Effect of Aeration on Organic Acid Production by *Propionibacterium shermanii*

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Propionibacteria have been reported as being oxygen sensitive bacteria. Nevertheless, in this work, we intend to show that *Propionibacterium shermanii* CDB 10014 is able to grow even at high volumetric oxygen transfer coefficients (K_La). Similar final cell concentrations were achieved in experiments at different K_La values, with increasing cell yield from 0.08 g/g cells in anaerobic conditions to 0.22 g/g at K_La 61 h^{-1}. The organic acid production pattern changes depending on the K_La. At K_La higher than 36 h^{-1}, propionic acid was not produced and acetic acid was the main product. At K_La higher than 20 h^{-1}, lactic acid began to be produced. In further studies, no important differences in the growth yield were observed in the pH range 5.5–7.0, while the cell production was enhanced by temperatures in the range 30–35 °C.

Introduction

Propionibacteria are used for vitamin B_{12} production (Florent, 1986), as a cheese-starter (Playne, 1985), in silage processing (Flores-Galagarza *et al.*, 1985), and as a probiotic (Mantere-Alhonen and Mäkinen, 1987). Propionibacteria also produce propionic acid, although this property has not yet been industrially used because the achieved acid concentration is low and the cell growth is inhibited by the products (Blanc and Goma, 1987b). Nevertheless, better productivities can be obtained by using high cell concentrations with a cell recycling system (Blanc and Goma, 1987a; Quesada-Chanto *et al.*, 1994a).

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Propionibacteria have been reported as being facultative anaerobes (Cummins and Johnson, 1992). They can grow in the presence of a very low oxygen concentration. De Vries *et al.* (1973) and Schwartz and Sporkenbach (1975) reported an anaerobic electron transport system in *Propionibacterium shermanii*, built up of components that almost attained the composition of a typical respiratory system of aerobes but was not adequate for a full aerobic function. These authors observed only a small respiration coefficient and concluded that this respiration was too small to abolish the anaerobic properties of the genus. It has also been reported that oxygen in concentrations close to zero could improve the cell yield (Schlegel, 1992) and affect the organic acid production (de Vries *et al.*, 1972).

These different reports are contradictory and difficult to evaluate, compare or repeat, since up to now no results have been reported in terms of volumetric oxygen transfer coefficient (K_La), partial oxygen pressure or oxygen concentration. The K_La is a useful tool to evaluate the effect of oxygen on the fermentation process (Pirt, 1975; Molwitz *et al.*, 1995). The aim of the present work was to study the effect of different K_La on growth and on the production of different organic acids by *Propionibacterium shermanii* and to compare this behaviour with the anaerobic culture. Furthermore, the effects of pH and temperature in aerobic conditions were also studied.

Material and Methods

Microorganism

Propionibacterium shermanii CDB-10014 (formerly *P. shermanii* PZ-3) was obtained from the Centro de Desenvolvimento Biotecnologico, SC, Brazil.

Media and fermentation conditions

The maintenance medium contained per litre deionized water: 20 g glucose, 2 g KH_2PO_4, 4 g $(NH_4)_2HPO_4$, 5 mg $FeSO_4 \times 7H_2O$, 10 mg $MgSO_4 \times 7H_2O$, 2.5 mg $MnSO_4 \times H_2O$, 10 mg $CaCl_2 \times 6H_2O$, 10 mg NaCl, 10 mg $CoCl_2 \times 6H_2O$, 10 g yeast extract (Oxoid, Brankstoke, England) and

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15 g agar, with a pH before autoclaving of 6.8–7.2. Except for the agar, the preculture medium had the same composition.

The fermentation medium contained per litre of deionized water: 70 g glucose, 1 g KH_2PO_4, 2 g $(NH_4)_2$ HPO_4, 5 mg $FeSO_4$ x $7H_2O$, 10 mg $MgSO_4$ x $7H_2O$, 2.5 mg $MnSO_4$ x H_2O, 10 mg $CaCl_2$ x 6H2O, 10 mg NaCl, 10 mg $CoCl_2$ x 6H2O, and 10 g yeast extract (Oxoid). The pH before autoclaving was 6.8–7.2.

