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CONTINUOUS-FLOW FERMENTATION OF SUGAR INTO ETHANOL USING IMMOBILIZED YEAST

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ABSTRACT

'Ipil-ipil' (*Leucaena leucocephala*) wood sawdust and shavings and kappa-carrageenan gel were used separately or in a mixture for immobilizing *Saccharomyces cerevisiae* BB 1945 and the immobilized yeast was utilized for the continuous-flow ethanol fermentation of sugarcane molasses. Kappa-carrageenan was the best carrier; at a yeast loading of 30 g/l gel a volumetric productivity of 11.5-16.6g ethanol per liter per hour was obtained during 67 days of continuous culture. Average values of fermentation efficiency and alcohol concentration in the 'beer' were 92% and 7.7% (w/v), respectively, at a fermenter residence time of six hours. Non-pasteurization of the molasses feed and the use of fertilizer grade ammonium phosphate as the only nutrient supplement reduced the ethanol productivity by less than 1 g/l-hr.

Banana pulp juice from ripe Cavendish banana fruits was extracted by either manual or mechanical pressing. The juice had a pH of 4.9-5.0 and contained 12.4-12.8% (w/v) total sugars. After supplementation with ammonium sulfate, sodium phosphate and magnesium sulfate the juice was fermented into ethanol using yeast immobilized on kappa-carrageenan gel. The volumetric productivity and fermentation efficiency were about 15 g/l-h and 94%, respectively. The concentrations of alcohol and residual sugar in the 'beer' were 54 g/l and 12.8-14.5 g/l, respectively.

Introduction

Biofuels from plant biomass represent one of the most promising alternative sources of energy. These plant-derived fuels are replenishable due to photosynthesis unlike fossil fuels, such as petroleum and coal, whose reserves are rapidly being exhausted. The most important liquid biofuel presently available is ethanol (or ethyl alcohol).

The fermentation of sucrose, glucose and fructose into ethanol in the presence of yeast follows the reaction:

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{invertase}} 2C_6H_{12}O_6 \xrightarrow{\text{glycolytic enzymes}} 4C_2H_5OH + 4CO_2$$

sucrose	glucose/fructose	ethanol
342 grams	2x180 grams	4x46 grams

Scheme 1

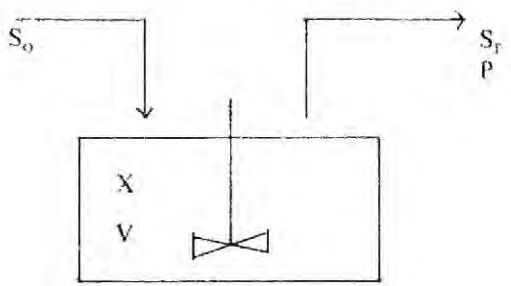
The conversion of sucrose to invert sugar is catalyzed by the enzyme invertase which is bound to the outer layers of the yeast cell while the fermentation of invert sugar into ethanol is catalyzed by the yeast's endocellular glycolytic enzymes. The stoichiometric product yield coefficient $Y_{p/s}$, which is the mass ratio of the product (ethanol) formed to the substrate consumed, is calculated from Scheme 1:

$$Y_{p/s} = \frac{\Delta P}{\Delta S} = \frac{4x46}{2x180} = 0.51 \text{ (based on glucose or fructose)}$$

$$Y_{p/s} = \frac{\Delta P}{\Delta S} = \frac{4x46}{342} = 0.54 \text{ (based on sucrose)}$$

The fermentation efficiency is calculated by dividing the actual mass ratio of ethanol produced to sugar consumed by the corresponding stoichiometric coefficient $Y_{p/s}$.

The fermentation of sugar into ethanol may be done either batchwise or in a continuous manner. In the first type, the fermenter is filled with inoculated wort or fermentation medium and then emptied completely (simple batch) or partially (semi-batch) of the 'beer' or fermented liquid. Subsequent fermentation batches are prepared by adding fresh wort to the fully or partially emptied fermenter. In the continuous process, the sugar solution is continuously pumped into the fermenter while the fermented product 'beer' is withdrawn such that the volume of the fermenting liquid remains constant, as shown below:



Scheme 2

The complete mass balance equation for the sugar during continuous alcohol fermentation is given by the equation (Wang *et al.*, 1979):

Sugar in	−	Sugar out	−	Biomass produced	−	Maintenance requirement	−	Ethanol formed	=	Sugar accumulated	
DS_o	−	DS_r	−	$\frac{\mu X}{Y_{x/s}}$	−	mX	−	$\frac{Q_p X}{Y_{p/s}}$	=	$\frac{dS}{dt}$	(1)

The parameters and their units are defined as follows:

D (hr^{-1}), dilution rate (ratio of flow rate F to fermenter working volume V)
S_r (g/l), residual sugar concentration in the 'beer'
μ (hr^{-1}), specific growth rate
X (g/l), yeast concentration (dry basis)
$Y_{x/s}$ (g/g), biomass yield coefficient
$Y_{p/s}$ (g/g), product (ethanol) yield coefficient
S_o (g/l), feed sugar concentration
m (g/g-hr), specific maintenance rate
Q_p (g/g-hr), specific ethanol production rate

The biomass yield coefficient $Y_{x/s}$ is equal to the mass ratio of yeast produced to sugar consumed. The specific maintenance rate is expressed as grams of sugar utilized per gram of dry yeast per hour and represents substrate utilization for cell functions other than growth and ethanol production. These functions include turnover of cell materials, osmotic work to maintain concentration gradients and cell motility. The specific ethanol production rate Q_p is defined as the ratio of the volumetric productivity dP/dt to the cell concentration X and is expressed as grams ethanol produced per gram of yeast (dry basis) per hour:

$$Q_p = \frac{1}{X}\frac{dP}{dt} \tag{2}$$

It can be seen after rearranging Eq. (2) that the volumetric productivity dP/dt is directly proportional to both the yeast concentration X and the specific productivity Q_p.

Under steady-state conditions, sugar does not accumulate in the continuous fermenter and Eq. (1) may be simplified and rearranged as:

$$Q_s = \frac{D(S_o - S_r)}{X} = \frac{\mu}{Y_{x/s}} + m + \frac{Q_p}{Y_{p/s}} \tag{3}$$

Q_s is the specific sugar uptake rate expressed as grams of sugar assimilated per gram of yeast per hour.

