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Extractive Fermentation of Ethanol Using Vacuum Fractionation Technique

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***Abstract*—**A vacuum fractionation technique was introduced to remove ethanol from fermentation broth. The effect of initial glucose and ethanol concentrations were investigated for specific productivity. The inhibitory ethanol concentration was observed at 100 g/L. In order to increase the fermentation performance, the ethanol product was removed as soon as it is produced. The broth was boiled at 35°C by reducing the pressure to 65 mBar. The ethanol/water vapor was fractionated for up to 90 wt% before leaving the column. Ethanol concentration in the broth was kept lower than 25 g/L, thus minimized the product inhibition effect to the yeast cells. For batch extractive fermentation, a high substrate utilization rate was obtained at 26.6 g/L.h and most of glucose was consumed within 21 h. For repeated-batch extractive fermentation, addition of glucose was carried out up to 9 times and ethanol was produced more than 8-fold higher than batch fermentation.

***Keywords*—**Ethanol, Extractive fermentation, Product inhibition, Vacuum fractionation.

I. Introduction

ETHANOL is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels. In addition, it is probably the most promising future fuel for transportation due to its high energy value and its simplicity of production process. Fermentation derived ethanol or "bio-ethanol" has received a wide popularity as a motor fuel additive. However, a major challenge in the production of ethanol is the separation including high energy cost associated with the distillation of ethanol from the large excess of water. In the case of ethanol fermentation from glucose, the limitation of conventional process comes from high initial glucose concentration and high ethanol concentration inhibition. When initial glucose concentration in the medium is over 300 g/L and ethanol concentration in the fermentation broth reaches 10-14% by weight, both of specific growth rate and specific production rates of yeast decline, the cell mass in the fermentation broth decreases and glucose cannot be converted completely to ethanol [1]. High substrate concentration suffers severe stresses on yeast cells including the high osmotic pressure from the fermentable sugar at the beginning, and the strong ethanol inhibition during the production stage. For single substrate and multiple by-products, the specific production rate (v) can be expressed in terms of inhibitions as followed [2],

$$v = v_{\max}\left[\frac{S}{K_S^{'} + S + \left(S^2 / K_i^{'}\right)}\right]\left[\Pi\left(1 - \frac{P}{P_m^{'}}\right)\right]^{ai} \quad (1)$$

where; v_{max} is the maximum specific production rate, S is substrate concentration, $K_S^{'}$ is the saturation constant, $K_i^{'}$ is the substrate inhibition constant, P is the product concentration, and $P_m^{'}$ is the maximum product concentration, respectively. In addition, the superscript ai represents the exponential constant of the inhibitory product. Ethanol fermentation coupling with *in situ* product separation has attracted considerable interests over the past few decades. It combines biochemical reaction with selective mass transport of ethanol from the reaction site resulting in an increase of the product yield [3]. In order to increase fermentation performance, different methods have been introduced to simultaneously separate ethanol from fermentation broths including pervaporation membrane bioreactor [4]-[6], membrane distillation bioreactor [7], [8], gas stripping [9] solvent extraction [10], and vacuum fermentation [11]-[14]. Nevertheless, the distillate or permeate ethanol products obtained from these techniques contain a large amount of water typically in the range between 20-40% by weight. As a result, additional distillation step is required in order to azeotrope mixture of ethanol solution prior to dehydration step.

In this work, extractive fermentation by using a vacuum fractionation technique was investigated. A convention bioreactor was equipped with a partial reflux condenser. The ethanol/water vapor mixture was fractionated allowing only high concentration of ethanol to leave the column. The consequence of this operation was not only to enhance yields and volumetric productivity, but also to obtain a high concentration of ethanol that can be directly supplied for dehydration without any further distillation. The system was studied for both batch and repeated batch fermentations.

II. Materials and Methods

A. Chemicals

All chemicals used in this study were of analytical grade. HYDRANAL® reagents for determination of water content were purchased from Sigma (Singapore). A commercially available dry distillery yeast (*Saccharomyces cerevisiae*) manufactured in Denmark was used as the ethanol producer.

