REFERENCES

Abate, C., Callieri, D., Rodriguez, E., and Garro, O. Ethanol production by mixed culture of flocculent strains of *Zymomonas mobilis* and *Saccharomyces sp.* Appl Microbiol Biotechnol. 45 (1996): 580-583.

Abdel-fattah, WR., Fadil, M., Nigam, P., and Banat, I.M. Isolation of thermotolerant ethanologenic yeasts and use of selected strains in industrial scale fermentation in an Egyptian distillery. Biotechnology and Bioengineering. 68 (2000): 531-535.

Allain, Eric J. Perspective cell-free ethanol production: the future of fuel ethanol. J Chem Technol Biotechnol. 82 (2007): 117-120.

Anderson, PJ., McNeil, K., and Watson, K. High-efficiency carbohydrate fermentation to ethanol at temperatures above 40°C by *Kluyveromyces marxianus* var. *marxianus* isolated from sugar mills. Appl Environ Microbiol. 51 (1986): 1314-1320.

Bai, F.W., Anderson, W.A., and Moo-Young, W. Ethanol fermentation technologies from sugar and starch feedstocks. Biotech. Adv. 26 (2008): 89-105.

Babat, IM., and Marchant, R. Characterization and potential industrial applications of 5 novel, thermotolerant, fermentative yeast strains. World J Microbiol Biotechnol. 11 (1995): 304-306.

Banat, IM., Nigam, P., and Marchant, R. Isolation of thermotolerant, fermentative yeasts growing at 52°C and producing ethanol at 45°C and 50°C. World J Microbiol Biotechnol. 8 (1992): 259-263.

Barron, N., Marchant, R., and McHale, AP. Studies on the use of a thermotolerant strain of *Kluyveromyces marxianus* in simultaneous saccharification and ethanol formation from cellulose. Appl Microbiol Biotechnol. 43 (1995): 518-520.

Blanch, H.W., and Clark, D.S. Biochemical Engineering. United States of America: Marcel Dekker, 1997.

Boyle, M., Barron, N., and McHale, AP. Simultaneous saccharification and fermentation of straw to ethanol using the thermotolerant yeast strain *Kluyveromyces marxianus* imb3. Biotechnol Lett. 19 (1997): 49-51.

Cáceres-Farfán, M., Lappe, P., Larqué-Saavedra, A., Magdub-Méndez, A., and Barahona-Pérez, L. Ethanol production from henequen (*Agave fourcroydes Lem.*) juice and molasses by a mixture of two yeasts. Bioresource Technology. 99 (2008): 9036-9039.

Chum, L.H., and Overend, R.P. Biomass and renewable fuels. Bioprocess Technol. 17 (2001): 187-195.

Dursun, A.Y., and Tepe, O. Internal mass transfer effect on biodegradation of phenol by Ca-alginate immobilized *Ralstonia eutropha*. J. Hazardous Materials. 126 (2005): 105–111.

Fleming, M., Barron, N., Marehant, R., McHale, L., and McHale, AP. Studies on the growth of a thermotolerant yeast strain, *Kluyveromyces marxianus* IMB3, on sucrose containing media. Biotechnol Lett. 15 (1993): 1195-1198.

Fu, N., and Peiris, P. Co-fermentation of a mixture of glucose and xylose to ethanol by *Zymomonas mobilis* and *Pachysolen tannophilus*. World J Microbiol Biotechnol. 24 (2008): 1091-1097.

Fu, N., Peiris, P., Markham, J., and Bavor, J. A novel co-culture process with *Zymomonas mobilis* and *Pichia stipitis* for efficient ethanol production on glucose/xylose mixtures. Enzyme and Microbial Technology. 45 (2009): 210-217.

Göksungur, Y., and Zorlu, N. Production of ethanol from beet molasses by Ca-Alginate immobilized yeast cells in a packed-bed reactor. Turk J. Biol. 25 (2001): 265-275.

Gough, S., and McHale, A.P. Continuous ethanol production from molasses at 45°C using alginate-immobilized *Kluyveromyces marxianus* IMB3 in a continuous-flow bioreactor. Bioprocess Engineering. 19 (1998): 33-36.

Guo, X., Zhou, J., and Xiao, D. Improved Ethanol production by mixed immobilized cells of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* from cheese whey powder solution fermentation. Appl Biochem Biotechnol (2008).

Junter, G.A., and Jouenne, T. Immobilized viable microbial cells: from the process to the proteome...or the cart before the horse. Biotechnol. Adv. 22 (2004): 633-658.

Kourkoutasa, Y., Bekatoroua, A., Banatb, I.M., Marchantb, R., and Koutinasa, A.A. Immobilization technologies and support materials suitable in alcohol beverages production: a review. Food Microbio. 21 (2004): 377-397.

Lark, N., Xia, YK., Qin, CG., Gong, CS., and Tsao, GT. Production of ethanol from recycled paper sludge using cellulose and yeast, *Kluyveromyces marxianus*. Bioomass Bioenergy. 12 (1997): 135-143.

Limtong, S., Sringiew, C., and Yongmanitchai, W. Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromyces marxianus*. Bioresource Technology. 98 (2007): 3367-3374.

Najafpour, G., Younesi, H., and Ku Ismail, KS. Ethanol fermentation in an immobilized cell reactor using *Saccharomyces cerevisiae*. Bioresource Technology. 92 (2004): 251-260.

Nffiez, M.J., and Lema, J.M. Cell immobilization: Application to alcohol production. Enzyme Microbio. Technol. 9 (1987): 642-651.

Nonklang, S., Abdel-Banat, Babiker M.A., Cha-aim, K., Moonjai, N., Hoshida, H., Limtong, S., Yamada, M., and Akada, R. High-Temperature ethanol fermentation and transformation with linear DNA in the thermotolerant yeast *Kluyveromyces marxianus* DMKU3-1042. Applied and environmental Microbiology. 24 (2008): 7514-7521.

Packed bed reactor [Online]. Available from: http://www.lsbu.ac.uk [2006, December 11].

Phisalaphong, M., Budiraharjo, R., Bangrak, P., Mongkolkajit, J., and Limtong, S. Alginate-Loofa as Carrier Matrix for Ethanol Production. Journal of Bioscience and Bioengineering. 3 (2007): 214-217.

Phisalaphong, M., Srirattana, N., and Tanthapanichakoon, W. Mathematical modeling to investigate temperature effect on kinetic parameters of ethanol fermentation. Biochemical Engineering Journal. 28 (2006): 36-43.

Ramakrishna, S.V., and Prakasham, R.S. Microbial fermentations with immobilized cells [Online]. Available from: http://tejas.serc.iisc.ernet.in/ currsci/jul10/ articles17.htm [2006, December 11].

Sánchez, O.J., and Cardona, C.A. Biotechnological production of fuel alcohol I: production from different feedstocks. Interciencia. 30 (2005): 671-678.

Singh, D., Banat, IM., Nigam, P., and Marchant, R. Industrial scale ethanol production using the thermotolerant yeast *Kluyveromyces marxianus* IMB3 in an Indian distillery. Biotechnol Lett. 20 (1998): 753-755.

Valach, M., Navrátil, M., Horváthová, V., Zigová, J., Šturdík, E., Hrabárová, E., and Gemeiner, P. Efficiency of a fixed-bed and gas-lift three-column reactor for continuous production of ethanol by pectate- and alginate-immobilized *Saccharomyces cerevisiae* cells. Chem. Pap. 60 (2006): 154-159.

Veliky, I.A., and Mclean, R.J.C. Immobilized biosystems theory and practical application. 1st ed. Great Britain: Galliard (Printers), 1994.

Verbelen, P.J., De Schutter, D.P., Delvaux, F., Verstrepen, K.J., and Delvaux, F.R. Immobilized yeast cell systems for continuous fermentation applications. Biotechnol Lett. 28 (2006): 1515–1525.

Vijaya Sarathi Reddy, O., and Basappa, S.C. Direct fermentation of cassava starch to ethanol by mixed cultures of *Endomycopsis fibuligera* and *Zymomonas mobilis*: synergism and limitations. Biotechnology Lett. 18 (1996): 1315-1318.

Wang, S.J., and Zhong, J.J. Bioreactor Engineering, Bioprocessing for Value-Added Products from Renewable Resources. (2007): 131-161.

Wilkins, M.R., Mueller, M., Eichling, S., and Banat, I.M. Fermentation of xylose by the thermotolerant yeast strains *Kluyveromyces marxianus* IMB2, IMB4, and IMB5 under anaerobic conditions. Process Biochemistry. 43 (2008): 346-350.

Yu, J., Zhang, X., and Tan, T. An novel immobilization method of *Saccharomyces cerevisiae* to sorghum bagasse for ethanol production. Journal of Biotechnology. 129 (2007): 415-420.

APPENDICES

APPENDIX A
EXPERIMENTAL METHODS

A-1 Agar slants preparation

In this study, Potato Dextrose Agar (PDA) was used as medium for stock cultures. For sterilization, TOMY SS-325 autoclave was used. The preparation steps of PDA agar slants in details are:

1. Mix 7.8 g PDA powder with 200 ml de-ionized (DI) water in 500 ml glass beaker.
2. Stir the solution with magnetic stirrer and heat it up until it is boiling.
3. Boil the solution for 1 minute or until all powder is dissolved as indicated by the formation of clear yellowish agar solution.
4. Transfer 4 ml agar solution into 16 x 150 mm screw cap culture tube by using 10 ml pipette.
5. Sterilize all agar containing tubes at 121°C for 15 minutes in autoclave. (Set the tube's cap to be rather loose before autoclaving to facilitate gas expansion inside the tube during sterilization.)
6. After sterilization, tighten the tube's cap and let the tubes to cool down before positioning them in slanted position to obtain agar slant inside the tubes.
7. Precautions:
    a) PDA agar powder is hygroscopic. Minimize exposure time of the powder to the ambient air to avoid excess water absorption.
    b) Sterilization is carried out at high temperature. Wear heat resistant gloves as protection when handling hot materials.
    c) When slanting the agar, provide enough space between tube neck and agar to minimize the risk of contamination from outside the tube.

A-2 Stock cultures preparation

Stock cultures were prepared by aseptic inoculation of the flocculating yeast *S. cerevisiae* M30 and *K. marxianus* DMKU 3-1042 on the PDA agar slants. The procedures are as follows:

1. Sterilize all equipments and agar slants with ultraviolet (UV) light with air flow for about 1 hour in the ISSCO VS-124 laminar flow hood.
2. After the UV lamp is turned off, clean all apparatus and the hood's compartment with alcohol 70% v/v solution to ensure asepticity.
3. Open the caps of source culture and fresh agar tubes then heat up the tubes' neck with an alcohol burner.
4. Heat up the inoculation loop thoroughly until it reds up.
5. Cool down the loop by contacting with fresh medium.
6. Transfer the yeast cells from source culture to fresh agar slant. Inoculate the cells on fresh agar by zigzag movement.
7. Heat the tube neck again before securing the cap.
8. Repeat step 4-8 again for other fresh medium until sufficient amounts of stock cultures is obtained.
9. Leave the stock cultures to grow at room temperature for 20-24 hours before use.
10. Precautions:
    a) Be cautious with the UV light as it is harmful for human eyes and skin.
    b) Wear protective gloves during inoculation for safety and aseptic reasons.

A-3 Medium preparation

There were 2 varieties of medium used in this study. One was designated for cell cultivation and other was for ethanol production.

A-3.1 Preculture medium precaration

Palm sugar was designated for cell cultivation. The main component of the medium in earlier experiments (until fermentation 3) was palm sugar which was used as carbon and energy source for the yeast. Palm sugar was dissolved to obtain sugar concentration of about 100 g/l for cell cultivation. The resulting sugar solution had a

brown color originated from the palm sugar. The color intensity increases with increasing sugar concentration. The amount of palm sugar required to achieve the target level of sugar was estimated from previous trial with 3,5-dinitrosalicylic acid (DNS) method (Section A-7).

For 1 liter of sugar solution, nutrients consisted of 0.5 g KH_2PO_4, 1.5 g $MgSO_4.7H_2O$, and 0.5 g $(NH_4)_2SO_4$ were added Limtong et al. (2007). The compositions were referred to the one which were used by ethanol producing industries. The pH value of the medium was adjusted to 5 with 0.1 M NaOH and HCl solution. The detailed procedures for medium preparation from palm sugar are listed in the following paragraph.

