ELSEVIER

Proton transfer in relation to growth of *Geotrichum candidum* and *Penicillium camemberti* in synthetic liquid media

Abdeltif Amrane, Florence Plihon, and Yves Prigent

Laboratoire des Procédés de Séparation (Unité associée I.N.R.A.), Université de Rennes I, I.U.T., Département Chimie, Rennes Cedex, France

Since continuous monitoring of mycelial growth in liquid media is still difficult, indirect ways of calculating on-line the total biomass concentration are of great interest. Growth of fungi like G. candidum *and* P. camemberti *(widely used in cheese ripening) in liquid media may induce large pH changes. When nitrogen is supplied by glutamic acid (also able to supply carbon), an alkalinization of the medium is recorded, irrespective of the presence of an extra carbon source; when nitrogen is provided by lysine (unable to supply carbon) supplemented by glucose, an acidification is observed. Depending on the amino acid used, the pH variation is linearly correlated with growth (glutamic acid) or not (lysine). A simple model predicts that the concentration of proton transferred vary in proportion to that of the total biomass: the former variable may be easily derived from the pH history and the buffer capacity of the medium. For all combinations of nitrogen and carbon sources tested, the model has been experimentally validated for both microorganisms during the main growth period: following a preliminary calibration, fungal growth may be monitored on-line by pH measurements.*

Keywords: Growth; proton transfer; *G. candidum*; *P. camemberti*; liquid media

Introduction

The mould *P. camemberti* and the yeast-like mould *G. candidum* are known to play an important role in cheese ripening, and especially in curd neutralization.[1–3] Growth of both microorganisms always results in an alkalinization of the rind, and the metabolization of lactic acid has been suspected to be the reason for this proton transfer;[1,4,5] but to our knowledge, this has not been clearly proven. Indeed, no mention has been found in the available bibliography of proton transfer in relation to the growth of *G. candidum* and *P. camemberti* in synthetic media containing lactic acid as carbon and energy sources. Moreover, both microorganisms are able to deaminate some amino acids[6–8] and ammonia resulting from this catabolism process might be involved in the alkalinization of the medium.[9,10]

Similar pH changes induced by fungal growth in liquid media have rarely been reported:

- twenty-one amino acids have been tested as a source of carbon for the growth of *P. roqueforti* with nitrate as nitrogen source,[11] resulting in a pH rise of 0–2.3 (initial pH = 6.0).
- In contrast, the metabolization of glucose by *G. lactis* in the presence of ammonium as a nitrogen source[12] and by *P. chrysogenum* (nitrogen supplementation: yeast extract + peptones + corn steep liquor)[13,14] led to a strong acidification of the medium. In the latter case, pH and biomass con-

Address reprint requests to Prof. Yves Prigent, Laboratoire des Procédés de Séparation (Unité associée I.N.R.A.), Université de Rennes I, I.U.T., Département Chimie, B.P. 1144, 35014 Rennes Cedex, France

Received 29 July 1998; revised 13 November 1998; accepted 1 December 1998

0141-0229/99/$ - see front matter

centration histories were determined after the filtration of samples, but the relationship between them was not further discussed.

The determination of the biomass concentration for mycelial cells is known to be a difficult problem: Colony Forming Units have been measured discontinuously by flow cytometry,[15] or by cultivation in specific media after careful crushing and homogenization;[16] the on-line monitoring of growth of filamentous microorganisms is still a difficult matter.[17,18]

For these reasons, an indirect and simple way of monitoring continuously a mycelial growth would be of great interest. This would be possible if the proton transfer associated with the metabolization of the nitrogen and/or the carbon source could be correlated with the fungal growth. With this in mind, a simple model will be developed.

So the main purpose of the present work is to clarify the effect on the culture medium pH of the metabolization by *G. candidum* and *P. camemberti* of some representative amino acids (one acting only as a nitrogen source, and the other as a source of both nitrogen and carbon) in the presence of different carbon sources. In addition, the above model will be tested for liquid cultures carried out in a fermenter equipped with a new turbidimetric system specially designed for on-line monitoring of mycelial growth.[19,20]

Theory

Let us assume that growth of both microorganisms proceeds at constant yields with respect to the carbon and nitrogen sources, i.e., Y_{XS1}, Y_{XZ1}, Y_{XS2}, Y_{XZ2} are constants.

In addition, it will be assumed that the metabolization of 1 g of both nutrients involve the transfer of a given number of protons, i.e., the stoichiometric coefficients m_1, n_1, m_2, n_2 are constants.

