Annals of Microbiology, **55 (4)** 273-278 (2005)

Effect of process parameters on the production of lactic acid bacteria in batch fermentation

Emanuele ZANNINI*, Sara SANTARELLI, Andrea OSIMANI, Luana DELL'AQUILA, Francesca CLEMENTI

Dipartimento di Scienze degli Alimenti, Università Politecnica delle Marche, Via Brecce Bianche 60131 Ancona, Italy

Abstract - The effect of aeration and type of neutralising agent on the growth of lactic acid bacteria, isolated from a typical Italian cheese, was investigated in laboratory fermenters, with the aim of defining process conditions for the production of autochthonous cultures to be used as starters in traditional cheese-making. Batch fermentation trials were carried out using six different bacterial species belonging to the genera *Lactobacillus*, *Lactococcus* and *Leuconostoc* on lactose or glucose based substrates, with sodium-hydroxide (NaOH) or ammonium-hydroxide (NH_4OH) as neutralisers and with or without micro-aeration 0.15 vvm. In most cases, optimal conditions for growth were observed in the presence of both ammonium and air supply. The specific growth rate (μ_{max}) values required for growth ranged from 0,16 to 0.39 h^{-1}.

Key words: lactic acid bacteria, autochthonous starter cultures, batch fermentation.

INTRODUCTION

Lactic acid bacteria (LAB) are widely used in food fermentation because of their ability to improve flavour, texture and safety of perishable raw materials such as milk, meat and vegetables (Caplice and Fitzgerald, 1999). The industrial exploitation of LAB have also attracted considerable attention due to their potential for production of lactic acid (Bibal *et al*., 1989) and bacteriocins, which can act as natural food preservatives against spoilage and pathogenic micro-organisms (Klaenhammer, 1993; Nettles and Berafoot, 1993; Messens and De Vuyst, 2002).

Starter cultures used in cheese-making can be classified into two groups: traditional mixed-strain starters and defined strain starters obtainable from commercial suppliers (Parente *et al*., 1997). In traditional Italian cheese-making, many sheep and goat cheeses are produced using raw milk without the addition of any starter culture in order to preserve the original microbial population responsible for the peculiar sensory characteristics. On the other hand, for these products it appears of utmost importance to find the right balance between traditional manufacturing conditions and hygiene demands (Cenci Goga *et al*., 1995). This goal can be accomplished through the use of autochthonous LAB cultures which can be isolated from traditional cheeses, reproduced in small-scale fermentations, and added to thermised or pasteurised milk, in place of the industrial starters (Coppola *et al.,* 1990; Cenci Goga *et al*., 1995; Clementi *et al*., 1998).

*Corresponding Author. Phone: +39 0712204985;
Fax: +39 0712204988; E-mail: e.zannini@univpm.it

The production of LAB in batch fermentations is considered as a well known technology, but the effects of some process parameters would not appeared to be fully elucidated. In particular, it is recognised for a long time that pH, temperature, medium composition, oxygen concentration and type of the neutraliser exert a strong effect on growth rate and biomass yield of LAB (Gilliland, 1985). The LAB group includes both mesophilic and thermophilic species. Consequently, fermentation temperature for biomass and lactic acid production vary from 26 to 42 °C, (Whitehead *et al.* 1993; Parente *et al.* 1997; Bury *et al.,* 1998) although most processes are carried out at 35-37 °C (Mulligan and Safi, 1991). The choice of medium is of fundamental importance as it influences down stream processes (biomass harvesting) and process costs. Tryptone-yeast extract-lactose media are largely used in the production of concentrated lactic acid bacteria cultures, due to low cost and absence of interference with the cell harvesting step (Bergere and Hermier, 1968; Peebles *et al*., 1969; Stahouder *et al*., 1969). In relation to aeration, lactic acid bacteria are known to be micro-aerophilic and do not tolerate high oxygen level. Accordingly, the addition of catalase (Teuber, 1993) or reducing compounds as ascorbic acid and ferrous sulphate (Whitehead *et al*., 1993) were proposed to remove the hydrogen peroxide produced by many strains and to maintain a low oxidation-reduction potential, respectively. In contrast, the supply of different amounts of oxygen was proven to increase lactic acid production by *Lactobacillus helveticus* (Tango and Ghaly, 1999) and the growth of *Leuconostoc mesenteroides* (Plihon *et al*., 1995; Champagne and Gardner, 2002) *Lactobacillus plantarum* (Yousten *et al*., 1975) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Schiraldi *et al*., 2003). Finally, since lactic

acid exerts an end-product inhibitory effect on the growth and survival of LAB (Bibal *et al*., 1989), different neutralising agents are used during fermentation (Mulligan and Safi, 1991; Rincòn *et al*., 1993; Champagne and Gardner, 2002) and found to exert either a positive or a negative influence on LAB growth (Peebles *et al*., 1969).

