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Process Biochemistry 36 (2001) 671–675

PROCESS BIOCHEMISTRY

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Effect of manganese on *Lactobacillus casei* fermentation to produce lactic acid from whey permeate

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Received 10 October 2000; received in revised form 13 October 2000; accepted 5 November 2000

Abstract

Batch fermentations were performed to investigate the effect of manganese addition, in the form of $MnSO_4 \cdot H_2O$, on the performance of *Lactobacillus casei* for producing L-lactic acid from whey permeate supplemented with yeast extract. There was a particular emphasis on evaluating how little yeast extract and $MnSO_4 \cdot H_2O$ is required while still obtaining high sugar conversion and lactic acid yield, as nutrient supplementation is a raw material cost and can lead to extra residual impurities remaining after fermentation. The addition of $MnSO_4 \cdot H_2O$ had a significant beneficial affect with the fermentation time being reduced from 120 to 24 h for permeate supplemented with 0.50%w/v yeast extract. Fermentations were performed with $MnSO_4 \cdot H_2O$ concentrations in the range of 0.001–0.03 g/l. From 0.005 to 0.03 g/l, the fermentation performance was very similar, however at the low concentration of 0.001 g/l, the fermentation was significantly slower. With $MnSO_4 \cdot H_2O$ addition, the yeast extract concentration was reduced to 0.30%w/v while still maintaining high sugar conversion and lactic acid yield, however the fermentation was slower at 37 h. At 0.1%w/v yeast extract supplementation, the fermentation performance was poor with only 67% sugar conversion after 150 h of fermentation.

Keywords: Lactic Acid Fermentation; Whey Permeate; Supplementation

1. Introduction

Whey permeate consists mainly of lactose in solution with ash and soluble nitrogen, and is produced as a by-product from whey protein concentrate [1], milk protein concentrate and casein production. There is continuing interest in ways of utilising this by-product. One alternative is the production of lactic acid by fermentation [2]. There is a major commercial interest in producing lactic acid biodegradable plastics using single isomer lactic acid derived by fermentation [3], however, lactic acid producers are concentrating mainly at producing lactic acid from sucrose and dextrose based media. Thus, there exists a challenge to make whey permeate a viable alternative substrate.

Some lactic acid bacteria, such as *Lactobacillus casei*, can convert the lactose in whey permeate homofermentatively to L-lactic acid. However, they require a nutrient supplement for complete conversion of lactose to lactic acid, otherwise, there is incomplete utilisation of lactose and the fermentation proceeds very slowly. Lactic acid bacteria have complex growth factor requirements including specific minerals, B vitamins, several amino acids, and purine and pyrimidine bases [4].

Manganese is an essential growth factor for *L. casei*, because of its role as a constituent of lactate dehydrogenase [5], and therefore, was added to the whey permeate in the form of $MnSO_4 \cdot H_2O$ to a concentration of 0.03 g/l. It has been reported that supplementation of whey hydrolysate with yeast extract and manganese ions was necessary to improve lactate productivity and lactose utilisation [6], and manganese supplementation in this work was 0.03 g/l of $MnSO_4 \cdot H_2O$. Another report also supplemented *L. casei* fermentations of whey permeate with 0.03 g/l of $MnSO_4 \cdot H_2O$ [7].

Adding a nutrient supplement is a raw material cost and also adds to the residual impurities remaining after

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0032-9592/01/$ - see front matter
PII: S0032-9592(00)00265-X

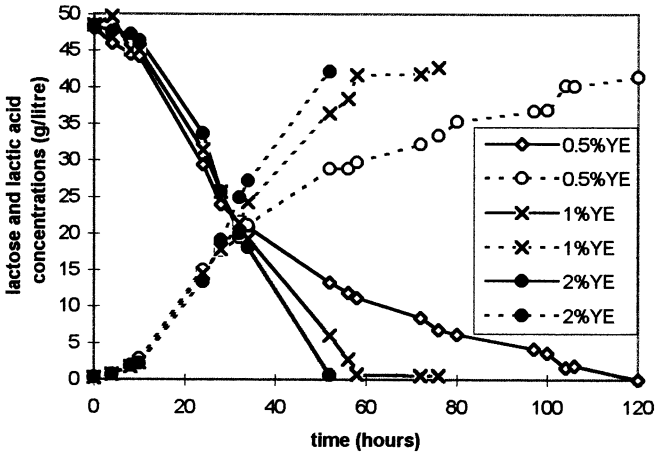

Fig. 1. Effect of yeast extract (YE%w/v) on lactose utilisation and lactic acid production when there is no manganese addition (solid lines represent lactose; dashed lines represent lactic acid).

