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Immobilised Sarawak Malaysia Yeast Cells for Production of Bioethanol

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Abstract: Bioethanol production using yeast has become a popular topic due to worrying depleting worldwide fuel reserve. The aim of the study was to investigate the capability of Malaysia yeast strains isolated from starter culture used in traditional fermented food and alcoholic beverages in producing Bioethanol using alginate beads entrapment method. The starter yeast consists of groups of microbes, thus the yeasts were grown in Sabouraud agar to obtain single colony called ST1 (tuak) and ST3 (tapai). The growth in Yeast Potatoes Dextrose (YPD) resulted in specific growth of ST1 at $\mu = 0.396\ h^{-1}$ and ST3 at $\mu = 0.38\ h^{-1}$, with maximum ethanol production of 7.36 g L^{-1} observed using ST1 strain. The two strains were then immobilized using calcium alginate entrapment method producing average alginate beads size of 0.51 cm and were grown in different substrates; YPD medium and Local Brown Sugar (LBS) for 8 h in flask. The maximum ethanol concentration measured after 7 h were at 6.63 and 6.59 g L^{-1} in YPD media and 1.54 and 1.39 g L^{-1} in LBS media for ST1 and ST3, respectively. The use of LBS as carbon source showed higher yield of product (Yp/s), 0.59 g g^{-1} compared to YPD, 0.25 g g^{-1} in ST1 and (Yp/s), 0.54 g g^{-1} compared to YPD, 0.24 g g^{-1} in ST3 . This study indicated the possibility of using local strains (ST1 and ST3) to produce bioethanol via immobilization technique with local materials as substrate.

Key words: Bioethanol, local yeast, calcium alginate, immobilisation, yeast fermentation, brown sugar

INTRODUCTION

Interest in bioethanol has been growing since it has considered as an alternative fuel for future due to depleting fossil fuel energy resources (Lin and Tanaka, 2006; Yu *et al*., 2009; Giordano *et al*., 2000). Ethanol can be produced by microbial fermentation using renewable substrates such as corn and sorghum, thus economic and profitable production emphasizing on substrates, processes and the microbes used deserved a proper attention from researchers (Ibeto *et al*., 2011; Millati *et al*., 2011; Sherief *et al*., 2010; Nadir *et al*., 2009).

Indigenous fermented foods are essential components of the diet and represent identity culture of the people or countries. Fermented food include alcoholic and non alcoholic beverages developed well traditionally with village-art methodologies that normally produced at household or cottage industry scales (Dung *et al*., 2007; Sujaya *et al*., 2002; N'Guessan *et al*., 2008; Valyasevi and Rolle, 2002). In Malaysia, various fermented foods being produced and amongst the popular are tapai and tuak. Tapai is a food made from tapioca (cassava), glutinous rice, rice or banana can be eaten raw after few days of fermentation with ragi. Similar to Indonesian tape ketan (black rice fermentation), Malaysian tapai is partially liquefied, sweet sour and mildly alcoholic rice paste served as dessert or snack. Whereas, tuak is rice wine for native peoples (especially in the state of Sarawak) usually serve during special and important occasions. Tuak is prepared similarly like tapai but in longer fermentation period, resulting in greater liquefaction of the rice and consumed as an alcoholic beverage. Both tapai and tuak produced by fermentation of rice from starter ragi, dry flattened circular cakes, about 3-5 cm in diameter, consisted of microflora of microorganism. These microbial exist in the traditional starter associated with the raw materials which is rice flour mixed with grounded spices (Gandjar, 2003; N'Guessan *et al*., 2008).

Traditionally, ethanol fermentation includes the usage of free cells of any suitable species and strains mainly involving *Saccharomyces cerevisiae* (Somda *et al*., 2011). The recuperation and reutilization of these cells require onerous steps (Rivaldi *et al*., 2008) resulted in separation of cells normally achieved by a unit procedure and discarded after the process ended. This also will escalate burden to a fermentation plant in order to discard such volume of biomass. The growing demands for bioethanol needing alternative optimization such as

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immobilization of cells in an inert support (Goksungur and Zorlu, 2001). Since the cells are entrapped inside the inert support, separation of bioethanol from the medium should be easier and cost saving since it is omitting a unit procedure than those of free cells. Apart from that, cells entrapment also allowing reutilization of entrapped cell, protection against adverse environmental conditions, utilizations of high cell densities that usually make higher processing rate and high dilution in continuous operation (Da Cunha *et al*., 2006).