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B. Experimental Setup

The schematic diagram of the experimental set up is illustrated in Fig. 1. A jacketed coil type partial reflux condenser (Kimble-Chase, USA) was placed on top of a 2.5 L glass bioreactor. Temperature of the fermentation broth (T_1) was controlled by using a water bath (Julabo ED, Germany). In order to generate a well-mixed condition, a magnetic stirrer was used to rotate a magnetic bar inside the bioreactor. A vacuum controller (Neuberger, Germany), and a vacuum pump (EYELA A-1000S, Japan) was employed to generate desired vacuum pressure (P_1). A flexible vacuum hose (Edward, United Kingdom) with a diameter of 3.0 cm was employed for a maximum flow of ethanol vapor. Concentration of the distillate ethanol vapor was controlled by adjusting the temperature (T_2) of a refrigerated thermostat (Grant TC120, United Kingdom). The distillate vapor product was totally condensed by using a series of cold traps, the first was placed in a -30°C cooling bath (LabTech, Taiwan), and the second was placed in a dewar containing liquid nitrogen.

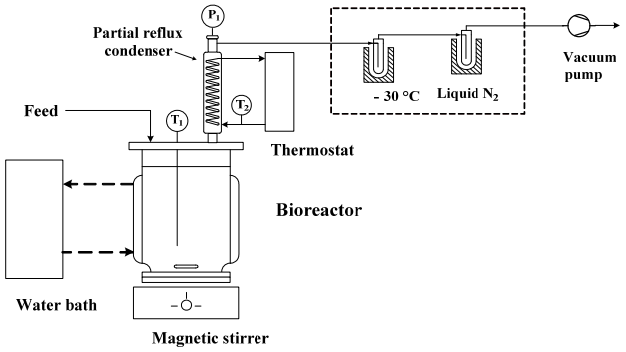

Fig. 1 Schematic Diagram for Extractive Fermentation of Ethanol using vacuum Fractionation Technique

C. Fermentations

The modified YM medium comprises of (per litre); 1.5 g of $(NH_4)_2SO_4$, 3.0 g of yeast extract, 3.0 g of malt extract, and 5.0 g of peptone. The pH was adjusted to 4.5 with citric acid prior to sterilization. Subsequently, glucose powder was added and the yeast was introduced into the fermentation medium at the concentration of 25 g/L. Fermentation was carried out at 35°C without aeration. For extractive fermentation using vacuum fractionation technique, the experiment started after 3 h of inoculation by lowering the pressure to 65 mBar by using a vacuum pump. The fermentation broth began to boil and the rising vapor of ethanol/water mixture was fractionated before leaving the column. Temperature of the thermostat was varied in the range between 0-35°C. As a result, the uncondensed ethanol/water vapor exited the system at different flow rates and different compositions according to the thermostat temperature. The vapor was totally condensed using a series of a condenser (-30°C) in connection with a glass cold trap containing liquid nitrogen. For repeated batch extractive fermentation, addition of glucose powder was carried out when the concentration in fermentation broth was lower than 0.5 g/L.

D. Analyses

Water content of the distillate ethanol was determined by using an automatic Karl Fisher's titration (TitroLine plus, Schott, Germany). Glucose concentrations were determined by using a glucose analyser (YSI, USA). Cell viability analysis was carried out by using the methylene blue test. Organic acids concentration was analysed by HPLC (Thermo Scientific, USA), and quantification by UV detection was made at the wavelength of 210 nm. The mobile phase consists of 1% acetonitrile + 9% 20 mM Na_2HPO_4 (pH 2) at a flow rate of 1 mL/min. The HPLC column was ZORBAX SB-Aq (4.6 mm × 150 mm). Ethanol concentration in the fermentation broth was analysed by using a gas chromatography (SRI Instrument, USA) equipped with a FID Detector. The GC column was a 30 m × 0.32 mm fused silica capillary column (Carbowax®, Restek, USA). The injector and detector were set at 200, and 250°C, respectively. The oven was operated at programmed temperature from 40 to 90°C with the rate of 10 °C/min.

