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Optimization of Bioethanol Production from Durian Skin by Encapsulation of *Saccharomyces cerevisiae*

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The optimum condition of durian skin using SSF process with encapsulation of *Saccharomyces cerevisiae* takes place at pH 5.0 at 37 °C. *S. cerevisiae* encapsulation can increase bioethanol production activity. Bioethanol production is produced at pH 4.5; 5.0 and 5.5 were 35.85, 41.25 and 39.89 g/L medium, respectively. While using free cells *S. cerevisiae*, bioethanol produced was 21.42, 34.94 and 28.15 g/L medium. The percentage of bioethanol produced by *S. cerevisiae* was 15.3 % at pH 5.0; 40.25 % at pH 4.5 and 29.43 % at pH 5.5. Encapsulation of *S. cerevisiae* cells can increase the resistance to process temperature by differences in bioethanol production between encapsulation with cells free of *S. cerevisiae* by 19.34 % at 37 °C, 24.02 % at 40 °C and 49.01 % at 45 °C. These results are higher compared to cells free of *S. cerevisiae.*

Keywords: Bioethanol, Encapsulation, Durian skin, *Saccharomyces cerevisiae*.

INTRODUCTION

Many researches have been done to optimize bioethanol productions. Due to increasing demand of clean energy, several steps for generating the sustainable and environmentally friendly energy sources are required [1]. Thus biofuels are now considered as eco-frendly alternative energy fuel as compared to conventional fossils. Sudiyani *et al.* [2] reported that the ethanol production using SSF process from TKKS and also the utilization of *Rhizopus orizae*. Millati *et al.* [3] achieved as much as 20 g/L ethanol from 50 g/L glucose. With different atmospheric conditions, ethanol production is also affected. Karimi *et al.* [4] investigated the effect of anaerob and aerob conditions, as much as 21.51 and 19.25 g/L ethanol was achieved, respectively.

Currently, the most advantegous method to produced bioethanol is the SSF process. SSF combined saccarification and fermentation process. However, saccarification and fermentation process have different optimum operation temperature. One of the aim of current study is to overcome this problem by cell encapsulation. Telebnia and Taherjadeh [5] found that encapsulated *S. cerevisiae* can increase the production of ethanol. Ylitervo *et al.* [6] also found that the fermentation using encapsulated *S. cerevisiae* produced ethanol in higher temperature than unencapsulated *S. cerevisiae*.

Durian skin considered as agricultural waste that contain 60.45 % α-cellulose, 13.09 % hemicellulose and 15.45 % lignin [7]. With these compositions, durian skin has high potential as natural sources of bioethanol production. The main purpose of this study is to increase the bioethanol production by investigating the optimum pH and temperature conditions for bioethanol production using SSF process with encapsulated *S. cerevisiae*, cellulase and β-glucosidase enzyme.

EXPERIMENTAL

The materials used in this research were durian skin (Pandenglang, Indonesia), cellulase enzyme ≥ 0.3 units/mg solid, β-glucosidase enzyme ≥ 6 U/mg solid, *S. cerevisiae* AM 12, Potato Dextrose Agar (PDA), Na-Alginat, yeast extrac, CH_3COOH (Sigma Aldrich) and H_2SO_4 5 mM (Sigma Aldrich).

The instrumentations used in this research were rotary shaker incubator, autoclave for sterilization, HPLC (Waters 2695, Milford, MA) to determine the ethanol concentration,

Aminex column HPX-87H (Bio-Rad, Richmond, CA, USA), RI detector (Waters 2414).

Pre-treatment: Pre-treatment were conducted to remove the lignin from the durian skin and also to increase the surface area of the particles [8]. First, durian skin was diced into small pieces and then soaked in NaOH 10 % solutions. Then, put the mixture inside pressurized reactor for 30 min at 150 °C. After 30 min, remove the mixture to be washed until neutral pH. Afterwards, separate the liquid and solid. Dry the solid in the oven and then grinds the solid until the solid reach the size of 30-60 mesh.

Analysis of cellulose, hemicellulose and lignin: WISE method was conducted to analyze the cellulose and hemicellulose concentration. First, sample was mixed with 3 M $NaClO_3$, 5 M CH_3COOH and distilled water. The mixture was then incubated with H_2O at 80 °C, followed by cooling and filtration. After that, the sample was rinsed using $(CH_3)_2CO$. The remaining solid on the filter was dried in oven at 105 °C and then weigh.

Modified klason lignin method was conducted to analyze lignin concentration. First, sample was mixed with 72 % H_2SO_4 and then heated in autoclave for 30 min at 121 °C. Heated sample then filtered using Whatman filter paper 41. Afterwards, the sample dried in oven for 1 h and then weigh.

***S. cerevisiae* cultivation:** *S. cerevisiae* was pre-cultured on Potato Dextrose Agar (PDA). A total of 0.5 g PDA were dissolved in 100 mL of distilled water and heated until all the ingredients dissolved. Then, sterilized by autoclave at 121 °C for 15 min. The culture of *S. cerevisiae* was isolated for 48 h.

