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ORIGINAL PAPER

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Substrate and metabolite diffusion within model medium for soft cheese in relation to growth of *Penicillium camembertii*

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Abstract *Penicillium camembertii* was cultivated on a jellified peptone—lactate based medium to simulate the composition of Camembert cheese. Diffusional limitations due to substrate consumption were not involved in the linear growth recorded during culture, while nitrogen (peptone) limitation accounted for growth cessation. Examination of gradients confirmed that medium neutralization was the consequence of lactate consumption and ammonium production. The diffusion of the lactate assimilated from the core to the rind and that of the ammonium produced from the rind to the core was described by means of a diffusion/reaction model involving a partial linking of consumption or production to growth. The model matched experimental data throughout growth.

Keywords *Penicillium camembertii* · Solid-state fermentation · Growth · Diffusion · Model

List of symbols

A, B	the growth- and the non-growth-associated coefficients (Eqs. 6, 7) A (dimensionless), B (day^{-1})
M	surface concentration (g cm^{-2})
D	diffusion coefficient (cm^{-2} day^{-1})
h	proton transfer concentration (g l^{-1})
p	ammonium concentration (g NH_4^+ l^{-1})
$\frac{dp}{dt}$	ammonium production rate (g NH_4^+ l^{-1} day^{-1})
q	specific production rate (day^{-1})
s	lactate concentration (g lactate anion l^{-1})
$\frac{ds}{dt}$	lactate consumption rate (g lactate anion l^{-1} day^{-1})
x	biomass concentration (g l^{-1})
$\frac{dx}{dt}$	growth rate (g l^{-1} day^{-1})
y	height of gel (cm)
z	peptone concentration (g peptone l^{-1})
μ	specific growth rate (day^{-1})

Subscripts

0	initial
res	residual
m	maximal

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Introduction

Camembert is one of the most famous cheeses in France. Thanks to its gustative quality, it has become a mass-market product. The ripening process plays an essential role in soft Camembert cheese manufacture because curd texturization and organoleptic characteristics responsible for the gustative properties of the final product appear during this phase.

For many years, it has been known that the texturization results from the neutralization of the curd during ripening [14, 19, 23, 32], which is a consequence, at least partially, of the growth of *Penicillium camembertii* on the surface of the cheese [16, 17]. This fungus utilizes lactic acid present in the curd as a carbon and energy source [14, 19], and peptides as nitrogen sources resulting in the release of ammonia, after amino acid deamination [16, 21].

The consumption of lactic acid by the microorganisms induces a diffusion of this compound from the core to the rind resulting in a concentration gradient. In a similar way, ammonium release at the surface of the curd induces a diffusion of this component from the rind to the core. These diffusional mechanisms appear

therefore as one of the main factors in soft cheese manufacturing.

In the available literature, there is a lack of information concerning the diffusional mechanisms, resulting from the concentration gradients of mineral elements, substrates or metabolites, induced by fungal growth. Some studies concerning the mineral migration of calcium and phosphorus [17] and some salts [15, 18], all under pH control, can be found. These authors have examined the mineral distribution between the rind, the under-rind and the centre of cheeses or model cheeses.

Obviously, a soft cheese during ripening is a complex system [15]. Moreover, there is no simple way to determine the biomass concentration at the surface of the cheese [24]. For a better understanding of the fundamental phenomena, it appears therefore more suitable, in a first approach, to mimic the real system (lactic curd during ripening) by cultures of the involved microorganisms at the surface of synthetic jellified medium.

With this aim, the diffusion gradients of glutamic acid were examined in a jellified model medium containing the amino acid as the sole nitrogen source, and described by means of a reaction/diffusion model [30]. The experimental gradients were fitted using the measured diffusion coefficient of glutamic acid in the culture medium and assuming a partial linking of substrate consumption to growth of *P. camembertii*.

The transposition of this theoretical approach to curd during ripening for its monitoring and control may be usefully preceded by similar work on a jellified model medium containing, among other things, tryptic casein peptone and lactate to simulate the aqueous phase of Camembert cheese [8]. The diffusion of lactate from the bottom of the gel to the upper surface or that of ammonium from top to bottom induced by their respective consumption and production at the surface of the gel due to *P. camembertii* growth in pure cultures was examined.

Materials and methods

Microorganisms

Freeze-dried spores of the commercial strain *P. camembertii* LV2 (Rhodia Food, Dangé St Romain, France) were stored at +7 °C.