III. Results and Discussion

A. Substrate and Inhibition Kinetics

Substrate and product inhibition kinetics were independently investigated in order to understand the effect of each compound on fermentation performance. For the application in the bio-ethanol production, it can be useful or necessary to apply fermentation media that have very high sugar concentrations. Such fermentations of high gravity media can increase downstream efficiency. However, high sugar concentrations may lead to growth inhibition, or increased formation of fermentation by-products such as glycerol, acetic acid, and higher alcohol leading to a reduction in ethanol yield. For the substrate inhibition kinetic, the experimental results and mathematical modelling of specific ethanol productivity are presented in Fig. 2 (a) as a function of initial glucose concentration ranging from 0-400 g/L. The values were determined by plotting ethanol concentration versus time at various glucose concentrations (data not shown). The period for rapid increasing in ethanol concentration was considered. All calculations were carried out by IBM computer based on the Marrquardt-Levenberg algorithm using the SigmaPlot program (Systat Software, USA). The mathematical modeling for substrate inhibition was in a good agreement with the experimental data at the r^2 value of 0.9462. The simulation result showed that the glucose inhibition effect on specific ethanol productivity was weak, partly due to the high yeast concentration used in fermentation process. The highest value of 4.08 $g_{EtOH}/g_{cell}/h$ was observed at glucose concentration of 100 g/L. The value was slowly decreased with the increasing glucose concentration. The saturation constant (K'_S) and the substrate inhibition constant (K'_i) of 8.92 and 620.71 g/L were reported in this work. The high value of K'_i implying that fermentation can be carried out at high substrate concentration. The high value of K'_i implying that fermentation can be carried out at a high substrate concentration (200-300 g/L) in which the size of the fermenter

could be reduced associated with a high volumetric productivity.

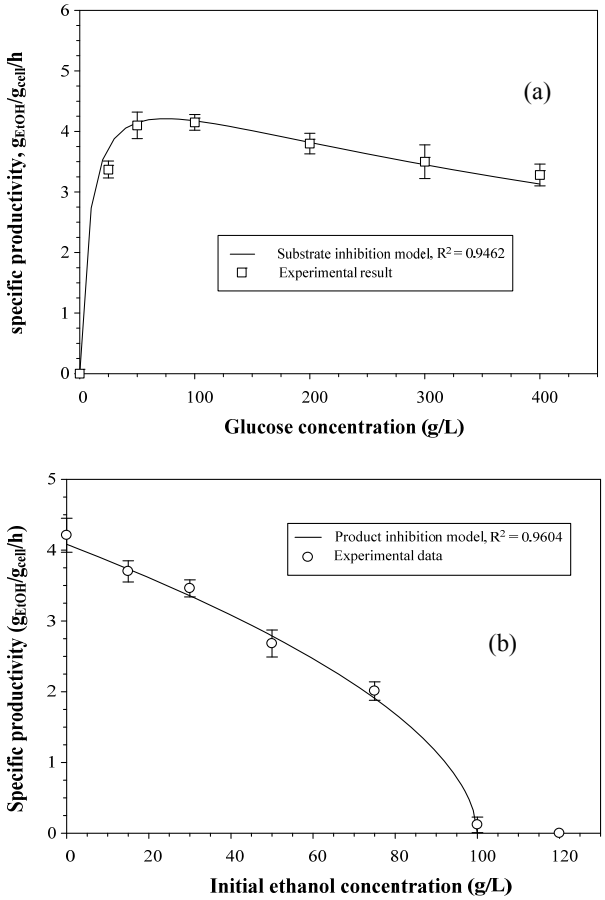

Fig. 2 Variation of the specific productivity as a function of the substrate concentration (a), and initial ethanol concentration (b)

TABLE I
EFFECT OF REFLUX TEMPERATURE ON DISTILLATE PROPERTIES

Temp. (°C)	Flow rate (kg/h)	Ethanol (wt%)	Water (wt%)	Impurities [a] (mg/L)
0	0.048	89.9	6.4	363.85
4	0.090	87.0	9.3	419.16
7.5	0.187	83.8	12.3	430.16
10	0.200	80.9	15.7	447.86
15	0.267	74.2	22.1	469.43
35	0.296	44.7	51.4	531.81

[a]Impurities in the distillate ethanol were the combination of propanol, butanol, iso-amyl alcohol, and n-amyl alcohol, respectively.