The volumetric productivity of a continuous alcohol fermenter is greater than that of the batch fermenter because the latter has a lag time before the yeast can maximally produce alcohol while the continuous fementer at steady state is always operating optimally. Furthermore, the batch process requires a "down time" or idle period for emptying, cleaning and filling of the fermenter in between batch runs. At the same productivity, a continuous fermenter is equivalent to a bigger batch fermenter. When employed at a high yeast concentration and with cell recycle,

which results in a rapid fermentation, the savings in capital investment can be substantial compared to the batch process.

As indicated in Eq.(2), the volumetric productivity may be maximized by selecting a productive yeast strain, i.e. with a high Q_p value and by working at a high yeast concentration X. The latter observation, which results in fermentation times of six hours or less, was earlier reported by Nagodawithana *et al.* (1974). Continuous-flow fermentation of saccharine substrates into ethanol at high yeast levels has been studied using flocculent yeast with or without cell recycle (Ghose and Tyagi, 1979; del Rosario *et al.*, 1979; Prince and Barford, 1982; Taniguchi *et al.*, 1983; Ramirez and Boudarel, 1983; Jones *et al.*, 1984; Comberbach and Bu'lock, 1984; Damiano and Wang, 1985; Netto *et al.*, 1985), as well as yeast which had been immobilized on solid supports (Ghose and Bandyopadhyay, 1980; Robinson *et al.*, 1981; Wada *et al.*, 1981; Cho *et al.*, 1981; Siva Raman *et al.*, 1982; Ryu *et al.*, 1982; Taguchi, 1982). Materials for yeast immobilization include pectin, gelatin, calcium alginate, polyacrylamide and carrageenan, as well as wood particles and porous inorganic supports.

The present paper describes the results of the continuous-flow ethanol fermentation of sugarcane molasses, which had been diluted and supplemented with nutrients, using yeast immobilized in wood particles and/or kappa-carrageenan. Similar studies were done on banana pulp juice from ripe Cavendish banana fruits using carrageenan-immobilized yeast. The various fermentation parameters were calculated and the prospects for large-scale process application are discussed.

Materials and Methods

Materials. Blackstrap sugarcane molasses was donated by Central Azucarera de Don Pedro, Nasugbu, Batangas. Alkali-treated kappa-carageenan was a gift of FMC (Philippines)/Marine Colloids, Cebu City. *Leucaena leucocephala* 'ipil-ipil' wood sawdust and shavings were obtained from the UPLB College of Forestry.

Banana fruit (Cavendish) rejects from a banana plantation in Davao were obtained through Eden Fruits and Vegetables Co., Inc. (Quezon City). The fruits at color-break stage 8, were used throughout this study. The fruit pulp juice was extracted using the processing steps outlined in Fig. 1. Due to lack of the necessary pieces of equipment, processing step no. 3 was done using a cassava chipper (UPLB-Engineering). However, processing step no. 5 was done using a hydraulic press (UPLB-Food Science & Technology). Manual pressing was also done using cotton sack cloth as filter. The banana fruit juice obtained was then kept in the walk-in freezer until needed. However, the peel and solids were dried at 105°C, milled and kept in plastic bags for further analysis.

Inoculum preparation and build-up. Saccharomyces cerevisiae BB1945 and BB1630 were selected from the culture collection of the National Institutes of Biotechnology and Applied Microbiology based on the evaluation of several local yeast isolates and yeast strains from foreign culture collections. Procedures for inoculum preparation and build-up followed those of Colle *et al.* (1982).

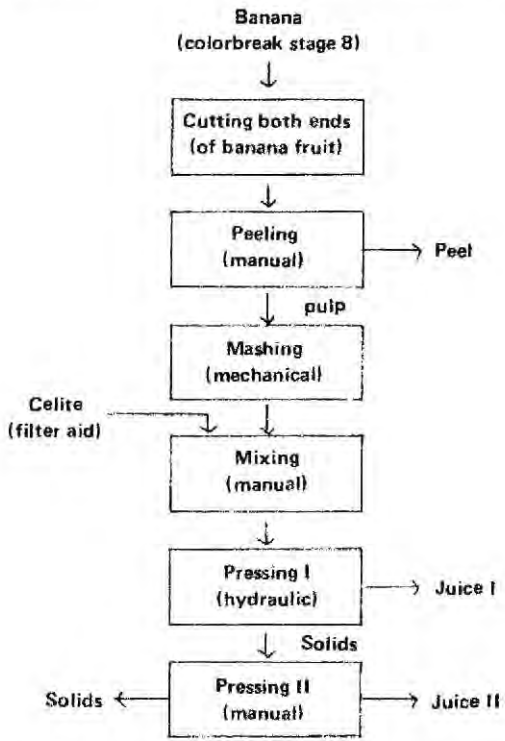


Figure 1. Block diagram for the extraction of pulp juice from ripe banana fruits.

Support preparation. The 'ipil-ipil' wood sawdust and shavings were boiled in tap water for two hours, soaked in 10% ethanol for two hours, boiled again in distilled water for two hours, washed with distilled water and finally dried and sterilized at 100°C for six hours. On the other hand, the alkali-treated kappa-carrageenan powder was mixed with distilled water in order to obtain a 1.5% solution and boiled in a water bath until a homogeneous mixture was obtained. It was then sterilized for 15 minutes at 121°C (15 psig) and cooled to about 40°C prior to mixing with yeast.

Yeast cell immobilization. For sugarcane molasses medium the following immobilizing systems were used per liter of fermenter volume, namely (1) 90 grams of 'ipil-ipil' wood shavings, (2) a mixture of 45 g each of 'ipil-ipil' wood sawdust and shavings, (3) 500 ml of 1.5% k-carrageenan and (4) 500 ml of 1.5% k-carrageenan and 1.5% 'ipil-ipil' wood sawdust mixture. Entrapment of a known number of yeast cells in the wood materials was done by incubation for 24 hours in the rotary shaker after addition of molasses medium containing 5% total sugars. Yeast entrapment in k-carrageenan gel material or in a mixture of this gel and ipil-ipil sawdust was done by direct mixing of yeast cells and support at 40°C and con-

version to pellets by dropwise extrusion (Fig. 2). A solution of 0.2M KC1 solution was the receiving medium for the bead-making process. The total yeast cell counts remaining in the molasses medium after immobilization and in the KC1 solution were determined in order to calculate the yeast loading (expressed as grams yeast on dry basis immobilized per liter of fermenter volume).