1. Mix palm sugar and nutrients. Add palm sugar until the desired sugar concentration (100 g/l for cell cultivation) is achieved.
2. Adjust the pH of the solution to 5 by adding NaOH or HCl solution.
3. Pour appropriate volume of medium (100 ml and 250 ml for inoculums development and ethanol fermentation respectively) through a sieve or screen into 500 ml Erlenmeyer flask.
4. Close each flask with cotton plug and wrap with aluminum foil before sterilization.
5. Sterilize the mediums with autoclave for 20 min at 121°C.
6. Precautions and notes:

   a) Avoid wetting the flasks' neck when pouring the solution as the heated solution may act as adhesive so that the plug is difficult to be removed after sterilization.

   b) The pH of the solution may be quite altered after sterilization.

   c) Some precipitates may be formed after sterilization from the sugar solution.

A-3.2 Fermentation medium preparation

For 1 liter of fermentation medium consisted 0.5 g KH_2PO_4, 1.5 g $MgSO_4.7H_2O$, and 0.5 g $(NH_4)_2SO_4$ and 220 g sugar from sugar cane molasses. The quantity of molasses needed to reach the intended sugar level was also estimated by DNS trial. Sugar cane molasses was diluted to obtain sugar concentration of about

220 g/l for ethanol fermentation. The procedures for preparing molasses based fermentation medium are follows:

1. Dilute the molasses with DI water until the desired sugar concentration (220 g/l) is achieved.
2. Mix the diluted sugar solution with appropriate amount of KH_2PO_4, $MgSO_4.7H_2O$, $(NH_4)_2SO_4$.
3. Adjust the pH of to 5 by adding NaOH or HCl solution.
4. Fill 500 ml Erlenmeyer flask with 250 ml medium.
5. Close each flask with cotton plug before sterilization.
6. Autoclave the medium for 15 min at 121 °C.
7. Precautions and notes are same with palm sugar based medium preparation.

A-4 Cell cultivation and harvesting

Cell cultivation was initiated with the transfer of cells from stock culture tube aseptically to Erlenmeyer flask containing fresh medium by using Gilson Pipetman auto pipette. Thus, sterile pipette tips should be prepared in advance by autoclaving or dry heat in hot air oven. Active yeast cells with generation time (age) 20-24 hours were used for cultivation purpose. After inoculation, cell cultivation was carried out in Innova 4330 Refrigerated Incubator Shaker for 20-24 hours at 200 rpm. After some time, the growing yeast cells could be noticed as brown colored suspended solids inside the sugar solution. The cells were then harvested and concentrated by medium draining. The complete steps are as follows:

1. Sterilize equipments and the laminar flow hood with UV and by wiping with alcohol 70% v/v solution.
2. Heat up the neck of stock culture tube and medium flask after removing the tube cap and cotton plug.
3. Heat up the inoculation loop evenly and then slightly deep it into the fresh medium in the Erlenmeyer flask to cool it down before touching the yeast cells.
4. Scratch the yeast culture on the tube to detach the cells from the surface of the agar using the loop.

5. Transfer the cell at the loop into the Erlenmeyer flask and then close the flask using cotton plug.
6. Repeat steps 3-5 for the other flasks.
7. Put all flasks in the incubator shaker and then operate the shaker at 200 rpm 33°C for a day before harvesting the cells.
8. Let the cells to settle for a while after incubation and then carefully take out 130 ml of the medium from each flask by using 10 ml of auto pipette.
9. Combine the concentrated cells suspension from several flasks by pouring it into one flask.
10. Further draining can be done to concentrate cells by the same method until the desired volume of concentrated cells suspension is obtained.
11. For mix culture, the proportion *S. cerevisiae/K marxianus* was adjusted volumetric ratio of 1:1.
12. Precautions and notes:
    a) Except the stock culture and the fresh medium, all equipments should be cleaned and sterilized using UV light and alcohol to ensure asepticity.
    b) Clean the outer surface of the tubes and flasks using alcohol before use.
    c) Keep the tube neck and flask opening hot by regular heating after removal of the cap or plug to prevent contamination originated from ambient air.

A-5 Cell immobilization

Immobilization of cells was investigated using two general methods. The first was the adsorption of cells onto the surface of thin shell silk cocoon. Thin shell silk cocoon and palm sugar medium were sterilized with autoclave for 15 minutes at 121°C prior to usage. Preparations of TSSC carrier were listed in the following paragraph.

1. Mix 10 ml of concentrated cell suspension with 250 ml of palm sugar medium.
2. Add the thin shell silk cocoon in the cell-mixture.
3. Incubated suspension mixture for 20-24 hours.
4. Precautions and notes:
    a) All procedures are conducted aseptically in laminar flow hood.
    b) All equipments are cleaned and sterilized before use.

The second was entrapment of cells in Alginate loofa matrix. To the entrapment of cells in Alginate loofa matrix, Alginate solution was made by dissolving Na-alginate powder in NaCl 9 g/l solution to obtain clear viscous solution with concentration of 30 g/l. Loofa sponge and alginate solution used for entrapment of cells were sterilized with autoclave for 5 minutes at 121°C prior to usage. Alginate solution was mixed first with concentrated cell suspension before gelation. Formation procedures of alginate-loofa were listed in the following paragraph.

i. Mix concentrated cell suspension with alginate solution with volumetric ratio of 1:10.
ii. Add the mixture using a syringe drop wisely into $CaCl_2$ 14.7 g/l solution to form alginate beads.
iii. For reinforced gel formation, dip the loofa sponge into alginate solution and then drop it into the $CaCl_2$ solution.
iv. Leave the gel to harden with mild stirring for 15 minutes.
v. Rinse the gel 3 times with NaCl 9 g/l solution.
vi. When storing is needed, keep the gels in NaCl 9 g/l solution at 4°C.
vii. Precautions and notes:
    1. All procedures are conducted aseptically in laminar flow hood.
    2. All equipments including the stirrer and syringe are cleaned and sterilized before use.

A-6 Ethanol fermentation

A-6.1 Batch fermentation

Sugar cane molasses was used as carbon source for the fermentation medium. The medium contained initial sugar concentration of 220 g/l, 0.05% w/v KH_2PO_4, 0.15% w/v $MgSO_4.7H_2O$, 0.05% w/v $(NH_4)_2SO_4$ and the initial pH was adjusted at 5.0. The volume of medium was adjusted to 250 ml in 500 ml Erlenmeyer flask in order to promote anaerobic condition which was favorable ethanol fermentation by yeast. Batch fermentation in shake flasks was performed in Innova 4330 Refrigerated Incubator Shaker (New Brunswick Scientific, USA) at 200 rpm, 33, 37, 40 and 45°C for 72 hours.

A-6.2 Continuous fermentation

The reactor column was sterilized by circulation of 70% v/v ethanol for 1 hour and then was kept under UV light overnight. The column was packed by immobilized cell (TSSC carrier) with working volume around 0.7 L. Temperature of the system is controlled at 37°C. The sterile molasses solution with the initial sugar concentration of 220 g/l and the initial pH at 5.0 was fed to the bottom of the fermentor continuously by means of a peristaltic pump through sterile silicon tubing. Effluent liquid overflowed from an outlet port at the top of the column, maintaining a constant level of fermentation broth in the column. The temperatures inside diameter of the column 5 position are measured by temperature sensors, which will recorded every 8 hours. The samples were harvested with volume of 5 ml every 8 hours from the 5^{th} port of the column.

A-7 Sugar analysis

Sugar (sucrose) concentration was determined using a modified DNS reagent method. All disaccharides in the samples and standard sucrose solutions were first hydrolyzed to their monomers by using acid solution at elevated temperature. The acid residue was then neutralized using a basic solution and the resulting precipitates were settled by centrifugation. After centrifugation, the supernatant was reacted with DNS reagent at high temperature resulting in the formation of brown colored solution. The solution was then diluted before being analyzed by using spectrophotometer. The absorbance of the sample was compared with standard sucrose solutions to obtain the corresponding sucrose concentration. Complete step by step procedures are provided in the following sections. The residual glucose concentration was also determined by the glucose analyzer YSI.

A-7.1 NaOH and HCl solution preparation

NaOH 20% w/v was prepared by dissolving 20 g of NaOH pellets in 100 mL of water. The reaction is highly exothermic so that the preparation should be done in water bath in order to avoid excess heat generation. Weighing time of NaOH pellets should be minimized because of the hygroscopic nature of NaOH. Solution of 37% w/v HCl was obtained by diluting concentrated HCl solution with DI water. Beware

of the acid vapor and wear protective gloves when preparing the solutions. Commercially available HCl 37% can be also be used directly.

A-7.2 DNS reagent preparation

DNS powder is toxic and easy to airborne so that it should be handled with caution. This powder should be added slowly in the mixing process because it is not easy to dissolve. After preparation, the resulting yellow colored reagent is best used in fresh condition so that it is not suggested to keep unused for long time (more than 1 month). The reagent is usually kept in brown bottle to protect it from degradation originated from light for example sun light. The complete preparation steps are:

1. Dissolve 1.633 g NaOH 98% w/w in 20 ml of water. Mix the solution with magnetic stirrer.
2. Under stirring, slowly add 1 g of 3,5-dinitrosalicylic acid powder into the solution.
3. Dilute by adding 50 ml of water. Stir until it is homogeneous.
4. Add 30 g Na-K tartrate & mix it thoroughly.
5. Adjust the volume to 100 ml.
6. Keep the reagent for 3 days before use.

A-7.3 Standard sucrose solution preparation

Standard sucrose solutions were prepared first by making the source solution which was the solution with the highest sucrose concentration as the upper limit. The source solution was then diluted with water so that a set of standard solution with increasing sucrose concentration (for instance 0, 6.25, 12.5, 18.75, and 25% w/v) was obtained. The detailed procedures are as follows:

1. Dry 3.0 g sucrose at 100-105°C in hot air oven for 2 hours.
2. Put the dried sucrose in desiccator for cooling.
3. Dissolve 2.5 g of the sucrose in 10 ml of water to obtain the source solution.
4. Prepare each 2 ml standard solution in small labeled bottle by serial dilution of suitable amount of source solution and diluting it with water as shown in detail in Table A-7. Use auto pipette for the transfer purpose.

Table A-7.3 Standard sucrose solution preparation

Sucrose concentration (% w/v)	Source solution (ml)	Water (ml)
0	0	2.0
6.25	0.5	1.5
12.50	1.0	1.0
18.75	1.5	0.5
25.00	2.0	0

A-7.4 Sample treatment I

In the first treatment, sample was hydrolyzed using HCl 37% in boiled water bath. After the hydrolysis reaction was stopped, NaOH was added into the solution. The sample was then centrifuged for removing suspended solids. Procedures of the first treatment are:

1. Mix 0.2 ml of sample with 0.8 ml DI water in screw cap tube.
2. Blend the sample with 0.5 ml HCl 37%.
3. Put the tubes in boiling water bath for 10 minutes.
4. Stop the reaction by placing the tubes in ice bath.
5. Add 0.5 ml NaOH 20% w/v and then mix with vortex mixer.
6. Add 10 ml DI water and then mix with vortex mixer.
7. Centrifuge the sample at 2000 rpm for 20 minutes.
8. Precautions and notes:
   a) Use vortex mixer for mixing the fluid in the tubes.
   b) Be cautious when handling the hot apparatus.
   c) The level of boiled water and ice bath must be sufficiently higher than the liquid level in the tubes to ensure good heating and cooling of the sample.

A-7.5 Sample treatment II

In treatment II, reducing sugar and residual glucose concentration were analyzed from supernatant obtained from treatment I. Supernatant obtained from treatment I was analyzed residual sugar concentration directly by the glucose analyzer (YSI). The reducing sugar concentration reacted with DNS reagent in boiled water

bath. The solution's color transformed from yellow to reddish brown in the course of reaction. The color intensity represents the corresponding sugar concentration. Solution with higher sugar content will have darker color. After the reaction was ended, the solution was diluted with sufficient amount of water until its absorbance spectrum obtained by spectrophotometer was well distributed along the range of concentration being considered (the absorbance measured was not more 0.7). Shimadzu UV-2450 UV-Visible spectrophotometer was used for absorbance measurement. Sample containing only water (0% sugar) which had been treated in the same manner as the other samples was used as blank. At every absorbance measurement, fresh standard solution should be used. Complete procedures are described in the following paragraph.

1. Mix 0.2 ml of supernatant obtained from treatment I with 1.0 ml DNS reagent in screw cap tube.
2. Boil the solution for 10 minutes using water bath.
3. Put the tubes in ice bath to stop the reaction.
4. Add 10 ml DI water and then mix with vortex mixer.
5. Measure the absorbance at 520 nm. Use sample with 0% sugar as blank.
6. Obtain the standard curve by plotting absorbance versus sucrose concentration of standard sucrose solution.
7. Use the standard curve to gain sugar concentration of the samples.