The drops in the concentrations of S and Z corresponding to the production of a biomass concentration $x_i - x_0$ are:

$$s_0 - s_i = \frac{x_i - x_0}{Y_{XSi}} \tag{1}$$

and

$$z_0 - z_i = \frac{x_i - x_0}{Y_{XZi}} \tag{2}$$

From the definition of the stoichiometric coefficients, the proton transfer resulting from the uptake of both nutrients are:

$$\Delta p_{Si} = m_i(s_0 - s_i) \tag{3}$$

$$\Delta p_{Zi} = n_i(z_0 - z_i) \tag{4}$$

Putting Eq. (1) into Eq. (3) and Eq. (2) in Eq. (4), it becomes:

$$\Delta p_{Si} = \frac{m_i}{Y_{XSi}}(x_i - x_0) \tag{5}$$

and

$$\Delta p_{Zi} = \frac{n_i}{Y_{XZi}}(x_i - x_0) \tag{6}$$

From Eqs. (5) and (6), the total proton transfer Δp_i varies in proportion to the concentration of produced biomass:

$$\Delta p_i = \Delta p_{Si} + \Delta p_{Zi} = \left(\frac{m_i}{Y_{XSi}} + \frac{n_i}{Y_{XZi}}\right)(x_i - x_0) \tag{7}$$

$$\text{or} \quad \frac{\Delta p_i}{x_i - x_0} = \left(\frac{m_i}{Y_{XSi}} + \frac{n_i}{Y_{XZi}}\right) = \text{constant} \tag{8}$$

From Eq. (8), the ratio R of the slope corresponding to *P. camemberti* and *G. candidum* is also constant.

$$R = \frac{\dfrac{\Delta p_2}{x_2 - x_0}}{\dfrac{\Delta p_1}{x_1 - x_0}} = \frac{\dfrac{m_2}{Y_{XS2}} + \dfrac{n_2}{Y_{XZ2}}}{\dfrac{m_1}{Y_{XS1}} + \dfrac{n_1}{Y_{XZ1}}} = \text{constant} \tag{9}$$

Materials and methods

Microorganisms

The strains *Geotrichum candidum* Geo17 and *Penicillium camemberti* LV2 (Texel, Dangé St. Romain, France) were used throughout this work. Freeze-dried spores were maintained at +7°C. Spore viability was periodically controlled:

- The total number of spores in a given volume of suspension was counted using a Thoma hemacytometer.
- The corresponding number of viable spores was determined by counting the Colony Forming Units having appeared after inoculation with successive decimal dilutions triplicates of pour plates of Yeast–Malt extract agar medium (Difco, Detroit, MI, USA) reconstituted at 38 g l^{-1} and incubated for 3 days at 20°C.

Media

Four media were used in this work; they had the following composition:

- glutamic acid: 15.76 g l^{-1}, medium *G*.
- glutamic acid (Acros, Geel, Belgium): 7.88 g l^{-1} and sodium L^+ lactate (Acros), 10 g l^{-1}: medium *Gl*.

- glutamic acid: 7.88 g l^{-1} and glucose (Merck, Darmstadt, Germany), 8.05 g l^{-1}: medium *Gg*.

These three media contained the same carbon concentration, 6.44 g l^{-1}.

- lysine (Acros): 3.91 g l^{-1}, instead of glutamic acid, corresponding to 0.75 g l^{-1} nitrogen, and 8.05 g l^{-1} glucose: medium *Lg*.

These media were supplemented with the following solutions:

- EDTA-chelated trace elements (TE): 50 ml l^{-1} at 20× final strength,[21] so the final trace elements concentrations were (mg l^{-1}): Mg, 25; Fe, 20; Ca, 18; Zn, 4.5; Mo, 2; Cu, 1.3.
- Inorganic phosphates (P_i): 500 ml l^{-1}, derived from the phosphate solution of Trinci:[21] this solution was prepared at 2× final strength and contained (g l^{-1}): KH_2PO_4, 12.52; Na_2HPO_4, 2 H_2O, 1.42.

Culture conditions

Batch fermentations were carried out in a 500-ml laboratory-made glass-blown fermenter, specially designed for the growth of filamentous fungi.[20] The fermenter was fed with 300 ml of sterile culture medium. The temperature was maintained at 25°C by circulation of thermostated water in a jacket. Air flow was 1.67 l h^{-1} (i.e., 0.093 v.v.m), and broth was magnetically stirred at 850 rev. min^{-1}, in order to minimize pellet formation. In these conditions, the maximum oxygen transfer rate, determined *in vitro* by suddenly replacing the air flow by an equivalent nitrogen flow[22] (0.045 g l^{-1} h^{-1}). Inoculation of culture medium was carried out by an aseptic addition of 5 ml of spore suspension in the same medium (corresponding to an initial density of $2–3 \times 10^8$ ml^{-1}). In order to obtain reliable time-lags, spores were left to germinate for approximately 1 h.

The fermenter was equipped with a sterilizable combination glass electrode (Ingold, Paris, France), and an aseptic recirculation loop involving a laboratory-made turbidimeter allowing on-line measurement of turbidity at $\lambda = 650$ nm and specially adapted to the case of filamentous fungi.[20] As turbidity was continuously recorded, the total biomass was calculated on-line after dry weight calibration: the observed standard deviation was ± 0.2 g l^{-1} (dry cellular weight range 0–6 g l^{-1}).

Buffer capacity

Buffer capacity was measured at 25°C, by titrating 25 ml of medium by 1 M NaOH or 1 M HCl (step = 0.02 ml), using an automatic system (Titrino Metrohm 702 SM).

Analyses

Glutamic acid and lysine concentrations were determined spectrophotometrically from the measurement of the primary amino groups by the trinitrobenzene sulfonic acid method.[23] Concentrations of L$^+$ lactate and glucose were determined enzymatically using the # 735-10 lactate Kit and # 510-DA glucose Kit, respectively (Sigma Diagnostics, St. Louis, MO, USA).

