BUTANOL RECOVERY FROM SYNTHETIC FERMENTATION BROTH VIA GAS STRIPPING

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Thesis submitted in partial fulfilment of the requirements
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ABSTRACT

ABE fermentation produces dilute acetone, butanol and ethanol products and required high energy consumption to separate the products during downstream process. In this study, gas stripping methods was used for butanol recovery process. Therefore, the objective of this experiment was to recover butanol from the synthetic fermentation broth via gas stripping based on the selected parameters. Two factorial design in Design-Expert® software was used to decide the range for each parameters selected. This research continued with the gas stripping experiment which was the main part for butanol recovery. Gas Chromatography (GC) with a flame ionization detector (FID) was used to analyze the samples. From the Design-Expert® software, the most contributing factor and interaction between the factors was analysed via two level factorial analysis. The ranking contribution factor of butanol recovery were butanol titre in feed > cooling temperature > stripping gas flow rate > feed temperature > gas stripping duration. Only butanol titre in feed (C) has negative effect meanwhile condenser cooling temperature (B) and interaction BC have positive effect on the butanol recovery. It is suggested to obtain the best condition for butanol recovery using Design-Expert® software testing on five factors related to gas stripping procedure which are: butanol titre in feed (20 g/L), feed temperature (60 ^{0}C), stripping gas flow rate (1 L/min), cooling temperature (-10 ^{0}C) and gas stripping duration (60 min). The results show that fractional factorial design is suitable in investigating the effect of large number of factors with a minimum number of experiments. Based on the experiment, the butanol recovery can be obtained until 88.18%. Thus, this project successfully achieved to recover butanol from the synthetic fermentation broth via gas stripping based on the predicted values of selected parameters.

ABSTRAK

Penapaian ABE menghasilkan cairan aseton, butanol dan produk etanol dan diperlukan penggunaan tenaga yang tinggi untuk memisahkan produk semasa proses hiliran. Dalam kajian ini, gas pelucutan kaedah telah digunakan untuk proses pemulihan butanol. Oleh itu, objektif eksperimen ini adalah untuk mendapatkan kembali butanol dari sup penapaian sintetik melalui pelucutan gas berdasarkan parameter yang dipilih. Dua reka bentuk faktorial dalam perisian Design-Expert® telah digunakan untuk menentukan julat bagi setiap parameter dipilih. Kajian ini diteruskan dengan pelucutan eksperimen gas yang merupakan bahagian utama untuk pemulihan butanol. Kromatografi gas (GC) dengan pengesan api pengionan (FID) telah digunakan untuk menganalisis sampel. Dari Desain-Expert®, faktor yang paling menyumbang dan interaksi antara faktor dianalisis melalui dua tahap analisis faktor. Faktor sumbangan ranking pemulihan butanol adalah butanol titre dalam makanan> suhu penyejukan> pelucutan kadar aliran gas> suhu feed> gas pelucutan tempoh. Hanya butanol titre dalam makanan (C) mempunyai kesan negatif sementara itu suhu kondenser penyejukan (B) dan interaksi BC mempunyai kesan positif ke atas pemulihan butanol itu. Adalah dicadangkan untuk mendapatkan keadaan yang terbaik untuk pemulihan butanol menggunakan pengujian perisian Design-Expert® kepada lima faktor yang berkaitan dengan gas pelucutan prosedur iaitu: butanol titer dalam makanan (20 g / L), suhu makanan (60 ^{0}C), pelucutan aliran gas Kadar (1 L / min), suhu penyejukan (-10 ^{0}C) dan gas pelucutan tempoh (60 min). Keputusan menunjukkan bahawa reka bentuk faktorial pecahan adalah sesuai dalam menyiasat kesan bilangan besar faktor dengan bilangan minimum eksperimen. Berdasarkan eksperimen, pemulihan butanol boleh diperolehi sehingga 88.18%. Oleh itu, projek ini berjaya mencapai pulih butanol dari sup penapaian sintetik melalui pelucutan gas berdasarkan nilai-nilai yang diramalkan parameter dipilih.

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LIST OF ABBREVIATION

ABE	Acetone butanol ethanol
DOE	Design of experiment
GC	Gas chromatography
FID	Flame ionization detector
OFAT	One factor at time
ANOVA	Analysis of variance
Spp	Species

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Butanol (butyl alcohol or n-butanol) is a saturated alcohol having the molecular formula of C_4H_9OH, has colourless liquid with a different odour and used as an intermediate in chemical synthesis. The production of butanol through biological routes has attracted increasing attention because butanol is highly requested in industries and the highest price in the chemical market (Green, 2011). In addition, Green (2011) found that butanol is a product chemical for paints, polymers and plastics. More attention is given to the alternative liquid fuels such as ethanol and butanol due to the global demand for fossil fuels rising. In fact, butanol has a potential as fuel substitute greater than ethanol in terms of chemical properties (Pang et al. 2016). Butanol is a chemical which has excellent fuel characteristics. It has a higher calorific value than ethanol, and a low freezing point (Qureshi and Blaschek, 2000). Butanol also has a better energy density and performance than ethanol that can fit the existing fuel infrastructure.

The ABE fermentation process is of interest for chemical or fuel production from renewable resources. There are many ways to produce ABE through fermentation by various *Clostridium* spp. (Qureshi et al. 2008) such as *C. acetobutylicum, C. beijerinckii, C.*

saccharobutylicum, and *C. saccharoperbutylacetonicum*. The most well-known strains used which are *C. beijerinckii* and *C. acetobutylicum* can help to improve the butanol tolerance and productivity (Nithyanandan et al. 2016). In China, the ABE fermentation process was started in the early 1950s and the total annual production of solvents reached 170,000 tons (Chiao and Sun 2007). Figure 1-1 shows the linked of synthetic butanol production costs and the price of crude oil in China during 2010.

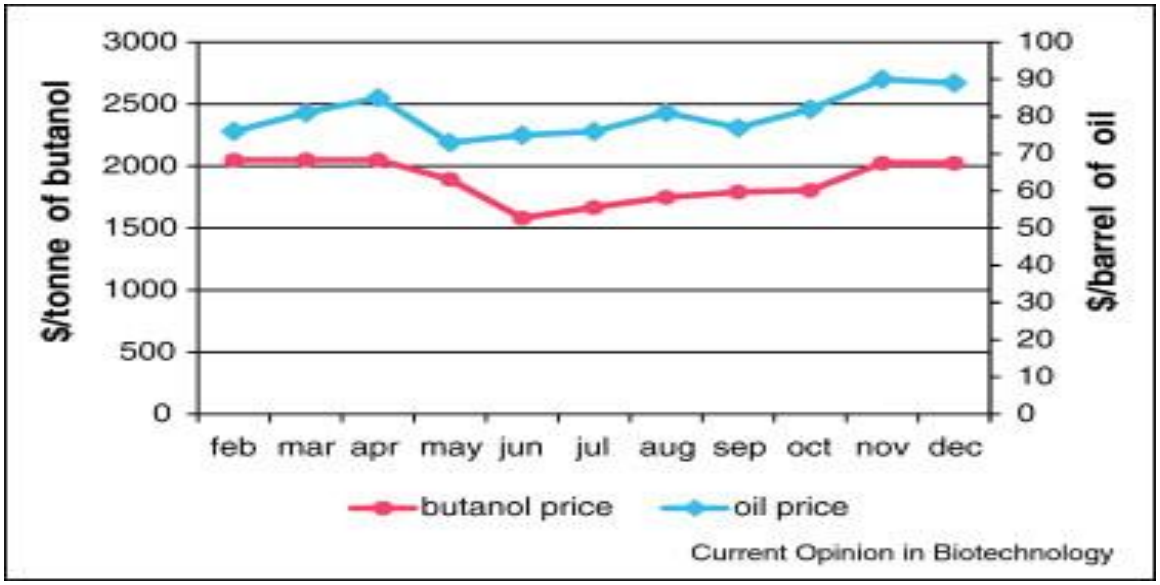

Figure 1-1: The relationship between crude oil and the synthetic butanol price on 2010 in China (Green, 2011)

However, the butanol is not currently produced by microbial fermentation over the years since the high cost of feedstock which is between 60% until 80% of the total production cost from corn-derived ethanol (Green, 2011). The ABE fermentation suffers from several limitations such as low product yields, low productivity, low final concentrations of products in the fermentation broth due to butanol toxicity, and high energy requirements to recover the products from the fermentation broth (Qureshi et al. 1992). Therefore, the gas stripping technique is used in removing selective products in order to solve the problem of butanol toxicity. Table 1-1 shows the concentration of ABE with and without product recovery. It clearly shown the butanol concentration increase when product recovery by stripping technique is applied.

