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Enzyme and Microbial Technology 31 (2002) 533–542

ENZYME and MICROBIAL TECHNOLOGY

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Growth of *Geotrichum candidum* and *Penicillium camembertii* in liquid media in relation with the consumption of carbon and nitrogen sources and the release of ammonia and carbon dioxide

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Received 14 November 2001; received in revised form 23 April 2002; accepted 10 May 2002

Abstract

The growth of the filamentous fungi *Geotrichum candidum* and *Penicillium camembertii* on peptones as a sole carbon and nitrogen source, and in the presence of a second carbon source, lactic acid, have been compared. On both media, *G. candidum* exhibited similar kinetics until the end of growth, since it preferentially metabolized peptones as a carbon source, and lactic acid only for cellular maintenance during stationary phase. Growth of *G. candidum* was then nitrogen limited (peptones). On the contrary, fewer amino acids were convenient carbon sources for *P. camembertii*, resulting in a simultaneous consumption of peptones and lactic acid, and a cessation of growth due to the complete consumption of lactic acid. Morever, a lower amount of ammonia was produced since this metabolite resulted from the deamination of only carbon and nitrogen source amino acids. The production of ammonia induced an alkalinization of the broth (from 4.5 to 7.1). Morever, it was demonstrated that lactic acid consumption contributed also to media alkalinization (final pH 8.4). In absence of lactic acid, the medium contained a lower amount of available carbon, resulting in the absence of stationary state, deceleration growth phase was immediately followed by the death phase.

Keywords: Liquid cultures; *Geotrichum candidum*; *Penicillium camembertii*; Growth; Substrates consumption; Products release

1. Introduction

The ripening of a Camembert-cheese is under tight control of the growth of two fungal populations, *Penicillium camembertii* and *Geotrichum candidum* [1–3]. The growth of both microorganisms always results in an alkalinization of the curd [4,5] which plays an important role in the development of texture. It has been long assumed that an increase in the pH of the medium results from the uptake of lactic acid by *P. camembertii* and *G. candidum* [6].

Numerous works dealing with amino acid uptake [7–10] and catabolism [11,12] by *G. candidum* and *P. camembertii* are available. Some amino acids can be deaminated by *G. candidum* and *P. camembertii*, and the ammonia resulting from this process may be involved in the pH increase [13,14].

Thus, the monitoring of the fungal biomass and the control of the growth of both fungi appear to be key-factors in soft cheese manufacturing. For this purpose, it seems necessary to identify physiological and nutritional mechanisms by monitoring the consumption of nutrients and the release of metabolites. As concerns the kinetics of carbon and nitrogen consumption, only few results may be found in the available bibliography [15].

These mechanisms may be favourably studied on liquid media when compared to solid media. Indeed, in liquid cultures, the medium is homogeneous and no mass transfer limitation (diffusional) of substrates and metabolites occurred. Moreover, cellular concentration (and pH of the medium) may be continuously measured since dry cellular weight has been proven to vary in proportion to broth turbidity [16–18].

The main purpose of the present study was to examine the relations between growth and the kinetics of carbon and nitrogen sources consumption and ammonia and carbon dioxide production for *G. candidum* and *P. camembertii* in pure cultures. Two media were compared: a peptone-based medium, since it has been previously shown that some amino acids can be metabolized not only as nitrogen sources but also as carbon and energy source [19]; and a peptone-based medium supplemented with lactic acid, as a second carbon source.

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PII: S0141-0229(02)00149-7

2. Materials and methods

2.1. Microorganisms

The strains *G. candidum* Geo17 and *P. camembertii* LV2 (Rhodia Food, Dangé St Romain, France) were selected because they are of special interest to the dairy industry. Freeze-dried spores were stored at +7 °C.

2.2. Media

Two media were used throughout this work for both microorganisms; they contained:

- Peptones: 5 g l^{-1} of tryptic casein peptones and 5 g l^{-1} of pancreatic casein peptones (Biokar, Pantin, France).
- Inorganics phosphates (P_i): 3.40 g l^{-1} of KH_2PO_4, and 3.45 g l^{-1} of $NaH_2PO_4{\cdot}H_2O$ [20].
- A mineral solution containing the following ion concentrations (mg l^{-1}) [20]: ethylene diamine tetra acetate (EDTA), 585; Mg, 25; Fe, 20; Ca, 18; Zn, 4.5; Mo, 2; Cu, 1.3.

One medium was also supplemented with 10 g l^{-1} of L(+) sodium lactate prepared by neutralization of L(+) lactic acid (Acros, Geel, Belgium). The pH of both media was then adjusted to 4.6 with 6 M HCl.

2.3. Culture conditions

Batch fermentations were carried out in a 700 ml laboratory-made glass-blown fermentor, specially designed for the growth of filamentous fungi [18]. The fermentor was filled with 300 ml of culture medium (the fermentor with the equipment and the media were sterilized at 120 °C for 20 min).

During the culture, the temperature was maintained at 25 °C by circulation of thermostated water in a jacket. The batch fermentor was continuously aerated with a constant air-flow of 2.00 l h^{-1} (i.e. 6.66 l of air/litre of medium/h). This rate was obtained with the help of a mass flow controller GFC17 (Aalborg, New York, USA) and the broth was magnetically stirred at 850 rev min^{-1}, in order to minimize pellet formation. A rate of 2.00 l h^{-1} was chosen since the aeration is low during the ripening of a camembert-cheese and to minimize foam formation. Inoculation of the culture medium was carried out by an aseptic addition of spore suspension (corresponding to an initial density of $2–3 \times 10^8$ spores ml^{-1}). In order to obtain reliable time-lags, spores were left to rehydrate and germinate approximately 1 h in the sterilized medium at room temperature before inoculation.