In contrary to substrate inhibition, the product inhibition effect of ethanol to fermentation performance was very sensitive. The specific productivity was investigated at various initial ethanol concentrations ranging from 0-120 g/L whilst the initial glucose concentration was fixed at 100 g/L. The specific ethanol productivity as a function of initial concentration is shown in Fig. 2 (b). Experimental data confirm that ethanol plays an important role on fermentation performance event at low concentrations. The maximum specific ethanol productivity was observed when none of ethanol was presented in the system. The value constantly decreased with the increase of initial ethanol concentration. At 75 g/L, the value reduced to approximately 50% and the value rapidly decreased to zero when the concentration approached 100 g/L. Almost no glucose consumption was also observed at this initial ethanol concentration. At 120 g/L ethanol concentration, there was no productivity and the experiment was not investigated beyond this concentration. The critical ethanol concentration (P'_m) refers to the concentration at which the fermentation performance is severely hampered. Therefore, the concentration of 100 g/L was set as P'_m in the (1). The correlation was best fitted with an *ai* value of 0.56 (R^2 = 0.9604). This model can be used to predict the inhibitory effect in a wide range of ethanol concentrations.

B. Effect of Partial Reflux Temperature

After a batch fermentation, the fermentation broth was also subjected to test for the vacuum fractionation experiment. The initial ethanol concentration in the feed was approximately 10 %wt. The purpose of this study was to determine the characteristic of distillate ethanol as the function of partial reflux temperature (T_2 of Fig. 1) using a vacuum fractionation technique. The boiling point of the fermentation broth was reduced to 35°C by decreasing the vacuum pressure to 70 mBar. The distillate ethanol was trapped with -30°C refrigerated bath and liquid nitrogen prior to analyse for its components. It is the fact that fermentation broth also contains several volatile impurities which can be presented in the distillate stream. These volatile impurities can be the group of aldehyde, higher alcohol, ester, and volatile organic acid, respectively. Changing in partial reflux temperature would affect the concentration of these compounds in the distillate ethanol product. The effect of partial reflux temperature ranging from 0-35°C on purity of the distillate ethanol was shown in Table I. It was found that the purity of the distillate ethanol was inversely proportional to the partial reflux temperature. The lower the temperature, the higher the purity obtained. At partial reflux temperature of 35°C, the result showed various impurities especially acetic acid (chromatogram not shown). This temperature was the same as in the fermentation broth; therefore, this distillate ethanol was not fractionated and almost represented the vapor-liquid equilibrium (VLE) between the liquid and gas phase. For the partial reflux temperature of 2.5°C, the chromatogram showed fewer impurities in comparison to the higher reflux temperature. It also showed that reducing the partial reflux temperature resulted in a decreasing in water concentration of the distillate ethanol as well as a reduction in mass flow rate. The mass flow rate of the distillate ethanol was inversely proportional to the ethanol concentration. For the temperature of 0°C, the flow rate of 0.048 kg/h, and ethanol concentration of 89.9 wt% were obtained. For the temperature of 35°C, the flow rate of 0.296 kg/h, and ethanol concentration of only 44.7 wt% were obtained. In addition, the amount of other impurities namely propanol, butanol, iso-amyl alcohol, and n-amyl alcohol were also significantly reduced at a lower partial

reflux temperature. The total amount of impurities at 531.81 mg/L was obtained, and this value reduced to 363.85 mg/L compared with the 0°C. In conclusion, fractionation of the distillate ethanol was effectively controlled with an optimal removal of vapor temperature resulting in a partial condensation of less volatile components. As a result, a high concentration of ethanol was obtained.

