Effects of different carbon sources for high level lactic acid production by *Lactobacillus casei*

Saber Salem Hassan\(^{1,a}\), Roslinda bt Abd Malek\(^{2,b}\), Asliaty Atim\(^{1,c}\), Suzi Salwah Jikan\(^{1,d}\), Siti Fatimah Zaharah Mohd Fuzi\(^{1,e}\)

\(^{1}\)Faculty of Science, Technology and Human Development, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia

\(^{2}\)Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia, 81310 Skudai, Johor

\(^{a}\)sabersalem79@gmail.com, \(^{b}\)roslinda@ibd.utm.com, \(^{c}\)Asliaty@uthm.edu.my, \(^{d}\)suzi@uthm.edu.my, \(^{e}\)fatimahz@uthm.edu.my

Keywords: carbon source, lactic acid, *Lactobacillus casei*, glucose, fermentation

Abstract. The fermentation process utilization to produce lactic acid has been studied from carbohydrate source and another source because of several significant reasons. Above all the production of biotechnology construction is found to be less costly compared to chemical synthesis. The production of biodegradable lactic polymer from lactic acid utilization of raw material can easily be obtained from industrial wastes such as pineapple waste. The process can positively affect the environment by reducing the environmental problems. The aim of this study is to estimate the effects of glucose concentration of pineapple wastewater as the carbon source on the volume of *Lactobacillus casei* (*L. casei*) subspecies in producing lactic acid. Five different glucose concentrations as carbon source are used for production of high lactic acid in the fermentation process using *L. casei*. *L. casei* could be ingesting the glucose presented within the levels tested and converts all into lactic acid. The result shows efficient yields of 0.09 g lactic acid/g glucose. The highest level of lactic acid is at 125.71 g/l and was obtained from 100% pineapple waste medium. When the carbon source is at 4 g/l, the level of lactic acid is decreased to 84.22 g/l. The fermentation time increases with the increment of sugars. It is more than double if the medium is composed of 100% of pineapple waste. Therefore pineapple waste is the best alternative as carbon source for bacteria growth because it is more cost effective.

Introduction

Factory canning of pineapple that are situated in tropical regions for example Malaysia, Thailand and Indonesia provide great quantity of liquid and solid waste in canneries where approximately 75% of the fruit in the form of peeled skin, core and also crown, etc are not utilized, being released as wastage, and create the problem of getting rid of and contamination [1]. The wastewater is coming from definite phases in the stages of the processing unit which yield varied forms, characteristics and waste quantities. The pineapple liquid waste is produced from the industrial activation, such as cleaning and separation process and also pineapple concentrate production. These various processing stages deliver a large number of pineapple wastewater, between 5000-7000m³ [2]. The liquid waste can be the reason for environmental contamination problems if not utilized because it still contains high content of carbohydrate, in addition to higher fiber and low protein contents [3]. However, pineapple waste may have the possibility for recycling; as raw material or for conversion into high value added products as well as raw material for other industries [4].
Lactic acid (2-hydroxypropionic acid) is an important chemical. It was first found by the Swedish chemist, Carl Wilhelm Scheele in 1780, who isolated the lactic acid from sour milk. It was first produced commercially by Charles, Avery at Littleton, Massachusetts USA in 1881 [5]. Lactic acid can be produced either through chemical synthesis or through carbohydrate fermentation. The traditional In addition, the oxirane function of ESO allows it to involve in cross-linking reaction with a suitable curing agent. Hence it will take a place in the cross linking reaction with epoxy resin and gives it an opportunity to be incorporated into matrix structure than just act as a plasticizer. Therefore, mechanical properties and curing particularity was improved using modified ESO as a part to consummate epoxy systems as reported by [6].

process for fermentative manufacturing of lactic acid (LA) was carried out in batch process that resulted in low productivity with high capital and working costs. It has the highest cost of conventional process for lactic acid production through lactose fermentation that depends on necessary splitting steps in order to achieve the standard quality of the food rank requirements [6]. Recently, lactic acid is utilized in the food, pharmaceutical and chemical industries and is a platform chemical to be used in these industries [7]. The utilization of sucrose as the carbon source in fermentation is economically unfavorable, due to its high cost, whilst lactic acid is a cheap product. This paper will study the effects of carbon source on high lactic acid production by L. casei.

