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Extraction of Volatile Fatty Acids from Leachate via Liquid-liquid Extraction and Adsorption Method

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Abstract: Volatile fatty acids (VFAs) are used in wide range of commercially-important chemicals. Treatment of leachate at landfills by fermentation process will produce VFAs (butyric acid, acetic acid, propionic acid, isovaleric acid and isobutyric acid) can be considered as a replacement for petroleum-based VFA due to their degradability, renewability and sustainability. Therefore, separation of VFAs residue after the leachate treatment is important and essential from the point of view of pollution control and recovery of useful material. The aim of this study is to compare the percentage of VFAs extracted between liquid-liquid extraction and adsorption method. The VFAs extracted in this study were acetic and butyric acids produced from the fermentation of leachate using *Clostridium butyricum*. Response surface methodology (RSM) was used using central composite design (CCD) to optimize the parameters that affect the extraction of acetic and butyric acids. Liquid-liquid extraction using petroleum ether (69/80) with optimum parameters (temperature: 35 ^{o}C, pH: 4.8, agitation: 175.4 rpm, incubation time: 16.8 h and volume of treated leachate: 14.1 %) showed that the acetic acid and butyric acid extracted were 28.1% and 88.8% respectively. On the other hand, adsorption method using activated carbon showed the highest extraction percentage of acetic acid, 87.4% and butyric acid 94.1% with the optimum parameters of pH 3.0, 19.8 % activated carbon weight, 40 ^{o}C, 9.5 h incubation time and 179.9 rpm agitation speed.

Keywords: Acetic acid, adsorption, butyric acid, chemical extraction, leachate, liquid-liquid extraction

1. Introduction

Leachate is a liquid that is formed due to the exposure of open landfills to the moisture and water like mist, rainfall and so on that penetrates the municipal solid waste (MSW) and being collected in the leachate pond [1]. Leachate contains a lot of dangerous materials that cannot be released to the environment [2]. In Malaysia, increase in the population also affects the MSW in industrial and agricultural byproducts being disposed to the landfill. Malaysian are generating about 5,781,600 tonnes of solid waste annually based on 2012 and it is expected that the amount of solid waste will be increased to double digits as the country is moving forward to be a developed nation in 2020 [3],[4]. Therefore, the appropriate MSW management is crucial. Biological treatment by fermentation process has attracted more interest due to its advantages includes variety of sources and the ease and speed which the microorganisms can be cultured and produced [5].

Clostridium butyricum is an anaerobic nature, acetic and butyric acids producing bacterium, gram positive, mesophilic, sporeforming and nitrogen-fixing bacterium [6], [7]. Currently, most of acetic and butyric acids rely on the petrochemical industries. Thus, by separating acetic and butyric acids from treated leachate gives a good competition to fulfill feedstock of these chemicals. Butyric acid ($CH_3CH_2CH_2COOH$) has been applied in many industries such as perfumes, pharmaceuticals, chemical intermediate, flavorings, and animal feeds [8], [9], [10]. Acetic acid (CH_3COOH) can be used in food, pharmaceutical and other industries [11]. Global Butyric Acid Market, 2015 reported that market price of butyric acid in 2014 is USD 124.6 million and expected to increase 15.1% for time period from 2014 to 2020. Acetic acid also had a higher market price which is USD 9,075.0 million in 2014 and was predicted to be USD 14,784.2 million by 2020 [12].

The general steps of the separation process can be seen in Fig 1. The first step is clarification method to separate cell debris from fermentation broth and several methods required to obtain a satisfactory separation of VFAs. Therefore, this study will be focusing on the extraction part from fermentation broth until primary recovery. Primary recovery method such as liquid-liquid extraction, adsorption, ultrafiltration, precipitation, direct distillation, reverse osmosis, electro dialysis, and anion exchange, have been employed to remove VFA from aqueous solution. However there has no study has been

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done to compare between the extraction methods which gives a significant effect in separating the VFAs.

Hence, the aim for this study is to compare percentage of VFAs extracted between liquid-liquid extraction and adsorption method after the treatment of leachate by *C.butyricum*. Liquid-liquid extraction mechanism is to separate compounds by their relative solubility in two different immiscible liquids, which holds an important status for separation of mixtures in the biochemical industry. Solvent used in liquid-liquid extraction is petroleum ether which is used as economic non-polar solvent. While, adsorption is highly recommended for removing of organic and inorganic pollutants, it requires a microporous adsorbent, capable of creating chemical bond and exchanging ions. Activated carbon adsorbents are used because it is frequently used in the extraction of chemical species in both gas and aqueous phases. This is because of their high adsorption capacity, their porous structure and accessibility of their surface. The parameters that affect the extraction of acetic and butyric acids for both methods were optimized by using response surface methodology.

