Penerbit Akademia Baru

Journal of Advanced Research in Materials Science
ISSN (online): 2289-7992 | Vol. 2, No.1. Pages 10-14, 2014

Effects of Different Carbon Sources for High-Level Lactic Acid Production by *Lactobacillus Casei*

S. S. Hassan[1,*,a], *R. Abd Malek*[2,b], *A. Atim*[1,c], *S. S. Jikan*[1,d], *S. F. Z. 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, Malaysia.
[*, a]*sabersalem79@gmail.com*, [b]*roslinda@ibd.utm.com*, [c]*asliaty@uthm.edu.my*, [d]*suzi@uthm.edu.my*, [e]*fatimahz@uthm.edu.my*

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

Keywords: Carbon source, lactic acid, Lactobacillus casei, glucose, fermentation

1.0 INTRODUCTION

Pineapple canning factories 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 are not utilized, released as wastage, and created problems for disposal and contamination [1]. The wastewater comes from definite phases in the stages of the processing units yields various forms, characteristics and quantities of waste. Pineapple liquid waste is produced from the industrial activation, such as cleaning and separation processes, and also pineapple concentrate production. These various processing stages deliver a large number of pineapple wastewater, between 5,000-7,000m³ [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 the 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 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.

Lactic acid is a carboxylic acid with a chemical formula of C3H6O3. The monomer, lactic acid (LA), is the smallest optical active organic compound present in nature. Due to the presence of a chiral carbon, LA exists in two optical isomers; L (+) and D (-) (Figure 1) [6].

Lactic acid is a versatile chemical used in food and chemical industries.. The conventional process for fermentative production of lactic acid is a batch process with low productivity and high capital and operating cost. However, the traditional process by chemical synthesis has the disadvantage that uses toxic solvents, and the LA produced is a racemic mixture of two optical isomers [7].

The process for fermentative manufacturing of LA is carried out in the batch process that resulted in low productivity with high capital and working cost. It has the highest cost of conventional process for LA production through lactose fermentation that depends on necessary splitting steps in order to achieve the standard quality of the food rank requirements [6]. Recently, LA is utilized in the food, pharmaceutical and chemical industries, and it is a platform chemical to be used in these industries [8]. The utilization of sucrose as the carbon source in fermentation is economically unfavorable due to its high cost, whilst lactic acid is a cheap option. This paper studies the effect of carbon source on high lactic acid production by L casei.

2.0 MATERIALS AND METHODS

2.1 Microorganism

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

2.2 Inoculum preparation

The inoculum preparation for this experiment was done by transferring 50 mL of liquid MRS into 250 mL of the Erlenmeyer flask. L. casei was grown in MRS shaken incubator for 24 h at 150 rpm. Then, the broth was centrifuged at 4,000 rpm for 10 min.

2.3 Fermentation medium

The prepared 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

Journal of Advanced Research in Materials Science
ISSN (online): 2289-7992 | Vol. 2, No.1. Pages 10-14, 2014

magnesium sulphate and 2 dipotassium sulphate, 0.05 manganese sulphate, 20 agar, with the initial pH of 6.5.

2.4 Different carbon sources for cultivation

In order to study the effect 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 production of Lactobacillus casei, the cultivation was conducted using 20, 40, 60, 80 and 100 g of carbon source in the shaking incubator with rotational speed of 150 rpm for 24 h.

2.5 Shake flask fermentation

The shake flask fermentation was then carried out by incubation in the incubator shaker. The free cell fermentation was carried out by transferring 10 mL of inoculum into a 125 mL of an Erlenmeyer flask containing 90 mL of fermentation medium. CaCO3 was added in the shake flask fermentation for pH adjustment. The flask was then incubated in the incubator shaker at 37°C for 24 h at 150 rpm in an aerobic condition. The fermentation broth was separated by centrifugation. The clear liquid was collected as lactic acid.

2.6 Sample preparation

During the fermentation process, 10 mL of lactic acid was 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.

Figure 1 shows the difference for different concentrations of carbon source. Different color with lactic acid yield was observed. As the concentration of carbon source increased, the yield of lactic acid also increased.