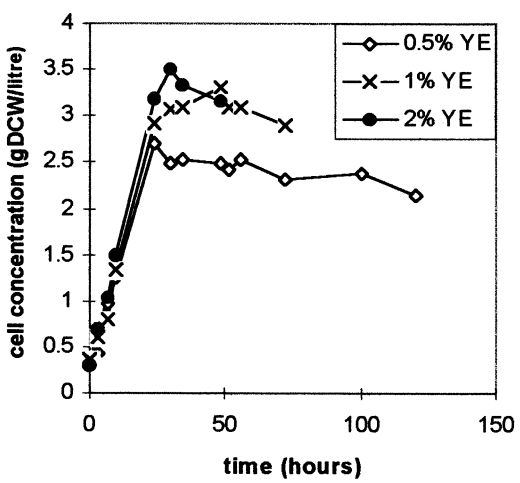

Fig. 2. Effect of yeast extract (YE%w/v) on cell growth when there is no manganese addition.

fermentation which may have to be removed by costly purification processes, for example in the production of polymer-grade lactic acid. Thus, the objective of this work is to investigate how little yeast extract and manganese sulphate supplements are required by *L. casei* to obtain high sugar conversion and lactic acid yield.

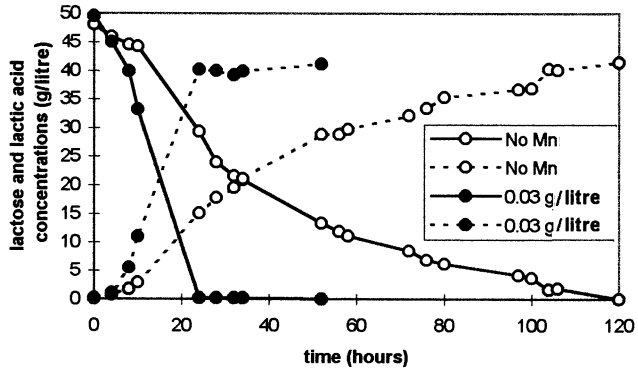

Fig. 3. Effect of $MnSO_4 \cdot H_2O$ addition to a concentration of 0.03 g/l on lactose utilisation and lactic acid production (solid lines represent lactose; dashed lines represent lactic acid). Yeast extract supplementation is 0.5%w/v.

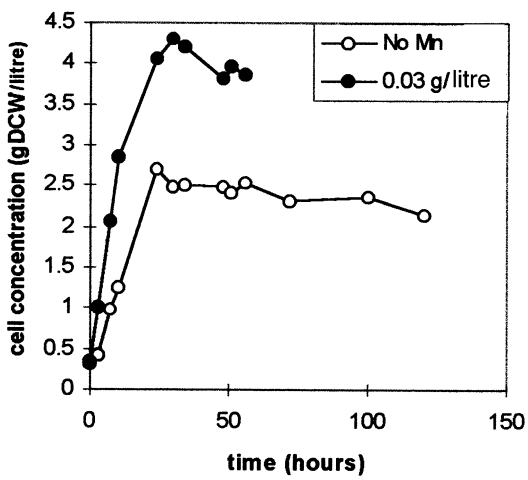

Fig. 4. Effect of $MnSO_4 \cdot H_2O$ addition to a concentration of 0.03 g/l on cell growth. Yeast extract supplementation is 0.5%w/v.

2. Materials and methods

2.1. Fermentation medium

The whey permeate was made up by reconstituting whey permeate powder (supplied by Kerry Foods, Listowel, Ireland) in distilled water. Yeast extract was obtained from Oxoid, Basinstoke, England, and manganese sulphate monohydrate (AnularR) from BDH Laboratory Supplies, Poole, England.

Table 1
Effect of yeast extract addition on fermentations without manganese supplementation

Yeast extract (%w/v)	Fermentation time (h)	Nitrogen beginning (%w/w)	Nitrogen end (%w/w)	Nitrogen used (%w/w)
0.5	120	0.072	0.045	0.027
1	72	0.122	0.091	0.031
2	52	0.233	0.192	0.041