Growth of *S. cerevisiae*: *S. cerevisiae* from stock was pre-cultured in 100 mL of sterilized medium. The medium consisted of glucose 10 g/L, yeast extract 100 g/L, KH_2PO_4 0.1 g/L, $MgSO_4.7H_2O$ 0.1 g/L and $(NH_4)_2SO_4$ 0.1 g/L were included in 200 mL of Erlenmeyer glass. Then, the mixture was incubated at 30 °C using an orbital shaker at 150 rpm speed.

***S. cerevisiae* encapsulation:** A total of 1 mL *S. cerevisiae*, which had grown in growth medium for 48 h was suspended into 20 mL solution of 1 % $CaCl_2$ solution. Afterwards, the culture suspension of *S. cerevisiae* was added using a syringe (drop by drop) into a 0.5 % Na-alginate solution and then stirred. The capsules formed were washed with distilled water and allowed to sit in a citrate buffer. Encapsulated cell of *S. cerevisiae* was then added to the growing medium at 30 °C.

Condition of SSF process: As much as 100 mL medium for SSF process that cointains 15 g of durian skin sample, nutritional medium, 0.05 M of citrate buffer to obtain a variation of pH 4.5; 5.0 and 5.5, β-glucosidase and cellulase (15FPU) and capsule *S. cerevisiae* were used. Nutritional medium consisted of 1.0 g/L $(NH_4)\ PO_4$, 0.05 g/L $MgSO_4{\cdot}7H_2O$ and 2 g/L yeast extract. Samples, nutrient medium and buffer were sterilized using autoclaves at 121 °C for 20 min, 15 FPU of enzyme solutions and *S. cerevisiae* were added after sterilization. Cultivation was taken and then put into 250 mL Erlenmeyer with a total volume of 100 mL. The saccharification and fermentation processes were carried out using orbital shakers at a speed of 150 rpm for 96 h at various temperatures *e.g.*, 37, 40 and 45 °C and at various pH *i.e.*, 4.5, 5 and 5.5 in anaerobic conditions. Sampling process was done at 24, 48, 72 and 96 h and free cell *S. cerevisiae* was also used as a comparison.

Determination of glucose and ethanol concentration: HPLC was used to determined the concentration of glucose and ethanol. Standard curve was made by plotting various standard concentrations 0.1, 0.3, 0.5, 0.7, 0.9, 1.1 and 1.3 g/L, respectively. Linear regression method was used to achieve the linear equation. Thus, the concentration of glucose and ethanol in sample can be determined.

RESULTS AND DISCUSSION

Durian skin composition: In general, the saccharification process for converting polysaccharides, especially cellulose into monosaccharides, is hampered due to the presence of lignin which are hydrophobic and has the characteristic to protect the cellulose. Lignin can be oxidized by alkaline solutions and other oxidizing agent. Initial treatment with NaOH solution was intended to help the cellulose saccharification process. Thus, resulting easier pathway for glucose to be fermented into bioethanol. Table-1 shows the analysis of cellulose content, hemicellulose and lignin obtained.

TABLE-1
DURIAN SKIN COMPOSITION

Composition	Percentage
Cellulose	63.95
Hemicellulose	10.45
Lignin	12.75

From Table-1, it can be seen that durian skin has high cellulose and hemicellulose composition. This result shows that durian skin has the potential to be processed into bioethanol as a renewable fuel. The results analysis of the durian skin content obtained showed almost the same results as those obtained by Khedari *et al.* [9], *i.e.* 60.45 % α-cellulose, 13.09 % hemicellulose and 15.45 % lignin.

Effect of pH variation on bioethanol production from durian skin with free cells of *S. cerevisiae*: Durian skin by SSF process using encapsulated *S. cerevisiae* was presented and compared to free cell of *S. cerevisiae*. The addition of cellulase enzyme and β-glucosidase was intended to help the saccharification process, converting cellulose to glucose. Cellulase enzymes used were 15 FPUs and 5 FPU β-glucosidase enzymes. Cellulase enzymes work specifically to break down the long chain of cellulose into glucose monomers. Crystalline cellulose has a rigid structure that is resistant to the action of individual cellulase. Effective breakdown of cellulose into glucose is only possible by the synergistic work of the following three cellulase subgroups [10]: (1) Endo-β-1,4-D-glucanase which breaks down the internal glucosidic bonds between intacted glucan chains; (2) Exo-β-1,4-D-glucanase/exo-β-1,4-D-cellobiohidrolase which breaks up the cellobiose dimer from the glucan chain and releases it into solution; (3) β-glucosidase which completes the hydrolysis of cellulose to glucose by breaking down the cellobiose into a glucose monomer.