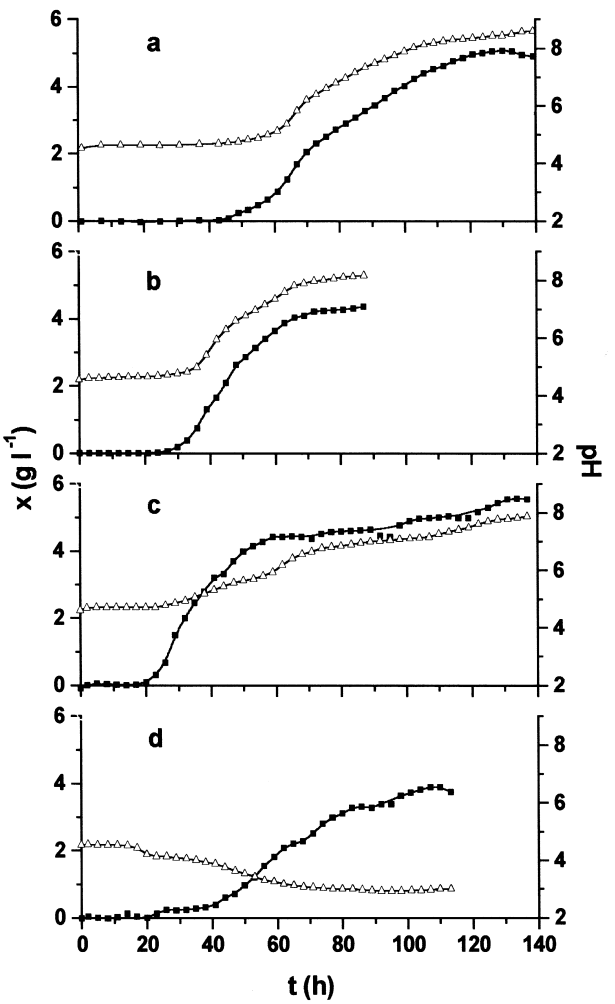

Figure 1 Biomass (■) and pH (△) histories for *G. candidum* growing on glutamate (a); glutamate +lactate (b); glutamate +glucose (c); and lysine +glucose (d) media

Calculations

Instantaneous rates of biomass production and pH variations were calculated from numerical derivation of the corresponding kinetics.

Results and discussion

Kinetics of growth and pH variations

For the batch cultures displayed in *Figure 1a–c*, glutamic acid was used as a nitrogen source; as shown in

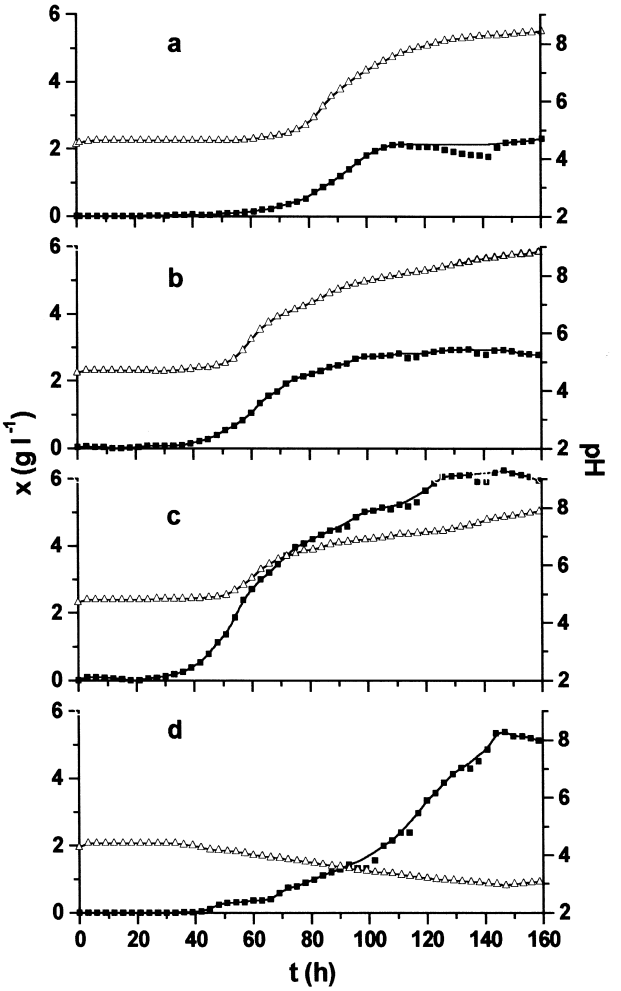

Figure 2 Biomass (■) and pH (△) histories for *P. camemberti* growing on glutamate (a); glutamate +lactate (b); glutamate +glucose (c); and lysine +glucose (d) media