The object of the present work was to investigate the effect of aeration and type of neutralising agent assessed in laboratory fermenters on the growth of six species of lactic acid bacteria isolated from Italian Pecorino cheese, with the aim of defining optimal process conditions for the production of autochthonous starter cultures for use in traditional cheese-making.

MATERIALS AND METHODS

Microorganisms. The strains *Lactobacillus casei* DiSA 27, *Lactococcus lactis* subsp. *cremoris* DiSA 6, *Lactobacillus plantarum* DiSA 33, *Lactobacillus brevis* DiSA 49, *Lactobacillus pentosus* DiSA 11 and *Leuconostoc* spp. DiSA 47, belonging to the Collection of lactic acid bacteria at the *Dipartimento di Scienze degli Alimenti* (DiSA) of our University were used. All the strains had been previously isolated from milk, curd and/or cheese samples withdrawn during manufacturing of a typical Italian Pecorino cheese (Zannini *et al*., unpublished results).

Equipment. A rotary shaker (model IKA-Labortechnik KS 250, Janke and Kunkel, Staufen, Germany) was used for precultures.

Fermentations were performed in 2 L (1.5 L max working volume) stirred tank BIOSTAT-B bioreactors (B-Braun Biotech International, Melsungen, Germany), comprising of a jacketed borosilicate glass vessel equipped with two six-bladed Rushton turbines and four vertical baffles. Depending on the experimental design, an air compressor (Fini-Zola Predosa, Bologna, Italy) was used to supply air to the bioreactors through a 0.20 µm filter (Cat. No. 000468; Midisart 2000, Sartorius Goettingen, Germany). Continuous control of pH was achieved using a peristaltic pump supplying a 5 N NaOH or NH_4OH as neutralising agents.

The Micro-MFCS software (B-Braun Biotech International) for Windows NT was used for monitoring and controlling process parameters during fermentation.

Growth media. Pre-cultures and fermentations were carried out using a substrate containing (%, w/v): 0.5, tryptone (casein peptone, Oxoid Basingstoke, UK); 0.5, yeast extract (Oxoid); 0.2, $(NH_4)_2C_6H_6O_7$; 0.2, K_2HPO_4; 0.02, $MgSO_4$; 0.005, $MnSO_4$ and 7.5, lactose. Lactose was replaced by the same concentration of glucose source for cultivation of the strain *Lactobacillus brevis* DiSA 49 which is unable to utilise lactose (Kandler and Weiss 1986).

The pH of pre-culture media was adjusted to 7.0 with 1 M NaOH prior to sterilisation. Both pre-culture and fermentation media were sterilised at 121 °C for 15 min.

All chemicals were analytical grade commercial products.

Culture conditions. Stock cultures were maintained at –80 °C in MRS broth supplemented with 25% (v/v) sterile glycerol. One hundred µL of the stock cultures were plated in MRS agar (Oxoid). After incubation for 24 h at 37 °C, a loop of the revitalised culture was utilised to inoculate 10 mL of MRS broth, which was incubated at 37 °C overnight. Six mL of this culture suspension were then inoculated into Erlenmeyer flasks filled at two thirds of their volume with 60 mL of the pre-culture medium. After incubation at 37 °C for 12-18 h and 140 rpm, the pre-culture broth was used as an inoculum in a further pre-culture step carried out under the same conditions. An aliquot of 130 mL of the above cited pre-culture broth was used to inoculate the 1.3 L of fermentation medium in the bioreactor.

In all fermentation trials, temperature was maintained at 35 ± 1 °C and agitation speed was set at 100 rpm, whereas two different conditions of aeration (0.00 or 0.15 vvm) were compared.

With the aim of verifying the effect of the neutralising agent on growth, each strain studied was comparatively cultivated using either NaOH or NH_4OH, with the exception of the strains *Lactobacillus lactis* subsp. *cremoris* DiSA 6 and *Leuconostoc* spp. DiSA 47 which were cultured in broths neutralised by NaOH only.

Analytical methods. Twenty mL aliquots of the culture broths were aseptically withdrawn from the bioreactor at intervals during fermentations and utilised for the assessment of growth and lactose consumption, as described below.