There are many support materials and carrier that is used in cell immobilization and give the different entrapment including alginate beads entrapment, biocapsules and PVA particles (Rakin *et al*., 2009; Peinado *et al*., 2006). Alginate in food, pharmaceutical, textile and paper production industry used for thickening, stabilizing, gel and film forming (Najafpour *et al*., 2004). Entrapment in calcium alginate beads has been one of the most used matrices for whole cell entrapment due to its simplicity and non-toxic character. This simple and mild immobilization technique involves the drop-wise addition of cells suspended in sodium alginate onto a solution of calcium chloride whereby the cells are immobilized in precipitated calcium alginate gel in the form of beads (Goksungur and Zorlu, 2001).

Local Brown Sugar (LBS), also known as 'gula merah' in Malay is the names of jaggery, a traditional unrefined sugar consumed in Asia, Africa, Latin America and Caribbean (Rathnasabapathy *et al*., 2009; Rajvanshi and Nimbkar, 1996). It is a natural sweetening substance made by concentrating sugarcane juice without any preservatives and colourings. LBS normally used in preparing cakes, syrups and desserts and sold cheaply at local market. There are many published studies on bioethanol production using sweet juice as a substrate from sorghum and sugar cane using *Saccharomyces cerevisiae* have been reported in producing bioethanol manipulating unsterilized juice substrate (Rajvanshi and Nimbkar, 1996; Yu *et al*., 2009). Thus, the usage of local yeast in production of ethanol has the potential to be explored. In this study, the ability of yeast isolated from cottage industry in Sarawak, Malaysia to produce ethanol using LBS and calcium alginate entrapment method were investigated.

MATERIALS AND METHODS

Microorganism: The yeast strain used in fermentation (2009) was isolated from two different of starter ragi which is used in making tuak and tapai are bought at the local market in Kuching, Sarawak (Malaysia). The starter ragi is dry flattened circular cakes, about 3-5 cm in diameter, prepared from rice flour and packed in small plastic bag. It was subcultured and screened from tapai fermentation followed the method of Sujaya *et al*. (2002) and isolated on Sabouraud agar (Koehler *et al*., 1999) to produce a single colony. The strain selected was named as ST1 (tuak) and ST3 (tapai) and was kept on YPD medium/agar at 4°C.

Substrates: The medium of Yeast-extract-peptone-dextrose (YPD) consisted of yeast extract 20 g L^{-1}, Peptone 10/L and dextrose 20 g L^{-1}. The local brown sugar used as a substrate in immobilisation fermentation was bought at the local supermarket in a packed plastic. The composition of the medium is LBS (20-50 g L^{-1}) which was diluted and filtered with addition of 5.19 g L^{-1} $(NH_4)_2SO_4$, 1.53 g L^{-1} KH_2PO_4 and 0.55 g L^{-1} $MgSO_4$ (Bravo and Gonzalez, 1991).

Cell immobilization: ST1 and ST3 cells were grown at 30°C for 10 h. Two hundred and fifty milliliter culture broths were harvested by centrifuge at 13000 rpm for 5 min. Fifty milliliter of this growth medium was mixed with an equal volume (1:1,v/v) of 4% (w/v) Na-alginate (Sigma, A-2033) solution (Goksungur and Zorlu, 2001). One hundred milliliter aliquot of alginate-cell suspension containing 2% Na-alginate (unless otherwise stated) was added drop wise to 1000 mL of 2% $CaCl_2$ with a syringe (Tataridis *et al*., 2005). Alginate drops solidified upon contact with $CaCl_2$, forming beads and thus entrapping yeast cells. The beads were allowed to harden for 30 min and then were washed with sterile saline solution (0.85% NaCl) to remove excess calcium ions and cells (Rakin *et al*., 2009). The fermentation using immobilized beads were executed in a 250 mL flask with agitation at 75 rpm to avoid bead breakage.

Analytical method: Ethanol and glucose concentrations were determined using biochemical analyzer, YSI Select (Yellow Spring Ltd.). The data were analyzed using Microsoft excel.