Medium and cultures

Cultures were carried out in Petri dishes of 18 cm (internal diameter), at 12 °C and 98% relative humidity (to simulate the operating conditions in a salting room during the ripening of Camembert cheese) in a cooling incubator (Friocell MMM 111 l: Bioblock, Illkirch, France). The presence of a saturated solution of K_2SO_4 in the cooling incubator maintained the relative humidity at 98%. The composition of the peptone—lactate based medium was (g l^{-1}): agarose, 20 (Biokar Diagnostics, Beauvais, France), tryptic casein peptone, 10 and sodium L(+)-lactate (Prolabo, Paris, France), 10. The medium was supplemented with the following solutions:

- 50 ml l^{-1} of EDTA chelated trace elements (TE) [31], the final TE concentrations were (mg l^{-1}): Mg, 25; Fe, 20; Ca, 18; Zn, 4.5; Mo, 2; Cu, 1.3.
- 500 ml l^{-1} of inorganic phosphates (P_i) [31], containing (g l^{-1}): KH_2PO_4, 3.4; Na_2HPO_4, 3.55.

The pH of both media was then adjusted to 4.5 with HCl 1 M.

Five hundred microlitre of medium were sterilized (121 °C, 20 min) and poured into the Petri dish (18 cm internal diameter), leading to a film of approximately 2 cm thick. After gelling, the surface of the medium was inoculated with 10 ml of the spore suspension, uniformly distributed at the surface of the Petri dish. The spore suspension was left to rehydrate and germinate 1 h in sterile medium at room temperature.

Analysis

During the fermentation, cores were sampled by stamping out the culture with a sterile hollow punch. By means of tweezers, the thin layers of biomass on the top of the cores were peeled off, dried and weighed; the total biomass concentration was expressed in gram of dry weight per volume of gel. An observed standard deviation of 0.5 g l^{-1} was observed, estimated from a set of four identical experimental runs.

The remainder of the solid samples was set in a microtome; horizontal slices of approximately 3 mm were then cut with a razor and weighed. Every slice was put into an Eppendorf tube and the pH was measured by sticking a 5 mm diameter combination pH electrode (Mettler Toledo, Viroflay, France) into the solid sample after homogenization. For lactate and ammonium analysis, every slice was dissolved by heating at 80 °C for 5 min in 10 ml distilled water. L(+)-lactate was then determined enzymatically (Sigma Diagnostics, St Quentin Fallavier, France) and ammonium by the Nessler method [29]. The observed standard deviations were ±0.4 g l^{-1} and ±6 mg l^{-1} for lactate and ammonium concentrations, respectively.

Total nitrogen was determined on a core by the Nessler method [29], after mineralization of the sample. Peptone concentrations were deduced by subtracting the ammoniacal nitrogen concentration in the core from the total nitrogen concentration, assuming that the nitrogen content of peptone was 12.5%. The observed standard deviation on peptone concentrations was ±0.4 g l^{-1}.

Determination of the diffusion coefficients

At the surface of a Petri dish containing 500 cm^3 of sterile culture medium lacking the component under

investigation, 25 ml of a solution of the component (5 mol l^{-1} for ammonium and 0.09 mol l^{-1} for L(+) lactate) was uniformly distributed. To induce an "instantaneous" source of the given component, the solution was put in contact with the surface of the gel for 5 min for ammonium and 30 min for L(+) lactate, and then poured off. The component will diffuse in the gel from the "rind" to the "core" by a plane one-dimensional diffusion mechanism. Cores were sampled at given times, cut in slices of approximately 3 mm, treated as above and then analysed for the investigated component. As with cultures, experiments were carried out at 12 °C and 98% relative humidity.

Results and discussion

In the case of a plane one-dimensional diffusion, induced by an instantaneous source, after a given time t, the second Fick relation has an analytical solution, which can be then linearized [10]:

$$\mathrm{Ln}(s(y)) = \mathrm{Ln}\left(\frac{M}{\sqrt{\pi Dt}}\right) - \frac{y^2}{4Dt}, \quad (1)$$

where y was the perpendicular axis to the surface of the gel ($y = 0$ corresponded to the surface), D the diffusion coefficient and M the surface concentration, which corresponded to the total amount of the investigated component in the gel related to the gel surface unit.

The coefficient D can be estimated from the slope of the straight line $\mathrm{Ln}(s(y))$ versus y^2 corresponded to $-1/4Dt$. The diffusion coefficient was determined by the minimization of the sum of the deviation squares, conducted simultaneously on all the concentration gradients. Values of 0.4 cm^2 day^{-1} for lactate and 0.8 cm^2 day^{-1} for ammonium have been found. More details on the determination of the diffusion coefficients can be found in a previous paper [30].

Mycelial growth can be described using the widespread logistic or Verlhust relation [13, 25–27] (Fig. 1):

$$x = x_0 x_m \frac{e^{\mu_m t}}{x_m - x_0 + x_0 e^{\mu_m t}}, \quad (2)$$

where x, x_0 and x_m were the biomass concentrations (g l^{-1}) at a given time t and its initial and maximal value respectively, μ_m is the maximal specific growth rate (day^{-1}). The optimized parameter values were 0.04 g l^{-1}, 3.4 g l^{-1} and 0.95 day^{-1} for x_0, x_m and μ_m.