Cellobiose actually exists in one component of a complex cellulase enzyme that functions to break down the cellulose into glucose. It is known that the presence of cellobiose enzyme in cellulase is only a little because it is dominated by endocellulase and exocellulase enzymes, so it is not optimal enough

if only relying on the cellulase contained in cellulase. The addition of an external cellulase enzyme is strongly recommended for enzymatic reactions to the breakdown of cellulose into glucose [11]. In the process of formation of bioethanol from durian skin, not only cellulose enzyme was added, β-glucosidase enzyme was also added to increase the process of breaking the cellulose into glucose. The glucose was simultaneously fermented into bioethanol by *S. cerevisiae*. From Fig. 1, it can be seen that the glucose concentration was increased in the first 24 h and then decreased until the end of the fermentation process (96 h). This shows the ongoing process of saccharification or hydrolysis, where cellulase and β-glucosidase enzyme were breaking the long chain of cellulose into glucose. The decreasing of glucose production shows that the glucose formed undergoes fermentation by *S. cerevisiae*. This proves that formation of bioethanol is increasing with increasing SSF processing time (Fig. 2).

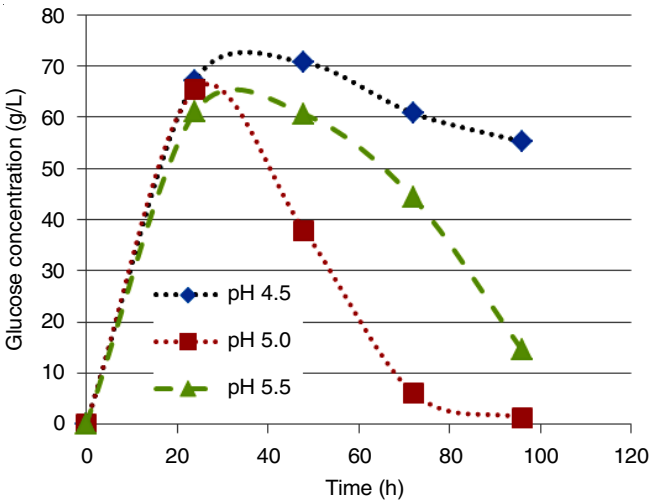

Fig. 1. Glucose concentration of SSF method with cellulose enzyme, β-glucosidase and *S. cerevisiae* free cells at various pHs

From Fig. 2, it can be seen at pH 5 the formation of bioethanol from durian skin is much higher than pH 4.5 and at pH 5.5. The higest bioethanol production at pH 5 reached a concentration of 34.94 g/L while at pH 4.5 and 5.5, the concentration of bioethanol only reached 21.42 and 28.16 g/L, respectively. These results indicate that pH 5.0 is the optimum pH formation of bioethanol from durian skin using *S. cerevisiae*. Previous studies also found the optimum pH for cellulase enzymes and yeast *S. cerevisiae* at pH 5 [12-14], because at pH 5 the cellulase enzyme was stable and more active (12). In the first 48 h, the production of bioethanol was significant. This phenomenon was also known as the logarithmic phase (high growth phase). However, after reaching 72 h, the increase of bioethanol production is not significant.

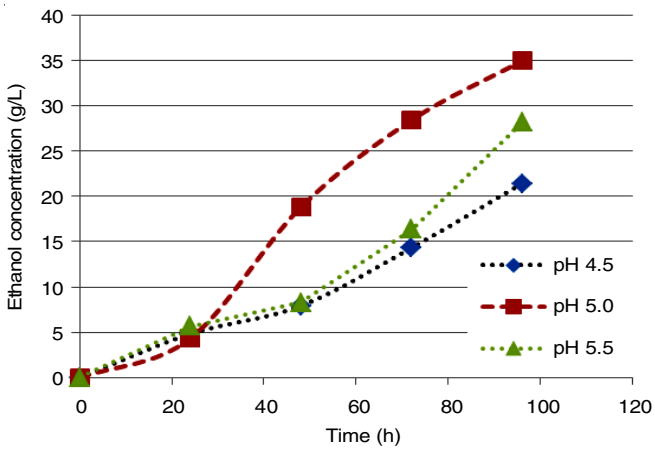

Fig. 2. Bioethanol concentration of SSF method with *S. cerevisiae* free cells at various pHs

Table-2 shows the concentration of bioethanol produced in the variation of pH, yield of bioethanol based on the content of cellulose in durian skin, yield g of bioethanol based on the dry weight of durian skin and the yield of theoretical bioethanol. The concentration of bioethanol obtained was not significant compared to theoretical calculations, *i.e.* 64.79 % at pH 5, 51.79 % at pH 5.5 and 39.41 % at pH 4.5. The results obtained using *S. cerevisiae* free cells showed that not all glucose formed was consumed effectively. Thus, it is important to make an improvement in order to enhance the bioethanol production. To improve the result, immobilization of *S. cerevisiae* free cells was needed to be done.

To prevent cells from reaching the stationary phase and also to avoid the formation of lactic acid when the source of glucose has been reduced, the SSF process of bioethanol formation was stopped at the 69 h because the glucose concentration had reached 5-10 % [15].