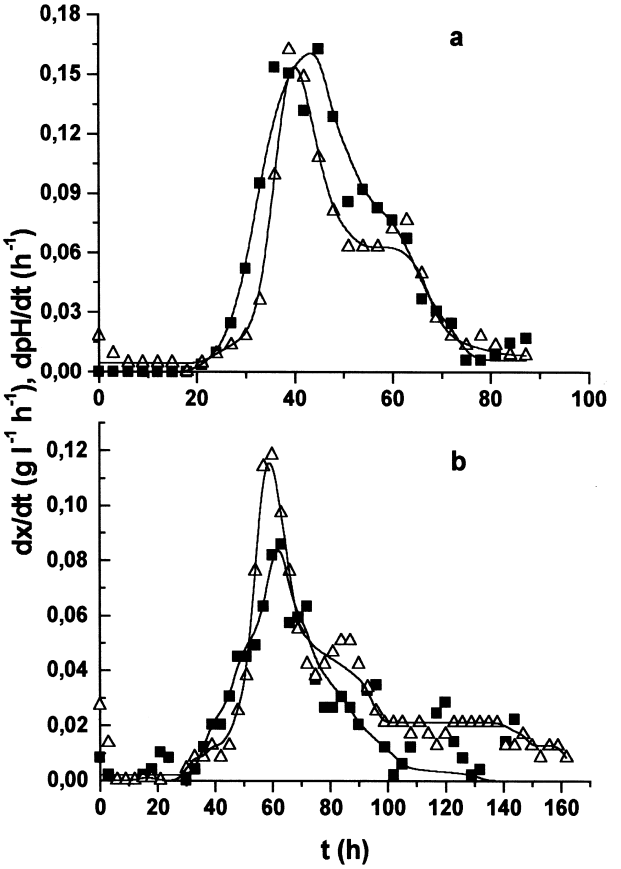

Figure 3 Rates of biomass production (■) and pH variations (△) for *G. candidum* (a); and *P. camemberti* (b) growing on glutamate + lactate medium

Figure 1a, a fair growth was observed on a medium containing this amino acid as the sole carbon source. For the batches of *Figure 1b* and *c*, lactate and glucose were added as an extra carbon source, respectively.

The length of the lag phases were 42, 21 and 17 h for the carbon sources glutamic acid, lactate and glucose. The final biomass concentrations were in the range 4.3–5.5 g l^{-1}.

Examination of the cultures of *Figure 1a–c* also showed that the growth of *G. candidum* always resulted in an alkalinization of the broth irrespective of the presence of lactate in the medium (*Figure 1a* and *b*). The metabolization of glutamic acid resulted in similar alkalinization kinetics, and close maximum alkalinization rates of 0.16 and 0.14 pH units h^{-1} were obtained in *Figure 1a and b*, respectively; this means that the role played by the lactate in the alkalinization process was not obvious. On the other hand, when glutamic acid and glucose were simultaneously metabolized, a low alkalinization rate was observed (0.08 pH units h^{-1} at the most) during the whole batch; this suggested that glucose metabolism resulted in proton efflux which reduced the alkalinization resulting from the metabolism of glutamic acid.

To check this assumption, *G. candidum* was cultivated on glucose supplemented with an amino acid assimilated as a nitrogen but not as a carbon source; these requirements were met by lysine (data not shown). As displayed in *Figure 1d*, the cultivation of *G. candidum* on such a medium resulted in a slightly lower growth than on *Gl* broth (final biomass concentrations 3.8 and 4.3 g l^{-1}, respectively); but the main effect of the substitution of lysine for glutamate was a clear acidification of the medium throughout the culture, leading to a final pH of approximately 3.

Therefore the results of *Figure 1a–d* appeared in fair accordance with the following explanations:

- The uptake of glutamate, acting as both carbon and nitrogen sources, results in an alkalinization of the medium, probably due to its deamination.[6–8]
- In opposition to the proton/glucose symport proved for some yeasts,[24–26] glucose metabolization by *G. candidum* seems to involve a proton/glucose antiport: so the uptake of lysine (used by this microorganism only as a nitrogen source) results in an acidification.

Obviously the rules found for glutamic acid and lysine have to be extended to the other amino acids metabolized by *G. candidum* as nitrogen or nitrogen and carbon sources: such work is in progress.

On the whole, the same conclusions also held true for *P. camemberti* (*Figure 2a–d*). With glutamic acid as a nitrogen source, the effect of the carbon source on the time lag was the same as in *Figure 1a–c*; but the growth of *P. camemberti* always started later than that of *G. candidum*.

The maximum alkalinization rates of *Figure 2a* and *b* were the same within experimental error (0.12 pH units h^{-1}), and slightly lower than those obtained for *G. candidum* on the same media. From *Figure 1a* and *b* and *Figure 2a* and *b*, irrespective of the medium and the microorganism used, the final pH stood in the range of 8–8.5; in addition, the maximum biomass concentrations of *P. camemberti* were lower than those obtained for *G. candidum*. On *Gg* medium (*Figure 1c and Figure 2c*), almost the same final pH (7.9) and maximum alkalinization rates (0.08 and 0.09 pH unit h^{-1}) were recorded for the two microorganisms, while *P. camemberti* gave the higher maximum biomass concentration (6 against 5.5 g l^{-1}) in opposition to the results of *Figure 1a* and *b* and *Figure 2a* and *b*. From *Figures 1* and *2d*, the growth of *P. camemberti* was larger than that of *G. candidum* (5.3 against 3.9 g l^{-1}) despite the fact that the final pH were practically identical ($pH_f = 3$).