As previously shown [3], lactate consumption was assumed to be involved in cellular biosynthesis as a carbon source and in energy supply for growth and cellular maintenance. From this, its consumption would appear as partially linked to growth and in order to take into account the substrate limitation at the end culture, an additional term $(1 - (s_{res}/s))$ was added:

$$-\frac{ds}{dt} = A\frac{dx}{dt} + Bx\left(1 - \frac{s_{res}}{s}\right) \quad (3)$$

with s_{res} being the residual lactate concentration.

The additional term in Eq. 3 was previously introduced to account for cessation of lactate consumption when it became limiting [6].

Amino acids [28] and peptides [11] can be in part assimilated as carbon sources by *P. camembertii*, in addition to nitrogen sources, for both biosynthesis and energy supply, leading to ammonia release [1] after amino acids deamination. Indeed, amino acids contain excess nitrogen in relation to their carbon content for fungi, leading to pH increase [12]. Ammonium production resulted therefore from both biosynthesis and viable cell maintenance, and then was also partially linked to growth:

$$\frac{dp}{dt} = A\frac{dx}{dt} + Bx\left(1 - \frac{z_{res}}{z}\right), \quad (4)$$

where p corresponded to the metabolite (ammonium) concentration, z_{res}, the residual peptone concentration (12.5×peptidic nitrogen).

The additional term $(1 - (z_{res}/z))$ in Eq. 4 was added to account for cessation of ammonium production due to nitrogen substrate limitation (peptones).

The optimized values for the associated and non-growth associated parameters A and B were 1.15 and 0.185 day^{-1} for lactate and 0.008 and 0.004 day^{-1} for ammonium, respectively. They were determined from the experimental data of lactate and peptones consumption and ammonium production (Fig. 1) and optimized using the EXCEL solver system.

Since the early work of Luedeking and Piret [22], the most common way to verify the partial association of a production with growth, was to draw the specific rate of production $(q_p = dp/x\,dt)$ versus the specific rate of growth $(\mu = dx/x\,dt)$. If consumption was considered, the specific rate of production was replaced by a specific rate of consumption $(q_s = -ds/x\,dt)$. Using the optimized A and B values (Eqs. 3, 4), calculated specific ammonium production rate q_p and calculated specific lactate consumption rate q_s were compared to the corresponding experimental values (Fig. 2). The partial association with growth, assumed for both lactate consumption and ammonium release, was confirmed at the examination of Fig. 2, since there was a fairly good agreement between the calculated and the experimental values, especially for the specific lactate consumption rate q_s versus the specific growth rate μ (Fig. 2a). On the contrary, a low amount of ammonium was produced resulting in a high error in the differentiation of experimental data [20], which accounted for the deviations observed for the experimental q_p values (Fig. 2b). Amino acids and peptides contain excess nitrogen in relation to their carbon content for fungi [12], so that ammonium is released during their metabolization as C and N sources [2]. However, *P. camembertii* assimilated large peptides but medium and small peptides, as it is the

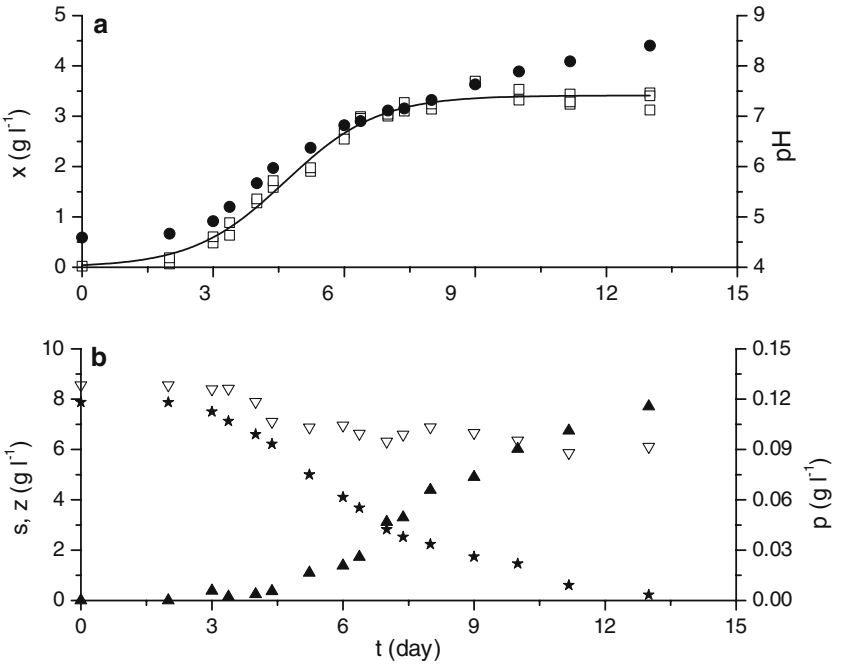

Fig. 1 Time courses of growth (*open square*) and alkalinization (*filled circle*) (**a**), peptone consumption (*open inverted triangle*), lactate consumption (*filled star*) and ammonium release (*filled triangle*) (**b**) for *P. camembertii* growing at the surface of the jellified medium. *Continuous line*: calculated biomass concentrations using the Verlhust model (Eq. 2)

case in the tryptic casein peptone, were less consumed [9]; and only few amino acids were previously reported to be convenient carbon sources for *P. camembertii* [28], accounting for the low amount of ammonium released throughout growth, following peptone consumption (Fig. 1b).

