ACETIC AND BUTYRIC ACIDS FROM FERMENTED LEACHATE BY VARIOUS EXTRACTION METHODS : OPTIMIZATION AND RECOVERY STUDIES

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UNIVERSITI SAINS MALAYSIA

2018

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by

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Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

August 2018

ACKNOWLEDGEMENT

Foremost, I would like to say thousands of thanks to my supervisor Dr Husnul Azan Tajarudin for the support on my Master study, for his guidance, encouragement and immense knowledge. Besides my main supervisor, I would like to sincere thanks to my co-supervisor Mohd Firdaus Yhaya for the knowledgeable advice and experimental suggestion.

I would also like to thanks the lab assistance Mr. Azmaizan Yaakub, Madam Najmah Hamid and all staff in School of Industrial Technology, USM for the great guidance and assistance on the laboratory machines.

Furthermore, I would like to sincere gratitude the financial support from USM through RU grant (1001.PTEKIND.811262) and the Mybrain15 scholarship funded by Ministry of Higher Education (MOHE) Malaysia.

I want to thank my lab mates who are the members of lab 407 and all postgraduate students of Bioprocess Technology Division, who always offered their help and guidance on my experimental study.

Last but not least, I am deeply grateful to my father Mr. Razali Jamil, my mother Madam Rosmawati Kassim and siblings for their understanding and supporting in all aspects in my life. Here, special thanks to my husband, Mr. Muhammad Aminuddin Ahmad for his patient and support on keeping me motivated throughout the study.

Yasmin Syafikah Binti Razali

August 2018

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATPS	Aqueous two phase system
BOD	Biochemical oxygen demand
C.butyricum	Clostridium butyricum
CCD	Central composite design
CMA	Calcium magnesium acetate
COD	Chemical oxygen demand
EPA	Environmental protect agency
FT-IR	Fourier transform infrared
GC	Gas chromatography
LCFA	Long chain fatty acid
MCFA	Medium chain fatty acid
MIBK	Isobutyl methyl ketone
MSW	Municipal solid waste
MTBE	Tert-butylether
PAC	Powdered activated carbon
PEG	Polyethylene glycol
PLBS	Pulau Burung Landfill Sites
PTA	Purified terephthalic acid
RSM	Response surface methodology
SCFA	Short chain fatty acid

SSF	Solid state fermentation
TBP	Tri-n-butylphosphate
TOPO	Tri-n-octylphosphine oxide
USD	United States dollar
VAM	Vinyl Acetate Monomer
VFA	Volatile fatty acid
VLCFA	Very long chain fatty acid
XRD	X-ray diffraction
XRF	X-ray fluorescence

LIST OF SYMBOLS

±	About
µm	Micrometer
3D	Three dimensional spaces
ɑ	Alpha
C	Carbon
$CH_3CH_2CH_2COOH$	Butyric acid
CH_3COOH	Acetic acid
E	East
Ka	Acid dissociation constant
KBr	Kalium Bromate
kHz	Kilohertz
mol	Moles
mV	Millivolts
N	North
Na_2SO_4	Sodium sulphate
^oC	Degree celcius
Pb^{2+}	Lead (II) ion
rpm	Revolution per minute
ß	Beta
Ɵ	Theta

ASID ASETIK DAN ASID BUTIRIK DARI LARUTAN BAHAN LARUT LESAP MELALUI PELBAGAI PROSES PENGEKSTRAKAN : KAJIAN PENGOPTIMUMAN DAN PEROLEHAN

ABSTRAK

Asid karboksilik adalah penting kerana mempunyai aplikasi yang luas dalam industri seperti industri makanan, farmaseutikal dan wangian. Rawatan bahan larut lesap dengan menggunakan proses fermentasi *Clostridium butyricum* mengandungi asid lemak meruap (VFA) yang berharga iaitu asid asetik dan asid butirik. Oleh itu, kajian ini memberi tumpuan kepada perolehan VFA dari larutan bahan larut lesap selepas proses fermentasi. Pengekstrakan asid lemak meruap ini dari larutan bahan larut lesap selepas proses fermentasi menggunakan tiga kaedah pengekstrakan; sistem dua fasa akueus (ATPS), pengekstrakan pelarut dan penjerapan telah dioptimumkan dan dibandingkan untuk menentukan kaedah pengekstrakan terbaik. Penjerapan dengan menggunakan karbon teraktif merupakan kaedah pengekstrakan terbaik pada dos 19.79% dalam setiap 100 ml sampel selama 9.45 jam pada pH 3.0 dan kelajuan agitasi 180 rpm. Asid asetik dan asid butirik yang berjaya diekstrak adalah masing-masing 87.91 ± 1.3% dan 98.32 ± 2.5%. Sementara itu, melalui kaedah ATPS hanya 43.66 ± 2.3% asid asetik dan 73.50 ± 4.5% asid butirik diekstrak. Asid asetik dan asid butirik yang diekstrak melalui kaedah pengekstrakan pelarut adalah masing-masing 28.10 ± 0.9% dan 91.92 ± 1.3%. Selain itu, mekanisme proses penjerapan dianalisis dengan menggunakan pendarfluor sinar-x (XRF), belauan sinar-x (XRD), *Fourier-transform* inframerah (FT-IR) dan keupayaan zeta. 73.99% komponen karbon yang dikesan dengan menggunakan analisis XRF dan kemunculan struktur amorf dalam karbon teraktif selepas proses penjerapan menunjukkan bahawa proses penjerapan berlaku. Sementara itu, FT-IR dan keupayaan zeta memberi kesimpulan bahawa proses penjerapan VFA pada permukaan karbon teraktif adalah disebabkan oleh jerapan kimia yang merupakan ikatan kovalen. Tambahan pula, penjerapan isoterma untuk kedua-dua asid asetik dan butirik adalah mengikut model Langmuir dengan nilai koefisien penentuan (R^2) 0.9624 untuk penjerapan isoterma asid asetik dan 0.9658 untuk penjerapan isoterma asid butririk yang menunjukkan penjerapan ekalapis oleh VFA pada permukaan karbon teraktif. Ikatan jerapan kimia juga dibuktikan oleh penjerapan kinetik yang menunjukkan kedua-dua asid asetik dan butirik adalah model pseudo tertib kedua yang R^2 ialah 0.9873 untuk asid asetik dan 0.9978 untuk asid butirik. Perolehan asid asetik dan butirik daripada karbon aktifan telah dikaji dalam proses pengenyahjerapan. Penggoncang adalah alat yang terbaik untuk digunakan dalam proses pengenyahjerapan jika dibandingkan dengan vorteks, bekas air sonik dan prob sonik. Sementara itu, bahan pencair yang menunjukkan peratusan perolehan yang tinggi untuk asid asetik (54%) dan asid butirik (29%) adalah air dan etanol dengan menggunakan penggoncang. Proses multitahap pengenyahjerapan digunakan untuk meningkatkan perolehan VFA. Oleh itu, perolehan terakhir adalah 89.1% untuk asid asetik dan 67.8% untuk asid butirik.