From the above results, it appeared that the proton transfer rate was under the control of both the microorganism and the composition of the medium used; the direction of the transfer (alkalinization or acidification) depended on the nitrogen and carbon sources of the medium; on the other hand, the final pH seemed practically independent of the microorganism cultivated. In case of an alkalinization of the broth, irrespective of media composition, a final pH of 8–8.5 was always achieved. Owing to the presence of residual concentrations of nitrogen and carbon sources of at least 4 g l^{-1} in the final supernatant, the final pH seemed to be growth-limiting.

The growth and pH variation rates observed during batch cultivation of *G. candidum* and *P. camemberti* on *Gl* medium are compared in *Figure 3a* and *b*, respectively: for both microorganisms, the growth and pH variation rates achieved their maximum at the same time (approx. 40 and 60 h, respectively); in addition, for a given species, both rate histories displayed similar shapes throughout the culture; these results suggest a possible coupling between growth and proton uptake or release.

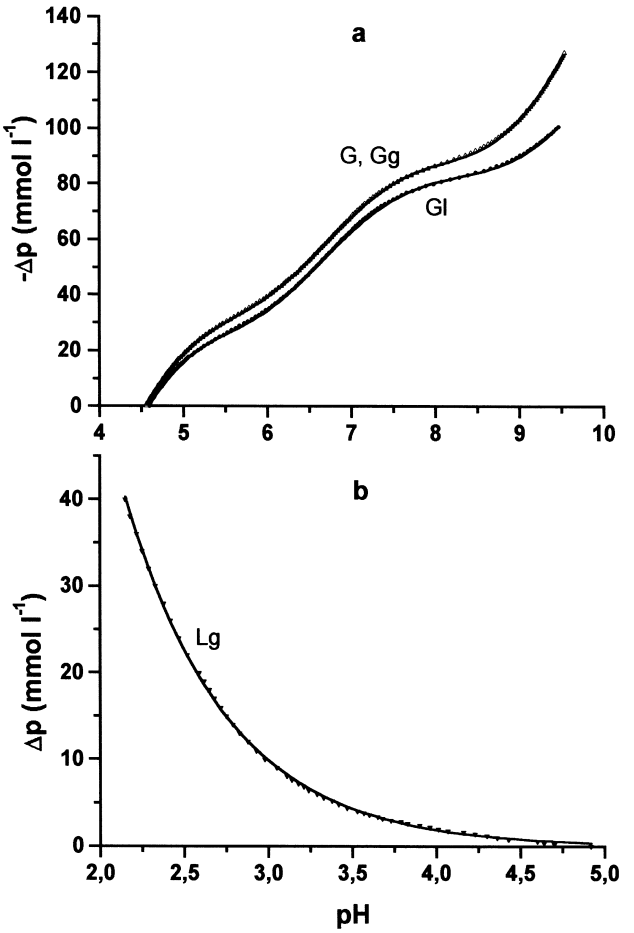

Figure 4 Buffer capacities of glutamate (*G*), glutamate + lactate (*Gl*) and glutamate + glucose (*Gg*) media (a); and lysine+glucose (*Lg*) medium (b)

Indeed the pH of the medium varied in proportion to the biomass concentration when both *G. candidum* and *P. camemberti* were cultivated in a glutamate-based medium (not shown); but this was not at all true for the lysine + glucose medium (not shown). The variable ΔpH did not appear as a general and simple means of monitoring growth.

Table 1 Parameters obtained from the fitting of the titration curves of the different media used

Medium	p_1	p_2	p_3	p_4	p_5	p_6	p_7	p_8	p_9	p_{10}
Glutamate, Glutamate + glucose[a]	−4.54	2.31	1.98	5.94	5.48	0.59	3.24	9.14	8.16	0.74
Glutamate + lactate[a]	0.39	1.78	4.19	6.44	5.47	0.47	4.02	9.35	8.30	0.83
Lysine + glucose[b]	2.15	1.01	0.61							

[a]Parameters obtained from the fitting by Eq. (10) (see text)
[b]Parameters obtained from the fitting by Eq. (11) (see text)

Buffer capacity

The buffer capacity of the media used was measured in the range of pH corresponding to *Figures 1 and 2*; these experiments were carried out on sterile media at 25°C, i.e., in the culture conditions.

The buffer capacity of *G* and *Gg* media were identical, since the glucose was not at all titrable, and titration curves of all the glutamate-based media were rather close (*Figure 4a*). To more easily calculate the proton release or uptake from the pH change, the titration curves were fitted by means of the following expressions:

$$[OH^-] = p_1 + (p_2 * pH) - \left[\frac{p_3 - p_4}{1 + \exp\left(\frac{pH - p_5}{p_6}\right)} - p_4\right] - \left[\frac{p_7 - p_8}{1 + \exp\left(\frac{pH - p_9}{p_{10}}\right)} - p_8\right] \quad (10)$$

where p_j ($j = 1....10$) are constants, for media containing glutamic acid (*Figure 4a*).

$$[H^+] = p_1 * \exp\left(-\frac{pH - p_2}{p_3}\right) \quad (11)$$

with p_j constants, for the medium containing lysine (*Figure 4b*).

The values for the constants p_j are given in *Table 1* for the different media used.