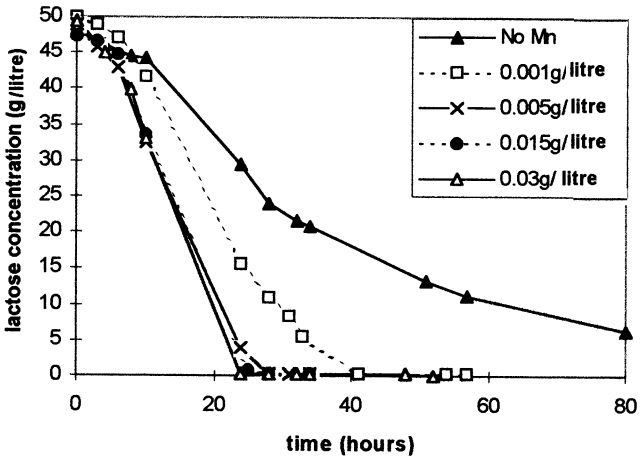

Fig. 5. Effect of $MnSO_4 \cdot H_2O$ addition on lactose utilisation. Yeast extract supplementation is 0.5%w/v.

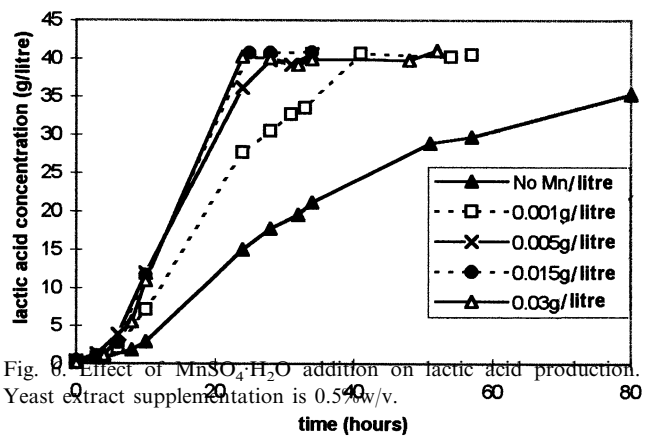

Fig. 6. Effect of $MnSO_4 \cdot H_2O$ addition on lactic acid production. Yeast extract supplementation is 0.5%w/v.

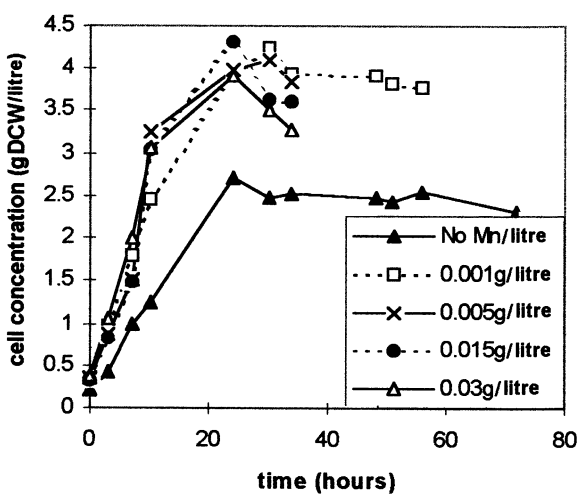

Fig. 7. Effect of $MnSO_4 \cdot H_2O$ addition on cell growth. Yeast extract supplementation is 0.5%w/v.

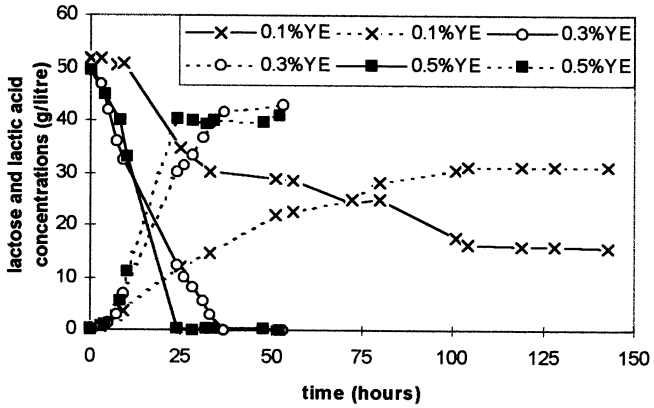

Fig. 8. Effect of yeast extract (YE%w/v) on lactose utilisation and lactic acid production when the fermentation is supplemented with 0.03 g/l $MnSO_4 \cdot H_2O$ (Solid lines represent lactose; dashed lines represent lactic acid).