Table 1-1: ABE fermentation with and without product recovery by stripping (Ezeji et al. 2007)

Parameters	Without product recovery (g/L)	With product recovery by stripping (g/L)
Acetone	4.3	7.7
Butanol	13.4	15.1
Ethanol	0.7	1.1

1.2 Motivation

There are many economically techniques in butanol recovery such as distillation, extraction, adsorption and pervaporation but the promising one is gas stripping. Gas stripping is an alternative technique which is less energy intensive to recover butanol from the fermentation broth. This is because gas stripping increased product concentration (Xue et al. 2014) and reduce butanol toxicity to cell. Generally, Liu and Fan (2004) found that gas stripping serves effectively in removing volatile components at the early stage of downstream processing. This pre-recovery method which is gas stripping seems as one of the more economic techniques than distillation, extraction, adsorption and pervaporation because it considers as an energy saving than distillation, extraction, adsorption and pervaporation (Xue et al. 2014). In the previous study, the application of gas stripping resulted in reduced butanol inhibition, thus it improves total solvent productivity and yield (Qureshi and Blaschek 2001).

Besides, most of the researcher used One-Factor-at-a-Time (OFAT) method. OFAT is a designing experiment method which involves only one factor studied at a time instead all factors simultaneously. Therefore, a design of experiments (DOE) is required in order to successfully analyse the test parameters of butanol recovery. According to Carton and Olabi (2010), the DOE randomly create the experiment run order output and statistically analyse the results. These results provide a clear relationship between the input parameters with the responses. On the other hand, the fractional factorial design is generally more efficient than conducting individual experiments on each factor (Collins et al. 2009).

1.3 Problem Statement

Theoretically, ABE fermentation via *Clostridium* spp. produces low product concentration and it is not easy to consume a high titer of butanol. Low butanol productivity from this process severely inhibits its potential industrial production (Yen and Li 2011). Hence, recovery of low product concentration has expensive cost as high amount of energy required during distillation process. It is expected to obtain the best condition for butanol recovery using Design of Experiment (DOE) throughout this work testing on five factors which are: butanol titer in feed, feed temperature, gas flow rate, cooling temperature and gas stripping period.

1.4 Objective

To recover butanol from the synthetic fermentation broth via gas stripping based on the selected parameters.

1.5 Scope of Study

The scopes are important in order to achieve the objective. These followings are the scope of the study:

1. Setting up the butanol recovery via gas stripping equipment.
2. Construct the experimental table run using Design-Expert® 7.1 software based on two-level factorial design with one centre point. There are five parameters in this study which are butanol titer in feed, the feed temperature, stripping gas flow rate, condenser cooling temperature and the period of gas stripping process. The ABE concentration at the collector and final feed are further analyzed as the responses.
3. Carry out the experimental run of butanol recovery process as suggested by DOE in the laboratory. Altogether there are 18 run numbers of experimental runs.

CHAPTER 2

LITERATURE REVIEW

2.1 Development of ABE Fermentation

Butanol is produced from the fermentation of carbohydrates in a process often referred to as the ABE fermentation, after its major chemical products are acetone, butanol and ethanol. Patakova et al. (2013) investigated the characteristics of butanol which it has high energy content per molecule, limited miscibility with water, low vapour pressure and less corrosive. Hence, butanol has a high potential to compete ethanol from the point of view of fuel. ABE production via clostridia has been widely studied especially during the oil crisis in 1973. This led to renewed interest in ABE fermentation from renewable resources and investigation into product recovery and the genetics of *Clostridium* species (Ezeji et al. 2004). In addition, it was found that in terms of thermal efficiency, ABE (6:3:1) might be much better suited for use as an alternative fuel compared to other different volumetric ratios which are ABE (3:6:1) and ABE (5:14:1) (Nithyanandan et al. 2016). Changing the ratio of the ABE components through fermentation can adjust the ABE fuel properties to suit internal combustion engine requirements. Moreover, the ratio of 6:3:1 is used as it helps in understanding the effect of increasing acetone and decreasing butanol. This statement is strengthened by providing the properties of fuel blends. Table 2-1 shows the fuel blends properties calculated using simple mixing rules. A mixing rule is used for data smoothing and evaluation. According to Huron and Vidal (1979), mixing rule also gives good data correlation and sometime avoiding false liquid-liquid immiscibility. Huang and Meagher (2001) discovered that even though the ABE

fermentation shows promise of industrial renewal, the recovery process by distillation from dilute fermentation broth makes the process uneconomic due to low product concentration.

Table 2-1: Properties of fuel blends (Nithyanandan et al. 2016)

Fuel type	Specific gravity (kg/m^3)	Lower heating value (MJ/kg)	Energy density (MJ/I)	Stoichiometric air/fuel ratio	Butanol (vol %)	Acetone (vol %)
Gasoline	0.739	43.44	31.68	14.65	0	0
ABE (6:3:1)	0.796	30.3	24.1	9.94	30	60
ABE (3:6:1)	0.802	31.45	25.22	10.36	60	30
ABE(5:14:1)	0.804	31.93	25.67	10.64	70	25
n-Butanol	0.81	33.1	26.81	11.06	100	0

2.1.1 Low Product Concentration via ABE Fermentation

Generally, anaerobic fermentation processes for production of fuels and chemicals, including ABE fermentation usually; suffer from a number of serious limitations including low yields, low productivity, and low final product concentration (Minton and Clarke 2013). According to Qureshi et al. (1992), an approach to solve these problems is to recycle the fermenter effluent to the fermenter, thus allowing residual sugar to be converted to product. Unfortunately, the ABE fermentation suffers from severe product inhibition so that product concentrations rarely exceed 20 g/L. Thus, recycling will be successful only if it is coupled to an effective product recovery technique to remove the inhibited products. The low product concentration affects the economics of recovery of the solvents from dilute fermentation broth by distillation, making the process unable to compete with the petroleum-based products.

2.1.2 Comparison of ABE Fermentation with Different Recovery Processes

Generally, several studies have been done on ABE fermentation with different product recovery processes. Table 2-2 shows the performance of different recovery processes which are liquid-liquid extraction, pervaporation, perstraction and gas stripping. Each *in situ*

recovery system has advantages and disadvantages that need to be examined thoroughly before incorporating with production systems at the industrial level.