A-8. Dry weight cell

A-8.1 Free cell concentration

Cell concentration was determined by separation of cell from its medium followed by dry weight cell. Dry weight of cell concentration was determined by separating the cells from their suspending liquid medium by centrifugation. The cells were then dried and their weight was measured as the representative of their concentration in the initial suspension. The procedures are:

1. Centrifuge the cell containing medium at 2000 rpm for 15 minutes.
2. Remove the supernatant (discarded or to be used for other analysis).
3. Add HCl 0.1 N to the cell pellet and mix with vortex mixer.

4. Centrifuge the suspension at 2000 rpm for 15 minutes.
5. Discard the supernatant.
6. Disperse the cell pellet with DI water.
7. Repeat step 4-6.
8. Transfer the cell suspension to a pre-weighted aluminum dish.
9. Dry the cell in hot air oven at 100°C for 24 hours.
10. Measure the weight of the cells.
11. Precautions and notes:
    a) The cells cake is fragile. Pour out all of the supernatant in one cycle instead of several cycles.
    b) Dry and measure the weight of aluminum dishes before use.
    c) The dry weight of the cells is obtained as the difference between the weight of the aluminum dish which contains cells and the weight of empty dish.

A-8.2 Immobilized cell concentration

Before the cell concentration could be measured, a measured amount of carrier should be dissolved to obtain cell suspension. The dissolution of TSSC was carried out using water. The thin shell silk cocoon was removed from the suspension after the gel was dissolved. The cells suspension was then treated with the same procedures as for free cells suspension in order to obtain its corresponding immobilized cell concentration. The complete procedures are as follows:

1. Cut the TSSC carrier in to the small size.
2. Dissolve appropriate amount of TSSC carrier with 10 ml water in 25 ml beaker.
3. Stir TSSC carrier in the beaker with magnetic stirrer for 30 minutes.
4. Remove the TSSC carrier from the suspension and continue with same procedures as step 2-9 of Section A-8.2.

APPENDIX B

EXPERIMENTAL DATA

B-1 Experimental data of suspension cell in batch fermentation

Table B-1.1 Data of batch fermentation of ethanol production in sugar cane juice by SS, SK and SM system at 33 ° C.

Time	Ethanol concentration (g/l)			Residual sugar concentration (g/l)		
(hours)	SS	SK	SM	SS	SK	SM
0	0.00	0.00	0.00	211.48	211.48	211.48
8	26.47	23.52	25.26	154.89	171.00	161.09
16	59.37	43.30	48.90	85.87	103.22	89.96
24	82.09	55.12	66.93	47.09	70.76	47.46
32	94.10	74.66	89.06	19.08	38.04	14.00
40	96.34	76.69	97.02	11.28	22.06	5.20
48	100.33	87.75	98.53	7.56	11.40	3.72
56	105.92	87.87	102.65	5.70	9.79	3.59
64	107.25	88.52	102.66	5.33	9.29	3.59
72	107.44	90.69	103.90	4.96	8.18	3.59

Table B-1.2 Data of batch fermentation of ethanol production in cane molasses by SS, SK and SM system at 33°C.

Time (hours)	Ethanol concentration (g/l)			Residual sugar concentration (g/l)		
	SS	SK	SM	SS	SK	SM
0	0.00	0.00	0.00	193.72	193.72	193.72
8	12.23	11.51	11.11	153.66	168.53	167.29
16	57.96	31.20	45.76	70.01	123.92	95.17
24	74.38	45.53	46.35	34.20	107.93	42.26
32	78.06	58.25	67.15	29.86	81.04	29.74
40	83.68	65.63	76.71	29.62	60.59	29.74
48	87.34	70.52	81.62	29.49	43.99	28.62
56	87.85	76.10	85.15	28.13	33.95	28.38
64	89.28	78.85	85.65	27.63	31.23	28.25
72	90.53	80.29	87.91	27.14	30.36	24.91

Table B-1.3 Data of batch fermentation of ethanol production in sugar cane juice by SS, SK, and SM system at 37 °C.

Time (hours)	Ethanol concentration (g/l)			Residual sugar concentration (g/l)		
	SS	SK	SM	SS	SK	SM
0	0.00	0.00	0.00	221.58	221.58	221.58
8	11.78	9.80	10.98	166.27	178.68	165.89
16	30.47	32.51	27.44	107.93	139.55	111.38
24	46.78	37.70	50.13	71.66	106.49	70.93
32	52.51	45.76	52.79	53.17	85.96	54.55
40	54.43	50.97	56.58	45.97	69.92	37.38
48	58.24	55.53	63.14	44.73	62.03	35.85
56	60.08	57.79	67.94	44.43	56.31	34.40
64	61.40	60.85	70.84	44.28	55.65	33.74
72	64.90	63.94	74.88	43.33	55.14	33.59

Table B-1.4 Data of batch fermentation of ethanol production in cane molasses by SS, SK and SM system at 37°C.

Time	Ethanol concentration (g/l)			Residual sugar concentration (g/l)		
(hours)	SS	SK	SM	SS	SK	SM
0	0.00	0.00	0.00	183.91	183.91	183.91
8	17.14	8.00	14.10	108.55	153.10	122.62
16	44.66	17.39	38.54	40.14	113.10	49.93
24	63.36	31.09	56.49	16.14	89.52	21.79
32	73.78	47.17	71.00	13.10	77.38	14.21
40	74.43	50.18	72.77	13.24	62.62	13.93
48	76.87	51.42	72.98	12.41	54.62	13.79
56	77.12	52.35	73.45	11.86	54.34	12.97
64	77.85	54.38	75.17	11.59	53.24	12.83
72	79.27	54.51	76.89	11.45	52.83	12.83

Table B-1.5 Data of batch fermentation of ethanol production in sugar cane juice by SS, SK and SM system at 40 ° C.

Time	Ethanol concentration (g/l)			Residual sugar concentration (g/l)		
(hours)	SS	SK	SM	SS	SK	SM
0	0.00	0.00	0.00	222.37	222.37	222.37
24	28.21	49.33	52.45	142.49	86.40	95.60
48	49.44	65.90	63.51	80.31	47.80	52.46
72	46.09	66.36	66.17	79.15	47.15	50.78

Table B-1.6 Data of batch fermentation of ethanol production in cane molasses by SS, SK and SM system at 40°C.

Time	Ethanol concentration (g/l)			Residual sugar concentration (g/l)		
(hours)	SS	SK	SM	SS	SK	SM
0	0.00	0.00	0.00	185.23	185.23	185.23
24	14.32	23.22	28.72	130.83	111.53	89.51
48	45.54	36.83	48.20	43.39	93.01	31.35
72	43.29	32.58	51.97	37.18	91.19	31.09

Table B-1.7 Data of batch fermentation of ethanol production in sugar cane juice by SS, SK and SM system at 45 ° C.

Time	Ethanol concentration (g/l)			Residual sugar concentration (g/l)		
(hours)	SS	SK	SM	SS	SK	SM
0	0.00	0.00	0.00	220.56	220.56	220.56
8	0.08	18.89	17.02	221.47	175.27	175.27
16	0.09	40.17	39.77	221.47	122.01	126.36
24	0.09	55.26	57.80	220.11	79.35	80.84
32	0.11	59.80	58.64	220.11	68.89	71.06
40	0.14	60.56	59.14	218.75	68.21	69.57
48	0.15	60.74	59.79	217.39	65.90	70.11
56	0.29	62.29	60.57	209.24	67.80	70.52
64	0.34	63.21	61.61	206.52	66.30	69.29
72	0.36	63.67	62.34	205.16	65.90	66.71

Table B-1.8 Data of batch fermentation of ethanol production in cane molasses by SS, SK, and SM system at 45 ° C.

Time	Ethanol concentration (g/l)			Residual sugar concentration (g/l)		
(hours)	SS	SK	SM	SS	SK	SM
0	0.00	0.00	0.00	186.59	186.59	186.59
8	0.30	5.23	7.41	180.71	171.20	165.76
16	0.76	16.59	21.48	179.35	158.97	148.10
24	0.93	25.84	31.17	180.71	131.39	132.88
32	0.98	32.91	36.98	177.99	120.92	123.51
40	1.07	34.67	38.26	179.35	114.54	126.22
48	1.40	34.67	38.81	176.63	116.98	125.14
56	1.49	37.94	38.99	173.91	117.93	124.46
64	2.74	38.40	39.04	171.20	118.75	123.91
72	3.47	38.67	39.74	169.84	114.13	118.48

B-2 Experimental data of immobilized cell in batch fermentation

Table B-2.1 Data of ethanol production from sugar cane juice by immobilized cell in batch fermentation of AL and TSSC carrier at 37°C

Time (hours)	Ethanol concentration (g/l)				Residual sugar concentration (g/l)			
	Mixed-AL	Mixed-TSSC	K-AL	K-TSSC	Mixed-AL	Mixed-TSSC	K-AL	K-TSSC
0	0.00	0.00	0.00	0.00	216.34	216.34	216.34	216.34
8	7.25	25.15	6.75	22.25	170.65	128.49	171.66	136.12
16	32.05	38.70	29.52	46.54	107.81	74.58	113.85	87.38
24	47.85	50.74	43.72	58.78	70.65	48.01	80.60	49.09
32	57.76	69.17	66.41	68.62	50.00	36.30	58.69	29.32
40	60.72	73.69	67.45	74.44	54.16	36.05	54.16	25.67
48	63.87	73.72	67.49	74.99	53.78	35.79	53.90	25.16
56	67.01	74.02	69.35	76.58	53.78	35.67	53.65	25.16
64	68.65	77.79	69.75	76.72	53.65	35.54	53.53	24.41
72	70.24	78.91	70.04	79.79	53.27	34.91	53.53	24.41

Table B-2.2 Data of ethanol production from cane molasses by immobilized cell in batch fermentation of AL and TSSC carrier at 37°C

Time (hours)	Ethanol concentration (g/l)				Residual sugar concentration (g/l)			
	Mixed-AL	Mixed-TSSC	K-AL	K-TSSC	Mixed-AL	Mixed-TSSC	K-AL	K-TSSC
0	0.00	0.00	0.00	0.00	177.62	177.62	177.62	177.62
8	1.89	5.81	2.04	3.06	170.03	155.14	122.92	147.75
16	26.05	36.51	13.08	18.53	132.24	88.01	83.28	99.05
24	48.83	57.05	31.97	31.87	72.29	43.68	52.21	82.46
32	66.92	69.16	35.91	37.25	34.76	20.00	44.18	75.53
40	70.60	75.10	36.19	36.52	29.60	7.41	30.05	68.12
48	70.65	78.79	46.80	40.18	28.59	7.28	17.52	57.09
56	79.02	80.56	48.75	43.40	28.46	7.15	10.39	52.25
64	79.29	80.60	50.14	43.75	28.46	6.90	4.74	51.17
72	80.35	80.65	56.08	45.02	26.45	6.65	3.73	50.97

Table B-2.3 Data of ethanol production from sugar cane juice by immobilized cell in batch fermentation of AL and TSSC carrier at 40°C

Time (hours)	Ethanol concentration (g/l)				Residual sugar concentration (g/l)			
	Mixed-AL	Mixed-TSSC	K-AL	K-TSSC	Mixed-AL	Mixed-TSSC	K-AL	K-TSSC
0	0.00	0.00	0.00	0.00	214.59	214.59	214.59	214.59
8	1.72	16.69	0.35	13.25	176.25	144.64	192.06	172.29
16	28.46	56.62	24.02	40.33	103.78	53.86	126.18	104.04
24	44.32	55.85	38.48	40.70	65.18	44.90	87.44	60.30
32	50.95	70.82	57.10	67.27	42.38	29.09	55.43	39.49
40	59.81	71.85	63.73	70.46	33.16	25.67	36.19	34.87
48	61.78	71.98	63.50	72.65	33.03	25.67	33.03	34.87
56	62.42	73.14	63.55	73.46	32.90	25.53	33.03	34.61
64	64.30	75.66	64.00	74.98	32.77	25.40	33.03	34.35
72	66.49	76.70	66.34	78.18	32.77	25.40	32.24	34.08

Table B-2.4 Data of ethanol production from cane molasses by immobilized cell in batch fermentation of AL and TSSC carrier at 40°C

Time (hours)	Ethanol concentration (g/l)				Residual sugar concentration (g/l)			
	Mixed-AL	Mixed-TSSC	K-AL	K-TSSC	Mixed-AL	Mixed-TSSC	K-AL	K-TSSC
0	0.00	0.00	0.00	0.00	186.47	191.28	186.47	186.47
8	0.13	2.86	0.30	7.77	162.29	180.74	172.83	158.88
16	14.35	24.45	3.91	31.67	126.46	153.07	154.39	118.17
24	39.71	40.52	29.05	47.39	77.18	100.37	114.47	81.81
32	55.85	60.60	45.01	62.71	47.67	58.21	72.70	47.55
40	56.80	69.74	57.59	66.97	41.21	44.90	45.56	35.03
48	57.36	70.03	58.25	67.26	41.08	44.11	41.74	34.64
56	57.74	70.62	60.31	68.25	40.82	43.98	41.48	34.51
64	60.50	70.81	62.44	68.74	40.55	43.72	40.82	34.51
72	60.64	71.84	64.67	69.11	40.29	43.45	40.29	33.98

B-3 Experimental data of continuous fermentation: non-control temperature

Table B-3.1 Experimental data of ethanol and residual sugar concentration in packed bed reactor of TSSC carrier with dilution rate of 0.10, 0.21, 0.30 and 0.41 h^{-1} for cane molasses medium, and with dilution rate 0.10, 0.21 and 0.30 h^{-1} for sugar cane juice medium.

