Production of Sorbitol from *Meranti* Wood Sawdust (MWS) using Immobilized Cells via Solid State Fermentation (SSF) Process: OFAT Study

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Abstract: Malaysia is the largest country that has produced many types of waste. One of it is *Meranti* wood sawdust (MWS). These wastes result in a significant environmental problem if not dispose of in the proper manner. The Agro-industrial like MWS has great potential as a substrate for sorbitol fermentation and others biochemical products. The objective of this study is to produce the high production of sorbitol via solid state fermentation (SSF) process from treated MWS using immobilization cells of bacteria (*Lactobacillus plantarum* sp. strain BAA 793) by entrapment technique (entrapped in sodium alginate). Besides that, one factor at a time (OFAT) were study for further process by solid state fermentation (SSF) process using immobilized cells and investigated the effect of relevant parameters (fermentation time, range: 2hours to 8hours, moisture content, range: 40% to 80%, substrate amount, range: 0.5gram to 2.5gram) to the solid-state fermentation (SSF) process in producing high yield of sorbitol production. The optimum fermentation time is at 4 hours after fixing the substrate amount at 2 g and moisture content at 50 % and the sorbitol production was about 8.396 g/L. Then, 50 % of moisture content will give optimum production of sorbitol, whereby the product was about 4.726 g/L after fixing the substrate amount at 2 g and fermentation time at 6 hours. The production of sorbitol is optimum at 1.0 g of substrate where is the production of sorbitol is about 12.210 g/L after fermentation time was maintained at 50% and 4 hours The highest product was obtained at 50% moisture content, at 4 hours of fermentation time and 1.0 gram of substrate amount whereby the concentrations of sorbitol was 12.21 g/L respectively. These results also indicate that the solid state fermentation (SSF) process will produce the high yield of sorbitol production using immobilized cells with control the important parameter.

Key words: Sorbitol production, solid state fermentation, *Lactobacillus plantarum* sp, immobilization cells

1. INTRODUCTION

Sorbitol or sometimes called as glucitol is an alcohol sugar that found in nature at high concentration in many fruits such as berries, cherries, apple and others. It is sugar alcohol with 6 carbon structure and the molecular formula of $C_6H_{14}O_6$. Normally, sorbitol is used in various food products because of health factors benefits. Sorbitol also has many applications such as in confectionary, chewing gums, candy, desserts, diabetics' food, ice cream and the wide range of food products. In addition, sorbitol also fulfills a role not only as a sweetener but also as a softener, humectants

and a texturizer [1]. In addition, the world market demand of sorbitol is increasing by constantly around 1-2% annually since the year 1997 [2].

The fermentation process to produce sorbitol can be divided into two types, namely solid state fermentation (SSF) which is still under intensive research and submerged fermentation (SmF), which is well established. Most of the industries, especially in Malaysia, rely on submerged fermentation where the bacteria or microorganisms are grown in liquid media and yet some industries also use the solid state fermentation process. Some authors such as Manpreet, (2005) have mentioned that SSF has a good option compared to the SmF process. Besides that, the process using SSF has been increasing nowadays because it is an important process and has applications in bio-pesticides, production of enzymes and aroma compounds, biopharmaceutical and the production of organic acids. The development of the SSF process was achieved sometime around 1950 to 1960 when steroid transformation was reported using fungal culture followed by mycotoxin production using the SSF process [3].

Solid state fermentation (SSF) can be described as the cultivation of microorganism in the absence of free water under control conditions. SSF has been utilized to convert moist agriculture polymeric substrate like soy, rice, sawdust, wheat and other substrates into fermented food products including industrial enzymes, fuel, and nutrient enriched animal feeds. In the Asia and Latin America, solid state fermentation (SSF) has a traditional process in productions of food. In addition, SSF process is versatile enough to be used in a wide variety of biotechnological process [4]. The solid state particle not only acts as a substrate but also serves as an anchorage for the cell [5].

