Characterization Of Raw Material (Glycerol Residue) And *Escherichia Coli* In Immobilized Cell For Succinic Acid Production

Nik Nor Aziati Abdul Aziz
Faculty of Chemical Engineering and Natural Resources,
University of Malaysia Pahang (UMP)
Kuantan Pahang, Malaysia
Tel: +6016-6668486, Fax: +60-095492825, E-mail: *atie_encat@yahoo.com*

Mimi Sakinah Abdul Munaim
Faculty of Chemical Engineering and Natural Resources
University of Malaysia Pahang (UMP)
Kuantan Pahang, Malaysia
Tel: +60-095492825, Fax: +60-095492825, E-mail: mimi@ump.edu.my

Abstract—The use of glycerol residue, a palm oil waste from Oleochemical industries as a raw material for the production of succinic acid with immobilized cell fermentation process was investigated. In this work, the raw material, immobilized cells to produced succinic acid were characterized by Fourier Transform Infrared Spectrometry (FTIR) and Scanning Electron Microscope instruments. Based on the results, the functional group appearing for treated and commercial glycerol is the hydroxyl, aromatic methoxyl, alkenes, soap, carbonyl and alcohol group. The hydroxyl group which is O-H appeared at a spectra value of 3394.27 $cm^{-1}$for the treated glycerol and 3299.96 cm^{-1} for the commercial group. Respectively. From the analysis of SEM for bead, it was found that majority of pores fall within range and the shapes was ellipsoid and a thick skin around strains was detectable, therefore indicating that immobilized cell prepared for fermnatation process is an attractive source for succinic acid applications.

Key words: succinic acid, immobilized cell, glycerol residue, characterization

1. INTRODUCTION

Over the last decade, succinic acid have been attracted a great deal of world attention with its excellent organic compound and key compound in producing more than 30 commercially important product which lead to many promising applications [1]. Succinic acid mostly produced by chemical processes which is using liquefield petroleum gas or petroleum oil as a starting material [2]. This may lead to a situation whereby the world would be threatened by the potential hazard and environmental pollution of succinic acid production. However, researchers have proposed that succinic acid can be produced by fermentation process by using microorganism and starting raw material is from glycerol [3]. In order to reduce the formation of by-product, the succinic acid can be produce by using glycerol as a carbon sources and by-product can affect the purification process such as acetic acid can negatively affect the purification process[3].

Recent production of palm–based Oleochemical industry processes has generated large amount of solid wastes especially the glycerol residue waste. These solid wastes are left unutilized on the fields, causing significant environment and disposal problems. One solution for this problem is to reuse this waste by pre-treatment method which is one of the most widely used materials due of its low cost process and about 70 % glycerol can be easily recovered by conventional chemical treatment [4]. To produced succinic acid with environmentally friendly, Succinic can be produce from glycerol waste by fermentation process by using microorganisms. The use of immobilized cells has proven to be effective in reduced cost of production, including the recycle the cells and increased production of succinic acid. By using the raw material as a waste and the immbolized cell has stimulated the interest in examining the feasibility of using cheaper raw materials which is effective and economic.

Nowadays, succinic acid produced from microbial fermentation has attracted interest of researchers because of its potential applications. Previously, research has revealed the effects of various microorganisms on succinic acid production [2]. For instance, Agarwal *et al*. studied the effects of different environmental and nutritional factors on succinic acid production using microbial *Enterococcus flavescens* by batch fermentation and enzyme involve in acid production. Gonzalez *et al*. proposed a kinetic model of succinic acid production by *Actinobaccilus sussinoges ZT-130*, and reported the details of fermentation mass balance[5].

