Jurnal Teknologi

Full paper

Potential Use of Liquid Pinapple Waste for Bioethanol Production by Immobilized Bakers' Yeast

Nor Azimah Mohd Zain[a*], Ng Sew Shen[a], Mohd Suardi Suhaimi[b], Nor Badzilah Hasan[a], Siti Nurbalqis Aziman[a]

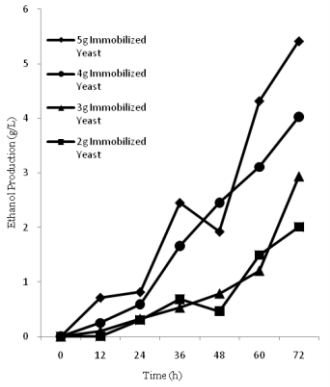
[a]*Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, 81310, UTM Johor Bahru, Johor Darul Ta'azim, Malaysia*
[b]*Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310, UTM Johor Bahru, Johor Darul Ta'azim, Malaysia*

*Corresponding author: azimah@fbb.utm.my

Article history

Received :1 August 2012
Received in revised form :7 Sept. 2012
Accepted :1 October 2012

Graphical abstract



Abstract

Bioethanol is the most extensively used biofuel for transportation in the world. Nowadays, researchers are focusing in producing bioethanol from crops and agricultural wastes. Malaysia contributes to 1% of world pineapple production which corresponds to an enormous amount of waste generation per year. Utilization of waste from pineapple processing industry for bioethanol production could help to reduce potential environmental issues. In this study, Bakers' yeast (*Saccharomyces cerevisiae)* was immobilized in modified PVA-alginate beads to produce bioethanol from liquid pineapple waste. The results revealed that by using 10 % (w/v) of the immobilized cells highest overall productivity of 0.0752 g/L/h and maximum production of 5.4179 g/L of bioethanol could be achieved. Although its maximum productivity of immobilized yeast was 0.0752 g/L/h which appreciably lower than that of the free cells, this deficiency is balanced by its overall productivity which is almost 50 % higher compared to that of free cells.

Keywords: Baker's yeast; immobilization; PVA-alginate beads; bioethanol production; liquid pineapple waste

Abstrak

Bioetanol adalah bahan api bio yang paling meluas digunakan dalam pengangkutan di dunia. Pada masa kini, usaha penyelidik tertumpu dalam menghasilkan bioetanol daripada tanaman dan bahan buangan pertanian. Malaysia menyumbang 1% daripada pengeluaran nanas dunia yang sepadan dengan jumlah penjanaan sisa yang sangat besar dalam masa setahun. Penggunaan bahan buangan dari industri pemprosesan nanas untuk pengeluaran bioetanol dapat membantu untuk mengurangkan masalah pencemaran alam sekitar. Dalam kajian ini, Yis Baker (*Saccharomyces cerevisiae*) telah disekat gerak di dalam manik alginat yang telah diubahsuai untuk menghasilkan bioetanol dari cecair sisa nanas. Keputusan menunjukkan bahawa dengan menggunakan 10% (w / v) sel-sel yang disekatgerak, produktiviti tertinggi 0.0752 g / L / h dan pengeluaran maksimum, 5.4179 g L / daripada bioetanol dapat dicapai. Walaupun produktiviti maksimum bagi sel tersekat gerak adalah 0.0752 g / L / h iaitu lebih rendah daripada sel-sel bebas, kekurangan ini diimbangi oleh produktiviti keseluruhan yang hampir 50% lebih tinggi berbanding sel-sel bebas.

Kata kunci: Yis roti; sekat gerak; manik PVA-alginat; pengeluaran bioetanol; cecair sisa nanas

1.0 INTRODUCTION

Bioethanol is one of the most well-known alternatives to fossil fuels which can be produced through biological process from a variety of biomasses like cellulose, lignocellulose, starch and sugar from various sources [1]. In Malaysia, available agricultural biomass is abundant and could be beneficially used for bioethanol production. Cultivation and processing of pineapple (*Ananas cosmosus*) is one of the main contributors of Malaysia's Gross Domestic Product (GDP). The statistics from the Board of Malaysian Pineapple Industry approximated that the annual overall production of pineapple in Malaysia is around hundreds thousands tones and Malaysia contributes to 1% of world pineapple production which corresponds to a massive amount of pineapple waste generation. This is because about 40 to 80% of the pineapple is discarded as waste, consisting of peel, cores and pomace [2]. Improper waste management may lead to serious environmental problems. High level of sucrose found in liquid pineapple waste also suggested that this waste could be an excellent substrate for bioethanol production.