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ABSTRACT

Carboxylic acids are important because of their wide application in the industries such as food, pharmaceutical and fragrance industries. Treatment of leachate is using fermentation process of *Clostridium butyricum* contains valuable volatile fatty acids (VFA) which is acetic and butyric acids. Thus, this research focuses on the recovery of acetic and butyric acids from fermented leachate. The extraction of these VFAs from fermented leachate by using three extraction methods; aqueous two phase (ATPS), solvent extraction and adsorption were optimized and compared to determine the best extraction method. Adsorption by using activated carbon was the best extraction method at a dosage of 19.79% per 100 ml sample within 9.45 h contact time, pH 3.0 and agitation speed of 180 rpm. The acetic and butyric acids extracted were 87.91 ± 1.3% and 98.32 ± 2.5%, respectively. Meanwhile, ATPS method extracted only 43.66 ± 2.3% of acetic acid and 73.50 ± 4.5% of butyric acid. Acetic and butyric acids extracted were 28.10 ± 0.9% and 91.92 ± 1.3%, respectively by using solvent extraction method. Moreover, the mechanisms of adsorption process was analyzes using x-ray fluorescence (XRF), x-ray diffraction (XRD), fourier-transform infrared (FT-IR) and zeta potential. The 73.99% of carbon component detected by using XRF analysis and appearance of amorphous structure in activated carbon after adsorption proses showed that the adsorption process was occurred. Meanwhile, FT-IR and zeta potential conclude that the adsorption process of VFAs on the surface of activated carbon was by chemisorption which was covalent bond. Furthermore, adsorption isotherm for both acetic and butyric acids fitted Langmuir model with the coefficient of determination value (R^2) of 0.9624 and 0.9658, respectively that indicate monolayer adsorption of VFAs on the surface of activated carbon. The chemisorption bonding was also proved by adsorption kinetics that showed both acetic and butyric acids fitted pseudo second-order model which the R^2 equal to 0.9873 for acetic acid and 0.9978 for butyric acid. The recovery of acetic and butyric acids from activated carbon were studied in desorption process. Shaker was the best instrument to be used in desorption process compared to vortex, waterbath sonicator and probe sonicator. Meanwhile, types of diluent that gave high recovery percentage of acetic (54%) and butyric (29%) acids were deionized water and ethanol respectively by using shaker. Multistage desorption process were applied to increase the VFAs recoveries. Thus, the final recoveries were 89.1% for acetic acid and 67.8% for butyric acid.

CHAPTER 1 : INTRODUCTION

1.1 Research Background

As a developing country, Malaysia has experienced a rapid growth, for example in technology. Thus, these transformations over the last decade eventually will generate such a tremendous quantity of wastes. The source of these wastes may come from residential, industrial or commercial areas which also known as Municipal Solid Waste (MSW). The MSW generation in Malaysia increased from 5.91 million tonnes in 2001 to 6.97 million tonnes in 2005 and this amount had been expected to increase with the population growth by the year 2020. Therefore, wise management of solid waste is crucial. Landfills are the facilities for the disposal of solid wastes. One of the problems in landfills is the management of leachate since they may lead to environmental problems such as water pollution due to their toxic effects (Raghab *et al.*, 2013). Leachate can be defined as a liquid that percolated through solid waste and contains heavy metals, organic and inorganic materials (Tarmudi *et al.*, 2009). Thus, treatment of leachate is important before it is being released to the environment.

Treatment of leachate by fermentation process is significant in green technology. A *Clostridium butyricum* is suitable for leachate fermentation. This is because *Clostridia* produces volatile fatty acids (VFA) from complex organic matter (Wu & Yang, 2003). Meanwhile, volatile fatty acids are important for the synthesis of compound used in the industries. Acetic acid is used in food industry as vinegar, manufacturing of Vinyl Acetate Monomer (VAM), Purified Terephthalic Acid (PTA), Acetic Anhydride, Acetate Esters and Calcium Magnesium Acetate (CMA) (Jin *et al.*, 2005). Meanwhile, butyric acid is important for perfumes, pharmaceuticals, chemical intermediate, flavorings, and animal feeds industries

(Dwidar *et al.*, 2012). Then, propanoic acid is important in cosmetic, pharmaceutical and plastics industries (Gonzalez-Garcia *et al.*, 2017). The market price of acetic and butyric acids are expected to increase to 15.1% and 62.9% respectively by the year 2020 (Global Acetic Acid Market, 2016; Global Butyric Acid Market, 2015) due to their wide applications in the industries. Thus, the recovery of acetic and butyric acids from fermented leachate is one of the methods to fulfill the industrial demand.

The aqueous two phase system (ATPS) method is a liquid-liquid extraction method that has been applied in separation of biomolecule, inorganic molecule and whole cells from bioproduction (Dan *et al.*, 2010). ATPS composed of between two different immiscible polymers or polymers with salt. It is characterized by low energy consumption and the extracting agent can be reused. The equilibrium in ATPS is determined by intermolecular forces, which are hydrogen bonds by adding salt to the system. The phase partition in the system is due to the salting out effect that acts as the separation method for volatile fatty acids. This is because ATPS is affected by addition of the salts. Thus, the targeted volatile fatty acids can be extracted selectively from fermentation broths. Solvent extraction is also a liquid-liquid extraction method that can be used for extraction of volatile fatty acid from aqueous solution. The addition of selected solvent in the aqueous volatile fatty acid solution will form two phases. Hence, the volatile fatty acids are transferred from the aqueous phase to the organic phase. Adsorption method is the unit operation that widely used in biotechnological and chemical industries because it is robust and easy to operate. Thus, separation of volatile fatty acids from aqueous solution is possible because of the efficient design and the operation of adsorption process including desorption process (Da Silva & Miranda, 2013).

This study will determine the optimum parameter for three extraction methods (ATPS, solvent extraction and adsorption) in order to extract acetic and butyric acids from fermented leachate by response surface methodology (RSM) by using software Design Expert 7.0®. After that, the highest percentage (%) of extracting acetic and butyric acids among these three methods is selected for the mechanism and recovery study. The mechanism of the selected extraction method is studied by adsorption isotherm, kinetics, X-ray fluorescence (XRF), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) and zeta potential. The recoveries of acetic and butyric acids are studied after the extraction process by using different types of laboratory instruments (vortex, waterbath sonicator, probe sonicator and shaker) and diluents (deionize water, iso-propyl ethanol, ethanol and petroleum ether). Finally, the design of adsorption and desorption process are developed to maximize the recovery of acetic and butyric acids.