The experimental values recorded at the beginning of cultures, namely at low biomass concentrations, can be regarded as of little significance, due to the high error in the differentiation of experimental data [20]; they have not been therefore reported in Fig. 2, especially for the graph q_p versus μ (Fig. 2b).

Initially, lactate and peptones were uniformly distributed in the gel at a concentration s_0 and z_0, respectively. From the initial time $t = 0$, the biomass grew uniformly at the surface of the gel ($y = 0$) following Eq. 2; at the upper surface of the gel (low y), the lactate concentration decreased and the ammonium

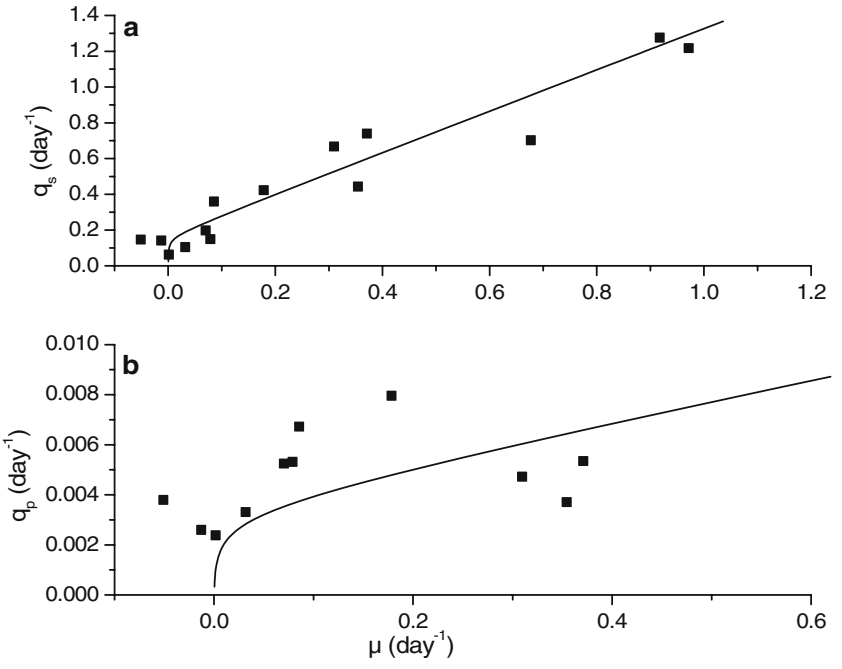

Fig. 2 Validation of the model assuming a partial association of lactate consumption (Eq. 3; $A = 1.15$ and $B = 0.185$ day^{-1}) (**a**) and ammonium release (Eq. 4; $A = 0.008$ and $B = 0.004$ day^{-1}) (**b**) with *P. camembertii* growing on jellified medium. *Symbols*: experimental data points; *continuous lines*: model

concentration increased but remained constant in a cross-section of the gel (plane one-dimensional diffusion). The second Fick relation with constant coefficient diffusion D had to be solved:

$$D\frac{\partial^2 w}{\partial y^2} - \frac{\partial w}{\partial t} = 0, \quad (5)$$

where w corresponded to the lactate concentration s or the ammonium production p.

Since lactate consumption and ammonium production are given by Eqs. 3 and 4, their flux across a section of 1 cm^2 of gel (at $y = 0$) are given by the following relation:

$$\text{For lactate: } y = 0, \quad \text{Flux} = -L\left(A\frac{dx}{dt} + Bx\left(1 - \frac{s_{res}}{s}\right)\right) \quad (6)$$

$$\text{For ammonium: } y = 0, \quad \text{Flux} = L\left(A\frac{dx}{dt} + Bx\left(1 - \frac{z_{res}}{z}\right)\right) \quad (7)$$

and a nil flux on the bottom and the side of the gel.

The numerical resolution of the above systems was carried out using the Galerkin Finite Element Method (PDease software) to give the concentration profiles of lactate $s(y)$ and ammonium $p(y)$ at different times (Fig. 3a, b, respectively).