Table 2-2: Performance of recovery process (Lee et al. 2008; Groot et al. 1989)

Recovery Technique	Efficiency	Capacity	Selectivity	State of Development	Scale	Operating Cost
Liquid-liquid extraction	High	High	High	Research	Laboratory	Medium
Pervaporation	High	Moderate	Moderate	Developed	Pilot	High
Perstraction	High	High	High	Research	Laboratory	Medium
Gas stripping	Medium	Moderate	Low	Research	Laboratory	High

2.2 Solvent Pre-Recovery Process

In order to produce butanol economically, the producing strain should be improved to produce a higher concentration of butanol. Otherwise, the concentration of butanol can be increased by choosing the right method of purifying products at downstream processing (Liu and Fan 2004). There are many methods for solvent recovery such as liquid-liquid extraction (Roffler et al. 1987), pervaporation (Fried1 et al. 1991), perstraction (Matsumura and Märkl 1987) and gas stripping (Qureshi, 2010). Figure 2-1 shows the integrated systems for fermentation and solvent recovery

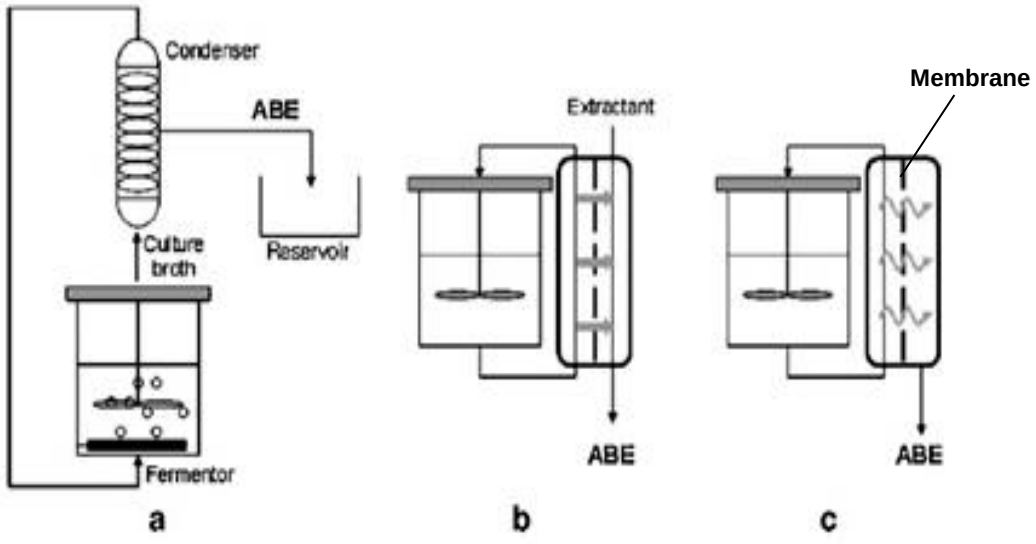

Figure 2-1: Integrated systems for fermentation and gas and solvent recovery: (a) gas stripping, (b) liquid-liquid extraction, (c) pervaporation (Lee et al. 2008)

2.2.1 Liquid-Liquid Extraction

Liquid-liquid extraction is a process where compounds are separated based on their relative solubility in two different immiscible liquids. A substance is extracted from one liquid phase into another liquid phase (Figure 2-2). This solvent extraction is used in nuclear reprocessing, the production of fine organic compounds, the processing of perfumes and other industries (Sahu et al. 2016).

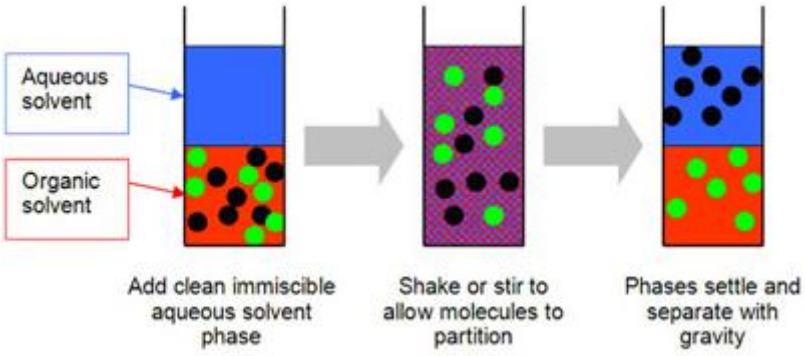

Figure 2-2: Principle of liquid-liquid extraction (Qureshi and Maddox, 1995)

Butanol removal by liquid-liquid extraction from the fermentation broth can be considered to be an important technique. Usually, a water-insoluble organic extractant is added to the fermentation broth and this causes the extraction of butanol from the aqueous into the organic phase because butanol is more soluble in the extractant. Oleyl alcohol is the extractant of choice among researchers because it is non-toxic and a good extractant. After butanol extraction the fermentation broth and the extractant can be easily separated because they are not immiscible. A big advantage of this method is the fact the substrates, water or nutrients are not removed during extraction hence it has high selectivity. However, liquid-liquid extraction has several problems such as loss of extraction solvent, extractant toxicity towards producing cell, formation of an emulsion and accumulation of cells in the extractant and fermentation broth interphase. A schematic diagram of ABE fermentation with integrated liquid-liquid extraction is shown in Figure 2-3.

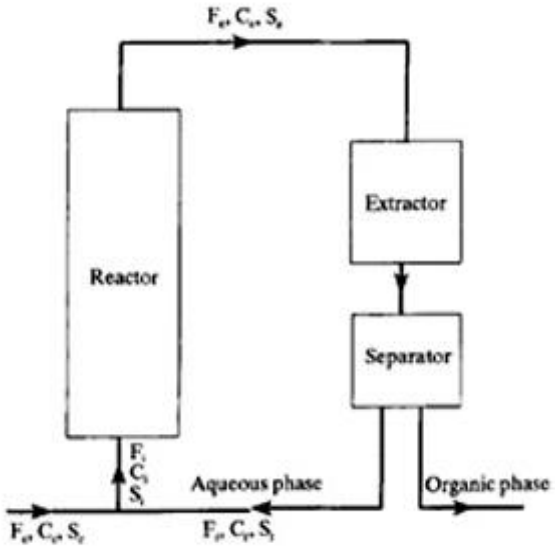

Figure 2-3: Schematic diagram of an integrated liquid-liquid extraction (Qureshi and Maddox, 1995)

2.2.2 Pervaporation

Pervaporation is another simple separation method of liquid mixtures by partial vaporization through a non-porous or porous membrane. Pervaporation is commonly used for the removal of organics from aqueous streams, for dehydration of organic solvents and for separation of heat sensitive products. In combination with ABE fermentation the membrane is placed in contact with the fermentation broth and the volatile or organic component selectively diffuses through the membrane as a vapour. There are two parameters in crucial factors for effectiveness of pervaporation which are selectivity (a measure of the selective removal of volatiles) and flux (the rate at which an organic/volatile passes through the membrane per m^2 membrane area). The main advantage of pervaporation is the major potential to save energy and has high selectivity. However, if the supply contains suspended matter or dissolved salts, then membrane failing may be encountered. Diagram of butanol recovery by pervaporation in fed-batch reactors is given in Figure 2-4.

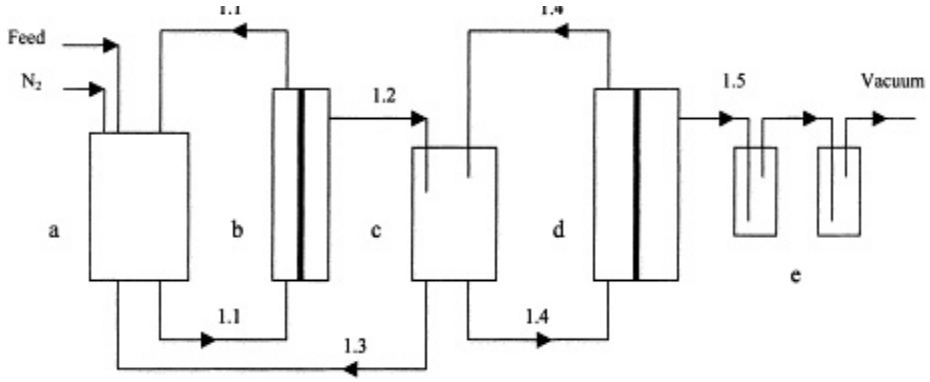

Figure 2-4: A schematic diagram of ABE production in fed-batch reactor and recovery by pervaporation. (a) fermentation reactor; (b) ultrafiltration membrane unit; (c) buffer tank; (d) prevaporation membrane unit; (e) cold traps (Fried1 et al. 1991)

2.2.3 Perstraction

Perstraction is a process which a liquid feed contacts one side of a nonporous membrane, so that a portion of the feed selectively dissolves into and diffuses across the membrane. The permeate molecules are removed from the second side of the membrane by sweeping the surface with a fluid that does not contain the permeated species. In this case, fermentation broth and extractant are separated by a membrane and the membrane provides surface area where the two immiscible phases can exchange butanol. Between the two phases is no direct contact so all the named problems are drastically reduced or eliminated. Butanol would diffuse across the membrane while fermentation intermediates and other components are retained in the aqueous phase. The membrane presents a physical barrier so it could be a limit to the rate of butanol extraction. This technique has high selectivity since butanol is more soluble in the extractant than in the fermentation broth, it is selectively concentrated in the extrant. There are also certain shortcomings in perstraction due to the toxicity of extractant to the cells and emulsion formation.