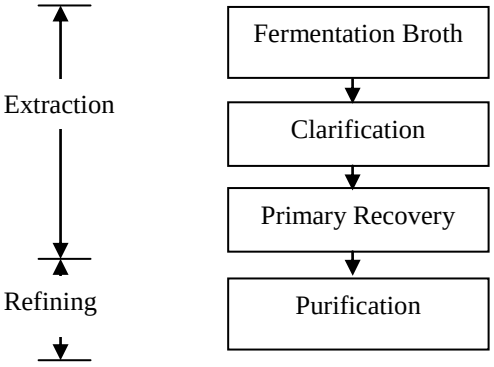

Fig. 1 Downstream process for the recovery of organic acids [8].

2. Materials

Leachate was taken at Pulau Burung Landfill Site (PBLS). PBLS is situated within the Byram Forest Reserve at 5.2065°N latitude and longitude 100.4254°E in Penang, Malaysia. *C.butyricum* strain sourced from National Collection of Industrial Food and Marine Bacteria (NCIMB Ltd) with the strain number NCIMB 7432, from Aberdeen United Kingdom. The inoculum for *C.butyricum* were prepared as previously in Table 1 [15].

Table 1 Formulate 1 liter of *C.butyricum* inoculum.

No	Chemical compound	Formula	Measurement
1	Yeast (Himedia, India)		10 g
2	Glucose (Systerm, Malaysia)	$C_6H_{12}O_6$	10 g
3	Ammonium sulfate (HmBG, Germany)	$(NH_4)_2SO_4$	10 g
4	Potassium phosphate	KH_2O_4	5 g
5	Resazurin (Sigma, USA)		0.1% (v/v)

Petroleum ether with boiling point 60 to 80 ^{0}C (Sigma, USA) was used as a chemical extractant. Commercial granular activated carbon (Bendosen Laboratory Chemicals) was used for adsorption method. Standard solutions of butyric acid (QReC^{TM}, New Zealand) and acetic acid glacial (QReC^{TM}, New Zealand) were used.

3. Methods

3.1 Clarification Process

Clarification process is the first step of downstream processing to separate cell debris from fermentation broths. The fermented leachate was centrifuged (Kubota, Japan) at 3000 rpm for 10 minutes [14]. Supernatant was stored at 4 ^{0}C for the next step of extraction.

Fermentation process is a biological treatment of leachate. The insoluble material was separated using Buchner funnel vacuum pump. Leachate undergoes pretreatment with limestone [15]. After that, leachate altered pH 6.5 was poured into anaerobic bottles and degassing using nitrogen gas. Then, it was autoclave at 121ºC for 15 minutes.

Medium was then adjusted to pH 6.5 and provided the oxygen-free environment by injecting nitrogen gas in the 50ml anaerobic bottle [14]. After autoclaving the medium, culture strain was transferred to new media by using aseptic technique and incubates at 37 ^{0}C for 12 hours. The size of inoculum used was 10% of the inoculum size. Fermentation process started once the inoculum being introduced to the leachate at 37ºC. Fermentation stopped when the growth of bacteria at stationary phase, it can be assumed by the absorbance reading (660 nm) taken for every hour.

3.2 Liquid-liquid Extraction

The five parameters that affect the extraction of acetic and butyric acids were temperature (A: 20-50^{0}C), pH of treated leachate (B: 2-7), incubation time (C: 6-24h), agitation (D: 50-200rpm) and volume of treated leachate (E: 10-50%) [9]. The extractant was aseptically added to the surface of the treated leachate in conical flask, Fig. 2. The experiment was followed by the design construct by the RSM using CCD to optimize the parameters, where all parameters were investigated at high (+1) and low (-1) levels consist of 50 runs. The acetic and butyric acids will be extracted at top phase. The top phase will be analyzed using gas chromatography (GC). The result will be calculated in percentage:

$$\text{Percentage of VFAs extracted (\%)} = \frac{\text{Concentration VFAs after extraction}}{\text{Concentration of VFAs before extraction}} \times 100 \quad (1)$$

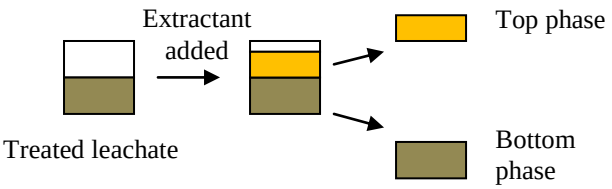

Fig. 2 Illustration of liquid-liquid extraction by petroleum ether.