Figure 5 displays the buffer capacity at the beginning and end of growth of *P. camemberti* on *G* medium: in the range of pH corresponding to the main part of the culture, i.e., from approximately 5 to 8.5, the curves were identical within experimental error. This result has also been checked for the other media (data not shown): this demonstrated that the variations of the buffer capacity during culture could be neglected.

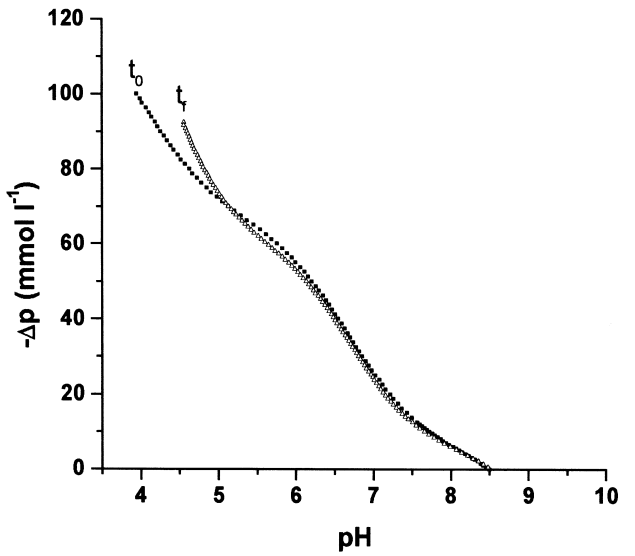

Figure 5 Buffer capacities of the glutamate medium at the beginning (△) and the end (■) of growth of *P. camemberti*

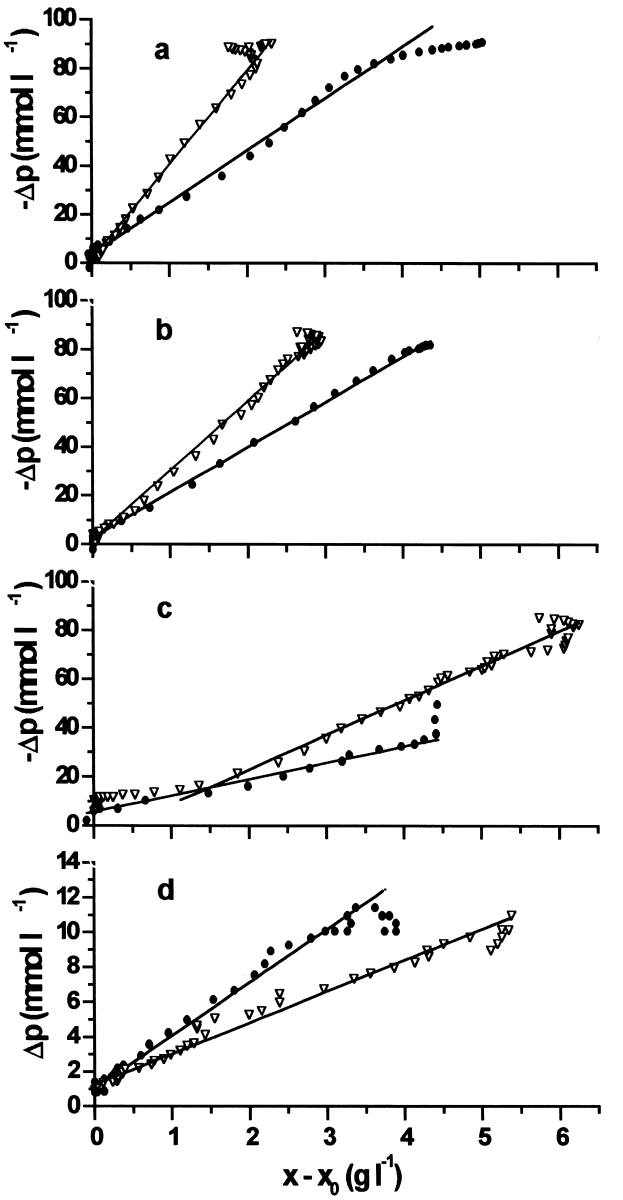

Figure 6 Test of Eq. (7) for *G. candidum* (●) and *P. camemberti* (▽) growing on glutamate (a); glutamate + lactate (b); glutamate + glucose (c); and lysine + glucose (d) media

Coupling of growth and proton transfer

Figure 6 shows that, irrespective of the medium and the microorganism used, the concentrations of proton transferred and that of biomass produced are linearly correlated during the main part of growth, as predicted by Eq. (7) and Eq. (8). This result is of particular

Table 2 Ratio of the slopes *P. camemberti/G. candidum* from *Figure 6*

Medium	Slopes (mol g^{-1})		R = *P. camemberti/ G. candidum*	
	G. candidum	*P. camemberti*		
Glutamate	0.023	0.040	1.74	
Glutamate + lactate	0.019	0.030	1.59	Mean = 1.7
Glutamate + glucose	0.008	0.013	1.66	S.D. = 0.1[a]
Lysine + glucose	0.0034	0.0020	0.59	

[a]Standard deviation

interest in the case of a culture on *Lg* medium (*Figure 6d*); as indicated above, the cultivations carried out in this medium led to a strong curvature of the plot ΔpH versus the concentration of biomass produced.

