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Kinetics of inhibition in propionic acid fermentation

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Abstract. The inhibitory effect of propionic acid P and biomass concentration X is studied in batch and continuous fermentations with cell recycle.

In batch fermentations, the specific growth rate decreases and cancels out at a critical propionic acid concentration Pc_1; the formerly decreasing specific production rate becomes constant after Pc_1 and cancels out when a second critical propionic acid concentration Pc_2 is reached.

In continuous fermentation with cell recycle, a similar inhibition is observed with biomass. The specific rates decrease and become constant at a critical biomass concentration Xc. They cancel out at different high biomass concentrations.

In both cases, the specific production rate can be related to the specific growth rate by the Luedeking and Piret expression: $v = \alpha\mu + \beta$, [1], where the constants α and β are determined by the fermentation parameters.

List of symbols

t	h	time
X	kg/m^3	biomass concentration
P	kg/m^3	propionic acid concentration
A	kg/m^3	acetic acid concentration
S	kg/m^3	lactose concentration
$\frac{dX}{dt}$	kg/(m^3 h)	instantaneous rate of cell growth
$\frac{dP}{dt}$	kg/(m^3 h)	instantaneous rate of propionic acid production
μ	h^{-1}	specific growth rate
v	h^{-1}	specific propionic acid production rate
D	h^{-1}	dilution rate

1 Introduction

Continuous cultures with cell recycle by ultrafiltration have greatly increased the performance of various fermentations [2, 3 and 4]. Propionibacteria have been suggested as potential producers of propionic acid from renewable ressources, such as orchard grass [5] or whey [6]. However, due to the strong inhibition of the metabolism by the propionic acid [7 and 8], the fermentation is not economically competitive.

We have shown that by increasing the biomass concentration through the use of a continuous culture with cell recycle by membrane process, the propionic acid productivity is increased [9]. We present results on the kinetics of P. acidi-propionici growth and propionic acid production, the object of our study being the inhibitions by the product and the biomass. It is observed that in both cases the specific rates μ and v are linked by the Luedeking and Piret relation.

2 Material and methods

2.1 Organism and culture medium

The organism used is Propionibacterium acidi-propionici, strain ATCC 4875. The culture medium is a complex medium containing the following components per dm^3 of water: 64 g of whey; 4 g of yeast extract; 2 g of $(NH_4)_2SO_4$; 1 mg of $CoCl_2$; 2 g of $MgSO_4$ and 0.5 cm^3 of antifoam.

2.2 Fermentation

Batch studies are conducted in a Setric fermentor of 2 dm^3 equipped with pH and temperature regulations. The agitation provided magnetically is fixed at 200 min^{-1}. When the substrate concentration becomes lower than 5 kg/m^3, 0.2 dm^3 of culture medium are removed and the same volume of concentrated new medium is added. The additions of medium are stopped when the propionic acid production ends.

Growth studies with cell recycle are conducted in a fermentor coupled to ultrafilters, type M_1 from SFEC (Bollène-France).

The cells are recycled with the permeate, and the ultrafiltrate containing the molecules (including volatile fatty acids, lactose and salts) is either recycled or removed with a peristaltic pump which fixes the dilution rate at the chosen value for the continuous runs.

The agitation is provided by the recirculation pump. The fermentation is first carried out batchwise, and the

continuous feeding is started when the substrate concentration becomes less than 5 kg/m^3. The medium volume in the fermentor is controlled by a level sensor coupled to a peristaltic pump.

In these two types of reactor the temperature is maintained at 35 °C and the pH-value of the medium is controlled at 6.6 by automatic addition of NH_4OH. The medium is initially autoclaved for 20 min at a temperature of 121 °C and then made anaerobic by bubbling pure nitrogen for 30 min. The fermentors are then inoculated with a 10% v/v growth culture.

2.3 Analysis of samples

Cell. concentration is estimated by dry weight measurement and numeration on a haemocytometer slide. Other analyses are made on supernatants of samples previously centrifuged at 15,000 min^{-1} for 15 min.

Residual lactose is determined by the anthrone method [10] Propionic and acetic acid concentrations are determined by injecting acidified supernatants into a Perkin-Elmer Sigma 3B gas chromatograph equipped with a flame ionization detector. Separation takes place in a column of 2.3 m length and a diameter of approx 3.2 mm, which is packed with Porapak Q 80–100 mesh over a length of 2 m and with Porapak R 80–100 mesh over the remaining length of 0.3 m. N_2 is used as carrier gas. Injector, detector and column temperatures are 300 °C, 320 °C and 190 °C, respectively. The analyses of chromatographic data are carried out on an Intersmat ICR 1B integrator.