Dilution rate (h^{-1})	Time (hours)	Sugar concentration (g/l)	Ethanol concentration (g/l)	$Y_{P/S}$ (%)
Feedstock :Cane molasses				
0.10	0	222.22	0.00	
0.10	8	107.71	21.99	0.19
0.10	16	50.59	50.24	0.29
0.10	24	34.25	64.54	0.34
0.10	32	29.15	68.63	0.36
0.10	40	29.54	68.78	0.36
0.10	48	22.48	67.97	0.34
0.10	56	29.15	72.56	0.38
0.10	64	29.80	71.75	0.37
0.10	72	29.02	71.12	0.37
0.10	80	31.24	71.87	0.38
0.10	88	32.16	71.33	0.38
0.10	96	31.63	67.35	0.35
0.21	104	58.56	63.06	0.39
0.21	112	65.88	48.11	0.31
0.21	120	52.03	59.30	0.35
0.21	128	52.81	60.72	0.36
0.21	136	55.82	64.23	0.39
0.21	144	50.85	64.38	0.38
0.21	152	53.20	63.06	0.37
0.21	160	55.03	63.16	0.38
0.21	168	55.16	65.20	0.39
0.21	176	59.61	55.66	0.34
0.30	184	75.82	48.10	0.33
0.30	192	74.90	47.49	0.32
0.30	200	74.25	49.44	0.33
0.30	208	72.81	51.07	0.34
0.30	216	68.50	48.27	0.31
0.30	224	70.59	50.75	0.34

Dilution rate (h^{-1})	Time (hours)	Sugar concentration (g/l)	Ethanol concentration (g/l)	$Y_{P/S}$ (%)
0.30	232	73.86	48.69	0.33
0.30	240	73.46	49.50	0.33
0.30	248	75.56	50.60	0.35
0.30	256	76.34	48.13	0.33
0.41	264	91.63	42.71	0.33
0.41	272	94.25	40.79	0.32
0.41	280	89.93	44.64	0.34
0.41	288	90.33	46.07	0.35
0.41	296	85.49	47.29	0.35
0.41	304	82.22	46.26	0.33
0.41	312	83.66	44.64	0.32
0.41	320	83.92	44.75	0.32
0.41	328	85.75	50.84	0.37
0.41	336	85.65	50.92	0.37
Feedstock :Sugar cane juice				
0.10	344	10.85	76.75	0.36
0.10	352	7.58	79.83	0.37
0.10	360	7.71	85.29	0.40
0.10	368	5.23	85.50	0.39
0.10	376	4.71	85.77	0.39
0.10	384	3.01	88.38	0.40
0.10	392	4.31	84.75	0.39
0.10	400	5.88	86.19	0.40
0.10	408	6.14	85.37	0.40
0.10	416	11.90	87.97	0.42
0.10	424	12.42	87.72	0.42
0.10	432	13.73	90.87	0.44
0.10	440	16.99	86.09	0.42
0.21	448	50.98	60.30	0.35
0.21	456	50.59	61.67	0.36
0.21	464	49.67	63.11	0.37
0.21	472	49.02	66.38	0.38
0.21	480	44.44	67.94	0.38
0.21	488	35.29	70.87	0.38
0.21	496	35.16	71.16	0.38
0.21	504	33.46	70.54	0.37
0.21	512	32.68	70.62	0.37
0.21	520	33.33	71.23	0.38

Dilution rate (h^{-1})	Time (hours)	Sugar concentration (g/l)	Ethanol concentration (g/l)	$Y_{P/S}$ (%)
0.30	528	68.37	53.62	0.35
0.30	536	67.97	53.76	0.35
0.30	544	65.36	56.07	0.36
0.30	552	63.14	57.83	0.36
0.30	560	73.59	54.85	0.37
0.30	568	63.27	55.96	0.35
0.30	576	69.54	57.65	0.38
0.30	584	73.20	50.35	0.34
0.30	592	71.24	52.83	0.35
0.30	600	72.55	52.96	0.35

Table B-3.2 Data of free cells concentration leaving the reactor.

Dilution rate (h^{-1})	Time (hours)	Free cell concentration (g/l)
Feedstock :Cane molasses		
0.10	0	0.20
0.10	8	0.20
0.10	16	0.20
0.10	24	0.40
0.10	32	0.45
0.10	40	1.00
0.10	48	1.10
0.10	56	1.15
0.10	64	1.00
0.10	72	1.40
0.10	80	1.40
0.10	88	1.40
0.10	96	1.40
0.21	104	1.40
0.21	112	1.40
0.21	120	1.40
0.21	128	1.60
0.21	136	1.80
0.21	144	2.00
0.21	152	2.10

Dilution rate (h^{-1})	Time (hours)	Free cell concentration (g/l)
0.21	160	2.00
0.21	168	2.15
0.21	176	2.13
0.30	184	2.10
0.30	192	2.20
0.30	200	2.10
0.30	208	1.80
0.30	216	2.00
0.30	224	2.00
0.30	232	2.00
0.30	240	2.00
0.30	248	2.00
0.30	256	1.80
0.41	264	4.20
0.41	272	4.50
0.41	280	5.20
0.41	288	5.00
0.41	296	6.60
0.41	304	5.40
0.41	312	6.20
0.41	320	6.40
0.41	328	4.80
0.41	336	6.00
Feedstock :Sugar cane juice		
0.10	344	3.20
0.10	352	3.60
0.10	360	3.20
0.10	368	4.00
0.10	376	4.00
0.10	384	2.20
0.10	392	3.20
0.10	400	2.60

Dilution rate (h^{-1})	Time (hours)	Free cell concentration (g/l)
0.10	408	2.20
0.10	416	3.80
0.10	424	1.80
0.10	432	2.80
0.10	440	2.40
0.21	448	1.80
0.21	456	2.60
0.21	464	2.80
0.21	472	2.00
0.21	480	3.20
0.21	488	2.60
0.21	496	2.00
0.21	504	2.80
0.21	512	2.80
0.21	520	2.20
0.30	528	2.20
0.30	536	2.60
0.30	544	3.00
0.30	552	2.80
0.30	560	3.40
0.30	568	4.40
0.30	576	3.00
0.30	584	3.40
0.30	592	3.80
0.30	600	4.00

Table B-3.3 Experimental data of ethanol productivity average at steady state in packed bed reactor of TSSC carrier with dilution rate of 0.10, 0.21, 0.30 and 0.41 h^{-1}.

Dilution rate (h^{-1})	Ethanol concentration (g/l)	Residual sugar Concentration (g/l)	$Y_{P/S}$ (%)	Productivity (g/l h)
Cane molasses				
0.10	71.73 (± 0.56)	30.27 (± 1.37)	37.41	7.17 (± 0.06)
0.21	64.00 (± 0.90)	54.01 (± 2.02)	38.10	12.80 (± 0.18)
0.30	49.20 (± 1.27)	73.61 (± 2.45)	33.16	14.76 (± 0.38)
0.41	45.61 (± 1.10)	85.93 (± 3.42)	33.52	18.24 (± 0.44)
Sugar cane juice				
0.10	86.33 (± 1.34)	21.81 (± 3.30)	43.00	8.63 (± 0.13)
0.21	70.88 (± 0.31)	40.82 (± 1.17)	40.97	14.18 (± 0.06)
0.30	56.47 (± 1.25)	81.98 (± 4.51)	40.36	16.94 (± 0.38)

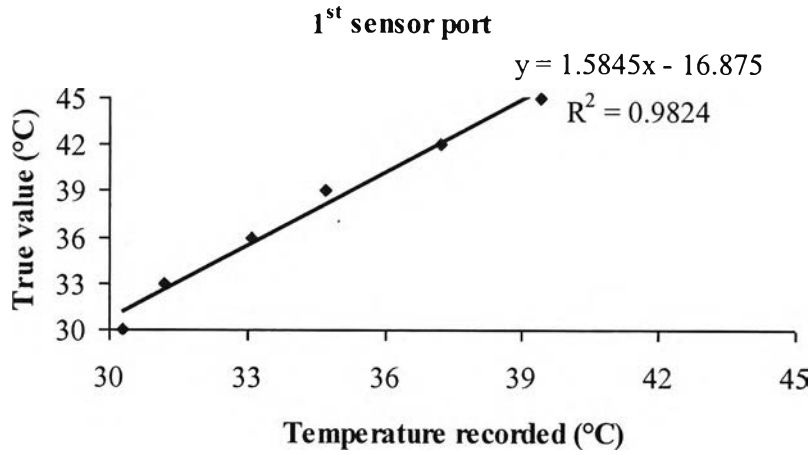


Figure B-3.1 Experimental data of standard curve for temperature; 1st sensor port

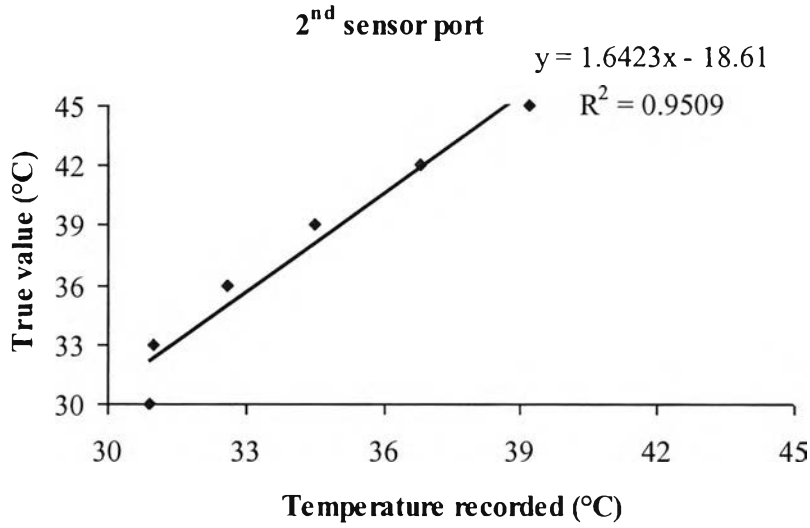


Figure B-3.2 Experimental data of standard curve for temperature; 2nd sensor port

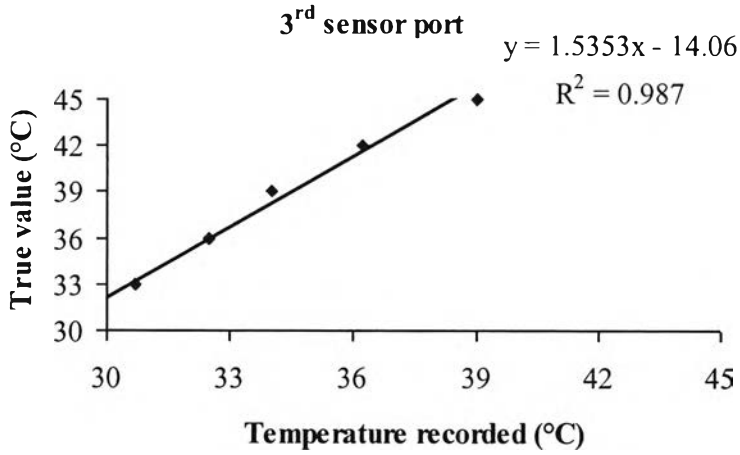


Figure B-3.3 Experimental data of standard curve for temperature; 3rd sensor port