Previous study has shown that liquid pineapple waste could be used in the production of bioethanol through fermentation

using *Zymomonas mobilis* [3]. The results suggested that pineapple juice and the waste material could be useful low-cost substrates for ethanol production by *Zymomonas mobilis* without supplementation of expensive organic nitrogen complexes. In addition, pH regulation is not required during fermentation, leading to the reduction in production cost. *Saccharomyces cerevisiae* is the most frequently employed microorganisms for bioethanol production as it has the capability to ferment hexoses, in particular glucose to bioethanol with up to 95% yield under anaerobic conditions [4]. In addition, *S. cerevisiae* is known capable to tolerate high concentration of ethanol, up to 150 g/L [5].

To date, not much data are available in the literature regarding the use of immobilized *S. cerevisiae* for the production of ethanol from pineapple cannery waste. In a study by Nigam [6], immobilized *S. cerevisiae* ATCC 24553 in K-carrageenan was used for ethanol production in a tapered glass column reactor employing pineapple cannery waste as substrate. This study attempts to utilize liquid pineapple waste to produce bioethanol by using *S. cerevisiae* immobilized in modified PVA-alginate beads. This study is the first attempt to produce bioethanol from liquid pineapple waste by using Baker's yeast (*S. cerevisiae*) immobilized in modified PVA-alginate (PI20081825).

2.0 MATERIALS AND METHODS

2.1 Materials

Polyvinyl alcohol (PVA) 60,000 MW and boric acid were purchased from Merck Schuchardt OHG, Darmstadt, Germany. Meanwhile, sodium alginate was obtained FlukaChemie GmbH, Buchs, sodium sulfate from GCE Laboratory Chemicals and calcium chloride from R&M Marketing, Essex, UK. Bakers' yeast (*Saccharomyces cerevisiae*) was purchased in dry yeast form from local market in Johor Bharu. The pineapple waste was obtained from Lee Pineapple Sdn. Bhd, Johor Bharu.

2.2 Pretreatment of Pineapple Waste

The concentration of reducing sugar in the waste was determined by using the Alkaline 3,5-dinitrosalicyclic acid (DNS) method proposed by Miller [7]. The pineapple waste contained undissolved compounds and suspended solids that interfere with the density measurement method. To flocculate the particles, the waste was boiled for 5 minutes and left to cool in room temperature prior to centrifuge using Sorvall® RC 5C Plus at 10000rpm for 15 minutes under refrigerated temperature (4°C). The supernatant was then filter sterilized through a 0.45µm nylon filter membrane (Advantec, no. 1 filter paper) prior to fermentation. The sterilized liquid pineapple waste was kept at -20°C and defrosted by using microwave oven immediately before used [8].

2.3 Rehydration of Active Dry Yeast

Rehydration of yeast was needed to reactivate the yeast. A 5g of Bakers' yeast (*Saccharomyces cerevisiae)* as added to 20mL of distilled water at 38°C. The mixture was stirred to dissolve the yeast. After 15 minutes, the formation of foam was observed indicated to adequate reactivation of the yeast cells.

2.4 Cultivation

Reactivated yeast was cultured on Potato Dextrose Agar (PDA) and incubated at 30°C for 2 days. A loopful of yeast colony was transferred into Potato Dextrose Broth (PDB) and incubated at 30°C with shaking at 200 rpm until the growth reached an absorbance of 0.8 at 600 nm. The culture was then used for immobilization in modified PVA-alginate beads prior to apply for bioethanol fermentation.

