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Abstract. The purpose of this study were to preliminary study of immobilization of*Zymomonas mobilis*in silica from rice husk ash. *Zymomonas mobilis* is an important microbe on bioethanol production. In this study, silica was contacted with inoculum of*Zymomonas mobilis* at various time, ratio of silica:cells and speed of shaker at room temperature. The results showed that *Zymomonas mobilis* can be immobilized in silica. The contact time and the ratio between the number of cells and the mass of the silica during immobilization affected the percentage of immobilized cells, while the speed of shaker was not. The optimum results achieved in this study were 38 %, achieved with the used of 0.2 g of silica matrix with 10 mL of a cell solution having an absorbance of 0.85 for a contact time of 30 min. Immobilized *Zymomonas mobilis* in silica could conduct the ethanol fermentation. These results suggest that silica werepotential matriks for immobilizing *Zymomonas mobilis*.

1. Introduction

The demand of bioethanol is increasing because of its very strategic role. Bioethanol is a renewable energy source as a substitute for fossil fuel. Bioethanol is also used as a solvent, raw material for industry, pharmaceuticals, cosmetics and liquor. The United States, Brazil, United of Europe, China and India are the largest bioethanol-producing countries in the world [1]. Bioethanol is obtained through the fermentation of sugar by microbes [2]. Saccharomyces, Aspergillus, Pichia and Zymomonas bacteria are the most common types of microbes used in bioethanol production. These microbes are able to change certain sugar monomers to ethanol, namely hexose sugar (C6) monomers derived from hydrolysis of starch, or pentose (C5) derived from hydrolysis of cellulose. *Saccharomyces cerevisiae* is the type of yeast that is most commonly used in bioethanol production [1, 3]. *Saccharomyces cerevisiae* theoretically produce 90% ethanol from glucose sugar, while yeast *P. stipitis, P. tannophilus* and *C. shehatae* are good at fermenting xylose [1]. Ethanol production using *Saccharomyces cerevisiae* gives good results at low temperatures and glucose concentrations [4]. *Zymomonas* has the advantage of being able to withstand high temperatures, faster fermentation times, theoretically the yield of ethanol produced is 97% [5].

The fermentation process can be carried out in batches or continuously. In a batch system, the medium and inoculum are inserted simultaneously into the bioreactor. Contact between the medium and cells occurs freely during the process of fermentation. Product retrieval can only be done at the end of fermentation. The advantage of a batch system is that the process is easier and simpler, can be

applied on a large scale. The disadvantage is that no substrate is added, bacterial growth decreases and a separation step is needed to separate the desired product from the cells. The continuous system can eliminate weaknesses in the batch system. The inoculum is placed in an inert matrix so that the process of adding media and taking products can be carried out continuously after maximum product concentration is affected by the media flow rate [6]. In continuous system, the cells are immobilised. The process of immobilization involves the absorption or entrapment of cells in an inert matrix, so that the cell is retained in the matrix. The matrix that has been used for cell immobilization is silica, carrageenan, chitosan, polyethylene glycol and Ca-alginate. *Zymomonas mobilis* immobilized in carrageenan can produce ethanol from xylose [7], *Zymomonas mobilis* immobilized on polyethylene glycol can ferment ethanol even in high glucose solutions [8]. *Bacillus sphaericus* immobilized on silica shows quite high activity when compared with other matrices such as polyutherane [9]. The purpose of this study was to preliminary study of immobilization of *Zymomonas mobilis* in silica from rice husk ash. Rice husk is an agricultural waste that is abundant and has very high silica content. The silica / SiO_2 content in it is about 90% [10]. The extraction of silica from rice husks is relatively simple and uses non-expensive reagents [11].

2. Materials and methods

2.1. Preparation of silica ash rice husk matrix

Rice husk was heated in a furnace for 1 hour, then the husk ash was added NaOH in a beaker glass and stirred with a magnetic stirrer for 1 hour at a temperature of 80^{o}C, so that a sodium silicate solution was obtained, then allowed to stand for a day and chocolate precipitate and a clear yellow solution are formed. The results were filtered, and filtrate and residues were obtained. The remaining residue can be washed with hot water. The filtrate was then added with concentrated sulfuric acid until the atmosphere becomes acidic then ammonium was added until alkaline, after which the gel was neutralized with the addition of distilled water. The separated gel was then dried into the oven [11].