1.2 Problem Statement

Commercial production of VFA accomplish by chemical routes but the use of non-renewable petrochemicals as the raw materials as the increasing price of oil have renewed the interest in biological routes of VFA production. The raises of the ethical concern on the use of food to produce chemicals can be solved by utilizing the organic-rich waste such as leachate generated from wastewater treatment plant (Lee *et al.*, 2014). Thus, such transformation of waste into VFA can provides an alternative route to reduce the increasing amount of waste generated. Therefore, eventhough the low percentage of the VFA recovery can reduce the amount of waste generate.

As being mentioned before, leachate has created problems involving the environment because of its hazardous materials. Thus, treatment of leachate is crucial. Recent studies on treatment of leachate using fermentation process reported that by using *Clostridia* bacteria, it will also produce volatile fatty acids (Bhatia & Yang, 2017; Liu *et al.*, 2006; Zhang *et al.*, 2009). Nevertheless, there is no study for a recovery of these volatile fatty acids after fermentation of leachate. The produced volatile fatty acids, by fermentation process, such as acetic, butyric and propionic acids have many applications in the industries. The extraction methods using aqueous two phase system, solvent extraction and adsorption are compared to find the best method to extract acetic and butyric acids since there is no study that compare these extraction methods.

1.3 Research Scope

This research focuses on recovery of acetic and butyric acids from fermented leachate collected from collected from pilot plant laboratory Bioprocess Technology Divison in School of Industrial Technology, Universiti Sains Malaysia. The leachate is from Pulau Burung Landfill Site (PBLS). The extraction methods (aqueous two phase system (ATPS), solvent extraction and adsorption) are compared by using response surface methodology (RSM) and the highest percentage of extracted acetic and butyric acids is selected for the recovery study. The recovery study involves the selection of instruments and diluents. Finally, the designs for the full process of extraction and recovery are developed.

1.4 Research Objective

The purpose of this study is to recover the acetic and butyric acids from fermented leachate. This study focuses on extraction and recovery methods for acetic and butyric acids.

Based on above, this study has been conducted with the following objectives:

1. To optimize on extraction methods (aqueous two phase system (ATPS), adsorption or solvent extraction) for acetic and butyric acids extraction by using Response Surface Methodology (RSM).
2. To identify the suitable parameters to recover acetic and butyric acid after extraction process.
3. To design the recovery method with target at least > 65 % recovery of acetic and butyric acids.

1.5 Thesis Outline

This thesis consists of five chapters which will be explained in details as follows:

Chapter 1 Introduction

This chapter presents the background of the study. It also includes problem statements, objectives and scope of the research.

Chapter 2 Literature Review

This chapter contains review from the previous study of the extraction process of volatile fatty acids from aqueous solution.

Chapter 3 Methodology

In this chapter, the details all procedures of the experiment were described. This chapter also includes materials and instruments used in the research.

Chapter 4 Result and Discussion

The findings and results from conducting research were compiled in this chapter. The research findings were compared and discussed in details.

Chapter 5 Conclusion and Recommendations

Findings from the research were summarized and several appropriate recommendations for future research were suggested.

CHAPTER 2 : LITERATURE REVIEW

2.1 Sanitary landfills in Malaysia

Annually, the municipal solid waste (MSW) being disposed into landfills was about 10.40 million tons in Malaysia (Fauziah & Agamuthu, 2010). Solid Waste and Public Cleansing Management Act 2007 were established because of pre-maturely landfills were closed due to the absence of waste management. Table 2.1 summarizes the classification of disposal sites and there are 12 sanitary landfills in Malaysia as shown in Table 2.2.

Table 2.1: Classification of landfill sites in Malaysia (Manaf *et al.*, 2009)

Level	Available facilities
I	Controlled landfill by perimeter drains and fencing
II	Controlled landfill by eliminating of informal scavenging and provision of environmental protection facilities, gas removal system, and separate unloading, working area, daily cover and enclosing bund (different in solid waste)
III	Sanitary landfill with leachate recirculation system
IV	Sanitary landfill with leachate treatment system

Table 2.2: Sanitary landfill in Malaysia (Fauziah & Agamuthu, 2010)

Landfill	Status of operating	State
Seelong Sanitary Landfill	Operating	Johor
Tanjung Langsat Sanitary Landfill	Operating	Johor
Bukit Tagar Sanitary Landfill	Operating	Selangor
Air Hitam Sanitary Landfill	Closed	Selangor
Jeram Sanitary Landfill	Operating	Selangor
Tanjung 12 Sanitary Landfill	Operating	Selangor
Pulau Burung Sanitary Landfill	Operating	Penang
Mambong Sanitary Landfill	Operating	Sarawak
Bintulu Sanitary Landfill	Operating	Sarawak
Sibu Sanitary Landfill	Operating	Sarawak
Miri Sanitary Landfill	Operating	Sarawak
Kota Kinabalu Sanitary Landfill	Operating	Sabah

2.1.1 Leachate

Leachate defined as water that has percolated through wastes, soil, groundwater contamination and emission of hazardous gases to the atmosphere

(Abbas *et al.*, 2009; Ojeda-Benítez & Beraud-Lozano, 2003). Leachate that generated from the landfill waste was collected and channelled to the leachate treatment facility to hinder the potential risk to environment. However, treatment of leachate in Malaysia is not well observed and monitored resulting the pollutions nearby water source (Vincent, 2017). Leachate can be treated either physically, chemically or biologically to remove BOD, COD, suspended solid, nitrogenous compound, colour and heavy metals (Yachiyo Engineering Co. & Ex Corporation, 2004). Table 2.3 shows the types of leachate treatment that comply with the effluent standard established by Department of Environment, Malaysia.

Table 2.3: Types of leachate treatment (Abbas *et al.*, 2009)

Treatment method	**Principle**
Biological Treatment	Biodegradation of organic waste by microbes using aerobic or anaerobic treatment
Physio-chemical Treatment	Removal of organic waste by flocculation, chemical precipitation, adsorption, oxidation, stripping or ion exchange
Physical Treatment	Removal of suspended solid, organic and inorganic compound by microfiltration, ultrafiltration, nanofiltration or reverse osmosis

2.1.2 Fermentation of leachate by using *Clostridium butyricum*

In the Kingdom Procaryota one of the largest genus is *Clostridium. Clostridium* is a strictly anaerobic and gram-positive bacteria. Their morphology is cylindrical-shape (Szymanowska-Powalowska *et al.*, 2014). *Clostridium* is a spore forming bacteria and strains can be isolated from soil, waste water, animal digestive systems and contaminated dairy products (Zigova & Sturdik, 2000). *Clostridium* is well known for producing bad smell. Optimal cultivation conditions for *Clostridium* are at temperature ranging from 35-37°C, pH range of 4.5-7.0 and an atmosphere of pure carbon dioxide (CO_2) or nitrogen (N_2) or N_2 and CO_2 mixture in the ratio of 1:9 (Zigova *et al.*, 1999). The product of *Clostridium* metabolism is CO_2, H_2, organic compounds (butyric, lactic, acetic and succinic acids) and solvents (butanol, acetone and isopropanol) (Szymanowska-Powalowska *et al.*, 2014).