2.5 Immobilization of Bakers' Yeast (*Saccharomyces cerevisiae*)

A 10mL of broth culture was added into a solution mixture consisting 90mL of 12.0% (w/v) PVA and 1.0% (w/v) sodium alginate solution. The mixture was then beaded into a 100mL of 5.0% (w/v) boric acid and 2.0% (w/v) calcium chloride solution using a syringe needle (21G x 4cm) and stirred for 30 to 50 minutes at moderate speed. The beads were stored at 4˚C for 24 hours prior to wash in 7.0% (w/v) boric acid solution for 30 minutes and treated with sodium sulfate solution for another 30 minutes. Finally, the beads were kept at 4˚C in distilled water for further use. These processes were performed under sterile conditions [9].

2.6 Bioethanol Production

Batch experiments were carried out in Erlenmeyer flasks containing 50mL of pretreated liquid pineapple waste. The temperature, pH, agitation speed were set at 30˚C, pH 5.0, and 200rpm respectively and the fermentation was carried out for 72 hours [10]. The initial inoculums sizes tested in the fermentation were 4, 6, 8 and 10% (w/v), respectively. All experiments were carried out in triplicate. Negative control experiment was also carried out using the same pretreated liquid pineapple except that the inoculum was excluded. The free suspended cell system of *S. cerevisiae* was carried out at the same condition with the immobilized cells. The results between immobilized and free cells were compared in terms of ethanol production and reducing sugar concentration. A 5mL sample from the fermentation was taken at 12 hours intervals throughout the experiment. The sample was centrifuged at 4000rpm for 15 minutes and the supernatant was used for the determination of ethanol and reducing sugar concentration.

2.7 Determination of Bioethanol Concentration

The concentration of ethanol in the samples was determined by using Agilent Technologies 6890N gas chromatography equipped with flame ionization detector (FID). Samples of culture supernatant (0.5mL) were acidified with a drop of 5.0% (v/v) HCl. The acidified samples were then extracted into an equal volume of dichloromethane. The mixtures were vigoriously vortexed for 30 seconds before it was centrifuged at 3000rpm for 30 minutes. The top layer of water was discarded while the bottom layer which contains extracted ethanol in dichloromethane was taken for further analysis.

2.8 Determination of Reducing Sugar Concentration

Reducing sugar is the sugar that contains aldehyde group that is oxidized to carboxylic acid. Alkaline 3,5-dinitrosalicylic acid (DNS) method was used to test the total concentration of reducing sugar and the presence of free carbonyl group in the sample [7]. The absorbance of each solution was read at 540nm.

3.0 RESULT AND DISCUSSION

3.1 Utilization of Reducing Sugar

Figure 1 represents the utilization of reducing sugars by both free and immobilized *S. cerevisiae*. All of the runs show patterns that are almost identical with an observed peak at 48 h and an abrupt dip afterwards. The decrease in reducing sugars concentration observed at 0-24 hours and 48-72 hours intervals is the result of glucose utilization by *S. cerevisiae* either for biomass or ethanol production. However, the observed increase in reducing sugars concentration during the 24-48 hours interval resulted from the conversion of sucrose to glucose and fructose by invertase secreted by *S. cerevisiae* [11]. This supposition was supported by DNS assay which is an analysis for detecting reducing sugar [7]. Liquid pineapple waste contains high concentration of sucrose, a substrate for invertase. The presence of sucrose would stimulate yeast to secrete invertase to hydrolyze it to glucose and fructose [11]. Via DNS assay, an increase in reducing sugar (glucose and fructose from sucrose hydrolysis by invertase) concentration was detected during the aforementioned interval.