The fermentor was equipped with a sterilizable pH electrode (Ingold, Paris, France), used for the on-line monitoring 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 [18]. Turbidity was calibrated from dry weight measurement of biomass at the end of culture. Carbon dioxide in the off-gas was also monitored on-line by an IR detector Rubis 3000 (Cosma, Igny, France) after desiccation in a column of calcium chloride. In order to detect any gas leak in the system, the flow rate at the outlet of the IR detector was measured by a Pelton-wheel flow-meter S110-3 (Mc Millan Co., Georgetown, TX, USA). The analogue pH, turbidity and carbon dioxide data were converted into eight bits words and stored in a four channel, 500 bytes data logger ENRE4 (Electrome, Bordeaux, France).

2.4. Analyses

Lactic acid was determined enzymatically: it was first oxidized in pyruvate in presence of lactate oxidase (LOD). Then, the produced hydrogen peroxide was measured after reduction to water by reaction with ABTS (AZINO-bis [3-ethylbenzthiazoline-6-Sulfonic acid]), in presence of peroxidase (POD) (all from Sigma Diagnostics, St Quentin Fallavier, France). A photometric detection was used.

Ammonium concentration was determined spectrophotometrically by the Nessler method [21]. Total nitrogen was determined in a 10401 digestion unit (Bioblock, Illkirch, France), after mineralization in a mixture of concentrated sulfuric acid and hydrogen peroxide [22], followed by a colorimetric measurement of the formed ammonium by the Nessler method [21].

The peptones concentration was obtained by subtraction of the ammonium concentration from the total nitrogen concentration.

Elementary compositions in carbon and nitrogen for peptones and cellular material were determined using a C, H, N analyser EA 1106 (Carlo Erba, Milano, Italy): a known mass of peptones or dried biomass was burnt in a flow of dry and carbon dioxide-free oxygen. C, H and N contents were measured by gas chromatography as carbon dioxide, water and nitrogen, respectively.

3. Results and discussion

3.1. Carbon and nitrogen balances

It was previously shown [18] that turbidity and total biomass concentration (dry weight calculated) were linearly correlated with an observed standard deviation of ±0.2 g l^{-1} (dry cellular weight: range 0–6 g l^{-1}).

The validation of the following discussion implied to draw the mass balances for the main elements constituting the system. For this purpose, elementary compositions of the cellular material of *G. candidum* and *P. camembertii* as well as that of the nitrogen source (peptones) are needed to establish the carbon and nitrogen balances, and are given in Table 1. It should be noticed that for each element, duplicates corresponded within ± 0.3%.

Table 1
Elementary composition of the cellular material of *Geotrichum candidum* and *Penicillium camembertii* and the mixture of peptones

	Carbon (%)	Nitrogen (%)	Ratio C/N
50% PCP + 50% TCP	40.26	11.90	3.38
G. candidum cellular material	38.40	6.56	5.85
P. camembertii cellular material	42.50	5.22	8.14

PCP: pancreatic casein peptone; TCP: tryptic casein peptone.

In Table 2, the mass of total nitrogen in the biomass and in the supernatant, as well as that of ammoniacal nitrogen in the supernatant, have been calculated from the concentrations of biomass and ammonia released displayed in Fig. 1a and b and the volume V_L of the medium remaining in the reactor at a given time. It has been assumed that the sole kinds of nitrogen in the medium were peptidic and ammoniacal, thus the amount of peptidic nitrogen was the difference between total and ammoniacal nitrogen in the supernatant. The right-hand column in Table 2 assesses the correctness of the global nitrogen balance, since the mean sum is 0.406 ± 0.007 g, namely a relative standard deviation of 1.7%.

For the three other experiments, similar results were obtained (details of calculation not shown): 0.412 ± 0.008 g for *G. candidum* on peptone-based medium, 0.418 ± 0.009 g and 0.441 ± 0.009 g for *P. camembertii* on lactic acid + peptones and peptone-based media, respectively.

In Table 3, the mass of total carbon in the biomass, in the supernatant and in the off-gas have been calculated from the data displayed in Fig. 1a and b, namely for *G. candidum* growing on lactic acid + peptones-based medium. It has been assumed that the sole kinds of carbon in the medium were that of lactic acid and peptones remaining in the reactor.

The right-hand column in Table 3 assesses the correctness of the global carbon balance, since the mean sum was 2.093 ± 0.079 g, namely a relative standard deviation of 3.8%. For the three other experiments, similar results were obtained (details of calculation not shown): 1.186 ± 0.093 g for *G. candidum* on peptones, 2.254 ± 0.037 and 1.443 ± 0.054 g for *P. camembertii* on lactic acid + peptones and peptone-based media, respectively. In a first approximation, the amount of CO_2 dissolved in the liquid phase was considered as negligible as previously shown [23]. This hypothesis was confirmed by the low relative standard deviations obtained for both microorganisms.

3.2. Growth, substrates consumption and metabolites production

3.2.1. Growth behaviour

Analysis of growth kinetics of *G. candidum* (Figs. 1a and 2a) and *P. camembertii* (Figs. 3a and 4a) showed that, in accord with previous results [3], for both media, lag phases were shorter for *G. candidum* (about 15 h) than *P. camembertii* (about 30 h). Then, after a short exponential phase (logarithmic plot not shown), a linear growth phase was observed, namely from about 20 h for *G. candidum* (Figs. 1a and 2a) and from 40 h for *P. camembertii* (Figs. 3a and 4a) on both media. The growth always remained linear for a short time (below 10 h). This linear rate occurred when oxygen supply became limiting as previously shown in similar conditions of aeration [24]. Since growth was limited by the low aeration (6.66 l of air/litre of medium/hour), the same linear rate was expected for each microorganism,

Table 2
Nitrogen mass balance during the growth of *Geotrichum candidum* on a lactic acid + peptone-based medium