Clostridium butyricum can be found in the soil, healthy humans and animal's intestine (Motomichi Takahashi *et al.*, 2018). The metabolism of *C. butyricum* involved acetic acid (main byproduct from butyrate production) been taken up by the bacteria and converted into butyrate. This pathway is inhibited by its end product which is butyrate. The fermentation of *C. butyricum* results in production of acetic acid, butyric acid, H_2 and CO_2. The fermentation of *C. butyricum* follows the Equation 2.1 below (Zhang *et al.*, 2009):

$$\text{Glucose} \rightarrow 0.8\ \text{butyrate} + 0.4\ \text{acetate} + 2\ CO_2 + 2.4\ H_2 \qquad (\text{ Equation 2.1})$$

2.2 Terminology of fatty acids

Fatty acids are important in metabolism of fuel as a storage and transport of energy, essential components of membranes and gene regulator (Rustan & Drevon, 2005). Fatty acids compounds contains hydrocarbon chain and one carboxyl group at the end of the molecule. Saturated fatty acids are straight hydrocarbon chain with a single carbon-carbon bond while unsaturated fatty acids have either one or more carbon-carbon double bonds. The numbers of carbons are between C_4 to C_{22}. Fatty acids can be classified either short chain fatty acid (SCFA), medium chain fatty acid (MCFA), long chain fatty acid (LCFA) or very long chain fatty acid (VLCFA) according to the chain length (Scrimgeour, 2005).

Volatile organic acids (VFAs) are organic acids that consist of six or less than six carbon atom (Bhatia & Yang, 2017). Examples of VFAs are acetic acid, butyric acid, propionic acid, isovaleric acid and isobutyric acid. Figure 2.1 shows the possible metabolic pathways for VFAs. There are many application of VFAs in the industries such as in food industry, textiles, pharmaceutical, cosmetics and bio-energy (Andersen *et al.*, 2015; Baumann & Westermann, 2016).

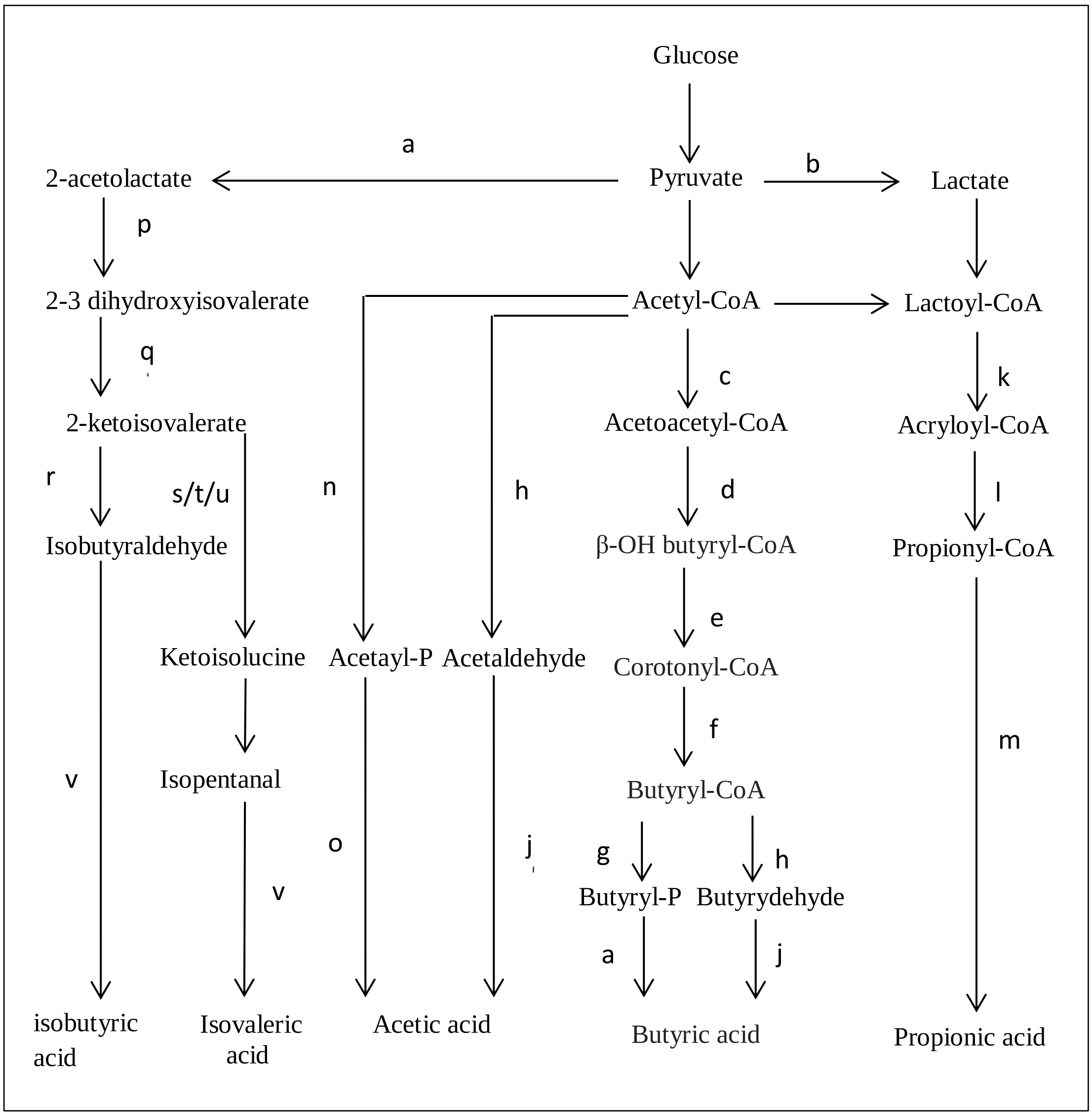

Figure 2.1: Schematic of possible metabolic pathways for VFAs. The enzymes coded by the genes are: acetolactate synthase (a), lactate dehydrogenase (b), thiolase (c), hydroxybutyryl-CoA dehydrogenase (d), crotonase (e), butyryl-CoA dehydrogenase (f), phosphotransbutyrylase (g), aldehyde dehydrogenase (h), butyrate kinase (i), aldehyde oxidoreductase (j), lactoyl-CoA dehydratase (k), acrylyl-CoA reductase (l), propionyl-CoA transferase (m), hosphotransacetylase (n), acetate kinase (o), keto-acid reductoisomerase (p), dihydroxy acid dehydratase (q), 2-keto acid decarboxylase (r), 2-isopropylmalate synthase (s), isopropylmalate isomerase complex (t), isopropylmalate dehydrogenase (u) and alcohol dehydrogenase (v) (Bhatia & Yang, 2017).

