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Immobilization of *Saccharomyces cerevisiae* in Jackfruit *(Artocarpus heterophyllus)* Seed Fiber for Bioethanol Production

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Abstract. Bioethanol is alternative renewable energy typically obtained from glucose through a fermentation process using *Saccharomyces cerevisiae*. In the bioethanol fermentation process using yeast, there are several inhibiting factors, such as a high concentration of substrate, ethanol as the product, and nutrients. The present study aimed to investigate the effect of fermentation time (12-72 hours), immobilized carrier size (sizes of 0.5 cm^3, 1 cm^3, and 1.5 cm^3), and medium pH (3.0, 4.0, and 5.0) on the ethanol fermentation process using immobilized yeast in jackfruit (*Artocarpus heterophyllus*) seeds and subsequently to compare its performance with a free cell system. The highest ethanol concentration (89.15 g/L) with a yield of 96.92% was obtained by immobilizing yeast in jackfruit seed at a fermentation time of 72 hours, carrier size of 0.5 cm^3, and medium pH of 5.0. When compared to the free cell system fermentation under identical operating conditions, immobilized yeast in jackfruit seed obtained 1.41 times higher ethanol concentration. Jackfruit seed also led to a higher ethanol concentration compared to other *S. cerevisiae* carriers. Altogether, our findings imply that jackfruit seed has great potential as a carrier of *S. cerevisiae* in the process of fermenting glucose into ethanol.

Keywords: ethanol fermentation, *Saccharomyces cerevisiae*, immobilization, jackfruit seed

INTRODUCTION

With the issue of depletion of fossil fuels and the rising demand for energy due to human population and activities growth in the forthcoming, the energy sector faces a serious and challenging problem. In addition, fossil fuels also play a significant role in global warming impact (Yanto et al. 2019). These challenges have stimulated interest in

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discovering sustainable alternative energy to substitute fossil energy (Ong et al. 2011). The topic of renewable energy has been extensively studied by many scientists over the planet and applied in some circumstances, one of the sustainable renewable energies is bioethanol (Megawati et al. 2022). The use of alternative energy as a substitute for fossil fuels can minimize the energy crisis and lower greenhouse gas emission production, which is excellent to assist in the realization of climate change targets (Jayed et al. 2011).

Bioethanol is an alcohol compound with a hydroxyl group derived from glucose. It is one of the potential alternative energies produced by biomass and fermentation processes (Li and Cheng 2020). Bioethanol is expected to have a critical role in the transportation sector as a substitute for gasoline in the upcoming. The use of bioethanol as a substitute for fuel has several advantages, including 35% higher oxygen content and lower emissions compared to fossil fuels (Verma et al. 2019). Its use has also been developed in over forty countries (Huang et al. 2020).

In general, bioethanol is produced by an anaerobic fermentation process that employs yeast (*Saccharomyces cerevisiae*) (Sudhakar et al. 2020). Bioethanol made from glucose substrate derived from food starch, agricultural waste, and algal biomass is known as first-generation, second-generation, and third-generation bioethanol, respectively (Megawati et al. 2021).

In the process of fermenting glucose into ethanol, there may be some inhibitions in *S. cerevisiae* cells. Nugraheni and Mastur (2017) reported that inhibition occurs while the concentration of glucose (as the main substrate) was more than 125 g/L, resulting in a decline in the amount of dissolved oxygen in the fermentation medium. Ethanol as a fermentation product can also inhibit the yeast cells themselves and cause yeast living cells to enter the death phase more quickly (Tesfaw and Assefa 2014). Immobilization is a technique for producing bioethanol in which the yeast cells will be trapped in a matrix or membrane so that the moving cells and their growth can be protected, and hence the substrate can be converted optimally into the product (Santos et al. 2018). Compared to free cells, immobilized cells were more resistant to inhibitory effects, medium pH at extreme ranges, and higher temperatures (Homaei et al. 2013). Research on the immobilization of *S. cerevisiae* has been broadly studied in bioethanol production. Yeast cell immobilization on sodium alginate films can increase bioethanol products compared to free cell yeast (Damayanti et al. 2021).

Besides, the immobilization method with sodium alginate produces a 17% greater ethanol yield than the free cell system (Kumoro et al. 2021). Meanwhile, the immobilization in the matrix made from biomass was reported that the concentration of bioethanol could be increased up to 1.4 times in the immobilization of yeast cells in the cashew apple bagasse compared to the free cell system (Pacheco et al. 2010) and 2.24 times higher than free cells system with immobilization of yeast cells in the sorghum bagasse (Yu et al. 2007).