Growth measurement. Viable lactic acid bacterial cells in the culture broth were assessed at 0, 6, 10, 12, 24 and 48 h of fermentations, except for the strains *Lactobacillus brevis* DiSA 49 and *Leuconostoc* spp. DiSA 47 which were monitored only at 0, 24 and 48 h. For all the microorganisms under study, viable counts were carried out on MRS agar with incubation at 37 °C for 48 h.

Dry weight (DW) was measured at 24 and 48 h after centrifugation of the culture broth samples at 4000 *x g* for 15 min followed by two washings with 0.95% (w/v) NaCl solution and final drying at 105 ± 1 °C for 24 h .

Lactose determination. The culture broth supernatants obtained by centrifugation as described above were subjected to a spectrophotometric assay using the Lactose/D-galactose enzymatic kit (Cat. No. 0176303, Boehringer Mannheim/R-Biopharm, AG, Darmstadt, Germany).

All the analytical determinations were carried out in duplicate.

RESULTS AND DISCUSSION

The effect of neutralising agent

The monitoring of neutraliser addition, automatically reported in a graph by the Micro-MFCS software, allowed the growth kinetics to be indirectly evaluated during fermentation since the quantity neutralising agent used was directly correlated to the viable count data, as shown in Fig. 1. From the Micro-MFCS graphs obtained during the different fermentation processes (data not shown), the lag phase of all the strains under study was comprised between 3 and 6 h. This was in accordance with the lag-time detected by Tango and Ghaly (1999) in fermentations carried out with *Lactobacillus helveticus* ATTC 15009 on pasteurised cheese whey, using a 10% (w/v) inoculum concentration, as in this work. In addition, the kinetics of neutraliser consumption showed that the log phase was completed within 24-30 h by most

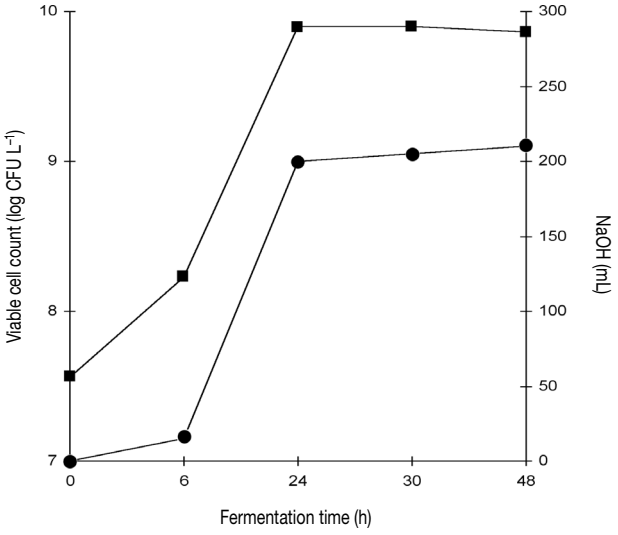

FIG. 1 – Viable cell counts (■) and the consumption of NaOH supply (●) during fermentation with *Lactobacillus casei* in the presence of air injected at rate of 0.15 vvm .

of the strains tested, with the exception of *Lactobacillus plantarum* DiSA 33 which was characterised by a significantly longer log phase of 45 h.

The data concerning the growth of LAB at 24 and 48 h under the different conditions tested are reported in Table 1.

Viable counts and dry weights suggest that the optimal conditions for growth of the lactic acid bacteria were achieved using NH_4OH as a neutralising agent and an air supply, at 0.15 vvm. With respect to the type of neutralising agent, NH_4OH was the neutralising agent of choice for the strains *Lactobacillus plantarum* DiSA 33 and *Lactobacillus brevis* DiSA 49 since they both reached the highest values of viable count (1 x 10^{10} and 1.2 x 10^{10} CFU mL^{-1} respectively after 48 h) in fermentations carried out with this neutraliser. The neutralising agent had small effect on the growth of strain *Lactobacillus pentosus* DiSA 11 which also reached the highest value of viable count at 24 h in the presence of NH_4OH, and the same count with NaOH, after 48 h. Likewise, the neutraliser did not significantly effect the growth of *Lactobacillus casei* DiSA 27. Though this strain seemed to prefer NaOH, attaining a count of 1.6 x 10^{10} CFU mL^{-1} after 24 h, a very similar result (1.0 x 10^{10} CFU mL^{-1}) was observed in the presence of NH_4OH, under the same conditions of aeration. Nothing can be noticed about the influence of neutralising agent on *Lactococcus lactis* subsp. *cremoris*, and *Leuconostoc* ssp. since for these strains the effect of this parameter was not taken into consideration. However, evidences are found in literature about the use of NaOH as neutraliser of choice for these species of LAB (Parente *et al*., 1994; Dols *et al*., 1997; Cheigh *et al*., 2002; Mataragas *et al*., 2004).

