The Optizmum Condition for Lactic Acid Yield From Pineapple Wastewater Using Lactobacillus Casei

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Abstract

For several reasons, the utilization of the fermentation process to produce lactic acid has been studied from carbohydrate sources and other sources. Accordingly, the use of biotechnology to produce lactic acid is found to be less costly compared to chemical synthesis. Lactic acid, which is the raw material for the production of biodegradable poly lactic acid, can easily be obtained from industrial wastes such as pineapple waste. The process can positively affect the environment by reducing the amount of liquid and solid waste that is harmful to the

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environment. This study aims to optimize the conditions for the lactic acid yield from pineapple wastewater using lactobacillus casei. The lactobacillus casei was propagated in MRS broth for three successive times before the fermentation of pineapple wastewater as the substrate, using the cheapest and simplest technique, namely shake flask fermentation in aerobic conditions at different temperatures (30°C, 37°C, 45°C), different inoculum sizes (5%, 10%, 15%) and pH. The viability of probiotics was determined spectrophotometrically at a 600nm wavelength. The results showed that the best bacterial growth to produce an optimum lactic acid concentration was obtained at 37°C and a 5% inoculum size at pH 6. The effect of the pineapple wastewater culture medium on bacterial growth was comparable to that of MRS.

Keywords: *inoculum size, lactic acid, Lactobacillus casei, fermentation, yield lactic acid*

1. Introduction

Factory canning of pineapple that is situated in tropical regions, for example, Malaysia, Thailand, and Indonesia generate a great quantity of liquid and solid waste in canneries where approximately 75% of the fruit in the form of peeled skin, core, and crown, etc. are not utilized, but released as wastage. This creates the problem of disposal and contamination [1]. The wastewater comes from definite phases in the stages of the processing, and yields varied forms, characteristics, and waste quantities. The pineapple liquid waste is produced from the industrial activation, such as cleaning and separation process, and concentrate production. These various processing stages deliver a large number of pineapple wastewater, between 5000-7000m^3 [2]. The liquid waste can be the reason for environmental contamination problems if not

utilized because it still contains high content of carbohydrates, 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-hydroxy propionic acid) is an important chemical. It was first discovered 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.

It is a carboxylic acid with a chemical formula of $C_3H_6O_3$. 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 the two optical isomers, L (+) and D (-) (Figure 1) [6].

L (+) Lactic acid **D (-) Lactic acid**

Figure(1) Two optical isomers, L (+) and D (-) of lactic acid

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

Process for fermentative manufacturing of lactic acid (LA) was carried out in the batch process that resulted in low productivity with high capital and working costs. It has the highest cost of the conventional process for lactic acid production through lactose fermentation that depends on necessary splitting steps to achieve the standard quality of the food rank requirements [8]. Recently, lactic acid is utilized in the food, pharmaceutical, and chemical industries and is a platform chemical to be used in these industries [9].

In this study, pineapple wastewater has been used as a substrate for the anaerobic fermentation process. The effects of temperature (T=30°C, 37°C, 45°C) and inoculum size be (5%, 10%, 15%) and at pH (5, 6, 6.5) on lactic acid production were investigated.

Material and method

Substrate

The fermentation media contained liquid pineapple wastewater obtained from RADA canny factory Sdn.Bhd,Batu Pahat,Johor, Malaysia.the pineapple wastewater was boiled for 5 min to remove existing enzymes and follow by filtering to remove the solid particles. It was also stored at -18°C [10].

Culture medium

Pineapple wastewater was substrate used to carry out to fermentation process, the De Man –Rogosa-Sharpe (MRS) medium was used as a reference [11].

Microorganism

The strain utilized is *L. casei* subspecies rhamnosus ATCA 11443 from American type culture collection ATCC, homo fermentative lactic acid produced. The microorganism lyophilized was transferred to 5 ml of deMan Rogosa and Sharpe (MRS) broth in the test tube 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 a 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 20g 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.