Time (h)	Volume of reactor (l)	N collected[a]	N biomass[b]	N supernatant[c]	N NH_4^+[d]	N peptides[e]	N total[f,g]
0	0.278	0.000	0.000	0.418	0.026	0.392	0.418
16	0.268	0.013	0.002	0.380	0.015	0.365	0.395
21	0.262	0.022	0.010	0.365	0.026	0.339	0.397
26	0.255	0.033	0.027	0.346	0.042	0.304	0.406
35	0.247	0.044	0.039	0.322	0.133	0.189	0.405
41	0.241	0.053	0.042	0.308	0.144	0.164	0.403
47	0.234	0.062	0.046	0.295	0.166	0.129	0.403
61	0.227	0.073	0.047	0.286	0.172	0.114	0.406
70	0.221	0.082	0.047	0.278	0.170	0.108	0.407
93	0.215	0.092	0.051	0.270	0.165	0.105	0.413
111	0.215	0.092	0.052	0.269	0.163	0.106	0.413

[a] Total nitrogen (g) withdrawn by sampling (N biomass + N peptides + N ammonia).
[b] Nitrogen (g) in the biomass remaining in the reactor, N biomass $= 0.0656 \times x \times V_L$; x denotes the concentration of biomass in liquid phase ($g\,l^{-1}$) and the coefficient 0.0656 corresponding to the ratio of nitrogen in *G. candidum* cells (Table 1).
[c] Total nitrogen (g) in the supernatant.
[d] Nitrogen (g) in the ammonia released in the medium, $N(NH_4^+) = (14/18) \times [NH_4^+] \times V_L$ where $[NH_4^+]$ was the concentration of ammonia released in the medium ($g\,l^{-1}$).
[e] Nitrogen (g) remaining in the reactor as peptides, N peptides = N supernatant − $N(NH_4^+)$.
[f] Total nitrogen (g), N total = N collected + N biomass + $N(NH_4^+)$ + N peptides.
[g] Average = 0.406; standard deviation = 0.007.

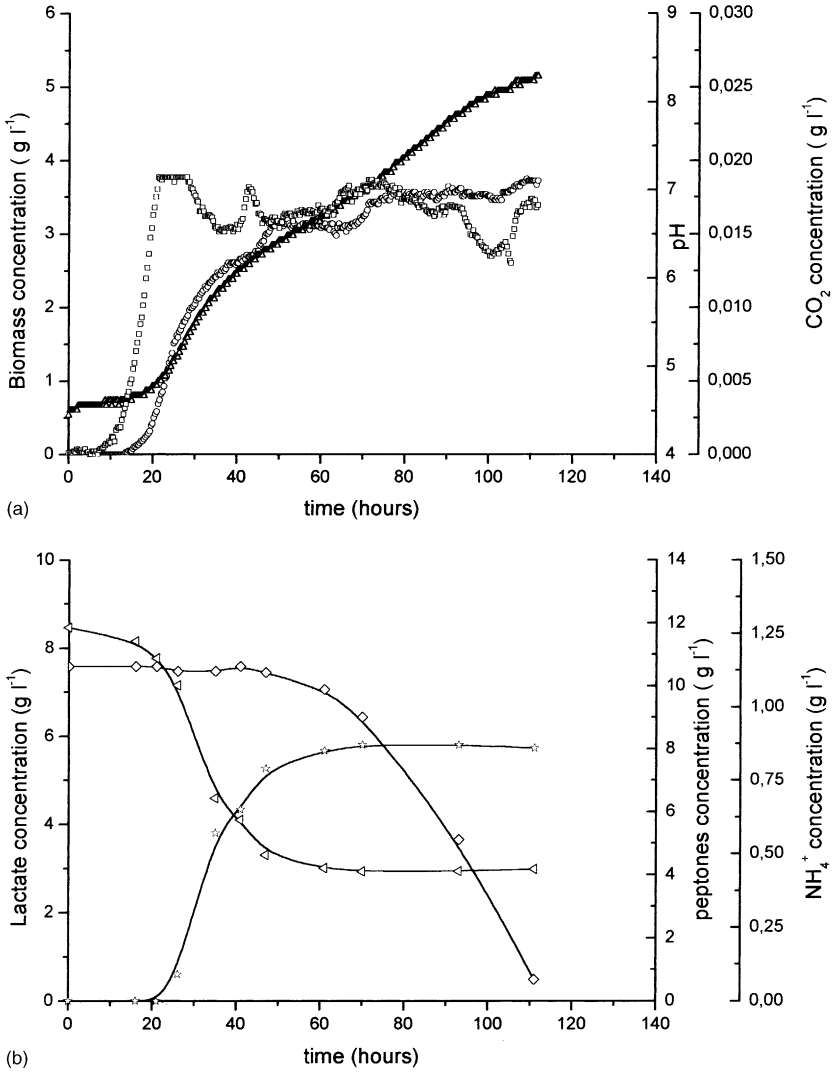

Fig. 1. Culture data of *Geotrichum candidum* growing on lactic acid + peptones based medium. On-line monitored parameters (a): (○) biomass concentration; (Δ) pH; (□) CO_2 concentration. Off-line parameters determined by periodical sampling (b): (◁) peptones concentration; (◇) lactic acid concentration; (☆) NH_4^+ concentration.

irrespective of the medium used. This assumption was experimentally supported since linear rates of 0.21 and 0.10 g l^{-1} h^{-1} were recorded for *G. candidum* and *P. camembertii*, respectively. At the end of the linear growth phase, the cellular concentration was approximately 1.5 g l^{-1} for both species growing on both media. During the deceleration phase, for both microorganisms, the decrease in the rates was more pronounced in absence of a second carbon source (lactic acid) in the medium. Moreover, in absence of lactic acid in the medium, lower maximum biomass concentrations were recorded for both species: 2.5 against 5.5 g l^{-1} for *P. camembertii* and 3.2 against 3.7 g l^{-1} for *G. candidum*. This was expected, due to the lower amount of available carbon.