2.2.4 Gas Stripping

The principle of gas stripping is the removal of the desired solvents through bubbling gases. Gas stripping is one of the simplest techniques to recover butanol from fermentation broth because it does not employ any expensive equipment. Gas stripping is normally used for waste water treatment or crude oil processing, but it is also an applicable technique for in situ butanol recovery during ABE fermentation. Gas stripping was found an attractive process option to improve both solvent titre and productivity (Green 2011). Many past researcher

claimed the gas stripping serves effectively in removing volatile components at the early stage of downstream processing (Liu and Fan 2004) and even simpler than simple distillation. The fed-batch with gas stripping has the potential to reduce at least 90% of energy consumption and water usage in butanol production from glucose when compared to conventional ABE fermentation. Gas stripping is an easily implemented and effective in recovery technique for butanol because it requires only a carrier gas to be sparged through the fermentation media. The hydrogen (H_2) and carbon dioxide (CO_2) gases are used in gas stripping to remove butanol. The gases are bubbled through the fermenter and then cooled in a condenser. As a result, bubbling through the fermentation broth ABE is captured and consequently condensed and collected in a receiver vessel. The cleaned gas is recycled back to the fermenter. Figure 2-5 shows three different possible applications of simultaneous fermentation and product recovery by gas stripping.

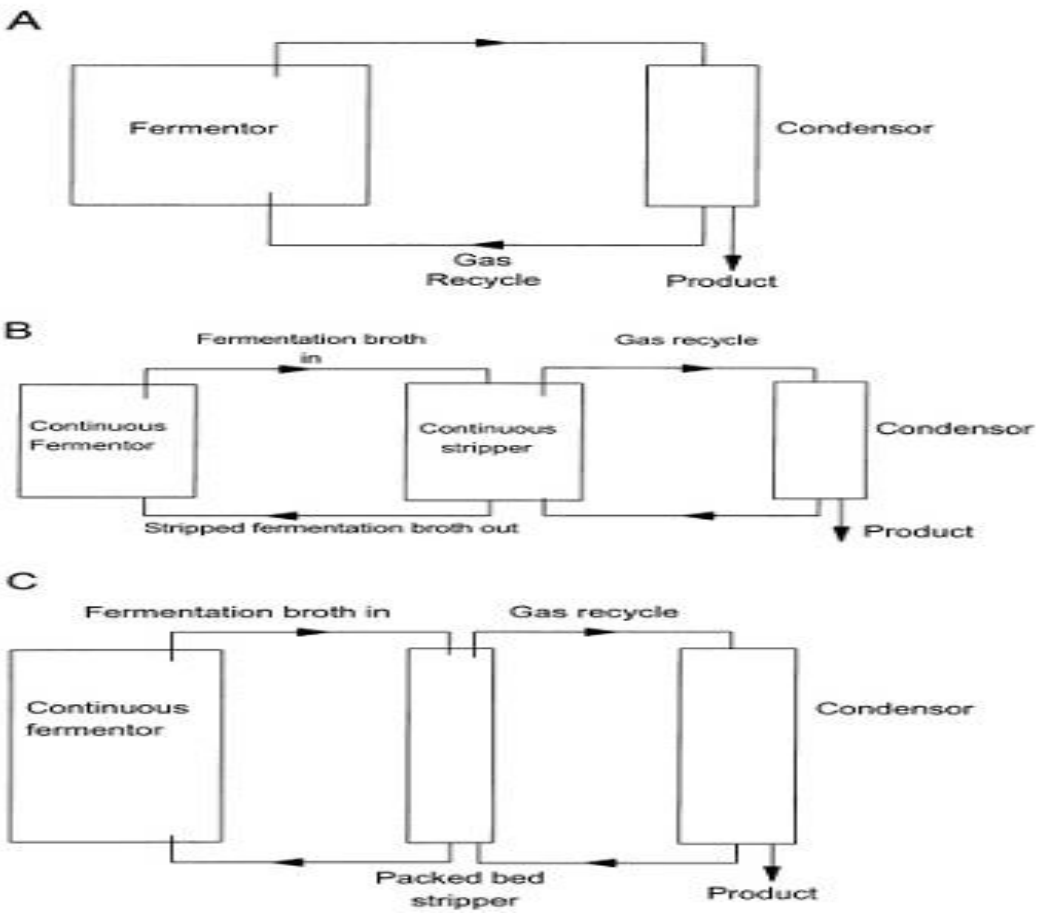

Figure 2-5: Schematic diagrams of butanol/ABE removal from fermentation broth by gas stripping. (A) Removal from the fermenter, (B) removal using a separate stripper, (C) removal using a separate packed-bed stripper (Qureshi and Blaschek, 2001)

2.3 Experimental Design by Design of Experiment (DOE)

One-Factor-at-a-Time (OFAT) approach is very intuitive and popular in companies across the globe when it comes to fixing complex quality issues. Parameters are changed and tested one at a time until the problem is fixed. In a Design of Experiments (DOE), the approach is completely different which all the parameters settings are changed together simultaneously. Therefore, it is better to perform DOE rather than OFAT because DOE also extremely useful in industrial research and development application. The application of a two-level factorial in design method made possible a fast and economical optimization of a separation and pre-concentration system based on solid phase extraction (Soylak et al. 2005). According to Holland and Cravens (1973), factorial design provides information on interaction among all variables and it is efficient because a maximum amount of information is obtained with a minimum number of experimental runs. Since there is no study yet being done by any researchers about using DOE in gas stripping, therefore the efficiency of DOE can be proofed by obtaining the best condition for butanol recovery in this work.

2.3.1 Advantages of DOE over OFAT

There are many benefits of using DOE for experimental design. Firstly, DOE can compare averages to other averages rather than individual values to other individual values. This allows the researcher to reach a much greater level of accuracy in the effect estimates for a given number of trials. Since experimental designs provide much more accurate estimates, this may also be helpful to compensate for a lack of accuracy in the measurement system, for example. In a standard experimental design, each factor is associated as many times with other factors at each level to provide a balanced combination levels. This enables one to estimate the effects of each factor independently. Thus, the estimations are not biased by other effects from other factors. In this study, it is essential to obtain the best condition for butanol recovery using DOE.