Jackfruit (*Artocarpus heterophyllus*) is a tropical agricultural plant widely grown in many tropical areas, such as Indonesia. Jackfruit production in Indonesia reaches 720,208 tons annually and produces between 57,600 and 108,000 tons of seeds annually (Jayus et al. 2016). Jackfruit seeds are frequently not regarded to have any economic value, and currently, in Indonesia,

they are only used as fertilizer base material, a little is used as animal feed, and even disposed of as agricultural waste. Jackfruit seeds are reported to have a good nanoporous structure (Chaudhary et al., 2020). This makes the interaction between the substrate and products among the jackfruit seed carrier easier. With great structure, it can be expected that jackfruit seeds can be used as a carrier for immobilization of the yeast *S. cerevisiae* in ethanol fermentation.

Hitherto, there is no research yet on the immobilization of yeast *S. cerevisiae* in jackfruit seeds in bioethanol production. The aim of the present study was to investigate the effects of immobilization of *S. cerevisiae* in jackfruit seeds and compare the result with the free cell system. Through this study, it is expected to obtain information about optimum conditions with a low production cost of bioethanol production as an attempt to develop environmentally-friendly sustainable renewable energy.

MATERIALS AND METHOD

Materials

The main materials used in the study were *S. cerevisiae*, jackfruit seeds, and glucose. *S. cerevisiae* was obtained from instant dry yeast producer "Fermipan®" (Indonesia). Jackfruit seeds were collected from plantation production around Semarang (Indonesia), and glucose as the substrate used for the fermentation process was purchased from Merck®(Germany).

Method

Immobilized carrier preparation

Jackfruit seeds were prepared, washed, and soaked in distilled water for an hour before being dried in an oven at 50 °C for two hours. Subsequently, the outer skins of the jackfruit seeds were stripped and cut into cubes (sizes of 0.5 cm^3, 1 cm^3, and 1.5 cm^3). The treated jackfruit seeds were then stored in a sealed container for the immobilization process.

Immobilization process

As many as 0.5 g of instant dry yeast of *S. cerevisiae* was inoculated in a medium of 125 mL distilled water comprised of 5 g/L yeast extract (Microgen®, India); 5 g/L glucose (Merck®, Germany); 5 g/L $(NH_4)_2SO_4$ (Merck®, Germany); 5 g/L $MgSO_4$ (Merck®, Germany); and 5 g/L peptones (Oxoid®, the USA). The medium was sterilized using an autoclave at 121 °C for 30 minutes. The Broth was incubated aerobically at 30 °C for 24 hours. After the inoculation process, the immobilization technique was then performed, which the procedure referred (Yu et al. 2007). 17 g of the treated jackfruit seeds were put in the inoculation broth, followed by the soaking process at a temperature of 30 °C for 24 hours.

Fermentation process

Jackfruit seeds that had been soaked in the inoculation broth were filtered and used for the fermentation in a 125 mL distilled water medium with 180 g/L glucose as the main substrate. The fermentation medium contained 2 g/L of peptone, 1 g of yeast extract, and 2 g/L of glucose. Like the preceding stage, the fermentation medium was sterilized in an autoclave at 121 °C for 30 minutes. Fermentation was carried out anaerobically by adding an airlock fermenter at a temperature of 30 °C for 72 hours, and samples were withdrawn at 12 hours intervals to analyze the concentration of ethanol and residual glucose.

Ethanol analysis

The ethanol concentration analysis was executed using the Conway method with a UV-Vis spectrophotometer (Sriariyanun et al., 2019). Absolute ethanol was dissolved in distilled water with a concentration of 0.5-6 g/L. 0.10 g of $K_2Cr_2O_7$ (Merck®, *Germany*) was dissolved in 2.5 mL of distilled water and 12.5 mL of H_2SO_4 (Merck®, Germany) in a 25 mL volumetric flask and cooled to room temperature. Subsequently, 1 mL of dichromate acid solution was diluted to 10 mL, and 5 mL of the solution was taken to be inserted into the center of the Conway dish. The outside of the Conway dish was filled with 5 mL of ethanol solution (0.5–6 g/L) and 1 mL of 20% Na_2CO_3 (Merck®, Germany) solution. The Conway was sealed and heated in the oven at 50 °C for two hours. To obtain a linear regression equation, a calibration curve regarding the relationship between the concentration of standard ethanol solution and the absorbance value was made. The absorbance value obtained in the fermentation sample was converted into a linear equation to determine the concentration of ethanol.

Glucose analysis

Glucose analysis referred to the phenol-sulfuric method (Masuko et al., 2005). 1 mL of standard glucose solution (0.1-0.5 g/L) was reacted in a test tube with 1 mL of 5% phenol (Merck®, Germany). Thereafter, the solution was added with 5 mL of H_2SO_4 (Merck®, Germany), stirred, and cooled at room temperature. The solution was heated using a water bath at 90 °C for five minutes. Absorbance was measured using a UV-Vis spectrophotometer with maximum wavelength. The absorbance result was drawn as a standard curve between glucose concentration and absorbance value to obtain a linear regression equation. The same treatment was carried out on the fermented samples and was read using a linear equation to acquire the value of glucose concentration.