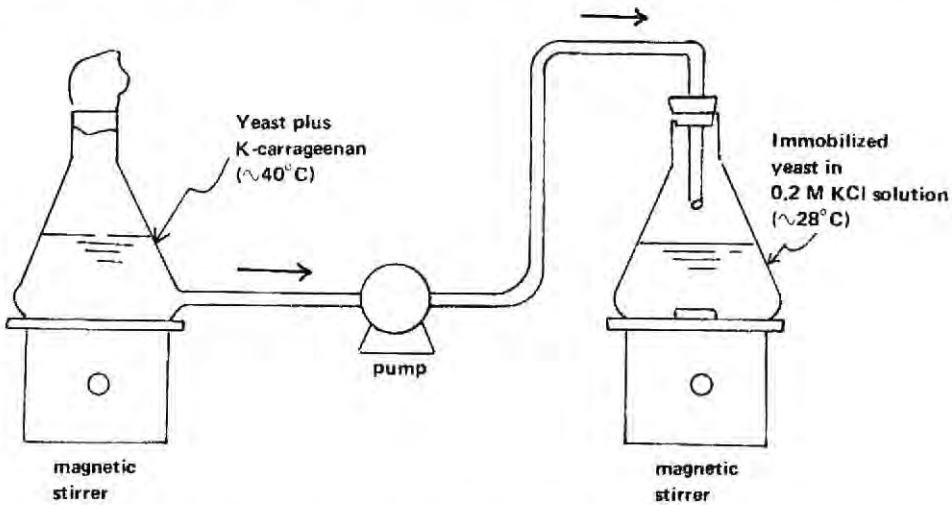


Figure 2. Set-up for immobilization of yeast cells in k-carrageenan.

The yeast cells, which were to be immobilized for fermenting banana fruit juice, were first grown in a 9-liter airlift fermenter for 24 hours at room temperature in a medium containing 5% total sugars (w/v) banana fruit juice supplemented with (a) 5.0 g/l $(NH_4)_2SO_4$, (b) 3.0 g/l Na_2HPO_4, (c) 0.01 g/l $MgSO_4.7H_2O$. The pH was adjusted to 4.5. Immobilized yeast cells were prepared under sterile conditions using kappa-carrageenan as follows:

(a) kappa-carrageenan (1.5% w/v, 40-42°C) was added to a required volume of concentrated yeast cells up to 350 ml in an Erlenmeyer feed flask.
(b) Using a Gilson pump (Fig. 2), the mixture obtained in (a) was added dropwise to gently-stirred 250 ml of 0.2 M KC1. The resulting carrageenan gel beads of about 4mm mean diameter contain the entrapped-yeast cells.
(c) Steps (a) to (b) were repeated as necessary.

Fermentation set-up for molasses medium. The same 9.5-liter airlift fermentor used in yeast build-up was utilized, except that the inner tube was removed and a stainless steel screen (20 mesh) plate was added at the upper part of the fermentor in order to prevent the outflow of immobilized material. As shown in Fig. 3, the fermentation apparatus consisted of (a) 20-liter substrate reservoir,

(b) water bath at 50°C for minimizing spoilage of the stored feed, (c) feed pasteurizer consisting of a stainless steel cylindrical tube wound with heating tape connected to a voltage regulator, (d) cooler with flowing water, (e) Gilson (minipuls' pump for substrate feeding, (f) fermentor wrapped with heating tape connected to a YSI temperature controller (Model YSI 73), (g) CO_2 bubbler which provided a visual estimate of the fermentation rate and (h) fermented product reservoir.

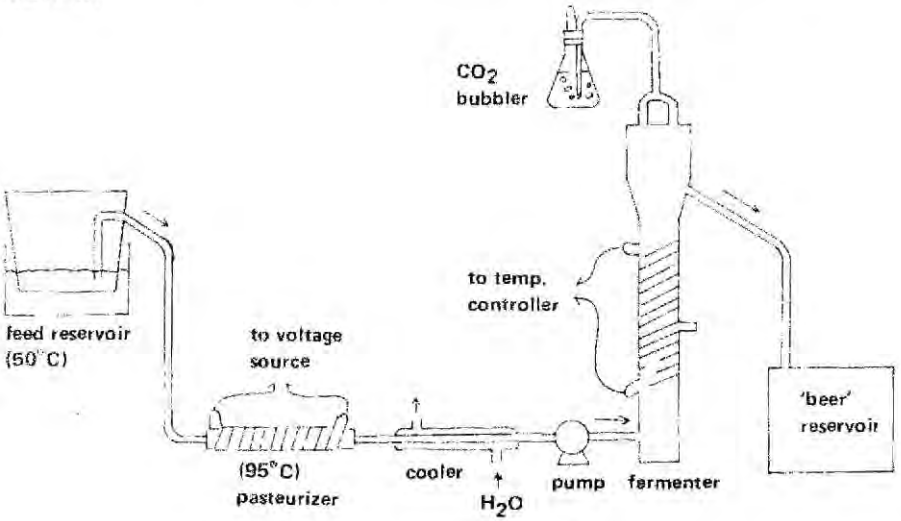


Figure 3. Set-up for continuous ethanol fermentation.

Continuous-flow fermentation of molasses. After transferring the immobilized material to the fermentor, molasses substrate was added for eight hours in a batch fermentation. The fermentor and substrate were sterilized with steam at 15 psig for 15 minutes. The substrate consisted of molasses diluted with tap water to a concentration of 200 g/l total sugars, supplemented with nutrients as previously described for inoculum build-up. No substrate sterilization was done for the subsequent continuous fermentation run.

Fresh molasses medium was prepared daily and fed to the fermentor. When no pasteurization was desired, the substrate from the reservoir was connected directly to the pump, by-passing the pasteurizer. Fertilizer grade $(NH_4)_2HPO_4$ was used, in place of the four nutrients previously used in order to test the effect of adding this low cost nutrient. Temperature adjustment at the fermentor was made possible using the YSI temperature controller.

For the two-stage fermentation, molasses medium containing 200 g/l total sugars was similarly used but it was sterilized prior to transferring into the sterile feed flask. The glass fermentors were also steam-sterilized before transferring the immobilized yeast. After eight hours of batch fermentation, the molasses medium was fed continuously into the fermentor.