After the deceleration phase, when the medium contained lactic acid, a stationary state was observed for both microorganisms for at least 35 h for *G. candidum* (Fig. 1a) and 15 h for *P. camembertii* (Fig. 3a) and no autolysis phase could be pointed out. On the contrary, the absence of lactic acid in the medium, namely a lower amount of available carbon, resulted for both microorganisms in a clear autolysis phase, almost immediately after the end of the deceleration growth phase (Figs. 2a and 4a).

3.2.2. Media alkalinization

In all experiments, as shown in Figs. 1a–4a, the growth of *P. camembertii* and *G. candidum* induced a pH increase, despite that media were buffered with species present in the media (phosphates, peptides, amino acids, ...), according to the previous results [25]. This alkalinization was the consequence of carbon sources metabolization as shown below (Section 3.2.4), and therefore for both microorganisms the pH increase began simultaneously with growth.

Irrespective of the microorganism used, the same final pH was obtained on a given medium: 7.1 ± 0.1 and 8.4 ± 0.1 in

Table 3
Carbon mass balance during the growth of *Geotrichum candidum* on a lactic acid + peptone-based medium

Time (h)	Volume of reactor (l)	C collected[a]	C lactic acid[b]	C peptides[c]	C biomass[d]	C CO_2[e]	C total[f,g]
0	0.278	0.000	0.851	1.324	0.000	0.000	2.175
16	0.268	0.071	0.823	1.233	0.011	0.012	2.150
21	0.262	0.117	0.805	1.147	0.059	0.047	2.175
26	0.255	0.175	0.770	1.026	0.156	0.097	2.224
35	0.247	0.225	0.748	0.638	0.230	0.181	2.022
41	0.241	0.267	0.738	0.555	0.246	0.232	2.038
47	0.234	0.304	0.706	0.436	0.271	0.287	2.004
61	0.227	0.346	0.648	0.384	0.274	0.410	2.062
70	0.221	0.380	0.575	0.365	0.275	0.496	2.091
93	0.215	0.409	0.317	0.355	0.298	0.713	2.092
111	0.215	0.409	0.042	0.360	0.307	0.867	1.985

[a] Total carbon (g) withdrawn by sampling (C biomass + C lactic acid + C peptides).
[b] Carbon (g) in the lactic acid remaining in the reactor, C lactic acid $= 0.4 \times [HL] \times V_L$; [HL] denotes the lactic acid concentration remaining in the medium ($g\,l^{-1}$) and the coefficient 0.4 corresponding to the ratio of carbon in lactic acid (Table 1).
[c] Carbon (g) in the peptides remaining in the supernatant with C peptides $= 3 \times$ N peptides (Table 1) and N peptides are given in the nitrogen mass balance (Table 2).
[d] Carbon (g) in the biomass remaining in the reactor, C biomass $= 0.384 \times x \times V_L$ with the coefficient 0.384 corresponding to the ratio of carbon in *G. candidum* cells (Table 1).
[e] Carbon (g) in the total CO_2 collected in the off-gas, C $(CO_2) = (12/44) \times F \times \int_0^t C(t)\,dt$; F denotes the air flow-rate ($l\,h^{-1}$) and $C(t)$ represents the concentration of carbon dioxide in the off gas ($g\,l^{-1}$).
[f] Total carbon (g), C total = C collected + C lactic acid + C peptides + C biomass + C(CO_2).
[g] Average = 2.093; standard deviation = 0.079.

absence and presence of lactic acid in the medium, respectively. This clearly indicated that the pH was not the factor limiting growth, at least for the peptone-based medium.

3.2.3. Substrates consumption

The sole nitrogen source in the media used was peptones. As expected, their consumption always started simultaneously with growth (Figs. 1–4). As previously shown [19], most of the amino acids could be metabolized as nitrogen sources by both species, and some of them were also convenient carbon and energy sources. According to previous results [19], this was especially the case for *G. candidum*, which therefore would be expected to metabolize more amino acids than *P. camembertii* on the peptone-based medium. Experimental data confirmed this result since 79.9% of peptones were metabolized by *G. candidum* against 64.4% by *P. camembertii* (Figs. 2b and 4b).

When a second carbon source, lactic acid, was added to the medium, both microorganisms exhibited different behaviours. Lactic acid consumption started simultaneously with the growth of *P. camembertii* (Fig. 3a and b), while for *G. candidum*, the consumption of lactic acid only became significant at the beginning of the stationary phase, corresponding to the cessation of peptones consumption (Fig. 1a and b). *G. candidum* metabolized 64.8% of the peptones against 44.9% by *P. camembertii*.

From this, the most probable explanation is a lactic acid consumption as an energy source for cell maintenance during stationary state. At the end of this phase, lactic acid was almost completely consumed (Fig. 1a and b). the measurement of the respiratory quotient RQ, may be informative to interpret the lactic acid consumption by *G. candidum* and to confirm this assumption. This will be done during subsequent runs.

Fairly close maximum peptones consumption rates (0.36 and $0.33\,g\,l^{-1}\,h^{-1}$) were recorded for *G. candidum* growing on the peptone-based medium supplemented or not with lactic acid, respectively (Figs. 1b and 2b). This result agrees with the fact that the consumption of lactic acid by *G. candidum* occurred only at the end of growth. Despite the presence of lactic acid in the medium, peptones remained the preferred carbon source.

For *P. camembertii* growing on a peptone-based medium, a maximum peptones consumption rate of $0.22\,g\,l^{-1}\,h^{-1}$was recorded. This value decreased sharply in presence of lactic acid in the medium ($0.05\,g\,l^{-1}\,h^{-1}$), since contrarily to *G. candidum*, both lactic acid and peptones were simultaneously metabolized as carbon sources.