2.2.1 Acetic acid

Acetic acid had a higher market price which was USD 9,075.0 million in 2014 and was predicted to be USD 14,784.2 million by 2020 (Global Acetic Acid Market, 2016). Acetic acid (CH_3COOH) is an important chemical in industry to manufacture Vinyl Acetate Monomer (VAM), Purified Terephthalic Acid (PTA), Acetic Anhydride, Acetate Esters and Calcium Magnesium Acetate (CMA) (Jin *et al.*, 2005). It also can be applies in food as the main component in vinegar (Verma, 2014). Vinegar has an antimicrobial property which can help in preventing spoilage in fermented food but it is not suitable for wine and beverages (Gullo *et al.*, 2014).

Diluted acetic acid (vinegar) was produced by an alcohol fermentation and after that, it was replaced by the dry distillation wood when the demand of acetic acid increasing in various fields. Later, this process was replaced by a synthetic process. The processes that can be used to produce acetic acid are methanol carbonylation, acetaldehyde oxidation, hydrocarbon oxidation and direct oxidation of ethylene processes (Sano, Uchida, & Wakabayashi, 1999). The current technique for the production of acetic acid is by using chemical synthesis and it is not suitable in future due to petroleum depletion. Despite of a using chemical process, acetic acid can be produced by the fermentation process using renewable biomass. The production of acetic acid by fermentation process can be done by using solid state fermentation (SSF) or liquid fermentation (submerged). However, the submerged system has advantages of having a high yield and faster process (Gullo *et al.*, 2014).

The major bacteria used in acetic acid production are Acetobacter species (aerobic) and Clostridium species (anaerobic). The Clostridium fermentation gives more advantages because it only requires one-step vinegar process, has a higher yield, low cost (because no aeration needed) and the ability to consume CO_2 and other one-carbon precursor permits their use in conversion of synthesis gases from waste. The anaerobic fermentation to produce acetic acid is more favorable compared to aerobic fermentation (Rogers *et al.*, 2006).

2.2.2 Butyric acid

Butyric acid ($CH_3CH_2CH_2COOH$) is a colorless oily liquid that has an extremely pungent smell. It has been applied in many industries such as perfumes, pharmaceuticals, chemical intermediates, flavorings, and animal feeds (Dwidar *et al.*, 2012). Global Butyric Acid Market, 2015 reported that the market price of butyric acid in 2014 was USD 124.6 million and expected to increase by 15.1% for time the period from 2014 to 2020.

The butyric acid production has evolved from time to time to keep up with the global demand. Butyric acid is produced from a chemical process called petroleum-based chemical synthesis. Chemical synthesis is more preferable because of its lower cost and large production scale (Dwidar *et al*., 2012; Zhu & Yang, 2004) Besides, extraction from butter also can be used to produce butyric acid. However, the downside of this method are the method is difficult and expensive to extract butyric acid compared to the chemical synthesis (Zigova & Sturdik, 2000).

Currently, butyric acid that obtained from microbial fermentation is more favorable compared to chemically synthesized butyric acid due to the environmental friendly, even though higher the cost of production is higher (Liu *et al.*, 2006; Zigová *et al.*, 1999) There are several bacteria that can produce butyric acid and they are strictly anaerobic. According to Zigova & Sturdik (2000), genera Clostridium, Butyrivibrio and Butyribacterium are commonly used bacteria to produce butyric acid but Clostridium is used the most for commercial production of butyric acid due to its stability and high productivity.

2.3 Separation process

Fermentation process using bacteria species is the biological routes for the industrial production of organic acids. There are a number of organic acids recoveries from fermentation broth have been studied in the literature. The recovery techniques for the separation process mainly include membrane separation, extraction, precipitation, distillation and chromatography (Hb̌ová *et al.*, 2004; Ren *et al.*, 2012; Zhou *et al.*, 2011). The general separation process for separation process can be seen in Figure 2.2 (Qian-Zhu Li *et al.*, 2015). The first step is the separation of cell debris from fermentation broth. Two or more steps of operation are required to obtain a satisfactory purification of organic acids.