Shake flask fermentation

The shake flask fermentation was then incubated in the incubator shaker. The free cell fermentation was carried out by transferring 5ml,10ml,15ml of inoculum into a 125 ml Erlenmeyer flask containing 85ml,90ml,95ml of fermentation medium. $CaCO_3$ was added to the shake flask fermentation for pH adjustment. The flask was then incubated in the incubator shaker at 30°C, 37°C, and 45°C for 24 hours at 150 rpm, in anaerobic conditions. The fermentation broth was separated by centrifugation. The clear liquid was collected as lactic acid.

Effect of temperature on the growth of *Lactobacillus casei*

The different temperatures to effect on fermentation were carried out at various temperatures of 30°C, 37°C, and 45°C. The growth of *Lactobacillus casei* strain was determined spectrophotometric at 600nm[12]

Effect of pH on the growth of *Lactobacillus casei*

The study of effect initial pH by conducting fermentation at various initial pH of 5, 5.5,6, with adding $caco_3$ to adjust it and in cubit at 37°C and the growth of *Lactobacillus casei* strain were determined spectrophotometric at 600nm.

Analytical techniques

The fermented broth was used for the determination of lactic acid, Lactic acid estimation was accomplished using a high-performance liquid chromatography (HPLC) system following the method. Samples were filtered through 0.20 μm membrane filters, 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_2SO_4, Temperature: 40°C, Flow rate: 0.6 m/min, Detection: UV 210 nm[13]

Results and discussion

Effect of temperature and growth of strain at a temperature condition

The temperature was monitored and controlled before starting the fermentation process at adjustment temperatures of 30°C, 37°C, and 45 °C with a fermentation time of 24 h. As shown in figure (1), the result

showed that the optimum temperature and time for lactic acid production was at 37 °C for 24 h. Temperature is the most important factor in nutrient utilization and cell viability. Higher temperature the sluggish growth of lactic acid bacteria (*L.casei*) resulted in pH6, and also showed an increase in the growth of the bacteria from 30°C to 37 °C; the decrease in the growth of *L.casei* started after 40°C at the same condition and consequently suppressed the growth of *L.casei*. The optimum temperature for its growth was at 37°C and was

Observed between 30°C and 40°C.

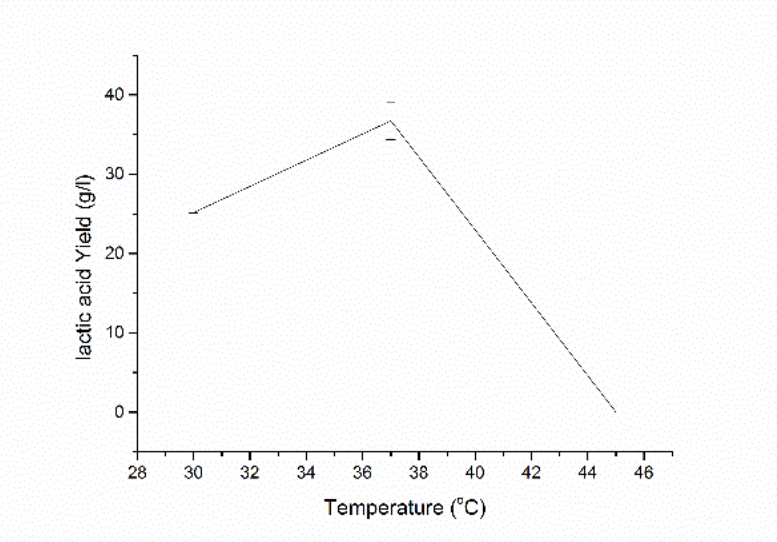

Figure (1) Different temperature on effect yield lactic acid at pH6

Figure (1) also view it showed the optimum of LA at 37°C and when the temperature was at 30°C the value of concentration LA reduce and when the temperature increased up to 37°C the microbe does not grow.