CHAPTER 3

MATERIALS AND METHODS

3.1 Overview

The schematic structure of the whole working process of this study is shown in Figure 3-1. The materials used for this experiment are acetone, butanol, ethanol, ethylene glycol and methanol. All the chemicals used are analytical grade and purchased from Sigma. Firstly, the range for each parameters selected will be decided beforehand by using two factorial design in Design-Expert® software and the response on solvent concentration will be investigated during the experiment. The parameter decisions are feed and cooling temperature, butanol titre in feed, gas flow rate and gas stripping period. The range selected parameters for feed temperature is 25 until 60°C, cooling temperature (-10°C - 15°C), butanol titre in feed (4 g/L - 20 g/L), gas flow rate (1 L/min - 5 L/min), and gas stripping period (20 min - 60 min). The maximum and minimum values of selected factors were determined as these values are necessary parameters in DOE. The equipment for gas stripping experiment will be set up in the laboratory. This research continued with the gas stripping process which is the main part for butanol recovery. The samples will be analyzed using Gas Chromatography (GC) prepared with a flame ionization detector (FID). The last step will be the experimental validation run. It is to confirm and to find the best condition to get high recovery of butanol.

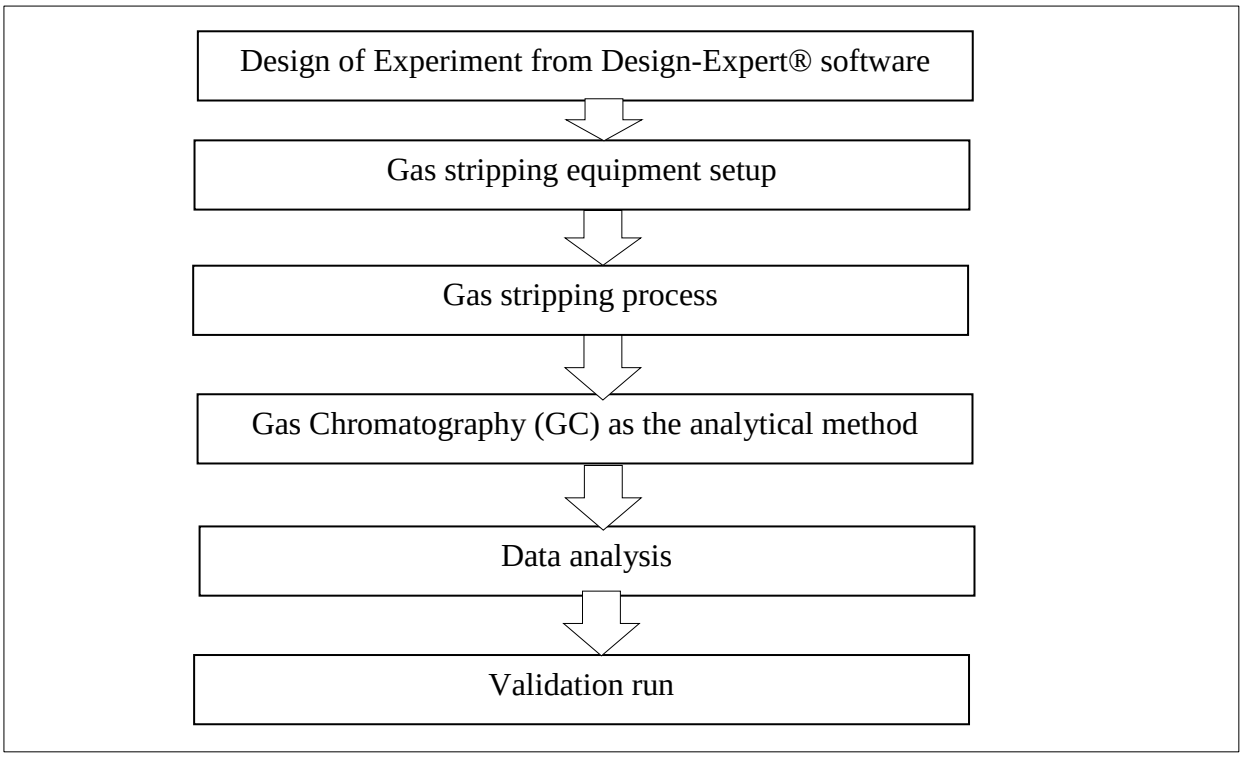

Figure 3-1: Flowchart process of the experiment

3.2 Design of Experiment (DOE) Method

Design of experiment theory is broadly related to the general theory of statistics and the general problem of experimental inference (Kempthorne and Oscar 1952). The experimental design was constructed using Design-Expert® software based on two-level factorial design of response surface methodology (RSM). The used of this method is to determine the influence of several factors on the response. During the process, non-significant variables were eliminated and minimum run of experiment were also provided. The factors selected for this study were feed and cooling temperature, butanol titer in feed, gas flow rate and gas stripping period while the response is the concentration of butanol condensed at the collector. According to Xue et al. (2014) the range of selected factors for feed temperature was 25 until 60°C, cooling temperature (-10°C - 15°C), butanol titre in feed (4 g/L - 20 g/L), gas flow rate (1 L/min - 6 L/min), and gas stripping period (20 min - 60 min). The maximum and minimum values of selected factors were determined as followed in Table 3-1 while center point will be resolved by the software. A total of 18 runs of experimental runs were suggested by using DOE as in the Table 3-2 and these values will be used in the experiment.

Table 3-1: Maximum and minimum values of selected factors

Parameter	Nomination in DOE	Minimum	Maximum
Feed temperature (^{o}C)	A	25	60
Condenser cooling temperature (^{0}C)	B	-10	15
Butanol titre in feed (g/L)	C	4	20
Gas flow rate (L/min)	D	1	5
Gas stripping duration (min)	E	20	60

Table 3-2: Experimental run suggested by using DOE software

Run	Feed temperature (^{o}C)	Condenser cooling temperature (^{o}C)	Butanol titre in feed (g/L)	Gas flow rate (L/min)	Gas stripping duration (min)
1	25.00	-10.00	20.00	1.00	20.00
2	60.00	-10.00	4.00	5.00	60.00
3	25.00	15.00	4.00	1.00	20.00
4	60.00	15.00	20.00	5.00	60.00
5	60.00	15.00	4.00	1.00	60.00
6	42.50	2.50	12.00	3.00	40.00
7	25.00	15.00	20.00	5.00	20.00
8	60.00	15.00	20.00	1.00	20.00
9	60.00	-10.00	20.00	1.00	60.00
10	60.00	-10.00	20.00	5.00	20.00
11	25.00	-10.00	4.00	1.00	60.00
12	25.00	-10.00	4.00	5.00	20.00
13	60.00	15.00	4.00	5.00	20.00
14	42.50	2.50	12.00	3.00	40.00
15	25.00	15.00	4.00	5.00	60.00
16	25.00	15.00	20.00	1.00	60.00
17	25.00	-10.00	20.00	5.00	60.00
18	60.00	-10.00	4.00	1.00	20.00

3.3 Gas Stripping Experiment Setup

The gas stripping experiment were performed at the Faculty of Chemical and Natural Resources Engineering Laboratory, University Malaysia Pahang. The apparatus setting for gas stripping as shown in Figure 3-2. The first thing to do is to ensure all ports of the vessel on the hot plate was closely tight except one inlet for stripping gas and one exhaust for the condenser connection. The vessel was used to fill the synthetic fermentation broth ABE. The stripping gas (N_2) will be passed through the inlet whereas the outlet from the vessel was connected to a condenser with a dimension of 36 cm height x 4 cm width. The thermometer was used to check the feed temperature following temperature suggested by DOE experimental run. Where applicable, the heating knob on hot plate will be adjusted to compliment the target temperature. The condenser was attached to a conical flask as the stripped liquid and gaseous flow through the outlet of the vessel into the condenser and end up into the collector while all gasses (fermentation gases and stripping gas) were passed through the exhaust. An anti-freeze liquid (60% ethylene glycol in water) flows into the condenser through its cooling coil to ensure the cooling temperature following the target temperature (-15°C, 2.5°C, 15°C). The cooling temperature was controlled by the chiller connected to the cooling coil of the condenser.

