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Characterization of Liquid Pineapple Waste as Carbon Source for Production of Succinic Acid

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Abstract

Pineapple cannery produces large amount of solid and liquid waste. The disposal of waste without an appropriate treatment can cause a great environmental pollution. Since pineapple waste contains some valuable components such as glucose, fructose and sucrose, the ability to convert this waste into higher value added product such as succinic acid would be advantageous. Therefore, in this study, liquid pineapple waste was characterized in order to investigate the possibility of succinic acid production via fermentation using liquid pineapple waste as a carbon source. The physical and chemical composition in the liquid pineapple waste such as cation, anion, pH, sugar content and soluble protein were determined. The dominant sugar in the liquid pineapple waste were glucose, fructose and sucrose and the total sugar content was more than 100 g/l. Result from the fermentation process proved that liquid pineapple waste can successfully produce succinic acid with almost the same amount as using glucose as carbon source, with the concentration of 6.26 g/l.

Keywords: Characterization; liquid pineapple waste; carbon source; succinic acid

1.0 INTRODUCTION

Nowadays, the economic activities such as chemical, petrochemical, agricultural and food industries have generated various types of waste. Pineapple canning industry is one of the many food industries producing substantial amount of solid and liquid waste. Normally, the solid pineapple waste from the cannery industry is sold to the farmers for animal feed or fertilizer [1-3]. On the other hand, liquid waste is discharged into the nearest stream without an appropriate treatment [2]. If these waste disposed to the environment untreated, they could cause a serious environmental problems.

Pineapple waste contains valuable components mainly sucrose, glucose, fructose, and other nutrients [4]. Therefore, there is a possibility for the pineapple waste to be used as raw material, or for conversion into useful and higher value added products such as fuels and chemicals. One of the potential chemicals that can be produced from pineapple waste is succinic acid, which has been recognized as an important platform chemical that can be produced from biomass. So far, there are no studies reported on the application of pineapple waste for the production of succinic acid.

Succinic acid is a 1,4-dicarboxylic acid with IUPAC name of butanedioic acid. It can be applied in many industries including food, chemical and pharmaceutical. The demand for succinic acid is expected to increase significantly, since it can be further converted to produce biodegradable polymer. Succinic acid also can be used to derive a wide range of commodity or specialty chemicals [5]. As a commodity chemical, succinic acid can replace many commodities based on benzene and other intermediate petrochemicals to produce polyester, solvents and other acids. Besides that, food ingredients, fuel additives and plant growth stimulator are examples of specialty chemicals from succinic acid.

Succinic acid is commercially produced by chemical process using petroleum as starting material. It is derived from maleic anhydride, which is produced from n-butane through ion process over vanadium-phosphorous oxide (VPO) catalyst [6-7]. Although this method of production is currently provide lower production cost than processing by fermentation, there are some significant weaknesses [8]. The commercial succinic acid produced through chemical process brings environmental pollution and the concerns of sustainable development [9-10]. Besides, facing shortage of crude oil supply and rising oil price, the fermentative production of succinic acid can be regarded as a green technology because renewable substrates are used. Besides, note that the fact CO_2 is assimilated during succinic acid fermentation can be considered as an environmental advantage.

Therefore, it is the objective of the present study to characterize the physical and chemical properties of the liquid pineapple waste to investigate the possibility of the waste to be used in succinic acid production. The characterization criteria include cation, anion, pH, sugar content and soluble protein. Apart from this, the fermentation process was also carried out to produce succinic acid from the liquid pineapple waste.