C. Batch Extractive Fermentation

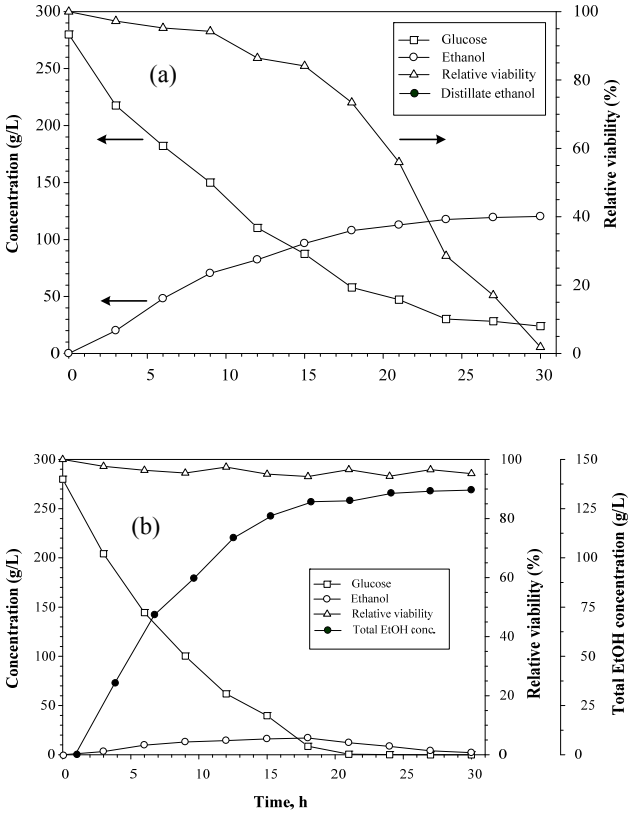

Fig. 3 Time course for glucose concentration, ethanol concentration, relative viability of yeast cells, distillate ethanol of batch fermentation (a), and batch extractive fermentation using vacuum fractionation technique (b)

From the experimental data of the fermentation kinetic, it is strongly advised that keeping the ethanol concentration at low level could results in high glucose consumption rate, high volumetric productivity, and low product inhibition effect to the yeast cells. Fig. 3 (a) illustrates the time course for glucose consumption, ethanol formation, and relative viability of the yeast cells during conventional batch fermentation. The concentration of glucose was rapidly decreased at the first 12 h of fermentation before the consumption rate gradually decreased. However, approximately 30 g/L of glucose still remained at the end of fermentation indicating that it was not completely consumed by the yeast cells. The decrease rate of glucose consumption was associated with the increasing ethanol concentration. The ethanol concentration rapidly increased at the first 15 h with a volumetric productivity of approximately 6.20 g/L/h. Subsequently, the value gradually increased until the maximum concentration of 119.7 g/L was reach corresponding to 93.6% of the theoretical yield. A decrease in volumetric productivity was observed at the ethanol concentration higher than 70 g/L. The main reason for a reduced product formation and substrate consumption rate was clearly due to the product inhibition effect as evidenced by the viability test. Since the ethanol was accumulated in the fermentation broth, the value of relative viability decreased since the fermentation was started. However, a sharp decrease was observed after 18 h where ethanol concentration higher than 100 g/L. At the end of fermentation process, there was no glucose consumption, ethanol formation, and most of the yeast cells lost their viability. For batch extractive fermentation using vacuum fractionation technique Fig. 3 (b), vacuum pressure was gradually applied to the system after 3 h of inoculation at the rate of 200 mBar/min until the value reached 65 mBar. Glucose concentration reduced constantly with the consumption rate of 26.6 g/L/h before it was completely consumed at 21 h of operation. This high consumption was attributed to continuous removal of the ethanol as the distillate. The average concentration of the distillate ethanol was 90% by weight and can be dehydrated without further distillation. The volumetric production was calculated at approximately 12.5 g/L/h and the value gradually reduced at the end of the process. This reduced rate of productivity was not a result of product inhibition, but it came from low glucose concentration in the system. In addition, the ethanol concentration in the fermentation broth was constantly low and never reached 25 g/L. When glucose was depleted, no more ethanol was produced and the ethanol concentration in the fermentation broth was still reduced to approximately 2.7 g/L. In conclusion, a recovery ratio of nearly 100% was obtained at the end of fermentation process. The total ethanol produced in this experiment was 268 g from 560 g of glucose (2 L of fermentation broth) which corresponds to 93.63% of theoretical yield. Although the value was in the same magnitude of batch fermentation, this system has advantages over conventional batch fermentation particularly the high relative viability of the yeast cells. More than 90% of the relative viability was observed through 30 h of operation. Some yeast cells were stained with the dye; however, the majority was still very active and some cells were in budding stage. This consequence indicated that more glucose can be added to produce more ethanol.