RESULTS AND DISCUSSION

Free cell: The experiment started with fermentation of free yeast cell ST1 and ST3 in 500 mL shake flask using Yeast extract-peptone-dextrose (YPD) as medium incubated at 30°C and Fig. 1a, b showed the early results with 12 h fermentation time. The growth rate, μ of ST1 and ST3 were 0.396 and 0.38 h^{-1}, respectively and the yield of ethanol production ST1 and ST2 in free cell fermentation were $Y_{p/s}$ 0.29±0.03 g ethanol/g substrate and 0.287±0.033 g ethanol/g substrate, respectively indicate that the choice of ST1 and ST3 used in this study were achieved. The

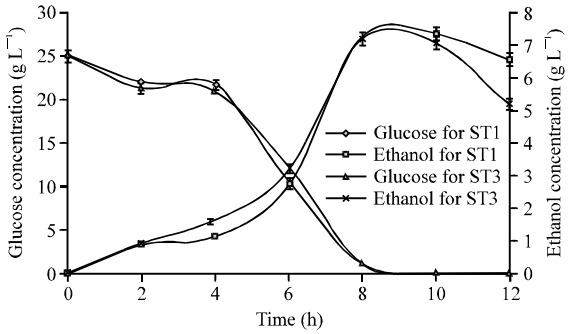

Fig. 1a: Growth of ST1 and ST3 in YPD medium using free-suspension system

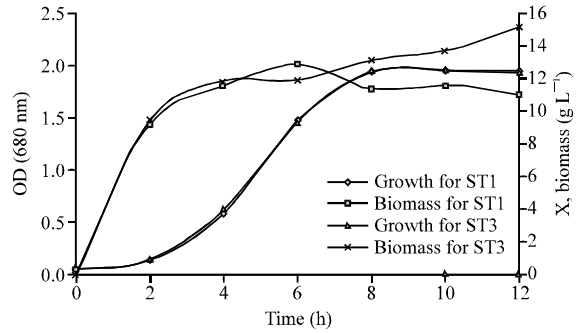

Fig. 1b: Glucose consumption and ethanol production profiles by ST1 and ST3 in YPD medium using free-suspension system

Table 1: Bioconversion of glucose to ethanol by ST1 and ST3 strain in free cell fermentation

Parameter	ST1	ST3
Growth rate (μ)	0.40±0.05 h^{-1}	0.38±0.07 h^{-1}
$Y_{P/S}$	0.290±0.030 g ethanol/g substrate	0.287±0.033 g ethanol/g substrate
$Y_{P/Xmax}$	0.63±0.045 g ethanol/g biomass	0.47±0.050 g ethanol/g biomass

Table 1 summarized the yield of ST1 and ST3 yeast cell, $Y_{p/s}$ for free cells fermentation. It is found that ST1 and ST3 produce similar results in term of μ and $Y_{p/s}$. These results suggest both strain has the capability to produce ethanol. It is expected since both starter ragi are used in food and alcoholic beverages (tuak and tapai).

The optical density data patterns were identical to both strains and the yeast cells adapted to the substrate in the first two hours. The maximum values reached at 8 h fermentation and correspond well with the maximum ethanol concentration (also at 8th h fermentation) values of 7.36 g L^{-1} while the biomass, X reached maximum at 10th h, at 15.1 g L^{-1} both in ST1 and ST3 strains showed in Fig. 2.

Figure 1b showed the sugar was quickly consumed and almost depleted after 10 h of fermentation. Sugar

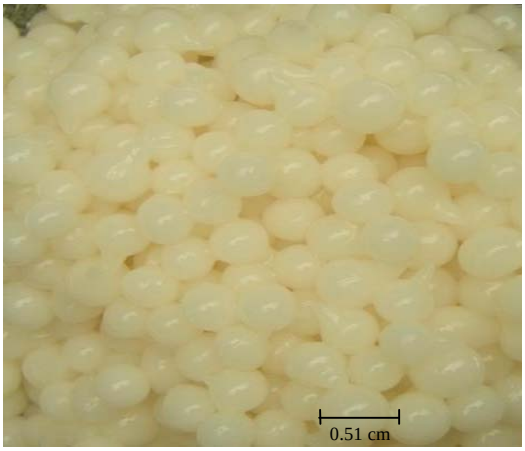

Fig. 2: Alginate beads

were utilized as energy source by yeast cell as during the fermentation to produce bioethanol. Therefore, the consumption rate of sugar can be related to the concentration of yeast cells (N'Guessan *et al*., 2008) and ethanol production. After 8 h fermentation, the production of ethanol declined since the sugar had almost 98% consumed. Moreover, there were factors which affect the production rate such as nitrogen limitation and ethanol inhibitory effect (Nikolic *et al*., 2009). The maximum ethanol production by ST1 and ST3 strain is 7.36 and 7.06 g L^{-1}, respectively. These results seemed to be similar as reported by Lin and Tanaka (2006) saying that the range of ethanol concentration produced by yeast cells is 2.4-91.8 g L^{-1}.

