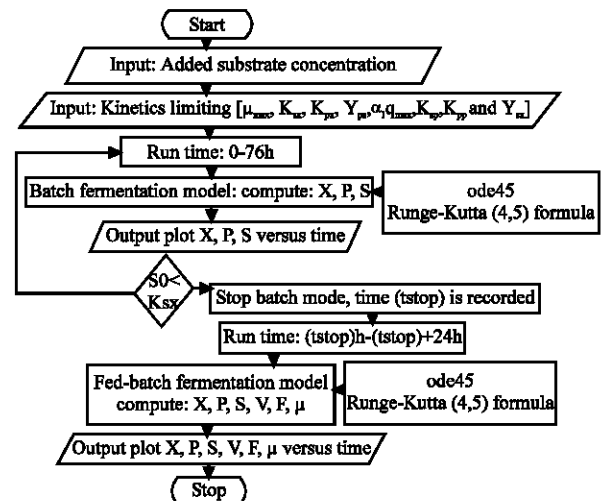
**Simulation tool:** MATLAB (Version 6.5, release 13, MathWorks) is used as simulation tool. Based on the fermentation kinetic models and estimated kinetic parameters, the lactic acid fermentation by *Lactobacillus casei* was simulated.

**Estimation of kinetics parameters:** The fermentation model parameters ( $\mu_{max}$ ,  $K_{sx}$ ,  $K_{px}$ ,  $Y_{ps}$ ,  $\alpha$ ,  $q_{max}$ ,  $K_{sp}$ ,  $K_{pp}$  and  $Y_{xs}$ ) were determined from literature which justification. The selected parameters were estimated from Lactobacillus group.

Table 1: Selected kinetics parameters for the simulation

$\mu_{max} = 0.462$	$K_{sx} = 3.36$	$K_{px} = 0.5$	$K_{sp} = 2.05$	$K_{pp} = 60$
$Y_{ps} = 0.948$	$\alpha = 1.332$	$q_{max} = 0.035$	$Y_{xs} = 0.247$	

**Simulation set-up:** The simulation programme was written in M-file of MATLAB, according to the following flow chart.



**RESULTS AND DISCUSSION**

The simulation result for variable volume fed-batch fermentation is shown in Fig. 1, where the biomass concentration and lactic acid concentration in the fermentor are decreased as soon as glucose is added. The rate of lactic acid concentration decreased more rapidly than the biomass concentration. This is because the dilution rate of the medium by the added feed is increased as the volume of the fermentor increased and fermentation time increased. Besides, the substrate level is maintained