Aeration condition

Air supply into the bioreactor clearly had a positive effect on the growth of *Lactobacillus plantarum* DiSA 33, *Lactobacil-*

TABLE 1 – Details of fermentations carried out under the different process conditions

Bacterial isolate	Neutralising agent	Trial*	Viable counts (CFU mL^{-1})		Dry weight (g L^{-1})		μ_{max} (h^{-1})
			24 h	48 h	24 h	48 h	
Lactobacillus plantarum DiSA 33	NaOH	A	1.6 x 10^9	3.9 x 10^9	nd	nd	nd
		B	2.8 x 10^9	6.1 x 10^9	nd	nd	0.300
Lactobacillus plantarum DiSA 33	$(NH_4)OH$	A	1.5 x 10^8	1.8 x 10^9	nd	nd	nd
		B	2.7 x 10^9	1.0 x 10^{10}	nd	nd	0.210
Lactobacillus casei DiSA 27	NaOH	A	8.0 x 10^9	7.3 x 10^9	nd	nd	0.226
		B	1.6 x 10^{10}	3.0 x 10^9	nd	nd	0.256
Lactobacillus casei DiSA 27	$(NH_4)OH$	A	8.5 x 10^9	4.5 x 10^9	nd	nd	0.243
		B	1.0 x 10^{10}	7.0 x 10^9	nd	nd	0.250
Lactobacillus pentosus DiSA 11	NaOH	A	1.0 x 10^9	4.7 x 10^9	1.36	1.80	0.208
		B	2.8 x 10^9	1.4 x 10^{10}	1.50	2.73	0.250
Lactobacillus pentosus DiSA 11	$(NH_4)OH$	A	1.4 x 10^{10}	6.0 x 10^9	5.20	6.50	0.187
		B	6.4 x 10^9	4.5 x 10^9	4.30	6.60	0.188
Lactobacillus brevis DiSA 49	NaOH	A	7.1 x 10^8	7.5 x 10^8	1.30	1.30	nd
		B	2.0 x 10^9	1.0 x 10^9	1.60	2.00	nd
Lactobacillus brevis DiSA 49	$(NH_4)OH$	A	6.9 x 10^8	1.2 x 10^{10}	0.84	1.92	nd
		B	4.0 x 10^9	1.2 x 10^{10}	2.61	3.46	nd
Leuconostoc spp DiSA 47	NaOH	A	7.0 x 10^9	3.2 x 10^9	3.60	3.30	nd
		B	1.0 x 10^{10}	6.4 x 10^{10}	5.00	5.10	nd
Lactococcus lactis subsp. *cremoris DiSA 6*	NaOH	A	2.8 x 10^9	4.7 x 10^9	nd	nd	0.242
		B	3.6 x 10^8	2.7 x 10^9	nd	nd	0.160

* A = fermentation without injected air; B = fermentation with injected air; nd = not detected; μ_{max} = specific growth rate

lus casei DiSA 27 and *Leuconostoc* spp. DiSA 47 which had the highest viable counts under micro-aeration conditions, at either 24 or 48 h. The opposite effect was observed for strain Lactococcus *lactis* subsp *cremoris* DiSA 6 since the viable counts which were significantly higher than those obtained in the presence of air at 0.15 vvm. For the strains *Lactobacillus pentosus* DiSA 11 and *Lactobacillus brevis* DiSA 49 the effect of air supply on maximum viable count values was not clearly defined since the highest data recorded resulted to be the same, under the two different aeration conditions tested (1.4×10^{10} and 1.2×10^{10} CFU mL^{-1} for the two strains, respectively).