The boiling point of broth at 65 mBar was 35°C where phase separation of ethanol/water vapor occurred. Due to the low ethanol concentration in the fermentation broth, its concentration at the gas/liquid interface was only approximately 20-25% by weight. When this vapor mixture entered the partial reflux condenser, the cooling liquid flowing inside the condenser caused fractionation of the vapor mixture where excessive water was condensed before flowing back into the reactor. As a result, the volume of the fermentation broth was relatively constant and substantial amount of water can be conserved for subsequent fermentation process. The concentration of the distillate ethanol can be controlled solely by the controlling of the thermostat temperature. Among

various techniques for extractive fermentation of ethanol, pervaporation membrane bioreactor was the most studied system; however, intrinsic problems associated with separation performance of the membrane made this system not technically viable; for example, the separation must be carried out at the temperature of 30-35°C resulting in a substantial low permeation flux of ethanol, some other fermentation by-product especially organic acids reduced the separation factor [15], and most importantly the permeate concentration of ethanol is low especially at lower ethanol concentration in the fermentation broth. The permeate is then subjected to further distillation prior to dehydration processes. Unlike the other extractive fermentation system, the separation performance of this particular system is not the limited by the ethanol concentration in the fermentation broth. Therefore, the distillate ethanol can be dehydrated accordingly, and the total product could be dramatically reduced because the expensive plate columns can be ignored

D.Repeated-Batch Extractive Fermentation

The overall benefit from this extractive bioreactor was extremely positive in that it was a one stage integrated process. Separation of the target product could be obtained in a concentrated form, and could results in an increase of the product formation. From the previous experiment, more than 90% relative viability at the end of the batch extractive fermentation suggesting that more glucose can be added into the system. In order to avoid substrate inhibition effect, the initial glucose concentration of 250 g/L was used for each cycle. Fig. 4 shows the time courses of glucose concentration, ethanol concentration, mass of distillate ethanol and relative viability during repeated-batch extractive fermentation. After 3.0 h for the addition of glucose and inoculation of yeast cells, the extractive fermentation was started. Experimental data showed that glucose concentration decreased for the first 15 h, and the consumption rate gradually decreased until glucose was completely consumed after 24 h. The produced ethanol was continuously fractionated from the system at the initial rate of 25 g/h with the concentration of approximately 93% by weight. Experimental results also revealed a constant ethanol concentration in the fermentation broth below 20 g/L. When the glucose concentration was low, the ethanol removal rate exceeded the production rate resulting in a decreasing of the ethanol concentration. The second addition of glucose was carried out when the glucose concentration depleted. The vacuum condition of the system was stopped and the glucose powder was introduced through the feeding port. This time interval took approximately 5 minute before a vacuum condition was applied to the system again. Since glucose was added in the form of solid powder, volume change of the fermentation broth was negligible. The addition of glucose was repeated for another 8 times, and system was very stable for 230 h. The total ethanol concentration was obtained at 995.2 g/L. After the ninth time of addition; however, the fermentation performance was significantly reduced since glucose consumption was poor. The experiment ceased after 250 h when glucose concentration was constant and no ethanol was produced. The consequence was accompanied by a substantial decrease of relative viability of the yeast cells. Finally, the sample was centrifuged, filtered and analysed using HPLC. The experimental result showed that approximately 70 g/L of lactic acid was found and this could be the reason to the death of the yeast cells. It is evidenced that lactic acid is among by-products generated during ethanol fermentation [15]. In conclusion, this experiment showed that the long continuation of fermentation activity was obtained as long as the concentration of inhibitory products was kept low. A high purity of ethanol was produced more than 8-fold in comparison to the conventional batch fermentation.

