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A N   I N T E R N A T I O N A L   J O U R N A L

Calculation of Fermentation Parameters from the Results of a Fed-Batch Test Taking Account of the Volume of Biomass in the Fermenting Medium

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ABSTRACT

The volume of biomass in the fermenting medium may significantly affect the values of fermentation parameters calculated from the measured concentrations of the substrates and/or products. The corrections proposed in this paper should be evaluated and, depending on their magnitude, considered in order to obtain more representative results. A numerical example is presented.

Key words: Biomass volume, corrections calculation, fed-batch fermentation, fermentation parameters, medium aqueous phase volume

INTRODUCTION

The values of the fermentation parameters (yield factors; efficiencies; productivities; production and consumption rates), usually calculated from the measured concentrations of the biomass, substrates and products, may be significantly influenced by several factors (e.g. evaporation and stripping losses, addition of the acid or base solutions to control the medium pH, addition of antifoam, production of substances that change the aqueous phase volume, biomass volume, samples withdrawal). Some aspects of the above topics have been discussed elsewhere (Borzani and Perego, 1976; Concone et al., 1978; Wang, 1979; Borzani and Baralle, 1983; Borzani, 1995; Borzani and Jurkiewicz, 1998; Borzani, 2003). The main purpose of this paper was to show to what extent the biomass volume in the fermenting medium could affect the values of the fermentation

parameters calculated from results of a fed-batch experiment.

Aqueous Phase Volume
Considering that the biomass concentrations are measured in the whole medium, and that the substrate and product concentrations are measured in the medium aqueous phase, that is, in the filtered (or centrifuged) medium, it is necessary to evaluate the aqueous phase volume in order to calculate the actual masses of the substrate and product in the fermenting medium. Equation (1), where V_a is the volume of the aqueous phase, V is the volume of the fermenting medium, X is the biomass concentration (dry matter), σ is the fraction of dry matter in the biomass, and ρ is the density of the microorganism, permits to calculate the above volume.

$$V_a = V \cdot \left(1 - \frac{X}{\sigma \cdot \rho} \right) \qquad (1)$$

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Otherwise, the volume of the fermenting medium in the reactor at time t ($t \leq \theta$, where θ is the duration of the reactor feeding phase) is calculated by Equation (2), where V_i is the volume of the inoculum, and F is the reactor feeding rate.

$$V = V_i + \int_o^t F \cdot dt \qquad (2)$$

Here it was assumed that F was constant and that V was not affected by the other operational conditions.

The actual masses of the biomass (M_X), substrate (M_S) and product (M_P) in the reactor were then calculated by Equations (3), (4) and (5), respectively, where S and P were, the concentrations of substrate and product respectively, measured in the aqueous phase of the fermenting medium.

$$M_X = X \cdot V \qquad (3)$$

$$M_S = S \cdot V \cdot \left(1 - \frac{X}{\sigma \cdot \rho}\right) \qquad (4)$$

$$M_P = P \cdot V \cdot \left(1 - \frac{X}{\sigma \cdot \rho}\right) \qquad (5)$$

It was proposed (Borzani, 2003) to replace S and P (measured in g/L aqueous phase), respectively, by S' and P' (measured in g/L whole medium) calculated by Equations (6) and (7).

$$S' = S \cdot \left(1 - \frac{X}{\sigma \cdot \rho}\right) \qquad (6)$$

$$P' = P \cdot \left(1 - \frac{X}{\sigma \cdot \rho}\right) \qquad (7)$$

Obviously, depending on both the value of $X / \sigma \cdot \rho$ and the uncertainty associated with the measured concentration, the difference between the measured and the corresponding calculated concentration could be insignificant (Borzani, 2003).

General Equations

The purpose of this section was to compare the values of a fermentation parameter calculated from the measured concentrations disregarding the biomass volume obtained taking account of the above volume. In the equations presented in this section, S_0 was the substrate concentration of the

feeding mash, and the subscript i and f indicate, respectively, the variable values at time $t = 0$ and $t = \theta$. If the value of a given parameter calculated disregarding the biomass volume was represented by Z, the value calculated taking account of the biomass volume would be represented by Z'.

Biomass yield factors (Y_X and Y_X')

$$Y_X = \frac{V_f \cdot X_f - V_i \cdot X_i}{F \cdot \theta \cdot S_0 + V_i \cdot S_i - V_f \cdot S_f} \qquad (8)$$

$$Y_X' = \frac{V_f \cdot X_f - V_i \cdot X_i}{F \cdot \theta \cdot S_0 + V_i \cdot S_i' - V_f \cdot S_f'} \qquad (9)$$

Equations (6), (8) and (9) show that: a) $Y_X > Y_X'$ when $S_i = 0$; b) $Y_X < Y_X'$ when $S_f = 0$; c) $Y_X = Y_X'$ when $S_i = S_f = 0$. Considering that S_i and S_f were usually relatively small, the difference between Y_X and Y_X' would be frequently insignificant.