The culture was transferred from a deep agar into an Erlenmeyer flask with 150 ml of the preculture medium and allowed to stand at 35 °C. After 72 h, 20 ml of this culture were inoculated in 150 ml of the preculture medium and allowed to stand at 35 °C. After 24 h, the bioreactor was inoculated with this preculture at a volume ratio of 15%.

The fermentations were carried out in batch mode in a Biostat Q system (B. Braun Biotech, FRG). The influence of the different K_La values, obtained by varying aeration and impeller speed, was evaluated at 35 °C and pH 6.5. In all cases, the pH was automatically controlled by adding 7 M NaOH. The effect of the pH and temperature was studied at K_La 20 h^{-1}. The studied pH range was 5.5–7.0, at 35 °C. Temperatures varying from 25 to 37 °C, at pH 6.5, were tested. In all runs, the fermentation time was limited to 75 hours.

K_La determination

The volumetric oxygen transfer coefficient (K_La) of each fermentation was estimated, before inoculation, by the method described by Pirt (1975).

Analytical methods

Cell growth was measured using optical density (560 nm) and cell dry biomass. Glucose and organic acid concentrations were measured by HPLC (Merck-Hitachi) equipped with an ORH-801 Column (Interaction Chromatography, USA) and an RI detector (Knauer, FRG). H_2SO_4 0.005 M was used as mobile phase.

Results and Discussion

Although oxygen has been considered detrimental to Propionibacteria, our results have shown that *Propionibacterium shermanii* CDB 10014 can grow aerobically even at high oxygen transfer coefficients (K_La). These results suggest that this strain may have a complete functional aerobic respiration system that is insufficient, however, to support growth on solid medium in a Petri dish, where the atmospheric oxygen pressure is toxic.

In an experiment with K_La = 9 h^{-1}, the oxygen partial pressure (pO_2) was promptly 0% and the glucose consumption was identical to that observed in anaerobiosis, suggesting a fermentative metabolism of the glucose (Table I). As K_La was increased to 20 h^{-1}, the pO_2 takes 20 h to achieve the 0%. With K_La $\geq$ 36 h^{-1}, the pO_2 was always higher than 50%. This fact and the similar degradation rate of the initial substrate suggest an oxidative metabolism of glucose.

Table I. Final cell and products concentrations in the fermentation of glucose by *Propionibacterium shermanii* CDB 10014, after 75 hours, at different K_La (temperature = 35 °C; pH = 6.5) (ΔS, glucose consumption; X, dry cell weight; HPr, propionic acid; HAc, acetic acid; HLac, lactic acid).

K_La [h^{-1}]	ΔS (%)	X [g/l]	HPr [g/l]	HAc [g/l]	HLac [g/l]	HPr/HAc Ratio
0	74	4.5	17.0	2.6	0	6.5
9	76	4.1	10.0	8.4	0	1.2
20	47	5.3	2.8	13.8	0.7	0.2
36	36	4.7	0	9.5	2.5	0
53	36	5.2	0	8.5	6.1	0
61	37	5.7	0	8.6	4.8	0

As already mentioned, Schlegel (1992) reported that low dissolved oxygen values can increase the cell yield in Propionibacteria. In our work, this effect occurs even at the high dissolved oxygen concentrations observed with intense oxygen supply, increasing from 0.08 g/g in anaerobic condition to 0.22 g/g at K_La 61 h^{-1} (Fig. 1). This correlation between cell yield and K_La reforzed the idea of a oxidative metabolism of glucose in aerobic conditions by propionibacteria.