Effect of pH variation on bioethanol production from durian skin with encapsulation of *S. cerevisiae*: Fig. 3 shows that glucose production in the first 24 h was significantly increased due to the saccharification of cellulose to glucose continues while the growth of *S. cerevisiae* spores was not as significant. After 24 h, the glucose concentration decreased due to the formation of bioethanol through a glucose fermentation reaction by *S. cerevisiae*. The results showed that the encapsulation of *S. cerevisiae* cells in Na-alginate capsules did not inhibit the growth of *S. cerevisiae* and still produce bioethanol. Encapsulation provides an artificial cell wall that creates cell protection from external influences. In general, encapsulation is carried out using polymers and biopolymers such as Na-alginate. Encapsulation that was made from polymers have the ability to be present in different phases such as liquid, gel or solid. This causes encapsulation to have strong mechanical and physical strength [16].

TABLE-2
PRODUCTION OF BIOETHANOL FROM DURIAN SKIN WITH *S. cerevisiae* FREE CELLS AT VARIOUS pHs

pH	Bioethanol production (g/L)	Yield of bioethanol based on cellulose content (g/g cellulose)	Yield of bioethanol based on dried durian skin weight (g/g durian skin)	Theoretic yield of bioethanol (%)
4.5	21.42	0.22	0.14	39.41
5.0	34.94	0.36	0.23	64.28
5.5	28.15	0.29	0.19	51.79

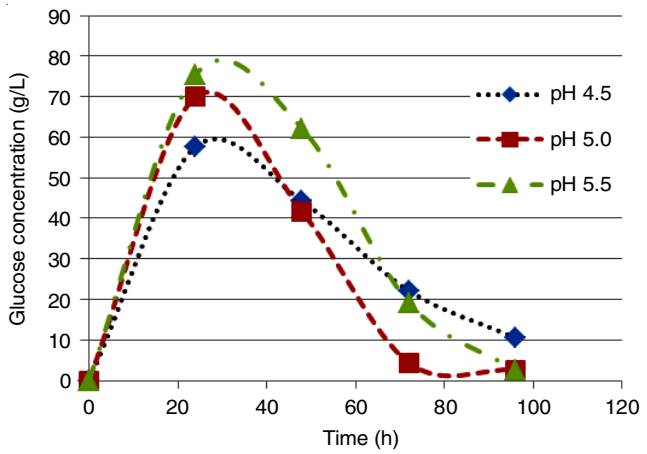

Fig. 3. Glucose concentration of SSF method with cellulase enzyme, β-glucosidase and encapsulated *S. cerevisiae* at various pHs

Encapsulated *S. cerevisiae* can still metabolize glucose. Glucose diffuse into the capsule and produced bioethanol through a fermentation process. Then, bioethanol will diffuse out of the capsule through the walls of Na-alginate. The right Na-alginate concentration can optimize the diffusion rate of glucose and bioethanol through the walls of Na-alginate [17]. Bioethanol was toxic to *S. cerevisiae*, thus encapsulation can also protect the cells from bioethanol to prevent self-poisoning.

Fig. 4 shows the production of bioethanol by encapsulation of *S. cerevisiae* cells. It can be seen that various pH was used to compare the bioethanol production; pH 4.5, pH 5.0 and pH 5.5 which resulted 35.85, 41.25 and 39.89 g/L, respectively. The optimum result was conducted at pH 5, reaching 41.25 g/L. The bioethanol yield produced from durian skin using *S. cerevisiae* encapsulation was presented in Table-3.

Table-3 shows the highest ethanol yield produced by encapsulation of *S. cerevisiae* cells was obtained at pH 5.0. This shows

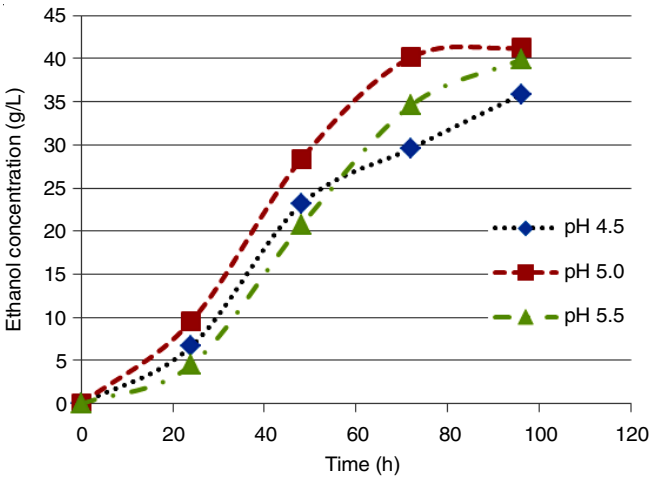

Fig. 4. Bioethanol concentration of SSF method with encapsulated *S. cerevisiae* at various pH

that pH 5.0 is the optimum pH for SSF process in bioethanol production from durian skin both with free cells and with cell encapsulation of *S. cerevisiae*. The yield of bioethanol produced by the encapsulation of *S. cerevisiae* cells was higher than *S. cerevisiae* free cells. At pH 5.0, the theoretical yield of bio-ethanol production by encapsulated *S. cerevisiae* reached 75.89 %.