Lactate (s) consumption started simultaneously with growth after about 2 days (Fig. 1), leading to non-negligible lactate diffusion gradients recorded after 3 days of culture. Lactate diffusion gradients, namely the concentration between the first (top of the gel) and the last slice (bottom of the gel) were expected to be linked to growth, as experimentally supported. Indeed, the gradient was maximum after 5–6 days of culture (2.9 g l^{-1}; Fig. 3a), namely at the end of the linear growth, recorded between the third day to the sixth day of growth (0.8–0.9 g l^{-1} day^{-1}; Fig. 1a); rate of lactate consumption remained also nearly constant at its maximal value during this lapse of time (1.2–1.3 g l^{-1} day^{-1}; Fig. 1b). The concentration gradients tended to homogenize along the longitudinal section of the gel only after the end of growth, namely after 9 days (Fig. 3a); after 13 days of culture lactate was almost completely exhausted (Fig. 1a). Lactate assimilation during stationary state corresponded to its use as an energy source for cell maintenance. Even at the end of growth, significant amount of lactate remained in the first slice (Fig. 3a), namely close to the top of the gel, indicating the absence of diffusional limitation by the lactate.

Diffusional peptone limitation was also, most likely, not involved in the linear growth. Peptone concentration gradients have not been determined, since they only give very global information, which cannot be helpful for the identification of the diffusional limitations, owing to the complexity of this substrate. However, the various peptides and amino acids contained in the peptone have obviously not the same rates of diffusion. From this, a diffusional limitation by the peptone would not result in a (long) linear growth, as was the case for *P. camembertii* growing on peptone and lactate. The linear growth recorded have to be therefore attributed to an oxygen limitation, as shown in liquid cultures on a similar medium under conditions of low aeration [5].

Resolution of Eqs. 5 and 6 by considering the optimized values of the parameters A and B (1.15 and 0.185 day^{-1}), as well as the diffusion coefficient of lactate into the gel D (0.4 cm^2 day^{-1}), gave the theoretical concentration gradients. As observed, the calculated gradients matched the experimental gradients from the beginning to the end of growth (9 days). Examination of the calculated gradients confirmed that diffusional limitation by the lactate was not responsible for the linear growth, since significant amount of lactate remained at the top of the gel up until the end of growth.

Ammonium release became significant after nearly 4 days of culture (Fig. 1b), as well as the diffusion gradients (Fig. 3b). This production is related to the use of peptides [11] and amino acids [2] from the peptone as carbon sources, in addition to nitrogen sources, and continued during stationary state, following the low peptone consumption also recorded (Fig. 1b). A low final amount of ammonium was produced (0.12 g l^{-1}), when compared to that recorded in absence of lactate in the medium (0.82 g l^{-1}; Aldarf, unpublished data), in agreement with previous results recorded in liquid culture [1]; in the presence of lactate in the medium, *P. camembertii* assimilated less peptone as a carbon source, leading to a lower amount of ammonium produced. In contrast to the behaviour observed for lactate, low ammonium gradients were recorded throughout growth, most likely due to the higher ammonium diffusion coefficient, 0.8 and 0.4 cm^2 day^{-1} for ammonium and lactate, respectively.

Resolution of Eqs. 5 and 7 by considering the optimized values of the parameters A and B (0.008 and 0.004 day^{-1}), as well as the ammonium diffusion coefficient D (0.8 cm^2 day^{-1}), gave the theoretical concentration gradients, which matched the experimental gradients except in the beginning of growth. The overestimation of the ammonium concentration gradients at the beginning of growth resulted from an overestimation of the growth-associated ammonium production, since the Verlhust model (Eq. 2) predicted slight growth during the lag phase (Fig. 1a).

From the beginning of growth (3 days), a high pH gradient was recorded (0.82 pH unit between the top and the bottom of the gel—Fig. 4). The pH increase was related to lactate consumption and ammonium production [14, 16]; maximum pH gradients were therefore expected to be linked to the maximum gradients of lactate and ammonium concentrations, as experimentally supported, since they were recorded during the linear growth: 1.1–1.2 pH unit between the top and the bottom of the gel from 4 to 6 days of culture (Fig. 4). It should be noted that the lactate was the main contributor to the pH increase; since after 13 days of