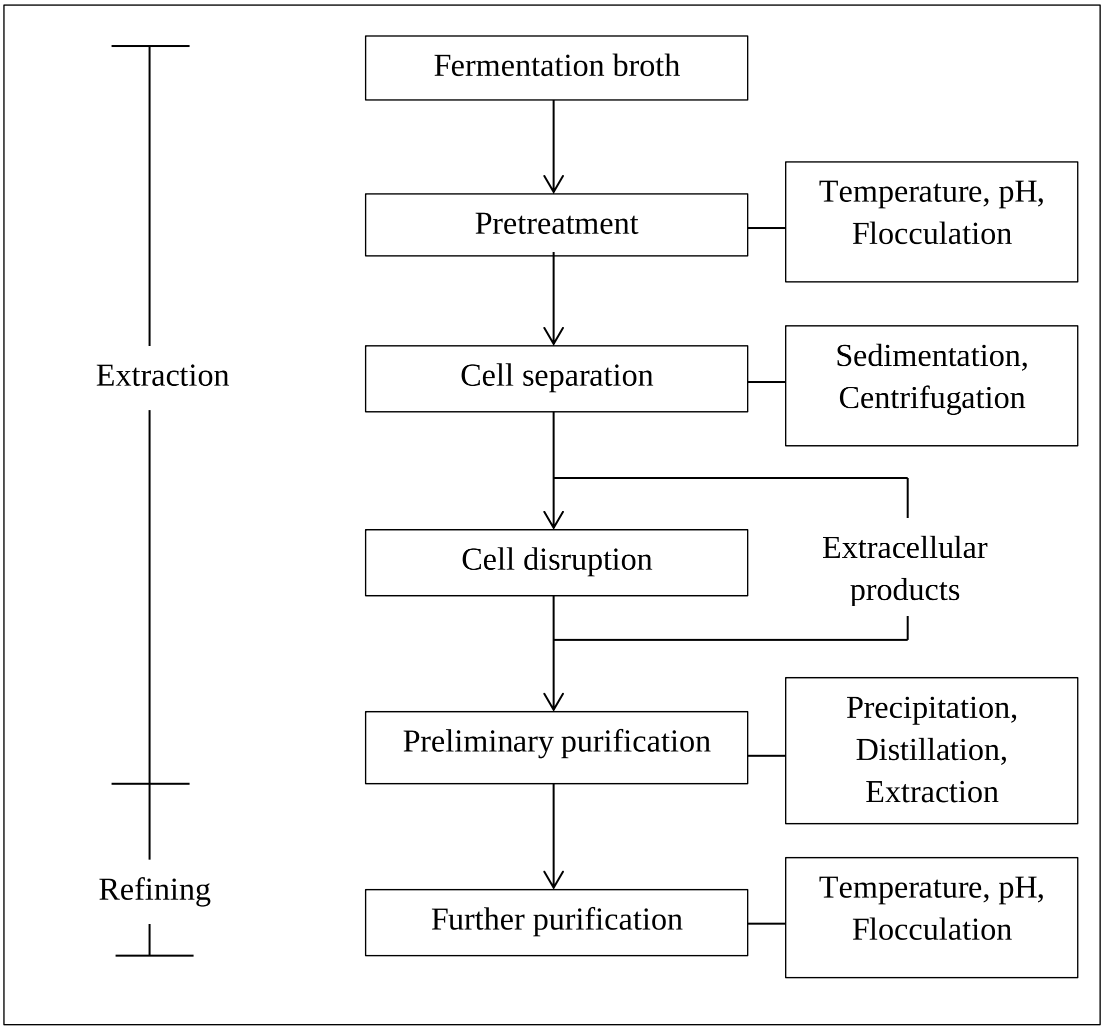

Figure 2.2: General flow for the separation process of organic acids from fermentation broth.

Extracellular products are mainly organic acids. The recovery of the extracellular products involving precipitation, distillation, membrane separation or extraction processes is mainly used in preliminary purification. Moreover, chromatography or crystallization is applied in the refining of organic acids. Table 2.4 shows the advantages and disadvantages of separation methods of organic acids from fermentation broth.

Table 2.4: Advantages and disadvantages of separation process for organic acids recovery (Kurzrock & Weuster-Botz, 2010).

Separation process	**Advantages**	**Disadvantages**
Precipitation	i) Highly selective for the target product. ii) Simple operation. iii) High product purity.	i) High operation cost
Liquid-liquid extraction (solvent extraction, reactive extraction and aqueous two phase system).	i) Highly efficient. ii) Low energy consuming.	i) Distillation process combination to purify the organic acids. ii) High cost operation
Distillation	i) Continuous reactive distillation has low energy consumption and higher selectivity	i) Not effective in high concentration.
Membrane separation (microfiltration, ultrafiltration, nanofiltration, reverse osmosis, pervaporation and electrodialysis)	i) Adaptability and selectivity. ii) High purity and yield of product.	i) High energy consuming ii) High cost of membrane. iii) Reduction of membrane efficiency with the increase of organic acids concentration. iv) Membrane pollution leading to by-product salt formation
Chromatography (Ion exchange resins and macroporous adsorption resins)	i) Good selectivity, high capacity and quick recovery. ii) Low energy consumption. iii) Does not produce the co-product (calcium sulfate). iv) High yield of product.	i) Large amount of liquor produced. ii) High consumption of salt in elution process.

2.3.1 Aqueous two phase system (ATPS)

Aqueous two phase system mechanism is the incompatibility of the two aqueous solutions causing separation between them. It can be either between two polymers or a polymer and salt at ionic strength. The most commonly used polymers are polyethylene glycol (PEG) and dextran. Due to the reason of the incompatibility, the polymers will form large aggregates when they are being mixed and the separation into two different phases is due to the steric exclusion (Dutra-Molino *et al.*, 2012). In ATPS, the soluble and particulate matter which is contaminant will partition to the lower phase which is more polar. Meanwhile, targeted volatile fatty acids will partition to the top phase, which is less polar and more hydrophobic phase, usually the PEG (Wu *et al.*, 2015).

There are wide application of the ATPS in recovery and purification of biological products including biopharmaceuticals such as monoclonal antibodies (Rosa *et al.,* 2010), bionanoparticles, proteins, genetic material, cells and organelles (Benavides & Rito-Palomares, 2007). According to Wu *et al.* (2015), ATPS also can be used in extraction of butyric acid from the fermentation broth by using PEG 6000 and sodium sulphate.

2.3.1(a)Phase diagram

Phase diagram is one of the important elements that can yield information about the scheme. The phase diagram describes the potential working area for a particular phase system. From a phase diagram, it can give information including the necessary concentration of the phase-forming components in order to form a two-phase system and the subsequent concentration of phase components in the both phases. Present in the phase diagram is a binodal curve as shown in Figure 2.3 (Raja *et al.*, 2012). Figure 2.3 shows the two regions of binodal curve which are the region of the component concentration that will form two immiscible aqueous phases (above the curve) and the region with one phase formation (below the curve) (Hatti-Kaul, 2000).

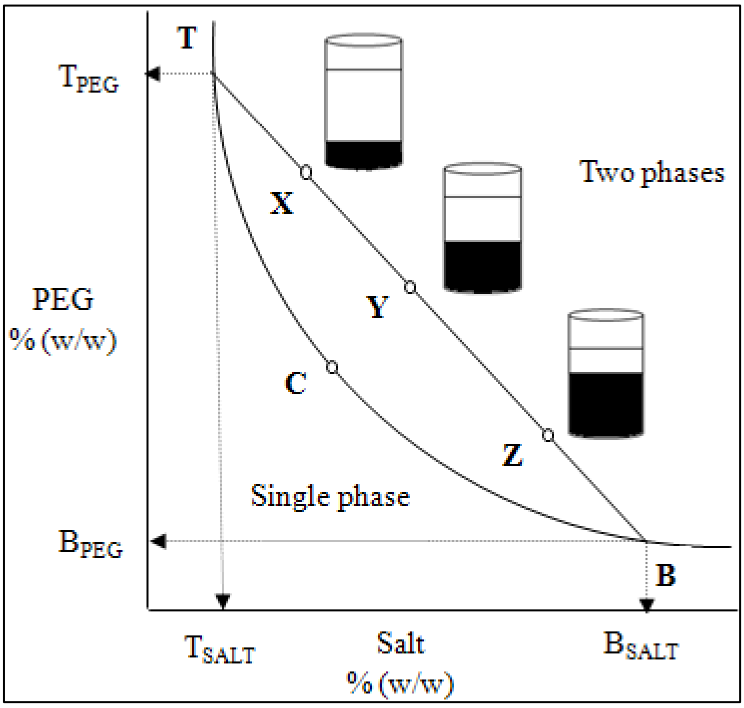

Figure 2.3: The binodal curve; TCB = Binodal curve, C = critical point, TB = Tie line, T = composition of the top phase, B = composition of the bottom phase, and X, Y and Z = total composition of ATPS.

