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Batch production of L(+) lactic acid from whey by *Lactobacillus casei* (NRRL B-441)

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Abstract: The effects of temperature, pH, and medium composition on lactic acid production by *Lactobacillus casei* were investigated. The highest lactic acid productivity values were obtained at 37 °C and pH 5.5. The productivity was 1.87 g dm^{-3} h^{-1} at 37 °C in shake flasks. In the fermenter, a productivity of 3.97 g dm^{-3} h^{-1} was obtained at pH 5.5. The most appropriate yeast extract concentration was 5.0 g dm^{-3}. Whey yielded a higher productivity value than the analytical lactose and glucose. Initial whey lactose concentration did not affect lactic acid productivity. $MnSO_4 \cdot H_2O$ was necessary for lactic acid production by *L casei* from whey. Product yields were approximately 0.93 g lactic acid g $lactose^{-1}$.

Keywords: L(+) lactic acid; *Lactobacillus casei*; whey; batch fermentation

1 INTRODUCTION

Having two optically active forms, D(−) and L(+), lactic acid has long been used in the food, chemical, textile, pharmaceutical and other industries. The worldwide production is 80 000 tons $year^{-1}$ and 90% of it is produced by lactic acid bacterial fermentation.[1] There is increased interest in production of L(+) lactic acid, since it is a potential feedstock for poly-L(+) lactic acid, which degrades in the environment and in the human body to lactic acid.[2,3] Chemical synthesis yields racemic (DL) lactic acid and most of the lactic acid bacteria produce DL or D forms. However, the species *Lactobacillus casei* produces predominantly the L(+) form.[4,5]

Whey is a major by-product of the dairy industry, containing approximately (w/v) 5% lactose, 1% protein, 0.4% fat, and some minerals. It has a high BOD content (40 000–60 000 ppm) which represents serious disposal problems.[6] For lactic acid production by lactic acid bacteria, whey is supplemented by appropriate nutrients since lactic acid bacteria have complex nutritional requirements, such as B-vitamins and peptides for effective growth.[7,8]

This study focuses on the utilization of whey lactose by *L casei* NRRL B441 and investigates several physical parameters to maximize lactic acid productivity. Results indicate that whey may be suitable as a substrate for industrial lactic acid production by *L casei*.

2 MATERIALS AND METHODS

2.1 Microorganism

Lactobacillus casei NRRL B-441 used throughout this study was kindly supplied by the US Department of Agriculture, National Center for Agricultural Utilization Research. The strain was maintained in litmus milk (Difco, Michigan, USA) and transferred to fresh medium every month.

2.2 Media

Whey powder containing 60–62% (w/w) lactose was obtained from PINAR Dairy Products, Inc, Turkey. It was dissolved to attain the desired initial substrate concentration (S_o). Unless otherwise indicated, whey powder was supplemented with (g dm^{-3}) yeast extract (10), K_2HPO_4 (0.5), KH_2PO_4 (0.5), $MgSO_4$ (0.2) and $MnSO_4 \cdot H_2O$ (0.05). In the carbon source study, whey was replaced by glucose or lactose. Sugar, yeast extract and salt solutions were sterilized separately at 121 °C for 15 min. All chemicals were purchased from Merck (Darmstad, Germany) except yeast extract, which was purchased from Oxoid (Hampshire, England).

2.3 Experimental methods

Fermentations were carried out batchwise in 250 cm^3 flasks with 100 cm^3 working volume in a temperature controlled flask shaker operated at 150 rpm and 37 °C. Sterile $CaCO_3$ (60% (w/w) of the initial lactose concentration) was added to the medium to neutralize the acid formed. Flasks were inoculated with 4–5 cm^3 litmus milk cultures that had been incubated at 37 °C for 24 h.

pH optimization studies were conducted in a 5 dm^3 fermenter (Bioengineering AG) with a 3 dm^3 working volume, and equipped with pH, temperature and stirring speed controllers. The pH value was maintained by automatic addition of 10 mol dm^{-3} NaOH. The stirring speed was adjusted to 200 rpm. The fermenter was inoculated with 375 cm^3, 24-h-old seed culture.

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The seed culture was grown in the flasks under the conditions described above. The initial lactose concentrations of the seed cultures were 50 g dm^{-3}. Due to the high inoculum concentration, the fermenter and the media did not need to be sterilized. Fermentations were conducted at least in duplicate and reproducible results were obtained. Here, results of representative fermentations are reported.