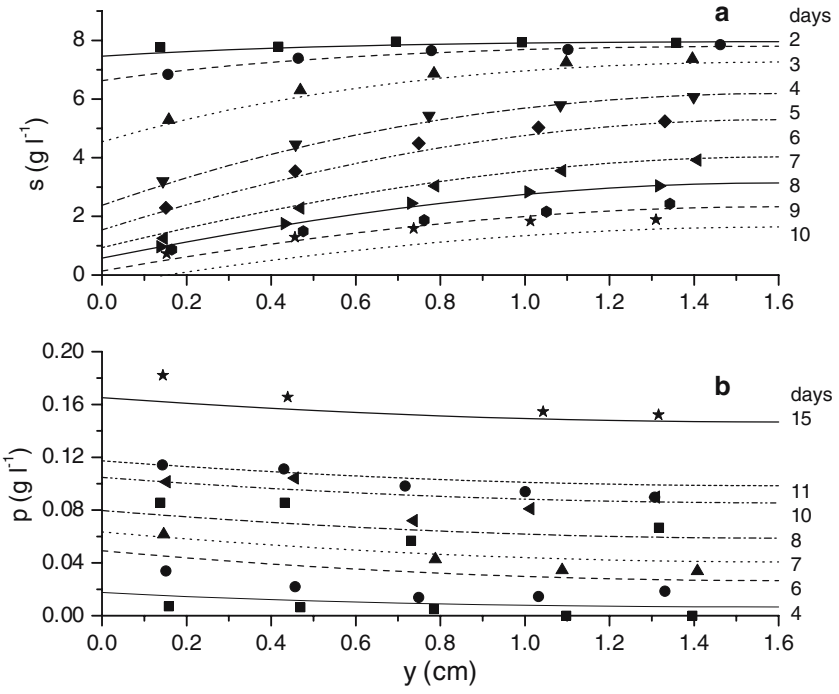

Fig. 3 Concentration gradients of lactate (**a**) and ammonium (**b**) induced by their respective consumption and production by *P. camembertii* growing at the surface of the jellified medium. *Symbols*: experimental data points; *continuous lines*: recalculated diffusion gradients (Eq. 6)

P. camembertii culture, 85.1 mM of lactate were assimilated while only 6.4 mM of ammonium were produced (Fig. 1b). Following lactate concentrations, pH gradients tended to homogenize along the longitudinal section of the gel only after 13 days of culture, namely after the end of growth (Fig. 4).

At the end of the linear growth (6 days), the mean pH value of the longitudinal section of the gel was 6.9, while the pH of the top of the gel was 7.5; end of linear growth also corresponded to a change in the rate of peptone consumption. The decrease of the growth rate may therefore be attributed to both factors, since the above results did not allow to discriminate in favour of pH or peptone limitation. On the contrary, cessation of growth cannot be attributed to pH, since a pH of 8.02 was measured at the top of the gel after 9 days of growth (Fig. 4), which was not inhibitory. Indeed, cessation of growth of *P. camembertii* on a yeast extract—lactate based medium was observed for pH above 8 [7]. In addition, it has been shown that total inhibition of growth was observed only at pH 8.6 (data not shown). The concentration of available nitrogen in the medium (peptone) was therefore the growth limiting factor, confirming a previous assumption [2].

Kinetics of proton uptake displayed in Fig. 5 were deduced from pH by means of the buffer capacities of the medium used, measured in the pH range recorded during *P. camembertii* culture [4]. Figure 5 shows that the molar sum of lactate assimilated and ammonium produced accounted well for proton transfer. This result agreed with those found in the literature, since curd neutralization during cheese ripening was assumed to result from lactic acid assimilation [14, 19] and ammonia release [16, 21]. Some discrepancies between the sum (lactate + ammonium) and the proton transfer could only be noted at the top of the gel after the linear growth, namely after 6 days of culture (the relative difference was 15% at the worst). These discrepancies might be attributed to the charge of peptides and amino acids. However, an analysis of the peptone effect on pH was too difficult for such a complex substrate. For instance, the initial pH value (4.6) was below the isoelectric pH of the majority of the amino acids, which were therefore positively charged at the beginning of culture and then assimilated in exchange for protons to maintain cellular electroneutrality. On the contrary, glutamic and aspartic acids, convenient nitrogen sources for *P. camembertii* [28] were negatively charged throughout culture.

Conclusions

It was confirmed that the observed linear growth was the consequence of an oxygen limitation, since a diffusional limitation by a complex substrate like peptone would not induce a linear growth. In addition, throughout growth significant amount of lactate remained at the top of the gel showing that its diffusion did not limit growth. Discrimination between a pH effect and a peptone limitation to account for the decrease of the growth rate following the linear growth has not been possible; however, the pH measured at the top of the gel at the end of growth (8.02) was clearly not inhibitory, confirming that growth ceased since the available nitrogen in the medium (peptone) became limiting. Lactate

