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Rice flour and white glutinous rice flour for use on immobilization of yeast cell in ethanol production

Kiky C. Sembiring[a,]*, Hani Mulyani[a], Irni Fitria A.[a], Deliana Dahnum[a], and Yanni Sudiyani[a]

[a]*Research Center for Chemistry of LIPI, Kawasan PUSPIPTEK Serpong Tangerang, 15314, Indonesia*

Abstract

Ethanol is a potential industrial chemical as a very promising biofuel to replace fossil fuels. Its capability to increase octane number and improve the emissions quality of gasoline drives the researches in efficient ethanol production. A rapid fermentation leading to high ethanol concentration; therefore a yeast strain with a good specific ethanol production rate becomes the main consideration. Due to several advantages presenting by the using of cell immobilization, such as increased productivity, reduced risk of contamination, biocatalyst recycling, rapid product separation and others, the interest of cell immobilization for ethanol production has increased in the last decades. The great importance to find cheap, abundant, and non-destructive immobilization supports, rice flour and white glutinous rice flour were tested as support for yeast immobilization in the present work. Three commercial yeast strains were used in the experiments, labelled A, B, and C. The fermentation runs were carried out with glucose as substrate in 250 mL Erlenmeyer flask containing 100 mL of medium and 5% of immobilized enzyme loading and statically incubated at 32^{o}C for 72 hours. Fermentations were carried out in duplicate and fermentations under the same conditions described above were also performed without support addition for comparison. The results showed that white glutinous rice flour was great potential for use as immobilization support during the ethanol production by the C yeast strain culture.

Keywords: Immobilization; yeast; rice flour; white glutinous rice flour.

* Corresponding author. Tel.: 022-2503051; fax: 022-2503240
E-mail address: kiky001@lipi.go.id.

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1. Introduction

Bioethanol is a form of renewable energy that can be produced from agricultural feedstock, i.e. sugar cane, potato, corn, and cellulose-content materials. Whichever substrate is chosen, the attention must be paid to the overall economics and energy consumption [1]. Efficient ethanol production requires a rapid fermentation to get high ethanol concentrations; therefore a yeast strain must have a good specific growth rate and good specific ethanol production rate at high ethanol concentration [2]. Fermentation step play a big role in ethanol production since many parameters can cause the decrease of specific rate of yeast growth, especially the inhibition caused by product or substrate concentration. Therefore, the interest in yeast cell immobilization for ethanol production has increased in the last decades due to several advantages presenting by the using of cell immobilization, such as increased productivity, reduced risk of contamination, biocatalyst recycling, rapid product separation and others [3]. The aim of the cell immobilization is to retain high cell densities inside the reactor.

The immobilization methods can be classified into 4 categories; they are carrier-binding, cross-linking, entrapping, and a combination of these 3 methods [4, 5]. Several natural polysaccharides have been reported to be excellent gel materials and widely used for entrapment, such as alginates, κ-carrageenan, agar, and agarose [6]. Delignified cellulosic material, chitosan [7, 8], natural zeolite [9], γ-alumina have also studied for cell immobilization. However, the development and testing of new carrier materials for microbial immobilization and its application in fermentation process is also an active research topic.The most common commercial yeast strain for ethanol production is *Sacharomycescerevisiae* due to their good fermentative capacity, high tolerance to ethanol and other inhibitors (that are formed during raw-materials pre-treatmentor produced during fermentation) and the capacity to grow rapidly under the anaerobic conditions. There are other microorganisms for alcoholic fermentations has been reported, namely *Zymomonasmobilis*, engineered *Escherichia coli*, and bacterium *Thermoanaerobacter*strain [10]. The aim of this study was to immobilize three types of commercial yeast strain into rice flour and white glutinous rice flour and to apply these immobilized yeasts for ethanol fermentation.

2. Materials and methods

2.1. Yeast strain

Three commercial yeast strains were used in the experiments, they are yeast strain from local market, commercial yeast strain widely known as fermipan, and yeast strain from Madukismo sugar factory in Yogyakarta; labeled A, B, and C respectively. Yeast strain A was in fine granule form, yeast strain B was in 1.5 cm flat round brittle dry yeast, while yeast C was culture stock maintained on YPG agar medium.

2.2. Immobilization

Sugarcane water was added gradually to the flour or glutinous white flour which previously has been stirred with pepper, chili, and onion powder in a certain dose to produce malleable dough. Yeast strain was then added to 5 w/w% final yeast content under gently stirring. Afterwards, the mixture was formed in 3 cm of flat round of immobilized yeast, incubated at 32^{o}C for 72 hours and dried at room temperature.