3 Discussion of results

3.1 Inhibition by propionic acid

The experimental data are summarized in Fig. 1. The value of the specific growth rate μ and of the specific propionic acid production rate v are calculated from the data (X; P) as follows:

$$\mu = \frac{1}{X} \cdot \frac{dX}{dt}$$

and:

$$v = \frac{1}{X} \cdot \frac{dP}{dt}.$$

The figures and the following discussion originate from the data in Fig. 1.

The effect of P on the values of μ and v appears in Fig. 2. It is obvious that propionic acid has a strong inhibitory effect on growth and metabolic activity. In a propionic acid fermentation, however, the accumulation of volatile fatty acids continues incessantly after the cessation of cell growth.

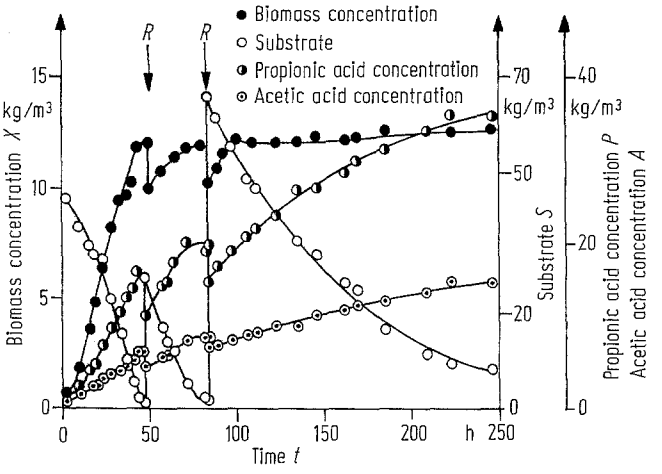


Fig. 1. Time variation of the biomass (X), substrate (S), propionic (P) and acetic (A) acid concentrations during a prolonged batch culture by replenishment of the medium. (R: replenishment of the medium)

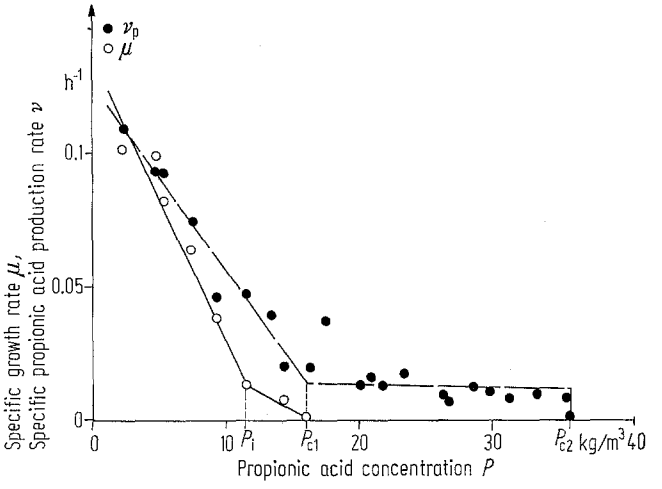


Fig. 2. Specific rates μ and v versus propionic acid concentration P

A critical concentration of propionic acid Pc_1 evidently prevents all growth, while a pseudocritical concentration Pi slows down the decrease of μ. The specific production rate, which was decreasing before Pc_1, remains constant from Pc_1 to Pc_2, which is the critical concentration of propionic acid for metabolic activity.

Thus, for this fermentation, the general equations are follows:

$$\mu = \mu_0 \cdot (1 - \varphi_1 \cdot K_x \cdot P) \cdot \varphi_2$$

$$v = v_0 \cdot (1 - K_p \cdot \varphi_3 \cdot P)$$

μ_0 and v_0 are the values of μ and v, respectively, when P is equal to zero. K_x and K_p are the specific growth rate and the specific production rate constants. φ_3 represents an apparent coefficient of metabolic activity and takes the

following values in each phase of fermentation:

If $0 < P < Pc_1$, then $\varphi_3 = 1$.

If $Pc_1 < P < Pc_2$, then $\varphi_3 = \frac{Pc_1}{P}$.