Continuous-flow fermentation of banana fruit juice. The fermentation set-up was prepared as shown in Fig. 4. The gel beads, which had been prepared as previously mentioned, were poured into the column and sterile banana fruit juice media [banana fruit juice + supplements (5.0 g/l $(NH_4)SO_4$, 3.0 g/l Na_2HPO_4, 0.01 g/l $MgSO_4H_2O$, 2.5 g/l KCl)] (pH 4.5) was pumped into the bottom of the column. The yeast cells entrapped in the gel beads were allowed to grow for about three hours or more. Then continuous feeding of banana juice media was began as soon as CO_2 bubbling became vigorous.

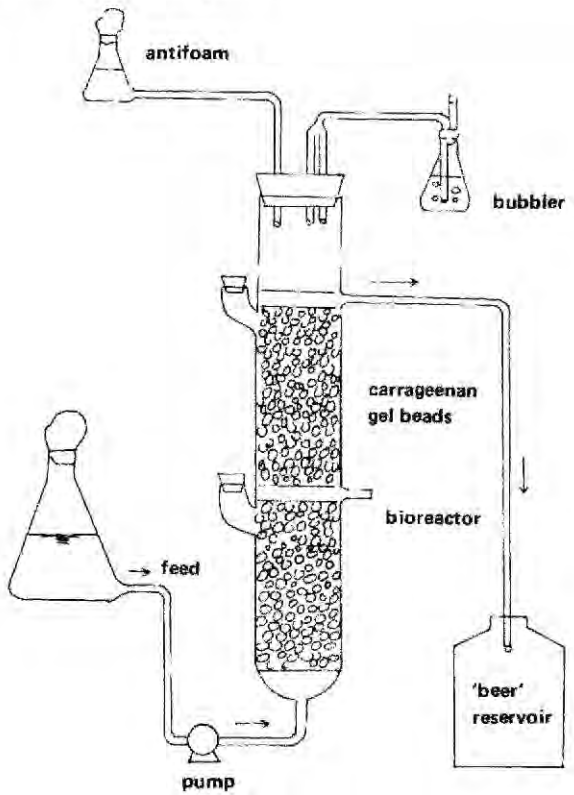


Figure 4. Set-up for continuous ethanol fermentation using immobilized yeast cells.

Analytical methods. Microscope counting of the yeasts was facilitated by using a haemocytometer. Yeast viability was determined using the viable plate count method (Colle *et al.*, 1982). The alcohol content of the fermented product 'beer' was analyzed with an ebulliometer and counterchecked by FID gas chromatography (after filtration using a GC Whatman filter paper). The Westphal balance was used to determine the density of the 'beer'. Total reducing sugars were determined on the 'beer', after inversion of sucrose with HCl (AOAC, 1975) using the dinitrosalicylic acid method (Miller *et al.*, 1960).

Results and Discussion

Fermentation of Molasses

Comparison of support materials. The results of the continuous-flow ethanol fermentation of nutrient-supplemented sugarcane molasses using four support materials are shown in Fig. 5 and summarized in Table 1. Kappa-carrageenan gave the highest ethanol concentration in the 'beer', namely 77 g/l (or 7.7% w/v), average volumetric productivity of 12.9 grams ethanol per liter per hour, and fermentation efficiency of 90.7%. These fermentation parameters were obtained at a yeast loading of 30 g/l and residence time of six hours (dilution rate of 0.167/hr). Unfortunately, when yeast loading was increased to 50 g/l the gel beds were easily ruptured, resulting in high yeast wash-out.

The better performance of carrageenan was due to the higher yeast loading, less cell wash-out and highly porous structure of the gel compared to wood or wood-plus-carrageenan. Inefficient yeast entrapment inside the wood particles resulted in low yeast loadings of approximately 17 g/l and yeast washout in the effluent of up to 5 x 10^7 cells/ml.

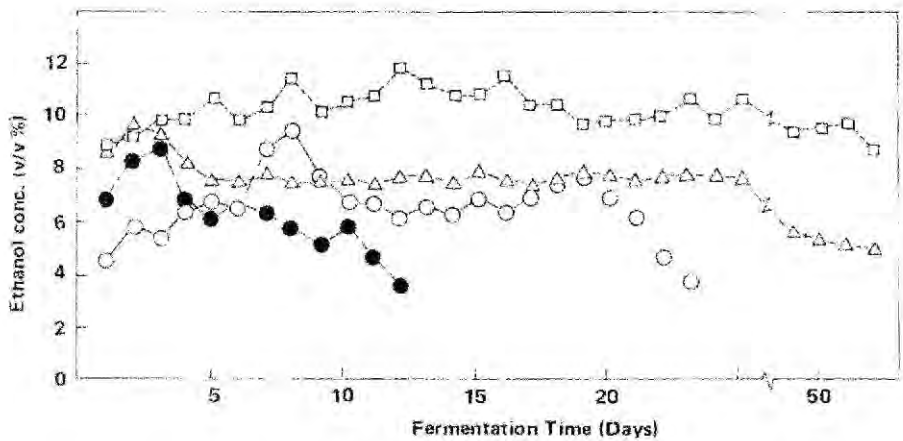


Figure 5. Effect of type of support material on continuous ethanol fermentation.
(● - - - ●) 50% sawdust + 50% shavings of 'ipil-ipil' wood-yeast loading = 17 g/l
(○ - - - ○) 'Ipil-ipil' wood shavings – yeast loading = 18 g/l
(△ - - - △) 50% Ipil-ipil wood sawdust + 50% K-carrageenan-yeast loading = 22 g/l
(□ - - - □) K-carrageenan – yeast loading = 30 g/l
(Sugars of molasses feed = 200 g/l)

Effect of feed pasteurization. It is seen in Fig. 6 and Table 1 that pasteurization of the molasses feed resulted in better performance of the carrageenan-immobilized yeast fermenter, especially in terms of fermentation efficiency and volumetric productivity. This may be explained by the adverse effects of contaminant microorganisms on yeast fermentation of sugar into ethanol.