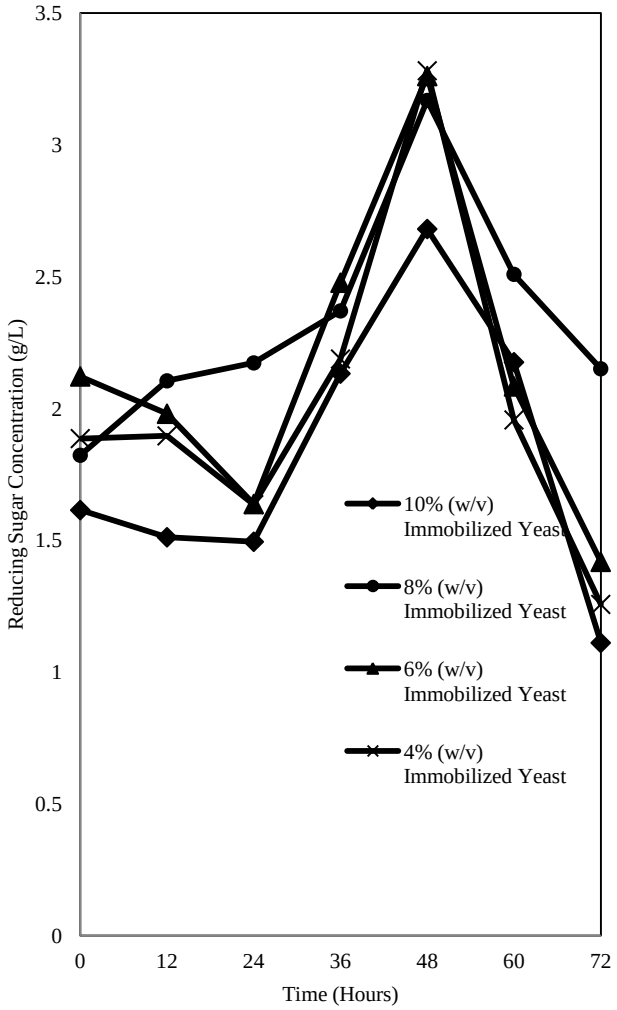


Figure 1 Reducing sugar production and consumption by immobilized *S. cerevisiae* in batch fermentation of pretreated liquid pineapple waste

Figure 2 indicates the comparison of reducing sugars utilized by immobilized and free *S. cerevisiae*. The immobilized cell system shows the highest value of reducing sugar consumed from the 48 to 72 hours of fermentation. In contrast, the highest reducing sugar consumption in free suspended cell system started much earlier during the fermentation (from 36 to 48 hour), though its consumption was found inhibited after 48 hours of fermentation. Apparently, the utilization of reducing sugars by free cells is faster than the immobilized cells. This could be due the better accessibility of sugars to free cell compared to immobilized cells where immobilization matrix could pose extra hindrance therefore reducing sugar mass transfer. This would also imply that the uptake of sugars by immobilized *Saccharomyces cerevisiae* might be hindered by PVA-alginate structure.

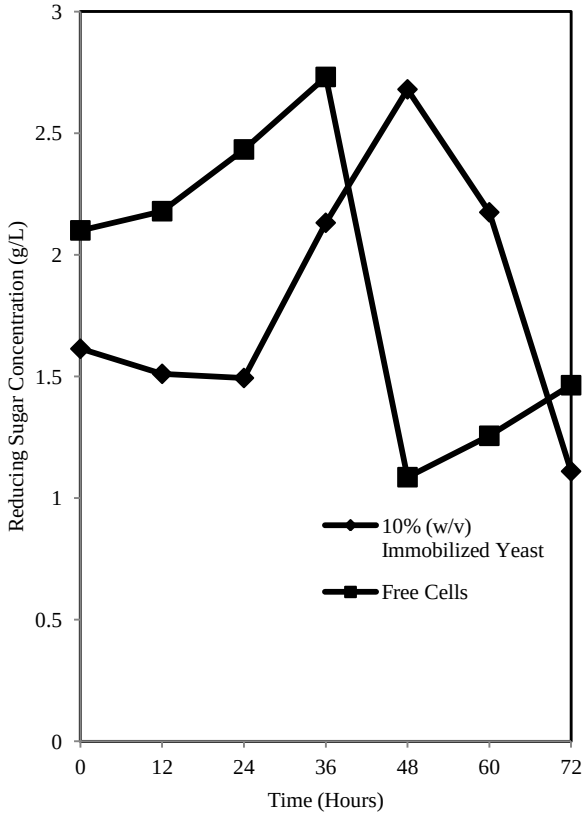


Figure 2 Comparison of reducing sugars utilized by immobilized *Saccharomyces cerevisiae* and free cells within 72 hours fermentation time