3.2.4. Ammonia production

Ammonia production resulted from the deamination of amino acids [11,12]. Therefore, the release of ammonia would be expected to start simultaneously with peptones consumption, and consequently with growth. Examination of Figs. 1–4 confirmed this assumption.

Ammonia release could be explained by a lower C/N ratio (in the range 1.3–5.15) for the amino acids metabolizable as carbon and nitrogen sources, than those found in the cells: 5.9 and 8.1 for *G. candidum* and *P. camembertii*, respectively (Table 1). Therefore, an excess of nitrogen was available resulting in amino acids deamination and then ammonia was released.

For both species growing on the peptone-based medium, the ratio of ammonia produced and peptones consumed

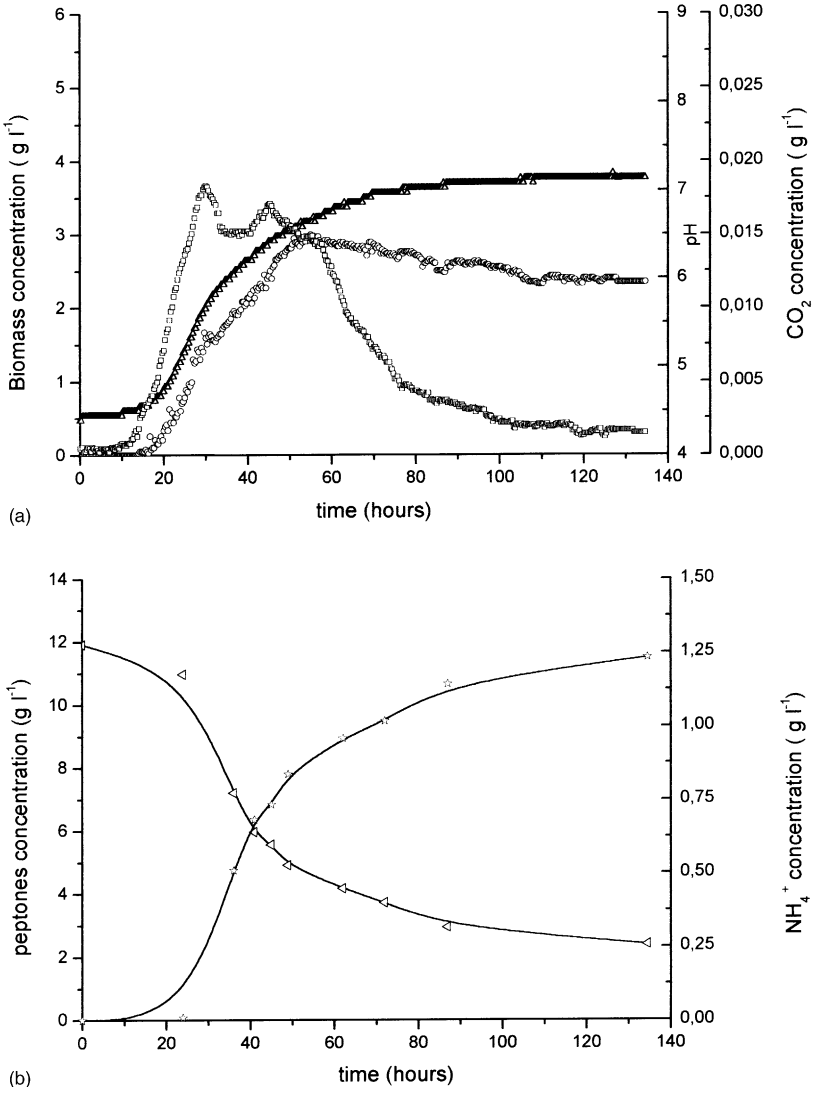

Fig. 2. Culture data of *Geotrichum candidum* growing on peptone-based medium. On-line monitored parameters (a): (○) biomass concentration; (△) pH; (□) CO_2 concentration. Off-line parameters determined by periodical sampling (b): (◁) peptones concentration; (☆) NH_4^+ concentration.

were identical: 0.14 for *G. candidum* and 0.13 for *P. camembertii*. This result indicated that when amino acids were the sole source of carbon and nitrogen, the same stoichiometry of deamination was observed for both microorganisms. On lactic acid + peptone-based medium, this ratio remained the same for *G. candidum* (0.13), since lactic acid consumption became noticeable only after the end of growth. However for *P. camembertii*, this ratio decreased (0.11), most likely since both carbon substrates (peptones and lactic acid) were simultaneously metabolized by *P. camembertii*. This observation confirmed the linking between peptones consumption and ammonia release.

On both media, close maximum rates of ammonia release were recorded for *G. candidum* (about 0.045 $g\,l^{-1}\,h^{-1}$). A similar maximum rate was recorded for *P. camembertii* in absence of lactic acid (0.044 $g\,l^{-1}\,h^{-1}$), while it decreased strongly in presence of lactic acid (0.006 $g\,l^{-1}\,h^{-1}$), as also observed and discussed for the peptones consumption (Section 3.2.3). Then, the similar values recorded for all experiments for the ratio of the maximum rates of ammonia production on peptones consumption were expected (0.13 ± 0.02).

For both microorganisms growing on a peptone-based medium, the deceleration growth phase was immediately followed by a death phase and no stationary state was observed (Figs. 2a and 4a). Examination of the possible factors involved in the growth limitation showed that:

- Phosphate and trace elements could not be suspected since they were available in a large excess; the higher maximum biomass concentrations achieved in presence of lactic acid in the medium for both species confirmed this assumption (Figs. 1a–4a).

