การผลิตกรดแลคติคแบบต่อเนื่องจากน้ำตาลไซโลส โดย *Lactococcus lactis*

Continuous Lactic Acid Production from Xylose by *Lactococcus lactis*

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บทคัดย่อ

Lactococcus lactis IO-1 (JCM 7638) ถูกเลี้ยงแบบต่อเนื่องโดยมีน้ำตาลไซโลส (10 กรัมต่อลิตร) เป็นแหล่งคาร์บอน ผลการทดลองพบว่า ผลได้ของโมลแลคเตทที่อัตราการเจือจาง 0.1 ถึง 0.5 ต่อชั่วโมง ไม่มีความแตกต่างกันอย่างมีนัยสำคัญ ขณะที่ผลได้ของโมลฟอร์เมทและอะซิเตทมีค่าสูงสุดที่อัตราการเจือจาง 0.2 ต่อชั่วโมง คาร์บอนที่ได้คืนมามีค่าระหว่าง 47 - 97 เปอร์เซ็นต์ การที่คาร์บอนที่ได้คืนมามีค่าไม่ถึง 100 เปอร์เซนต์อาจเป็นเพราะคาร์บอนถูกเปลี่ยนไปเป็นผลิตภัณฑ์อื่นในไพรูเวทเมตาบอลิซึม ซึ่งไม่ได้ถูกวัดในการศึกษานี้

Abstract

Under xylose-limited (10 g l^{-1}) continuous culture of *Lactococcus lactis* IO-1 (JCM 7638), the molar product yield of lactate did not show any significant difference at dilution rate of 0.1 to 0.5 h^{-1} while the molar product yields of formate and acetate were highest at dilution rate of 0.2 h^{-1}. Carbon recovery ranged from 47 - 97%. This was possibly due to part of the utilized carbon being converted to other products of pyruvate metabolism which were not quantified in this study.

คำสำคัญ: น้ำตาลไซโลส, *Lactococcus lactis*, การเลี้ยงแบบต่อเนื่อง

Keywords: Xylose, *Lactococcus lactis*, Continuous culture

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Introduction

Lactic acid has been produced commercially by fermentation process since 1881 and its fermentative production accounts for half of the world production (Vickroy, 1985). Lactic acid is used as an acidulant, flavour and preservative in food, pharmaceutical, leather and textile industries. In addition it is also used for the production of basic chemicals and for polymerization to biodegradable polylactic acid (PLA) which is used for medical applications such as sutures and clips for wound closure or posthetic devices (Hofvendahl and Hahn-Hägerdal, 2000).

There is considerable interest in the use of hydrolyzed lignocellulose from crop residues as fermentation substrates because such hydrolysates are rich in both glucose and xylose (Kanagachandran et.al., 1997; Aristidou and Penttilä, 2000). Thus, it is important that any potential fermentation organism can utilize both carbon substrates to maximize carbon conversion to product and to minimize the difficulties of effluent treatment.

Previous works reported that *Lactococcus lactis* IO-1 is such a bacterium which can produce L-lactate at the expense of both glucose and xylose but that xylose utilization is suppressed by the presence of glucose (Kanagachandran et.al., 1997; Ishizaki et. al., 1992; Ishizaki et. al., 1993). To facilitate the development of xylose utilization it is necessary to elucidate major physiological factors influencing sugar catabolism and product formation. The aim of this work was to study the effect of dilution rate on product formation in xylose continuous cultures.

Materials and methods

Microorganism

Lactococcus lactis IO-1 (JCM 7638) from stock culture was transplanted into a sterile Borosilicate culture tube containing 9 ml of sterile thioglycolated medium (Difco, USA) at two weeks intervals and stored in a refrigerator at 4°C.

Medium

The basal medium was composed of (per litre of distilled water) 5.0 g yeast extract (Oxoid, England), 5.0 g peptone (Oxoid) and 5.0 g NaCl (BDH, England). This medium was supplemented with 10 g xylose l^{-1}.