Furthermore, the increasing demand for succinic acid is expected as the uses of succinic acid is extended to the synthesis of glycerol. This is partially due to the high conversion cost of maleic anhydride to succinic acid by the chemical process, which limits the use of succinic acid for the wide range of applications. On the other hand, recent analysis showed that fermentative production of succinic acid from renewable resources can be more cost-effective than the petroleum-based processes[6]. Recently, this succinic acid has demonstrated that it can produce commercially by catalytic hydrogenation of petrochemical derived maleic acid or maleic anhydride[7]. As reported by Zeikus *et al*, succinic acid to be produces and accumulated by anaerobic microorganism as the major product of their metabolisms. Thus, attempts are being made worldwide to screen anaerobic microorganisms for succinic acid production.

2. MATERIAL AND METHODS

A.Material

The glycerol residue collected from a local oleochemical companies in Selangor was used as starting material in this work. The bead was produced using the natrium alginate.

B.Pre-treatment sample and immobilized cell preparation

The pre-treatment process was prepared by using the recovery method [8] . Initially, the materials were characterized by using FTIR method [4]for pre-treatment process. The immobilized cell was prepared by using the natrium alginate and using the entrapment method.

C.Sample Characterization

(FTIR) is a one of the methods to determine the functional group of the samples. The glycerol samples were prepared to determine its functional group. For this type of glycerol (liquid) it was determined by liquid film (neat) and the absorbance was by wave number (cm^{-1})[4].

A Scanning Electron Microscope (SEM) was used in this study. The structural of the raw material and the produced activated carbons were observed at magnification of 10 to 100,000 times with virtually unlimited depth of field. SEM images are very useful to obtain accurate adsorption details of adsorbent before and after the activation process [9].

.

3. RESULTS AND DISCUSSIONS

A FTIR (Substrate)

Treated glycerol and commercial glycerol were analyzed using the Fourier Transform Infrared Spectrometry (FTIR) to determine the functional group. The functional group is a group of atoms that replace the hydrogen in the organic compound. The structure of organic family compounds and their properties are defined by organic compounds. Table 1 shows the comparison data for the glycerol residue, recovered glycerol and commercial glycerol.

Table 1: Comparing the functional group for treated glycerol and commercial glycerol

unctional Group	Spectra Value (cm^{-1})	
	Treated glycerol	nmercial glyce
Iydroxyl (O-H)-	3394.2700	3299.9600
atic methoxyl ((	2950	2934.1100
Alkenes (C=C)	N/A	3022
Soap (COO)	1365.4100	1455
arbonyl (-C=O)	1645.6200	NA
lcohol (C-OH)	1015.2800	1038.5400

Based on the results, the functional group appearing for treated and commercial glycerol is the hydroxyl, aromatic methoxyl, alkenes, soap, carbonyl and alcohol group. The hydroxyl group which is O-H appeared at a spectra value of 3394.2700 $cm^{-1}$for the treated glycerol and 3299.9600 cm^{-1} for the commercial group. This common hydroxyl group has water in its contents and also has a percentage of water [10]. The presence of soap (COO functionality) was indicated by the absorption frequency of 1565.4100 cm^{-1} which was present in treated glycerol and 1455 cm^{-1} in commercial glycerol.

Groups that contained oxygen were carbonyl (C=O) and alcohol (C-OH) with each bonding present for a different activity based on the location and also with a hybridization of C-O bond. The presence of carbonyl (C=O) did appear in treated glycerol at 1645.6200 cm^{-1} but not in commercial glycerol. This is caused by some impurities during product oxidation of glycerol for example glyceraldehydes, dihydroxyacetone and also free fatty acids [10]. The alcohol group (C-OH) also appears in treated and commercial glycerol at a spectra value of 1015.2800 cm^{-1} and 1038.5400 cm^{-1}.

The glycerol molecule has three polar hydroxyl groups which contributes to water solubility and hygroscopic characteristics. Usually the glycerol is completely soluble in water and alcohol, this is due to glycerol having three molecules in the hydroxyl group. The primary hydroxyl group is more reactive compared to the secondary internal hydroxyl group.