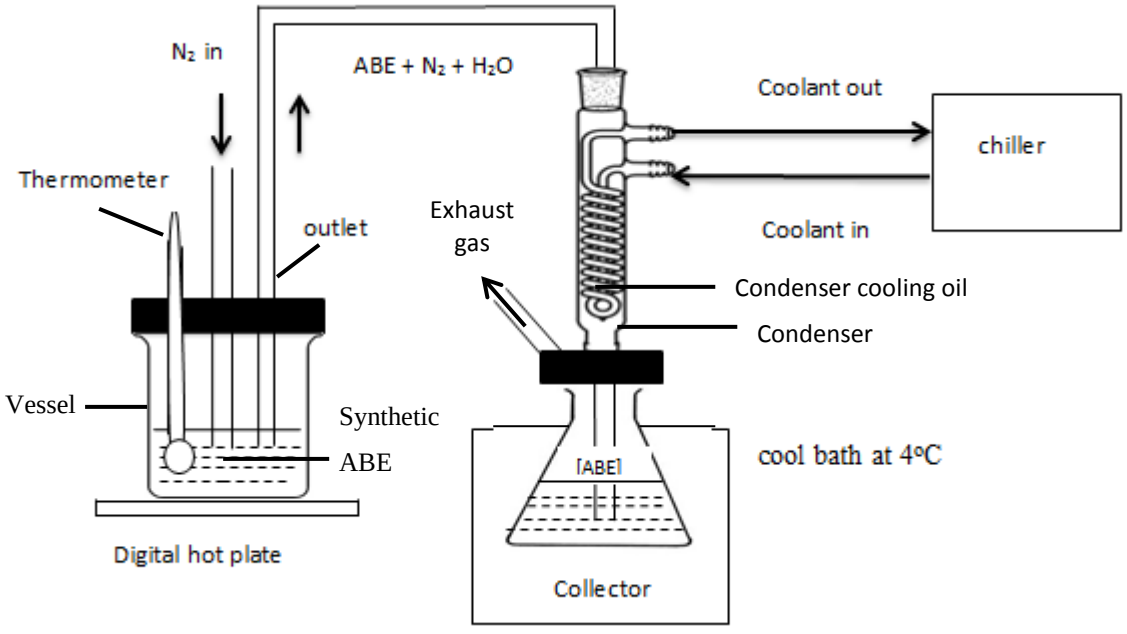

Figure 3-2: Schematic diagram of butanol recovery via gas stripping

3.4 Gas Stripping Process for Butanol Separation

The separation of butanol by gas stripping was started with the 500 mL glass vessel of synthetic fermentation broth with the butanol, acetone and ethanol ratio of 6:3:1. The total butanol in the broth was following concentration suggesting by DOE where the maximum will 20 g/L and minimum will 4 g/L. The solution contains of 20 g/L butanol at 25^{o}C with nitrogen air at a flow rate of 1 L/min was prepared for the first experimental run. The stripping gas passed through the condenser where a coolant with cooling temperature of -10^{o}C was allowed to cool the stripping gas. Next, the condensate was collected in a flask as the final feed. The gas stripping experiment steps were repeated following the experimental run generated by DOE software. Finally, the impacts of process parameters on solvent concentration at collector and final feed were investigated. The gas stripping performance was calculated based on the butanol recovery calculated using Equation 3-1.

$$\text{Butanol recovery: } \left|\frac{\text{initial solvent} - \text{final solvent}}{\text{initial solvent}}\right| \text{x } 100\%$$

Equation 3-1

3.5 Quantification of the solvent

The concentration of acetone, butanol and ethanol will be determined using gas chromatography equipped with a flame ionization detector (FID) and a 60 m fused silica column (HP-Innowax, 0.25 μm film thickness and 0.25 mm ID). An internal standard method using 10 g/L of methanol was mixed at 1 to 1 ratio with the sample's supernatant liquid. The operating GC occurs at 220^{o}C injection temperature with 1 μm of mixture was injected. The initial column temperature was set at 150^{o}C for 20 min, and then increased at a constant rate which was 15^{o}C/min to 180^{o}C with a 15 min final hold.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Design of experiment for factorial analysis

The experimental design for factorial analysis was done by using Design Expert Software. The two-level factorial design was used to analyze five factors that were found to be affecting the butanol recovery from synthetic fermentation broth. These factors were feed temperature (A), condenser cooling temperature (B), butanol titer in feed (C), stripping gas flow rate (D) and the period of gas stripping (E). This factorial analysis was carried out at certain ranges of value that obtained from the other's. The design of experiment was applied at 2^5 full factorial design (FFD). This design suited for factorial analysis because it allowed the investigation of large number of factors at the initial of experiment. In FFD, it has the advantages to identify the significant factors with minimum number of experiments (Chang et al. 2011). It also can determine the effect for the main factors and the interactions effect between the factors (Golshani et al. 2013). From the design, 18 runs of experiments were generated. The sequence of experiments was randomized in order to minimize the experimental error and the effects of uncontrolled factors. Table 4-1 shows the experimental design and the result of butanol concentration at collector for factorial analysis.

Table 4-1: Experimental design and butanol concentration for factorial analysis

Run	A	B	C	D	E	Butanol concentration, [B] (g/L)
1	25	-10	20	1	20.00	11.82
2	60	-10	4	5	60.00	3.50
3	25	15	4	1	20.00	3.89
4	60	15	20	5	60.00	8.08
5	60	15	4	1	60.00	2.42
6	42.5	2.5	12	3	40.00	8.71
7	25	15	20	5	20.00	5.93
8	60	15	20	1	20.00	7.93
9	60	-10	20	1	60.00	12.97
10	60	-10	20	5	20.00	9.76
11	25	-10	4	1	60.00	3.89
12	25	-10	4	5	20.00	3.77
13	60	15	4	5	20.00	1.90
14	42.5	2.5	12	3	40.00	5.48
15	25	15	4	5	60.00	1.91
16	25	15	20	1	60.00	4.75
17	25	-10	20	5	60.00	10.50
18	60	-10	4	1	20.00	3.56

Based on the Table 4-1, the result showed the lowest butanol concentration was 1.90 g/L at this following condition: butanol titre in feed (4 g/L), feed temperature (60 ^{0}C), stripping gas flow rate (5 L/min), cooling temperature (15 ^{0}C) and gas stripping duration (20 min). Meanwhile the highest concentration was 12.97 g/L at this following condition: butanol titre in feed (20 g/L), feed temperature (60 ^{0}C), stripping gas flow rate (1 L/min), cooling temperature (-10 ^{0}C) and gas stripping duration (60 min).

4.2 Statically modelling and ANOVA for factorial analysis

The independent and dependent variables were analysed to obtain the regression model for linear equation. From the equation, the factors can be determined whether it gave positive or

negative effect to the butanol concentration at collector. The equation obtained from this analysis as shown in Equation 4-1.

$$[B] = 6.03 + 0.23A - 1.44B + 2.93C - 0.37D - 0.031E + 0.49AC + 0.51AE - 0.86BC + 0.36DE$$

Equation 4-1

Where A, was feed temperature, B was condenser cooling temperature, C was butanol titer in feed, D was stripping gas flow rate and E was the period of gas stripping. A, B, C, D and E were referred as the main effects while AC, AE, BC, and DE were the interaction effects involves in the butanol recovery process by gas stripping. When the coefficient of main factors gave the positive values, it showed positive impact which is increased butanol yield while negative values showed the negative impact (Chang et al. 2011). In this study, the factors which were condenser cooling temperature (B) gave negative effect on the butanol concentration. The stripped butanol concentration was decreased when the cooling temperature changed from -10 ^{0}C to 15 ^{0}C. However, the butanol titre in feed (C) showed the positive effect. When the butanol titre in feed changed from 4 g/L to 20 g/L, the stripped butanol concentration at the collector increased.