As is well known, in anerobiosis, propionic acid is the main fermentation product. The presence of oxygen, however, leads to changes in the organic acid formation pattern (Table I). With K_La values up to 20 h^{-1}, decreasing propionic acid and increasing acetic acid final concentrations were observed, resulting in a drop of the propionic/acetic acid ratio (HPr/HAc). In a previous work, Que-

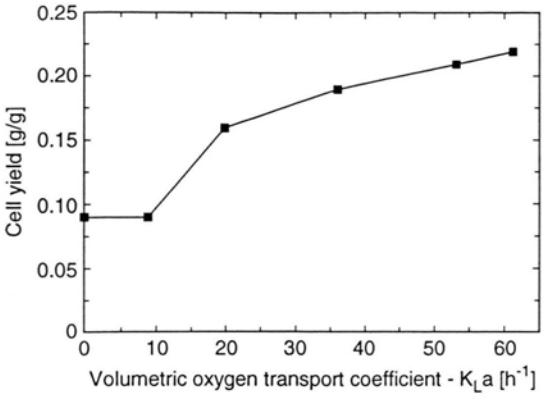

Fig. 1. Effect of the oxygen supply (K_La) on the cell yield (g dry cell mass/g glucose consumed) of *P. shermanii* CDB 10014 after 75 h.

sada-Chanto *et al.* (1994b) reported the same behaviour for *Propionibacterium acidipropionici* DSM 8250. The fact that they used other strain and a low aeration rate may be the reason why they did not achieve a point where no propionic acid was produced, as shown in the present work for $K_La \geq 36\ h^{-1}$. Lactic acid was also produced in fermentations with $K_La \geq 20\ h^{-1}$. When K_La was raised, significant final concentrations of lactic acid were achieved. It is interesting to mention that, in the specialised literature, only one reference to the production of this acid by Propionibacteria is found (Georgopapadakau *et al.*, 1976). These researchers used a mutant strain in anaerobic conditions.

We also tested the effect of pH and temperature on the process in aerobic conditions at a K_La value of 20 h^{-1}. This K_La was chosen because in this condition the three organic acids are produced and, thus, the effect of these variables could be evaluated not only on the growth but also on the formation of acids.

In the pH range tested, although no important difference in the cell growth has been observed after 75 hours, the highest cell yield (0.25 g/g) was achieved at pH 6.0. The results have also shown that an increase in the pH value enhances propionic acid production and, consequently, the HPr/HAc ratio (Table II).

Table II. Final cell and products concentrations in the fermentation of glucose by *Propionibacterium shermanii* CDB 10014, after 75 hours, at different pH (K_La = 20 h^{-1}; temperature = 35 °C).

pH	ΔS [g/l]	X [g/l]	HPr [g/l]	HAc [g/l]	HLac [g/l]	HPr/HAc Ratio
5.5	29.0	4.7	1.4	11.8	0	0.12
6.0	27.0	5.4	3.3	11.7	0	0.28
6.5	38.0	5.3	6.2	13.8	0.7	0.45
7.0	30.0	5.1	8.2	7.0	0.8	1.17

For abbreviation see legend of Table I.

Table III. Final cell and products concentrations in the fermentation of glucose by *Propionibacterium shermanii* CDB 10014, after 75 hours, at different temperatures (K_La = 20 h^{-1}; pH = 6.5).

Temperature [°C]	ΔS (%)	X [g/l]	HPr [g/l]	HAc [g/l]	HLac [g/l]	HPr/HAc Ratio
25	24.0	3.8	0.5	6.6	0	0.08
30	20.0	5.0	1.4	14.5	0	0.10
35	24.4	6.1	3.6	14.8	2.8	0.24
37	35.0	5.3	6.8	13.3	3.4	0.51

For abbreviation see legend of Table I.

In the temperature range studied, one can observe that at 25 °C the achieved cell concentration was the lowest one, while the highest one was obtained at 35 °C (Table III). The highest cell yield (0.25 g/g) was obtained at 30 and 35 °C. In anaerobic conditions Quesada-Chanto, *et al.* (1997) achieved the highest cell yield also in this temperature range. The results in Table III also show that increasing the temperature led to higher propionic acid concentrations, increasing, consequently, the HPr/HAc ratio.