Inoculum

The stock culture was revived by incubation in 10 ml thioglycolated medium for 18 h at 37°C in a static incubator. The 18-h culture (10 ml) was then transferred into 100 ml of basal medium containing 10 g xylose l^{-1} and incubated at 37°C with agitation 150 rpm for 3 h. An inoculum (5% by volume) was used to initiate the continuous cultures.

Culture conditions

All continuous fermentations throughout this work were conducted in a Bioflow III (New Braunswick, USA) 2.5-litre fermenter with the working volume of 800 ml. The culture was agitated at 400 rpm without aeration at a temperature of 37°C. The pH of the cultures was monitored using an in situ pH probe and maintained at pH 6.0 by automatic addition of NaOH 2.5 mol l^{-1}. Steady-state conditions were indicated by stable of biomass, substrate and product levels.

Analytical methods

Bacterial growth was monitored by spectrophotometric measurements at 562 nm (Unicam 5625 spectrophotometer, UK) and converted to cell dry weight from a standard calibration curve. Determination of xylose and products (lactate, formate and acetate) from fermentation supernatant was performed by HPLC using an Aminex HPX 87H$^+$ column (300 mm (78 mm, Bio-Rad Lab, CA, USA) under the following conditions: a temperature of 50°C, mobile phase H_2SO_4 5 mmol l^{-1}, a flow rate of 0.57 ml min^{-1}, and a refractometer detector (refracto Monitor II, Milton Roy, England).

Results

In continuous culture using 10 g xylose l^{-1} as a carbon source, the dilution rate was varied between 0.05 and 0.5 h^{-1}. The results show that *Lactococcus lactis* IO-1 produced at least three organic acids; acetate, formate and lactate (Fig. 1). It was observed that wall growth occurred after around 120 h of the operation and at the highest dilution rate, wall growth appeared early at around 70 h of the culture. It was also observed that after the appearance of wall growth cells did not only attach at the surface of the fermenter but they also flocked in liquid medium even though a pre-coating on fermenter jar, 2% solution of dimethyl-dichlorosilane in octamethylcyclotetra siloxane (BDH), was used. The molar product yields (mol product mol^{-1} of xylose utilized) of lactate, formate and acetate at steady-state were calculated and are summarized in Fig. 2. Under steady-state conditions the molar product yields of lactate, formate, and acetate at a dilution rate of 0.05 h^{-1} were lower than those at dilution rates of 0.1 and 0.2 h^{-1} (Fig. 2). The molar product yields of formate and acetate were increased with increasing dilution rate up to 0.2 h^{-1} and then markedly decreased at the highest dilution rate while the molar product yields of lactate did not show any significant difference between dilution rates of 0.1 and 0.5 h^{-1}. In addition at dilution rates of 0.2 and 0.5 h^{-1}, the cell concentration was higher than at 0.05 and 0.1 h^{-1} (Table 1). The carbon recovery at dilution rates of 0.05, 0.1, 0.2 and 0.5 h^{-1} was 47, 79, 97 and 70% respectively (Table 1).

Discussion

The results show that *L. lactis* IO-1 produced mixed acids (lactate, formate and acetate) under xylose limited conditions. The product formation profile in this study was similar to that of *L. lactis ssp. lactis* 65.1, ATCC 19435 and AS 211 which were grown in continuous culture under maltose limited conditions (Sjäberg et.al., 1995). It has also been reported that a range of product patterns, from mixed acids to more pronounced lactate formation, was found with mutants defective in enzymes important for transport and metabolism (Crow and Thomas, 1984) and maltose-assimilating *L. lactis ssp. lactis* 65.1 (Sjäberg and Hahn-Hägerdal, 1989). The mixed acid production of *L. lactis ssp. lactis* from maltose has been suggested to occur in order to maintain intracellular adenosine nucleotide concentration at the desired level (Sjäberg et.al., 1995). Carbon recovery (Table 1) was less than 100%. This was possibly due to part of the utilized carbon being converted to other products in pyruvate metabolism such as ethanol, diactyl, acetoin, acetaldehyde and 2, 3-butanediol (Cocaign-Bousquet et.al., 1996), which were not detected in this study. Biomass yields at dilution rate of 0.2 and 0.5 h^{-1} were higher than those of 0.05 and 0.1 h-1, indicating the dilution rate has an effect on cell concentration in the system.