The analysis of variance (ANOVA) was done to determine the significance of the model. Table 4.2 shows the results of ANOVA. The significance of a regression equation was checked by using F-values while the p-value was used to check the significance of each coefficient (Wang et al. 2012). The p-value tests the null hypothesis that data from the experiment with the identical means. If the p-value was less than 0.05, the null hypothesis was rejected. The null hypothesis was failed to reject when the p-value higher than 0.05. From the ANOVA, the F-value for the model was 17.47 and the p-value was 0.0005. The corresponding coefficient was more significant when the p-value is small (Zou et al. 2011). Besides, the p-value for B (condenser cooling temperature), C (butanol titer in feed), D (gas flow rate) and interaction BC showed value less than 0.05. It indicated the contribution of the model was significant (Wang et al. 2012). The F-value for B, C, and BC were higher than other factors. It showed these factors gave the strong effect on the butanol recovery. The R-squared (R^2) from the ANOVA was used to indicate how close the data to the fitted regression line. R^2 should more than 80% for a good fitting model (Karazhiyan et al. 2011).

The R^2 obtained from factorial analysis was 95.74% which shows that the model was a good fit.

Table 4-2: ANOVA for factorial analysis

Source	Sum of Square	Df	Mean square	F value	p-value Prob > F	
Model	195.58	9	21.73	17.47	0.0005	Significant
A	0.85	1	0.85	0.68	0.4356	
B	33.08	1	33.08	26.59	0.0013	
C	137.66	1	137.66	110.66	<0.0001	
D	2.14	1	2.14	1.72	0.2307	
E	0.016	1	0.016	0.013	0.9140	
AC	3.79	1	3.79	3.05	0.1244	
AE	4.16	1	4.16	3.34	0.1101	
BC	11.79	1	11.79	9.48	0.0178	
DE	2.08	1	2.08	1.67	0.2369	
Residual	8.71	7	1.24			
Cor Total	206.28	17				

Values of "prob>F" less than 0.05 indicate model are significant.

4.3 Main effect for factorial analysis

One of the aspects that was studied in the factorial analysis was the main effect analysis. This analysis was studied in order to determine the factors that most contributed to the butanol recovery by gas stripping. Table 4-3 shows the contribution of each main factor to butanol recovery.

Table 4-3: The contribution of main factors to butanol recovery

Factor	Contribution, %
A	0.41
B	16.04
C	66.74
D	1.04
E	0.007566

From the Table 4-3, factor C (butanol titer in feed) proved to be the most contributing factor with 66.74% with positive effect. Positive effect means as the factor contribution increased from minimum to maximum value, the response will also increase. In this case, as the butanol titer in feed increased from 4 g/L to 20 g/L, the respond which is the butanol recovered, also increased. This seems legit as butanol titer in feed play an important part for the butanol recovery by gas stripping. Generally, when butanol titer in feed solution increased, efficiency of gas stripping increased due to less amount of water being stripped with butanol. Besides, more butanol existed in the feed solution also can increase the mass transfer of butanol. Butanol has a strong polar solvent cause strong cohesion effect on water due to strong hydrogen bonding. The coupling effect on stripping between water and butanol during the absorption on the surface of gas bubble can be increased by having a higher concentration of butanol in the feed solution (Xue et al. 2014). Thus, in order to obtain the high concentrated butanol in condensate and full energy saving potential of gas stripping, it is important to conduct gas stripping at a butanol titer higher than 4 g/L in the feed solution.

Another factor that showed high contribution was factor B (condenser cooling temperature) with 16.04 % contribution with negative effect. Negative effect means as the factor contribution increased from minimum to maximum value, the response will do inversely. In this case, as the condenser cooling temperature increased from -10 ºC to 15 ºC, the respond which is butanol recovered increased. This factor was proven to be one of the major factors that influence the butanol recovery. Lower cooling temperature would be better for condensing and recovering butanol vapor in a more concentrated state because of higher selectivity over water. In fact butanol is much easier to condense due to its lower vapor pressure (Oudshoorn et al. 2009).

Factor D (gas flow rate) showed low contribution with 1.04% negative effect. If more water was stripped off and condensed in the collector, the butanol in the condensate may be diluted at higher gas flow rate (Xue et al. 2014). Factor A (feed temperature) showed a contribution as much as 0.41% to the butanol recovery. It gave less effect on butanol recovery. Out of all factors involved, factor E (gas stripping duration) showed the lowest contribution on butanol recovery with only 0.007566% contribution.

4.4 Interaction between factor for factorial analysis

The interaction effect plot was generated to represent the result of the regression analysis. It was represented the deviation of the average between the high and low levels for each factors. The effect for the factor was positive when the butanol recovery increased as the factor change from low to high level. However, it was negative when the butanol recovery decreased from low to high level. When the lines of two factors were unparalleled, the factors were interacting. On the contrary, when the lines were parallel to each other, it shows there was no interaction between the factors (Chang et al. 2011). The significant interactions between the factors were showed in Figure 4-1 and 4-2.

Figure 4-1 shows the two-factor interaction plot between condenser cooling temperature (B) and butanol titer in feed (C). These plot clearly indicated that the interaction between condenser cooling temperature and butanol titer in feed (BC) was stronger than the others two-factor interaction. The interaction between BC gave high contribution for the butanol recovery at 5.72%. At high butanol titre in feed (C=20 g/L), butanol recovery significantly decreased as the condenser cooling temperature (B) increased. At low butanol titre in feed (C=4 g/L), butanol recovery slightly decreased as the condenser cooling temperature (B) increased. The amount of butanol condensed at the collector was 12.97 g/L and 8.08 g/L when the condenser cooling temperature at -10 °C and 15 ^{0}C respectively. Gas stripping was efficient when the butanol titer in the feed solution was more than 4 g/L. This will give the butanol recovery of 83.45% to 88.18%.

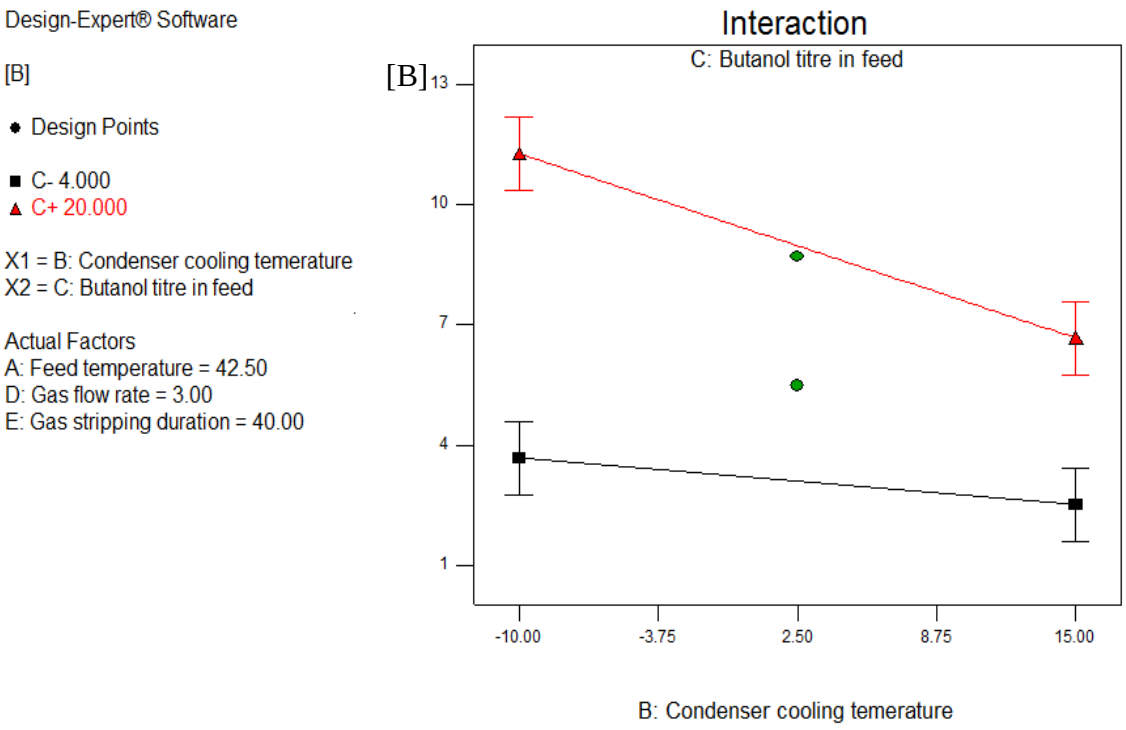

Figure 4-1: Two-factor interaction between B (condenser cooling temperature) and C (butanol titer in feed)

