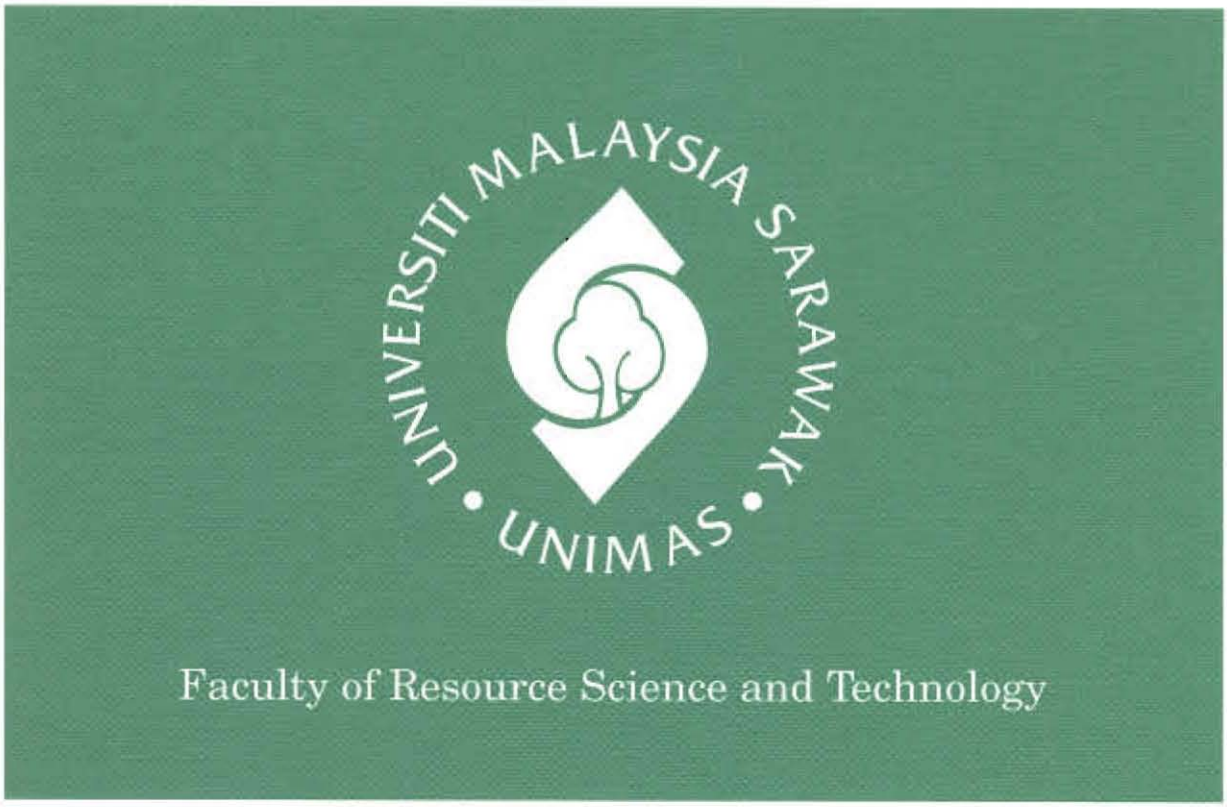

THE UTILIZATION OF CALCIUM CARBONATE AS GROWTH PROMOTER IN LACTATE FERMENTATION

Sadi Bin Sujang

QR
92
B3
S124
2004

Bachelor of Science with Honours
(Resource Biotechnology)
2004

Pusat Khidmat Maklumat Akademik
UNIVERSITI MALAYSIA SARAWAK
94300 Kota Samarahan

P.KHIDMAT MAKLUMAT AKADEMIK
UNIMAS
1000126465

THE UTILIZATION OF CALCIUM CARBONATE AS GROWTH PROMOTER IN LACTATE FERMENTATION

Sadi Bin Sujang

This project is submitted in partial fulfilment of the requirements for the degree of Bachelor of Science with Honours (Resource Biotechnology)

Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2004

CONTENTS

The Utilization of Calcium Carbonate as Growth Promoter in Lactate Fermentation

SADI BIN SUJANG

Resource Biotechnology Program, Faculty of Resource Science and Technology, UNIMAS
94300 Kota Samarahan, Sarawak, Malaysia.

ABSTRACT

A homofermentative bacterium, *Lactococcus lactis* IO-1, produces purely L-lactate on glucose. Iu present studies, Calcium Carbonate ($CaCO_3$) was utilized as a growth promoter in order to enhance lactate productivity. Addition various concentration (w/v) of $CaCO_3$ to culture at the 8 h of fermentation process had a positive effects on both biomass and lactate production. After 24 hrs, pH of the fermentation culture was between 5.0 – 6.0 and the highest lactate production was 32.08 g/L with the glucose consumption of 25.49 g/L .