Product yield factors (Y_P and Y_P')

$$Y_P = \frac{V_f \cdot P_f - V_i \cdot P_i}{F \cdot \theta \cdot S_0 + V_i \cdot S_i - V_f \cdot S_f} \qquad (10)$$

$$Y_P' = \frac{V_f \cdot P_f' - V_i \cdot P_i'}{F \cdot \theta \cdot S_0 + V_i \cdot S_i' - V_f \cdot S_f'} \qquad (11)$$

If $S_i = S_f = P_i = 0$, Equations (6), (7), (10) and (11) lead to Equation (12).

$$\frac{Y_P'}{Y_P} = 1 - \frac{X_f}{\sigma \cdot \rho} \qquad (12)$$

Process productivities (Q and Q')

$$Q = \frac{V_f \cdot P_f - V_i \cdot P_i}{V_f \cdot t_f} \qquad (13)$$

$$Q' = \frac{V_f \cdot P_f' - V_i \cdot P_i'}{V_f \cdot t_f} \qquad (14)$$

If $P_i = 0$, Equations (7), (13) and (14) give:

$$\frac{Q'}{Q} = 1 - \frac{X_f}{\sigma \cdot \rho} \qquad (15)$$

Rates during the reactor filling phase
The rates of biomass growth (R_X), product formation (R_P and R_P') and substrate consumption (R_S and R_S') during the reactor filling phase may be calculated by equations (16) to (20).

$$R_X = D \cdot X + \frac{dX}{dt} \qquad (16)$$

$$R_P = D \cdot P + \frac{dP}{dt} \qquad (17)$$

$$R_P' = D \cdot P' + \frac{dP'}{dt} \qquad (18)$$

$$R_S = D \cdot (S_0 - S) - \frac{dS}{dt} \qquad (19)$$

$$R_S' = D \cdot (S_0 - S') - \frac{dS'}{dt} \qquad (20)$$

Equations (7), (17) and (18) give:

$$R_P' = \left(1 - \frac{X}{\sigma \cdot \rho}\right) \cdot R_P - \frac{P}{\sigma \cdot \rho} \cdot \frac{dX}{dt} \qquad (21)$$

Otherwise, Equations (6), (16), (19) and (20) lead to Equation (22).

$$R_S' = R_S + \frac{S}{\sigma \cdot \rho} \cdot R_X + \frac{X}{\sigma \cdot \rho} \cdot \frac{dS}{dt} \qquad (22)$$

Numerical Example
Table 1 shows the results obtained during the reactor filling phase of a fed-batch ethanol fermentation of sugar-cane molasses medium carried out under the following experimental conditions (Koshimizu, 1984; Koshimizu *et al.*, 1984): $V_i = 4.00$ L, $S_i = P_i = 0$, $S_0 = 247$ g/L, $F = 1.17$ L/h and $\theta = 5$ h.

Table 1 – Biomass (X), substrate (S) and ethanol (P) concentrations during the reactor filling phase.

t(h)	X (g/L)	S (g/L)	P (g/L)
0	33.1	0	0
1	28.5	18.3	15.7
2	24.5	28.2	26.2
3	22.8	31.1	34.8
4	22.2	29.7	43.8
5	21.9	26.2	51.3

In the case of ethanol fermentation, the concentration of intracellular ethanol would be equal to the concentration of ethanol in the medium aqueous phase (Pamment and Dasari, 1988). Hence, if the purpose of the test is to study the process performance taking account of both the intracellular and the extracellular ethanol, the values of P, P_f and P_i could be used in Equations (11), (14) and (18) instead of P', P_f' and P_i', respectively. If, however, the purpose would be to study the process performance taking account of only the extracellular ethanol, since only the medium aqueous phase is distilled, Equations (11), (14) and (18) must be used. Calling Y_P'', Q'' and R_P'' the ethanol yield factor, the process productivity and the ethanol production rate,

respectively, calculated taking account of both the extracellular and the intracellular ethanol, Equation (23) to (25) must be used.

$$Y_P'' = \frac{V_f \cdot P_f - V_i \cdot P_i}{F \cdot \theta \cdot S_0 + V_0 \cdot S_i' - V_f \cdot S_f'} \qquad (23)$$

$$Q'' = \frac{V_f \cdot P_f - V_i \cdot P_i}{V_f \cdot t_f} = Q \qquad (24)$$

$$R_P'' = D \cdot P + \frac{dP}{dt} = R_P \qquad (25)$$

Table 2 shows the calculated values of V, D, S' and P' during the reactor feeding phase. The

values of σ and ρ were 0.300 and $1.10 \cdot 10^3$ g/L, respectively.

The values of Tables 1 and 2 permit to calculate the biomass yield factors ($Y_X = 0.182$ g/g; $Y_X^{'} = 0.179$ g/g), the ethanol yield factors ($Y_P = 0.426$ g/g; $Y_P^{'} = 0.392$ g/g; $Y_P^{''} = 0.420$ g/g) and the process productivities ($Q = Q^{''} = 10.3$ g/L.h; $Q^{'} = 9.6$ g/L.h).