The processes of solid state fermentation (SSF) can involve pure culture of microorganisms or mixture of pure strains. In this research, solid state fermentation is preferred than liquid state fermentation/submerged fermentation, SmF because of simple technique, low waste water output (liquid waste is not produced) besides fewer chances of contamination, low capital investment (cheaper), lower levels of catabolite repression, better product recovery, less time consuming and high quality of production. In order to start the solid state fermentation process, many important aspects must be considered before proceed, such as selection of suitable microorganism and substrate, optimization of process parameters, isolation, and purification of the product [3]. Solid state fermentation (SSF) and submerged (liquid state) fermentation can be used to produce sorbitol.

Currently, production of biochemical such as lactic acid, sorbitol, bioethanol and others biochemical product providers about 50% of the world supply through free cell fermentation, but productivity is very low in the fermentation process. However, employing cell immobilization technique that provides high density can increase the productivity in biochemical products. Besides that, the immobilization process is one of the most attractive methods of maintaining high cell concentration in the bioreactor for process biochemical products[6], [7]. Generally, the whole cells of immobilization were described as localization of intact cells to a certain defined region of space with preservation of some desired activity [8]. The immobilization of whole cells process and their application in bioprocessing has been interesting for 30 years [9]. Besides that, immobilization also consists immobilizing cells of microorganism inside or on the surface of a carrier in a way that preserving their catalytic activity [10,11]. The immobilization cell system offers the great advantages in several industrial processes such as easy to handle biocatalyst, easy separation of biological material from the reaction medium, employs high cell loading capacity, improve the production rate of products and others advantage [12,13].

2. MATERIAL AND METHOD

A. Microorganisms:

The microorganism that used for this study was *Lactobacillus plantarum* sp. (BAA 793) and was purchased from America type Culture Collection (ATCC). The *Lactobacillus plantarum* was maintained in MRS medium. The MRS agar and MRS broth were prepared according to the formula of [14, 15].

B. Inoculum Preparation for Immobilized Cells:

The cells for immobilization were obtained by transferring one loop of bacteria (*Lactobacillus plantarum*) from the agar plate into 100ml of MRS medium then incubate for 24 hours at 30 °C. After 24 hours of cultivation, then these inoculums cells were used for immobilization process (entrapped in sodium alginate). The bacteria (*Lactobacillus plantarum*) cells that were grown in MRS broth were mixed with an equal medium volume of 2% Na-alginate solution

(1:1, v/v). Then, after its mix, the alginate-cell solution was dropped slowly into 0.2% of calcium chloride ($CaCl_2$) solution using syringe needle. After that, the alginate solidified when its contact with $CaCl_2$ solution and forming gels beads thus entrapping the bacteria cells. The beads were allowed to harden in $CaCl_2$ solution for 30 minutes. Then the beads were washed with 0.85% of NaCl solution and distil water to remove excess calcium ion and cells. The beads were stored at 4 °C for further experiment. To improve the immobilization results, the ratio of $CaCl_2$ and NaCl that used in solution preparation was 1:1, v/v (Modification from: [16],[17], [18]).

C. Substrate:

The *Meranti* wood sawdust (MWS) was obtained from Kilang Kayu Aman Sdn Bhd, Gambang Kuantan, Pahang. The MWS is of a hardwood type that contains a high cellulose content compared to the other types. MWS was taken by bulk from the sawmill plant in order to make sure the quality of the materials is same until the end of the research. The substrate that used in this study was cellulose recovery after the process of pre-treatment MWS.

D. Solid state fermentation (SSF) process using immobilized cells (entrapment technique):

In the solid state fermentation process, several parameters play more important role in order to produce a high yield of sorbitol. For this part of OFAT study, the parameters such as fermentation time, moisture content and substrate amount were studied. The effect of fermentation time, moisture content and substrate amount on the sorbitol production were evaluated by varying the fermentation time from 2 hours to 8 hours, the moisture content of substrate from 40 % to 80 %, and substrate from 0.5 gram to 2.5 gram.