Calculation ethanol yield

The results of the fermentation of glucose into ethanol can be calculated as the production yield. Ethanol yield can be expressed as the value of ethanol concentration per concentration of total glucose as substrate and is expressed in Eq. (1):

$$yield\ (\%) = \frac{Concentration\ of\ ethanol\ (\frac{g}{L})}{0.511\ x\ Concentration\ of\ total\ glucose\ (\frac{g}{L})} x100\% \quad (1)$$

RESULTS AND DISCUSSION

Effect of fermentation time

In the current study, the effect of fermentation time on the ethanol concentration and glucose residue was studied. The fermentation process using the immobilization size of 1 cm^3 jackfruit yeast was incubated at 30°C and pH 5.0 for 72 hours. The effects of fermentation time on glucose and ethanol concentrations are depicted in Figure 1.

Figure 1 shows that with the prolonged fermentation time, the glucose concentration gradually decreased to 13.31 g/L at the end of fermentation. This phenomenon may be linked with the possibility of glucose as the main substrate was being consumed by the trapped yeast *S. cerevisiae* in the jackfruit seed matrix for metabolism and growth. Significant glucose depletion occurred in the first 12 hours of fermentation. This may be due to the fact that the yeast cell was already in the log phase, so the yeast cell grew rapidly

and consumed glucose as the main substrate and converted it into ethanol. This condition was also supported by the remarkable increase of ethanol concentration up to 80.19 g/L with a yield of 87.16%.

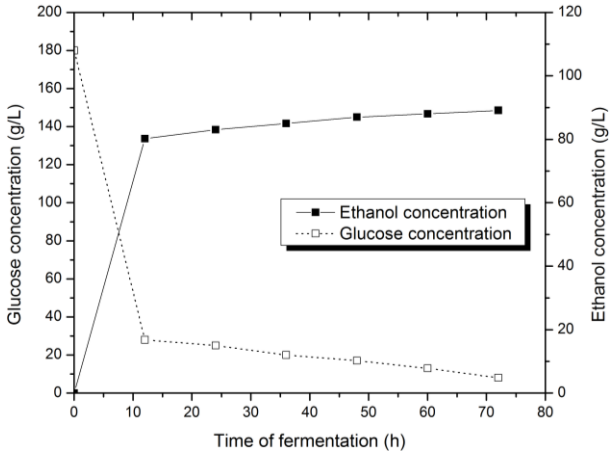

Fig. 1: The effects of fermentation time on ethanol concentration using immobilized yeast.

At the end of the fermentation time, the ethanol concentration obtained was up to 89.12 g/L with a yield of 96.81%. Meanwhile, in the 12-72 hours of fermentation, the ethanol concentration as the product tended to be constant, which was possibly caused by yeast cells having entered the stationary phase due to the depletion of glucose as the main substrate (Mohd Azhar et al. 2017). Moreover, ethanol as a fermentation product from glucose is one of the cell growth inhibitors and can reduce fermentation activity (Zhang et al. 2015). However, the immobilized yeast cells can protect against ethanol and increase the number of living cells compared to the free cell system (Zhu et al. 2018). This finding is also in line with the research conducted by Martini et al. (2011) on the fermentation of sugar by *S. cerevisiae* immobilized in rice hulls and Malik et al. (2021) on cotton stalk lignocellulosic for bioethanol production.

Effect of carrier size on ethanol concentration and residual glucose

Jackfruit seed size used for carrier *S. cerevisiae* was also investigated for its effect on the concentration of ethanol and residual glucose. At this stage, the seed size varied from cubical 0.5 cm^3, 1 cm^3, and 1.5 cm^3. Figure 2 depicts the effects of jackfruit seed on ethanol and glucose concentration.

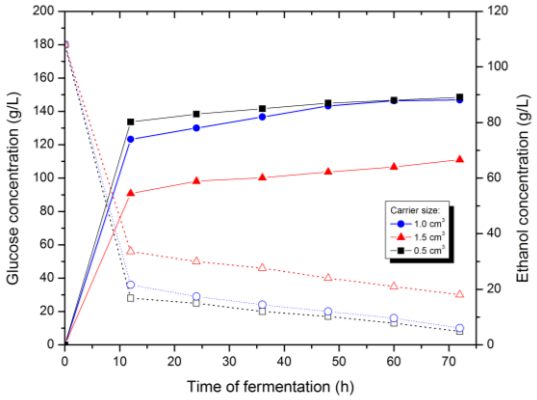

Fig. 2: The effects of the size of carrier jackfruit seeds on ethanol concentration using immobilized yeast