2.0 MATERIALS AND METHODS

2.1 Materials

Liquid pineapple waste (LPW) was obtained from Lee Pineapple Co. Pte. Ltd located in Johor Bahru, Malaysia. Acetonitrile (99.9% assay) as high performance liquid chromatography (HPLC) mobile phase, Bovine serum albumin (BSA) (98% assay) as protein standard, and Bradford reagent were purchased from Sigma Aldrich. Luria broth (LB) agar, Glycerol (99% assay), and Magnesium Sulfate Heptahydrate ($MgSO_4.7H_2O$) were used in culture medium and purchased from Merck. In addition, glucose, di-Potassium Hydrogen Phosphate (K_2HPO_4) (99% assay), Potassium DihydrogenPhosphaste (KH_2PO_4) (98% assay), and Ammonium Sulphate ($(NH_4)_2SO_4$) (99.5%) were purchased from R&M Chemicals. Meanwhile, Sodium Hydroxide (NaOH) (98% assay), and phosphoric acid were purchased from J. T. Baker and Qrec respectively. All these reagents and solutions were used directly as received without further purification.

2.2 Characterization Method of LPW

Prior to characterization, the solution was pretreated through the following steps; firstly, the solution was boiled for 5 minutes. Then, it was centrifuged (HettichZentrifugen, model EBA 20) at 4000 rpm for 15 minutes where the supernatant was collected while the pelt was discarded.

The sugar content was measured using HPLC (Waters 2998) with the following parameters; 300 mm × 4 mm ID µ Bondapak/Carbohydrate column (Waters) with RI detector. The eluent was a mixture of acetonitrile: water (80:20) at flow rate of 2ml per minute and temperature of 25°C. Soluble protein is measured using Bradford assay [11]. Cations were determined using the Direct Air-Acetylene Flame method using the Atomic Absorption Spectrometer (AAS) model PU 9200. This method measures amount of light absorbed by the atomized element in the flame. In this measurement, the hallow cathode lamp was used where each specific lamp was selected for each element being measured. For anion content determination, Ion Chromatography model LC20 with Dionex DX 500 Column and electric chemical detector ED40 was used. The LPW solution was passed through the column, where the ions were separated. Then, the different anion concentrations in the sample were determines according to the retention time.

2.3 Fermentation Procedure of LPW

The succinic acid production was carried out using *E. coli* AFP 184 (ATCC PTA-5132). The bacterium was cultivated on Luria Broth (LB) agar at 37°C for 24 hours. Cultures were stored at –80°C in 30% glycerol stocks.

Then, the inoculum was prepared through the following steps; firstly, four 100 ml shake flask containing 40 ml medium solution was prepared. The medium consist of 1.4 g/l of K_2HPO_4, 0.6 g/l of KH_2PO_4, 3.3 g/l of $(NH_4)_2SO_4$, 0.4 g/l of $MgSO_4.7H_2O$, and 15 g/l of yeast extract in distilled water. Then, the medium was sterile in autoclave (HIRAYAMA, Model HVE 50) at 121°C for 20 minutes. After that, 0.2 ml of glycerol stock culture was added into each flask. The inoculated flask was then transferred into an incubator shaker (SASTEC, Model ST 100C) and incubated for 24 h at 37°C and 200 rpm. After 24 h, the inoculum was combined into one flask which will be used in fermentation process.

The succinic acid production was done in batch fermentations. First of all, 40 ml of fermentation medium was prepared in 100 ml shake flask. The medium used for the fermentation is the same as used for the inoculum. Besides that, 10 ml of carbon sources was prepared in universal bottle. Then, the prepared medium and carbon sources were sterilized at 121°C for 20 minutes. After that, the carbon source with 5 ml of inoculum was added into the medium. The pH of the solution was modified between 6.6 and 6.7 by the addition of NaOH. The final solution was then incubated for 24 h at 37°C and 200 rpm. The carbon source in the fermentation system is compared between 26 g/l of glucose and liquid pineapple waste and the experiment was done in duplicates for each carbon source.

2.4 Identification of Succinic Acid

Samples were taken for the succinic acid analysis using HPLC (Waters 2998). A Purospher STAR C_{18} (250 mm x 4.6 mm, 5 µm) column with UV detector were used. A 10% phosphoric acid at a flow rate of 0.35 mL/min were used as the mobile phase. Sample for acid analysis were centrifuged at 40,000 rpm for 15 minutes. The supernatant is filtered througha 0.45 µm syringe filter (Teknokroma).