The inoculum was prepared in MRS broth. In this part, all the experiment should be conducted in laminar flow space in order to avoid contamination. 100 ml of MRS medium was transferred into 250 ml Schott bottle and one loop full of bacteria *(Lactobacillus plantarum*) from agar plate was transferred into 100 ml of MRS medium. After that, nitrogen gas (N_2) was purged into Schott bottle that was contained MRS medium and bacteria (*Lactobacillus plantarum*) in order to remove oxygen gas (O_2) inside Schott bottle and to maintain the anaerobic condition during cultivation process. Then, it was kept in the incubator at 30 ^{o}C. After 24 hours cultivation, the optical density of inoculums was checked using UV-Vis Spectrophotometer. Setting the optical density at UV-Vis spectrophotometer equal to 600 nm (OD_{600}). The values of OD_{600} should be less than 0.4 or (0.1-0.2) [19]. Then, the bacteria (*Lactobacillus plantarum*) cells that were grown in MRS broth were mixed with an equal medium volume of 2% Na-alginate solution (1:1, v/v). Then, the alginate-cell solution was dropped slowly into 0.2% of calcium chloride ($CaCl_2$) solution and forming gels beads thus entrapping the bacteria cells. The beads were allowed to harden in $CaCl_2$ solution for 30 minutes before washed.

After that, 2 grams of samples were put into the 100 mL Erlenmeyer conical flask, and then it was moistened with 50 % of distilled water. All the apparatus and materials were sterilized at 121°C for 15 minutes to avoid contamination (Modification from: [20], [21], [19], [20]). Besides that, this experiment was conducted in the laminar flow to avoid contamination and loss of viability. After the sterilizing process, the samples were cooled. Then, 10% of beads of immobilized cells were put into the samples (Modification from: [22],[18], [17],[16]). The samples were purged with nitrogen gas (N_2) in order to replace oxygen gas to maintain the anaerobic condition and then it was incubated at 35°C for range time: 2 to 10 hours, moisture content range: 40% to 80% and substrate amount range: 0.5 to 2.5 gram. In this part of the experiment, which varies only one factor or variable at a time and while keeping others parameter fixed. All the experiments were carried out in 3 sets to get the average values.

3. RESULT AND DISCUSSION

A. Effect of Fermentation Time on Sorbitol Production:

Figure 1 shows the effect of fermentation time on sorbitol production using immobilized cells of *Lactobacillus plantarum* strain (BAA793) via solid state fermentation (SSF) process whereby cellulose treated used as the substrate and the cellulose remaining during the fermentation process. From Figure 1, it can be seen that the sorbitol production was slow at the beginning of 2 hours fermentation (7.842 g/L) and then increased smoothly with the optimum time of 4 hours when the sorbitol production was about 8.396 g/L after fixing the substrate amount at 2 g and moisture content at 50 %. This is because the immobilized cells of bacteria had already interacted optimally with the substrate for 4

hours. After 4 hours, the production of sorbitol slowly decreased beginning from 6 hours until 8 hours whereby the production sorbitol was about 7.382 g/L and 6.654 g/L respectively. This is because the bacteria cells in gel bead had started to be inactive at that point in a solid state condition that is why the sorbitol production was low.