The above values showed that Y_X was 1.7% higher than $Y_X^{'}$, Y_P was 1.4% higher than $Y_P^{''}$ and 8.7% higher than $Y_P^{'}$, and $Q = Q^{''}$ was 7.3% higher than $Q^{'}$. It was then possible to conclude that, in this example, the volume of the biomass in the fermenting medium: a) did not affect the calculated value of the biomass yield factor; b) did not affect the calculated values of the ethanol yield factor and process productivity when the intracellular ethanol was also considered; c)

affected the values of the ethanol yield factor and process productivity when only the extracellular ethanol was considered.

From the curves representing the variation of S, P, $S^{'}$ and $P^{'}$ during the filling phase, the values of R_S, R_P, $R_S^{'}$ and $R_P^{'}$ were calculated (see Table 3). The numerical differentiation method (Sinclair and Cantero, 1990) was used to calculate the dS/dt, dP/dt, $dS^{'}/dt$ and $dP^{'}/dt$.

Assuming that the difference $R_S - R_S^{'}$ (or $R_P - R_P^{'}$) could be considered as negligible if $R_S / R_S^{'}$ (or $R_P / R_P^{'}$) belonged to the interval 0.980 to 1.020, Table 3 showed that the volume of the biomass in the fermenting medium affected the value of the ethanol production rate. The above volume, however, did not affect the value of the substrate consumption rate when $t \geq 1.75$ h.

Table 2 – Calculated values of the fermenting medium volume (V), dilution rate (D) and concentrations of substrate [$S^{'}$, Equation (6)] and ethanol [$P^{'}$, Equation (7)] during the reactor filling phase.

t (h)	V (L)	D (h⁻¹)	S' (g/L)	P' (g/L)
0	4.00	0.292	0	0
1	5.17	0.226	16.7	14.3
2	6.34	0.184	24.1	24.2
3	7.51	0.156	28.9	32.4
4	8.68	0.135	27.7	40.8
5	9.85	0.119	24.5	47.9

Table 3 - Substrate consumption rates (R_S and $R_S^{'}$) and ethanol production rates (R_P and $R_P^{'}$) during the reactor filling phase

t (h)	R_S (*)	$R_S^{'}$ (*)	$\dfrac{R_S}{R_S^{'}}$	R_P (*)	$R_P^{'}$ (*)	$\dfrac{R_P}{R_P^{'}}$
0.25	45.6	47.8	0.954	19.9	17.7	1.124
0.75	39.7	41.2	0.964	15.8	14.7	1.075
1.25	36.3	37.5	0.968	14.9	13.8	1.080
1.75	34.8	35.4	0.983	15.2	13.8	1.101
2.25	34.3	34.9	0.982	13.4	12.6	1.063
2.75	33.2	33.6	0.988	13.7	12.9	1.062
3.25	33.4	32.9	1.015	14.0	13.1	1.069
3.75	31.9	32.5	0.981	14.1	13.3	1.060
4.25	31.8	31.4	1.013	14.3	13.4	1.067
4.75	30.4	30.6	0.993	14.5	13.6	1.066

(*) g/L.h

NOMENCLATURE

D	dilution rate (h^{-1})
F	reactor feeding rate (L/h)
M_P	mass of product in the fermenting medium (g)
M_S	mass of substrate in the fermenting medium (g)
M_X	mass of biomass in the fermenting medium (g)
P	product concentration measured in the aqueous phase (g/L)
P'	product concentration calculated by Equation (7) (g/L)
Q	process productivity (*) (g/L.h)
Q'	process productivity (**) (g/L.h)
R_P	product production rate (*) (g/L.h)
R'_P	product production rate (**) (g/L.h)
R_S	substrate consumption rate (*) (g/L.h)
R'_S	substrate consumption rate (**)(g/L.h)
R_X	biomass growth rate (g/L.h)
S	substrate concentration measured in the aqueous phase (g/L)
S'	substrate concentration calculated by Equation (6) (g/L)
S_0	substrate concentration of the feeding mash (g/L)
t	time (h)
V	volume of the fermenting medium (L)
V_a	volume of the aqueous phase (L)
V_i	volume of the inoculum (L)
X	biomass concentration (dry matter) (g/L)
Y_P	product yield factor (*) (g/g)
Y'_P	product yield factor (**) (g/g)
Y_X	biomass yield factor (*) (g/g)
Y'_X	biomass yield factor (**) (g/g)
θ	duration of the reactor feeding phase (h)
ρ	density of the microorganism (g/L)
σ	fraction of dry matter in the biomass (-)
i	subscript indicating the value at t = 0
f	subscript indicating the value at t = θ
(*)	calculated disregarding the biomass volume
(**)	calculated taking account of the biomass volume

RESUMO

O volume da biomassa no meio em fermentação pode afetar significativamente os parâmetros do processo calculados a partir das medidas das concentrações de substratos e/ou produtos. Correções propostas neste trabalho deveriam ser avaliadas e, dependendo de seus valores, consideradas a fim de obter resultados mais representativos. Apresenta-se um exemplo numérico.

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