3.0 RESULTS AND DISCUSSION

3.1 Characterization of LPW

The physical and chemical composition of LPW is tabulated in Table 1. The results showed that amount of sugar are 40.23 g/l, 26.33 g/l, and 40.27 g/l for sucrose, glucose and fructose respectively. This indicates that the total sugar composition was quite high, which is more than 100 g/l, hence, should be suitable to be used in fermentation process which generally would require sugar between 0.2 to 25% [12]. Moreover, result of the sugar composition in this study is higher than similar study conducted by Abdullah and Mat [13], where 73.76 g/l total sugar was determined in the LPW.

Results showed that the amount of protein is very little with only 0.025 g/l, compared to 1.13 g/l by Abdullah and Mat [13]. Protein is required in the fermentation media to supply nitrogen, as well as carbon and energy. Since there is little amount of protein, supplement might be required from protein hydrolysates and peptones.

According to the result, liquid pineapple waste can provide a good culture condition as it also contain monovalent (Potassium (K) at 2118 mg/l) and divalent cations such as Magnesium (Mg) (84.8 mg/l) and Calcium (Ca) (75.56 mg/l) as well as other elements including Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Cadmium (Cd), and Sodium (Na). These elements function to catalyze many reactions, vitamin synthesis and cell wall transport [12].

Table 1 The characteristics of liquid pineapple waste

Sugars (g/l)	
Sucrose	40.23
Glucose	26.33
Fructose	40.27
Protein (g/l)	
Soluble protein	0.025
Cations (mg/l)	
Iron (Fe)	2.973
Calcium (Ca)	75.56
Manganese (Mn)	2.398
Magnesium (Mg)	84.8
Zinc (Zn)	2.327
Copper (Cu)	0.966
Cadmium (Cd)	0.023
Sodium (Na)	29.97
Potassium (K)	2118
Anions (mg/l)	
Sulphate (SO_4^{2-})	48.944
Phosphate (PO_4^{3-})	130.759
Nitrate (NO^{3-})	18.757
Chloride (Cl^{1-})	231.548
pH	5.13

Anion composition in the liquid pineapple waste was also characterized. Results show that the chloride ion was the highest which is 231.55 mg/l, followed by phosphate (130.76 mg/l), sulphate (48. 94 mg/l) and nitrate (18.76 mg/l). High amount of Chloride meets the requirement for the bacteria to grow as it is the principle extracellular anion in organism. Other anions are the essential elements as energy source during the cultivation of microorganism.

The pH of pineapple waste is 5.3. This pH can be easily modified to neutral pH since the organism that will be utilize for succinic acid production is *E. coli*, which is a neutrophile organism and grow best at near neutral pH as attempted by Andersson [14].

3.2 Fermentation of LPW

The result of this study showed that fermentation of liquid pineapple waste produce 6.26 g/l of succinic acid, similar amount to the fermentation of glucose which produce which produce 6.53 g/l. the result proved that liquid pineapple waste has high possibility to be used as carbon source in succinic acid fermentation. However, the amount of succinic acid produce in this study is lower than previous study reported by Andersson *et al*. [15] which is 25-40 g/l. One of possible the reasons for the big difference in the yield of succinic acid are the stages of fermentation involved. Andersson *et al*. [15] used dual phase fermentation; consist of aerobic and anaerobic phase. On the other hand, this study only focus on aerobic phase to prove that liquid pineapple waste also can be used as carbon source. In future study, it might be possible to conduct fermentation process in dual phase system to produce higher amount of succinic acid.

■4.0 CONCLUSION

Liquid pineapple waste contains high amount of sugar, which is more than 100 g/l as well as other macro and micronutrients which is essential during cell cultivation. Therefore, it can potentially be used as substrate for succinic acid production. Result from fermentation process proved that liquid pineapple waste can successfully produce succinic acid with almost the same amount as using glucose as carbon source.

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