Comparison of bioethanol production with encapsulation and free cells at various pH variations using SSF process: From Fig. 5, it can be seen that the production of bioethanol from the durian skin fermentation process using encapsulated *S. cerevisiae* was higher than free cells *S. cerevisiae*. The production of ethanol by encapsulation of *S. cerevisiae* at pH 4.5; 5.0 and 5.5 respectively were 35.85, 41.25 and 39.89 g/L medium. This result is 15.3-40.25 % higher when compared to ethanol production using free cells *S. cerevisiae*. In the variation of pH, it can be seen that the ethanol production has the same tendency between free cells with cell encapsulation, which has the highest ethanol production at pH 5.0 and lower ethanol production is obtained at pH 5.5 and 4.5. So it can be concluded that pH 5.0 is the optimum pH for the fermentation process of durian skin by using encapsulated free cells or *S. cerevisiae*. At the optimum pH *S. cerevisiae* will make metabolic processes faster to consume glucose, the more glucose consumed, the more ethanol produced.

Fig. 5 also showed the effect of pH variation to ethanol production. It can be seen in the fermentation process using free cell, pH change between pH 5.0 to pH 4.5 reduced the bioethanol production by 38.7 %. Meanwhile, fermentation process using encapsulation of *S. cerevisiae* only decrease ethanol production by 13.18 % with the same pH change.

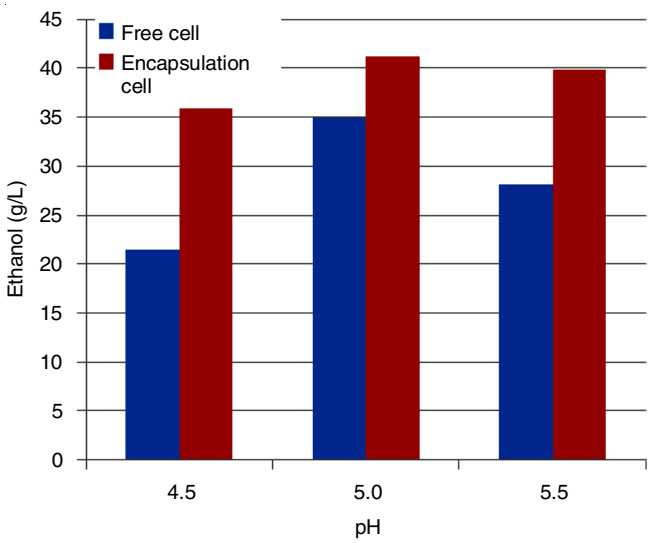

Fig. 5 Comparison of bioethanol concentration with free cell and encapsulated *S. cerevisiae* at various pH

TABLE-3
BIOETHANOL PRODUCTION FROM DURIAN SKIN WITH *S. cerevisiae* ENCAPSULATION IN pH VARIATIONS

pH	Bioetanol concentration (g/L)	Yield of bioethanol based on cellulose content (g/g cellulose)	Yield of bioethanol based on dried durian skin weight (g/g durian skin)	Theoretical yield of bioethanol (%)
4.5	35.85	0.37	0.24	65.95
5.0	41.25	0.43	0.28	75.89
5.5	39.89	0.42	0.27	73.39

Cell encapsulation increases cell resistance to changes in surrounding conditions; in this case, the various pH change of the fermentation process. The cell encapsulation process provides cell protection with artificial cell walls and provides resistance to cells from acidic conditions in solution. High pH (base condition) can cause stress on microorganisms that will affect its metabolism. While low pH (acidic condition) will make the process of metabolism run slower. The process that uses free cell *S. cerevisiae* is vurnerable to changes in pH, slight change in pH reduced the production of ethanol produced (Fig. 5). Whereas the process carried out using cell encapsulation is more resistant to changes in pH. Changes in pH to 5.5 decrease the production of ethanol produced by *S. cerevisiae* encapsulation. In addition, the increase in ethanol production using encapsulated cells was also caused by the encapsulated cells that were more anaerobic in nature compared to *S. cerevisiae* free cells. Encapsulated cells also prevent the contact of the cell with oxygen, so that the anaerobic process takes place better.

Fig. 6 shows the difference in ethanol production between encapsulated and free cells of *S. cerevisiae*. It can be seen that with pH change affect the ethanol production. At pH 5.0 the difference in ethanol production between cell encapsulation and free cells was 15.3 %. This indicates the production of ethanol produced in the fermentation with *S. cerevisiae* encapsulation was 15.3 % higher than fermentation with *S. cerevisiae* free cells. The same tendency was also happening at pH 4.5 and 5.5, the difference in ethanol production between free cells and cell encapsulation was 40.25 and 29.43 %, respectively. These results indicate the fermentation process using cells free of *S. cerevisiae* is more vulnerable to changes in pH compared to the process of using encapsulated *S. cerevisiae*.