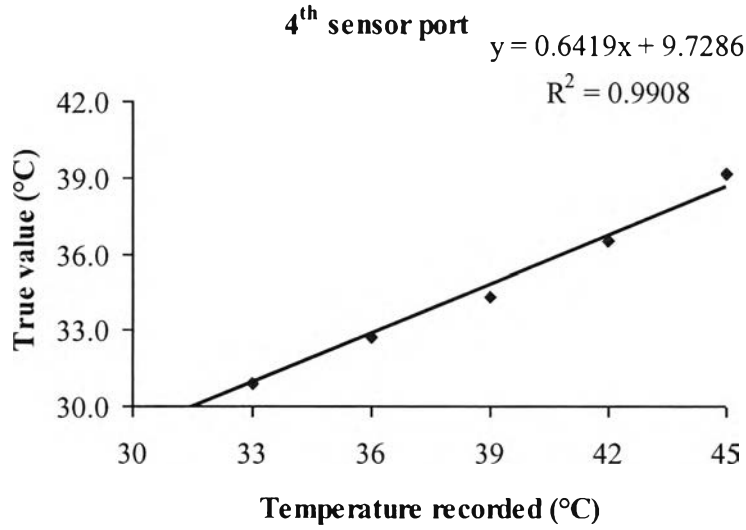


Figure B-3.4 Experimental data of standard curve for temperature; 4th sensor port

Table B-3.4 Experimental data of temperature profiles

Time (hours)	Dilution rate (h^{-1})	1st sensor port (°C)	2nd sensor port (°C)	3rd sensor port (°C)	4th sensor port (°C)	5th sensor port (°C)
0	0.10	31.45	36.41	33.5	37.37	31.23
8	0.10	33.20	33.94	35.0	34.15	29.95
16	0.10	32.40	35.26	35.8	35.22	30.46
24	0.10	30.82	33.12	34.5	33.38	29.63
32	0.10	31.29	31.97	33.9	32.46	29.24
40	0.10	31.93	33.12	34.6	33.53	29.69
48	0.10	32.24	33.62	35.0	34.15	29.95
56	0.10	31.93	32.63	34.4	33.23	29.56
64	0.10	31.29	32.30	34.1	32.77	29.43
72	0.10	32.40	33.45	34.9	33.84	29.88
80	0.10	31.93	32.96	34.7	33.53	29.76
88	0.10	30.82	31.64	33.7	32.15	29.18
96	0.10	32.56	33.78	35.1	34.30	30.01

Time (hours)	Dilution rate (h^{-1})	1st sensor port (°C)	2nd sensor port (°C)	3rd sensor port (°C)	4th sensor port (°C)	5th sensor port (°C)
104	0.21	32.72	33.78	35.1	33.84	29.95
112	0.21	33.67	35.09	35.8	34.76	30.33
120	0.21	35.89	37.89	37.5	37.22	31.42
128	0.21	34.78	36.57	36.8	35.99	30.85
136	0.21	33.20	34.93	35.8	34.46	30.27
144	0.21	36.21	38.38	37.8	37.68	31.62
152	0.21	35.41	37.23	37.1	36.76	31.23
160	0.21	34.15	35.75	36.3	35.38	30.65
168	0.21	36.05	38.38	37.8	37.53	31.62
176	0.21	35.57	37.39	37.1	36.76	31.36
184	0.30	34.78	36.24	36.4	35.22	30.72
192	0.30	36.21	38.38	37.8	37.07	31.75
200	0.30	35.73	38.21	37.7	36.76	31.75
208	0.30	34.78	37.06	36.9	35.53	31.23
216	0.30	36.05	38.21	37.8	37.07	31.75
224	0.30	35.89	38.21	37.6	35.99	31.68
232	0.30	35.10	37.56	37.2	34.92	31.49
240	0.30	36.68	39.53	38.4	36.60	32.20
248	0.30	37.31	38.54	38.4	36.76	32.20
256	0.30	36.05	38.38	37.8	34.76	31.68
264	0.41	37.63	39.20	38.0	34.46	31.81
272	0.41	35.57	38.21	37.6	33.38	31.10
280	0.41	35.41	37.89	37.1	32.46	30.91
288	0.41	36.21	39.36	38.1	34.15	31.42
296	0.41	35.89	38.71	37.7	33.69	31.10
304	0.41	35.73	37.89	37.2	32.92	30.85
312	0.41	37.47	39.86	38.3	34.76	31.36
320	0.41	36.68	39.03	37.8	33.69	31.30
328	0.41	36.68	38.71	37.8	33.07	30.85

Time (hours)	Dilution rate (h^{-1})	1st sensor port (°C)	2nd sensor port (°C)	3rd sensor port (°C)	4th sensor port (°C)	5th sensor port (°C)
336	0.41	30.66	32.63	37.4	36.45	30.65
344	0.10	29.87	31.48	34.7	35.38	29.88
352	0.10	30.34	31.97	34.0	36.14	30.14
360	0.10	30.18	31.32	34.3	35.68	29.95
368	0.10	29.87	30.82	33.8	35.07	29.82
376	0.10	31.61	32.63	34.0	37.53	30.53
384	0.10	30.82	31.48	34.5	36.91	30.21
392	0.10	30.03	30.66	33.9	36.14	29.76
400	0.10	30.98	31.81	33.2	36.30	30.33
408	0.10	31.14	31.81	33.9	36.91	30.27
416	0.10	31.93	32.63	33.9	36.30	30.14
424	0.10	31.93	32.63	34.3	35.68	29.82
432	0.10	33.51	34.93	34.2	34.46	30.08
440	0.10	35.26	37.23	35.7	36.30	31.04
448	0.21	34.78	34.60	35.8	34.30	30.01
456	0.21	34.30	35.75	36.4	33.99	30.08
464	0.21	37.00	39.20	38.5	37.37	31.49
472	0.21	35.73	37.23	37.2	35.68	30.78
480	0.21	35.57	36.90	37.0	35.22	30.65
488	0.21	37.47	39.20	38.4	37.68	31.49
496	0.21	36.21	37.56	37.5	36.91	31.10
504	0.21	35.57	36.90	37.2	35.53	30.72
512	0.21	38.11	39.69	38.7	36.14	31.23
520	0.21	38.11	39.36	38.3	35.07	30.65
528	0.30	37.79	39.53	38.6	35.07	29.88
536	0.30	38.74	40.35	38.9	35.84	30.14
544	0.30	37.95	39.20	38.5	34.92	29.95
552	0.30	37.95	38.87	38.4	35.38	30.91
560	0.30	38.11	39.20	38.2	35.53	30.98

Time (hours)	Dilution rate (h^{-1})	1st sensor port (°C)	2nd sensor port (°C)	3rd sensor port (°C)	4th sensor port (°C)	5th sensor port (°C)
568	0.30	38.58	40.02	38.9	36.30	31.55
576	0.30	38.11	39.53	38.3	35.68	31.49
584	0.30	37.31	39.03	37.8	35.38	31.55
592	0.30	38.42	38.71	38.3	36.60	31.87
600	0.30	38.90	39.20	38.7	37.53	32.13

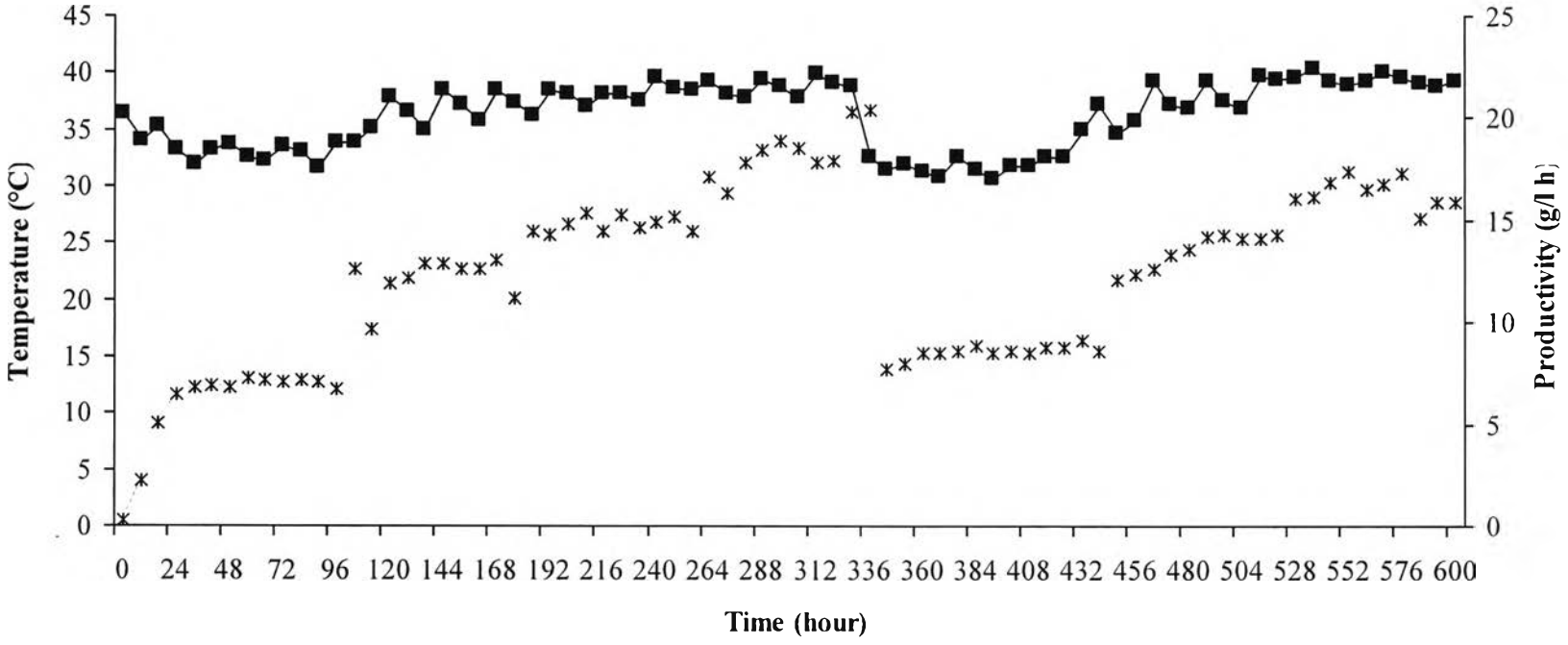


Figure B-3.5 Temperature profile at the 2nd sensor port as compared to productivity; ■, Temperature (°C) and *, Productivity (g/l h)

B-4 Experimental data of continuous fermentation: at 37 °C

Table B-4.1 Experimental data of ethanol and residual sugar concentration in packed bed reactor of TSSC carrier with dilution rate of 0.11, 0.18, 0.20, 0.30 and 0.40 h^{-1} for cane molasses medium.