Figure 2 illustrates that the size difference in the carrier of yeast *S. cerevisiae* affects the ethanol concentration produced. The carrier size 0.5 cm^3 produced the highest ethanol up to 89.12 g/L during 72 hours of fermentation. The difference in ethanol concentration at the size of 1 cm^3 carrier was insignificant ($p<0.05$) compared to the 0.5 cm^3 carrier size. On the other hand, glucose consumption in the 0.5 cm^3 and 1 cm^3 carrier size was also very rapid, and the final glucose concentration for each carrier size at the end of fermentation was 8.01 g/L and 10.06 g/L, respectively. Nevertheless, at the carrier size of 1.5 cm^3, the difference in ethanol concentration was slightly significant ($p>0.05$) and recorded the lowest ethanol concentration. This condition was also corroborated by glucose as the main substrate consumed by yeast, which

decreased by 25%.

Generally, glucose is the main component as a nutrient for yeast cells to grow and reproduce the cell. This may be due to the smaller particle size of the jackfruit seed as a carrier, which reduces the contact surface area and makes it easier for the medium to enter the carrier matrix so that yeast cells will be more grow rapidly and active in consuming glucose into ethanol product (Hussain et al. 2015). Thus, the trapped yeast will lack nutrients and cannot grow optimally. This finding aligns with research conducted by Adelabu et al. (2019) on bioethanol production from corn straw substrate with yeast immobilization in *Mucuna urens* matrix, which found that the larger the size of the beads resulted in a reduction in the productivity of ethanol up to 27%. However, the smaller carrier size was not investigated in this study.

Effect of medium pH on the fermentation process

In this stage, the effect of medium pH (3.0-5.0) on the bioethanol fermentation process was studied. The yeast cells trapped at 0.5 cm^3 were employed anaerobically at 30°C for 72 hours. The effect of medium pH on the concentration of bioethanol and residual glucose is illustrated in Figure 3.

Figure 3 demonstrates that the highest glucose concentration reached up to 86.67% within 12 hours at the medium pH of 5.0. Research conducted by Lee et al. (2011) also found that *S. cerevisiae* immobilized on calcium alginate could consume up to 90% glucose in just 19.5 hours. This indicates that at optimum medium pH conditions, glucose as the main substrate could be consumed rapidly for the growth of the cell.

Besides, the medium pH also affects the productivity of ethanol. This experiment's results showed that the highest ethanol concentration occurred at the medium pH of 5.0 with a concentration of 89.15 g/L or when the yield reached 96.92%. The ethanol concentration decreased along with the decrease in the pH of the fermentation medium. In the research conducted by Bouaziz et al. (2020) using date seed as substrate, the highest ethanol concentration obtained was 21.57 g/L after six hours of fermentation. At the pH of 5.0, the medium also produced ethanol rapidly in the first 12 hours of fermentation time. This may be correlated with enzymes in yeast cells that play an essential role in the fermentation process.

Yeast *S. cerevisiae* has optimum conditions for growth in the pH range of 4.0-5.0 (Liu et al. 2015). At a medium pH of 5.0, glucose could be rapidly consumed by cells for their growth and metabolism processes and converted into ethanol and carbon dioxide. In a more acidic medium pH (3.0), hydrogen ions could change cell membrane structure, causing not optimal enzyme functioning (Lin et al. 2014).

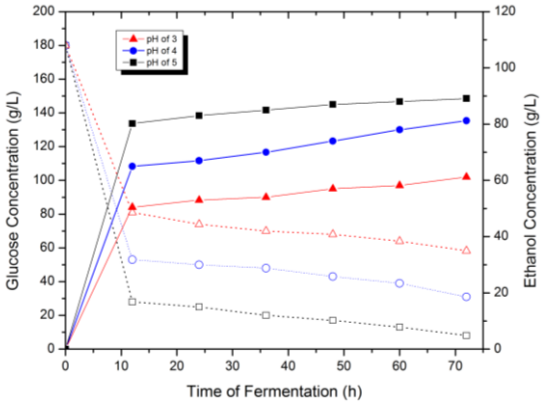

Fig. 3: The effects of pH medium on ethanol and glucose concentration

Furthermore, under extreme conditions, enzymes composed of proteins could be easily denatured and cause damage to yeast

cells (Ezugwu et al. 2015). With the immobilization of cells into jackfruit seed, yeast will be more resistant to acidic conditions and can still carry out growth and metabolism, as evidenced by glucose which can still be converted into ethanol. This is in accordance with several pertinent studies about immobilized cells, which are more resistant in acidic or alkaline pH mediums than free cells (Beniwal et al. 2018).