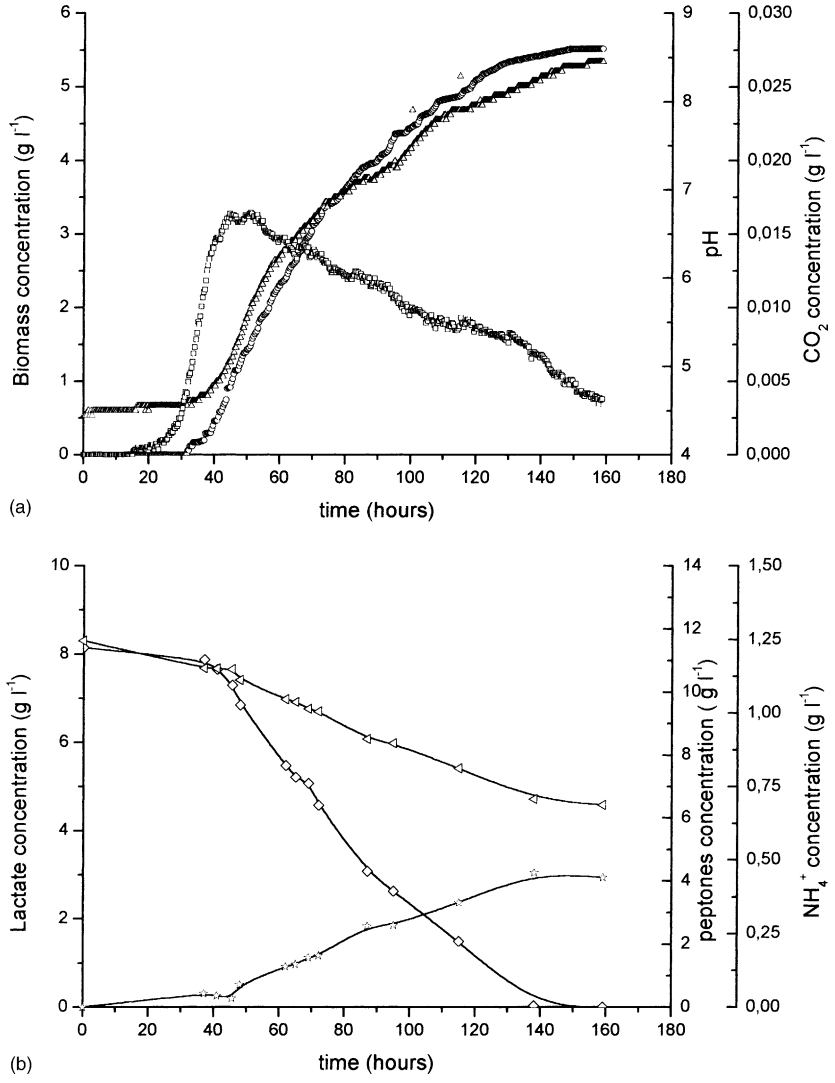

Fig. 3. Culture data of *Penicillium camembertii* growing on lactic acid + peptones based medium. On-line monitored parameters (a): (○) biomass concentration; (△) pH; (□) CO_2 concentration. Off-line parameters determined by periodical sampling (b): (◁) peptones concentration; (◇) lactic acid concentration; (☆) NH_4^+ concentration.

- As has been previously demonstrated, group B Vitamins, purine and pyrimidine base supply were not necessary for growth of *G. candidum* and *P. camembertii* [24].
- An inhibition due to the amount of ammonia released cannot be suspected since its production continued during autolysis.
- The final pH (about 7) was not involved since on peptone + lactic acid-based medium, growth ceased at a higher pH (above 8).

Therefore, the sole factor which could be suspected was peptones remaining in the medium.

On peptone + lactic acid-based medium, peptones were also most probably the limiting factor, since their consumption ceased simultaneously with growth of *G. candidum* (Fig. 1a and b). Then, the apparition of the stationary phase probably indicated a nitrogen limitation (peptones). This result appeared tedious to prove, and may be pointed out in a more simple way, by cultivation of *G. candidum* on synthetic media, containing only one amino acid as a nitrogen source: such a work is in progress in the laboratory.

On the contrary, for *P. camembertii*, peptones were obviously not the limiting growth factor, since at the end of growth their concentration was lower than those observed in absence of lactic acid, 52.2 and 79.5% on both media, respectively. Moreover, lactic acid was completely exhausted at the end of growth (Fig. 3a and b), and therefore caused cessation of growth.

For both microorganisms, the alkalinization of the medium from pH 4.5 ± 0.1 to 7.1 ± 0.1 on a peptone-based medium was the consequence of ammonia production. But in presence of lactic acid, further increase (8.4 ± 0.1) observed during the growth of *G. candidum* resulted from the