2.4 Analytical methods

Lactose, glucose and lactic acid were analyzed by HPLC (Perkin Elmer, USA) with an Aminex HPX-87H column (Biorad Laboratories, USA) operated at 45 °C using a refractive index detector (Perkin Elmer, USA). H_2SO_4 (5 mmol dm^{-3}) was used as the eluent at a flow rate of 0.6 cm^3 min^{-1}.

3 RESULTS AND DISCUSSION

3.1 Effect of temperature

L casei was adapted to five temperatures (27, 32, 37, 42 and 47 °C) during three successive generations in litmus milk. Culture growth, as determined by color change, was observed in 1 day at 32 and 37 °C while it took 2 days for the cultures grown at 27 and 42 °C. Growth was very poor at 47 °C; therefore, no fermentation runs were carried out at this temperature.

As seen in Fig 1, the fastest production of lactic acid was obtained at 37 °C although the effect of temperature was not significant between 32 and 42 °C. The product formation was quite slow at 27 °C and the fermentation was completed in 45 h at this temperature. The overall productivity was 1.87 g dm^{-3} h^{-1} at 37 °C. The lactic acid production rate was found to be very similar at the temperatures of 32 and 42 °C.

These results confirmed those of Hujanen and Linko[9] who found that the best production temperature for *L casei* NRRL B-441 was 37 °C. They obtained 80 g dm^{-3} lactic acid in 24 h from 90 g dm^{-3} by using high amounts of yeast extract and inoculum (2.2% and 20%, respectively). Krischke *et al*[10] also determined the optimum temperature for lactic acid fermentation of whey permeate by immobilized *L casei* subsp *casei* to be 37 °C. Temperature optimization investigations by Tuli *et al*[11] showed no significant influence of temperature between 40 and 50 °C for lactic acid production from whey permeate by immobilized *L casei*. Temperatures of 35 and 55 °C reduced the product formation.[11]

3.2 Effect of pH

Optimum pH studies were carried out for the pH values of 5.0, 5.5, 6.0 and 6.5 for *L casei* in the fermenter. When pH values of 5.0 and 5.5 were investigated, the pH values were allowed to decrease to the specified values itself, which means no acid was added to adjust the pH to low values. The level of pH is maintained at the desired values by the automatic addition of 10 mol dm^{-3} NaOH. At most, 200 cm^3 of NaOH was added to 3 dm^3 of fermentation media in order to maintain the pH, thus fermentation volume did not increase more than 6.6%.

All lactose was utilized in all fermentations and product yield values were about 93 g lactic acid g^{-1} lactose. Complete utilization of lactose at pH 5.0 was achieved in 23 h as can be seen in Fig 2. At other pH values similar trends were obtained within 12 h. Although no significant difference in productivity was observed for the pH values between 5.5 and 6.5, the highest productivity value of 3.97 g dm^{-3} h^{-1} was obtained at pH 5.5.

The results confirmed those of Tuli *et al*,[11] who determined the optimum initial pH for lactic acid production from whey permeate by immobilized *L casei* to be 5.5. Krischke *et al*[10] found that best growth and product formation occurred between pH 6.0 and 6.5 for immobilized *L casei* subsp *casei* grown on whey permeate.

3.3 Effect of yeast extract

Providing amino acids, vitamins and co-factors required for cell maintenance and lactic acid production, yeast extract supplementation is one of the

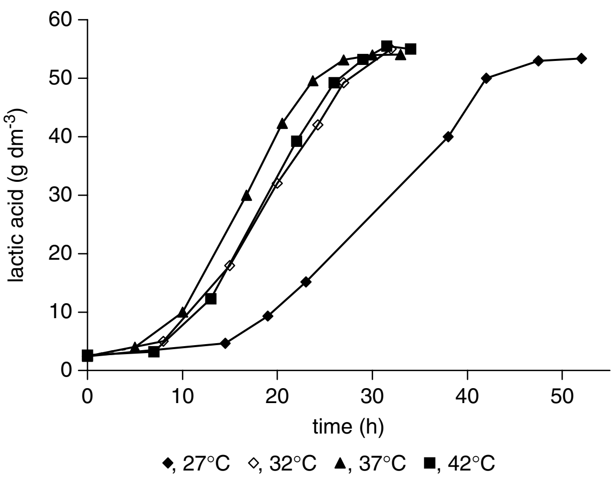

Figure 1. Lactic acid production at different temperatures (S_o = 54 g dm^{-3}, yeast extract = 10 g dm^{-3}).