3.3 Adsorption

The parameter involved were pH (3-9), activated carbon weight (A: 1-20%), temperature (B: 20-40^{0}C) , time (C: 1-24h) and agitation (D: 50-200 rpm) [14]. Design of experiment was construct by the RSM using CCD to optimize the parameters, where all parameters were investigated at high (+1) and low (-1) levels consist of 30 runs. pH was not included in the optimization experiment, instead the experiment conducted by single factor affecting acetic and butyric acids extracted. The VFAs extracted were analyzed using gas chromatography (GC). The result will also be calculated in percentage (1).

3.4 Response Surface Methodology (RSM)

Central Composite Design (CCD) was used to develop a mathematical model by identifying significant factors combination for the design of the optimization experiment. The design was contained two analyses for acetic acid (AA) and butyric acid (BA) extracted. The Design Expert 7.00 (Stat-Ease Inc., Minneapolis, USA) software was used to find out the interactive effects between parameters. For the validation in actual experiment, the parameters were set according to the optimal point suggested by the software. The percentage of acetic and butyric acids extracted were calculated and compared to find the best method for the extraction.

The determination of acetic and butyric acids concentrations was carried out by gas chromatography (Shimadzu, Japan). The detector applied was flame ionized detector (FID) and the column applied was BP21 FFAP column (SGE Analytical Science, Australia) with the internal diameter (ID) 0.53mm, film thickness 0.5µm, length 30 m and the temperature limit from 35˚C to 250˚C. The part number for this column was 054477. The procedure for detecting of acetic acid and butyric acid contained in fermentation products were followed the standard examination of water and wastewater with the method number 5560D. Standard graph for pure acetic and butyric acids were plotted to calculate the concentration of these acids in the fermentation broth.

4. Results and Discussion

4.1 Optimization Parameters of Liquid-liquid Extraction

Optimization using CCD design showed that quadratic model is obtained for both acetic and butyric acids extracted, based on Model Summary Statistic (Table 2, Table 3). Thus, the design of experiment is accepted. From the table, standard deviations (AA: 2.25, BA: 0.48) were low enough and acceptable. R-squared (AA: 0.9773, BA: 0.9975) showed that the model was acceptable. Analysis of variance (ANOVA) report for Response Surface Quadratic Model implies that both analyses were significant by F value (AA: 42.92, BA: 575.48). The mathematical models for acetic acid extracted (2) and butyric acid extracted (3) fit the second order polynomial equation.

$$Y\,[AA\,(\%)] = 24.1 - 1.75A - 2.71E + 3.72AB + 2.03BC - 2.98BE - 3.96CE - 1.99DE - 6.66A^2 - 9.62C^2 \quad (2)$$

$$Y\,[BA\,(\%)] = 93.36 + 1.17D + 0.47AB + 0.38AD - 0.54AE + 0.77BC + 0.42BD + 1.57CD - 1.25DE - 4.25A^2 - 2.89B^2 - 4.84C^2 - 1.98D^2 - 2.03E^2 \quad (3)$$

Table 2 Fit summary analysis (Model Summary Statistics) for acetic acid extracted

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	9.46	0.1208	0.0209	-0.1368	5094.88	
2FI	8.35	0.4705	0.2369	-0.0259	4597.88	
Quadratic	2.25	0.9673	0.9448	0.8953	469.27	Suggested
Cubic	1.84	0.9894	0.9629	0.8451	694.07	Aliased

Table 3 Fit summary analysis (Model Summary Statistics) for butyric acid extracted

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	7.65	0.0207	-0.0906	-0.1884	3122.46	
2FI	8.38	0.0902	-0.3112	-0.8019	4734.45	
Quadratic	0.48	0.9975	0.9958	0.9932	17.74	Suggested
Cubic	0.56	0.9983	0.9942	0.8954	274.94	Aliased

The single factor for pH was done before run the optimization for other parameters. From the data obtained in Table 4 showed that pH 3 and pH 8 had highest VFA extracted (%). Acetic acid extracted was 5.03% higher when treated leachate adjusted to pH 3 rather than pH 8. Meanwhile, when treated leachate adjusted to pH 8, the butyric acid extracted was 0.57% higher than pH 3. Thus pH 3 was chosen because the significant effect on acetic acid extracted.