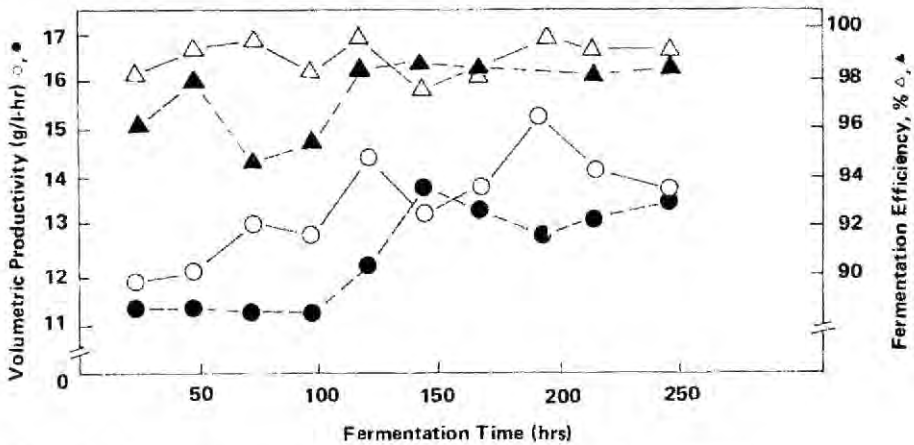


Figure 6. Continuous ethanol fermentation of pasteurized (○—○), (△ - - △) and Non-pasteurized molasses feed (● ●), (▲ - - - ▲).

Effect of nutrient supplementation. A significant improvement in fermentation characteristics was observed after supplementation of diluted sugarcane molasses with one or more nutrients (Fig. 7 and Table 1). Addition of fertilizer-grade ammonium phosphate resulted in greater enhancement of the fermentation compared to further addition of reagent grade K_2HPO_4, $(NH_4)_2SO_4$ and $MgSO_4$; this indicates that the molasses used was deficient in the nutrients that were added.

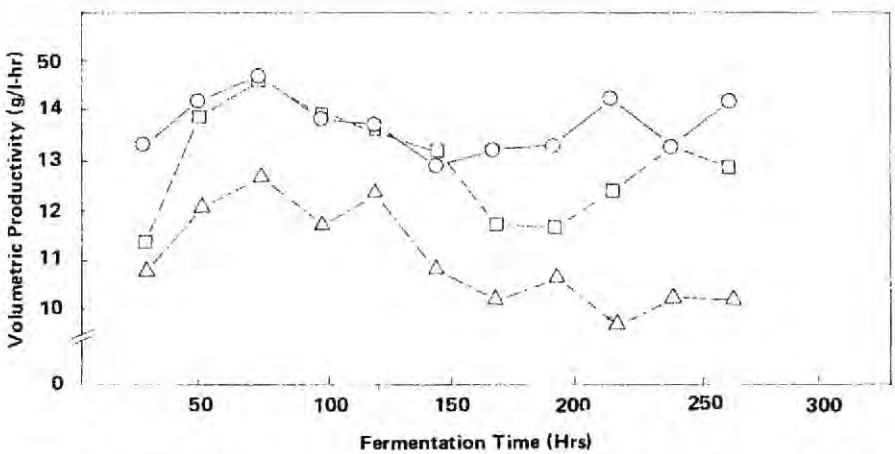


Figure 7. Effect of nutrient supplementation on continuous ethanol fermentation (○ - - - ○) Reagent grade: KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$ – 1.4, 1.0, 0.25 g/1, resp.;
(□..□
(□—□) Fertilizer grade: $(NH_4)_2HPO_4$
(△—△) Without nutrient Sugar conc. 200 g/1

Effect of temperature. Poorer fermentation performance was observed at 42°C compared to 30°C as shown in Fig. 8 and Table 1. Although the yeast strain used (BB 1945) is heat tolerant (Colle *et al.*, 1982), it performed better at the lower temperature; this could be due to enhanced inhibition effects of ethanol at elevated temperatures (Nagodawithana *et al.*, 1974; Nagodawithana and Steinkraus, 1976).

Two-stage continuous fermentation. Results of the sequential fermentation of nutrient-supplemented sugarcane molasses showed a slightly greater average volumetric productivity of 13.4 g/l-hr compared to the value of 12.9 g/l-hr for the single-stage continuous run at the same dilution rate of 0.167/hr. As previously mentioned, the ethanol concentration in the "beer" and sugar utilization were enhanced at lower dilution rates.

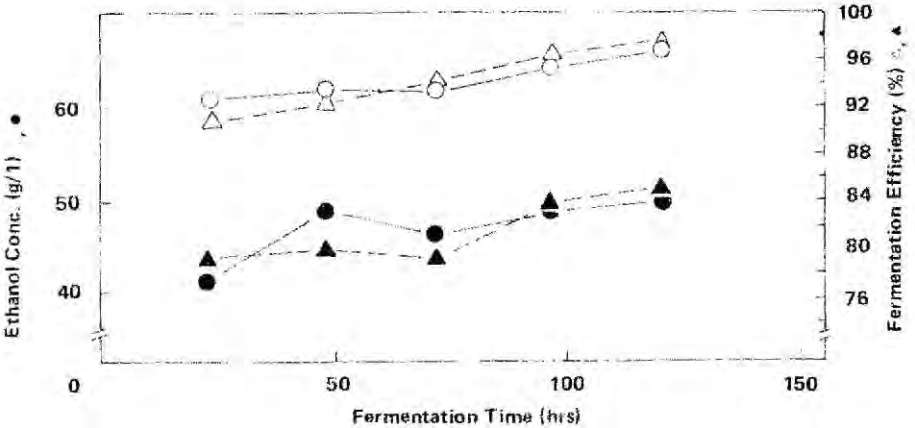


Figure 8. Continuous ethanol fermentation at 30°C and 42°C by BB 1945. Sugar conc. = 150 g/l.
30°C – (○–○), (△- -△)
42°C – (●–●), (▲- -▲)

Comparison of the results with literature values. The maximum volumetric productivity of approximately 13 g ethanol/l-hr obtained in the present study is lower than values obtained on nutrient-supplemented cane molasses by other workers namely 40 (Navarro *et al.*, 1984) and 107-250 g/l-hr (Siva Raman *et al.*, 1982) using yeast immobilized on a polymeric matrix. However, some restrictions and/or operational problems were observed in these literature reports such as reduction of productivity with prolonged fermentation and feed sugar concentration of only 10%. A productivity of 15 g/l-hr was obtained by Ramirez and Boudarel (1983) on beet juice using a flocculant yeast. Cho *et al.* (1981) obtained a productivity of 21 g/l-hr and 94% conversion of sugar into ethanol using alginate-immobilized yeast in a fluidized bed reactor. The productivity was only 10 g/l-hr for the corresponding packed bed reactor. For both types of reactors the maximum alcohol concentration in the 'beer' was only 50 g/l.