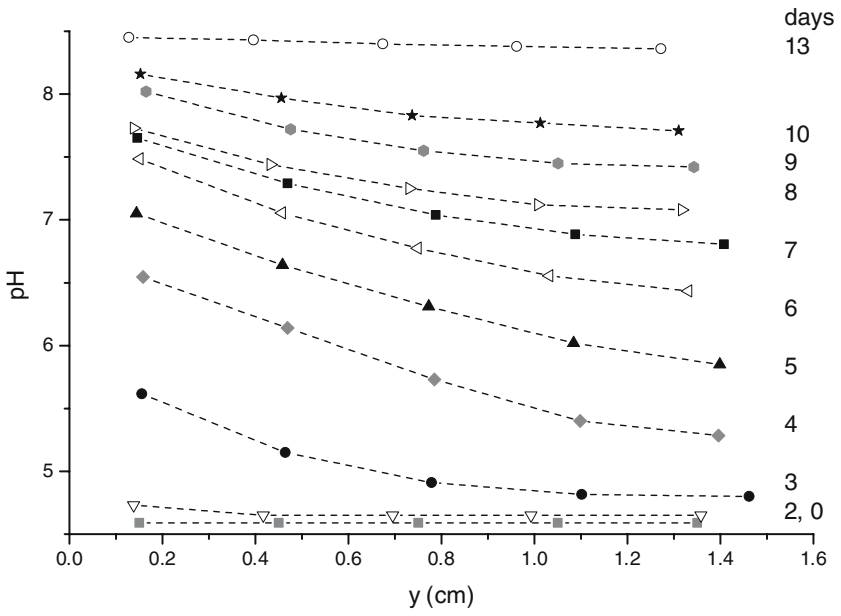

Fig. 4 pH gradients recorded during *P. camembertii* growth on a peptone—lactate based medium

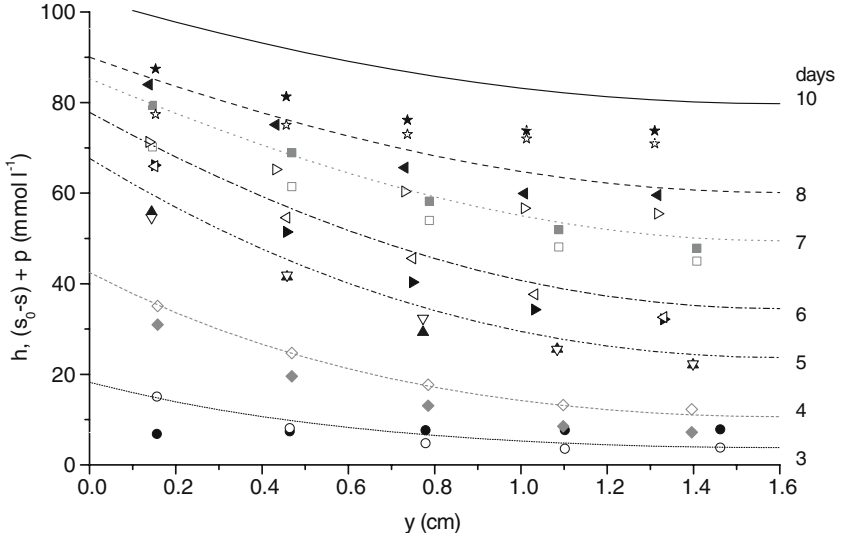

Fig. 5 The sum of the gradients of the assimilated lactate and the released ammonium, experimental data: *closed symbols*, and recalculated gradients: *continuous line*; experimental gradients of proton uptake (*open symbols*) induced by the growth of *P. camembertii* at the surface of a peptone—lactate based medium

consumption and ammonium release accounted for medium neutralization, since the sum of their gradients corresponded rather well to the proton transfer gradients throughout growth.

The gradients of substrate consumption (lactate) or metabolite release (ammonium) were rather well described by means of a reaction/diffusion model, based on a partial linking between growth and substrate consumption or metabolite release. The validation of the model on synthetic jellified medium can be considered as a preliminary step, which has to be followed by similar work on the real medium, lactic curd, in view of a deeper understanding of the mechanism of curd neutralization, responsible for texturization development.

References

1. Adour L, Couriol C, Amrane A, Prigent Y (2002) Growth of *Geotrichum candidum* and *Penicillium camemberti* in liquid media in relation with the consumption of carbon and nitrogen sources and the release of ammonia and carbon dioxide. Enzyme Microb Technol 31:533–542
2. Aldarf M, Amrane A, Prigent Y (2002) Carbon and nitrogen substrates consumption, ammonia release and proton transfer in relation with growth of *Geotrichum candidum* and *Penicillium camembertii* on a solid medium. J Biotechnol 95:99–108
3. Aldarf M, Amrane A, Prigent Y (2002) Reconstruction of the biomass history from carbon and nitrogen substrate consumption, ammonia release and proton transfer during solid cultures of *Geotrichum candidum* and *Penicillium camembertii*. Appl Microbiol Biotechnol 58:823–829