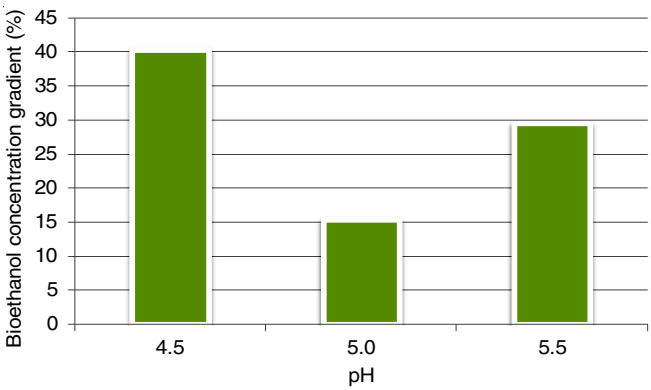

Fig. 6. Differences of bioethanol concentration with free cell and encapsulated cell at various pH

Effect of temperature variation on bioethanol production from durian skin with free cells of *S. cerevisiae*: One of the important things in the growth of microorganisms including *S. cerevisiae* is temperature. Figs. 7 and 8 show the saccharification process of cellulose to glucose and fermentation of glucose to bioethanol at various temperatures 37, 40 and 45 °C. The increase of process temperature caused the enhancement of glucose concentration in the first 24 h, the production of glucose at 45 °C is higher than at 37 and 40 °C. However, bioethanol production decreases when the temperature is raised to 40 °C and decreases when the temperature is raised to 45 °C.

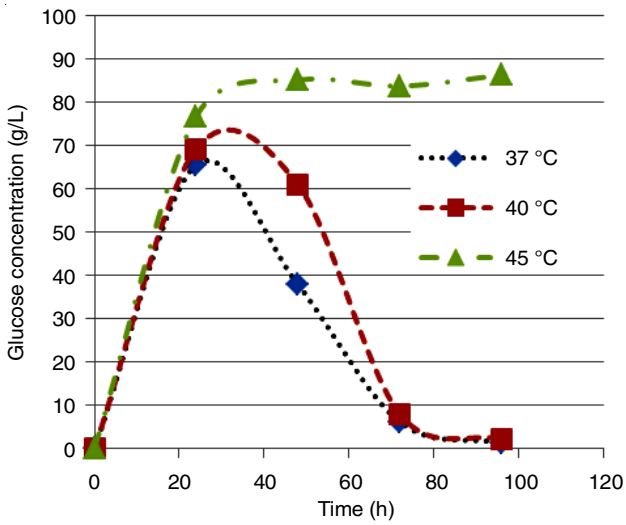

Fig. 7. Glucose concentration of SSF method with cellulase enzyme and β-glucosidase, *S. cerevisiae* free cells at various temperatures

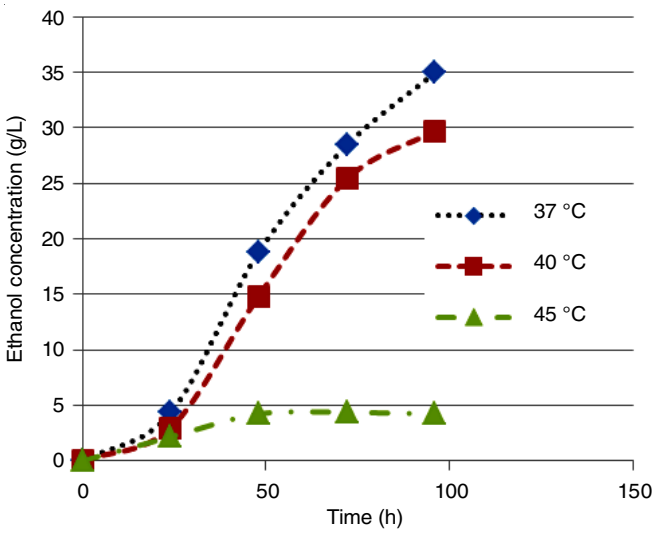

Fig. 8. Bioethanol concentration of SSF method with encapsulated *S. cerevisiae* at various temperatures

From Fig. 6, it can be seen that the highest bioethanol production was obtained at an optimum temperature of 37 °C, which was 34.94 g/L media. At this temperature *S. cerevisiae* is less optimal in consuming glucose, so not all glucose that is formed is converted to bioethanol.

The yield of bioethanol produced at various temperatures was shown in Table-4. The highest bioethanol production was produced at 37 °C with the yield of 0.36 g/g cellulose with 64.28 % of theoretic yield of bioethanol. With the increasing of the process temperature, production of biothenaol was decreased.