2.3.1(b)Factors affecting ATPS partitioning

In ATPS partitioning, there are several factors that will influence the behavior of the system which lead to a more efficient step of volatile fatty acids extraction. These factors can be manipulated in order to extract the desired volatile fatty acids more efficiently.

The molecular weight of the polymer will influence the biomolecule partitioning by changing the number of interactions between the polymer-biomolecule. As the molecular weight of the polymer increases, the partition between the polymer-biomolecule decreases. This is because higher the molecular weight of the polymer will increase the chain length of the polymer and the exclusion effect, thus reducing the free volume (Ratanapongleka, 2010). According to Wu *et al.* (2015), a high PEG molecular weight leads to a higher molecular hydrophobicity and hindrance which resulting in a higher amount of butyric acid extracted in PEG-rich phase.

According to Raja *et al.* (2012), the higher the molecular weight of the polymers, the lower the required polymer concentration for phase separation. The concentration of the systems affects the phase hydrophobicity. As the molecular weight of the polymer increases, the hydrophilic groups will decrease which lead to increase in hydrophobicity. The hydrophobic resolution is the ability of the system to discriminate between the target compound and contaminants according to their hydrophobicities (Asenjo & Andrews, 2011).

Since the pH of the system may alter the ratio of charge molecules, pH will give effect on the partitioning of the system. Thus, the pH of the system may be manipulated to promote a better selective separation (Asenjo & Andrews, 2011; Raja *et al.*, 2012). The increase in salt concentration can extract more volatile fatty acids from fermentation broth. This is due to the water content in the PEG-rich phase is reduced and the water content of the salt-rich phase was increased (Wu *et al.*, 2015).

Next, the temperature of the system also affects the separation in ATPS. It affects the ATPS viscosity and at optimum temperature, the volatile fatty acid entered PEG-rich phase with strong hydrophobicity (Wu *et al.*, 2015). Meanwhile, at high temperature, the water solubility of the PEG increased and resulting the accumulation of PEG in salt-rich phase. According to Wu *et al.* (2015), the volume of fermentation broth also affect the extraction of volatile fatty acid using ATPS.

2.3.2 Solvent extraction

Solvent extraction is one of the examples of in situ product removal. It is considered as the conventional methods to extract fatty acids from fermentation broth. The extraction is obtained depends on the solubility of a substance in the two different immiscible liquids. The used materials are usually organic solvent (organic phase) and water (aqueous phase). Basically, fatty acids are extracted from a medium into an organic phase.

Nowadays, the extraction or pertraction method is integrated into the fermentation process in order to separate the products. Reactive extraction has higher efficiency in isolation of products compared to physical extractions. Reactive liquid-liquid extraction has the advantages that can increase bioreactor productivity and simple operation in reactor pH control without any usage of base addition. This method also recovers the fermentation product in a continuous step, thus reducing the purification cost and downstream processing load (Wasewar, 2012). In reactive extraction, an organic phase has its reactant. This reactant acts as a carrier and a physical transporter for the acid to extract into the organic phase.

There is a study that investigated the recovery of propionic acid by using tri-n-butylphosphate (TBP) in three different diluents (n-heptane, petroleum ether and toluene). TBP-toluene system was found to give the highest distribution coefficient from 0.14 to 3.54. However, this system is not applicable to extract propionic acid from fermentation broth due to its toxicity and high water co-extraction values. The TBP-n-heptane system is preferable to be used (Keshav *et al.*, 2009).

For the study which is conducted by Zigova *et al.* (1999), his team integrated the extraction and pertraction systems into the fermentation process to produce butyric acid. The organic solvent was Hostarex A327 (20% w/w), while its diluent was oleyl alcohol. The distribution coefficient of butyric acid between organic and aqueous phase was 13.0, but it could be decreased to 3.0 with the increase of pH of the fermentation process. A direct stripping was recommended in the process as it was a faster process because it was faster. Thus, it did not disturb the fermentation process. The total butyric acid concentration also increased from 7.3 g/L in the control to 10.00 g/L in extraction fermentation and 20.00 g/L in pertraction system. Meanwhile, the yield of butyric acid increased from 0.24 g/g in control to 0.30 g/g in pertraction system.

According to Bilgin *et al.* 2006, the reactive extraction is conducted by tri-n-octylphosphine oxide (TOPO). TOPO is an organophosphorus compound that is more environmentally friendly than amine. Recently, it was dissolved in several different solvents, like isoamyl alcohol, oleyl alcohol, toluene, methyl tert-butylether (MTBE), cyclohexyl acetate, kerosene and isobutyl methyl ketone (MIBK) to observe the recovery yield of propionic and butyric acids from an aqueous phase. The result showed that the distribution coefficients of propionic and butyric acids between the organic phase and aqueous phase live up to 51.22 and 12 times, respectively when TOPO was used. Methyl tert-butyl ether (MTBE) is very preferably because the highest distribution coefficient of both acids is obtained.

As mention above, phosphorus-bonded oxygen donor extractant such as tri-n-octyl phosphine oxide (TOPO) promotes higher distribution coefficient and it is not environmentally friendly when compared to amine. Furthermore, an aliphatic amine is more effective and less expensive than organophosphorus compound. The amine interacts with acid to form an acid-amine complex that provides high distribution of acid (Wasewar & Yoo, 2012). Further improvement in term of yield can be achieved through a screening process to obtain the optimum temperature, pH and others for extraction ability (Keshav *et al.*, 2009).

2.3.2(a)Types of extractant

The extraction process is analyzed by the means of distribution coefficient and the degree of extraction (Zigova *et al.*, 1999). There are important considerations while choosing the suitable organic solvent. Solvent extraction can be conducted by using three types of extractant, which are carbon-bonded oxygen-bearing (hydrocarbon) extractant, phosphorus-bonded oxygen-bearing (organophosphorus) extractant and aliphatic amines.

For oxygen-bearing extraction systems, the extractability of the organic acid is measured depends on its acid strength in the aqueous solution and hydrogen bond strength in the organic phase. Hydrocarbon extractants also are more soluble in water than the other two extractant categories (Tugtas, 2011a).

Phosphorus-bonded oxygen-bearing extractant has stronger Lewis base group, more water immiscible and more extractable than carbon-bonded oxygen-bearing extractant. Hence, the organophosphorus extractant is more effective to extract weak organic acids compared to the carbon-bonded oxygen-bearing extractant under the same condition. The examples of organophosphorus extractant are tributylphosphate (TBP) and trioctylphosphine oxide (TOPO) (Tugtas, 2011).