Ethanol Fermentation of Banana Fruits

Per kilogram of ripe cavendish fruit pulp, which had been manually separated from the peel, 0.50-0.71 liter of juice was obtained. This banana juice had a pH of 4.9-5.0 and contained 12.4-12.8% (w/v) total sugars. The pulp-peel ratio was in the range 1.6-2.1 (w/v). This value is less than 3.4 which had been previously reported (Pontiveros *et al.*, 1978) for *unripe* Giant Cavendish bananas. Sugar recovery in the juice was calculated from the ratio of the sugar extracted in the juice, which was equal to juice volume and sugar concentration, to the total sugar content of the fruit pulp. The latter value was found to be 16%. Sugar recoveries in the juice were 39.8 and 55.3% for runs I and II, respectively. These low recoveries may be explained by the inefficiency of both mechanical extraction using a hydraulic press and manual pressing. The use of suitable filter press is expected to substantially improve the juice extraction process.

The yields of ethanol from ripe bananas are equal to 0.037-0.051 liters of 94.5% alcohol per kilogram fresh pulp and 24.8-31.6 liters of 94.5% alcohol per metric ton of fresh fruits (using only the fruit pulp as alcohol raw material). These low ethanol yields are mainly due to low sugar recoveries during juice extraction from banana pulp, which was at most 55.3%. A 90% sugar recovery in the juice extraction process would correspond to a yield of approximately 52 liters of 94.5% alcohol from the pulp of one metric ton of fresh fruits.

The average values of the fermentation parameters at room temperature are given in Table 2. Run I was conducted at a yeast loading of 6.4g dry yeast per liter of column bed volume and an average dilution rate of 0.18/hr corresponding to a residence time of 5.6 hrs, while run II had a greater yeast loading of 13.4 g/l and an average dilution rate of 0.28/hr (3.6 hrs residence time). The average values were calculated after at least 24 hours of fermentation for both runs in order to establish steady-state conditions. Comparing these values, there are no significant differences for the two runs in terms of the concentrations of ethanol and residual sugar in the fermented product 'beer' as well as sugar utilization. However, run II had a greater volumetric ethanol productivity DP (14.9 g/l-hr) but a lower specific sugar uptake rate Q_s (2.34 g/g-hr) and specific ethanol production rate Q_p (1.11 g/g-hr) compared to run I. For the latter, the corresponding values of DP, Q_s and Q_p are 9.5, 3.09 and 1.49, respectively.

Conclusions

The present study has shown that for molasses medium kappa-carrageenan is a better immobilizing material than *Leucaena leucocephala* 'ipil-ipil' wood sawdust or shavings. The volumetric productivities of carrageenan-and wood-immobilized yeast systems were approximately 13 and 7 g/l-hr, respectively. The lower productivity of the wood system is due mainly to yeast wash-out from the fermenter. This problem was less pronounced with the carrageenan system and steady-state condi-

tions could be maintained for at least three months. The gel-immobilized yeast had a fermentation efficiency of up to 94%, an alcohol concentration of about 80 g/l and the lowest value of sugar concentration (17.9 g/l) in the 'beer' using 200 g/l feed sugar concentration.

For the extracted juice of banana fruit pulp, which contained about 126 g/l total sugars, fermentation by carrageenan-immobilized yeast resulted in a volumetric productivity of almost 15 g/l-hr, fermentation efficiency of about 94%, alcohol concentration of approximately 54 g/l and 12.8-14.5 g/l residual sugar in the 'beer'.

The characteristics of the continuous-flow fermentation systems described in the present study, which are denoted as BIOTECH Processes 2 and 3 for wood-and carrageenan-immobilized yeast, respectively, are compared with the traditional batch process in Table 3. A previously-studied process using flocculent yeast (del Rosario *et al.*, 1983), denoted as BIOTECH Process 1, and a semi-commercial continuous-flow process developed by Alcon Biotechnology Limited of the United Kingdom (Guidoboni, 1984; A. Thomas, personal communication, 1981) are also included in the Table. BIOTECH Process 1 had a volumetric ethanol productivity of 18 g/l-hr but yeast viability could not be maintained for more than two weeks even with limited aeration. Process 3 is definitely more promising in terms of large-scale applications than Processes 1 and 2 and has more than six times the productivity of the traditional batch process used in most Philippine distilleries. Needless to say, further research and development work is needed in order to scale-up and cost-evaluate Process 3 for Philippine conditions.

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Table 1. Continuous-flow ethanol fermentation parameters for nutrient-supplemented sugarcane molasses

Condition*	Initial Yeast Loading (g/l) X	Sugar Concentration of Feed (g/l) S_o	Total Fermentation Time (days)	Dilution Rate (hr^{-1}) D	Alcohol Concentration (g/l) P	Residual Sugar Concentration (g/l) S_r	Sugar Utilization (%) $\frac{S}{S_o}$	Ethanol Yield Coefficient (g/g) $Y_{p/s}$	Fermentation Efficiency (%) $\frac{Y_{p/s}}{0.51}$	Volumetric Productivity (g/l-hr) DP	Specific Sugar Uptake Rate, Q_s (g/g-hr) $\frac{D(S_o-S_r)}{X}$	Specific Ethanol Production Rate, Q_p (g/g-hr) $\frac{DP}{X}$
A. Type of Support												
1. K-carrageenan	30	200	67	0.167	77.2	33.1	83.4	.463	90.7	12.9	0.93	0.43
2. Sawdust and k-carrageenan (1:1)	22	200	66	0.147	58.9	38.9	80.6	0.366	71.7	8.7	1.08	0.39
3. 'Ipil-ipil' Shavings and Sawdust (1:1)	17	200	13	0.124	58.5	57.9	71.0	0.412	80.7	7.2	1.04	0.42
4. 'Ipil-ipil' Sawdust	18	200	24	0.136	53.8	73.5	63.2	0.425	83.4	7.3	0.96	0.41
B. Feed Pasteurization												
1. Pasteurized	30	200	9	0.167	80.1	17.9	90.0	0.481	94.3	13.4	0.90	0.44
2. Non-pasteurized	30	200	9	0.167	78.5	31.4	84.8	0.453	88.8	12.8	0.87	0.42
C. Nutrient Addition												
1. Reagent grade nutrients**	30	200	11	0.167	81.5	31.4	84.3	0.478	93.7	13.6	0.93	0.45
2. 1.0 g/l $(NH_4)_2HPO_4$ (fertilizer grade)	30	200	11	0.167	77.1	36.2	81.9	0.470	92.2	12.9	0.91	0.43