Effect of temperature variation on bioethanol production from durian skin with free cell encapsulation of *S. cerevisiae* Figs. 9 and 10 shows the effect of *S. cerevisiae* encapsulation on cell resistance at various process temperature. It shows that the increase in temperature also decreases the production of bioethanol as well as in free cells. This shows that the encapsulation provides resistance to pH change and also resistance to process temperature change. The highest bioethanol production at 37 °C was 43.32 g/L medium. While bioethanol produc-

TABLE-4
BIOETHANOL PRODUCTION FROM DURIAN SKIN WITH *S. cerevisiae* FREE CELLS AT VARIOUS TEMPERATURES

Temp. (°C)	Bioetanol concentration (g/L)	Yield of bioethanol based on cellulose content (g/g cellulose)	Yield of bioethanol based on dried durian skin weight (g/g durian skin)	Theoretic yield of bioethanol (%)
37	37.94	0.36	0.23	64.28
40	29.73	0.31	0.20	54.70
45	4.36	0.045	0.03	8.02

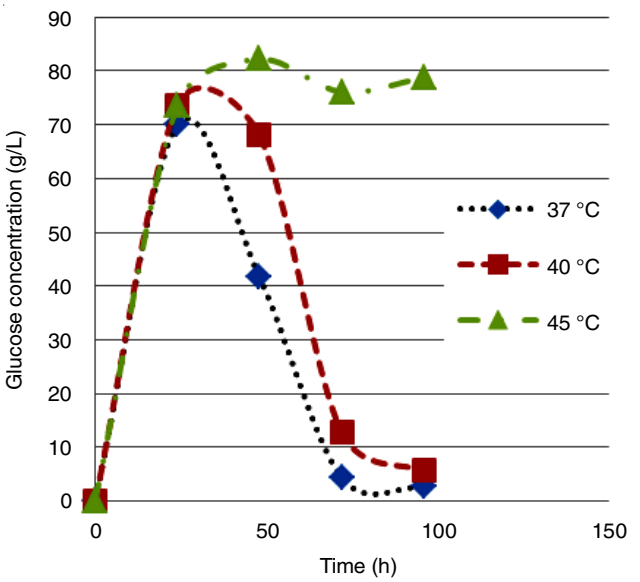

Fig. 9. Glucose concentration of SSF method with cellulase enzyme, β-glucosidase and encapsulated *S. cerevisiae* at various temperatures

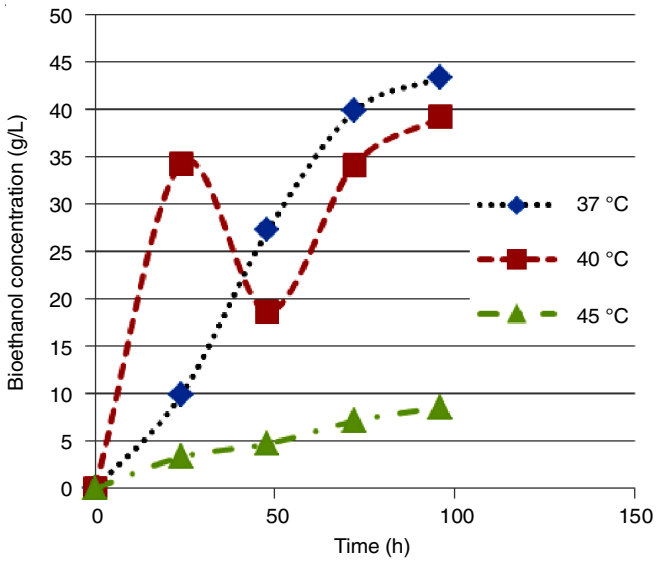

Fig. 10. Bioethanol concentration of SSF method with *S. cerevisiae* cell encapsulation at temperature variations

tion at 40 °C was 39.13 g/L medium and at 45 °C resulted significant drop to 8.55 g/L medium.

From Table-5, it was shown that the highest bioethanol production carried out at 37 °C, which resulted higher yield and theoretical yield of bioethanol production; 0.45 g/g cellulose 79.70 %, respectively. At 40 °C, the yield and theoretical yield of bioethanol production is 0.41 g/g cellulose and 71.99 %, respectively. While at 45 °C, the production of bioethanol has decreased significantly, which is 0.09 g/g cellulose and 15.73 % for theoretical yield of bioethanol production.

Comparison of SSF results with encapsulated and free cells *S. cerevisiae* at various temperatures: Fig. 11 showed that the highest bioethanol production is obtained at 37 °C. When the fermentation process was carried out at 40 °C, ethanol production produced both in cell encapsulation and with *S. cerevisiae*-free cells decreased. Similarly, when the process temperature was raised to 45 °C, there was a significant decrease in ethanol production. It can be seen that the encapsulation of *S. cerevisiae* cells produces higher ethanol production than the fermentation process using *S. cerevisiae*-free cells at a higher temperature change.