2.3. Ethanol fermentation

Glucose was subjected to ethanol fermentation by yeast stain for both of non-immobilized and immobilized yeast under anaerobic condition (pH 4.8; 32^{o}C; mixing rate: 150 rpm). To the fermentation

medium, 2.5% of yeast strain was added. The fermentation was performed in 250 ml flasks which contained 100 ml of medium in scientific orbital shaker for 72 hours.

2.4. Alcohol analysis

In the end of the fermentation period, the fermented mixture was distilled to concentrate the alcohol. Alcohol content was analyzed using densitometer.

3. Results and discussion

3.1. Yeasts train immobilization

Physical appearance of non-immobilized and immobilized yeast strain A, B, and C in rice flour and white glutinous rice flour is presented in Fig. 1. It can be seen in Fig 1 that yeast strain A, B, and C were reformed to 3 cm in diameter flat round immobilized cells. All the immobilized yeast strains were similar in the shape and color. However, the structure of yeast strains in white glutinous rice flour support was more brittle compare to yeast strain in rice flour.

3.2. Fermentation using commercial yeast strain

Three commercial yeast strains, labeled A, B, and C were used for glucose fermentation. Meanwhile, immobilized those three kinds of commercial yeast strains in both of rice flour support and white glutinous rice flour support were also applied for ethanol fermentation. Comparison of alcohol content resulted from the fermentation processes are showed in table 1.

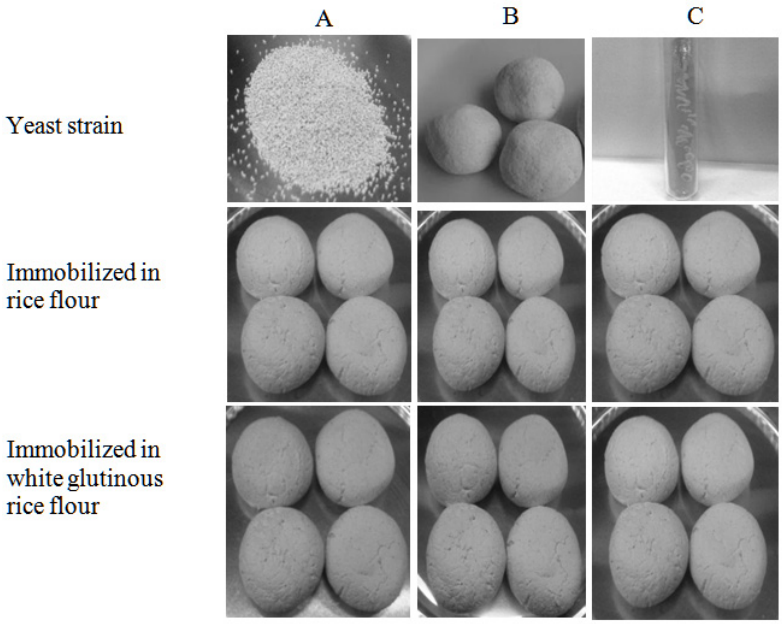

Fig. 1.Yeast strain A, B, C were immobilized in rice flour and white glutinous rice flour

Table 1.Comparison of alcohol content in fermentation using non-immobilized and immobilized commercial yeast strain[a]

Non-immobilized yeast strain	Alcohol content (%)	Immobilized yeast strain	Final pH	Alcohol content (%)
A	4.71	AR[b]	4.09	4.5
		AG[c]	3.98	3.8
B	6.83	BR[b]	3.43	3.0
		BG[c]	4.04	5.0
C	6.58	CR[b]	3.93	4.9
		CG[c]	4.16	5.4

[a]substrate: glucose; pH: 4.8; T: 32ºC; mixing rate: 150 rpm; t: 72 h; [b]R: yeast strain in rice flour support; [c]G: yeast strain in white glutinous rice flour support

Table 1.shows that alcohol fermentation using non-immobilized yeast strain resulted in higher alcohol content compare to the processes using immobilized yeast strain.The ethanol concentration was determined based on thedensity of alcohol distillate at 16ºC and expressed in volume %(v/v).The highest alcohol content was produced by yeast strain B with 6.83 % of ethanol concentration. Several theories have been proposed to explain the reduction in final ethanol concentration during the fermentation using the immobilized yeast strain due to the formation of a protective layer by the support may act to restrict the microorganism to reach the substrate [11, 12].