Dilution rate (h^{-1})	Time (hours)	Ethanol Concentration (g/l)	Residual sugar Concentration (g/l)	$Y_{P/S}$ (%)
0.11	0	0	221.35	
0.11	8	27.25	148.97	37.65
0.11	16	43.03	47.59	24.76
0.11	24	54.20	36.83	29.37
0.11	32	54.79	40.97	30.37
0.11	40	52.12	41.66	29.01
0.11	48	54.01	44.41	30.52
0.11	56	52.85	48.14	30.51
0.11	64	52.89	46.62	30.27
0.11	72	52.88	44.69	29.93
0.11	80	52.88	50.48	30.95
0.11	88	50.79	46.07	28.98
0.11	96	51.15	60.12	31.72
0.18	104	46.67	59.31	28.80
0.18	112	36.93	90.07	28.13
0.18	120	36.32	90.07	27.67
0.18	128	35.66	78.07	24.89
0.18	136	40.32	73.38	27.25
0.18	144	40.62	71.45	27.10
0.18	152	38.82	73.79	26.31
0.18	160	38.65	71.45	25.78
0.18	168	36.49	78.48	25.54
0.20	176	47.85	54.48	28.68
0.20	184	39.19	74.90	26.76

Dilution rate (h^{-1})	Time (hours)	Ethanol Concentration (g/l)	Residual sugar Concentration (g/l)	$Y_{P/S}$ (%)
0.20	192	42.33	63.31	26.78
0.20	200	42.57	60.14	26.41
0.20	208	53.21	43.03	29.84
0.20	216	58.34	38.62	31.93
0.20	224	44.93	50.12	26.24
0.20	232	48.40	56.69	29.39
0.20	240	54.37	49.38	31.62
0.30	248	57.92	52.28	34.26
0.30	256	41.12	74.62	28.02
0.30	264	49.02	63.59	31.07
0.30	272	46.66	57.10	28.41
0.30	280	46.69	61.79	29.26
0.30	288	46.69	65.38	29.94
0.30	296	46.65	67.31	30.28
0.30	304	43.24	73.79	29.30
0.30	312	41.47	69.10	27.24
0.40	320	38.37	80.41	27.22
0.40	328	37.35	76.14	25.72
0.40	336	33.46	85.52	24.63
0.40	344	34.80	84.94	25.51
0.40	352	35.99	71.03	23.94
0.40	360	27.26	105.38	23.51
0.40	368	27.26	98.35	22.16
0.40	376	30.25	91.03	23.21
0.40	384	30.65	88.00	22.98
0.11	408	53.21	36.55	28.79
0.11	432	40.91	54.76	24.56
0.11	456	32.32	70.07	21.36
0.11	480	30.81	78.76	21.61
0.11	504	37.93	64.41	24.17

Dilution rate (h^{-1})	Time (hours)	Ethanol Concentration (g/l)	Residual sugar Concentration (g/l)	$Y_{P/S}$ (%)
0.11	528	38.04	54.62	22.82
0.11	552	44.83	54.62	26.89
0.11	576	37.13	61.52	23.23
0.11	600	57.71	38.48	31.56
0.11	624	54.64	35.31	29.37
0.11	648	57.27	34.76	30.69
0.11	672	54.09	36.14	29.20
0.11	696	54.01	20.12	26.84

Table B-4.2 Data of free cells concentration leaving the reactor.

Dilution rate (h^{-1})	Time (hour)	Free cell concentration (g/l)
0.11	0	0.00
0.11	8	1.40
0.11	16	1.40
0.11	24	1.00
0.11	32	1.40
0.11	40	1.40
0.11	48	1.40
0.11	56	1.80
0.11	64	1.40
0.11	72	1.60
0.11	80	1.60
0.11	88	1.40
0.11	96	1.40
0.18	104	1.20
0.18	112	1.80
0.18	120	1.40
0.18	128	1.20
0.18	136	1.60
0.18	144	0.80
0.18	152	1.00
0.18	160	1.00
0.18	168	0.60

Dilution rate (h^{-1})	Time (hour)	Free cell concentration (g/l)
0.20	176	0.80
0.20	184	0.40
0.20	192	1.20
0.20	200	1.00
0.20	208	1.10
0.20	216	1.20
0.20	224	1.40
0.20	232	1.00
0.20	240	0.60
0.30	248	1.00
0.30	256	0.60
0.30	264	0.60
0.30	272	0.80
0.30	280	0.80
0.30	288	0.40
0.30	296	0.40
0.30	304	1.40
0.30	312	1.20
0.40	320	1.20
0.40	328	1.00
0.40	336	1.00
0.40	344	1.00
0.40	352	1.00
0.40	360	1.50
0.40	368	1.60
0.40	376	1.40
0.40	384	1.00
0.11	408	1.00
0.11	432	1.20
0.11	456	1.20
0.11	480	1.00
0.11	504	1.30

Dilution rate (h^{-1})	Time (hour)	Free cell concentration (g/l)
0.11	528	1.20
0.11	552	1.00
0.11	576	1.10
0.11	600	1.10
0.11	624	1.50
0.11	648	1.30
0.11	672	1.20
0.11	696	1.40

Table B-4.3 Experimental data of ethanol productivity in packed bed reactor of TSSC carrier with dilution rate of 0.11, 0.18, 0.20, 0.30 and 0.40 h^{-1}, at temperature of 37°C

Dilution rate (h^{-1})	Ethanol concentration (g/l)	Productivity (g/l)
0.11	53.33 (± 0.90)	5.82 (± 0.00)
0.18	39.60 (± 1.01)	7.13 (± 0.18)
0.20	55.78 (± 3.63)	10.37 (± 1.05)
0.30	46.67 (± 0.02)	14.14 (± 0.31)
0.40	28.86 (± 1.85)	11.54 (± 0.74)

; Ethanol concentration was calculated from the average at steady state.

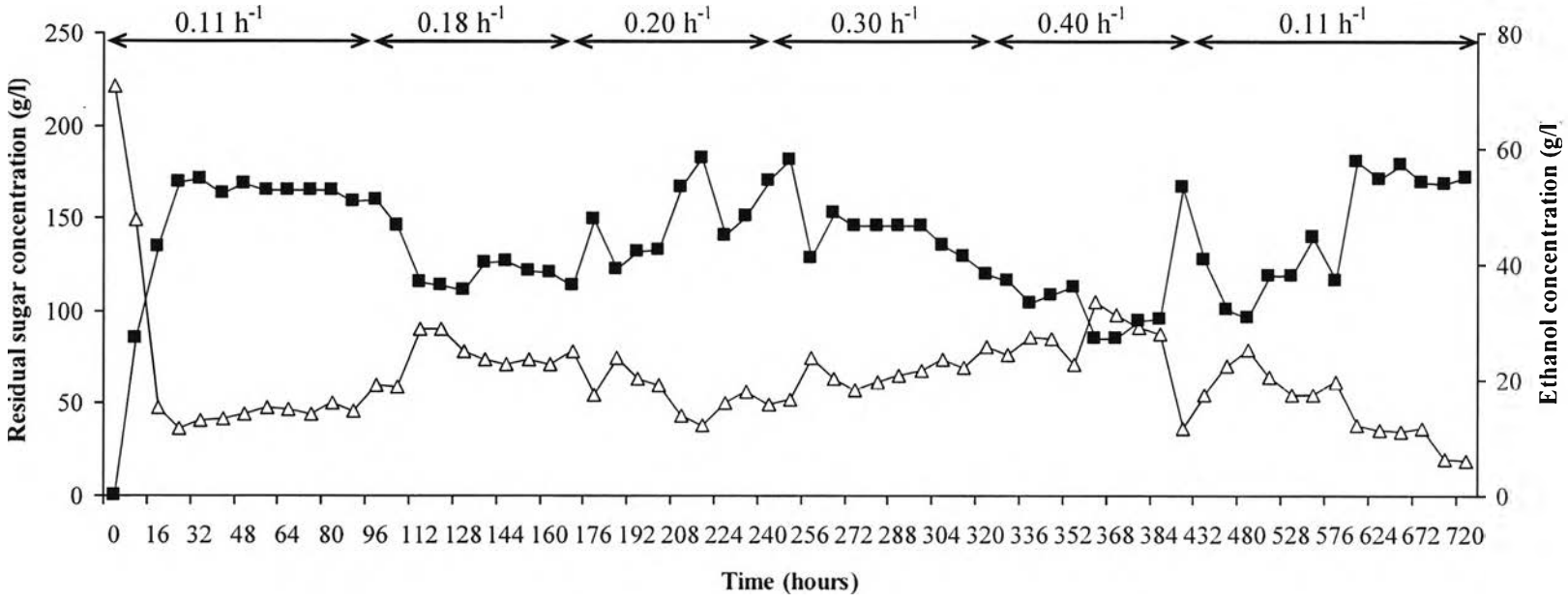


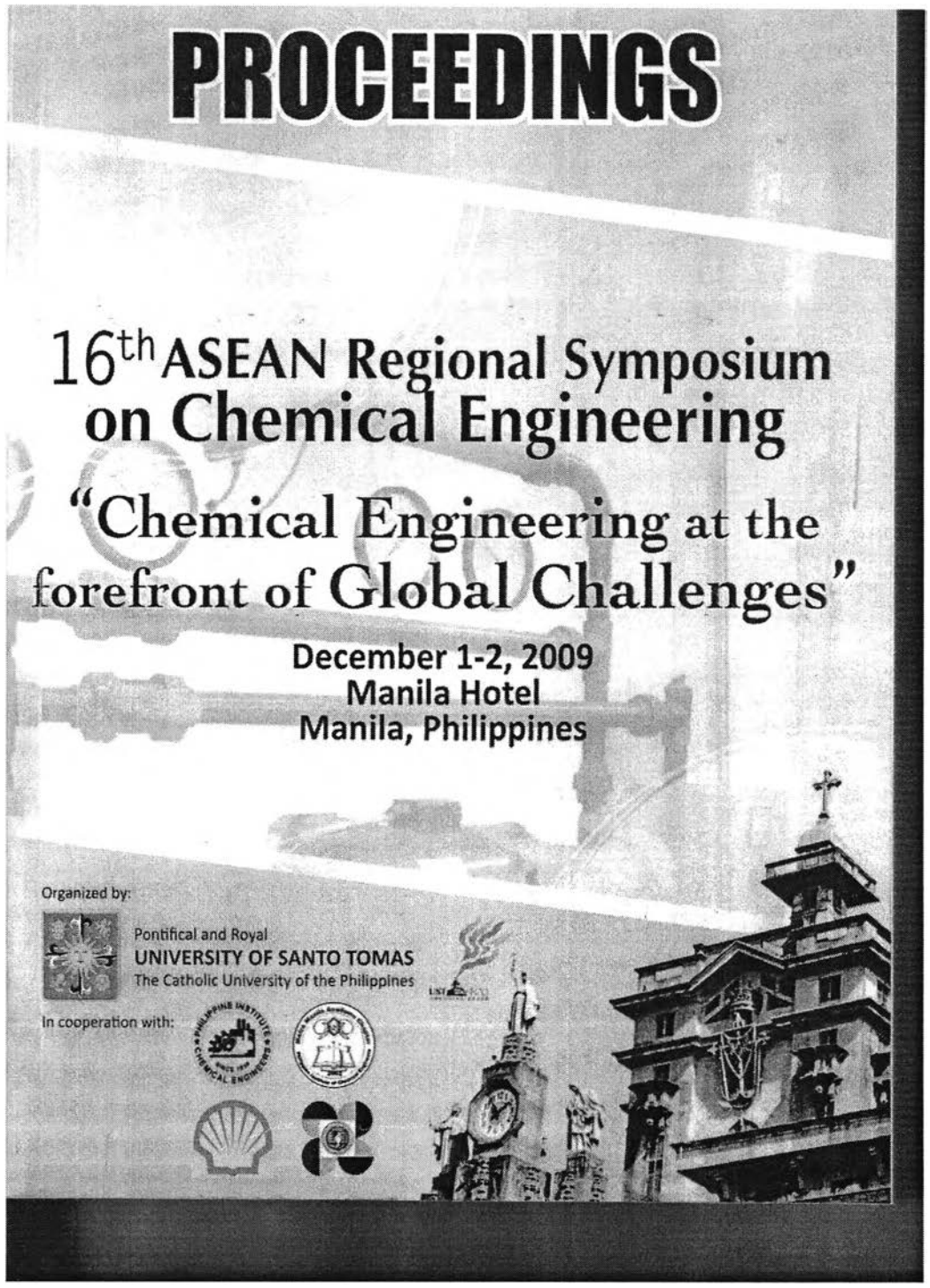
Figure B-4.1 Continuous fermentation in a PBR at dilution rate of 0.11, 0.18, 0.20, 0.30 and 0.40 h^{-1} ;
Δ, Residual sugar concentration and ■ , Ethanol concentration

APPENDIX C
LIST OF PUBLICATION

International conference

1. Akekasit Eiadpum, Anuchit Rattanapan, Jirawan Mongkolkajit, Savitree Limtong and Muenduen Phisalaphong, "Ethanol productivity from sugar cane juice and cane molasses by mixed cultures of *Kluyveromyces marxianus* DMKU 3-1042 and *Saccharomyces cerevisiae* M30", Proceeding for The 16th ASEAN Regional Symposium on Chemical Engineering "Chemical Engineering at the forefront of Global Challenges", December 1 – 2, 2009 Manila Hotel, Manila, Philippines, Paper Code 3Pro-Biotech7.
   *He has received finalist in poster competition and won as second place.