Figure 4-2 shows the second interaction which involved the factor A (feed temperature) and C (butanol titer in feed). The butanol titer in feed greatly affected the butanol recovery. At high butanol titre in feed (C=20 g/L), butanol recovery significantly increased as the feed temperature (A) increased. At low butanol titre in feed (C=4 g/L), butanol recovery slightly decreased as the feed temperature (A) increased. The butanol condensed at the collector was slightly increased from 7.93 g/L to 9.76 g/L when the temperature increased from 25 ^{0}C to 60 ^{0}C. This trend indicated that more water vaporized at the higher temperature resulted on diluted condensate (Lu et al., 2013). The butanol recovery was between 80.16 % to 82.51 %.

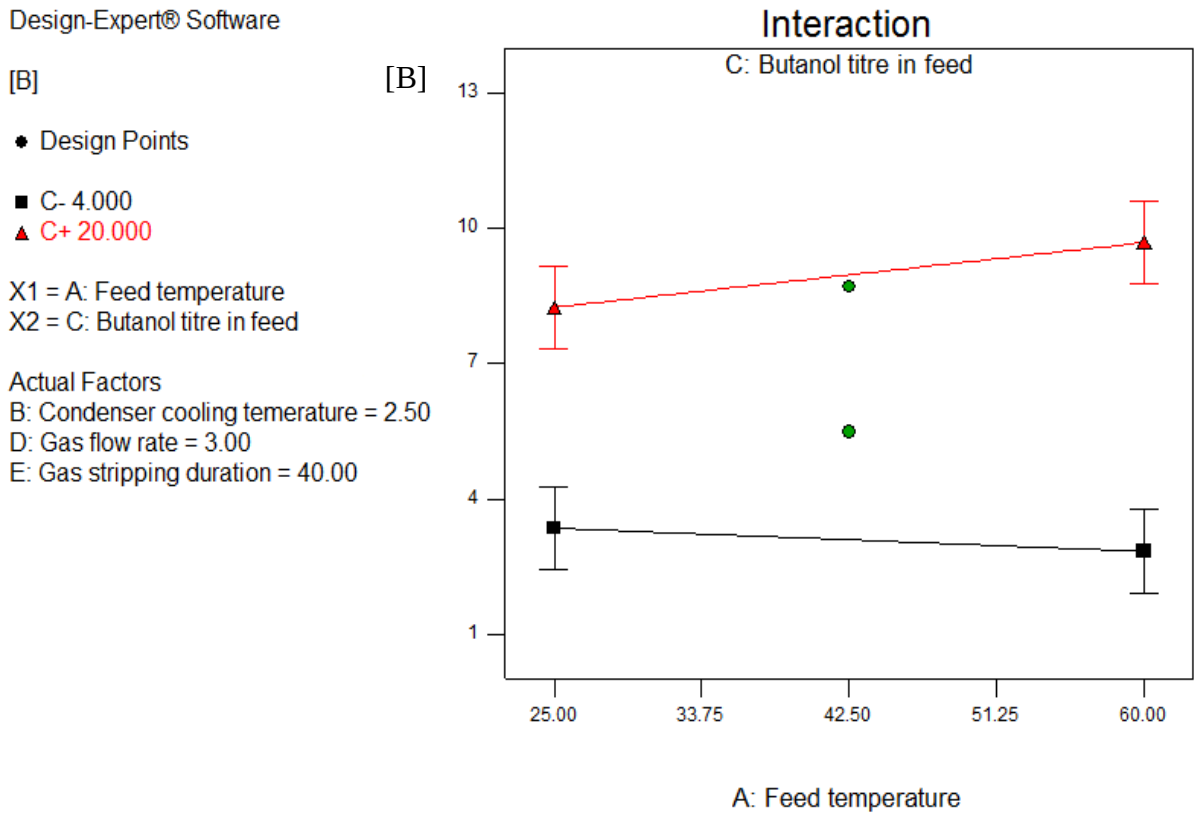

Figure 4-2: Two-factor interaction between A (feed temperature) and C (butanol titer in feed)

The main concern for this project is the amount of butanol that can be recovered from the synthetic fermentation broth by using gas stripping. The percentage of butanol recovery obtained was tabulated in Table 4-4.

Table 4-4: Percentage of butanol recovery

Run	Initial [B] at vessel, g/L	Final [B] at vessel, g/L	Butanol Recovery (%)
1	20	4.10	79.49
2	4	1.17	70.87
3	4	0.97	75.87
4	20	2.37	88.18
5	4	1.10	72.41
6	12	1.54	87.14
7	20	3.40	83.00
8	20	3.97	80.16
9	20	3.31	83.45
10	20	3.50	82.51
11	4	1.21	69.83
12	4	0.88	78.03
13	4	0.74	81.51
14	12	1.90	84.14
15	4	0.77	80.77
16	20	3.55	82.25
17	20	3.60	82.02
18	4	0.98	75.51

All the values in Table 4-4 were calculated from Equation 3-1. The butanol recovery percentage indicates the performance of gas stripping. The higher the percentage of butanol recovery, the more efficient of the gas stripping method. Based on the Table 4-4, the maximum of butanol recovery was 88.18%. Last but not least, it is important to obtain the best condition for butanol recovery using Design-Expert® software. This software testing on five factors related to gas stripping procedure and predicted the values which are: butanol titre in feed (20 g/L), feed temperature (60 °C), stripping gas flow rate (1 L/min), cooling temperature (-10 °C) and gas stripping duration (60 min). However, due to the time constraint, there are no verification results. It is believes that these predicted values will give a high amount of butanol condensed at the collector and thus giving high percentage of butanol recovery.

CHAPTER 5

CONCLUSION AND RECCOMENDATION

The process parameters of gas stripping including butanol titre in feed, feed temperature, stripping gas flow rate, cooling temperature and gas stripping duration were crucial for the performance of butanol recovery from ABE fermentation. Design-Expert® 7.1 software was used to construct experimental table where all the factors was randomized. From the same software, the most contributing factor and interaction between the factors was analysed via two level factorial analysis. The ranking contribution factor of butanol recovery were butanol titre in feed > cooling temperature > stripping gas flow rate > feed temperature > gas stripping duration. From the factors, only butanol titre in feed (C) was significant factor with negative effect meanwhile condenser cooling temperature (B) and interaction BC were significant factor with positive effect on the butanol recovery. Based on the predicted values from Design-Expert® software, it was suggested to obtain the high concentration of butanol at the collector, thus contribute to high butanol recovery. The results show that full or fractional factorial design is suitable in investigating the effect of large number of factors with a minimum number of experiments. Thus, the efficiency of gas stripping methods could be proved for butanol recovery process from synthetic fermentation broth.

As the recommendation, the validation run should be conducted to prove the reliability of the models proposed by Design-Expert® software. Using the same software, further study can be done to optimize the selected factor. Factors that can be selected for optimization are butanol titre in feed, condenser cooling temperature and gas stripping flow rate as these factors have high contribution. Optimization is a process of regulating the important factors so that the result becomes optimal. Therefore, the optimum condition for high butanol recovery from synthetic fermentation broth via gas stripping process can be achieved.

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