With respect to dry weight (DW) data, the highest values were obtained after 48 h of fermentation and ranged from 0.84 g L^{-1} for *Lactobacillus brevis* DiSA 49, to 6.6 g L^{-1} for *Lactobacillus pentosus* DiSA 11. The DW values continued to increase or remained quite stable also when the viable counts decreased, thus indicating that cell lysis did not occur under the operating conditions applied. A comparison between the biomass DW values obtained with or without air supply during fermentation was made for all the strains tested, with the exception of *Lactobacillus casei* DiSA 27 and *Lactococcus lactis* subsp. *cremoris* DiSA 6. All the strains were positively affected by the air inlet, which gave DW values which were always higher than those corresponding values in the absence of micro-aeration. Moreover, the comparison of DW data obtained for *Lactobacillus pentosus* DiSA 11 and *Lactobacillus brevis* DiSA 49 in presence of either NH_4OH or NaOH clearly showed that both these strains were able to reach higher values of final biomass dry weight when NH_4OH was used.

The maximum specific growth rates (μ_{max}) were calculated, on the basis of the viable count data at 0, 6, 10, 12 and 24 h of fermentation (data not shown). The μ_{max} values, ranged from 0.160 and 0.390 h^{-1}, are comparable with those (ranging from 0.334 to 0.392 h^{-1}) reported for *Lactococcus lactis* subsp. *cremoris* in media containing from 5 to 40 g L^{-1} of lactose, under process conditions similar to those applied in this work (Cheigh *et al*., 2002). Our results also agree with the specific growth rates (from 0.118 to 0.217 h^{-1}) obtained by Tango and Ghaly (1999) for *Lactobacillus helveticus* in media containing from 50 to 150 g L^{-1} of lactose, at 0.00 to 0.15 vvm. The latter authors reported a specific growth rate of 0.212 h^{-1} in a medium with 75 g L^{-1} of lactose and 0.15 vvm of air flow rate, as in this work.

As the effect of the process parameters on the μ_{max} is concerned, no considerable differences were observed for the strains *Lactobacillus casei* DiSA 27 and *Lactobacillus pentosus* DiSA 11 with and without air inlet, whereas air inlet exerted a negative effect on the μ_{max} of *Lactococcus lactis* subsp. *cremoris* DiSA 6, as already revealed from the viable count data. By contrast, the effect of type of neutralising agent did not significantly effect on the μ_{max}.

Carbohydrate consumption

The kinetics observed for the sugar consumption in the different strains resulted in very different profiles (Fig. 2). The strains *Lactobacillus plantarum* DiSA 33 and *Lactobacillus casei* DiSA 27 completed the utilization of the carbohydrate source, whereas the highest values of residual lactose (> 50% of the original) at the end of fermentation were detected with *Lactococcus lactis* subsp. *cremoris* DiSA 6. The findings for viable count and DW were confirmed. Both parameters had a certain influence on all the strains tested, though to a different extent for the different strains. When applicable, all the strains resulted to be advantaged in the consumption of the C-source when both air inlet and ammonium were used, whereas in fermentations with strains *Lactococcus lactis* subsp. *cremoris* DiSA 6 and *Lactobacillus brevis* DiSA 49, performed only with sodium hydroxide, the positive influence of the sole aeration was proved.

Our findings concerning the effect of aeration and ammonium on the production of lactic acid bacteria biomass are in accordance with those of several authors. Although LAB growth can occur in non-aerated conditions, better growth was observed in different media sparged with air, even at very low flow rate (Condon, 1983; Tango and Ghaly, 1999; Champagne *et al*., 2002; Schiraldi *et al*., 2003). Slight improvements in growth were obtained by increasing the air flow rates; whereas pure oxygen at 120 L h^{-1} increased growth and cell yield by more than 50% (Plihon *et al*., 1995).

The positive influence of ammonium is in accordance with the earliest findings of Snell (1952) and Gyorgy (1954) who asserted that the use of this compound allows higher viable counts to be obtained, probably due to its stimulatory effect onto the cultures. Other authors (Zarlengo and Abrams, 1963) supposed that, after crossing the cell membranes by simple diffusion, the ammonium ion neutralises some of the intracellular acids inside the cell, thus raising the intracellular pH. The use of NH_4OH ion as a neutralising agent was also reported to provide an additional nitrogen source suitable to