Growth of probiotics *Lactobacillus casei* in medium

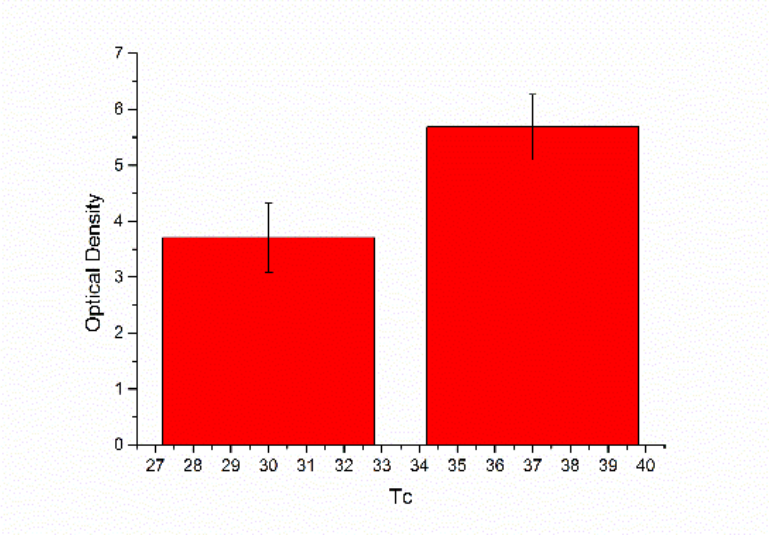

Figure (2) growth the *Lactobacillus casei* in different temperature

Figure (2) shows the comparison of *Lactobacillus casei* growth at optimum temperature 30°C, 37°C between pineapple waste-medium and the commercial MRS, the result sowed the temperature 37°C was suitable to growth strain.

Effect of initial pH on lactic acid yield

In this section, the effects of different pH levels on the production of batch fermentation were investigated. A study was performed on the effect of pH on cell growth and substrate metabolism. The effect of initial pH was conducted in a 250ml Erlenmeyer flask with a working volume of 100 ml at 37 °C using liquid pineapple waste. The initial pH of the fermentation medium was controlled by using $CaCO_3$ 3% (w/v) as a pH control agent, and the initial pH of the medium was maintained at 5.5, 6, and 6.5, respectively. Fermentation ended after 24 h of fermentation, and it was observed that either glucose was completely depleted or there was

no change in glucose concentration to lactic acid, and the cell growth had almost ceased**.**

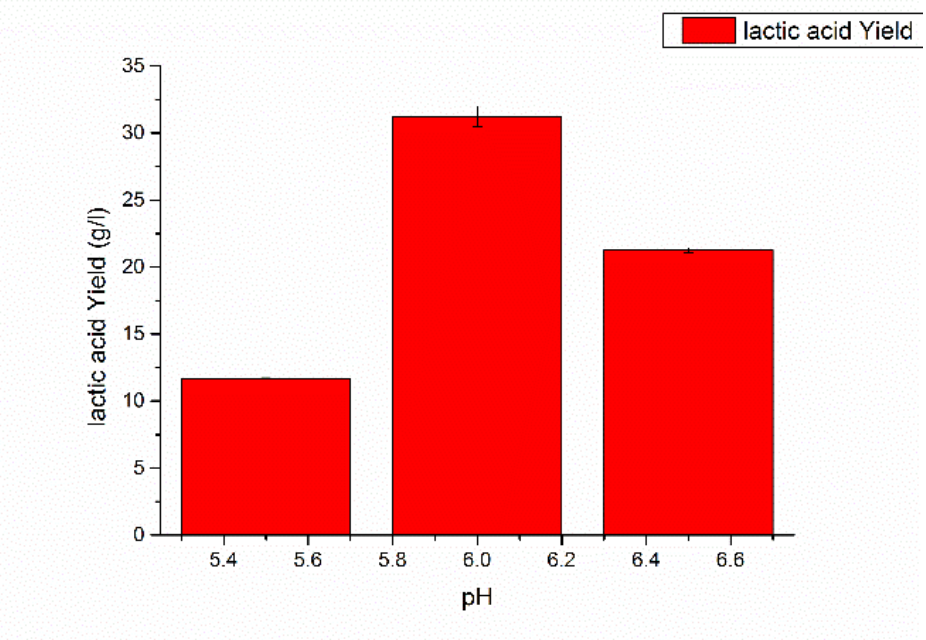

Figure (3) views the effect different the PH on LA yield.