φ_1 and φ_2 are the apparent coefficients of growth activity and take the following values:

If $0 < P < P_i$, then $\varphi_1 = 1$
and $\varphi_2 = 1$.

If $P_i < P < Pc_1$, then $\varphi_1 = \frac{P_i}{P}$
and $\varphi_2 = \frac{Pc_1 - P}{Pc_1 - P_i}$.

If $P > Pc_1$, then $\varphi_2 = 0$.

Thus, a reasonably close correlation is obtained:

$$v = \alpha\mu + \beta, \qquad \text{(Fig. 3)}$$

which well known as the Luedeking and Piret relation for the lactic acid fermentation, with

$$\alpha = \frac{v_0 \cdot K_p \cdot (Pc_1 - P_i)}{\mu_0 \cdot (1 - K_x \cdot P_i)}$$

and

$$\beta = v_0 \cdot (1 - K_p \cdot P_{c1})$$

in our case.

3.2 *Inhibition by the biomass*

The experimental data are summarized in Fig. 4. The values for the specific growth rate and for the specific propionic acid production rate are calculated from the data (D; X; P) as follows:

$$\mu = \frac{1}{X} \cdot \frac{dX}{dt}$$

$$v = \frac{1}{X} \cdot \left(D \cdot P + \frac{dP}{dt} \right).$$

In prolonged fermentations a steady state is never achieved. Thus all calculated rates are instantaneous. The figures and the following discussion originate from the data in Fig. 4. If the decrease of the specific rates during the batch run (Fig. 5) is seemingly due to product concentration, the elimination of the inhibitory effect by pumping the product during the continuous run may be evident.

The specific growth rate, however, decreases linearly with increasing biomass until the latter reaches a critical concentration X_c. Between X_c and a high biomass concentration X_e, it remains roughly constant. Above X_e the specific growth rate decreases and finally cancels out at X_m, which is the maximal concentration of biomass. A

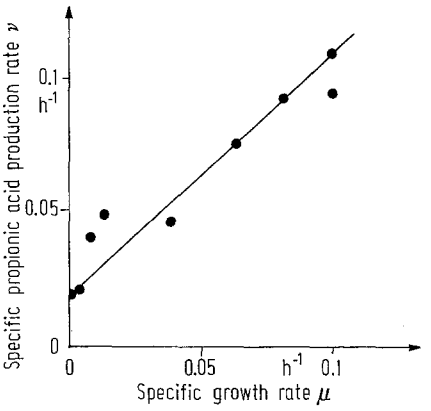


Fig. 3. Specific propionic acid production rate v versus specific growth rate μ

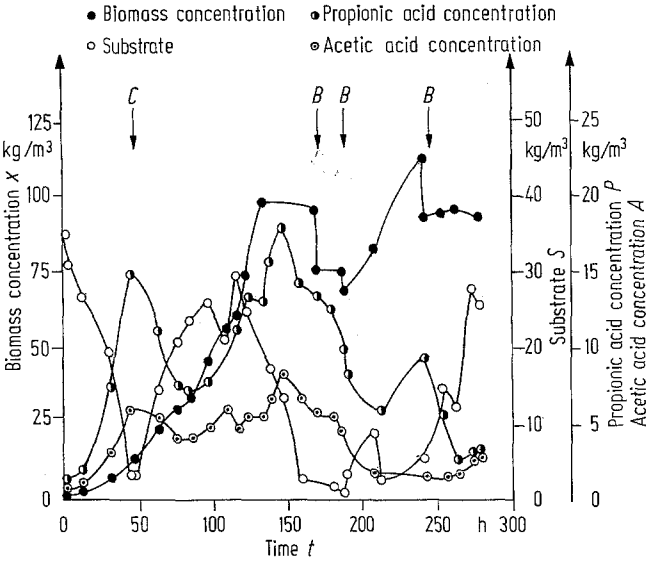


Fig. 4. Time variation of the biomass (X), substrate (S), propionic (P) and acetic (A) acid concentrations during a continuous culture with cell recycle. (C: continuous feed, B: bleeding of biomass)

bleeding of biomass lets the growth start again, but growth cancels out definitively when X_m is reached. A second bleeding does not start the growth again.