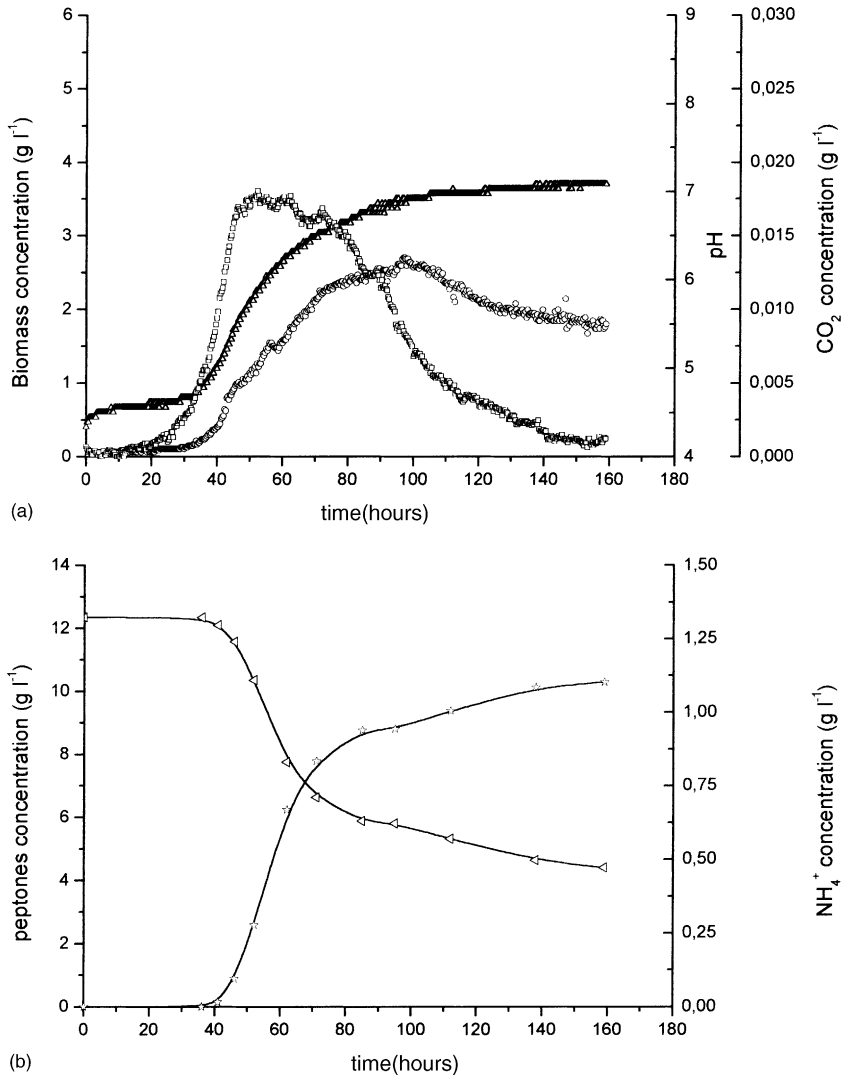

Fig. 4. Culture data of *Penicillium camembertii* growing on peptone-based medium. on-line monitored parameters (a): (○) biomass concentration; (△) pH; (□) CO_2 concentration. Off-line parameters determined by periodical sampling (b): (◁) peptones concentration; (☆) NH_4^+ concentration.

uptake of lactic acid. For *P. camembertii* the alkalinization from pH 4.5 ± 0.1 to 8.4 ± 0.1 resulted from the simultaneously uptake of lactic acid and release of ammonia.

3.2.5. Carbon dioxide emission

It should be pointed out that the liquid phase was closed to mass exchange with the surrounding (batch process). In contrast, the gaseous phase, which acts as a continuous reactor, was open to mass exchange.

As previously shown [23], the mass of carbon dioxide produced per litre of medium, could be deduced by integration of the measured CO_2 concentration in the gaseous phase. Therefore, the CO_2 concentration has been compared to the growth rate and both maxima have been found to be nearly concomitant for the four experiments, since the carbon dioxide emission resulted from aerobic respiration [26]. For each microorganism, the same maximum of CO_2 concentration was observed irrespective of the medium used (0.019 and 0.017 g l^{-1} for *G. candidum* and *P. camembertii*, respectively) (Figs. 1a–4a).

For *P. camembertii*, the CO_2 concentration in the off-gas decreased sharply during the deceleration phase and this decrease continued until the end of culture (below 0.005 g l^{-1}) (Figs. 3a and 4a). This remark led to the conclusion that the main part of the released CO_2 resulted from biosynthesis. Only a few part resulted from cellular maintenance.

For *G. candidum*, the CO_2 concentration recorded at the end of growth on peptone-based medium (0.014 g l^{-1}) (Fig. 2a), corresponded to that observed on peptone + lactic acid medium (0.016 g l^{-1}) (Fig. 1a). On the other hand, during the stationary phase, observed only during the culture on medium containing lactic acid, the energy required for cellular maintenance was important. Indeed, carbon dioxide emission was important (Fig. 1a), according to previous results [23], and higher than that needed for *P. camembertii* cell maintenance.

For both microorganisms, the death phase observed in absence of lactic acid in the medium, indicated a high decrease of the number of viable cells, and thus a decrease in the CO_2 emission (Figs. 2a and 4a).

4. Conclusion

On both media, *G. candidum* exhibited faster growth kinetics than *P. camembertii*, according to previous results [3]. Peptones were used as a nitrogen source, but also as a carbon source, since it has been previously shown that most of the amino acids could be metabolized as nitrogen source and some as a carbon source as well [19]. Since peptones were the sole nitrogen source, their consumption began simultaneously with growth, and therefore also the resulting ammonia production. This component resulted from the deamination of only C and N source amino acids. This deamination was caused by an excess of available nitrogen, due to the lower C/N ratio of these amino acids if compared to those found in both microorganisms.

More amino acids were metabolized as C and N sources by *G. candidum* [19]; the consequence was that more peptones (the limiting substrate) were consumed on the peptone-based medium, resulting in a higher maximum biomass concentration and a higher amount of ammonia released, if compared to *P. cammberti*. On this medium, for both species, growth was followed by a clear death phase, since too low amount of metabolizable carbon remained in the medium.

Even in presence of a second carbon source in the medium, lactic acid, *G. candidum* preferred peptones as a carbon source, and lactic acid consumption became significant only at the end of growth, when a nitrogen limitation appeared. The presence of lactic acid did not result in a really significant increase of the maximum biomass. Lactic acid was mainly used for cellular maintenance and a long stationary state was observed. On the contrary, *P. camembertii* metabolized lactic acid and peptones simultaneously. Therefore, in presence of lactic acid, its growth resulted in a higher maximum biomass and a lower amount of peptones consumed, leading to a lactic acid (carbon) limitation and a lower amount of ammonia produced.

A pH increase (7.1) was observed on peptones due to the ammonia release, which was more pronounced in presence of a second carbon source, lactic acid, in the medium (8.4). Aerobic respiration of microorganisms resulted in carbon dioxide emission. Therefore, the maximum CO_2 concentration was expected to be concomitant to the maximum growth rate; this assumption has been experimentally supported. *P. camembertii* biosynthesis resulted in a higher amount of CO_2 produced if compared to *G. candidum*, While it was the opposite during cellular maintenance.