Immobilisation: The dropwise method of forming the alginate bead is shown in Fig. 2. Small beads size is favorites because of high surface area thus facilitated better mass transfer of substrate (Margaritis and Kilonzo, 2005; Goksungur and Zorlu, 2001). Using this application, the average sizes obtain was 0.51 cm. But the smaller the alginate beads, it is also prone to breakage. At this diameter and alginate concentration used (2 wt%), the beads were fully active, flexible and hard enough to stand mild agitation and have a good stability (Najafpour *et al*., 2004). Also, we observed that the method produced that the method produced bud-like shape due to the dragging of the sodium alginate solution. The effect of bud-like shape of alginate bead on the surface area was not investigated and all beads were assumed to be spheres.

Observations by electronic micrographs were taken from the fresh ST1 strain is shown in Fig. 3. These micrographs were used as to observe the yeast cells entrapment in the alginate beads. The cells were found to

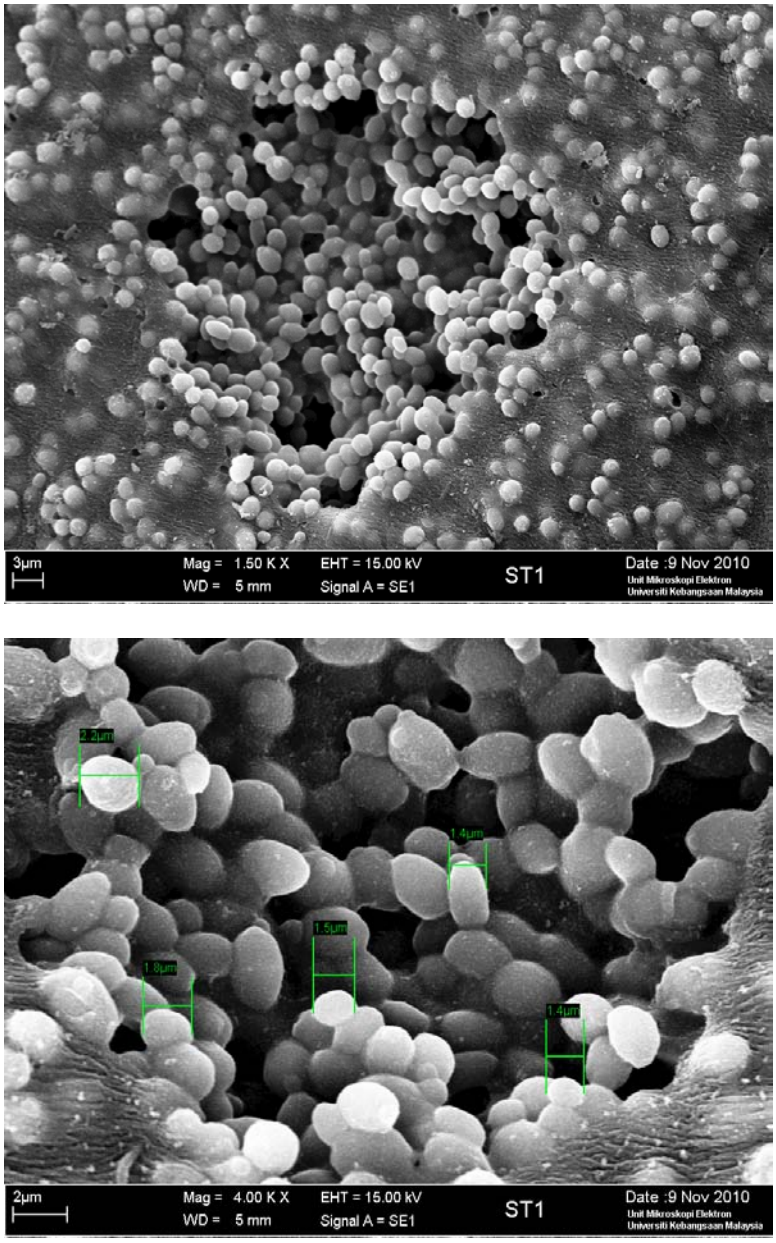

Fig. 3: Scanning electron microscopy of immobilized ST1-yeast cell

be attached close to the surface of the alginate beads. Although, it has been reported that there were no cell in the centre of particle (Ogbonna *et al*., 1989; Giordano *et al*., 2000) but from our SEM photograph indicated that the yeast cells were distributed equally throughout the beads.