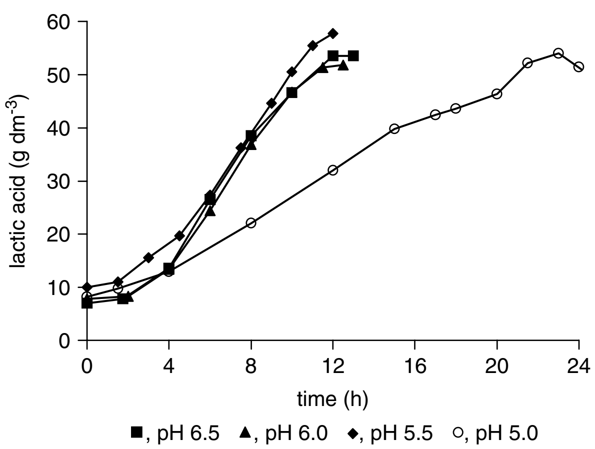

Figure 2. Lactic acid production at different pH values (S_o = 47.4–49.0 g dm^{-3}, yeast extract = 10 g dm^{-3}).

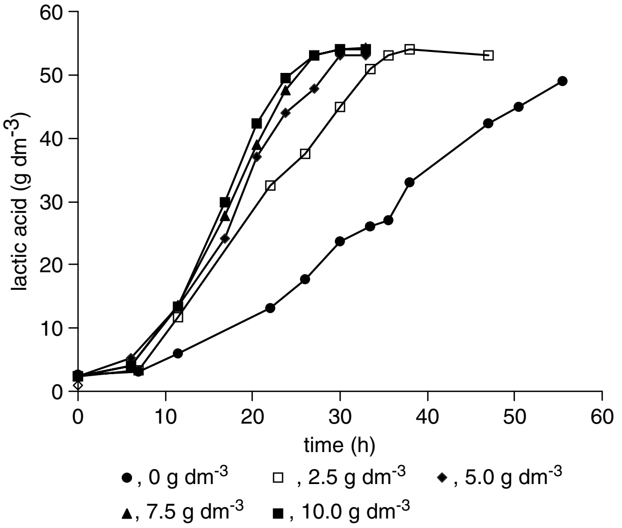

Figure 3. Lactic acid production with different yeast extract concentrations ($S_o = 54\,g\,dm^{-3}$).

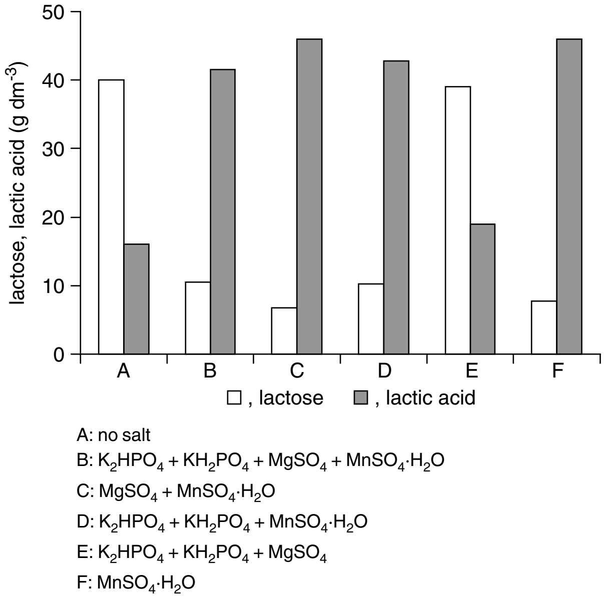

Figure 4. Lactic acid and lactose concentrations after 24 h fermentation with different salt contents (S_o = 50–53 g dm^{-3}, yeast extract = 5 g dm^{-3}).

key factors in lactic acid fermentation. However, the high cost of yeast extract limits its usage in the fermentations, and in industrial processes the yeast extract concentration is mostly suboptimal. Therefore, the effect of yeast extract was examined in this study by conducting fermentations in shake flasks with different concentrations of yeast extract (0, 2.5, 5.0, 7.5 and 10 g dm^{-3}) to find the optimum concentration.