It should be noticed that a reliable calculation of the coefficients m_i, n_i requires a lot of additional experiments with various carbon and nitrogen sources, and some extra assumptions: the corresponding work is in progress.

Nevertheless, some interesting conclusions may be deduced from the slopes of the lines displayed in *Figure 6a–c*. In these figures, glutamate was always used as a nitrogen source with a yield Y_{XZ} of approximately 5; in *Figure 6a–c* the carbon sources were glutamate, glutamate and lactate, and glutamate and glucose, respectively: for all these carbon sources, the yield Y_{XS} were close to 0.5. Since Y_{XZ} was approximately 10 times greater than Y_{XS}, the main part in the constant coefficient in Eq. (7) was undoubtedly the term m_i/Y_{XS}; from this the direction of the proton transfer is controlled by the sign of the stoichiometric coefficient m_i of the carbon source. So the stoichiometric coefficients of these carbon sources would vary in order of the slopes displayed in *Table 2*: $m_{i\ \mathrm{Glu}} < m_{i\mathrm{lac}} < m_{i\mathrm{glc}}$.

In addition, *Table 2* highlights an encouraging result: for the different media containing glutamate, a constant value $R = 1.7 \pm 0.1$ has been found for the ratio of the slopes *P. camemberti/G. candidum*, as predicted by Eq. (9).

Conclusion

When *G. candidum* or *P. camemberti* are cultivated on synthetic liquid media, large pH variations are observed.

These pH changes seem in close relation to growth, and the direction of proton transfer (alkalinization or acidification) depends mainly on the carbon and nitrogen sources available in the medium:

- When the amino acid used as a nitrogen source may also be metabolized to supply for carbon and energy, as is the case for glutamic acid, a strong alkalinization of the medium (ΔpH = 3.5–4) is observed.
- When carbon and energy are provided by glucose, and nitrogen by an amino acid unable to play the role of carbon source (lysine for instance), a final pH of 3 is recorded.

For the media containing glutamic acid, during the main part of the cultivation, the observed pH change is linearly correlated to the concentration of the biomass produced, but this result is not of general scope (lysine + glucose medium).

A simple theory shows that the number of protons transferred during growth vary in proportion to the concentration of biomass, under the condition that the stoichiometric coefficients of the carbon and nitrogen sources, as well as their biomass on substrate yields are constants.

By means of the buffer capacities of the media, the pH variations have been converted into proton concentration changes, and the above model has been validated for all the media tested.

So, at the expense of a preliminary calibration of a given culture medium, the main part of growth of *G. candidum* and *P. camemberti* can be monitored by recording the corresponding pH history.

Nomenclature

Subscripts:	
0:	initial
i	= 1: *G. candidum* 2: *P. camemberti*
f:	final
Glu:	glutamic acid
Lys:	lysine
glc:	glucose
lac:	lactic acid
X:	biomass
x:	biomass concentration (g l^{-1})
S:	carbon source
s:	carbon source concentration (g l^{-1})
Z:	nitrogen source
z:	nitrogen source concentration (g l^{-1})
$Y_{XSi} = \frac{x_i - x_0}{s_0 - s_i}$:	biomass formation yield with re-

spect to the carbon source consumption;

$Y_{XZi} = \frac{x_i - x_0}{z_0 - z_i}$: biomass formation yield with respect to the nitrogen source consumption;

Δp_{Si} = proton transfer corresponding to the carbon source concentration drop $s_0 - s_i$ (mol l^{-1});

Δp_{Zi} = proton transfer corresponding to the nitrogen source concentration drop $z_0 - z_i$ (mol l^{-1});

$\Delta p_i = \Delta p_{Si} + \Delta p_{Zi}$: total number of proton transferred per litre of culture medium (mol l^{-1}) ($\Delta p_i < 0$: proton uptake, $\Delta p_i > 0$ proton release);

$m_i = \frac{\Delta p_{Si}}{s_0 - s_i}$: stoichiometric coefficient for S (mol g^{-1}, sign as Δp_{Si});

$n_i = \frac{\Delta p_{Zi}}{z_0 - z_i}$: stoichiometric coefficient for Z (mol g^{-1}, sign as Δp_{Zi});

$R = \frac{\Delta p_2 * (x_1 - x_0)}{\Delta p_1 * (x_2 - x_0)}$: ratio of the slopes corresponding to *P. camemberti* and *G. candidum* in *Figure 6*.

Acknowledgments

We wish to thank Ms A. Copeland for correcting this manuscript.