The results of ethanol production and glucose consumption profiles for immobilisation system of ST1 and ST3 strains are shown in Fig. 4a, b for YPD and LBS, respectively with additional fermentation in medium LBS with two different concentrations showed in Fig. 4c. The maximum ethanol concentration was achieved after 7 h in the immobilised fermentation for both media and ST1 and ST3 strains achieve complete fermentation in same time with 98% glucose consumed well.

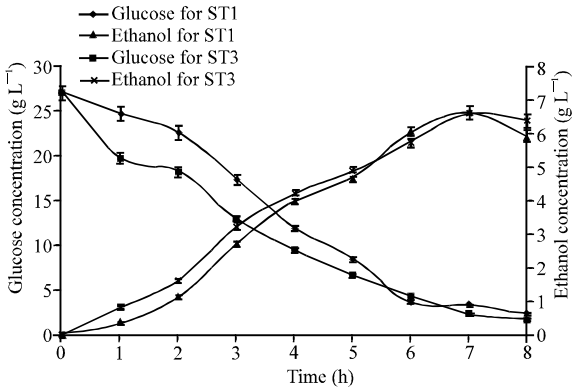

Fig. 4a: Production of ethanol and glucose consumption profile of ST1 and ST3 in YPD by immobilized cell system

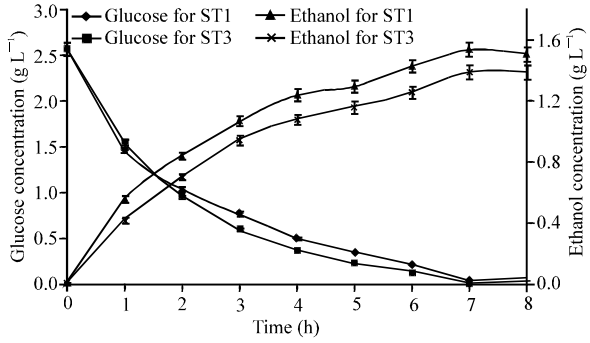

Fig. 4b: Production of ethanol and glucose consumption profile of ST1 and ST3 in LBS using immobilized cell system

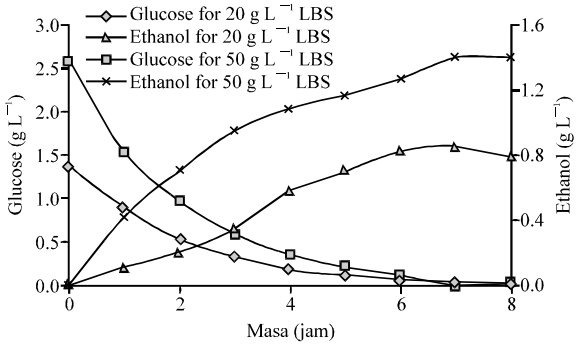

Fig. 4c: Production of ethanol and glucose consumption profile of ST1 in 20 and 50 g L^{-1} LBS using immobilized cell system

Ethanol production in YPD media is higher as 6.59 g L^{-1} in this immobilized fermentation. In LBS media, the maximum glucose concentration observed at Fig. 4b only 1.54 g L^{-1} but when we measured the concentration of glucose in LBS at the beginning of the fermentation (2.57 g L^{-1}) and it was actually much lower than that in YPD. In the concentration of LBS media, 50 g L^{-1} resulted higher ethanol concentration than 20 g L^{-1} LBS media showed in Fig. 4c since the initial concentration of glucose is high. The initial glucose concentration might have affected on the yield of ethanol. The yields $Y_{P/S}$ were 0.244 and 0.54 g g^{-1} for YPD and LBS, respectively showed in Table 2, suggesting that LBS can be used as suitable cheap substrate for production of ethanol. Selection of cheap products as substrate for ethanol production has been investigated thoroughly such as cellulose, cellobiose and xylose but has to overcome of pre treatment methods to obtain the sugar (Fukuda *et al*., 2009).