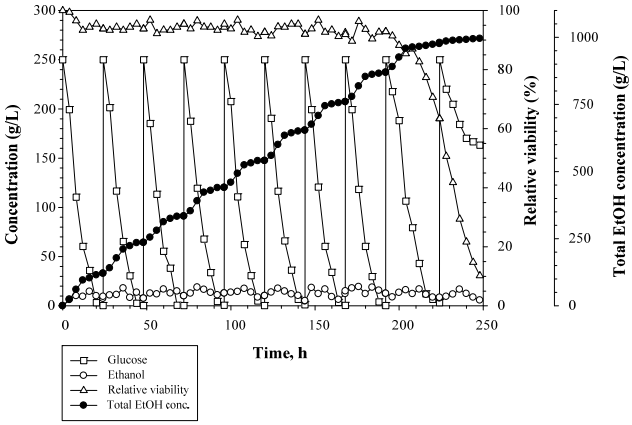

Fig. 4 Time course for glucose concentration, ethanol concentration, relative viability of yeast cells, and distillate ethanol during repeated-batch extractive fermentation experiment

E. Comparison between Vacuum Fermentation Techniques

Cysewski et al. investigated vacuum fermentation with cell recycling for continuous ethanol reduction [11]. The highest ethanol productivity of 82 g/L/h was obtained when the cell concentration reached 124 g/L. The ethanol concentrations in fermentation broth and in the condenser were 3.5 % by weight and approximately 30% by weight, respectively. Ghose et al. studied simultaneous saccharification and fermentation of lignocellulosic materials to ethanol under vacuum cycling and step feeding. Rice straw treated with cellulase and β-glucosidase was intermittently fed into the bioreactor. The ethanol productivity of 4.5 g/L/h was reported and the maximum ethanol concentration of 13.6% by weight was collected [12]. Lee et al. examined a vacuum fermentation of ethanol by using *Zymomonas mobilis*. A high productivity of 85 g/L/h was observed in the continuous cell recycle experiment. Condensate ethanol concentration was obtained for up to 40% by weight [13]. Nguyen et al. studied a continuous vacuum fermentation integrated with separation process. A fermentation-separation column was filled with yeast cells immobilized on bioreactors. During a quasi-steady state, volumetric productivity of 4.8 g/L/h and the average distillate ethanol concentration of 33.2% by weight were obtained [14]. For the repeated batch extractive fermentation presented in this work, an average productivity of 10.97 g/L/h and the average ethanol concentration of 93% by weight were

obtained throughout the 230 h operation. Unlike other works, the high concentration of ethanol obtained in this work requires no further distillation, and can be dehydrated directly in order to produce fuel grade ethanol.

IV. Conclusion

The extractive fermentation using a vacuum fractionation technique was successfully developed to have a great potential in enhancing the productivity of ethanol production process. The removal of 90% by weight ethanol from fermentation broth is the key to successful application of this approach. The integration of fermentation and separation process has a positive impact on the ethanol productivity. The high concentrate of ethanol removal was achieved by the controlling of the thermostat temperature (T_2) at 0^oC under pressure approximately 65 mbar for condensed excessive water of the rising vapor back into the bioreactor. In repeated-batch mode of extractive fermentation, a long operation time and a high ethanol yield were attributed to minimized product inhibition effect to the yeast cells. This particular system has advantages over conventional fuel ethanol process in term of simpler system design, longer life of the yeast, and lower water discharge. Still, cheaper raw materials feeding and more steady process operation should be further improved for a larger scale experiment.

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