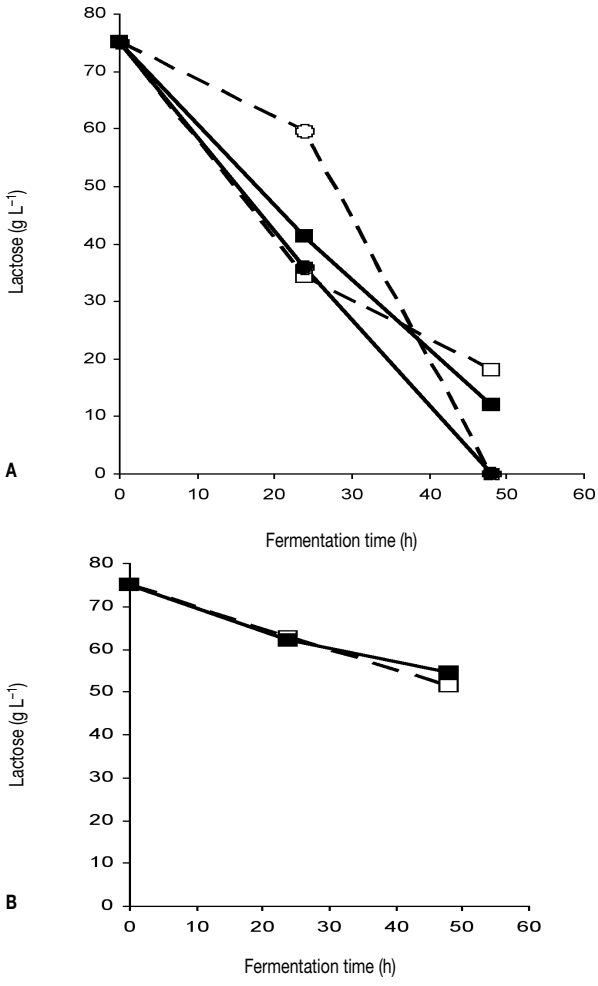

FIG. 2 – (*Continued*).

FIG. 2 – (*Follow the previous page*).