Table 2: Yield, $Y_{P/S}$ for YPD and LBS media

	YPD	LBS
ST1	0.25±0.02 g ethanol/g substrate	0.59±0.04 g ethanol/g substrate
ST3	0.24±0.03 g ethanol/g substrate	0.54±0.05 g ethanol/g substrate

Table 3: Summary of theoretical yields of ST1 and ST3 yeast cells in free cell and immobilization fermentation

		Immobilisation (%)	
	Free cell (%)	YPD	LBS
ST1	57.6±0.030	48±0.045	85.0±0.050
ST3	55.2±0.033	47±0.050	94.5±0.050

The theoretical yield of ethanol production was calculated as the actual ethanol divided by the theoretical maximum on the basis that 1 mol of glucose able to produce 2 mol of ethanol×100 (Goksungur and Zorlu, 2001). Summary of the theoretical yields for ST1 and ST3 yeast cells using these two sugar medium are showed in Table 3. The ST1 yeast cells yielded the highest theoretical yields, 94.5% of bioethanol in immobilised cells fermentation. This indicates that the imobilised yeast cell able to produce high ethanol, as also reported by Lin and Tanaka (2006), saying that the production of bioethanol using immobilised cells were doubled as compared in free cells system.

CONCLUSION

From the results we found that the local isolate, both ST1 and ST3 immobilised-cells have the ability to produce ethanol using both commercial ($Y_{P/S}$; 0.25 and 0.24 g g^{-1}, respectively) and local substrate ($Y_{P/S}$; 0.59 and 0.54 g g^{-1}, respectively). These yields can be increased by studying the optimization parameters involve in the fermentation of ethanol using immobilized system which is ongoing.

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REFERENCES

Bravo, P. and G. Gonzalez, 1991. Continuous ethanol fermentation by immobilized yeast cells in a fluidized-bed reactor. J. Chem. Technol. Biotechnol., 52: 127-134.

Da Cunha, M.A.A., A. Converti, J.C. dos Santos and S.S. da Silva, 2006. Yeast immobilization in lentikats: A new strategy for xylitol bioproduction from sugarcane bagasse. World J. Microbiol. Biotechnol., 22: 65-72.

Dung, N.T.P., F.M. Rombouts and M.J.R. Nout, 2007. Characteristic of some traditional Vietnamese starch-based rice wine ferementation starters (men). Food Sci.Technol., 40: 130-135.

Fukuda, H., A. Kondo and S. Tamalampudi, 2009. Bioenergy: Sustainable fuels from biomass by yeast and fungal whole-cell biocatalysts. Biochem. Eng. J., 44: 2-12.

Gandjar, I., 2003. TAPAI from cassava and cereals. Proceedings of the 1st International Symposium and Workshop on Insight into the World of Indigenous Fermented Food for Technology Development and Food Safety, Aug. 13-15, Bangkok, pp: 152-157.

Giordano, R.L.C., P.C. Hirano, L.R.B. Goncalves and W.S. Netto, 2000. Study of biocatalyst to produce ethanol from starch. Applied Biochem. Biotechnol., 84-86: 643-654.

Goksungur, Y. and N. Zorlu, 2001. Production of ethanol from beet molasses by Ca-alginated immobilized yeast cells in a pack-bed bioreactor. Turk. J. Biotechnol., 25: 265-275.

Ibeto, C.N., A.U. Ofoefule and K.E. Agbo, 2011. A global overview of biomass potentials for bioethanol production: A renewable alternative fuel. Trends Applied Sci. Res., 6: 410-425.

Koehler, A.P., K.C. Chu, E.T.S. Houang and A.F.B. Cheng, 1999. Simple, reliable and cost-effective yeast identification scheme for the clinical laboratory. J. Clin. Microbiol., 37: 422-426.

Lin, Y. and S. Tanaka, 2006. Ethanol fermentation from biomass resources: Current state and prospects. Applied Microbiol. Biotechnol., 69: 627-642.

Margaritis, A. and P.M. Kilonzo, 2005. Production of Ethanol Using Immobilised Cell Bioreactor System. In: Application of Cell Immobilisation Biotechnology, Nedovic, C. and R. Willaert (Eds.). Springer, Netherland, pp: 375-405.