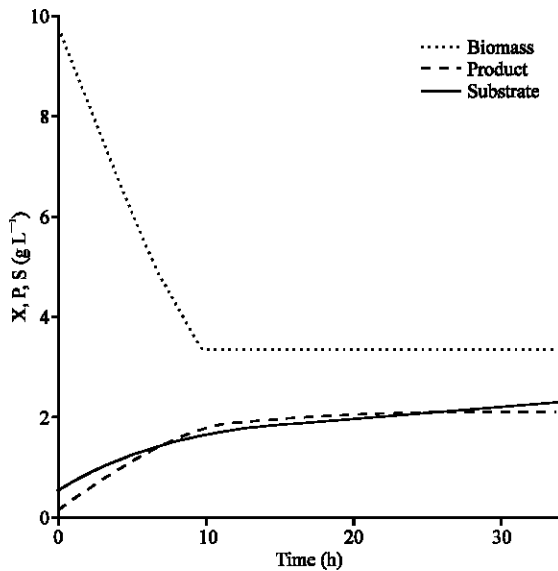


Fig. 1: Variable volume fed-batch fermentation

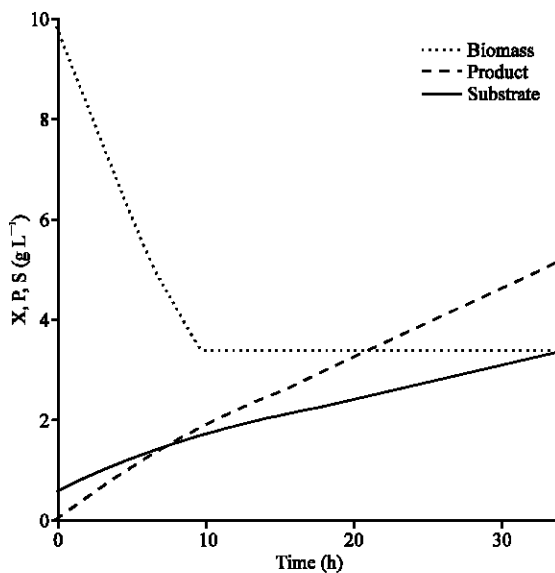


Fig. 2: Intermediate fed-batch fermentation

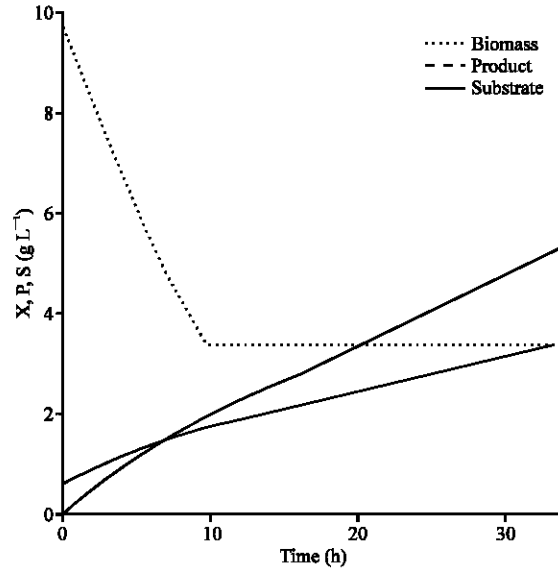


Fig. 3: Fixed volume fed-batch fermentation

at 3.36 g L<sup>-1</sup> along the fermentation, the biomass production rate is higher than lactic acid formation rate after 25 h of fermentation. When the volume of the fermentor reached 2 L, the total biomass is 2.8 g, which is about 1.40 times higher than the achievement of batch mode. Total required amount of glucose is 20g. However, it is not feasible because biomass yielded per glucose used is only 0.14, which is lower than batch mode (0.20). This due to at the end of fermentation, there were still 6.72g of unconsumed glucose in fermentor.

Figure 2, clearly shows that both biomass concentration and lactic acid concentration increase with fermentation time. But the product formation rate is increasing relatively fast; therefore the biomass production rate is inhibited by the product, hence slowed down.

Among the three fed-batch modes, intermediate fed-batch fermentation is the only approach that was able to produce about 2 times higher total biomass than batch mode. Besides, all fed-batch fermentation approaches has lower biomass yield per glucose supplied than batch mode. This is, because there was glucose still remained in the fermentor unconsumed to maintain the glucose concentration, 6.72 g left in variable volume approach, 3.42 g left in fixed volume approach and 3.51g left in intermediate approach. This shows that variable volume fed-batch fermentation required less glucose to produce biomass because 13.28g of glucose is utilized from supplied 20g of glucose. This may due to the dilution which helps in the reduction of product inhibition effects.

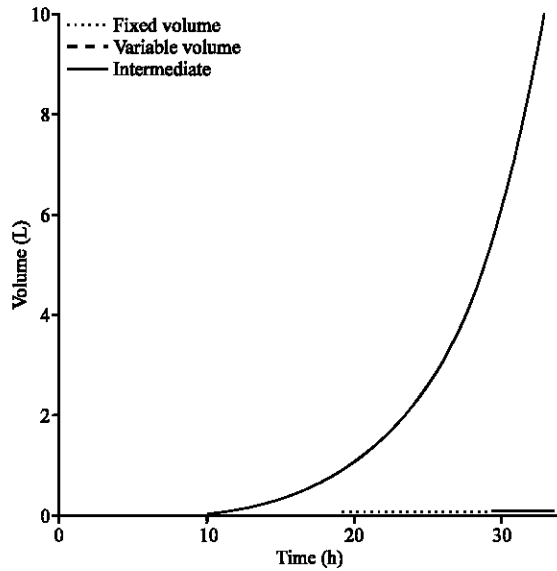


Fig. 4: Volume changes of the fermentor

### CONCLUSION

Simulation results showed that fed-batch fermentation is a good approach that can overcome the problem of initial substrate inhibition, substrate limitation and toxic by-product built up due to excessive substrate. This is because fresh feed is added to the fermentation broth according to the substrate required for the cell growth and product formation. Therefore, fed-batch fermentation can improve the biomass production.

### NOMENCLATURE

- D : Dilution rate ( $\text{h}^{-1}$ )
- $K_S$  : Substrate limitation constant ( $\text{g L}^{-1}$ )
- $K_P$  : Product inhibition constant ( $\text{g L}^{-1}$ )
- P : Product concentration ( $\text{g L}^{-1}$ )
- $q_{p,\text{max}}$  : Maximum specific product formation rate. ( $\text{gg}^{-1}\text{h}^{-1}$ )
- S : Substrate concentration ( $\text{g L}^{-1}$ )
- $S_0$  : Initial substrate concentration ( $\text{g L}^{-1}$ )
- X : Biomass concentration ( $\text{g L}^{-1}$ )
- $Y_{PS}$  : Product yield based on substrate utilized ( $\text{gg}^{-1}$ )
- $Y_{XS}$  : Biomass yield based on substrate utilized ( $\text{gg}^{-1}$ )
- $\beta$  : Non-growth associated constant in Luedeking-Piret model ( $\text{gg}^{-1}\text{h}^{-1}$ )
- $\alpha$  : Growth associated constant in Luedeking-Piret model ( $\text{gg}^{-1}\text{h}^{-1}$ )
- M : Specific growth rate ( $\text{h}^{-1}$ )
- $\mu_{\text{max}}$  : Maximum specific growth rate ( $\text{h}^{-1}$ )
- F : Feed flow rate ( $\text{Lh}^{-1}$ )

- Xi : Initial biomass concentration in the added feed ( $\text{g L}^{-1}$ )
- Pi : Initial product concentration in the added feed ( $\text{g L}^{-1}$ )
- LS : Concentration of the limiting substrate in the added feed ( $\text{g L}^{-1}$ )
- V : Volume of the fermentor (L)

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