Comparison of immobilized yeast and free cells system in the fermentation process

A comparison of immobilized cells and free cells system in the fermentation of glucose into bioethanol was investigated in this study. The glucose substrate used for fermentation was 180 g/L, operating conditions at pH of 5.0 and 30 °C for 72 hours.

As seen in Figure 4, fermentation using immobilized yeast on jackfruit seeds produced 43.16% higher ethanol concentration than the free cell system at the end of the fermentation process. In addition, the immobilized yeast will convert glucose into ethanol more rapidly, as evidenced by the faster glucose concentration depletion occurs. This difference may be due to the yeast trapped in the jackfruit seed carrier, which is more resistant to the conditions of the fermentation medium. According to Rattanapan et al. (2011), immobilized yeast cells can protect cells from the possible presence of toxins in the fermentation medium. Other than that, Maiorella et al. (1983) reported that a high concentration of glucose feed substrate could also inhibit yeast *S. cerevisiae*, resulting in less optimum cell work. In the entrapped yeast cell, glucose will be absorbed gradually in the jackfruit seed carrier so that the yeast cell work and cell growth will be more optimum. Ethanol, as the main product in the fermentation process, also inhibits yeast cells which cause cells to go into the death phase more rapidly (Zhang et al., 2015). With a carrier that protects cells from contact with ethanol caused, the cells can still grow and develop optimally. A similar result was reported by Kumoro et al. (2021), in which the fermentation process using yeast immobilized on sodium alginate beads led to a greater ethanol concentration of 17% than the free cell system. Meanwhile, Zhu et al. (2017) found 18% greater ethanol yield using immobilized *S. cerevisiae* in polyethyleneimine grafted collagen fibers for synthetic glucose fermentation. In general, the yeast *S. cerevisiae* immobilized in jackfruit seeds has excellent potential to be developed on a larger scale in the bioethanol production process with higher productivity ethanol and decreased contamination or inhibition.

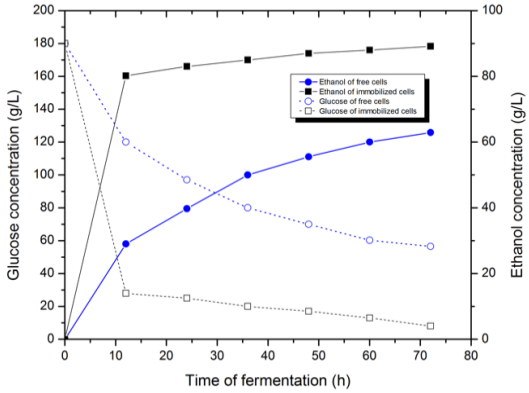

Fig. 4: Comparison of immobilized yeast and free cells system on glucose fermentation.

Comparison of immobilized *S. cerevisiae* in jackfruit carrier with another carrier

The results of the optimum immobilization of ethanol yield in this study were compared with other carrier types using similar yeast (*S. cerevisiae*) and glucose as the main substrates. The comparison of carrier types is presented in Table 1.

Table 1. Comparison of various carrier types in ethanol fermentation immobilization

Carrier type	Ethanol yield
Jackfruit seed (this study)	96.92 %
Rice hulls (Martini et al., 2011)	62.7%
Ceramics (Diana et al., 2014)	86.20 %
Sodium alginate (Kumoro et al., 2021)	96.68 %
Microtube array membrane (Chen et al., 2015)	48.00 %

According to Table 1. the carrier of jackfruit seeds in the ethanol fermentation process with the immobilization technique produces the highest yield of 96.92%. This indicates that carriers with biomass as materials can work more effectively when compared to carriers with non-biomass materials. Desimone et al. (2003) observed that high carbohydrate concentrations in carrier types could protect the hydrate layer around yeast from ethanol. Jackfruit seed contains carbohydrates of roughly 19 g/100 g of dry base (Waghmare et al. 2019). The ethanol yield in the treatment of using a jackfruit seed as the carrier in the present study was better than a carrier with non-biomass materials. Not less important, careers with non-biomass materials require relatively high processing costs, so the ethanol fermentation process using immobilization with jackfruit seed can be a new exciting finding with low-cost production and optimum yield and at the same time, support sustainable agricultural waste treatment.

CONCLUSION

Our results revealed that the jackfruit seed has great potential and is feasible to be a carrier of *S. cerevisiae* in the process of fermenting glucose (180 g/L) into ethanol. The results showed that immobilizing yeast in jackfruit seed at a 72 hours fermentation time, carrier size of 0.5 cm^3, and pH medium of 5.0 obtained the highest ethanol concentration of 89.15 g/L or yield of 96.92%. Yeast immobilization in jackfruit seed had been shown to protect yeast cells from moderately acidic environmental conditions. Immobilized yeast in jackfruit seed produced ethanol 1.41 times higher than the free cells system. Our finding is expected to assist in the production of bioethanol as alternative renewable energy while at the same time utilizing agricultural waste. Additionally, the less pretreatment needed in the career makes the cost of producing ethanol more effective on a larger scale without sacrificing ethanol productivity.