References

1. Lenoir, J. Degradation of proteins during cheese maturation. *Comptes rendus de l'Académie Agricole*. 1962, **48,** 392–399
2. Vassal, L. and Gripon, J.-C. Bitterness of cheeses of the Camembert type: role of rennet and *Penicillium caseicolum*, means of its control. *Lait* 1984, **643–644**, 397–417
3. Molimard, P., Lesschaeve, I., Bouvier, I., Vassal, L., Schlich, P., Issanchou, S., and Spinnler, H. E. Bitterness and nitrogen fractions of mold ripened cheese of Camembert type: impact of the association of *Penicillium camemberti* with *Geotrichum candidum*. *Lait* 1994, **74,** 361–374
4. Lenoir, J. The surface flora and its role in the ripening of cheese. *Int. Dairy Fed. Bull.* 1984, **171,** 3–20
5. Fox, P. F., Lucey, J. A., and Cogan, T. M. Glycolysis and related reactions during cheese manufacture and ripening. *Crit. Rev. Food Sci. Nutr.* 1990, **29,** 237–253
6. Hemme, D., Bouillanne, C., Métro, F., and Desmazeaud, M.-J. Microbial catabolism of amino acids during cheese ripening. *Sci. des Aliments*. 1982, **2,** 113–123
7. Cerning, J., Gripon, J.-C., Lamberet, G., and Lenoir, J. Biochemical activities of *Penicillium* used in cheese making. *Lait* 1987, **67,** 3–39
8. Gueguen, M. and Schmidt, J. L. Yeasts and *Geotrichum candidum*. In: *Microbial Groups of Interest for the Dairy Industry* (Hermier, J., Lenoir, J., and Weber, F., Eds.). CEPIL, Paris, 1992, 165–219
9. Karahadian, C. and Lindsay, R. C. Integrated roles of lactate, ammonia and calcium in texture development of mould surface ripened cheese. *J. Dairy Sci.* 1987, **70,** 909–918
10. Lucey, J. A. and Fox, P. F. Importance of calcium and phosphate in cheese manufacture: a review. *J. Dairy Sci*. 1993, **76,** 1714–1724
11. Gottlieb, D. The utilization of amino acids as a source of carbon by fungi. *Arch. Biochem*. 1946, **9,** 341–351
12. Duran, A., Uruburu, F., and Villanueva, J. R. Morphogenetic and nutritional studies of *Geotrichum lactis* cells. *Arch. für Mikrobiol*. 1973, **88,** 245–256
13. Pusztahelyi, T., Pócsi, I., Kozma, J., and Szentirmai, A. Aging of *Penicillium chrysogenum* cultures under carbon starvation: I: morphological changes and secondary metabolite production. *Biotechnol. Appl. Biochem*. 1997, **25,** 81–86
14. Pusztahelyi, T., Pócsi, I., and Szentirmai, A. Aging of *Penicillium chrysogenum* cultures under carbon starvation: II: protease and *N*-acetyl-β-D-hexosaminidase production. *Biotechnol. Appl. Biochem*. 1997, **25,** 87–93
15. Dumain, P.-P., Desnouveaux, R., Bloc'h, L., Leconte, C., Fuhrmann, B., De Colombel, E., Plessis, M.-C., and Valery, S. Use of flow cytometry for yeast and mould detection in process control of fermented milk products: the ChemFlow system — a factory study. *Biotechnol. Forum Eur*. 1990, **3,** 224–229
16. Molimard, P., Vassal, L., Bouvier, I., and Spinnler, H. E. Growth of *Penicillium camemberti* and *Geotrichum candidum* in pure and mixed cultures on experimental mold ripened cheese of Camembert-type. *Lait* 1995, **75,** 3–16
17. Flynn, D. S. Instrumentation and control of fermenters. In: *The Filamentous Fungi* (Smith, J. E., Berry, D. R., and Kristiansen, B., Eds.). Fungal Technology Vol. 4, Edward Arnold, 1983, 77–100
18. Viniegra-González, G., Larralde-Corona, C. P., and López-Isunza, F. A new approach for modelling the kinetics of mycelial cultures. In: *Advances in Bioprocess Engineering* (Galindo, E. and Ramirez, O. T., Eds.). Kluwer Academic Publishers, Dordrecht, the Netherlands, 1994, 183–189
19. Amrane, A. and Prigent, Y. Comparison of growth parameters for *Geotrichum candidum* Geo 17 and *Penicillium camemberti* LV2 in solid and liquid cultures. *Lait* 1997, **77,** 641–648
20. Amrane, A. and Prigent, Y. A new turbidimetric device for on-line monitoring of growth of filamentous microorganisms. *J. Microbiol. Methods* 1998, **33,** 37–43
21. Trinci, A. P. J. A kinetic study of the growth of *Aspergillus nidulans* and other fungi. *J. Gen. Microbiol*. 1969, **57,** 11–24
22. Wernau, W. C., and Wilke, C. R. A new method for evaluation of dissolved oxygen response for $k_L a$ determination. *Biotechnol. Bioeng*. 1973, **15,** 571–578
23. Satake, K., Okuyama, T., Ohashi, M., and Shinoda, T. The spectrophotometric determination of amine, amino acid and peptide with 2,4,6-trinitrobenzene 1-sulfonic acid. *J. Biochem*. 1960, **47,** 654–660
24. Postma, E. and Van Den Broek P. J. A. Continuous-culture study of the regulation of glucose and fructose transport in *Kluyveromyces marxianus* CBS 6556. *J. Bacteriol*. 1990, **172,** 2871–2876
25. Castillo, F. J. Lactose metabolism by yeasts. *Bioprocess Technol*. 1990, **5,** 297–320
26. Spencer-Martins, I. Transport of sugars in yeasts: implications in the fermentation of lignocellulosic materials. *Bioresource Technol*. 1994, **50,** 51–57