3.2 Bioethanol Production

Figure 3 represents the bioethanol production by immobilized *S. cerevisiae*. Results showed that the liquid pineapple waste inoculated with 10% (w/v) immobilized *S. cerevisiae* produced the highest ethanol concentration of 5.4179g/L. This could be due to the higher concentration of cells in the system with 10% (w/v) immobilized cells thus inducing high rate of cellular metabolism to convert reducing sugar to ethanol in the fermentation. At the condition where induction of metabolic rates occur, increased in substrate-enzyme binding would be achieved for higher production of metabolite. At 10% (w/v) immobilized cells, the chance of substrates to bind to their respective enzymes is much

higher than those at lower concentration (4-6% (w/v)) of immobilized cells.

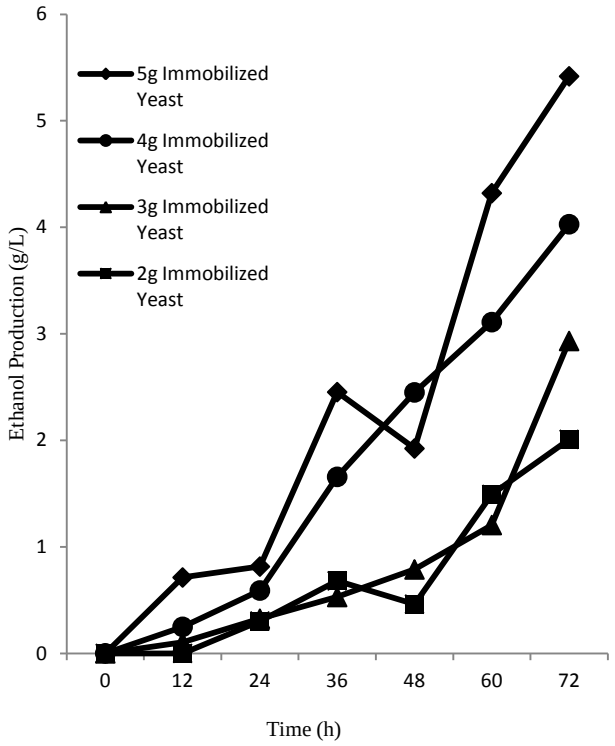


Figure 3 Bioethanol production by immobilized *Saccharomyces cerevisiae* within 72 hours fermentation time

In the fermentation with 4% (w/v) and 10% (w/v) of immobilized *S. cerevisiae*, the concentration of bioethanol decreased at the halfway of fermentation period. This could be attributed to the conversion of ethanol to aldehyde. According to Olga *et al.* [12], alcohol dehydrogenases are oxidoreductases that facilitate the interconversion between alcohols and aldehydes or ketones with the concomitant reduction of NAD^+ or $NADP^+$.

Figure 4 shows the comparison of bioethanol production by immobilized and free suspended *S. cerevisiae* cells. For the free suspended cell system, ethanol production achieved the highest concentration at the 48 hour though ethanol production was found inhibited thereafter. In contrary, the production of ethanol in the fermentation with 10% (w/v) immobilized cells was gradually increased throughout the fermentation period. The same patent of ethanol production was also observed by Lee *et al.* (2011) [13]. Maximum concentration of ethanol (approximately 5.3 g/L) was observed at 72 hour of fermentation with 105 (w/v) immobilized cells. This scenario may be due to the mass transfer limitation imposed by PVA-alginate beads since the bead size used in this study was 1.0 mm. The bead size of 1.1 ± 0.2 mm may show decrease in activity of immobilized cells due to mass transfer limitation [14]. Based on the study reported by Knezevic *et al.* (2002) [14], activity of immobilized cells decreases with increasing bead size due to mass transfer limitation.

The overall productivity of bioethanol fermentation is showed in Table 1. In the case of immobilized cells, the highest overall bioethanol productivity of 0.0752 g/L/h was obtained from fermentation with 10% (w/v) immobilized *S. cerevisiae* . However, the maximum bioethanol productivity of 0.1095 g/L/h obtained from the free suspended cell fermentation is far higher than that obtained from fermentation with 10% (w/v) immobilized cell.