Key words: Glucose, fermentation, calcium carbonate, lactate.

ABSTRAK

Homofermentatif bakteria, Lactococcus lactis IO-1, menghasilkan L-laktat tulen melalui fermentasi glukosa.. Dalam kajian yang telah dijalankan, kalsium karbonat ($CaCO_3$) digunakan sebagai agen penggalak pertumbuhan bakteria disamping meningkatkan penghasilan laktat.. Penambahan berbagai (w/v) kepekatan kalsium karbonat ke dalam kultur fermentasi pada masa ke 8 jam proses fermentasi,menunjukkan kesan positif terhadap jumlah biomass dan penghasilan laktat. Selepas 24 jam, pH kultur fermentasi didapati pada paras di antara 5.0 – 6.0 dengan jumlah tertinggi laktat sebanyak 32.08 g/L dan penggunaan glukosa sebanyak 25.49 g/L.

Kata kunci : *Glukosa, fermentasi, kalsium karbonat, laktat.*

1.0 INTRODUCTION

Lactate fermentation is the most important of commercial fermentation processes. Since lactate fermentation from pyruvate is not accompanied by the release of carbon dioxide, lactate fermentation has a major advantage over all other fermentation from the viewpoint of the release of carbon dioxide to environment (Ishizaki & Vontaveesuk, 1996). For the most part of industrial fermentation, homofermentative lactic acid bacteria are the main source of industrial lactic acid. Lactate occupies important role in wide range of industrial application, for instance, food and beverages industries, pharmaceuticals, cosmetics and biopolymers production.

The strain of *Lactococcus lactis* IO-1 (JCM 7638) has been employed for the production of L-lactic acid in enzyme hydrolyzed sago starch (Ishizaki *et al.*, 1990). *L. lactis* IO-1 is a mesophillic L-lactate producing coccus.. The optimal temperature for the growth as well as fermentation was 37°C and the strain tolerant in 6.5% natrium chloride (NaCl).

The strain fermented various carbohydrates to produce L-lactate with high conversion rate and no other volatile fatty acid was detected. Starches were reported as suitable medium for fermentation, for instance sago starch. Starch is first enzymatically liquefied and saccharified to glucose, which fermented by microorganisms to lactic acid (Yan *et al.*, 2001). Moreover, lactate fermentation using sago starch as a carbon source which produces polylactate shows a great potential (Ishizaki, 2000).

In batch fermentation, the initial pH is set before cultivation started. According to Mirdamadi *et al.* (2002) fermentation without pH control, the pH of fermentation broth dropped to below 3, and the volumetric productivity was slowed by the inhibitory effect of lactic acid. In addition, $CaCO_3$ is the best neutralizing agents, due to the highest glucose consumption rate and lactic acid production yield (Mirdamadi *et al.*, 2002). Besides that, it can enhance cell adsorption (Sonomoto, Chinachoti, Endo, & Ishizaki, 2000). On the other hand, the addition of Ca^{2+} to medium showed a stimulating effect on the production of nisin Z by *Lactococcus lactis* (Matsusaki, Endo, Sonomoto, & Ishizaki, 1996). Sirisansaneeyakul *et al.* (2003) reported that $CaCO_3$ has been utilized in preparation of the starch slurries during the enzymatic hydrolysis of cassava starch as a fermentation media. Furthermore, calcium is required for the growth of many bacteria species and it can stabilize cell wall structure by bridging carboxyl groups in peptidoglycan chain (Weinberg, 1977). Hence, $CaCO_3$ was utilized as a growth promoter in lactate fermentation.

2.0 OBJECTIVES

The objectives of this study were to establish $CaCO_3$ as growth promoter in lactate fermentation due to enhance lactate production.

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Sago Starch

Industrial grade fine sago starch was obtained from local market.

3.1.2 *Lactococcus lactis*

The microorganisms *Lactococcus lactis* IO-1 JCM 7638 (Japanese Collection of Microorganism) was used throughout the experiments.The strain tolerant in 6.5% natrium chloride (NaCl) with optimum growth at temperature 37°C (Ishizaki *et al.*, 1990)

3.1.3 Enzymes for Enzymatic Hydrolysis

The enzymes used for hydrolysis was Termamyl-120L (Thermostable amylase from *Bacillus licheniformis*, 120 KNU/g) and Dextrozyme (a mixture of glucoamylase from *Aspergillus niger* and pullulanase from *Bacillus acidopullulyticus*, 225/75L) supplied by Novo Nordisk.