Aliphatic amine consists of three group compounds, which are long chain aliphatic amine, secondary amine and tertiary amine. These extractants have been applied widely due to their high distribution coefficients for carboxylic acids and to their low water immiscible characters. The main drawback of amine based extractant is acidic pH condition need to be adjusted before the extraction, in order to convert acid to its dissociated form (Tugtas, 2011). Tertiary amines is preferably used to extract the undissociated form of carboxylic acid (Hong & Hong, 2005). This method is reversible reaction between extractant and extracted fatty acid. Acid dissociation constant (Ka) and pH condition are the two main parameters in order to extract specific acid by using tertiary amines (Song & Lee, 2006).

Petroleum ether is the volatile and highly flammable hydrocarbon solvent. It is existed from petroleum refinery between lighter naphtha and kerosene. The recorded specific gravity is recorded between 0.6 to 0.8. There are many types of petroleum ether according to their distillation fraction, which are 30°C to 40°C boiling point, 60°C to 80°C boiling point and 80°C to 100°C boiling point (Verma, 2014).

2.3.3 Adsorption

In recent years, researchers have been using an adsorption process due to its easy application and cost effectiveness. Adsorption is one of the physiochemical processes that uses the physical properties of medium to separate the contaminants (volatile fatty acids) (Aziz *et al.*, 2014). Adsorption is a process where matters are attracted to the surface and being attached to it via physical or chemical bonding of a solid adsorbent. The adsorption capacity depends on the pore size distribution and the surface chemistry of adsorbent (Zhang *et al.*, 2010).

2.3.3(a)Activated carbon

Adsorption using activated carbon has been cited by United States Environmental Protect Agency (EPA) as the best available environmental control technology (Alves *et al.*, 2014). The wide applications of granular and powdered activated carbon include wastewater treatment, food, pharmaceutical and chemical industries (Moreno-Castilla, 2004). Activated carbons are produced from a carbonaceous materials that are from biomass sources such as low grade plants, agricultural residues, forest residues and municipal solid wastes (Kiliç *et al.*, 2013). Activated carbon is a mesoporous materials (Hu & Srinivasan, 2001). Therefore, activated carbon from biomass sources are renewable, widely available, environmentally friendly and cheap.

The previous researchers were using activated carbon for adsorption of organic acid from aqueous solution. According to Morad *et al.* (2014), 16.5% with an adsorption capacity of $3.30E-03\ \mathrm{mol/g}$ of acetic acid was being adsorbed on the surface of powdered activated carbon (PAC) at 4 h contact time, 25 ^{o}C and 0.2 mol/L acetic acid with 10 g/L PAC.

2.3.3(b)Desorption process

The adsorption process can be designed together with the desorption process for the recovery of volatile fatty acids. Desorption method is when a substance is released from a surface or through the surface. The desorption occur when an equilibrium condition is altered.

Activated carbon synthesized from sugarcane bagasse is impregnated with 40% v/v zinc chloride showing the highest individual volatile fatty acids adsorption which is 60%, 48% and 21% of acetic, propionic and butyric acids respectively (Bautista-Carrizosa *et al.*, 2014). The process is continued to recover the volatile fatty acids from activated carbon using desorption process by using sonication, heating or both of them. The result shows that the highest percentage of the desorption process by using sonication which is 38.02% acetic acid recovered. According to Da Silva & Miranda (2013), multistages of desorption process is required to increase the recovery of volatile fatty acids. The work shows the importance of addressing both adsorption and desorption processes. The recovery of propionic acid from activated carbon using n-propanol as a desorbent is above 90% when the multistages desorption process is applied.

2.3.3(c)Adsorption dynamic behavior

The adsorption behavior can be detected by using Fourier-transform infrared (FT-IR), x-ray diffraction (XRD) and scanning electron microscope (SEM).

The research on the removal of rhodamine B dye and Pb^{2+} ions from aqueous solution by using hybrid ion-exchanger shows that the FT-IR analysis peaks are shifted, indicating there is an interaction between synthesized ion exchanger and rhodamine B after adsorption process (Saruchi & Kumar, 2016). FT-IR is an analytical technique used to identify structure and molecular components by measuring the absorption of infrared radiation of the sample material versus wavelength. According to Vijayakumar *et al* (2012), the slight reduction in the spectrum for the FT-IR analysis but the peak position did not change are indicated the physical forces instead of chemical combination for the adsorption process.

The XRD analysis is used to detect the presence crystalline structure in a material. According to Saruchi & Kumar (2016), the XRD analysis used to compare the crytallinity between *Gum tragacanth* and synthesized ion exchanger. The result shows that *Gum tragacanth* is less crystalline rather than synthesized ion exchanger.

The signals that derive from the interaction between electrons and sample by using SEM analysis gives an information about the external morphology (texture) of sample. The research on the removal of rhodomine B dye an Pb^{2+} ions by adsorption method shows that the folded structure of synthesized hybrid ion exchanger which may provide a large surface area to adsorb rhodomine B dye and Pb^{2+} ions (Saruchi & Kumar, 2016). The activated carbon that shows rough surfaces, pores and cavities on the carbon samples will hold more adsorbate on the surface of adsorbent (Babu Rajendran *et al*, 2015).

2.3.3(d)Adsorption isotherm

The definition of adsorption isotherm is the relationship between the amount or concentration of adsorbate that accumulates on the adsorbent and the equilibrium concentration of dissolved adsorbate (Hamidi *et al.*, 2010). The most frequent adsorption isotherms used and cited are Langmuir and Freundlich isotherms as a ideal experimental data analysis (LeVan & Vermeulen, 1981).

The langmuir isotherm describes the dependence of surface coverage of an adsorbed gas on the pressure of the gas above the surface at a fixed temperature. The Langmuir model assumption is a finite number of active sites that homogeneously distributed over the surface of adsorbent (Vijayakumar *et al.*, 2012). It is based on the four hypotheses which are the uniform surface of adsorbent as all adsorption sites are equal, adsorbed molecules do not interact, all adsorptions take place through the same mechanism and lastly, only at maximum adsorption, a monolayer is formed.

The Freundlich isotherm applies to adsorption on heterogenous surfaces with the interaction between adsorbed molecules and formation of multilayers (Vijayakumar *et al.*, 2012). The assumption of this model is when the adsorbate concentration increasing, the concentration of adsorbate on the adsorbent surface also increases and vice versa.

2.3.3(e)Adsorption kinetics

The kinetics data is generally used to elucidate the adsorption mechanism and to understand the dynamics of sorption reaction in terms of rate constant. These kinetic models include the pseudo first-order and pseudo second-order.

Pseudo first-order adsorption model is describe by non-reversible equation. The five assumption for the model are sorption only occurs on localized sites that involves no interaction between the sorbed ions, adsorption energy is not dependent on the surface coverage, maximum adsorption corresponds to a saturated monolayer adsorbates on the adsorbent surface, adsorbate concentration is considered constant and the metal ion uptake is governed by a first-order rate equation (Largitte & Pasquier, 2016). The kinetics of ions removal are described by pseudo second-order.