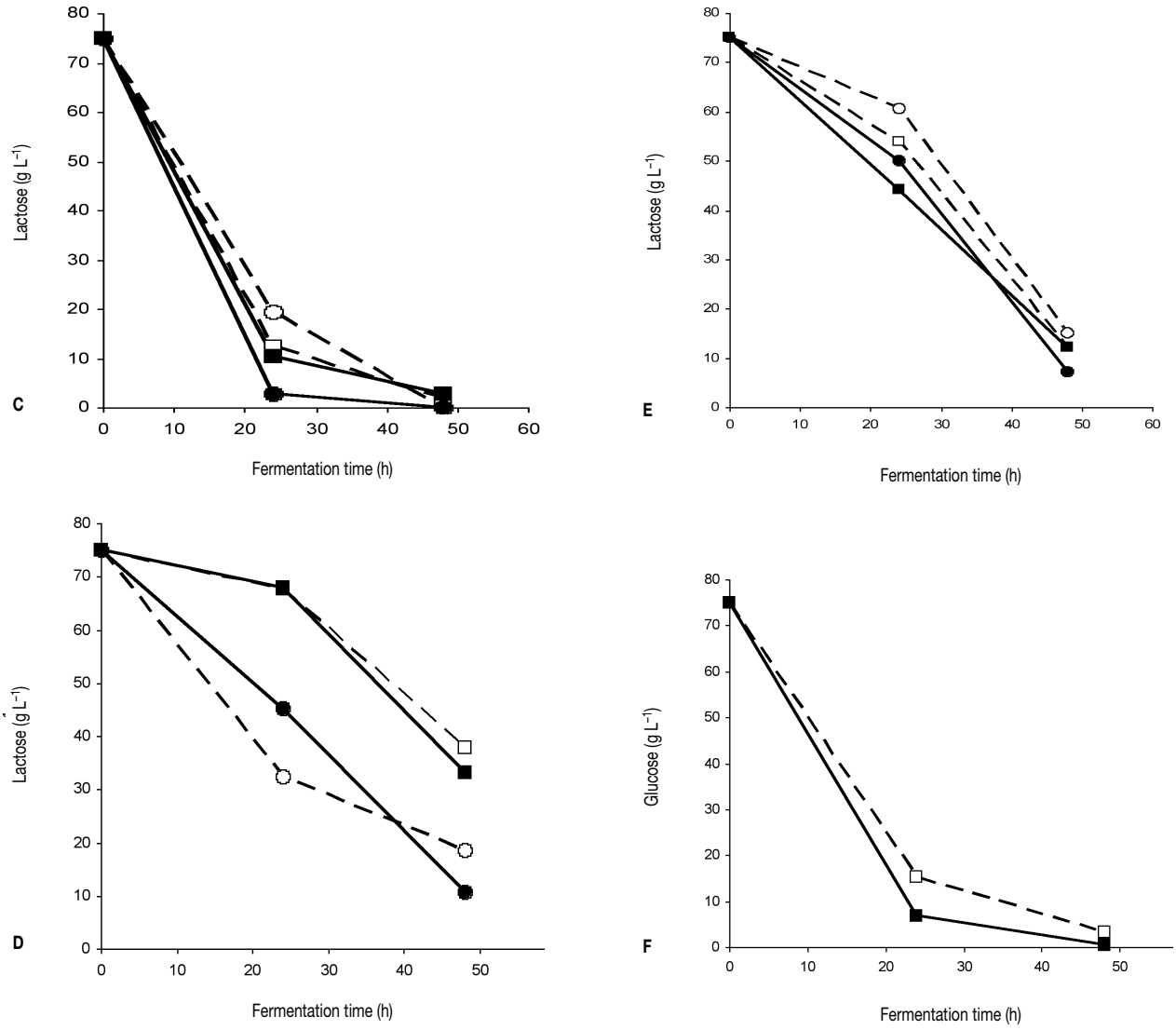

FIG. 2 – Consumption of the C-source by *Lactobacillus plantarum* DiSA 33 (A), *Lactococcus lactis* subsp. *cremoris* DiSA 6 (B), *Lactobacillus casei* DiSA 27 (C), *Lactobacillus pentosus* DiSA 11 (D), *Leuconostoc spp*. DiSA 47 (E) and *Lactobacillus brevis* DiSA 49 (F) in fermentations carried out using NaOH (■□) or NH_4OH (●○) as a neutralising agent with (closed symbols and solid lines) or without (open symbols and dotted lines) aeration.

be introduced into certain metabolic reactions such as the formation of glutamic acid or glutamine (Peebles *et al*., 1969). However, an inhibitory effect of the Na^+ ion, observed by Sirny *et al*. (1954), could also explain the negative effect observed in this study when sodium hydroxide was used in place of ammonium.

Aknowledgements

The present research work was commissioned by the RASA S.p.A (Manfredonia-FG-Italy), within the project no. 12/cluster C08-B "Valorization of Dairy Products", of the Manfredonia municipality (FG-Italy), granted by the Ministry of University and Research-Italy (MURST).

REFERENCES

Bergere J.L., Hermier J. (1968). Mass production of cells of lactic streptococci. Growth of *Streptococcus lactis* in medium at constant pH. Lait, 48: 13-30.

Bibal B., Kapp C., Goma G., Pareilleux A. (1989). Continuous culture of *Streptococcus cremoris* on lactose using various medium conditions. Applied Microbiology and Biotechnology, 32:155-159.

Bury D., Jelen P., Kimura K. (1998). Whey protein concentrate as a nutrient supplement for lactic acid bacteria. Int. Dairy J., 8: 149-151.

Caplice E., Fitzgerald G.F. (1999). Food fermentation: role of microorganisms in food production and preservation. International Journal of Food Microbiology, 50: 131-149.

Cenci Goga B.T., Clementi F., Di Antonio E. (1995). Behaviour of lactic and non lactic microflora during the production and ripening of farm-manufactured Pecorino cheese. Annali di Microbiologia ed Enzimologia, 45: 219-235.

Champagne C.P., Gardner N.J. (2002). Effect of process parameters on the production and drying of *Leuconostoc mesen-*

teroides cultures. Journal of Industrial Microbiology and Biotechnology, 28: 291-296.

Cheigh C.I., Choi H.J., Park H., Kim S.B., Kook M.C., Kim T.S., Hwang J.K., Pyun Y.R. (2002). Influence of growth on the production of a nisin-like bacteriocin by *Lactococcus lactis* subsp. *lactis* A164 isolated from kimchi. Journal of Biotechnology, 95: 225-235.

Clementi F., Cenci Goga B.T., Marinucci T.M., Di Antonio E. (1998). Used of selected starter cultures in the production of farm manufactured goat cheese from thermized milk. Italian Journal of Food Science, 10: 44-45.

Condon S. (1983). Aerobic metabolism of lactic acid bacteria. International Journal of Food Science and Technology, 7: 15-25.

Coppola S., Villani F., Coppola R., Parente E. (1990). Comparison of different starter systems for water-buffalo mozzarella cheese manufacture. Le Lait, 70: 411-423.

Dols M., Chraibi W., Simeon Remaud M., Lindley N.D., Monsan P.F. (1997). Growth and energetics of *Leuconostoc mesenteroides* of various sugar and their consequences for dextransucrase production. Applied and Environmental Microbiology, 63: 2159-2165.