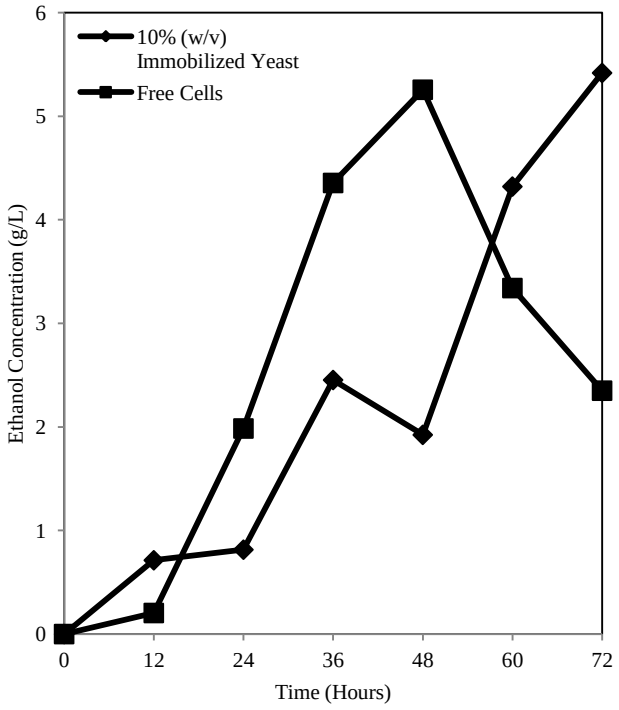


Figure 4 Comparison of bioethanol production by immobilized *Saccharomyces cerevisiae* and free cells within 72 hours fermentation time

In terms of maximum productivity, free cells system recorded the highest value at 0.1095 g/L/h. This could be due to the ease of accessibility for the reducing sugars to reach the cells compared to that of immobilization system. This observation also indicates that the mass transfer limitation can also affect the productivity of the immobilization system.

Table 1 Profile of bioethanol production by immobilized *Saccharomyces cerevisiae* and free cells

Sample	Maximum Production (g/L)	Fermentation Time (Hours)	Maximum Productivity (g/L/h)	Fermentation Time (Hours)	Overall Productivity (g/L/h)
10 % (w/v) Immobilized Yeast	5.4179	72	0.0752	72	0.0752
8 % (w/v) Immobilized Yeast	4.0291	72	0.0560	72	0.0560
6 % (w/v) Immobilized Yeast	2.9328	72	0.0407	72	0.0407
4 % (w/v) Immobilized Yeast	2.0093	72	0.0279	72	0.0279
Free Cells	5.2558	48	0.1095	36	0.0326

The overall productivity for 10% (w/v) immobilized *Saccharomyces cerevisiae* is the highest among the immobilization system. During fermentation, accumulation of inhibitors and toxic metabolic by products, changes in pH and temperature may affect the growth of *S. cerevisiae*. These factors

could contribute to the observed sharp decrease in bioethanol production in the case of free cells which can be explained by Figure 4. However, bioethanol production from ferementation with 10% (w/v) immobilized *S. cerevisiae* increased steadily until the end of fermentation period. This could be attributed to PVA-alginate matrix that can provide protection to the cells from the unfavourable conditions and exposure to toxic materials accumulated during batch fermentation system [15]. *Saccharomyces cerevisiae* will utilize reducing sugars to produce bioethanol during the fermentation process causing the concentration of reducing sugars to increase with a corresponding increase in bioethanol concentration. However, not all of the reducing sugars will be converted to bioethanol. This is because the cells of *S. cerevisiae* also metabolize sugars for biomass and energy production for cell maintenance.