Effect of controlled pH on lactic acid production is given in figure (3) the optimal pH for production of lactic acid was found to be at 6 with lactic acid production and yield at 54.97 mg/l If the pH increased to 6.5, lactic acid production and yield obtained would decrease to 21.88, For PH 5.5 however, lactic acid production and yield were 11.59 respectively. The Bactria *L.casei* seems to be a growth medium neutral environmental with an initial pH in the region of 5.5-7. But best at initial pH6, an environmental, which is too acidic and alkaline condition.

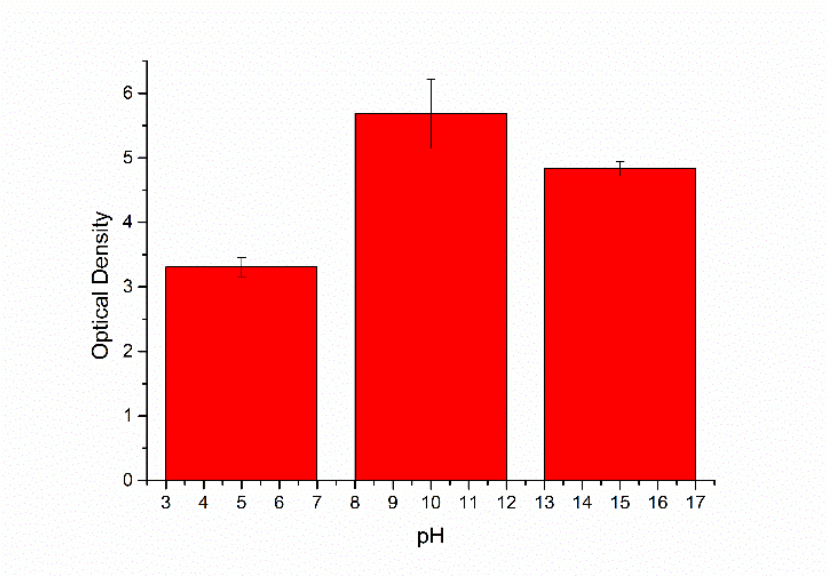

Figure (4) growth of the *Lactobacillus casei* in different pH

The exponential growth rate at initial pH6 was the fastest compared with the other initial pH values in the beginning at prolonged lag initial pH 5.5 the Bactria exhibited a prolonged lag Phase and the Bactria did not grow as well as higher initial pH value, as the initial pH above 5.5 the cell growth increase. The initial pH 6.5 its growth rate was decreased.

Effect of Inoculum Size on lactic acid yield

The influence of inoculum size and lactic acid production was studied different inoculum sizes (5%, 10%, 15 % (w/w), were separately added to the optimized medium. Bacterial growth and along lag phase is disadvantageous because it is time-consuming and the medium is used in maintaining a viable culture before growth. The length of the lag phase is affected by the size of the inoculum and its physiological condition. The maximum concentration of lactic acid and biomass can be achieved depending on the quantity or size of inoculum added to the substrate. In

this study the inoculum sizes of (5%, 10%, 15%) v/v were used, effect of bacterial growth on inoculum followed by 5%, 10%, 15%.of inoculum at 24 h respective.