B.SEM (Immobilized cells)

The microstructure of the immobilized cell which is bead was prepared at surface and 1 bead are shown in Fig.3. For the surface bead, the micrograph showed clearly the structure, which is good texture for production sucinic acid because the substrates can easily contact with the inside surfaces. From the SEM micrographs, it can be seen that morphological characteristics shown the shapes was ellipsoid and a thick skin around strains was detectable. The SEM images reveal the nature of its surface. The good structure for the bead means good contact the bacteria with the substrate and also good production of succinic acid. Immobilized microorganism is different in their growth rates and show altered morphological forms of colonies. The paper Immobilization of yeast and bacteria cells in alginate microbeads coated also using SEM method to investigated the texture of the bead [9].

Cell immobilization technique is the most efficient method to contribute the high cell growth rates and long term stability in the reactor fermentation. One can see the immobilized cell had significant effect on the substrate concentration prepared. Normally the productivity of the product is proportional to the cell density in the reactor fermentation. The surface structure of the immobilized cell is generally characterized by the SEM method and the test (plate count method) was done to check the leakage of the bacteria. One of the advantages of cell immobilization is cell can be recycling to increased the cell density and product concentration of the continuous cultures. In addition it also can reduce the formation of inhibitory end-products resulting from the lower concentrations of the substrates due to the higher dilution rate applied. From the observation of the outer surface of immobilization bead there are no significant contamination and the bead was in spherical shapes.