Material and method

Microorganism:
The strain utilized is L. casei subspecies rhamnosus ATCA 11443 from American type culture collection ATCC, homofermentation lactic acid produced. The microorganism lyophilized was transferred to 5 ml of deMan Rogosa and Sharpe (MRS) broth in the test tub and then incubated at 37 °C for 18 hours in static culture.

Inoculum preparation:
The inoculum preparation for this experiment was transferring 50 ml liquid MRS into 250 ml Erlenmeyer flask. The L. casei was grown in MRS shaken incubator for 24 hours, at 150 rpm. Then the broth was centrifuged at 4000 rpm for 10 minutes.

Fermentation medium:
Preparation medium contained the following constituents (g/l): 10 peptone, 10 beef extract, 5 yeast extract and glucose, 1 polysorbate 80 and 2 ammonium citrate, 5 sodium acetate, 0.1 magnesium sulphate and 2 dipotassium sulphate, 0.05 manganese sulphate, 20 agar, with initial pH of 6.5.

Cultivation Different carbon source
In order to study the effects of carbon source type on bacterial L. casei production of high lactic acid, the glucose in the culture medium was replaced with other carbon sources. For the Lactobacillus casei production the cultivation of carbon source were 20, 40, 60, 80 and 100 g in the shaking incubator with rotational speed of 150 rpm for 24 hours.

Shake flask fermentation
The shake flask fermentation was then incubated in the incubator shaker. The free cell fermentation was carried out by transferring 10 ml of inoculum into a 125 ml Erlenmeyer flask.
containing 90 ml of fermentation medium. CaCO$_3$ was added in the shake flask fermentation for pH adjustment. The flask was then incubated in the incubator shaker at 37°C for 24 hours at 150 rpm, in an aerobic condition. The fermentation broth was separated by centrifugation. The clear liquid was collected as lactic acid.

**HPLC analysis**

During the fermentation process, 10 ml of the lactic acid concentration were taken to the centrifuge. After that it was filtered by a filtration paper of 0.45 μm in order to get the clear liquid that determined the level of lactic acid used. HPLC Waters 2690 Alliance Separations Module with Waters 996 Photodiode Array Detector, Column: Hi-Plex H, 300 x 7.7 mm with guard column, Mobile Phase: 0.005N H$_2$SO$_4$, Temperature: 40°C, Flow rate: 0.6 m/min, Detection: UV 210 nm.

**Results and discussion**

The effectiveness of lactic acid production is shown in Figure 1. The sample of carbon source consumption changes depending on different concentration. The carbon source is necessary for cell growth and lactic acid production. In order to investigate the effects of carbon source in lactic acid production, this study has used different concentrations of carbon source (glucose).

![Figure (1) Effects of different glucose concentration on lactic acid production](image)

This study has shown the highest relative yield of lactic acid is 122.71 mg/l as the lactic acid increased significantly. The optimum of lactic acid concentration level was obtained at the value of 80 g/l carbon source. The application of 40 g/l carbon source showed a decrease in the value of lactic acid concentration (84.99 mg/l), whilst a 60 g/l glucose has increased the lactic acid concentration to 108.71 mg/l, source and when the carbon source was 100 g/l, the concentration of lactic acid decreased at 103.99. Table (1) shows statistical view of the lactic acid production.
Table (1) Statistical view of the lactic acid production

<table>
<thead>
<tr>
<th>N total (no. of sample)</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Sum</th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>109.2184</td>
<td>16.34115</td>
<td>546.092</td>
<td>84.99</td>
<td>108.71</td>
<td>122.712</td>
</tr>
</tbody>
</table>

Conclusion
The suitable carbon source (glucose) for bacteria used in the fermentation process to produce lactic acid is *L. casei*. It shows that the highest value of lactic acid concentration was at the amount of 80 g/l of carbon source. The optimum value of lactic acid concentration was 122.71 mg/l when the value of carbon source was 80 g/l.

Acknowledgments
The authors are thankful for the monetary support provided by the Graduate Research Assistance Incentive Grant (GIPS-ORICC), Universiti Tun Hussein Onn Malaysia.

References