Table 1. Continuous-flow ethanol fermentation parameters for nutrient-supplemented sugarcane molasses

*Condition**	*Initial Yeast Loading (g/l) X*	*Sugar Concentration of Feed (g/l) S_O*	*Total Fermentation Time (days)*	*Dilution Rate (hr^{-1}) D*	*Alcohol Concentration (g/l) P*	*Residual Sugar Concentration (g/l) S_r*	*Sugar Utilization (%) $\Delta S / S_O$*	*Ethanol Yield Coefficient (g/g) $Y_{p/s}$*	*Fermentation Efficiency (%) $\frac{Y_{p/s}}{0.51}$*	*Volumetric Productivity (g/l-hr)*	*Specific Sugar Uptake Rate, Q_s (g/g-hr) $\frac{D(S_O-S_r)}{X}$*	*Specific Ethanol Production Rate, Q_p (g/g-hr) $\frac{DP}{X}$*
3. No Added nutrient	30	200	11	0.167	63.9	42.6	78.7	0.469	92.0	11.1	0.87	0.36
D. Effect of Temperature												
1. 42°C	22.6	150	5	0.167	46.4	48.4	67.7	0.456	89.4	7.8	0.75	0.34
2. 30°C	22.6	150	5	0.167	62.4	16.8	88.8	0.469	92.0	10.4	0.98	0.46
E. Effect of Dilution Rate	30	200	5	0.125	95.4	4.2	97.9	0.487	95.5	11.9	0.82	0.40
	30	200	5	0.131	89.0	12.7	93.6	0.475	93.2	11.7	0.82	0.38
	30	200	5	0.167	80.4	30.8	84.6	0.475	93.2	13.4	0.94	0.45
	30	200	5	0.250	62.8	49.6	75.2	0.416	81.9	15.7	1.25	0.52

*Except for A, all experiments used k-carrageenan as support material.
11.4 g/l KH_2PO_4, 1.0 g/l $(NH_4)_2SO_4$, 0.25 g/l $MgSO_4$

Table 2. Fermentation parameters for ethanol production from ripe banana fruit pulp

*Fermentation Run**	*Dilution Rate* (hr^{-1}) D	*Alcohol Conc.* (g/l) P	*Residual Sugar Conc.* (g/l) S_r	*Sugar Utilization* (%) $\frac{\Delta S}{S_o}$	*Ethanol Yield Coefficient* $Y_{p/s}$	*Fermentation Efficiency* (%) $Y_{p/s/0.51}$	*Volumetric Productivity* (g/l-hr) DP	*Specific Sugar Uptake Rate* Q_s (g/g-hr) $\frac{D(S_o-S_r)}{X}$	*Specific Ethanol Production Rate* Q_p (g/g-hr) $\frac{DP}{X}$
I	0.175±0.020	54.6±1	14.5±1.0	88.7±0.8	0.481±.007	94.4±1.4	9.5±0.6	3.09±0.22	1.49±0.10
II	0.283±0.019	53.1±3.0	12.8±1.8	89.7±1.4	0.477±.031	93.6±6.2	14.9±1.0	2.34±0.20	1.11±0.07

*Run I: Working Volume – 1100 ml; Liquid Volume – 420 ml; Bead Volume – 680 ml

Fermentation Medium: Total Sugar – 12.8% (w/v); pH after sterilization – 4.6

Cell Loading: $\frac{10.3\text{g dry yeast}}{\text{L bead volume}}$ or $\frac{6.4\text{g dry yeast}}{\text{L column bed volume}}$

Run II: Working Volume – 1145 ml; Liquid Volume – 572 ml; Bead Volume – 573 ml

Fermentation Media: Total Sugar – 12.4% (w/v); pH after sterilization – 4.6

Cell Loading: $\frac{26.7\text{g dry yeast}}{\text{L bead volume}}$ or $\frac{13.4\text{g dry yeast}}{\text{L volume bed volume}}$

Table 3. Comparative characteristics of ethanol processes

System/Process	*Feedstock*	*Organism*	*Residence time (hr)*	*Sugar concentration*		*Yeast concn.*		*Ethanol concn. (g/l)*	*Volumetric productivity (g/l-hr)*
				Initial (g/l)	*Final (g/l)*	*(g/l)*	*(cells) ml*		
Traditional batch	molasses	*S. cerevisiae* Magnac strain	25	120	32	<1	$<10^8$	50	2.0
Melle-Boinot batch with yeast recycle	molasses	*S. cerevisiae* (with recycle)	12 (total cycle time)	130		>1	$>10^8$	60	5.0
Alcon (U.K.) continuous-flow	sugarcane juice	*S. cerevisiae* (flocculent)	7	100	2	3	$4x10^8$	67	9.6
BIOTECH Process 1 continuous-flow	molasses	*S. diastaticus* (flocculent)	4	220	36	30	$4x10^9$	72	18
BIOTECH Process 2 continuous-flow	molasses	*S. cerevisiae* (heat-tolerant immobilized on wood particles)	7	200	50	15	$2x10^9$	60	8.6
BIOTECH Process 3 continuous-flow	molasses	*S. cerevisiae* (heat-tolerant immobilized on carrageenan)	6	200	33	30	$4x10^9$	77	12.8
	ripe banana pulp juice		3.6	124	13	13	$2x10^9$	53	14.7

Perfecto Q. Flor, Discussant

Continuous fermentation of sugar into ethanol using immobilized yeast is basically a new process in the field of fermentation. The use of wood sawdust and shavings, and K-carrageenan has been reported by several workers from Canada, Japan and U.S.A.. However, the work of Dr. del Rosario *et al.* is supposed to be the first in the Philippines. The use of ipil-ipil sawdust and shavings showed to be poor in containing the yeast cells, precisely because we have to depend only on the amount of yeast cells that are physically trapped in the wood pores. Wood sawdust and shavings could be a good immobilizing agent depending on the pretreatment process. One is by mild alkali or acid treatment process. This is to improve the texture, to remove some of the protein materials if present, and to loosen up the fibers to give way for a better entrapment.