The process temperature has great effect on the growth of *S. cerevisiae* or microorganisms in general. High temperatures damage the cells including membrane or cell wall damage, protein denaturation and cell aggregation [18]. Encapsulation is also proven to provide resistance to temperature rise. The decrease in bioethanol production was due to changes in process

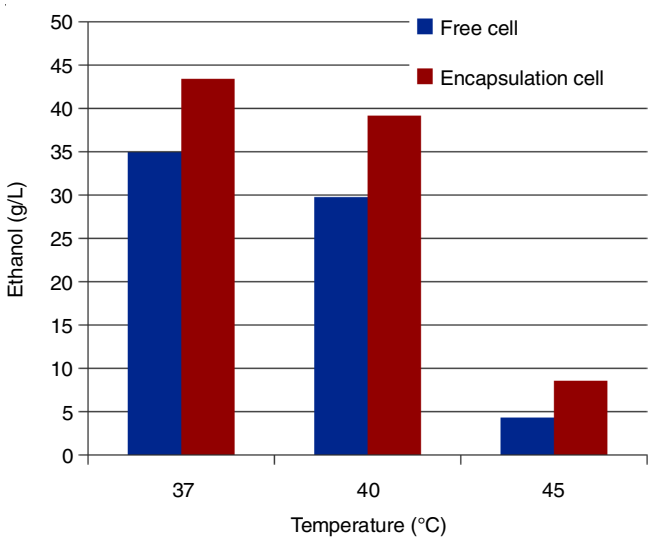

Fig. 11. Comparison of bioethanol concentration with free cell and encapsulated *S. cerevisiae* at various temperatures

TABLE-5
BIOETHANOL PRODUCTION FROM DURIAN SKIN WITH *S. cerevisiae* ENCAPSULATION AT VARIOUS TEMPERATURES

Temp. (°C)	Bioetanol concentration (g/L)	Yield of bioethanol based on cellulose content (g/g cellulose)	Yield of bioethanol based on dried durian skin weight (g/g durian skin)	Theoretic yield of bioethanol (%)
37	43.32	0.45	0.29	79.70
40	39.13	0.41	0.26	71.99
45	8.55	0.09	0.06	15.73

temperatures (Fig. 11). Changing the fermentation temperature from 37 to 40 °C and 45 °C appears that production of ethanol by free cells significantly decreased than the production of ethanol by cell encapsulation. A rise in temperature from 37 to 40 °C decrease the ethanol production by 14.9 % in free cells, while in encapsulated cell only reached 9.7 %.

Fig. 12 shows the differences in bioethanol production by fermentation processes with free cells and encapsulated *S. cerevisiae* at various temperatures. The fermentation process using encapsulated *S. cerevisiae* at 37 °C was higher 19.34 % than that of fee cell *S. cerevisiae*. The increase in process temperature causes a decrease in the production of ethanol. The difference in ethanol production increased at 40 °C, encapsulated *S. cerevisiae* produced 24.02 % higher ethanol than free cells and 49.01 % higher at 45 °C. The greater difference in data shows that the free cells *S. cerevisiae* is more vulnerable to temperature change, while encapsulated *S. cerevisiae* was more resistant to it. This study shows that at higher temperature, encapsulated *S. cerevisiae* can produce higher ethanol compared to free cells. This was also shown that encapsulated *S. cerevisiae* has heat resistance compared to free cells. Encapsulation provides an artificial cell wall in cells trapped inside, thus providing heat protection from the environment around the cell. Analysis of membrane composition in cell encapsulation showed that fatty acid content such as phospholipid and sterols were increased, resulting enhance protection from the surrounding environment [19].

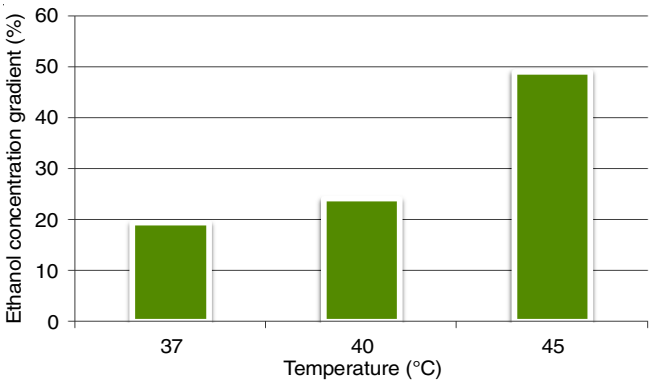

Fig. 12. Differences of bioethanol concentration with free cell and encapsulation of *S. cerevisiae* at various temperatures

Conclusion

The yield of bioethanol from durian skin produced by the encapsulation of *S. cerevisiae* cells was higher than *S. cerevisiae* free cells. At pH 5.0, theoretical yield of bioethanol production by encapsulated *S. cerevisiae* reached 0.43 g/g cellulose with 75.89 % and at 37 °C with the yield of 0.36 g/g cellulose with 64.28 %.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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