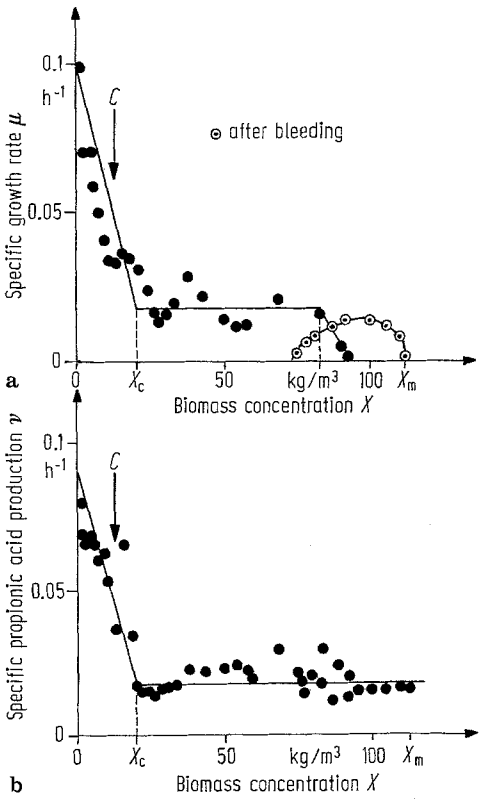
The specific production still decreases after the beginning of the continuous run, and when the critical concentration of biomass X_c is reached, v becomes constant until X_m. Bleeding of biomass does not change the specific production rate.

For this fermentation, the general equations are as follows:

$$\mu = \mu_0' \cdot (1 - K_x' \cdot \varphi_1' \cdot X)$$

$$v = v_0' \cdot (1 - K_p' \cdot \varphi_1' \cdot X)$$

μ_0' and v_0' are the values of μ and v, respectively, where X is equal to zero. K_x' and K_p' are the specific growth rate and specific production rate constants. φ_1' represents an appa-



Figs. 5a and b. Specific rates μ and ν versus biomass concentration X. (C: continuous feed)

rent coefficient of cell activity:

If $0 < X < X_c$, then $\varphi_1' = 1$

If $X_c < X < X_e$, then $\varphi_1' = \dfrac{X_c}{X}$.

Beyond X_e bleeding of biomass makes it impossible to follow the fermentation equations, although ν stays constant. In this case there also results a reasonably close correlation following the Luedeking and Piret expression: $\nu = \alpha\mu + \beta$ (Fig. 6) is obtained, with

$$\alpha = \frac{\nu_0'}{\mu_0'} \cdot \frac{K_p'}{K_x'}$$

and

$$\beta = \nu_0' \cdot \left(1 - \frac{K_p'}{K_c'}\right).$$

4 Conclusion

Luedeking and Piret have found that during lactic acid fermentation, the instantaneous rate of acid formation $\dfrac{dP}{dt}$ can be related to the instantaneous rate of bacterial growth dX/dt and to the bacterial density X by the

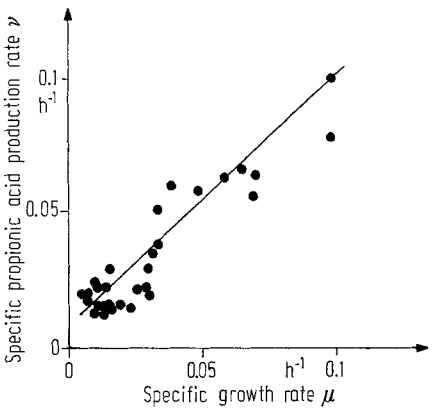


Fig. 6. Specific propionic acid production rate ν versus specific growth rate μ

expression $\dfrac{dP}{dt} = \alpha \cdot \dfrac{dX}{dt} + \beta \cdot X$. Asai and Kono [11] presented general equations, where the growth rate and production rate depended on the cell concentration at the beginning of a constant growth phase during production of maridomycin by streptomycetes.

As far as propionic acid fermentation is concerned, the growth and the metabolic activity are inhibited by the propionic acid and the biomass. The general equations are:

$$\mu = \mu_0 \cdot (1 - \varphi_1 \cdot K_x \cdot P) \cdot \varphi_2$$

and

$$\nu = \nu_0 \cdot (1 - \varphi_3 \cdot K_p \cdot P),$$

where K_x and K_p are specific growth rate and specific production rate constants. φ_1, φ_2 and φ_3 represent apparent coefficients of cell activity, which take different values in the various phases of fermentation. Nevertheless, for all phases the Luedeking and Piret expression $\nu = \alpha\mu + \beta$ is followed.

Acknowledgement

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