2.2. Inoculum of Zymmomonas mobilis

Zymomonas mobilis was carried out aseptically by inoculating pure bacteria into the nutrient broth medium and was incubated for 24 hours at 30^{o}C. The number of cells in inoculum of *Zymomonas mobilis* was measured as absorbance at 660 nm. The absorbance value is proportional with the number of the cells, modified from [7].

2.3. Immobilized of Zymmomonas mobilis on silica

A total of 10 mL of *Zymomonas mobilis* inoculum was contacted with amount of silica matrix at variations in contact time, ratio of silica:cell and speed of shaker at room temperature. After filtration, the residue *Zymomonas mobilis* was immobilized in silica and filtrate contained remaining cells. Absorbance of inoculum of *Zymomonas mobilis*was as absorbance of free cells, and absorbance of filtrate was as absorbance after immobilization, modified from [12]. The study was conducted in triplicate. The value of % immobilized was conducted with formula:

$$\% \text{ immobilized} = \frac{\text{absorbance of free cells} - \text{absorbance after immobilization}}{\text{absorbance of free cells}} \; x \; 100\% \qquad (1)$$

2.4. Zymomonas mobilis immobilized ability test to conduct ethanol fermentation

A total of 0.05 grams *Zymomonas mobilis* immobilized in silica were pour into 10 mL of glucose 20 ppm, then incubated for 18 hours at room temperature. The fermentation result was analyzed by qualitatively test i.e. $K_2Cr_2O_7$ [13] and Lucas test [14] to identify the ethanol as product of fermentation.

3. Results and Discussion

Cell immobilization used for continuous fermentation has several advantages: wash out of cells from the beads can be eliminated, protects cells from toxic environment, loading of biomass is high, ease of separation, cost effective method due to the possibility of reusing the cells [15]. In this study *Zymomonas mobilis* were immobilized in silica matrix that were extracted from rice husk ash. The silica matrix were in the form of white powder (Figure 1), has a large surface area and a certain porosity, chemically composed of functional groups silanol (-SiOH) and siloxane (Si-O-Si) (Figure 2) [12]. The silanol group allows various chemical interactions such as hydrogen bonds, van der Waals and electrostatic interactions between silica with various molecules including bacterial cell surfaces, so that cells can be absorbed on the surface of silica matrix. Affinity of microbial cells towards the support is primarily depends on the chemical nature and age of the cells [15]. Thus, preparation of fresh bacterial cell inoculum solutions was needed to obtain cells that have high viability. Under these conditions it were expected that the immobilization process will be effective.

Figure 1. Silica powder from rice husk ash [12].

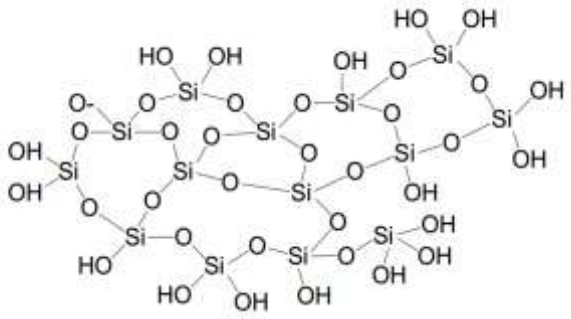

Figure 2. General structure of silica [16].

The result showed that the longer contact time between silica and *Zymommonas mobilis* inoculum the higher value of% immobilized as shown in Table 1.The value of % immobilization increased from 24% at 15 minutes to 31% at 30 minutes This showed that sufficient time was needed for the interaction between the outer surface of the cell and the functional group on the surface of the silica. In this study, optimum contact time was not obtained for the comparison of silica and the number of cells studied. It was estimated that the immobilization of *Zymommonas mobilis* in silica increased at contact time above 30 minutes.