2. Akekasit Eiadpum, Anuchit Rattanapan, Jirawan Mongkolkajit, Savitree Limtong and Muenduen Phisalaphong, "Ethanol productivity from sugar cane juice by immobilized *Kluyveromyces marxianus* DMKU 3-1042", Proceeding for ChemBiotech'09-10, January 28th - 29th, 2010 Department of Chemical & Biomolecolar Engineering , National University of Singapore,


PROCEEDINGS
16th ASEAN Regional Symposium on Chemical Engineering
"Chemical Engineering at the forefront of Global Challenges"
December 1-2, 2009
Manila Hotel
Manila, Philippines
Organized by:
Pontifical and Royal
UNIVERSITY OF SANTO TOMAS
The Catholic University of the Philippines
In cooperation with:

Pontifical and Royal

UNIVERSITY OF SANTO TOMAS

The Catholic University of the Philippines

CERTIFICATE

This is to certify that

AKEKASIT EIADPHUM, ANUCHIT RATTANAPAN, JIRAWAN MONGKOLKAJIT, SAVITREE LIMTONG and MUENDUEN PHISALAPHONG

"Ethanol productivity from sugarcane juice and cane molasses by mixed cultures of Kluyveromyces marxianus DMKU 3-1042 and Saccharomyces cerevisiae M30"

has participated as

FINALIST in poster competition and won as SECOND PLACE

in the

16th ASEAN Regional Symposium on Chemical Engineering (RSCE 2009)

with the theme *"Chemical Engineering at the Forefront of Global Challenges"*

held on 01 – 02 December 2009 at the Manila Hotel

and the University of Santo Tomas, Manila Philippines

Engr. Cezar S. de la Cruz
National President,
PIChE

Prof. Philipina A. Marcelo, Ph.D.
16th ASEAN RSCE Secretariat
Chair, Technical Committee

Prof. Maria Natalia R. Dimaano, Ph.D.
Chair
16th ASEAN RSCE

16TH ASEAN REGIONAL SYMPOSIUM ON CHEMICAL ENGINEERING
December 1-2, 2009, Manila Hotel, Philippines 3Pro-Biotech7

Ethanol Productivity from Sugarcane Juice and Cane Molasses by Mixed Cultures of *Kluyveromyces marxianus* DMKU 3-1042 and *Saccharomyces cerevisiae* M30

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ABSTRACT: In this study, ethanol production by the mixed cultures of *Kluyveromyces marxianus* DMKU 3-1042 and *Saccharomyces Cerevisiae* M 30 was compared to the single cultures. Sugarcane juice and cane molasses were used as a carbon and energy source for the fermentation at the initial sugar concentration of 220 g/l. Ethanol production were carried out in shaking flasks at 200 rpm at 37, 40 and 45°C, for 72 hours. *S. cerevisiae* M 30 is well-established fermenting strain for high ethanol production from molasses at 30-35°C but its growth and activities were suppressed at temperatures >40°C. *K. marxianus* DMKU 3-1042 was found to be the better strain for high-temperature (40- 45 °C) especially in the sugarcane juice medium. It was found that the mixed culture manifested the highest activity for ethanol production for all conditions. After the fermentation, the ethanol production from molasses using the mixed cultures at temperature of 37, 40 and 45°C were 76.9, 51.2 and 39.7 g/L, respectively whereas, the ethanol production from sugarcane juice were 56.7, 66.2 and 62.3 g/L, respectively.

Keywords: ethanol production, mixed cultures, sugarcane, molasses

INTRODUCTION

Fossil fuels reserves are rapidly diminishing. The fuel sources to replace fossil fuels must be renewable, sustainable, efficient, cost-effective, convenient and safe (Najafpour et al., 2004). Therefore, ethanol industry has grown dramatically in the last few years for the supply of ethanol as the clean renewable fuel. However, in Thailand, ethanol production by yeast fermentation in hot climates has generally been found uneconomic without government subsidy. This is because of the high energy input required to maintain the fermentation process temperature between 25°C and 35°C to maximize ethanol production (Banat et al., 1992). Ethanol fermentation at high temperature has received much attention for effective ethanol production in topical counties where average day-time temperatures are usually high throughout the year (Limtong et al., 2007). The advantages associated with the production of ethanol at high temperature include increased rate of productivity, reduced cooling costs, reduced risk of contamination and suitability for use in tropical countries. The well-known yeast producers, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are candidates for ethanol production. *Saccharomyces cerevisiae* has an advantage for its tolerant to high sugar and ethanol concentration. It has previously been demonstrated that *Saccharomyces cerevisiae* M30 is capable of producing ethanol at temperature ranging from 30 to 33°C when grows on media containing cane molasses (Phisalaphong et al., 2006). Recently it has been demonstrated that the thermotolerant yeast strain *Kluyveromyces marxianus* DMKU 3-1042 was an effective strain that could be employed for ethanol production at elevated temperature up to 45 °C when sugar cane juice was used as a raw material (Limtong et al., 2007). In order to improve the ethanol fermentation process, the use of a mixed culture of these strains, *S. cerevisiae* M30 and *K. marxianus* DMKU 3-1042 is applied in this study. The batch fermentation by the mixed suspension culture is evaluated using shake flasks.

MATERIALS AND METHODS

Microorganisms

K.marxianus DMKU 3-1042 and *S.cerevisiae* M30 strains were kindly provided by Assoc. Prof. Dr. Savitree Limthong, (Department of Microbiology, Kasetsart University, Bangkok).

Culture media and cell preparation

Starter culture were prepared by transferring cells from stock PDA slants to 100 ml of sterilized medium followed by incubation at 37, 40, and 45°C, 200 rpm for 24 h. The cultivation medium for the starter culture contained 10% w/v sugar from palm sugar, 0.05%w/v $(NH_4)_2SO_4$, 0.05%w/v KH_2PO_4, and 0.15%w/v $MgSO_4.7H_2O$. The initial pH of the medium was adjusted at 5.0. After that, the obtained cell suspension was concentrated by decantation and then transferred to the main culture.

Batch fermentation

Sugarcane juice and cane molasses were used as carbon source for the fermentation medium. The medium contained initial sugar concentration of 220g/l, 0.05%w/v $(NH_4)_2SO_4$, 0.05%w/v KH_2PO_4, and 0.15%w/v $MgSO_4.7H_2O$ and the initial pH was adjusted at 5.0. Experiments were initialed by transferring prepared cell suspension into 500 ml Erlenmeyer flasks containing 250 ml of the fermentation medium in order to promote anaerobic condition which was favorable ethanol fermentation. Fermentation flasks were then shaken in the incubator at 200 rpm. The experiments were carried out for 72 hour in isothermal conditions at 37, 40 and 45°C and monitored by removing 5 ml samples every 8 hour for cell, sugar and ethanol analyses.

Analytical Methods

For cell dry weight determination; 5 ml sample of the fermentation broth was centrifuged at 20,000 rpm for 15 min.

The cell pellet was resuspended in 0.1N HCl and washed twice with distilled water and then dried at 90°C for 24 h and then weighted. The residual glucose concentration was determined by the YSI (Model 2700 SELECT Biochemistry Analyzer, Yellow Springs, Ohio 45387 USA). Concentrations of ethanol were determined by gas chromatography using a Shimadzu Model GC 7 AG equipped with a flame ionization detector. A column with length of 2 m, outer diameter of 3.3 mm, and packed with Porapak Q 80-100 mesh was used with N2 as carrier gas. Flow rate of N2 is 50 ml/min. The oven and detector temperature are 190°C and 240°C respectively. The samples are injected with volume of 1µL and injection temperature of 240°C.

RESULTS AND DISCUSSION

Batch fermentation in shake flasks for ethanol production was carried out in duplicate for 72 h at constant temperatures of 37, 40 and 45°C. The effect of temperature on ethanol fermentation in a basal sugar cane juice and cane molasses medium by *S.cerevisiae*, *K.marxianus* and the mixed culture of *S.cerevisiae* and *K.marxianus*, was investigated. Fig. 1 demonstrates the experimental results at the end of the fermentation, the ethanol concentrations from sugar cane juice medium using the mixed culture at constant temperature of 37, 40 and 45°C were 56.72, 66.17 and 62.34 g/l respectively, which were higher or equivalent in comparison to those of *S.cerevisiae* or *K.marxianus* monoculture system. Moreover, the residual sugar concentrations from the systems using the mixed culture were lower than those of the monoculture systems. The ethanol concentrations by the mixed culture system using cane molasses medium were 76.89, 51.97 and 39.74 at constant temperature of 37, 40 and 45°C respectively, which were higher or at least combatable to those of the monoculture systems.

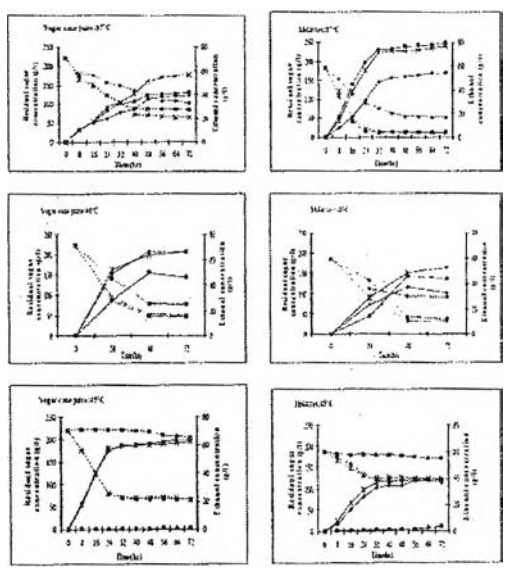

Figure 1. Ethanol and residual sugar concentration during the fermentation S.cerevisiae M30 (■), K.marxianus DMKU 3-1042 (♦) and mixed culture of S.cerevisiae and K.marxianus (×) using sugar cane juice or cane molasses medium by reciprocating flask cultivation at 37, 40 and 45°C ; the residual sugar concentration (----), Ethanol concentration (—). The proportion of S.cerevisiae and K.marxianus in mixed culture was 1:1.

The summary of ethanol fermentations of the mixed culture and the monocultures in sugar cane juice and cane molasses medium is shown in **Table 1.** The results demonstrated that ethanol fermentation by using the mixed culture system achieved the highest production rate under various controlled conditions regarding the final ethanol yield and the residual sugar concentration. Moreover, the mixed culture offered some important advantages such as high stability under wide operating temperature control range and high fermentation efficiency on various substrates.

Table 1. Ethanol fermentation by *S. cerevisiae* M.30, *K. marxianus* DMKU 3-1042 and the mixed culture of *S. cerevisiae* and *K. marxianus* in sugar cane juice and cane molasses medium at various temperatures for 72 hour.

System	37°C		40°C		45°C	
	Ethanol (g/l)	Conversion yield (%)	Ethanol (g/l)	Conversion yield (%)	Ethanol (g/l)	Conversion yield (%)
Sugar cane juice						
S.cerevisiae	42.30	31.90	45.09	36.22	9.36	1.03
K.marxianus	39.66	32.33	66.36	30.51	65.67	41.56
S.cerevisiae-*K.marxianus*	56.72	33.76	66.17	44.11	62.34	45.22
Cane Molasses						
S.cerevisiae	79.27	48.00	35.54	33.49	3.47	0.82
K.marxianus	51.51	30.90	56.83	17.44	38.67	40.50
S.cerevisiae-*K.marxianus*	76.89	46.45	51.97	37.37	39.74	46.77

CONCLUSION/S AND RECOMMENDATION/S

The mixed culture of *S.cerevisiae and K.marxianus* exhibited highly desirable properties in ethanol fermentation under various controlled conditions. The mixed culture system achieves higher ethanol productivity with the higher conversion yield than those from the fermentations by the monocultures. The high temperature stability of the mixed culture in sugar cane juice and cane molasses medium was observed.

ACKNOWLEDGEMENTS

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REFERENCES

Abate, C., Callieri, D., Rodrý´guez, E., & Garro, O. (1996). Ethanol production by mixed culture of flocculents strains of *Zymomonas mobilis* and *Saccharomyces* sp: *Appl Microbiol Biotechnol, 45*, 580-583.

Allain, Eric J. (2007). Cell-free ethanol production: the future of fuel ethanol? : A literature review. *J Chem Technol Biotechnol, 82*, 117-120.

Bai, F.W., Anderson, W.A., & Moo-Young, W. (2008). Ethanol fermentation technologies from sugar and starch feedstocks: *Biotech. Adv, 26*, 89-105.

Banat, I.M., Nigam, P., & Marchant, R. (1992). Isolation of thermotolerant, fermentative yeasts growing at 52°C and producing ethanol at 45°C and 50°C: *World Journal of Microbiology and Biotechnology, 8*, 259-263.

Cáceres-Farfán, M., Lappe, P., Larqué-Saavedra, A., Magdub-Méndez, A., & Barahona-Pérez, L. (2008). Ethanol production from henequen (*Agave fourcroydes* Lem.) juice and molasses by mixture of two yeasts: *Bioresource Technology, 99*, 9036-9039.

Chum, L.H., & Overend, R.P. (2001). Biomass and renewable fuels: *Bioprocess Technol, 17*, 187-195.