An unstructured model has been recently validated for the reconstruction of biomass history from carbon dioxide emission during batch cultures of *G candidum* [23].

In a similar way, the above results may be helpful in view of the reconstruction of biomass history from the kinetics of carbon and nitrogen sources consumption, ammonia production and proton transfer (deduced from pH measurement by means of the measurement of the buffer capacity of the media used). It should be remembered that fungal biomass monitoring is one of the key factors in soft cheese manufacturing. The above results can be also helpful for the determination of the contribution of both lactic acid consumption and ammonia production in the amount of protons exchanged. Both contributions are the main factors involved in curd neutralization, one of the key factors of the texturization of soft Camembert-type cheeses. The corresponding works are in progress in the laboratory.

References

[1] Fox PF, Law J, Mc Sweeney PLH, Wallace J. Biochemistry of cheese ripening. In: Fox PF, editor. Cheese: chemistry, physics and microbiology, major cheese groups, vol. 2. 2nd ed. Glasgow: Chapman & Hall, 1993. p. 389–438.

[2] Gripon J-C. Mould-ripened cheeses. In: Fox PF, editor. Cheese: chemistry, physics and microbiology, major cheese groups, vol. 2. 2nd ed. Glasgow: Chapman & Hall, 1993. p. 111–36.

[3] Amrane A, Prigent Y. Comparison of growth parameters for *Geotrichum candidum* Geo 17 and *Penicillium camemberti* LV2 in solid and liquid cultures. Le Lait 1997;77:641–8.

[4] Fox PF, Lucey JA, Cogan TM. Glycolysis and related reactions during cheese manufacture and ripening. Crit Rev Food Sci Nutr 1990;29:237–53.

[5] Engel E, Tournier C, Salles C, Le Quéré JL. Evolution of the composition of a selected bitter camembert cheese during ripening: release and migration of taste-active compounds. J Agric Food Chem 2001;49:2940–7.

[6] Lenoir J. Degradation of protides during cheese maturation. Compt Rend Acad Agric 1962;48:392–9.

[7] McEvoy JJ, Murray JR. Amino acid transport in germinated arthrospores of *Geotrichum candidum*. Arch Mikrobiol 1972;86:101–10.

[8] Duran A, Uruburu F, Villanueva JR. Morphogenetica and nutritional studies of *Geotrichum lactis* cells. Arch Mikrobiol 1973;88:245–56.

[9] Leuschner RG, Heidel M, Hammes WP. Histamine and tyramine degradation by food fermenting microorganisms. Int J Microbiol 1998;39:1–10.

[10] Bonnarme P, Arfi K, Dury C, Helinck S, Yvon M, Spinnler HE. Sulfur compound production by *Geotrichum candidum* from L-methionine: importance of the transamination step. FEMS Microbiol Lett 2001;205:247–52.

[11] Mc Sweeney PLH, Sousa MJ. Biochemical pathways for the production of flavour compounds in cheeses during ripening: a review. Le Lait 2000;80:293–324.

[12] Yvon M, Rijnen L. Cheese flavour formation by amino acid catabolism. Int Dairy J 2001;11:185–201.

[13] Karahadian C, Lindsay RC. Integrated roles of lactate, ammonia and calcium in texture development of mould surface ripened cheese. J Dairy Sci 1987;70:909–18.

[14] Lucey JA, Fox PF. Importance of calcium and phosphate in cheese manufacture: a review. J Dairy Sci 1993;76:1714–24.

[15] Mc Intyre M, Berry DR, Mc Neil B. Response of *Penicillium chrysogenum* to oxygen starvation in glucose and nitrogen limited chemostat cultures. Enzyme Microb Technol 1999;25:447–54.

[16] Thatipamala R, Rohani S, Hill G. On-line monitoring of a wide range of biomass concentrations based on a new equation using a spectrophotometer: process control applications. J Biotechnol 1994;38:33–42.
[17] Castro GR, Andribet EP, Ducrey LM, Garro OA, Sineriz F. Modelling and operation of a turbidity-meter for on-line monitoring of microbial growth in fermenters. Process Biochem 1995;30:767–72.
[18] Amrane A, Prigent Y. A new turbidimetric device for on-line monitoring of growth of filamentous microorganisms. J Microbiol Methods 1998;33:37–43.
[19] Plihon F, Le Doujet S, Amrane A, Prigent Y. Effects of amino acids on the growth of submerged cultures of *Geotrichum candidum*. J Food Mycol 1998;1:203–10.
[20] Trinci APJ. A kinetic study of the growth of *Aspergillus nidulans* and other fungi. J Gen Microbiol 1969;57:11–24.
[21] Rodier J. Ammoniacal nitrogen analysis. In: Water analysis, vol. 1. Paris: Dunod, 1975. p. 116–20 [in French].
[22] Hach CC, Bowden BK, Kopelove AB, Brayton SV. More powerful peroxide Kjeldahl digestion method. J Assoc Off Anal Chem 1987;70:783–7.
[23] Couriol C, Amrane A, Prigent Y. A new model for the reconstruction of biomass history from carbon dioxide emission during a batch cultivation of *Geotrichum candidum*. J Biosci Bioeng 2001;91:170–5.
[24] Amrane A, Plihon F, Prigent Y. Kinetics of growth and medium de-acidification for *Geotrichum candidum* and *Penicillium camemberti* cultivated on complex liquid media. World J Microbiol Biotechnol 1999;15:489–91.
[25] Amrane A, Prigent Y. Growth of *Geotrichum candidum* and *Penicillium camemberti* cultivated on liquid media correlated with ammonia and methanethiol emission. Acta Biotechnol 2001;21: 283–90.
[26] Roger B, Desobry S, Hardy J. Respiration of *Penicillium camemberti* during ripening and cold storage of semi-soft cheese. Le Lait 1998;78:241–50.