Besides that, the Figure 1 below also represented cellulose that remained during fermentation sorbitol using immobilized cells via solid state fermentation (SSF). The actual amount of cellulose after treated MWS was about 1.9937g. Then, after (2, 4, 6, 8) hour of fermentation, the amount of cellulose that remained were (0.286, 0.158, 0.038, 0.021) g respectively. The cellulose content decreased when fermentation time increased. In addition, the bacteria cells that immobilized in gel bead were contacted with substrate and in the same time it used cellulose as carbon sources in order to produce sorbitol

B. Effect of Moisture Content on Sorbitol Production:

Figure 2 shows the results of the OFAT study for the effect of moisture content (in percentage form) using immobilized cells of *Lactobacillus plantarum* strain (BAA793) via solid state fermentation (SSF) process using cellulose from pre-treated MWS. From the results, the moisture content in solid state fermentation was quite significant and it depends on the type of microorganism that was immobilized and also substrate that are used in the solid state fermentation process. From the Figure 2, 50 % of moisture content will give optimum production of sorbitol, whereby the product was about 4.726 g/L after fixing the substrate amount at 2 g and fermentation time at 4 hours. This means that the formation of some products such as sorbitol will be influenced by water activity where the optimal moisture content depends on the cultivation temperature and optimal growth *of Lactobacillus plantarum* (BAA793). This can be proved from the equation below describing water-activity-based growth dependence [23].

$$\mu FW = 1.053\exp(-131.6aw3 + 94.99aw2 + 214.219\ \ aw - 177.668) \quad (1)$$

where μFW = Fractional specific growth rate based on water activity and aw= Fermenting solid water activities.

Based on the equation 1, the fractional specific growth rate based on water activity increased when the fermenting solid water activities increased. Then, 60% of moisture content will give about 4.557 g/L, 70% was about 4.437 g/L, and 80% will be about 4.357 g/L of sorbitol production respectively. This is because immobilized cells of bacteria *Lactobacillus plantarum* (BAA793) was not active to react to the high moisture content of the substrate and that is why water activity is significant in the production of sorbitol. The equation of moisture content is shown as below:

$$MC = (\text{Weight of water}/\ \text{Weight of samples}) \times 100\% \quad (2)$$

In addition, the moisture content also has relations with water activity (aw) and relative humidity (RH). Actually, the water activity (aw) of the moist solid substrate is the ratio of the vapor pressure of water above the substrate in the closed system to the vapor pressure of the pure water at the same temperature. For the pure water, the water activity (aw) has 1.0 and aw will decrease after addition of the solutes. The water activity (aw) is measured by relative humidity divided by 100 [3]. The equation for water activities and relative humidity was showed below:

$$aw = p/po = \%\ RH/100 \quad (3)$$

where, p = Vapor pressure of water in above the substrate, po = Vapor pressure of water at the same temperature and RH = relative humidity.

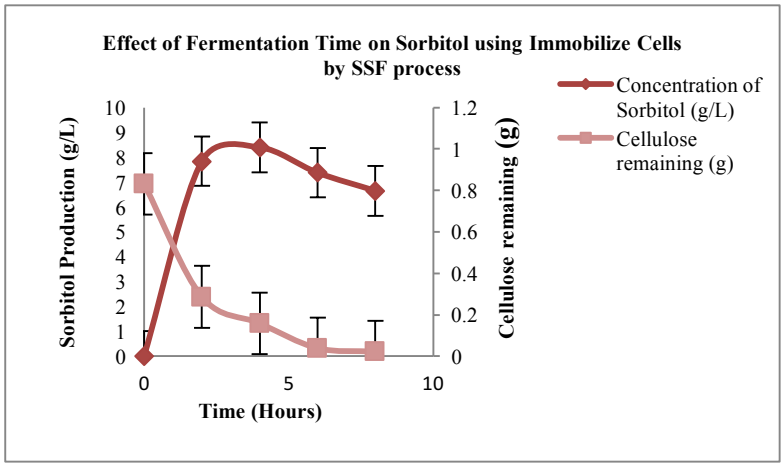

Figure 1: Effect of fermentation time using immobilized cells in the SSF process and cellulose content during fermentation sorbitol using immobilized cells via solid state fermentation (SSF)