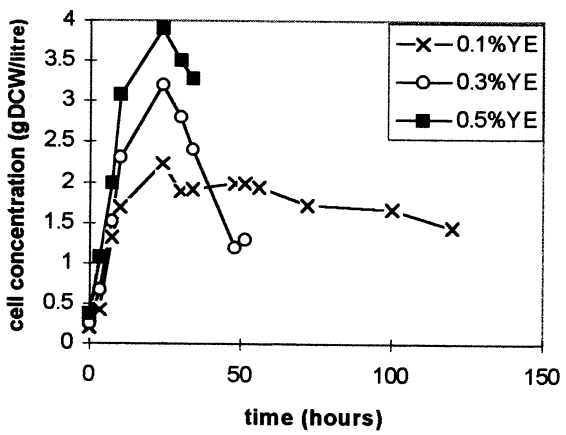

Fig. 9. Effect of yeast extract (YE%w/v) on cell growth when the fermentation is supplemented with 0.03 g/l $MnSO_4 \cdot H_2O$.

2.2. Microorganism

L. casei (strain 01) was obtained from Chr. Hansen's Laboratory, Cork, Ireland, and was stored at −80°C. The culture was defrosted under sterile conditions, and 0.1 ml of culture was added to 9 ml of sterile MRS broth and incubated for 14 h at the fermentation temperature. 1 ml of this broth was added to 100 ml of fermentation medium and incubated for a further 9 h. This was subsequently used to inoculate the fermentor at a level of 5%v/v.

2.3. Batch fermentation

The fermentor was a two litre autoclavable glass vessel with disc turbine impeller and automatic pH and temperature control. The fermentation procedure consisted of adding the permeate, yeast extract and manganese sulphate into the glass vessel and autoclaving it for 15 min at 121°C. After cooling, the fermentation temperature was set to 38°C and the fermentor was

Table 2
Effect of yeast extract addition on fermentations supplemented with 0.03 g/l $MnSO_4 \cdot H_2O$

Yeast extract (%w/v)	Fermentation time (h)	Nitrogen beginning (%w/w)	Nitrogen end (%w/w)	Nitrogen used (%w/w)
0.1	150	0.031	0.017	0.014
0.3	37	0.049	0.027	0.022
0.5	24	0.07	0.036	0.034

inoculated. The pH was set to pH 5.4 and controlled by addition of 4 M NaOH. Samples were taken throughout the fermentation for analysis.

2.4. Analysis

Lactose, glucose, galactose, and sodium lactate concentrations were measured with a HPLC system (Spectra series P100, USA) using an ion exclusion column (Aminex HPX-87H, BioRad, USA). Nitrogen concentrations were measured by the Kjeldahl method using the Kjeltec system (Tecator, Sweden). Cell concentration is expressed as gram dry cell weight per litre (g/l). Cells were centrifuged, washed with distilled water and dried at 105°C. Cell concentration was measured optically at 620 nm and converted to g/l using a calibration curve.

Sugar conversion is defined as mass of sugars utilised during fermentation divided by mass of initial sugars. The mass of sugars is the sum of the masses of lactose, glucose and galactose. Lactic acid yield is defined as mass of lactic acid produced during fermentation divided by mass of sugars utilised.

3. Results and discussion

3.1. Effect of yeast extract without manganese addition

Fermentations were performed initially without manganese addition in order to highlight the poor performance of *L. casei* in the absence of manganese ions. Fig. 1 illustrates the long fermentation times required to achieve high sugar conversion (98–99%) and lactic acid yield (88–93%). Increasing the yeast extract supplementation from 0.5 to 2%w/v increased cell growth (Fig. 2) and reduced the fermentation time from 120 to 52 h, however, this is still a long time. In addition, most of the nitrogen in the media remained unused (Table 1), which represents an additional source of impurities as well as being costly.

3.2. Effect of manganese addition with 0.5%w/v yeast extract supplementation

The 0.5%w/v yeast extract fermentation was supplemented with 0.03 g/l of $MnSO_4 \cdot H_2O$, which has been reported in the literature [5–7], in order to verify its beneficial effect on the fermentation. Fig. 3 illustrates this significant beneficial effect whereby the fermentation time required to obtain around 98% sugar conversion and 90% lactic acid yield, is reduced from 120 to 24 h. Fig. 4 shows that manganese has a major beneficial affect on cell growth rate and the maximum cell concentration obtained.

Further fermentations were then performed to investigate how the amount of $MnSO_4 \cdot H_2O$ added affected the fermentation. Fermentations were conducted in a range from 0.001 to 0.03 g/l reported in the literature.