4. Amrane A, Plihon F, Prigent Y (1999) Proton transfer in relation to growth of *Geotrichum candidum* and *Penicillium camembertii* in synthetic liquid media. Enzyme Microb Technol 24:561–568
5. Amrane A, Plihon F, Prigent Y (1999) Kinetics of growth and medium de-acidification for *Geotrichum candidum* and *Penicillium camembertii* cultivated on complex liquid media. World J Microbiol Biotechnol 15:489–491
6. Amrane A (2001) Batch cultures of supplemented whey permeate using *Lactobacillus helveticus*: unstructured model for biomass formation, substrate consumption and lactic acid production. Enzyme Microb Technol 28:827–834
7. Amrane A, Prigent Y (2001) Growth of *Geotrichum candidum* and *Penicillium camembertii* cultivated on liquid media correlated with ammonia and methanethiol emission. Acta Biotechnol 21:283–290
8. Boutrou R, Gaucheron F, Piot M, Michel F, Maubois JL, Léonil J (1999) Changes in the composition of juice expressed from Camembert cheese during ripening. Le lait 79:503–513
9. Boutrou R, Aziza M, Amrane A (2005) Enhanced proteolytic activities of *Geotrichum candidum* and *Penicillium camembertii* in mixed culture. Enzyme Microb Technol:DOI:10.1016/j.enzmictec.2005.1011.1003 (in press, 1017 pp)
10. Crank J (1975) The mathematics of diffusion. 2nd edn. Clarendon Press, Oxford, p 414
11. Da Silva MC, bertolini MC, Ernandes JR (2001) Biomass production and secretion of hydrolytic enzymes are influenced by the structural complexity of the nitrogen in *Fusarium oxysporum* and *Aspergillus nidulans*. J Basic Microbiol 41:269–280
12. Deacon JW (1997) Modern mycology, 3rd edn. Blackwell, Oxford, p 303
13. Diaz C, Lelong P, Dieu P, Feuillerat C, Salome M (1999) Online analysis and modelling microbial growth using a hybrid system approach. Process Biochem 34:39–47
14. Fox PF, Lucey JA, Cogan TM (1990) Glycolysis and related reactions during cheese manufacture and ripening. Crit Rev Food Sci Nutr 29:237–253
15. Gaucheron F, Le Gräet Y, Michel F, Briard V, Piot M (1999) Evolution of various salt concentrations in the moisture and in the outer layer and centre of a model cheese during its brining and storage in an ammoniacal atmosphere. Le Lait 79:553–566
16. Karahadian C, Lindsay RC (1987) Integrated roles of lactate, ammonia, and calcium in texture development of mold surface-ripened cheese. J Dairy Sci 70:909–918
17. Le Gräet Y, Lepienne A, Brulé G, Ducruet P (1983) Mineral migration in soft cheese during ripening. Le Lait 629–630:317–332
18. Le Gräet Y, Brulé G (1988) Macro and trace elements migration in Camembert soft cheese during ripening. Le Lait 68:219–234
19. Lenoir J (1984) The surface flora and its role in the ripening of cheese. Int Dairy Fed Bull 171:3–20
20. Levenspiel O (1962) Chemical reaction engineering. Wiley, New york, p 668
21. Lucey JA, Fox PF (1993) Importance of calcium and phosphate in cheese manufacture, a review. Crit Rev Food Sci Nutr 76:1714–1724
22. Luedeking R, Piret EL (1959) A kinetic study of the lactic acid fermentation. Batch process at controlled pH. J Biochem Microbiol Technol Eng 1:393–412
23. Molimard P, Lesschaeve I, Bouvier I, Vassal L, Schlich P, Issanchou S, Spinnler HE (1994) Bitterness and nitrogen fractions of mold ripened cheese of Camembert type: impact of the association of *Geotrichum candidum* and *Penicillium camembertii*. Le Lait 74:361–374
24. Molimard P, Vassal L, Bouvier I, Spinnler HE (1995) Growth of *Penicillium camemberti* and *Geotrichum candidum* in pure and mixed cultures on experimental mold ripened cheese of Camembert-type. Le Lait 75:3–16
25. Moraine RA, Rogovin P (1966) Kinetics of polysaccharide B-1459 fermentation. Biotechnol Bioeng 8:511–524
26. Norton S, Lacroix C, Vuillemard JC (1994) Kinetic study of continuous whey permeate fermentation by immobilised *Lactobacillus helveticus* for lactic acid production. Enzyme Microb Technol 6:457–466
27. Pandey A, Soccol CR, Mitchell D (2000) New developments in solid state fermentation: I -bioprocesses and products. Process Biochem 35:1153–1169
28. Plihon F, Le Doujet S, Amrane A, Prigent Y (1998) Effect of amino acids on the growth of submerged cultures of *Geotrichum candidum* and *Penicillium camembertii*. J Food Mycol 1:203–210
29. Rodier J (1975) Ammoniacal nitrogen analysis. In: Water analysis, vol 1. Dunod, Paris, pp 116–120
30. Stephan J, Couriol C, Fourcade F, Amrane A, Prigent Y (2004) Diffusion of glutamic acid in relation to growth of *Geotrichum candidum* and *Penicillium camembertii* at the surface of a solid medium. J Chem Technol Biotechnol 79:234–239
31. Trinci APJ (1969) A kinetic study of the growth of *Aspergillus nidulans* and other fungi. J Gen Microbiol 57:11–24
32. Vassal L, Gripon JC (1984) Bitterness of cheeses of the camembert type: role of rennet and *Penicillium caseicolum*, means of its control. Le Lait 643–644:397–417