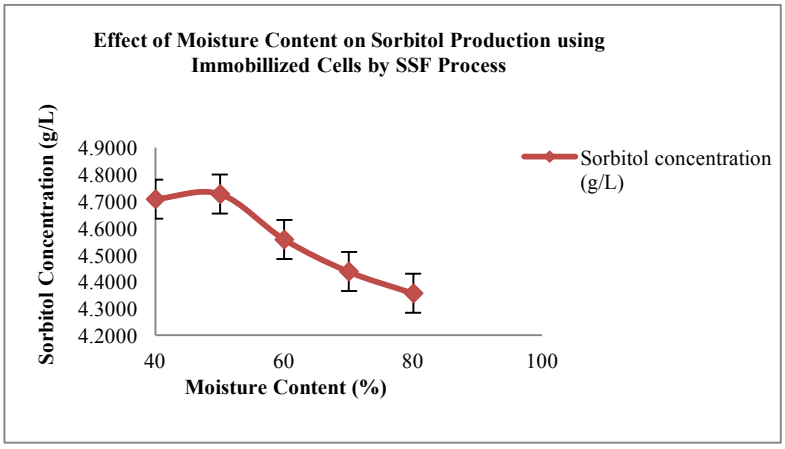

Figure 2: Effect of moisture content using immobilized cells in SSF process

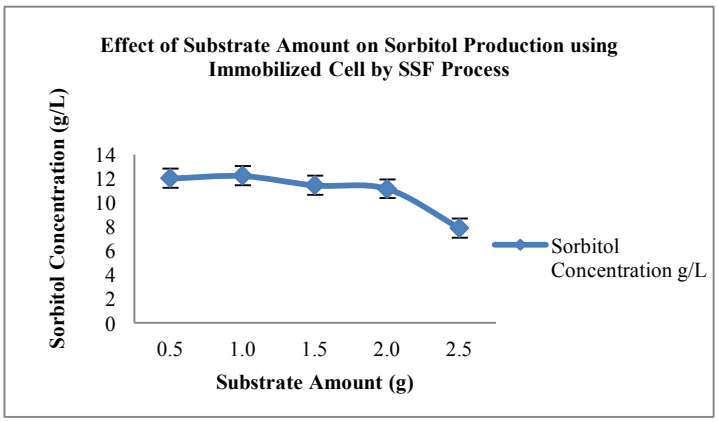

Figure 3: Effect of substrate amount on sorbitol production using immobilized cells in the SSF process

C. Effect of Substrate Amount on Sorbitol Production:

For Figure 3, the result shows the effect of the substrate amount for the OFAT study using immobilized cells of *Lactobacillus plantarum* strain (BAA793) via solid state fermentation (SSF) process. For this study, the substrate amount that used was 0.5 g/L to 2.5 g/L in order to check the optimum point of the substrate amount for sorbitol production while the moisture content and fermentation time were maintained at 50% and 4 hours. The graph plotted from Figure 5.4 shows that the starting substrate amount of 0.5 g gave production of sorbitol which is about 12.002 g/L and the production of sorbitol increased until substrate amount reached 1.0 g where is the production of sorbitol was about 12.210 g/L respectively. This high production of sorbitol is due to the bacteria *Lactobacillus plantarum* BAA793 that entrapped in Na-alginate was out/leak from the gel were contacted and reacted optimally with the substrate at 1.0 g. Besides that, the production of sorbitol decreased starting at 1.5 g (11.428 g/L) of substrate until 2.5 g (7.854 g/L) because the immobilized cells of bacteria *Lactobacillus plantarum* (BAA793) lost its viability and was destroyed by lysis

4. CONCLUSION

In conclusion, solid state fermentation (SSF) process will produce the high yield of sorbitol at 4 hours of fermentation time, 50% of moisture content and 1.0 gram of substrate amount (12.21 g/L) using immobilized cells of *Lactobacillus plantarum* sp. The parameters that influencing sorbitol production such as (fermentation time, moisture content and substrate amount) should be control in order to get the maximum production of the product. Those three parameters play an important role in solid state fermentation (SSF) process.

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