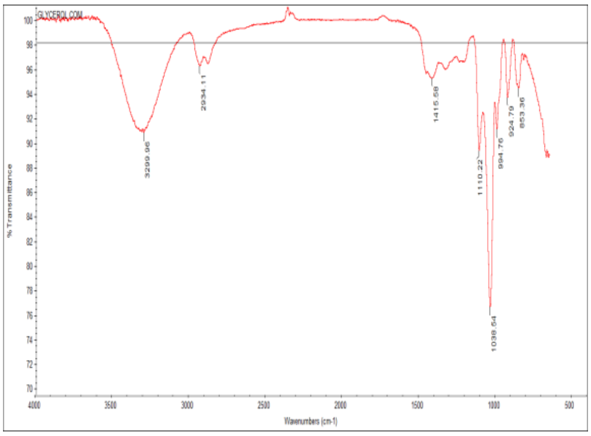

Figure 1: The FTIR Analysis for commercial glycerol

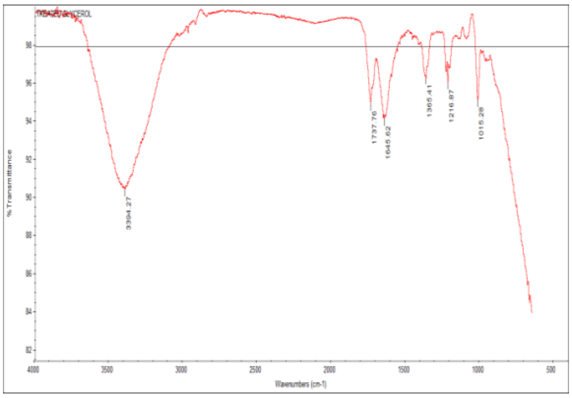

Figure 2: The FTIR Analysis for treated glycerol

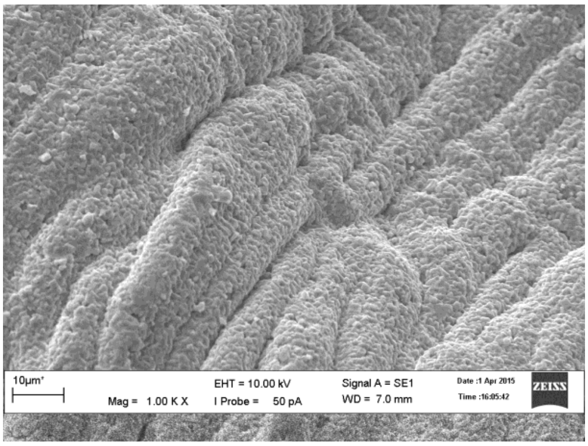

Figure 3: The surface of bead observe after immobilized cell process

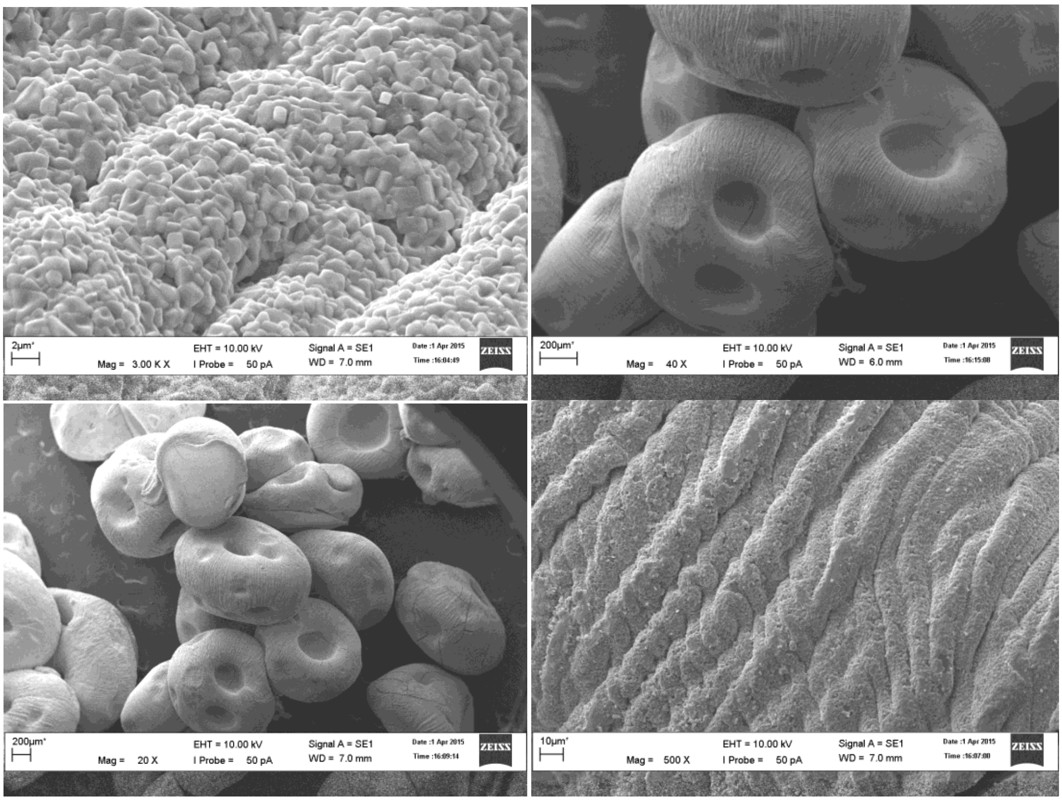

Figure 4: SEM micrographs of *Escherichia coli* cells immobilized on bead (alginate)

These micrographs of *Escherichia coli* cell in bead which is the cell inside the calcium alginate. By using the alginate polysaccharide there have an advantaged due cell immobilization and polymerization. Furthermore, can give a good stability of the bead or gel matrix under mild condition at ambient temperature. Figure 4 showed the cell was entrapped in the rigid alginate matrix and there was observed the rigid network of the outer cell which can prevent the leakage of the *Escherichia coli* cell and also can prevent the diffusion of the cell to the liquid medium while still allowing the cell ferment the substrate.