The higher amounts of matrices were used, the more cells were immobilized as shown in Table 2. In used from 0.05 grams to 0.2 grams the value of % immobilization continues to increase from 21.3% to 38.1%.Butthe increase between 0.15 and 0.2 g tend to be less significant.

The speed of shaker was not significantly affecting the number of immobilized cells as shown in Table 3. The % immobilized was obtained at 20.4% at 50 rpm, then 21.9% at 100 rpm, while at speeds

of 150 and 200 rpm, the % immobilized only became 24.6 and 24.5%. Based on this, the optimum speed for immobilization of *Zymomonas mobilis* on silica was used at 150 rpm.

Table 1. Effect of contact time to *Zymomonas mobilis* immobilization percentage on silica surface.

No	Contact time (minutes)	Absorbance after immobilization	Absorbance of free cells	% imobilised
1	15	0.676±0.010	0.890	24.0± 1.35
2	20	0.668±0.008	0.890	24.9±1.13
3	25	0.640±0.014	0.890	28.0±1.93
4	30	0.609±0.017	0.890	31.0±2.38

Note: ± Standar deviation from triplicate

Table 2. Effect of matrix mass to *Zymomonas mobilis* immobilization percentage on silica surface.

No	Matrix mass (g)	Absorbance after immobilization	Absorbance of free cells	% imobilized
1	0.05	0.680±0.004	0.865	21.3±0.58
2	0.10	0.600±0.026	0.865	30.6±3.72
3	0.15	0.537±0.035	0.865	37.9±5.01
4	0.20	0.535±0.024	0.865	38.1±3.45

Note: ± Standar deviation from triplicate

Table 3. Effect of shaker speed to *Zymomonas mobilis* immobilization percentage on silica surface.

No	Shaker speed(rpm)	Absorbance after immobilization	Absorbance of free cells	% imobilized
1	50	0.660±0.005	0.830	20.4±0.77
2	100	0.648±0.011	0.830	21.9±1.65
3	150	0.625±0.014	0.830	24.6±2.12
4	200	0.626±0.018	0.830	24.5±2.69

Note: ± Standar deviation from triplicate

To confirm whether the potential of silica for *Zymomonas mobilis* immobilisation has been conducted, the ability of immobilised *Zymomonas mobilis* to fermented glucose was carried out. The results showed that in the results of glucose fermentation by immobilized *Zymomonas mobilis* for 18 hours it contained alcohol because it was positive on the $K_2Cr_2O_7$ test and negative with Lucas test such as ethanol as standar. The $K_2Cr_2O_7$ test in a sample containing alcohol will produced a bluish green color because the reduction of dichromate by alcohol in an acidic condition: $2CrO_4^{2-} + 3C_2H_5OH + 10H^+ \rightarrow 2Cr^{3+} + 3CH_3CHO\uparrow + 8\ H_2O$. $K_2Cr_2O_7$ test on fermented products even though it does not produce a clear bluish green color but changes in color from orange of dichromate turns to light purple (Tabel 4), has a strong expectation that there has been a reduction of dichromat ions to chrome ions by alcohol compound in fermentation product. The Lucas test showed that there was no cloud on the tube wall and the solution remains clear after being left within 1 hour (Table 4).This indicates that alcohol in fermented products is a primary alcohol same as ethanol [14]. Considering that *Zymomnas mobilis* performs ethanol fermentation, it was strongly assumed that the fermentation product was ethanol. GC-MS analysis was carried out to prove that statement.

Table 4. Observations of qualitative tests on glucose fermentation results by *Zymmomonas mobilis* immobilized on silica.

Sample	$K_2Cr_2O_7$Test	Lucas test
Alcohol	Positive	Negative
Fermentation product		
	Positive	Negative

4. Conclusion

The optimum results achieved in this study were 38% *Zymomonas mobilis* immobilized in silica, achieved with the use of 0.2 g of silica with 10 mL of a cell solution having an absorbance of 0.85 for a contact time of 30 min. Immobilized *Zymomonas mobilis* in silica could conduct the ethanol fermentation. These results suggest that silica has potential matrix for immobilizing *Zymomonas mobilis*.

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