The lactic acid production curves were similar with yeast extract concentrations of 5.0, 7.5 and 10.0 g dm^{-3} (Fig 3). In about 27 h of fermentation all the lactose was consumed by *L casei* and more than 52 g dm^{-3} lactic acid was produced with 7.5 and 10.0 g dm^{-3} yeast extract. In order to produce the same amount of lactic acid without yeast extract in the medium, the fermentation had to continue for up to 60 hs. With 2.5 g dm^{-3} yeast extract, 38 h was needed. The lactic acid productivity increased with increasing yeast extract concentration. The overall productivity values were 1.87 g dm^{-3} h^{-1} for both 7.5 and 10 g dm^{-3}, and 1.65 g dm^{-3} h^{-1} for 5.0 g dm^{-3} yeast extract concentration. The productivity value decreased to 1.36 g dm^{-3} h^{-1} with 2.5 g dm^{-3} yeast extract. In all fermentations lactose was totally utilized. Product yields were not very different and all were between 92 and 94%. In the subsequent experiments, 5 g dm^{-3} yeast extract was used.

For batch fermentation of whey permeate by *L casei* subsp *casei*, Krischke *et al*[10] concluded that the best growth comparable to MRS medium occurred with a combination of yeast extract (5 g dm^{-3}) and hydrolyzed whey retentate (50 g dm^{-3}). Guoqiang *et al*[12] determined that increasing yeast extract concentration (0–10 g dm^{-3}) increased lactic acid production. Aeschlimann and von Stockar[13] obtained an increase in lactic acid conversion from 0.34 to 0.92 g g^{-1} using *L helveticus* by increasing yeast extract concentration from 0.0 to 3.0 g dm^{-3}. They, however, needed 10 g dm^{-3} yeast extract for efficient production in continuous culture; 10 g dm^{-3} was found to be the optimum yeast extract concentration by Göksungur and Güvenç,[14] and Norton *et al*[15] for *L delbrueckii* and *L helveticus*, respectively. Hujanen and Linko[9] showed that the type and initial concentration of the nitrogen source influenced lactic acid production.

3.4 Effect of salts

In the study, the effect of salts was studied by discarding the salt(s) of interest from the medium formulation. Fermentations with all salts and none of the salts were the controls. Figure 4 shows the effect of different salts on the fermentations of *L casei* using whey lactose supplemented with 5 g dm^{-3} yeast extract. Both lactose and lactic acid concentrations after 24 h of fermentation are given in the figure.

When $MnSO_4{\cdot}H_2O$ was discarded, the results were the same as the one without any salts. The other salts showed no or very small effects. It should be noted that the values were taken at 24 h and fermentations had not been finished at that time. Therefore, it was concluded that only $MnSO_4{\cdot}H_2O$ was essential for the lactic acid fermentation of whey by *L casei* and that Mn^{2+} in the form of $MnSO_4{\cdot}H_2O$ was the ion that *L casei* needed. Whey may have provided some other ions as well.

Fitzpatrick *et al*[16] and Krischke *et al*[10] have shown the beneficial effect of Mn^{2+} for lactic acid production by *L casei*, due to its role as a constituent of lactate dehydrogenase.

3.5 Effect of initial substrate concentration

The effect of initial substrate (whey lactose) concentrations was investigated using concentration values of 48.7, 64.0, 79.0 and 102.9 g dm^{-3}. The results of lactose utilization and lactic acid production are given in Fig 5(a and b respectively). As seen in Fig 5(a),