Table 4 Varies pH of treated leachate to VFA extracted by adsorption method.

pH of Treated Leachate	Acetic Acid Extracted (%)	Butyric Acid Extracted (%)
Unaltered pH	61.78	94.33
3	79.48	99.21
4	75.88	99.26
5	72.10	99.41
6	70.07	99.63
7	67.43	99.67
8	74.45	99.78
9	75.62	94.33

Quadratic model is obtained for both of analyses result by CCD as the result of optimization of parameters involve in extraction of acetic and butyric acids (Table 5, Table 6). The standard deviations (AA: 0.71, BA: 0.58) and R-squared (AA: 0.9941, BA: 0.9955) showed in tables were acceptable. F value in ANOVA (AA: 181.92, BA: 238.98) showed the model is significant.

The mathematical models for both analyses which were acetic acid extracted (4) and butyric acid extracted (5) fit the second-order polynomial equation as given below:

$$Y\,[AA(\%)] = 68.50+1.84A+1.83B+4.69D+ 5.11A^2+ 9.11B^2 – 3.89D^2 \quad (4)$$

$$Y\,[BA(\%)] = 78.68+1.34A+2.32B+4.19D+ 1.30AB+1.16AC+1.03BD+5.16A^2+8.74B^2–4.38D^2 \quad (5)$$

4.3 Verification of Predicted Optimal Point by CCD in Actual Experiment

The optimal condition predicted using mathematical model generate by RSM and suggested points were verified experimentally (Table 7). The percentages of acetic and butyric acids extracted obtained experimentally were compared to the value predicted by RSM. The suggested point liquid-liquid extraction (A: 34.95 ^{0}C, B: 4.81, C: 16.78 h, D: 175.36 rpm, E: 14.09%) and adsorption (A: 19.79%, B: 40 ^{0}C, C: 9.45h, D: 179.89 rpm) showed that the experiment were acceptable due to the similarities above 95%. Table 7 also showed that adsorption method is the best method of extraction as the actual percentage in extracting acetic and butyric acid were higher than liquid-liquid extraction method.

Table 5 Fit summary analysis (Model Summary Statistics) for acetic acid extracted

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	5.48	0.4119	0.3178	0.1876	1037.4	
2FI	6.15	0.4366	0.1401	-0.7685	2258.27	
Quadratic	0.71	0.9941	0.9887	0.9779	28.18	Suggested
Cubic	0.77	0.9967	0.9864	0.8128	239.05	Aliased

Table 6 Fit summary analysis (Model Summary Statistics) for butyric acid extracted

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	5.28	0.3918	0.2944	0.1515	970.58	
2FI	5.72	0.4556	0.1691	-0.6185	1851.39	
Quadratic	0.58	0.9955	0.9914	0.9848	17.41	Suggested
Cubic	0.67	0.9973	0.9887	0.9684	36.15	Aliased

Table 7 Percentage of VFA extracted (%) between predicted value from RSM and actual experiment by the suggested point optimization.

Primary Recovery Method	Predicted (%)		Actual (%)		Similarities (%)	
	Acetic	Butyric	Acetic	Butyric	Acetic	Butyric
Liquid-liquid extraction	29.44	92.7091	28.10603	88.84556	95.46884	95.83262
Adsorption method	88.9438	98.5345	87.35831	94.19143	98.21742	95.59234

5. Summary

The optimum parameter and comparison between two methods of primary recovery (liquid-liquid extraction and adsorption) were conducted using response surface methodology. It showed that the optimum parameters for liquid-liquid extraction were 34.95 ^{0}C, pH 4.81, agitation speed 175.36 rpm, 14.09% volume of treated leachate at incubation time 16.78 h will extracting 28.11% acetic acid and 88.85% butyric acid. Meanwhile, adsorption method showed the highest extraction percentage of acetic acid, 87.4% and butyric acid, 94.1% with the optimum parameters of 19.8 % activated carbon weight, 40 ^{o}C, pH 3.0, 9.5 h incubation time and 179.9 rpm agitation speed. This study proves that adsorption method using activated carbon gives the highest extraction percentage rather than using liquid-liquid extraction method.

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