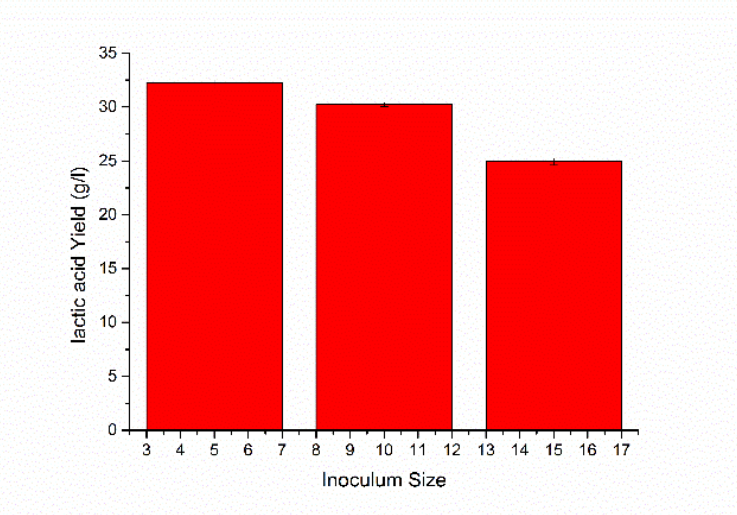

Figure (5) Effect of inoculum sizes on growth and lactic acid production by bacterial *Lactobacillus casei*

The lactic acid production increased with the increase in inoculum size up to 5 %(v/v), therefor no improvement in both the function was observed. The maximum lactic acid production of 33.96 mg/l was observed with 5 %(v/v).It had almost stopped inoculum of bacterial culture. The low lactic acid production at 15% (v/v) inoculum level could be attributed to the low density of starter culture. The use of 10% (v/v) inoculum for lactic acid production has been reported in earlier studies also. However, from the above observations, an inoculum of 5% (v/v) could be considered optimal for achieving maximum lactic acid production using 24 h old inoculum; however, 5 % inoculum size was used in the subsequent studies.

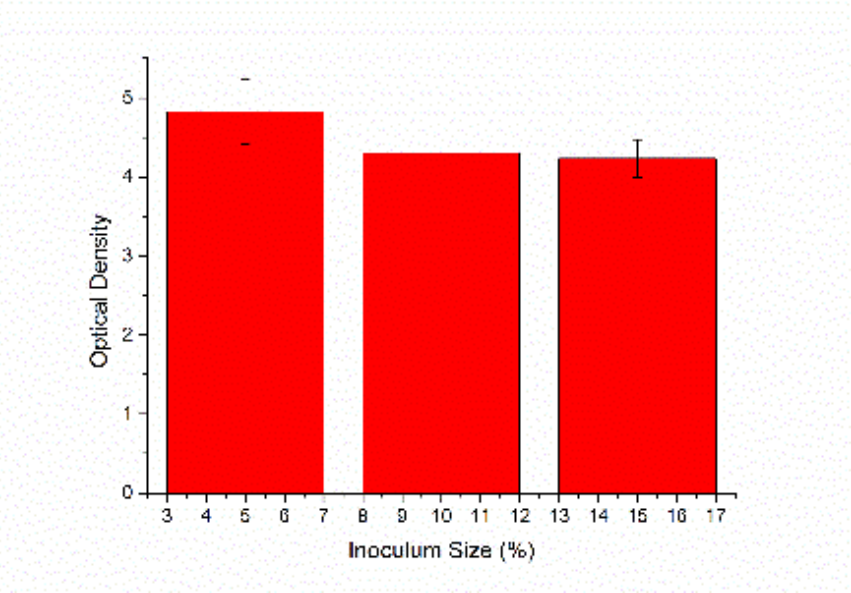

Figure (6) growth the *Lactobacillus casei* in different inoculum size

The exponential growth rate at inoculum size was the fastest compared with the other inoculum size values in the beginning at prolonged lag inoculum size 5% the Bactria exhibited a prolonged lag phase and the Bactria grow higher at the inoculum size, as the inoculum size above 5% the cell growth rate was decreased.

Conclusion

The result obtained showed that lactic acid could be produced from renewable resources from pineapple wastewater, experimental fermentation results indicated that the substrate to produce lactic acid is at temperature 37°C with 5% of inoculum size and at pH 6. the growth of the Bactria on pineapple wastewater medium was comparable to that of commercial MRS medium.

Acknowledgments

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