Many researchers have studied in immobilized cell and their findings are quite conclusive. Same as Gorbani *et al*., also study about the cane molassess fermentation using immobilization process by *saccharomyces cerevisiae*. Furthermore, these studies also include the outer surface observation of the bead used in the fermentation process. Thus, the method used for observation is via electronic microscopic scanning of immobilized cells. However, very few literatures have been found on work involving the use of bead cell as immobilized cell to produced valuable product. Due to this reason, there is a need to perform research on this promising immobilized cell for generating the product. The focus of this work was to evaluate the characteristics immobilized cell prepared from alginate solution using entrapment method.

4. CONCLUSION

Glycerol residue, waste from Olechemical industries, is a potential source for the production of high quality of succinic acid by fermentation process. The fermentation of immobilized cell has significant influence on immobilized cell to produced high concentration of succinic acid. The immobilized cell with huge specific surface area give the significant effect on the production this is to make sure the bacteria are not leakage. In addition, the characterization studies to its bead immobilized are developed that made to make sure suitable for adsorption application and to prevent the leakage. It is concluded that the characterization for pre-treatment FTIR (raw material) and fermentation (immobilized bead) could be exploited for commercial applications in the tertiary level treatment of glycerol residue as well as industrial effluents to produced valuable product.

REFERENCES

[1] L. Agarwal, J. Isar, and R. K. Saxena, "Rapid screening procedures for identification of succinic acid producers," *J. Biochem. Biophys. Methods*, vol. 63, no. 1, pp. 24–32, 2005.
[2] H. Song and S. Y. Lee, "Production of succinic acid by bacterial fermentation," *Enzyme Microb. Technol.*, vol. 39, no. 3, pp. 352–361, 2006.
[3] P. C. Lee, W. G. Lee, S. Y. Lee, H. N. Chang, and Y. K. Chang, "Fermentative Production of Succinic Acid from Glucose

and Corn Steep Liquor by Anaerobiospirillum succiniciproducens," *Biotechnol. Bioprocess Eng.*, vol. 5, pp. 379–381, 2000.

[4] a H. Hazimah, T. L. Ooi, and a Salmiah, "Recovery of Glycerol and Diglycerol From Glycerol Pitch Recovery of Glycerol and Diglycerol From Glycerol Pitch," *J. Oil Palm Res.*, vol. 15, no. 1, pp. 1–5, 2003.

[5] R. I. Corona-González, A. Bories, V. González-Álvarez, and C. Pelayo-Ortiz, "Kinetic study of succinic acid production by Actinobacillus succinogenes ZT-130," *Process Biochem.*, vol. 43, no. 10, pp. 1047–1053, 2008.

[6] A. Microbiologybiotechnology, M. K. Jain, K. Science, and T. Corporation, "Biotechnology of Succinic Acid Production and Markets for Derived Industrial Products," no. APRIL 1999, pp. 545–552, 2016.

[7] J. G. Zeikus, M. K. Jain, and P. Elankovan, "Biotechnology of succinic acid production and markets for derived industrial products," *Appl. Microbiol. Biotechnol.*, vol. 51, no. 5, pp. 545–552, 1999.

[8] T. L. Ooi, K. C. Yong, K. Dzulkefly, W. M. Z. Wan Yunus, and A. H. Hazimah, "Crude Glycerine Recovery From Glycerol Residue Waste From a Palm Kernel Oil Methyl Ester Plant," *J. Oil Palm Res.*, vol. 13, pp. 16–22, 2001.

[9] E. Callone, R. Campostrini, G. Carturan, A. Cavazza, and R. Guzzon, "Immobilization of yeast and bacteria cells in alginate microbeads coated with silica membranes: procedures, physico-chemical features and bioactivity," *J. Mater. Chem.*, vol. 18, no. 40, p. 4839, 2008.

[10] K. C. Yong, T. L. Ooi, K. Dzulkefly, W. M. Z. Wanyunus, and a H. Hazimah, "Characterization of glycerol residue from a palm kernel oil methyl ester plant," *J. Oil Palm Res.*, vol. 13, no. 2, pp. 1–6, 2001.