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REFERENCES

Adelabu, B. A., Kareem, S. O., Oluwafemi, F., & Abideen Adeogun, I. (2019). Bioconversion of corn straw to ethanol by cellulolytic yeasts immobilized in Mucuna urens matrix. *Journal of King*

Saud University - Science, *31*(1), 136–141. https://doi.org/10.1016/j.jksus.2017.07.005

Beniwal, A., Saini, P., Kokkiligadda, A., & Vij, S. (2018). Use of silicon dioxide nanoparticles for β-galactosidase immobilization and modulated ethanol production by co-immobilized K. marxianus and S. cerevisiae in deproteinized cheese whey. *LWT*, *87*, 553–561. https://doi.org/10.1016/J.LWT.2017.09.028

Bouaziz, F., Abdeddayem, A. Ben, Koubaa, M., Barba, F. J., Jeddou, K. Ben, Kacem, I., Ghorbel, R. E., & Chaabouni, S. E. (2020). Bioethanol Production from Date Seed Cellulosic Fraction Using Saccharomyces cerevisiae. *Separations 2020, Vol. 7, Page 67*, *7*(4), 67. https://doi.org/10.3390/SEPARATIONS7040067

Chaudhary, R., Maji, S., Shrestha, R. G., Shrestha, R. L., Shrestha, T., Ariga, K., & Shrestha, L. K. (2020). Jackfruit Seed-Derived Nanoporous Carbons as the Electrode Material for Supercapacitors. *C*, *6*(4), 73. https://doi.org/10.3390/c6040073

Chen, C. C., Wu, C. H., Wu, J. J., Chiu, C. C., Wong, C. H., Tsai, M. L., & Lin, H. T. V. (2015). Accelerated bioethanol fermentation by using a novel yeast immobilization technique: Microtube array membrane. *Process Biochemistry*, *50*(10), 1509–1515. https://doi.org/10.1016/J.PROCBIO.2015.06.006

Damayanti, A., Kumoro, A. C., & Bahlawan, Z. A. S. (2021). Review Calcium Alginate Beads as Immobilizing Matrix of Functional Cells: Extrusion Dripping Method, Characteristics, and Application. *IOP Conference Series: Materials Science and Engineering*, *1053*(1), 012017. https://doi.org/10.1088/1757-899x/1053/1/012017

Desimone, M. F., Degrossi, J., D'Aquino, M., & Diaz, L. E. (2003). Sol-gel immobilisation of Saccharomyces cerevisiae enhances viability in organic media. *Biotechnology Letters 2003 25:9*, *25*(9), 671–674. https://doi.org/10.1023/A:1023481304479

Diana, B., Scherbaka, R., Patmalnieks, A., & Rapoport, A. (2014). Effects of yeast immobilization on bioethanol production. *Biotechnology and Applied Biochemistry*, *61*(1), 33–39. https://doi.org/10.1002/BAB.1158

Ezugwu, A. L., Eze, S. O. O., & Chilaka, F. C. (2015). A study of the optimal conditions for glucoamylases obtained from Aspergillus niger using amylopectin from cassava starch as carbon source. *African Journal of Biotechnology*, *14*(36), 2693–2702. https://doi.org/10.5897/ajb2015.14857

Homaei, A. A., Sariri, R., Vianello, F., & Stevanato, R. (2013). Enzyme immobilization: an update. *Journal of Chemical Biology*, *6*(4), 185. https://doi.org/10.1007/S12154-013-0102-9

Huang, J., Khan, M. T., Perecin, D., Coelho, S. T., & Zhang, M. (2020). Sugarcane for bioethanol production: Potential of bagasse in Chinese perspective. *Renewable and Sustainable Energy Reviews*, *133*(August), 110296. https://doi.org/10.1016/j.rser.2020.110296

Hussain, A., Kangwa, M., Yumnam, N., & Fernandez-Lahore, M. (2015). Operational parameters and their

influence on particle-side mass transfer resistance in a packed bed bioreactor. *AMB Express 2015 5:1*, *5*(1), 1–8. https://doi.org/10.1186/S13568-015-0138-Z

Jayed, M. H., Masjuki, H. H., Kalam, M. A., Mahlia, T. M. I., Husnawan, M., & Liaquat, A. M. (2011). Prospects of dedicated biodiesel engine vehicles in Malaysia and Indonesia. In *Renewable and Sustainable Energy Reviews* (Vol. 15, Issue 1, pp. 220–235). Elsevier Ltd. https://doi.org/10.1016/j.rser.2010.09.002