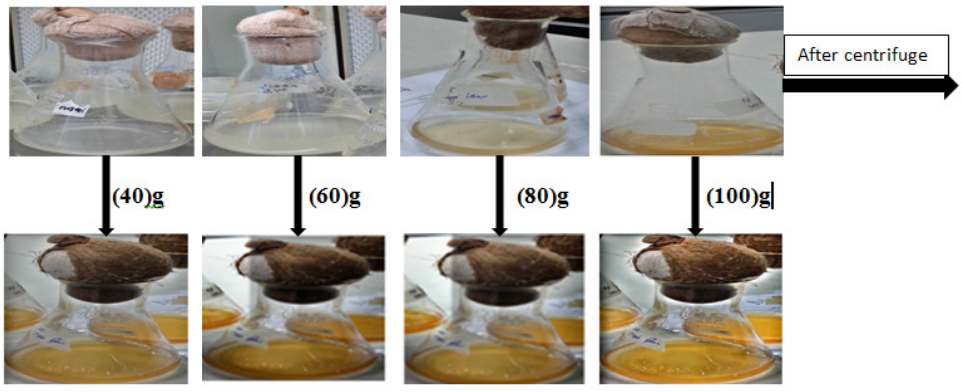

Figure 1: Different view of samples before and after centrifugation

2.7 Analytical techniques

The fermented broth was used for the determination of lactic acid. Lactic acid estimation was accomplished using high performance liquid chromatography (HPLC) system following the method of [9] with some modification. Samples were filtered through 0.20 µm membrane filters. The parameters/conditions for the HPLC analysis are as follows: 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 H2SO4; Temperature: 40□C; Flow rate: 0.6 m/min, and Detection: UV 210 nm.

3.0 RESULTS AND DISCUSSION

The effectiveness of lactic acid production is shown in Figure 2. The sample of carbon source consumption changes depending on different concentrations. The carbon source is necessary for cell growth and lactic acid production. In order to investigate the effect of carbon source in lactic acid production, this study has used different concentrations of carbon source (glucose).

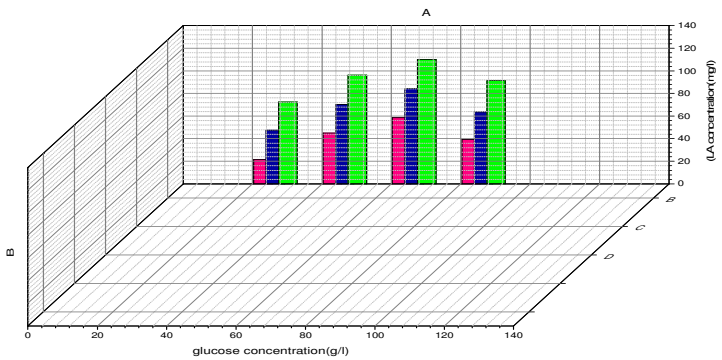

Figure 2: Effects of different glucose concentrations on lactic acid production

This study has shown that the highest relative yield of lactic acid was 122.71 mg/L as the lactic acid increased significantly. The optimum 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 60 g/L glucose increased the lactic acid concentration to 108.71 mg/L. For the carbon source of 100 g/L, the concentration of lactic acid decreased to 103.99 mg/L. Table 1 shows statistical view of the lactic acid production.

On the other hand, the concentration of lactic acid increased as the concentration of carbon source increased.

Table 1: Statistical view of lactic acid production

N total (no. of sample)	Mean	Standard Deviation	Sum (mg/L)	Minimum (mg/L)	Median (mg/L)	Maximum (mg/L)
4	109.2184	16.34115	546.092	84.99	108.71	122.712

4.0 CONCLUSION

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

ACKNOWLEDGEMENT

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

REFERENCES

[1] A.M. Busairi, Effect of Nitrogen Source and Initial Sugar Concentration on Lactic Acid Fermentation of Pineapple Waste using Lactobacillus Delbrueckii, Teknik 31(2010) 31-34.

[2] A. Sutanto, M. Metro, M. Lampung, L. Cair, B. Baku, P. Nata, D.P. Penelitian, Pineapple Liquid Waste as Nata Depina Raw Material, Makara Teknologi 16 (2012), 63-67.

[3] H. Pyar, M. Liong, K.K. Peh, Potentials of Pineapple Waste as Growth Medium for Lactobacillus species, International Journal of Pharmacy and Pharmaceutical Sciences 6 (2014), 1-4.

[4] H.M. Abdullah, Solid and Liquid Pineapple Waste Utilization for Lactic Acid Fermentation, Reaktor 11 (2007), 50-52.

[5] A. Sheeladevi, N. Ramanathan, Lactic Acid Production Using Lactic Acid Bacteria under Optimized Conditions, International Journal of Pharmaceutical & Biological Archive 2 (2011), 1686-1691.

[6] T. Ghaffar, M. Irshad, Z. Anwar, T. Aqil, Z. Zulifqar, A. Tariq, S. Mehmood, Recent trends in lactic acid biotechnology: A brief review on production to purification, Journal of Radiation Research and Applied Sciences,7 (2014), 222-229.

[7] M.I. González, S. Alvarez, F.A. Riera, R. Álvarez, (2008). Lactic acid recovery from whey ultrafiltrate fermentation broths and artificial solutions by nanofiltration, 228 (2008) Desalination 84-96.

[8] H.Y. Choi, H.K. Ryu, K.M. Park, E.G. Lee, H. Lee, S.W. Kim, E.S. Choi, Direct lactic acid fermentation of Jerusalem artichoke tuber extract using Lactobacillus paracasei without acidic or enzymatic inulin hydrolysis, Bioresource Technology 114 (2012), 745-747.

[9] P.S. Panesar, J.F. Kennedy, C.J. Knill, M. Kosseva, Production of L (+) Lactic Acid using Lactobacillus casei from Whey, Brazilian Archive Biology and Technology 53 (2010), 219-226.