Gilliland S.E. (1985). Concentrated starter cultures. In: Gilliland S.E., Bacterial Starter Culture for Food. CRC Press, Inc., Boca Raton, USA, pp.145-157.

Gyorgy P. (1954). Its occurrence in milk from different species and in other natural products. In *Bifidus* factor. Archives of Biochemistry and Biophysics; 48: 202-208.

Kandler O., Weiss N. (1986). Regular, non-sporing Gram-positive rods. Genus *Lactobacillus*. In: Sneath P.H.A., Hair N.S., Sharpe M.E., Holts J.G., Eds, Bergey's Manual of Systematic Bacteriology, Vol. 2, Williams and Wilkins Co., Baltimore, p. 1208.

Klaenhammer T.R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiology Review, 12: 39-86.

Mataragas M., Drosinos E.H., Tsakalidou E., Metaxopoulos J. (2004). Influence of nutrients on growth and bacteriocin production by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442. Antonie van Leeuwenhoek, 85: 191-198.

Messens W., De Vuyst L. (2002). Inhibitory substances produced by *Lactobacilli* isolated from sourdough – A review. International Journal of Food Microbiology, 72: 31-43.

Mulligan C.N., Safi B.F. (1991). Continuous production of ammonium lactate by *Streptococcus cremoris* in a three-stage reactor. Biotechnology and Bioengineering, 38: 1173-1181.

Nettles C.G., Barefoot S.F. (1993). Biochemical and genetic characteristics of bacteriocins of food-associated lactic acid bacteria. Journal of Food Protection, 36: 338-356.

Parente E., Ricciardi A., Addario G. (1994). Influence of pH on growth and bacteriocin production by *Lactococcus lactis* subsp. *lactis* 140NWC during batch fermentation Applied Microbiology and Biotechnology, 41: 388-394.

Parente E., Rota M.A., Ricciardi A., Clementi F. (1997). Characterization of natural starters cultures used in the manufacture of pasta filata cheese in Basilicata (Southern Italy). International Dairy Journal, 7: 775-783.

Peebles M.M., Gilliland S.E., Speck M. L. (1969). Preparation of concentrated lactic *Streptococcus* starters. Applied Microbiology, 17: 805-810.

Plihon F., Taillandier P., Strehaiano P. (1995). Oxygen effect on batch cultures of *Leuconostoc mesenteroides*: relation between oxygen uptake, growth and end-products. Applied Microbiology and Biotechnology, 43: 117-122.

Rincòn J., Fuertes J., Moya A., Monteagudo J. M., Rodrìguez L. (1993). Optimization of the fermentation of whey by *Lactobacillus casei*. Acta Biotechnologica, 13:323-331.

Schiraldi C., Adduci V., Valli V., Maresca C., Giuliano M., Lamberti M., Carteni M., De Rosa M. (2003). High cell density cultivation of probiotics and lactic acid production. Biotechnology and Bioengineering, 82: 213-222.

Sirny R.J., Braekkan O.R., Klungsøyr M., Elvehjem C.A. (1954). Effects of potassium and sodium in microbiological assay media. Journal of Bacteriology, 68: 103-109.

Snell E.E. (1952). The nutrition of the lactic acid bacteria. Bacteriological Reviews, 16: 235-241.

Stahouder J., Jansen J.A., Hap G. (1969). Preservation of starters mass production of starter bacteria. Netherlands Milk and Dairy Journal, 23: 182-199.

Tango M.S.A., Ghaly A.E. (1999). Amelioration of lactic acid production from cheese whey using micro-aeration. Biomass and Bioenergy, 17: 221-238.

Teuber, M. (1993) Lactic acid bacteria. In Biotechnology. Vol.1. Biological fundamentals. pp. 325-366, VCH Ltd., Weinheim, New York, Basel, Cambridge, Tokyo.

Whitehead W.E., Ayres J.W., Sandine W.E.(1993). Symposium. Recent developments in dairy starter cultures: microbiology and physiology. Journal of Dairy Science, 76: 2344-2353.

Yousten A.A., Johnosn J.L., Salin M. (1975). Oxygen metabolism of catalase-negative and catalase-positive strains of *Lactobacillus plantarum*. Journal of Bacteriology, 123: 242-247.

Zarlengo M., Abrams A. (1963). Selective penetration of ammonia and alkylamines into *Streptococcus fecalis* and their effect on glycolysis. Biochimica et Biophysica Acta., 71: 65-77.