This work presents preliminary but important results, that demonstrate that propionibacteria, in this case *P. shermanii* CDB 10014, can grow under high oxygen supply conditions in contrast to most of the results cited in the literature.

Acknowledgements

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Blanc P. and Goma G. (1987a), Propionic acid fermentation: improvement of performances by coupling continuous fermentation and ultrafiltration. Bioprocess Eng. **2,** 137–139.

Blanc P. and Goma G. (1987b), Kinetics of inhibition in propionic acid fermentation. Bioprocess Eng. **2,** 175–179

Cummins C. S. and Johnson J. L. (1992), The Genus *Propionibacterium*. The Prokaryotes. (Balow A. ed.), Vol. **1**. Springer Verlag, New York, Berlin, Heidelberg, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest. pp. 834–849.

de Vries W., van Wijck-Kapteijn W. M. C. and Stouthamer A. H. (1972), Influence of oxygen on growth, cytochrome synthese and fermentation pattern in propionic acid bacteria. J. Gen. Microbiol., **71**, 515–524.

de Vries W., van Wijck-Kapteijn W. M. C. and Stouthamer A. H. (1973), Generation of ATP during cytochrom-linked anaerobic electron transport in propionic acid bacteria. J. Gen. Microbiol., **76**, 31–41.

Florent J. (1986), Vitamins. Biotechnology (Rehm H. J. and Reed, R. eds.), Vol **4**, VCH, Weinheim, pp. 119–158.

Flores-Galagarza R. A., Glatz B. A., Bern C. J. and Fossen L. D. (1985), Preservation of high-moisture corn by microbial fermentation. J. Food Protection **48**, 407–411.

Georgopapadakou N. H., Petrillo J., Scott A. I. and Low B. (1976), Tetrapyrrole biosynthesis: N-methyl-N'-nitrosoguanidina induced mutants of *Propionobacterium shermanni*. Genet. Res. 28, 93–100.

Mantere-Alhonen S. and Mäkinen E. (1987), A new type of sour milk with Propionibacteria. Meijeritieteellinen Aikakauskirja **45,** 49–61.

Molwitz M., Silva S. S., Ribeiro J. D., Roberto I. C., Felipe M. G. A., Prata A. M. and Mancilha I. M. (1996), Aspects of the Cell Growth of *Candida Guilliermondii* in sugar cane bagasse hydrolysate. Z. Naturforsch., **51c,** 404–408.

Pirt S. J. (1975), Principles of Microbe and Cell Cultivation. Blackwell Scientific Publications. Oxford.

Playne M. J. (1985), Propionic and butiric acid. Comprehensive biotechnology, (Moo-Young, M., Editor) vol 3. Pergamon Press, pp 731–759.

Quesada-Chanto A., Afschar A. S. and Wagner F. (1994a), Microbial production of propionic acid and vitamin B_{12} using molasses or sugar. Appl. Microbiol. Biotechnol., **41**, 378–383.

Quesada-Chanto A., Afschar A. S. and Wagner F. (1994b), Optimization of a *Propionibacterium acidipropionici* continuous culture utilizing sucrose. Appl. Microbiol. Biotechnol., 42, 16–21.

Quesada-Chanto A., Schroeder A. G., Schmid-Meyer A. C., López J. A., Silveira M. M. and Jonas R. (1997), Organic acid production by *Propionibacterium shermanii*: effect of pH, temperature and vitamin-nitrogen source. Z. Naturforsch. **52c,** 193–196.

Schlegel H. G. (1992), Allgemeine Mikrobiologie. 7 ed. Georg Thieme Verlag, Stuttgart New York, pp. 304–307.

Schwartz A. C. and Sporkenbach J. (1975), The electron transport system of the anaerobic *Propionibacterium shermanii*. Cytochrome and inhibitor studies. Arch. Microbiol. 102, 261–273.