During xylose metabolism by the phosphoketolase pathway one mole of pyruvate and one mole of acetate are produced per mole of xylose utilized (Kandler, 1983). Thus, xylose metabolism by the phosphoketolase pathway produces acetate regardless of how pyruvate is further metabolized. Metabolism of pyruvate via pyruvate dehydrogenase (PDH) and pyruvate formate lyase (PFL) pathways could produce additional acetate resulting in the generation of supplementary ATP. Hence, xylose fermentation via the phosphoketolase pathway would produce a molar yield of acetate greater than or equal to one utilized (Kandler, 1983). Below 0.5 h^{-1} dilution rate, the molar product yields of acetate were high and at 0.1 and 0.2 h^{-1} dilution rates the molar product yields of acetate were equal to or greater than one respectively (Fig. 2). This could be due to xylose metabolism by the phosphoketolase pathway as explained above.

The molar product yield of lactate was low at all dilution rates studied (Fig. 2) demonstrating a diminished LDH (lactate dehydrogenase) pathway. It appeared that *L. lactis* IO-1 generated additional ATP

by the formation of acetate under these conditions. Furthermore, the diminished LDH pathway would not have sufficient ability to recycle all the coenzymes necessary and other pathways (to produce ethanol) or enzymes (NADH oxidizing) involved in coenzyme recycling would have had to be operational to maintain energetic equilibrium.

The molar product yield of acetate was less than one at 0.05 and 0.5 h^{-1} dilution rates (Fig. 2) which could not be explained by the phosphoketolase pathway of xylose metabolism. It appeared that under these conditions xylose metabolism in *L. lactis* IO-1 deviated from the predicted pathway. However this work did not examine the pathway of xylose utilization by *L. lactis* IO-1 under these conditions.

The observations from carbon-limited continuous culture suggest that further studies into the control of product formation in *L. lactis* IO-1 is required. Carbon-limitation was proposed to be the controlling factor for pyruvate metabolism in continuous culture and it was claimed that the mechanism of pyruvate metabolism was co-ordinated by both the intracellular level of glycolytic intermediates and by enzymes involved in pyruvate production and metabolism (Cocaign-Bousquet et.al., 1996).

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Table 1 Biomass yields and carbon recovery in steady-state continuous cultures of *Lactococcus lactis* IO-1 in xylose (10 g l^{-1}) at various dilution rates.

Dilution rate (h^{-1})	Biomass yield (g dry cell weight g^{-1} xylose utilized)	Carbon recovery (%)
0.05	0.09±0.03	46.8±0.10
0.10	0.09±0.03	79.3±0.09
0.20	0.14±0.05	97.4±0.09
0.50	0.14±0.04	69.7±0.08

: The results were expressed as mean and standard deviations for 4 values.