■4.0 CONCLUSION

This study has significantly showed that the immobilized cells of *S. cerevisiae* were capable of producing bioethanol from liquid pineapple waste. In addition, immobilized cells at all concentrations tested consistently show its advantages mainly in providing cells with protection toward toxic effect from the accumulation of some compounds in the liquid pineapple waste as well as those produced as metabolic by products during fermentation. Fermentation with 10% (w/v) immobilized *Saccharomyces cerevisiae* give the highest overall productivity (0.0752 g/L/h) with maximum productivity of 0.0752 g/L/h and maximum production of 5.4179 g/L of bioethanol. Even though free cells system gives the highest maximum productivity of 0.1094 g/L/h, its overall productivity is almost 50 % lower than that of the 10% (w/v) immobilized *S. cerevisiae* (0.0752 g/L/h). At 0.0752 g/L/h, the overall productivity of 10% (w/v) immobilized *Saccharomyces cerevisiae* is the highest.

Acknowledgement

Financial support from the Universiti Teknologi Malaysia through the research university grant (RUG) vote Q.J130000.7135.02J29 is gratefully acknowledged.

References

[1] Ibeto, C. N., Ofoefule, A. U. & Agbo, K. E.. 2011. A. Global. Overview of Biomass Potentials for Bioethanol Production: A Renewable Alternative Fuel. *Trends Appl Sci Res*. 6: 410–425.

[2] Ban-Koffi, L., Han, Y. W. 1990. Alcohol Production from Pineapple Waste. *World J Microb Biot*. 6: 281–284.

[3] Tanaka, K., Hilary, Z. D., Ishizaki, A. 1999. Investigation of the Utility of Pineapple Juice and Pineapple Waste Material as Low-cost Substrate for Ethanol Fermentation by *Zymomonas Mobilis*. *J. Biosci. Bioeng*. 87: 642–646.

[4] Cardona, C. A., Sánchez, Ó. J. & Gutiérrez, L. F. 2009. Microorganisms for Ethanol Production. *Process Synthesis for Fuel Ethanol Production.* CRC Press.

[5] Claassen, P. A. M., Van Lier, J. B., Lopez Contreras, A. M., Van Niel, E. W. J., Sijtsma, L., Stams, A. J. M.. 1999. *et al*. Utilisation of Biomass for the Supply of Energy Carriers. *Appl. Microbiol. Biotechnol*. 52: 741–755.

[6] Nigam, J. N. 2000. Continuous Ethanol Production from Pineapple Cannery Waste Using Immobilized Yeast Cells. *J Biotechnol*. 80: 189–193.

[7] Miller, G. 1959. Use of DNS Reagent for Determination of Reducing Sugars. *Anal Chem* . 31: 426–428.

[8] Idris, A. and Suzana, W. 2006. Effect of Sodium Alginate Concentration, Bead Diameter, Initial Ph and Temperature on Lactic Acid Production From Pineapple Waste Using Immobilized *Lactobacillus Delbrueckii*. *Process Biochem*. 41: 1117–1123.

[9] Zain, N. A. M.2009. *Modification of PVA- Alginate Immobilization Matrix to Immobilize Invertase.* Doctor of Philosophy. Universiti Teknologi Malaysia.

[10] Liu, R., Shen, F. 2008. Impacts of Main Factors on Bioethanol Fermentation From Stalk Juice of Sweet Sorghum by Immobilized *Saccharomyces Cerevisiae* (CICC 1308). *Bioresour. Technol*. 99: 847–854.

[11] Drapcho, C. M., Nhuan, N. P., Walker, T. H. 2008. *Biofuels Engineering Process Technology*. United States of America. McGraw-Hill Companies.

[12] Olga, D. S., James, C. D. P., Jacobus, A. 2008. The Alcohol Dehydrogenases of *Saccharomyces Cerevisiae*: A Comprehensive Review. *FEMS* 8: 967–978.

[13] Knezevic, Z., Bobic, S., Milutinovic, A. 2002. Obradovic B, Mojovic L, Bugarski B. Alginate-Immobilized Lipase by Electrostatic Extrusion for the Purpose o Palm Oil Hydrolysis in Lecithin/Isooctane System. *Process Biochem.* 38: 313–318.

[14] Lee, K. H., Choi, I. S., Kimb, 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 Technol.* 102: 8191–8198.

[15] Kourkoutas, Y., Kanellaki, M., Koutinas, A., Tzia, C. 2005. Effect of Fermentation Conditions and Immobilization Supports on the Wine Making. *J Food Process Eng*. 69: 115–123.