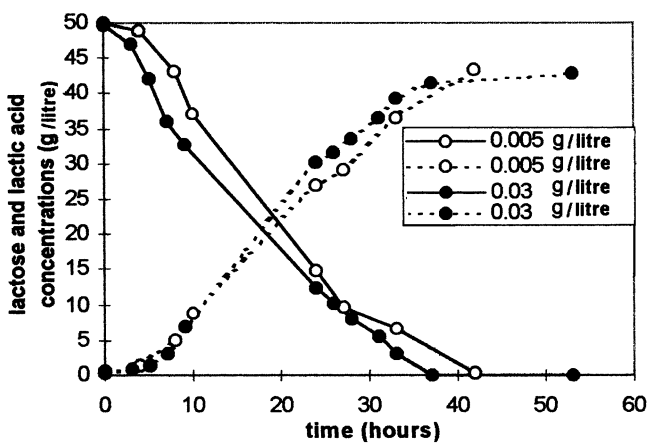

Fig. 10. Comparison of the effect of 0.005 and 0.03 g/l of $MnSO_4 \cdot H_2O$ addition on lactose utilisation and lactic acid production when the fermentation is supplemented with 0.3%w/v yeast extract (solid lines represent lactose; dashed lines represent lactic acid).

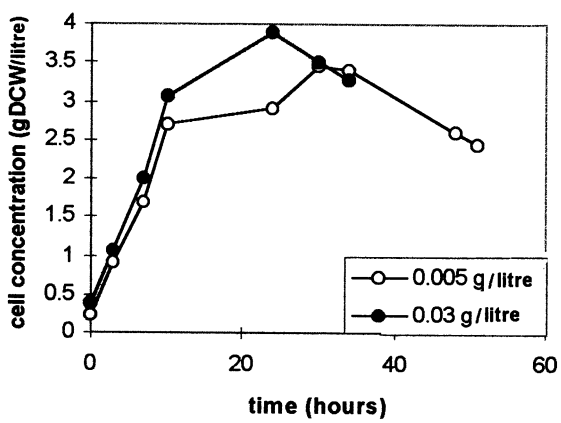

Fig. 11. Comparison of the effect of 0.005 and 0.03 g/l of $MnSO_4 \cdot H_2O$ addition on cell growth when the fermentation is supplemented with 0.3%w/v yeast extract.

The concentration of $MnSO_4 \cdot H_2O$ added could be lowerd to 0.005 g/l without any significant reduction in fermentation performance (Figs. 5 and 6). However, at 0.001 g/l $MnSO_4 \cdot H_2O$, the fermentation time increased to 41 h, even though the cell concentration during the fermentation was similar to the other fermentations supplemented with higher amounts of $MnSO_4 \cdot H_2O$ (Fig. 7). These experimental results show that lower concentrations of $MnSO_4 \cdot H_2O$ could be used than those reported in the literature [5–7] while still obtaining similar fermentation performance.

3.3. Effect of manganese addition on reducing yeast extract requirement

Fermentations were also performed to investigate if the amount of yeast extract added could be lowered in fermentations supplemented with 0.03 g/l $MnSO_4 \cdot H_2O$. Fig. 8 shows that the yeast extract supplementation could be lowered to 0.3%w/v while maintaining around 98% sugar conversion and 90% lactic acid yield, although the fermentation time was increased from 24 to 37 h. At 0.1%w/v yeast extract, the fermentation performance was very poor with only 67% sugar conversion after 150 h. Fig. 9 illustrates the expected beneficial effect of yeast extract addition on cell growth. Table 2 illustrates the advantage of using lower yeast extract in terms of the lower amount of unused nitrogen impurities remaining after fermentation.

Finally, a fermentation was performed at 0.3%w/v yeast extract and 0.005 g/l $MnSO_4 \cdot H_2O$ and its fermentation performance is compared with the fermentation supplemented with 0.03 g/l $MnSO_4 \cdot H_2O$ and the same yeast extract (Figs. 10 and 11). The performance of both fermentations is very similar with the higher $MnSO_4 \cdot H_2O$ addition performing slightly better.

4. Conclusions

Manganese addition has a very significant beneficial effect on the fermentation of whey permeate by *L. casei*. Addition of $MnSO_4 \cdot H_2O$ to a concentration of 0.03 g/l has been reported in the literature, however, the results provided in this work show that lower concentrations can be used while still obtaining a similar fermentation performance. Fermentations performed at 0.005 g/l $MnSO_4 \cdot H_2O$ gave a similar performance to 0.03 g/l. Addition of manganese allowed a lowering in the amount of yeast extract required. The yeast extract concentration was reduced to 0.3%w/v, while maintaining high sugar conversion and lactic acid yield.

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