3.1.4 Culture Medium

The basal medium for fermentation was glucose broth consists of 5 g/L NaCl (UNIVAR Australia), 5 g/L polypeptone, 5 g/L yeast extract (Difco, USA) 60 g/L hydrolysed sago starch and distilled water. The same medium with 10 g/L glucose was used for inoculum preparation and 5% (v/v) of inoculum was used for a fermentation (Ishizaki & Ohta, 1989).

3.1.5 Calcium Carbonate ($CaCO_3$)

Calcium Carbonate ($CaCO_3$) used throughout the experiments was in powder form. $CaCO_3$ (PC laboratory reagent, $CaCO_3$ M=100.09 g/mol) obtained from UNIMAS Biochemistry Laboratory.

3.1.6 Fermentor

Fermentation carried out in 250 mL shake flask with a working volume of 200 mL for 24 at 100 rpm in a Water Bath Shaker (Shel. Lab® Sheldon Manufacturing Inc.) at 37°C.

3.2 METHODS

3.2.1 Enzymatic Hydrolysis of Sago Starch

The protocol of the enzymatic hydrolysis of sago starch done based on methods reported by Bujang, Adeni and Jolhery (1999, 2001). Sago starch was hydrolysed utilizing commercial enzyme such as Termamyl and Dextrozyme (as stated in previous paragraph). The hydrolyzed sago starch (HSS) then centrifuged with centrifugal model KUBOTA 8800. The HSS centrifuged for 15 minutes at temperature 4°C and rotation rate was 6000 rpm. After that, HSS was autoclaved at 121°C for 15 minutes and stored in oven at temperature 70°C before being use.

3.2.2 Stock Culture Activation

Stock culture (stored in –80°C) was thawed in room temperature then incubated in Thioglycollate (TGC) medium without dextrose (MERCK, Germany) at pH 6.0 for 18 hours at temperature 37°C.

3.2.3 Parameters and Medium Preparation

The medium was prepared in shake flask. The initial pH of medium was set at 6.0 and was adjusted using 2 M sulphuric acid (H_2SO_4) and 1 M natrium hydroxide (NaOH). Medium then autoclaved at 121°C, 15 psi for 15 minutes. 1 mL starter culture transferred to 19 mL seed culture at pH 6.0 in universal bottle and incubated at 37°C for 6 hours prior to start the fermentation process.

3.2.4 The Addition of Calcium Carbonate ($CaCO_3$)

The treatments of $CaCO_3$ was conducted in two experiments. First experiment, the addition of $CaCO_3$ was done at 0 hour (initial stage), mixed together in the fermentation medium. Second experiment, $CaCO_3$ was added at 8 hours (exponential stage) of fermentation process and was sterilized prior to add in the fermentation culture. The concentration of $CaCO_3$ added into fermentation culture was 1% w/v, 2% w/v and 3% w/v. All the experiments were run parallel in triplicates.

3.2.5 Sampling

Fermentation process was operated for 24 hours. Aliquots, 10 mL of broth taken from shake flask every 6 hours in order to determine the glucose uptake, cell growth and lactic acid production.

3.2.6 Reducing Sugar Analysis

The reducing sugar assay conducted based on dinitrosalicyclic acid (DNS) method. Samples are diluted at 600 times of dilution factor. 3 mL of DNS (3, 5-dinitrosalicyclic

acid) reagent added to 3 mL aliquot of test solution. The mixture then heated for 15 minutes in boiling water. The test tube quickly cooled under chilled water and followed by the addition of 1 mL 40% Rochelle salts (potassium sodium tartarate - to stabilize the color) before being measured by optical absorbance at 575 nm on a spectrophotometer (JENWAY). A standard curve was obtained using standard glucose to estimate glucose equivalent values.

3.2.7 Dry Cell Weight Determination

10 mL sample taken from the fermentation medium was centrifuged twice at 6000 rpm for 15 minutes at 4°C in centrifuge tube (weight known) in centrifugal model KUBOTA 8800. The supernatant then collected in a universal bottle while the cells resuspended in steriled water. The cells centrifuged again at 6000 rpm for 15 minutes at 4°C and then water was discarded. The cells dried in oven at 70°C overnight, until the weight is constant. The equation for Dry Cell Weight (DCW) determination were as follows;

$$\text{DCW (g/L)} = \frac{\text{Weight of dried tube + cells (g)} - \text{Weight of tube (g)} \times 10^3}{\text{Sample volume (mL)}}$$

3.2.8 Lactic Acid Determination

The supernatant from fermentation was analyzed using High Performance Liquid Chromatography (HPLC) method with UV Detector (Water 2487 Dual λ Absorbance Detector). 1.0 mM H_2SO_4 was used as a mobile phase and the flow rate was set at 6.0 mL/minute and column temperature was set at 60°C . 20.0 µL of the sample was injected into the column using autosampler.