Jayus, Setiawan, D., & Giyarto. (2016). Physical and Chemical Characteristics of Jackfruit (Artocarpus Heterophyllus Lamk.) Seeds Flour Produced Under Fermentation Process by Lactobacillus Plantarum. *Agriculture and Agricultural Science Procedia*, *9*, 342–347. https://doi.org/10.1016/j.aaspro.2016.02.148

Kumoro, A. C., Damayanti, A., Bahlawan, Z. A. S., & Melina, M. (2021). Bioethanol Production from Oil Palm Empty Fruit Bunches Using Saccharomyces cerevisiae Immobilized on Sodium Alginate Beads. *Periodica Polytechnica Chemical Engineering*. 1–12. https://doi.org/10.3311/PPch.16775

Lee, K. H., Choi, I. S., Kim, Y. G., Yang, D. J., & Bae, H. J. (2011). Enhanced production of bioethanol and ultrastructural characteristics of reused Saccharomyces cerevisiae immobilized calcium alginate beads. *Bioresource Technology*, *102*(17), 8191–8198. https://doi.org/10.1016/J.BIORTECH.2011.06.063

Li, J., & Cheng, W. (2020). Comparison of life-cycle energy consumption , carbon emissions and economic costs of coal to ethanol and bioethanol. *Applied Energy*, *277*(July), 115574. https://doi.org/10.1016/j.apenergy.2020.115574

Lin, Y., Zhang, W., Li, C., Sakakibara, K., Tanaka, S., & Kong, H. (2014). Factors affecting ethanol fermentation using Saccharomyces cerevisiae BY4742. *Biomass and Bioenergy*, *47*, 395–401. https://doi.org/10.1016/j.biombioe.2012.09.019

Liu, X., Jia, B., Sun, X., Ai, J., Wang, L., Wang, C., Zhao, F., Zhan, J., & Huang, W. (2015). Effect of Initial PH on Growth Characteristics and Fermentation Properties of Saccharomyces cerevisiae. *Journal of Food Science*, *80*(4), M800–M808. https://doi.org/10.1111/1750-3841.12813

Maiorella, B., Blanch, H. W., & Wilke, C. R. (1983). By-product inhibition effects on ethanolic fermentation by Saccharomyces cerevisiae. *Biotechnology and Bioengineering*, *25*(1), 103–121. https://doi.org/10.1002/BIT.260250109

Malik, K., Salama, E. S., El-Dalatony, M. M., Jalalah, M., Harraz, F. A., Al-Assiri, M. S., Zheng, Y., Sharma, P., & Li, X. (2021). Co-fermentation of immobilized yeasts boosted bioethanol production from pretreated cotton stalk lignocellulosic biomass: Long-term investigation. *Industrial Crops and Products*, *159*, 113122. https://doi.org/10.1016/J.INDCROP.2020.113122

Martini, E., Andriani, D., GobiKrishnan, S., Kang, K., Bark, S.-T., Sunwoo, C., Prasetya, B., & Park, D.-H. (2011). Immobilization of Saccharomyces Cerevisiae in Rice Hulls for Ethanol Production. *Makara Journal of Technology*, *14*(2), 61–64. https://doi.org/10.7454/693

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S. I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Analytical Biochemistry*, *339*(1), 69–72. https://doi.org/10.1016/j.ab.2004.12.001

Megawati, Damayanti, A., Putri, R. D. A., Bahlawan, Z. A. S., Mastuti, A. A. D., & Tamimi, R. A. (2022). Hydrolysis of S. platensis Using Sulfuric Acid for Ethanol Production. *Materials Science Forum*, *1048*, 451–458. https://doi.org/10.4028/WWW.SCIENTIFIC.NET/MSF.1048.451

Megawati, Bahlawan Z. A. S., Damayanti, A., Putri, R. D. A., Triwibowo, B., & Prasetiawan, H. (2021). Comparative study on the various hydrolysis and fermentation methods of Chlorella vulgaris biomass for the production of bioethanol. *International Journal of Renewable Energy Development*, *11*(2). https://doi.org/10.14710/ijred.2022.41696 10.14710/IJRED.2022.41696

Mohd Azhar, S. H., Abdulla, R., Jambo, S. A., Marbawi, H., Gansau, J. A., Mohd Faik, A. A., & Rodrigues, K. F. (2017). Yeasts in sustainable bioethanol production: A review. In *Biochemistry and Biophysics Reports* (Vol. 10, pp. 52–61). Elsevier B.V. https://doi.org/10.1016/j.bbrep.2017.03.003

Nugraheni, S. D., & Mastur, M. (2017). Perbaikan Bioproses Untuk Peningkatan Produksi Bioetanol Dari Molase Tebu / Bioprocess Improvement for Enhanching Bioethanol Production of Sugarcane Molase. *Perspektif*, *16*(2), 69. https://doi.org/10.21082/psp.v16n2.2017.69-79