Millati, R., R. Wikandari, E.T. Trihandayani, M.N. Cahyanto, M.J. Taherzadeh and C. Niklasson, 2011. Ethanol from oil palm empty fruit bunch via dilute-acid hydrolysis and fermentation by *Mucor indicus* and *Saccharomyces cerevisiae*. Agric. J., 6: 54-59.

N'Guessan, K.F., S. Aka, N.T. Djeni and K.M. Dje, 2008. Effect of traditional starter inoculation rate on sorghum beer quality. J. Food Technol., 6: 271-277.

Nadir, N., M. Mel, M.I.A. Karim and R.M. Yunus, 2009. Comparison of sweet sorghum and cassava for ethanol production by using *Saccharomyces cerevisiae*. J. Applied Sci., 9: 3068-3073.

Najafpour, G., H. Younesi and K.S.K. Ismail, 2004. Ethanol fermentation in an immobilized cell reactor using *Saccharomyces cerevisiae*. Bioresour. Technol., 92: 251-260.

Nikolic, S., L. Mojovic, D. Pejin, M. Rakin and V. Vucurovic, 2009. Improvement of ethanol fermentation of corn semolina hydrolyzates with immobilized yeast by medium supplementation. Food Technol. Biotechnol., 47: 83-89.

Ogbonna, J.C., Y. Amano and K. Nakamura, 1989. Elucidation of optimum conditions for immobilization of viable cells using calcium alginate. J. Fermentation Bioeng., 67: 92-96.

Peinado, R.A., J.J. Moreno, J.M. Villalba, J.A. Gonzalez-Reyes, J.M. Ortega and J.C. Mauricio, 2006. Yeast biocapsules: A new immobilization method and their applications. Enzyme Microb. Technol., 40: 79-84.

Rajvanshi, A.K. and N. Nimbkar, 1996. Sweet sorghum R and D at the Nimbkar Agricultural Research Institute (NARI). Proceedings of First European Seminar on Sorghum for Energy and Industry, Apr. 1-3, Toulouse, France, pp: 356-362.

Rakin, M., L. Mojovic, S. Nikolic, M. Vukasinovic and V. Nedovic, 2009. Bioethanol production by immobilized *Sacharomyces cerevisiae* var. ellipsoideus cells. Afr. J. Biotechnol., 8: 464-471.

Rathnasabapathy, C.S., S.M. Basha and R. Dhanasekar, 2009. Enhanced production of Glutathione from *Candida utilis* using Palm Jaggery. Int. J. ChemTech. Res., 1: 1137-1144.

Rivaldi, J.D., B.F. Sarrouh and S.S. da Silva, 2008. An evaluation of different bioreactor configurations with immobilized yeast for bioethanol production. Int. J. Chem. Reactor Eng., 6: A115-A115.

Sherief, A.A., N.E.A. El-Naggar and S.S. Hamza, 2010. Bioprocessing of lignocellulosic biomass for production of bioethanol using thermotolerant *Aspergillus fumigates* under solid state fermentation conditions. Biotechnology, 9: 513-522.

Somda, M.K., A. Savadogo, C.A.T. Ouattara, A.S. Ouattara and A.S. Traore, 2011. Improvement of bioethanol production using amylasic properties from *Bacillus licheniformis* and yeasts strains fermentation for biomass valorization. Asian J. Biotechnol., 3: 254-261.

Sujaya, I.N., S. Amachi, K. Saito, A. Yokota, K. Asano and F. Tomita, 2002. Specific enumeration of lactic acid bacteria in *ragi tape* by colony hybridization with specific oligonucleotide probes. World J. Microbiol. Biotechnol., 18: 263-270.

Tataridis, P., P. Ntagas, I. Voulgaris and E.T. Nerantzis, 2005. Production of sparkling wine with immobilized yeast fermentation. Electron. J. Sci. Technol., 1: 1-21.

Valyasevi, R. and R.S. Rolle, 2002. An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: Scope for their improvement. Int. J. Food Microbiol., 75: 231-239.

Yu, J., X. Zhang and T. Tan, 2009. Optimization of media conditions for the production of ethanol from sweet sorghum juice by immobilized *Saccharomyces cerevisiae*. Biomass Bioenergy, 33: 521-526.