Dursun, A.Y., & Tepe, O. (2005). Internal mass transfer effect on biodegradation of phenol by Ca-alginate immobilized *Ralstonia eutropha*: *J. Hazardous Materials, 126*, 105–111.

Kourkoutasa, Y., Bekatoroua, A., Banath, I.M., Marchanth, R., & Koutinasa, A.A. (2004). Immobilization technologies and support materials suitable in alcohol beverages production: a literature review. *Food*

Microbio, 21, 377-397. Limtong, S., Sringiew, C., & Yongmanitchai, W. (2007). Production of fuel ethanol at high temperature from

sugar cane juice by a newly isolated *Kluyveromyces marxianus*: *Bioresource Technology, 98*, 3367-3374.

Gough, S., and McHale, A.P. (1998). Continuous ethanol production from molasses at 45°C using alginateimmobilized

Kluyveromyces marxianus IMB3 in a continuous-flow bioreactor: *Bioprocess Engineering, 19*, 33-36.

Guo, X., Zhou, J., & Xiao, D. (2008). Improved Ethanol production by mixed immobilized cells of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* from cheese whey powder solution fermentation: *Appl Biochem Biotechnol.*

Najafpour, G., Younesi, H., & Ku Ismail, K.I. (2004). Ethanol fermentation in an immobilized cell reactor using *Saccharomyces cerevisiae*: *Bioresource Technology, 92*, 251-260.

Nonklang, S., Abdel-Banat, Babiker M.A., Cha-aim, K., Moonjai, N., Hoshida, H., Limtong, S., Yamada, M., & Akada, R. (2008). High-Temperature ethanol fermentation and transformation with linear DNA in the thermotolerant yeast *Kluyveromyces marxianus* DMKU3-1042: *Applied and Enevironmental Microbiology, 24*, 7514-7521.

Paul Peiris & Nan Fu. (2008). Co-fermentation of a mixure og glucose and xylose to ethanol by *Zymomonasmobilis* and *Pachysolen tannophilus*. *World J Microbiol Biotechnol, 24*, 1091-1097.

Phisalaphong, M., Srirattana, N., & Tanthapanichakoon, W. (2006). Mathematical modeling to investigate temperature effect on kinetic parameters of ethanol fermentation: *Biochemical Engineering Journal, 28*, 36-43.

Wilkins, M.R., Mueller, M., Eichling, S., & Banat, I.M. (2008). Fermentation of xylose by the thermotolerant yeast strains *Kluyveromyces marxianus* IMB2, IMB4, and IMB5 under anaerobic conditions. *Process Biochemistry, 43*, 346-350.

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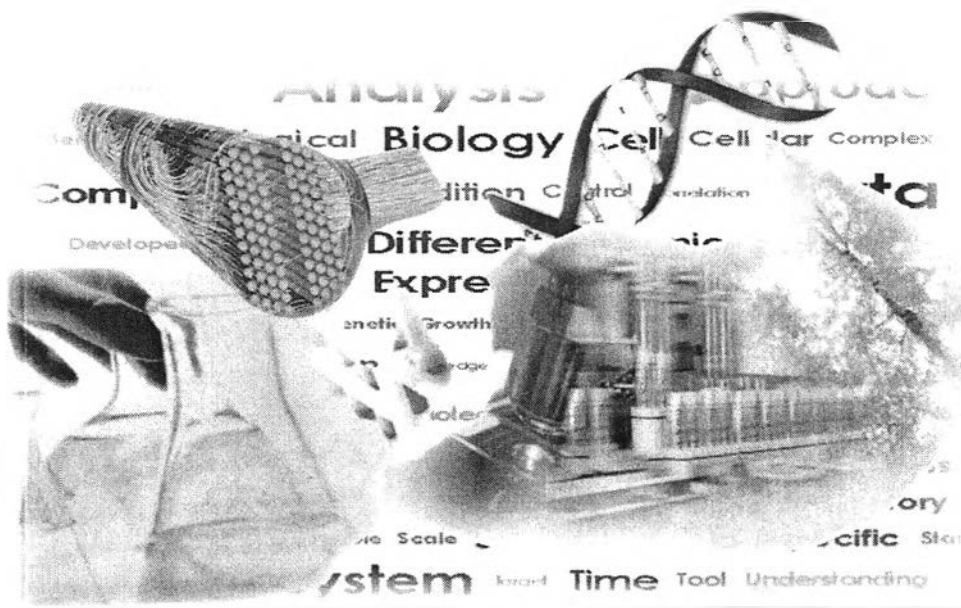

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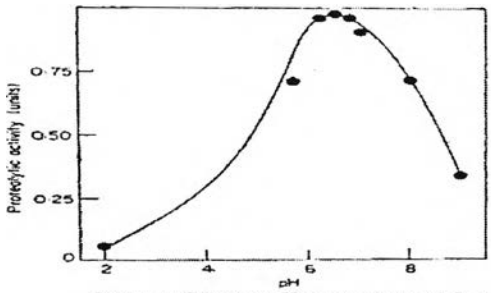

Figure 1: Effect of pH on enzyme activity

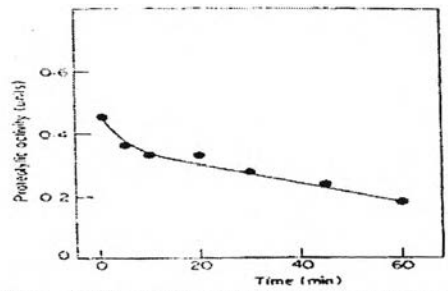

Figure 2: Effect of heat treatment on enzyme activity

Keywords: Protease, Neem leaves

Ethanol productivity from sugar cane juice by immobilized kluyveromyces marxianus dmku 3-1042

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Ethanol fermentation at high temperature has received much attention for effective ethanol production in tropical countries where average day-time temperatures are usually high throughout the year (Limtong *et al.*, 2007). The advantages associated with the production of ethanol at high temperature include increased rate of productivity, reduced cooling costs, reduced risk of contamination and stability for use in tropical countries. To achieve high temperature

fermentation, it is necessary to use an efficient yeast strain that can tolerate high temperature. The *Kluyveromyces marxianus* DMKU 3-1042 is an effective strain that could be employed for ethanol production at elevated temperature (Limtong *et al.*, 2007). On the other hand, ethanol production using an immobilized cell system offers many advantages such as higher productivity and protection of cells from inhibitions. In the present work, we selected ethanol production by batch fermentation using *Kluyveromyces marxianus* DMKU 3-1042 immobilized within the alginate-loofa matrix, which was then examined and compared with that using suspended cell.

In these studies, the system of cell immobilization by entrapment in alginate-loofa was compared with the systems of free cells. The fermentation was carried out with 220 g/l of initial sugar concentration from sugar cane juice as a carbon source for *K. marxianus* DMKU 3-1042. Batch fermentations were performed in the shaking incubator at 200 rpm, at constant temperature of 37°C and 40°C for 72 hours. The results of the fermentation are summarized in Table 1.

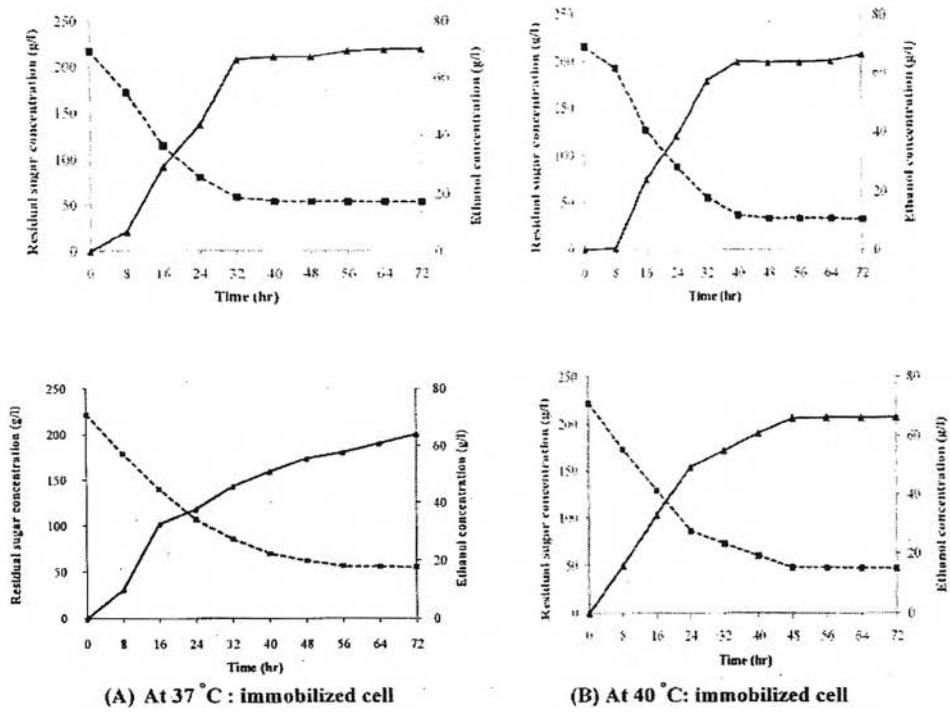
Table 1. Yield and end products of batch ethanol fermentation for 72 hr using the cultures of suspended cells (SC) and Aginate-loofa-matrix-immobilized cells (ALM)

Temperature	Culture system	P(g/l)	X_F	X_I	$Y_{P/S}$ (g/g)
37°C	SC	63.9	3.4	-	0.38
	ALM	70.0	2.7	4.8	0.43
40°C	SC	66.4	8.8	-	0.38
	ALM	66.3	1.5	3.4	0.36

X_I is the immobilized cell concentration; X_F is the free cell concentration. Ethanol yield ($Y_{P/S}$) is the ratio of ethanol accumulation ($P-P_0$) to the sugar consumption (S_0-S).

The effect of temperature for systems of immobilized cell was studied by the comparison of the samples at 37 °C and 40°C. At the end of the fermentation, the ethanol concentrations were 70 and 66.3 g/l respectively, which were higher and equivalent in comparison to the free cells system (63.9 and 66.4 g/l); Moreover, the residual sugar concentration and the fermentation time were lower and shorter in the immobilized cell system than in the free cells system. As shown in

Figure 1, the concentration of sugar gradually decreased while the ethanol concentration increased for the duration of 72 hours. The final residual sugar concentration of the free cells and the immobilized cell system were 55.1, 47.2 and 53.5, 32.2 g/l, respectively. A comparison of experimental results showed that the sugar consumption and ethanol production trend of the immobilized cell system was initially lower than that of the free cells system for the duration of 24 hours but they become higher after that. On the other hand, the productivities of the immobilized cells system were stable in comparison to the free cells system after 48 hrs of fermentation. The cell activities of the immobilized cell system were better than the free cell system which might be attributable to the negative effect of high ethanol concentration on cell activity and viability in the free cell system.



(A) At 37 °C : immobilized cell

(B) At 40 °C: immobilized cell

Figure 1

Figure 1:

Ethanol and residual sugar concentration during the fermentation free cells of *K.marxianus* DMKU 3-1042 and immobilized cell of *K.marxianus* DMKU 3-1042 using sugar cane juice medium by reciprocating flask cultivation at 37 and 40 °C ; the residual sugar concentration (---), Ethanol concentration (—).

REFERENCES

Abate, C., Callieri, D., Rodrý'guez, E., & Garro, O. (1996). Ethanol production by mixed culture of flocculents strains of *Zymomonas mobilis* and *Saccharomyces* sp: *Appl Microbiol Biotechnol, 45*, 580-583.

Allain, Eric J. (2007). Cell-free ethanol production: the future of fuel ethanol? : A literature review. *J Chem Technol Biotechnol, 82*, 117-120.

Bai, F.W., Anderson, W.A., & Moo-Young, W. (2008). Ethanol fermentation technologies from sugar and starch feedstocks: *Biotech. Adv, 26*, 89-105.

Limtong, S., Sringiew, C., & Yongmanitchai, W. (2007). Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromyces marxianus*: *Bioresource Technology, 98*, 3367-3374.

Phisalaphong, M., Budiraharjo, R., & Bangrak, P. (2007). Alginate-Loofa as carrier matrix for ethanol production. *Journal of Bioscience and Bioengineering, 104*, 214-217.

Phisalaphong, M., Srirattana, N., & Tanthapanichakoon, W. (2006). Mathematical modeling to investigate temperature effect on kinetic parameters of ethanol fermentation: *Biochemical Engineering Journal, 28*, 36-43.

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