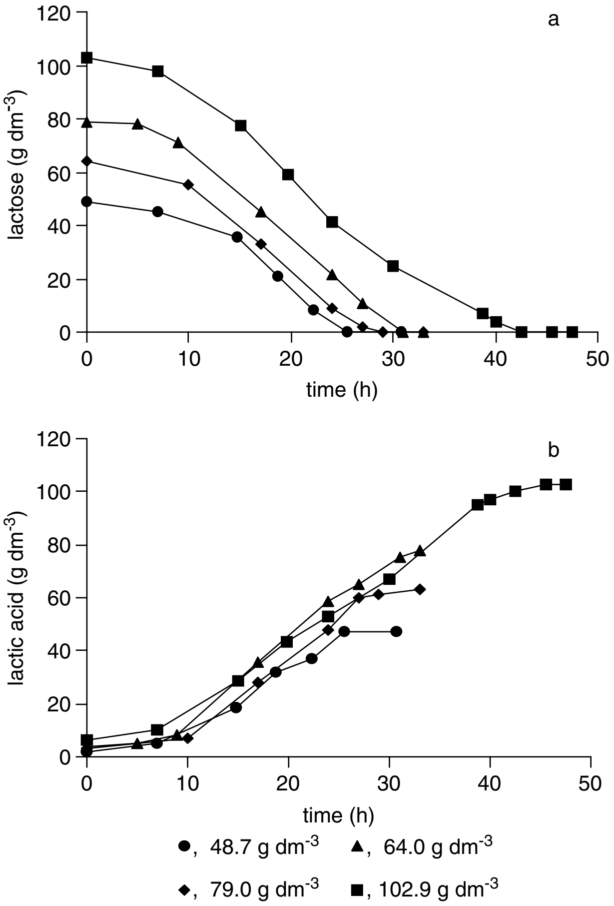

Figure 5. (a) Lactose consumption and (b) lactic acid production with different initial whey lactose concentrations (yeast extract = 0.5 g dm $^{-3}$, $MnSO_4 \cdot H_2O$ only).

lactose consumption curves were parallel and the time needed for the *L casei* to utilize lactose completely depended on the initial substrate concentration. In all concentrations, *L casei* could utilize the sugar completely. Lactic acid production curves followed nearly the same pattern, but in each case the maximum amount of lactic acid produced was obviously different due to the substrate concentration. Lactic acid yields were 0.93–0.94 g per g lactose at all initial substrate concentrations. The overall productivity values were similar and around 2.2 g dm^{-3} h^{-1} except that of 1.77 g dm^{-3} h^{-1} for the initial substrate concentration of 48.7 g dm^{-3}. These showed that there was no substrate inhibition at these initial sugar concentrations.

The results confirmed those of Vaccari *et al*,[4] who obtained similar volumetric productivity values between 6, 8, 10 and 12% glucose. Tuli *et al*,[11] on the other hand, found that the rate of lactic acid production increased up to 4.5% (w/v) initial lactose concentration and decreased thereafter.

3.6 Effect of carbon source

Lactose and glucose synthetic media containing 5 g dm^{-3} yeast extract showed similar lactic acid production and sugar utilization patterns. Whey lactose was utilized more rapidly than the synthetic lactose and glucose, so the productivity was higher.

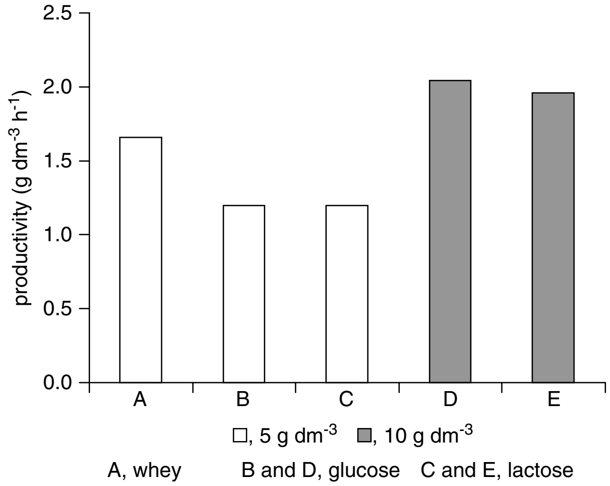

Figure 6. Overall productivity values for fermentations with different substrates with 5 g dm^{-3} or 10 g dm^{-3} yeast extract (S_o = 50–51 g dm^{-3}, $MnSO_4 \cdot H_2O$).

Productivity values for lactose and glucose media were around 1.45 g dm^{-3} h^{-1} and for whey medium it was approximately 1.75 g dm^{-3} h^{-1}. In the second set of experiments, yeast extract concentrations of synthetic lactose and glucose media were raised to 10 g dm^{-3}. In that case, fermentations were complete within nearly 30 h, while the 5 g dm^{-3} yeast extract needed a longer fermentation time to utilize all sugars. The productivity values were about 1.47 g dm^{-3} h^{-1} for synthetic lactose and glucose media that were supplemented with 5 g dm^{-3} yeast extract, while with 10 g dm^{-3} yeast extract productivity values were approximately 30% higher (around 1.9 g dm^{-3} h^{-1}) (Fig 6). It was concluded that the complex composition of whey could have provided additional nutrition to the organism.