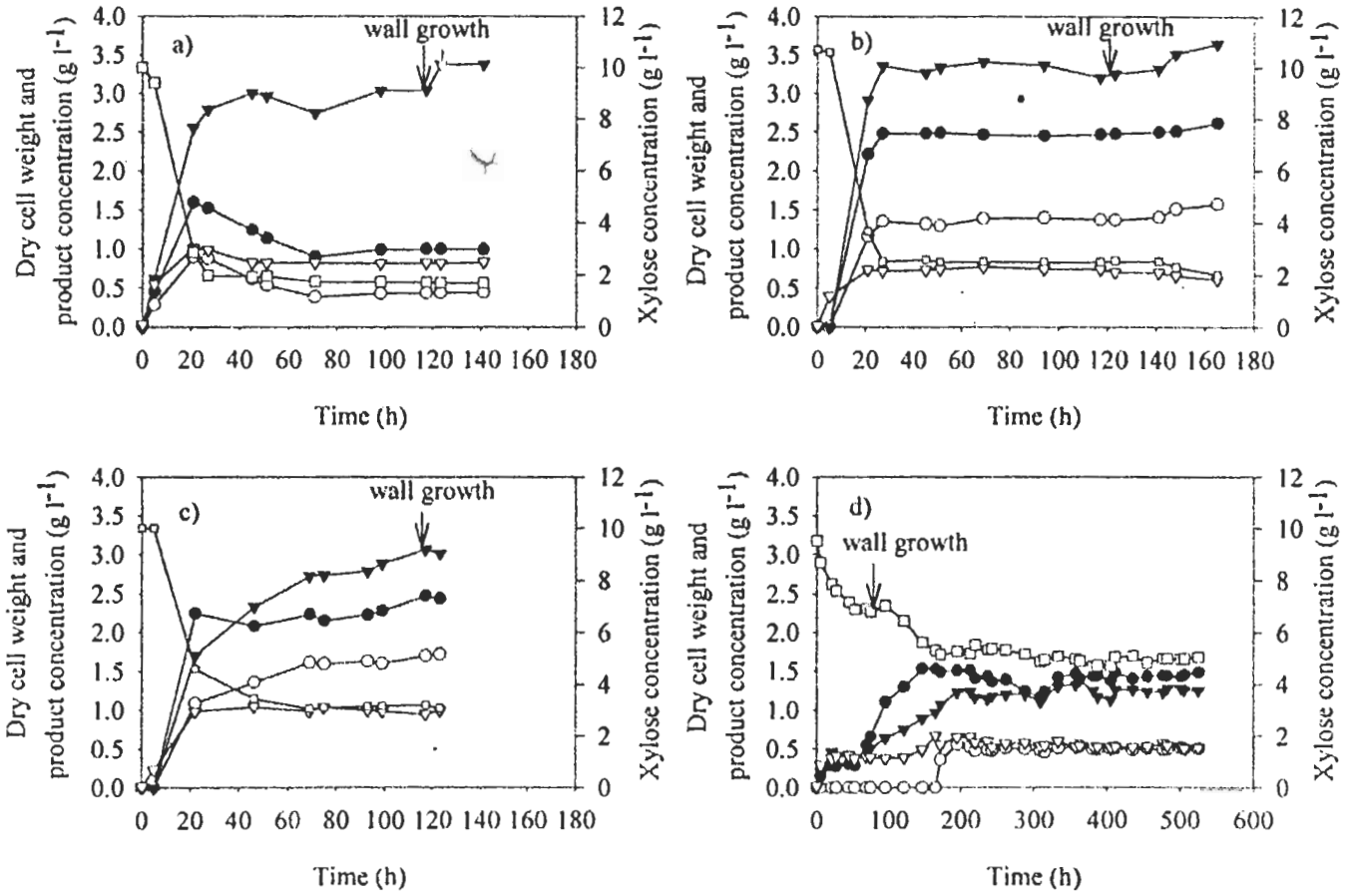

Fig. 1 Product and dry cell weight profiles in continuous culture of *Lactococcus lactis* IO-1 on xylose medium (basal medium supplement with 10 g xylose l^{-1} at dilution rat of a) 0.05, b) 0.2 and d) 0.5, b 0.1, c) 0.2 and d) 0.5 h^{-1}. Symbols: lactate (●). formate (○), acetate (▼), dry cell weight (▽) and xylose (□)

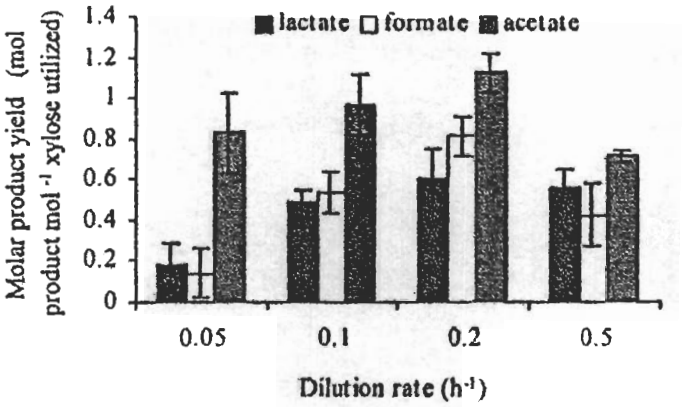

Fig. 2 Effect of dilution rate on molar product yields at steady-state contionuous cultures of *Lactococcus lactis* IO-1.