K-carrageenan gel is undoubtedly a very good matrix for yeast entrapment. In fact this material could withstand rigid environmental conditions making advantageous for yeast immobilization. The disadvantage of this medium is that it allows the yeast cells to grow or multiply causing the beads to break, and in the long run freeing the cells into the fermentation medium.

The economics for continuous fermentation operation should be more carefully studied especially on the energy side of the process. We cannot go into continuous fermentation operation process unless the system is closed, i.e. everything must be sterilized. Continuous fermentation by immobilized yeast would undeniably help in preventing contamination because of high cell loading and fact fermentation.

Lydia M. Joson, Discussant

Fermentative ethanol production can be accomplished by either batch or continuous process. Most distilleries, however, not only in the Philippines but throughout the world operate by batch fermentation process. The process has not changed much from what had been successfully practiced thousands of years ago and handed down from generation to generation.

Although the batch process is beset with many problems (i.e., low cell concentration leading to long fermentation time; high volume of low concentration of saccharine material to be fermented to overcome substrate and end product inhibition; high energy consumption in the distillation due to low alcohol content of the beer; and the continual start up and shut down inherent in the process, which all leads to low productivity) alcohol yields of more than 80% can be attained even fermentations are carried out in apparently primitive ways.

It was only in the 1930's when improvement in the process was made. To overcome the low cell concentration, Boinot of Melle Distillery in France recovered the yeast and partially recycled it after treatment with acid to a new batch to

provide a more concentrated cell mass. The pioneering work of Boinot gave birth to the Melle Boinot process extensively used in Brazilian Distilleries.

As given in Table 4 of Dr. del Rosario's, productivity was increased from 2 g/l/hr in the traditional process to 5 g/l/hr in the Melle-Boinot process. This, however, could still be increased two- to four-fold by the application of continuous fermentation process as in the Alcon process and Biotech Process I, respectively.

Guidobani (1984) reviewed the commercially available continuous processes into two major headings:

A. Simple Tank Fermenters

1. Alcon Process – maintains the yeast in high concentration (15 to 45 g/l day matter) by cell recycle. The fermenters are stirred by pump recirculation to provide adequate mass transfer between yeasts and feedstock in use.
2. Tate and Lyle Process – is very similar to the Alcon process. The basis of the process is a single-stage fermenter with yeast separation and recycle without the use of any moving parts (presumably a gravity settler) made possible by the use of yeast strains which are highly flocculant and exhibit high alcohol tolerance.
3. Inter-loop process – a single fermenter followed by two gravity decanters in series.
4. ATPAL (Atkins Power Alcon Process) features a novel, fully integrated fermentation and distillation system that operates continuously. Only about one fourth of the energy equivalent is consumed in the integrated process.
5. Biostill Process of Alfa-Laval – another integrated fermentation/distillation process using concentrated feedstocks.

B. Cascade Fermenters in Series – a bridge between traditional batch and truly continuous Cell Sedimentation Stirred Tank Reactor fermentation process. Fermentation plant usually consists of between five and seven production fermenters connected in series.

1. Vogelbusch
2. Technipetrol S.p.a.

The constraint of cell wash-out in the continuous process using free living cell, is encountered when high dilution rate is used to attain high productivity. This is eliminated when cells are immobilized as in the BIOTECH Processes 2 and 3. Carrageenan and alginic acid which are easily available in the Philippines are two carriers usually employed in cell immobilization. The former is obtained from *Eucheuma cottonii* while the latter is from *Sargassum*.

The productivity of Dr. del Rosario's process could still be improved by modification in the immobilization process. Wang and Hettner (1982) were able to further increase the productivity by 50% by trapping tricalcium phosphate crystals into the immobilized yeast cell in K-carrageenan. The co-existence of yeast cell and

tricalcium phosphate in the material resulted in sustained viability through internal pH control, increased cell loading, greater settling velocity and enhanced production. On the other hand, Fang *et al.* (1983) immobilized *Saccharomyces formosensis* in sodium alginate and sands (2:1) and obtained maximum productivity of 116.34 g/l/hr at a dilution rate of $4.2h^{-1}$.

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Pham Binh Chay, Discussant

Alcohol has long been produced from molasses for industrial use. In developing countries, significant improvement in ethanol production technology are necessary to reduce production cost and make ethanol available in large amount as fuel and chemical feed stock. Various innovative techniques like continuous fermentation with recycling and cell recycling have been investigated.

Adoption of some of these techniques in the Philippines, where batch fermentation is being used for ethanol production, would require complete overhauling of the present set up of distilleries resulting in enormous capital investment.

In this context, the technique of continuous flow fermentation in which yeast can be maintained either by immobilization or natural sedimentation (cell recycling) was undertaken by Dr. E. del Rosario's studies on ethanol fermentation to exploit its potential using *S. cerevisiae* for increase ethanol production.

There are many valuable data given in the study for the application of the fermentation processes and design criteria for scale-up. First, the optimization of the process using two important agricultural products, banana peels and molasses, an agricultural waste product and an agricultural by-product, respectively. Second, the use of locally available nutrients instead of the imported ones and third, the different modes of preparation of the substrate and also the use of the different types of bioreactors.

The author was able to study in detail valuable process parameters and data evaluation primarily for design and scale-up. Although the continuous process has shown promise, it has few industrial applications for ethanol. It may be useful for laboratory scale only as it is not used in industrial scale. The industrial fermentation process industries of Brazil use instead the fed-batch process for ethanol production.

I still have some doubt about the BIOTECH process (Figure 4). If this process will eventually scale-up, the energy consumption is very high for maintaining this process. Another observation is in BIOTECH process 3 (Figs. 5, 6). If this process will eventually scale-up to for example 50,000 l/day of anhydrous alcohol, the maintenance of the tank containing the substrate after sterilization may pose a problem due to contamination. According to Figures 5 and 6, this tank also contains nutrient supplements for the substrate. This may cause the reduction of sugar concentration as nitrogen components of the supplement would interact with sugars resulting to browning and consequently reduce sugar concentrations.

It was shown in the results that residual sugars in process 3 is very high which means that the ethanol produced must have inhibited further conversions of sugars to the ethanol produce. Therefore, I suggest that economic feasibilities of fermentation system should be undertaken before scale-up of BIOTECH process 3 should be done.