4 CONCLUSIONS

Lactic acid could be produced from a cheap, abundant crude feedstock, whey, which is a pollutant when it is not utilized or processed, with a yield comparable to the other carbon sources. However, when selecting a carbon source for the fermentation productivity cannot be the sole parameter, because the complex composition of whey leads to higher purification cost than pure sugars such as glucose, lactose and sucrose. So, the raw material cost and purification cost should be considered as well as the productivity.

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REFERENCES

1 Hofvendahl K and Hahn-Hagerdal B, Factors affecting the fermentative lactic acid production from renewable resources. *Enzyme Microbiol Technol* **26**:87–107 (2000).

2 Datta R, Tsai SP, Bonsignore P, Moon SH and Frank JR, Technological and economic potential of poly (lactic acid) and lactic acid derivatives. *FEMS Microbiol Reviews* **16**:221–231 (1995).

3 Martin AM, Fermentation process for the production of lactic acid, in *Lactic Acid Bacteria: Current Advances in Metabolism, Genetics and Applications*, ed, by Bozoglu TF and Ray B. Springer Verlag, New York, pp 269–301 (1997).

4 Vaccari G, Gonzales-Vara A, Campi AL, Dosi E, Brigidi P and Matteuzzi D, Fermentative production of L-lactic acid by *Lactobacillus casei* DSM 20011 and product recovery using ion exchange resins. *Appl Microbiol Biotechnol* **40**:23–27 (1993).

5 Senthuran A, Senthuran B, Mattiasson B and Kaul R, Lactic acid fermentation in a recycle batch reactor using immobilized *Lactobacillus casei*. *Biotechnol Bioeng* **55**:841–853 (1997).

6 Irvine DM and Hill AR, Cheese technology, in *Comprehensive Biotechnology*, Vol 3, ed by Moo-Young M. Pergamon Press, New York, pp 523–566 (1985).

7 Atkinson B and Mavituna F, Industrial microbial process, in *Biochemical Engineering and Biotechnology Handbook*. Stockton, New York, pp 1111–1220 (1991).

8 Amrane A and Prignet Y, Lactic acid production from lactose in batch culture: analysis of the data with the help of a mathematical model; relevance for nitrogen source and preculture assessment. *Appl Microbiol Biotechnol* **40**:644–649 (1994).

9 Hujanen M and Linko YY, Effect of temperature and various nitrogen sources on L(+)-lactic acid production by *Lactobacillus casei*. *Appl Microbiol Biotechnol* **45**:307–313 (1996).

10 Krischke W, Schroder M and Trosch W, Continuous production of L-lactic acid from whey permeate by immobilized *Lactobacillus casei* subsp. *casei*. *Appl Microbiol Biotechnol* **34**:573–578 (1991).

11 Tuli A, Sethi RP, Khanna PK and Marwaha SS, Lactic acid production from whey permeate by immobilized *Lactobacillus casei*. *Enzyme Microbiol Technol* **7**:164–168 (1985).

12 Guoqiang D, Kaul R and Mattiasson B, Evaluation of alginate-immobilized *Lactobacillus casei* for lactate production. *Appl Microbiol Biotechnol* **36**:309–314 (1991).

13 Aeschlimann A and von Stockar U, The effect of yeast extract supplementation on the production of lactic acid from whey permeate by *Lactobacillus helveticus*. *Appl Microbiol Biotechnol* **32**:398–402 (1990).

14 Göksungur Y and Güvenç U, Batch and continuous production of lactic acid from beet molasses by *Lactobacillus delbrueckii* IFO 3202. *J Chem Technol Biotechnol* **69**:399–404 (1997).

15 Norton S, Lacroix C and Vuillemard JC, Reduction of yeast extract supplementation in lactic acid fermentation of whey permeate by immobilized cell technology. *J Dairy Sci* **77**:2494–2508 (1994).

16 Fitzpatrick JJ, Ahrens M and Smith S, Effect of manganese on *Lactobacillus casei* fermentation to produce lactic acid from whey permeate. *Proc Biochem* **36**:671–675 (2001).