Results presented in Table 1. for immobilized yeast strains indicated that the cells immobilized in white glutinous rice flour yielded higher alcohol concentration than that of immobilized in rice flour except for yeast strain A. The highest alcohol content was reached by yeast strain C with 5.4% of ethanol concentration.

It is also can be seen that the decreasing of pH in the end of the fermentation period affected the alcohol content. At the constant pH about 4.8, the cells can perform their optimum activity. However it is found that the pH decreased during fermentation reaction. The higher the final pH, the higher the alcohol content in the fermentation medium. It might be caused by formation of carboxylic acid derivatives resulted in lessen of alcohol content.

3.3. Screening of immobilized yeast strain

Based on the previous results of immobilized yeast strain in ethanol fermentation, immobilized yeast strain C in white glutinous rice flour was chosen for the further experiment to investigate time profile of alcohol formation during fermentation. The time profile of alcohol fermentation is presented in Fig. 2.

The time profile of glucose consumption and ethanol formation during fermentation process (Fig. 2.) shows the glucose concentration gradually decreased during the fermentation process from 10 % concentration in the initial reaction and completely consumed during 48 hours. Meanwhile, the ethanol production reached 5.4% v/v during 48 hours fermentation time and relatively stable until the end fermentation period at 72 hours.

Furthermore, pH condition and the number of viable cells during the fermentation process are summarized in Table 2.

Table 2.The pH and the number of viable cells during fermentation process using yeast strain C immobilized in white glutinous rice flour

Time (h)	pH	Σ cells (x 10^9)
0	4.89	1.08
24	3.7	6.28
48	4.08	2
72	4.78	1.32

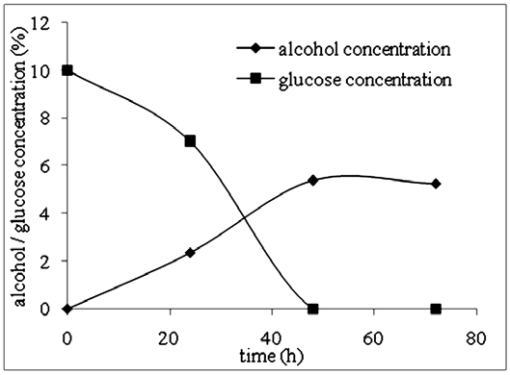

Fig 2. The ethanol and glucose concentration during the fermentation of glucose with yeast strain C immobilized in white glutinous rice flour. Process condition: pH 4.8; 32ºC; 150 rpm; initial glucose concentration 10.

The number of viable cells increased significantly in the initial fermentation process to reach 6.28 x 10^9 cells at 24 hours. The existence of enormous yeast cells in the fermentation media boosted sugar conversion into alcohol. However, decreasing glucose content and other nutrition in the fermentation media over time caused cells lose viability in the late fermentation period. Therefore in the end of fermentation process, cells number was 1.32 x 10^9cells.

Regarding pH evolution, during the first 24 hours, the pH decrease from 4.89 to 3.7, then it increased up to 4.78 during the 48 following hours. The pH decrease is assumed to be correlated to the nitrogen consumption. It is known that during the fermentation, the consumption of nitrogen by yeasts produces H^+ions which were released in solution, as was studied further by Won et al, Kotyk and Sigler et al, and also Hernandez-Orte et al [13]. Between 24 and 72 hours fermentation, the ethanol concentration increased in the medium which can explain the increasing of pH during this period.

4. Conclusion

Immobilization of three commercial yeast strains was carried out using rice flour and white glutinous rice flour. For the immobilization result, the structure of yeast strains in white glutinous rice flour support was more brittle compare to yeast strain in rice flour. The cells immobilized in white glutinous rice flour yielded higher alcohol concentration than that of immobilized in rice flour with highest alcohol content was reached by yeast strain C with 5.4% of ethanol concentration. Applying the immobilized yeast strain C in white glutinous rice flour for fermentation process resulted in 5.4% ethanol concentration. Although the final ethanol content produced by immobilized yeast strain was still lower compareto the non-

immobilized yeast strain due to the formation of a protective layer by the support that may act to restrict the microorganism to reach the substrate, several advantages presenting by the using of cell immobilization, such as productivity increasing, contaminate on risk reducing, biocatalyst recycling, and rapid product separation are still be the main reason to develop cell immobilization. All the results in this work can be used for further study to increase the activity of immobilized cells by optimizing process reaction.

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