Ong, H. C., Mahlia, T. M. I., & Masjuki, H. H. (2011). A review on energy scenario and sustainable energy in Malaysia. In *Renewable and Sustainable Energy Reviews* (Vol. 15, Issue 1, pp. 639–647). Pergamon. https://doi.org/10.1016/j.rser.2010.09.043

Pacheco, A. M., Gondim, D. R., & Gonçalves, L. R. B. (2010). Ethanol production by fermentation using immobilized cells of Saccharomyces cerevisiae in cashew apple bagasse. *Applied Biochemistry and Biotechnology*, *161*(1–8), 209–217. https://doi.org/10.1007/S12010-009-8781-Y

Rattanapan, A., Limtong, S., & Phisalaphong, M. (2011). Ethanol production by repeated batch and continuous fermentations of blackstrap molasses using immobilized yeast cells on thin-shell silk cocoons. *Applied Energy*, *88*(12), 4400–4404. https://doi.org/10.1016/J.APENERGY.2011.05.020

Santos, E. L. I., Rostro-Alanís, M., Parra-Saldívar, R., & Alvarez, A. J. (2018). A novel method for bioethanol production using immobilized yeast cells in calcium-alginate films and hybrid composite pervaporation membrane. *Bioresource Technology*, *247*, 165–173. https://doi.org/10.1016/J.BIORTECH.2017.09.091

Sriariyanun, M., Mutrakulcharoen, P., Tepaamorndech, S., Cheenkachorn, K., & Rattanaporn, K. (2019). A Rapid Spectrophotometric Method for Quantitative Determination of Ethanol in Fermentation Products. *Oriental Journal of Chemistry*, *35*(2), 744–750. https://doi.org/10.13005/ojc/350234

Sudhakar, M. P., Arunkumar, K., & Perumal, K. (2020). Pretreatment and process optimization of spent seaweed biomass

(SSB) for bioethanol production using yeast (Saccharomyces cerevisiae). *Renewable Energy*, *153*, 456–471. https://doi.org/10.1016/j.renene.2020.02.032

Tesfaw, A., & Assefa, F. (2014). Current Trends in Bioethanol Production by Saccharomyces cerevisiae : Substrate, Inhibitor Reduction, Growth Variables, Coculture, and Immobilization . *International Scholarly Research Notices*, *2014*, 1–11. https://doi.org/10.1155/2014/532852

Verma, P., Stevanovic, S., Zare, A., Dwivedi, G., Van, T. C., Davidson, M., Rainey, T., Brown, R. J., & Ristovski, Z. D. (2019). An overview of the influence of biodiesel, alcohols, and various oxygenated additives on the particulate matter emissions from diesel engines. *Energies*, *12*(10). https://doi.org/10.3390/en12101987

Waghmare, R., Memon, N., Gat, Y., Gandhi, S., Kumar, V., & Panghal, A. (2019). Jackfruit seed: an accompaniment to functional foods. *Brazilian Journal of Food Technology*, *22*, 2018207. https://doi.org/10.1590/1981-6723.20718

Yanto, H., Rofiah, A., & Bahlawan, Z. A. S. (2019). Environmental Performance and Carbon Emission Disclosures: A case of Indonesian Manufacturing Companies. *Journal of Physics: Conference Series*, *1387*(1), 12005. https://doi.org/10.1088/1742-6596/1387/1/012005

Yu, J., Zhang, X., & Tan, T. (2007). An novel immobilization method of Saccharomyces cerevisiae to sorghum bagasse for ethanol production. *Journal of Biotechnology*, *129*(3), 415–420. https://doi.org/10.1016/J.JBIOTEC.2007.01.039

Zhang, Q., Wu, D., Lin, Y., Wang, X., Kong, H., & Tanaka, S. (2015). Substrate and product inhibition on yeast performance in ethanol fermentation. *Energy and Fuels*, *29*(2), 1019–1027. https://doi.org/10.1021/ef502349v

Zhu, D., Li, X., Liao, X., & Shi, B. (2017). Immobilization of Saccharomyces cerevisiae using polyethyleneimine grafted collagen fibre as support and investigations of its fermentation performance. *Http://Mc.Manuscriptcentral.Com/Tbeq*, *32*(1), 109–115. https://doi.org/10.1080/13102818.2017.1389302

Zhu, D., Li, X., Liao, X., & Shi, B. (2018). Immobilization of Saccharomyces cerevisiae using polyethyleneimine grafted collagen fibre as support and investigations of its fermentation performance. *Biotechnology and Biotechnological Equipment*, *32*(1), 109–115. https://doi.org/10.1080/13102818.2017.1389302/SUPPL_FILE/TBEQ_A_1389302_SM5733.PDF